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ABSTRACTS

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Contents

Plenary Session I

OP1 *Trans*-acting gene regulation drives the omnigenic patterns of trait inheritance. Y. Li*, *Department of Human Genetics, University of Chicago, Chicago, IL, USA.*

Early genome-wide association studies (GWAS) led to the surprising discovery that, for typical complex traits, most of the heritability is due to huge numbers of common variants with tiny effect sizes. Previously, we argued that new models are needed to understand these patterns and proposed the Omnigenic model. In this talk, I provide a formal model in which genetic contributions to complex traits are partitioned into direct effects from core genes, and indirect effects from peripheral genes acting in *trans*. We propose that most heritability is driven by weak *trans* eQTL SNPs, whose effects are mediated through peripheral genes to impact expression of core genes. In particular, if the core genes for a trait tend to be co-regulated then the effects of peripheral variation can be amplified such that nearly all of the genetic variance is driven by weak *trans* effects. Thus our model proposes a framework for understanding key features of the architecture of complex traits.

Key Words: genome-wide association studies (GWAS)

OP2 Can molecular genetics help improve breeding for complex traits? H. Simianer*, *Animal Breeding and Genetics Group, Center for Integrated Breeding Research, University of Goettingen, Goettingen, Germany.*

Complex traits, or, more precisely, traits with a complex genetic background are typically improved by classical breeding, being a combination of 3 key elements: performance testing, breeding value estimation and selection. Molecular genetics have made a major contribution toward improving efficiency of breeding by providing tools for genomic selection. Most theoretical approaches are based on Fisher's 'infinitesimal' model and attempts to conceptually reduce the number of effective loci to a smaller number (e.g., by using Bayes'ian alphabet or conditional methods) have led, if at all, only to marginal improvements at the cost of increased computation time and reduced robustness. With the advent of efficient gene editing methods like CRISPR/Cas9 it was suggested that editing of selection candidates at a set of top targets may further increase efficiency of breeding programs. We showed in a simulation study, that the critical step in this concept is the identification of suitable targets for editing, which will not be easy to overcome. Recent results from studies of adaptation point to a high non-additivity in the pathways underlying complex traits, including phenomena like epistasis, canalization, genetic redundancy and buffering, or even an 'omnigenic' inheritance. It will be illustrated with an example that even if a gene is identified by GWAS, is physiologically plausible, and is functionally validated (e.g., by a knockout model), this does not mean that editing such a gene will have a predictable and desired outcome on the phenotype under a complex genetic architecture. While molecular genetic approaches including gene editing will continue to play a major role in breeding for monogenic traits and improving our understanding the genetic architecture underlying complex traits, it is hard to foresee another 'quantum leap' in breeding efficiency comparable to the genomic revolution for complex traits.

Key Words: complex traits, genetic architecture, gene editing

OP3 Genome editing tools—Update and novel applications. L. Montoliu*, *CNB-CSIC and CIBERER-ISCIII, Madrid, Spain.*

Genome editing has boosted the field of genetic modification of animals. Initially through Zinc-Finger Nucleases and TALEN and, in the last 6 years, through the use of CRISPR-Cas9 tools, genome editing approaches have revolutionized all aspects of biology, including animal genetics. It is now possible to move selected traits, known to be associated with certain genetic variants, between strains, directly, editing on the genome of the intended recipient strain to be modified and implementing the aimed allelic difference detected in the donor strain, thereby avoiding the tedious and inefficient process of moving a trait between strains through standard breeding techniques, an endless project full of problems and suboptimal outcomes. Genome editing is therefore providing a simpler, more direct and more effective way of modifying an animal genome at will in just one generation, microinjecting ribonucleoprotein CRISPR-Cas9 complexes into 1-cell embryos and detecting the intended changes in the progeny. Of course, the process is not free of unexpected variability. The on-target and/or off-target genome modifications can occur and result in genetic noise and/or the birth of mosaic individuals carrying multiple alleles, among which there will always be the planned allele. On-target and off-target will normally be segregated through meiosis in subsequent generations. The systematic genotyping of all individuals obtained from F_1 would enable to select those carrying the desired modified allele, while discarding the rest. In addition, once the edited allele has been fixed, any other off-target allele should be segregated away and, if co-linked, a sufficient number of progeny should be analyzed to be able to come across of at least a single individual where the planned mutation and the potentially confounding off-target mutation have been effectively separated. In this talk, I will illustrate the process with graphics and will provide numerous examples of genome-edited animals, their uses, advantages and limitations.

Animal Epigenetics

OP4 Comparative analyses of cattle DNA methylome provide insights into tissue specific patterns, epigenomic evolution, and complex traits. G. Liu*, *Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD, USA.*

Although sperm DNA methylation has been studied in humans and other species, its status in cattle is largely unknown. Using whole-genome bisulfite sequencing (WGBS), we profiled the DNA methylome of cattle sperm through comparisons with human and mouse sperm, as well as with 3 cattle somatic tissues (mammary gland, brain, and blood). Large differences between cattle sperm and somatic cells were observed in the methylation patterns of global CpGs, pericentromeric satellites, partially methylated domains (PMDs), hypomethylated regions (HMRs), and common repeats. The sperm-specific HMRs also targeted to distinct spermatogenesis-related genes, including BOLL, MAEL, ASZ1, SYCP3, CTCFL, MND1, SPATA22, PLD6, DDX4, RBBP8, FKBP6, and SYCE1. By comparing sperm DNA methylomes and large-scale genome-wide association signals (GWAS) between human and cattle, we aimed to examine the DNA methylome evolution and its associations with complex phenotypes in mammals. We validated our findings using sperm-retained nucleosome, preimplantation transcriptome, and adult tissue transcriptome, as well as sequence evolutionary features, including motif binding sites, mutation rates, recombination rates and evolution signatures. Additionally, we showed that DNA methylation alterations induced by aging impacted male fertility in cattle. In conclusion, our results demonstrate importance roles of epigenome evolution in shaping the genetic architecture underlying complex phenotypes, hence enhance signal prioritization in GWAS and provide valuable information for human neurological disorders and livestock genetic improvement.

Key Words: sperm DNA methylation, epigenome evolution, hypomethylated region, large-scale GWAS, cattle complex traits

OP5 Identification of orthologous tissue-specific enhancer-gene pairs across chicken, pig and cattle. M. M. Halstead*[1], C. Kern[1], Y. Wang[1], X. Xu[1], G. Chanthavixay[1], P. Saelao[1], S. M. Waters[1], J. F. Medrano[1], A. L. Van Eenennaam[1], M. E. Delany[1], H. H. Cheng[2], C. K. Tuggle[3], C. W. Ernst[4], H. Zhou[1], P. J. Ross[1], *[1]University of California Davis, Davis, CA, USA, [2]USDA, ARS, ADOL, East Lansing, MI, USA, [3]Iowa State University, Ames, IA, USA, [4]Michigan State University, East Lansing, MI, USA.*

Distal regulatory elements, such as enhancers, contribute extensively to phenotypic differences between species. However, efforts to identify enhancers and link them to their target genes have proven difficult, as enhancers tend to evolve rapidly and their target genes can be hundreds of kilobases away. In mammals, Villar et al. (2015) observed that conservation of enhancer activity generally did not correlate with overall sequence conservation, although enhancers with conserved activity did show enrichment for shorter motifs, such as transcription factor binding sites (TFBS) – especially for transcription factors with tissue-specific functions. In liver, the subset of enhancers that demonstrated conserved activity across 20 mammals were enriched for liver-specific TFBS and flanked genes with liver-specific functions. It remains unclear why some enhancers are more conserved than others, given that most are not subject to strong evolutionary constraint. Here we identify orthologous tissue-specific enhancer-gene pairs across chicken, cattle, and pig in 5 tissues – adipose, brain, liver, lung, muscle and spleen. Active enhancers were predicted based on the presence of H3K27ac, H3K4me1, and chromatin accessibility. To narrow down the search space for potential target genes, topologically associated domains (TADs) were predicted from CTCF binding sites, determined from ChIP-seq, and tissue-specific enhancers were linked to orthologous genes with conserved tissue-specific expression within the same TADs. Nearly all orthologous genes with tissue-specific expression occurred in TADs containing tissue-specific enhancers, which were then characterized for TFBS enrichment and synteny. Broadly, tissue-specific genes and enhancers tended to co-occur in the same TADs. TADs containing more genes with conserved tissue-specific expression were enriched for enhancers with specific activity in the same tissue. These results indicate that conserved enhancers are not limited to mammals, and span more broadly across vertebrates. The functional conservation of this subset of enhancers over more than 300 million years of evolution suggests that they may be integral for modulating tissue-specific expression of orthologous genes.

Key Words: epigenetics, enhancer, conservation, livestock

OP6 Whole-genome DNA methylation profiles in the central nervous system of sheep naturally infected with scrapie. A. Hernaiz*[1], S. Sentre[1], R. Bolea[2], O. López-Pérez[1,2], A. Sanz[1], P. Zaragoza[1], J. J. Badiola[2], J. M. Toivonen[1], H. Filali[2], and I. Martín-Burriel[1,2], *[1]LAGENGIO, Faculty of Veterinary, IA2, University of Zaragoza, Zaragoza, Spain, [2]CIEETE, Faculty of Veterinary, IA2, University of Zaragoza, Zaragoza, Spain.*

Scrapie is a transmissible spongiform encephalopathy (TSE) that affects sheep and goats and it is considered a good natural animal model to study prion diseases. Although changes in DNA-methylation occur in the central nervous system (CNS) in many neurodegenerative diseases, potential DNA-methylation alterations have not been investigated in any TSE models or naturally infected cases. We present here a whole-genome sequencing analysis of bisulfite treated DNA (WGBS) obtained from thalamus of 4 naturally scrapie infected sheep and 4 controls. All animals were female, carried the ARQ/ARQ genotype for the *PRNP* allele and were sacrificed with similar age (4 to 6 years old). No differences in the genomic percentage of methylated cytosines (5mC) were observed between the scrapie group and the control. As expected, the highest genomic percentage of 5mC was found in the CpG regions. Although genomes displayed similar average methylation levels, we identified 39 differentially methylated positions (DMP) in promoter regions and a total of 8,907 differentially methylated regions (DMR) of which 7,511 were located in intron regions, 2,426 in exon regions and 955 in promoter regions. Gene Ontology enrichment revealed that hypomethylated DMRs were enriched in genes involved in transmembrane transport and cell adhesion whereas hypermethylated DMRs were related with intracellular signal transduction genes. The cellular prion protein (PrP^C) seems to act as an important regulator of cell adhesion and membrane barrier function. Therefore, the enrichment observed in these cellular processes when PrP^C has lost its function after the conversion to PrP^{Sc} could be indicative of an epigenetic regulation of these mechanisms. KEGG Pathway Enrichment Analysis of DMP or DMR related genes displayed Calcium signaling and ABC transporters as the most enriched pathways for hypermethylated DMR; and calcium binding, circadian entrainment and cAMP signaling pathways for hypomethylated DMR. The effect of differential methylation on the expression of some of these genes will be confirmed in further studies as well as the functional implication that the methylation changes could have.

Key Words: genome sequencing, DNA methylation, prion, brain, sheep

OP8 The genome-wide RNA-chromatin interactions revealed by GRID-seq in skeletal muscle of three pig breeds. L. Fu, J. Li*, Y. Liao, P. Zhou, X. Li, and S. Zhao, *Key Laboratory of Pig Genetics and Breeding, MOA China, Huazhong Agricultural University, Wuhan, Hubei Province, China.*

Chromosomes of eukaryotes are bound by a large number of coding and non-coding RNAs, providing an additional layer of epigenomic information. Therefore, the ability to identify these RNAs from actively transcribing genes associated with enhancers and promoters will advance understanding of the relationship between TF binding, chromatin status and the regulation of gene expression. Global RNA interactions with DNA by deep sequencing (GRID-seq) is a recently developed technique used to capture the entire repertoire of chromatin interacting RNAs and their respective binding sites. In this study, we use GRID-seq to capture the whole collection of chromatin-binding RNAs of 2-week skeletal muscle tissues in 3 pig breeds, Duroc, Large White and Enshi Black, focusing on the breed-specific landscape of the identity and binding-sites of essential functional RNAs during muscle growth. The number of detected RNAs varied in 3 breeds, with 189 novel RNAs highly enriched on chromatin in Enshi Black, while 300 and 287 novel RNAs were identified in Duroc and Large White, respectively. To further characterize these newly identified chromatin-interacting RNAs, we categorize their chromatin-interactions into local (±10 Kb from their encoding genes), cis (in the same chromosomes), and trans (across different chromosomes) and noted that the majority of RNAs exhibited local and cis-interacting preference, only a few exceptions of specific RNAs were highly engaged across the genome. However, compared with Large White and Duroc, we observed a much lower degree of global trans-interaction preference in Enshi Black, 37% compared with 42% of Duroc and 54% of Large White, suggesting potential regulatory differences of skeletal muscles between Asian and European breeds. Furthermore, single-nucleotide polymorphisms (SNPs) were also integrated to reveal potential key variants of RNA-interacting sites. Together, these findings demonstrate a novel RNA-chromatin interacting model in skeletal muscle tissue, where a large set of newly identified RNAs could be further characterized with LncRNA identification and histone modification.

Key Words: pigswons

OP9 Tissue-specific characterization of the ovine methylome. A. J. Caulton*[1,2], R. Brauning[2], B. M. Murdoch[3], and S. M. Clarke[2], *[1]University of Otago, Dunedin, New Zealand, [2]AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand, [3]University of Idaho, Moscow, ID, USA.*

Over the past decade, the application of genomic technologies to animal breeding has significantly advanced the profitability and sustainability of agricultural breeding programs by facilitating selection

of genetically superior animals. However, genomic information alone explains only part of the phenotypic variance in quantitative traits. A portion of this so called 'missing variance' is embedded within the epigenome, a promising yet relatively unexplored resource to identify missing causality and heritability in complex traits and diseases. As one of the most common and stable epigenetic marks, DNA methylation plays a fundamental role in the regulation of growth and development in mammals. While the importance of DNA methylation is well established, tissue specific signatures of methylation have not been extensively characterized at the genomic level. We have employed a combination of whole genome bisulfite sequencing (WGBS) and 2 reduced representation methylation profiling assays to achieve single nucleotide resolution of DNA methylation across blood, muscle and skin tissue in sheep. This work sheds light on the level of conservation of DNA methylation between tissues and individuals and highlights the technical considerations when choosing an appropriate methylation profiling technology for research or commercial applications. In addition, as part of the Ovine Functional Annotation of Animal Genomes (FAANG) Project, we will be extending the WGBS work to characterize the methylome of over 60 tissue samples from a Rambouillet ewe whose DNA has previously been used for the de novo construction of the ovine reference genome (Oar_rambouillet_v1.0). The methylation data sets will be superimposed with gene expression profiles and chromatin architecture analyses to enhance functional annotation of the sheep genome and obtain a comprehensive picture of the ovine epigenetic landscape. Determining the functional potential of genetic variants will inform our understanding of the biological processes underlying phenotype and has application in animal breeding for use in weighted genomic selection predictions and the construction of genomic relatedness matrices using causative variants.

Key Words: methylation, sheep, functional annotation, animal breeding, epigenetics

OP10 Regulatory polymorphisms associated with allele-specific expression (aseQTL) in Nelore (*Bos indicus*) muscle. J. J. Bruscadin[1,2], M. M. de Souza[3], K. S. de Oliveira[1], A. Zerlotini Neto[4], and L. C. A. Regitano*[1], *[1]Embrapa Southeast Livestock, São Carlos, São Paulo, Brazil, [2]Graduate Program on Evolutionary Genetics and Molecular Biology, Federal University of São Carlos, São Carlos, São Paulo, Brazil, [3]Department of Animal Science, Iowa State University, Ames, IA, USA, [4]Embrapa Informatics Agriculture, Campinas, SP, Brazil, [5]Department of Animal Science, University of São Paulo, Piracicaba, São Paulo, Brazil.*

Identification of regulatory mutations is important to elucidate the molecular mechanism responsible for phenotypic variation. In previous studies we identified single nucleotide polymorphisms (SNPs) showing allele-specific expression (ASE) in the *Longissimus dorsi* muscle of Nelore cattle (*Bos indicus*). This expression pattern may result from aseQTLs (allele-specific expression quantitative trait loci), which are supposed to be cis-acting regulatory SNPs associated with ASE. Thus, this study identified aseQTLs within 1Mb of the SNPs with ASE (ASE SNPs). For this, the candidate SNPs were tested for their relationship to allelic expression, associating the genotypes to the measure of allelic expression imbalance in each ASE SNP for each animal. In addition, possible regulatory mechanisms involved with ASE were investigated, such as the location of these aseQTLs overlapping methylation in CpG islands, regions of topological association domains (TAD) and transcription factor binding sites (TFBS), particularly the CCCTC-Binding factor (CTCFs), which can promote long-range regulation in TADs. We identified 547 aseQTLs, associated with 47 different ASE SNPs. Of these, 9 aseQTLs were methylated in our population and 515 aseQTLs were within TADs. Furthermore, we found 35 different significant TFBS, present in 231 aseQTLs from which 8 were located within CTCFs biding sites. Comparison against the Cattle QTL Database revealed that 434 aseQTLs were within 302 QTL regions. These results indicate potential regulators distributed in the genome and associated with ASE that can act through specific regulatory mechanisms to influence the expression of the parental alleles. This approach is interesting for future studies aiming at the modulation of phenotypes such as animal production and meat quality.

Key Words: ASE, imprinting, SNP, bovine

OP11 Can we predict an animal biological age: A study on DNA methylation from bovine tail hair and liver tissues? L. T. Nguyen*, E. M. Ross, and B. Hayes, *Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, Australia.*

DNA methylation occurs predominantly on the cytosine nucleotide in CpG dinucleotides. In humans, the methylation states of CpG sites changes with age and can therefore be utilized as an accurate biomarker for aging. In cattle, biological age prediction based on methylation status could provide key information for genetic improvement programs. For instance, this could help to avoid the difficultly of recording chronological age (time since birth) in large animal populations for association studies. Additionally, comparing chronological age with biological age (based on methylation status) can provide important information about the stress an animal has been under during its lifetime. However, relatively little is known about DNA methylation patterns in livestock. We aimed to gain a better understanding of genome wide DNA methylation in cattle, to ultimately derive age predictions from methylation patterns. To achieve this goal, we apply a combination of different techniques including long-read Nanopore sequencing, reduce representation bisulfite sequencing, whole genome bisulfite sequencing (coverage > 30x) and Human Methylation EPIC array (>850K methylation sites) to study genome-wide bovine DNA methylation patterns. Seven tail hair and 2 liver tissue samples from cattle with known chronological age were selected and used for all 4 approaches. The set of CpG sites generated will now be used to perform age prediction analyses. Furthermore, high-resolution DNA methylation maps of bovine tail hair and liver can also provide critical insights for further epigenome studies.

Key Words: DNA methylation, whole genome bisulfite sequencing, reduced representation bisulfite sequencing, Oxford Nanopore sequencing, human methylation EPIC array

OP12 Allele-specific chromatin accessibility and histone modifications in an F_1 cross of MD resistant and susceptible chicken lines. C. Kern*[1], Y. Wang[1], P. Saelao[1], K. Chanthavixay[1], M. E. Delany[1], H. H. Cheng[2], P. Ross[1], and H. Zhou[1], *[1]Department of Animal Science, University of California, Davis, Davis, CA, USA, [2]USDA-ARS, Avian Disease and Oncology Laboratory, East Lansing, MI, USA.*

Marek's disease (MD), a T cell lymphoma induced by the highly oncogenic Marek's disease virus (MDV), is estimated to cost the worldwide poultry industry between $1 and $2 billion per year. As part of the FAANG pilot project at UC Davis, 8 tissues were collected from 2 male F_1 crosses of 2 highly inbred chicken lines with relative susceptibility and resistance to MD. Data were collected using RNA-seq, DNase-seq for open chromatin regions, and ChIP-seq to identify H3K4me3, H3K4me1, H3K27me3, and H3K27ac histone modifications as well as CTCF binding sites, and then analyzed to detect allele-specific epigenetic regulation of gene expression. A χ^2 test with FDR correction (q-value < 0.01) found 39,746 SNPs with allele-specific gene expression and 1,868 SNPs with allele-specific chromatin accessibility. For allele-specific histone modifications, 1,499 SNPs for H3K4me3, 73 for H3K27me3, 279 for H3K27ac, and 171 for H3K4me1 were identified. Finally, 369 SNPs were found with allele-specific CTCF binding. Using DNA sequencing of the parental lines, the allelic bias of each SNP was attributed to the maternal (resistant) or paternal (susceptible) line. Bias toward the maternal allele accounted for 80% of the allele-specific RNA-seq SNPs, and between 60% and 70% of the epigenetic-associated SNPs. Previous research into allele-specific expression of the same F_1 cross line under MDV infection (Perumbakkan et al., 2013) identified 4,528 SNPs of which 75% were identified from at least one epigenetic assay in our analysis using a p-value cut-off of 0.05. Future

work will identify allele-specific epigenetics unique to each tissue collected and correlate allele-specific epigenetics with tissue-specific and allele-specific expression.

Key Words: epigenetics, histone modification, Marek's disease, allele-specific, poultry

OP13 Epigenome-wide skeletal muscle DNA methylation profiles at the background of distinct metabolic types and ryanodine receptor variation. S. Ponsuksili*[1,2], N. Trakooljul[1,2], S. Basavaraj[1,2], F. Hadlich[1,2], E. Murani[1,2], and K. Wimmers[1,2], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Institute for Genome Biology, Dummerstorf, Germany.*

Epigenetic variation may emerge from selective breeding of traits related to metabolic processes or as an adaptive response to exogenous stressors. In particular, the methylation state of specific loci may be driven by a genetic variant, linking epigenetic and genetic variation. We examined DNA methylation in skeletal muscle of pig breeds differing in metabolic type, Duroc and Pietrain. More than 2000 differently methylated sites were found between these breeds. We found evidence that DNA methylation variation could be related to metabolic type (fat or lean), as shown by the change in methylation profiles and associated expression pattern of *METRNL, IDH3B, COMMD6*, and *SLC22A18*, genes involved in lipid metabolism. We included F_2 crosses of these breeds to get a first clue to DNA methylation sites that may contribute to breed differences. Moreover, we compared DNA methylation in muscle tissue of Pietrain pigs differing in genotypes at the gene encoding the Ca^{2+} release channel (*RYR1*) that profoundly affects muscle physiology. Alternative genotypes at *RYR1* exhibited 1060 differently methylated sites in functionally related genes, such as *CABP2* and *EHD*, which play a role in buffering free cytosolic Ca^{2+} or interact with the Na^{+}/Ca^{2+} exchanger. This study shows that DNA methylation differs between these specific breeds and that there are distinct differences in metabolic type and genetic background. The divergence in the level of methylation between breeds is probably the result of the long-term selection process for quantitative traits involving an infinite number of genes, or it may be the result of a major gene mutation that plays an important role in muscle metabolism and triggers extensive compensatory processes.

Key Words: DNA methylation, pig, RRBS, genetic variation, RYR

Animal Forensic Genetics

OP7 Comparing two commercial domestic dog (*Canis familiaris*) STR genotyping kits for forensic identity calculations in a mixed breed dog population sample. S. Kanthaswamy*, *Evolutionary and Forensic Genetics Laboratory, Arizona State University, Glendale, AZ, USA.*

Abstract not provided.

OP14 Application of mtDNA for determining species of forensic traces. M. Natonek-Wisniewska* and A. Radko, *National Institute of Animal Production, Balice, Poland.*

The aim of the study was to present the possibility of using mitochondrial DNA to determine the species origin of animal traces.The study included biological traces of unknown animal in the form of blood spots, which were secured on the car of a person suspected of taking part in a theft of pig.DNA was isolated in several replication using the method specific to the DNA matrix.DNA of sample was amplified using the PCR reaction and starters which ware specific for mammalians. The resulting DNA extracts were characterized by concentrations under 25ng / Î1/4l with a purity of A260/280 from 1.7 to 2.2.For all DNA samples, reaction products were obtained, which then were sequenced using an ABI Prism BigDye Terminator v1.1 Cycle Sequencing Kit and subjected to capillary electrophoresis with an automated ABI Prism 3130 sequencer (Applied Biosystems). The obtained sequences were compared with those available in GenBank, using BLAST.The sequencing reaction gave consensus forward and reverse sequences and enabled a common consensus sequence of 512 bp for the mammals primers. The sequence was 99% homologous to the bovine sequence (Bos taurus). The results allowed excluding the analyzed sample as the sample coming from pig and showed the suitability of mitochondrial DNA for the analysis of forensic traces.

Key Words: forensics, genetic identification, DNA sequencing

OP15 Multiplex PCR assay for identification and quantification of bovine and equine in minced meats using species-specific nuclear DNA sequences. X. Zhou*, W. Wang, and B. Liu, *Huazhong Agricultural University, Wuhan, Hubei, China.*

The 2013 horsemeat scandal demonstrated the importance and significance of constantly developing new analytical methods to accurately identify and quantify bovine and equine components in meats. The species-specific nuclear DNA sequences were screened based on comparative genome analysis of different species. The primers and probes designed from bovine- and equine-specific nuclear DNA sequences were critically analyzed against 50 species through available bioinformatics software, and then detected against 23 species through PCR to evaluate the specificity. The conservation of primers and probes binding regions was tested in 44 different individuals of bovine or equine. There was no cross reaction between the primers and probes, and the limit of detection is 0.05 ng in conventional PCR and real-time PCR. Real-time PCR confirmed bovine- and equine-specific nuclear DNA sequences are present as fixed copy number in the genome. A multiplex conventional PCR method was established to effectively discriminate bovine (223 bp) and equine (197 bp) DNA. A multiplex real-time PCR was developed and confirmed in 5 binary mixtures of cattle and horse DNA, exhibiting adequate parameters of trueness (R.E. $\leq$ 9.35%) and precision (R.SD $\leq$8.88%). Its application to 4 artificial meat samples comprising different weight ratios of cattle and horse showed our method holds good result and potential in the detection of meat products in real life. The method and strategy developed in this study will play an important role in combating adulteration and maintaining a fair market.

Key Words: comparative genome analysis, meat, bovine, equine, multiplex PCR

OP16 Patterns of SP-InDels for mammalian species identification in forensic casework. M. L. López Díaz, E. J. Santillana, and D. Parra*, *Departamento de Medio Ambiente, Servicio de Criminalística, Dirección General de la Guardia Civil, Madrid, Madrid, Spain.*

Species determination is an important issue in Forensic DNA Labs to determine the donor specie of the sample under investigation. The SP-InDel assay (Species Identification by Insertions/Deletions) is a fast test for identification of mammalian species. It is based on the analysis of the mitocondrial ribosomal RNA genes (Pereira et al., 2010; Carneiro et al., 2012) by the simultaneous study of 6 highly conserved regions that contains events of insertion/deletion (InDels) which are very polymorphic between species. Genotyping is based in a one-step multiplex PCR of 17 primers (6 of them labeled with 6-FAM) and fragments are electroforetically analyzed as the microsatellite typing avoiding Sanger sequencing as the study of Cytochrome *b* or COI. Each sample is identified by a pattern of a numeric profile of the combination of the length of the hypervariable regions, allowing the species discrimination. The characteristic patterns of >25 species of casework and wildlife forensic species was determined. These included domestic species as dog (*Canis familiaris*), cat (*Felis catus*), horse (*Equus caballus*),

donkey (*Equus asinus*), cow (*Bos taurus*), water buffalo (*Bubalus bubalis*), sheep (*Ovis aries*), goat (*Capra hircus*), swine (*Sus scrofa*), rabitt (*Oryctolagus cunniculus*), alpaca (*Lama pacos*), game species as red deer (*Cervus elaphus*), roe deer (*Capreoulus capreoulus*) and fallow deer (*Dama dama*), wild autochthonous species as lynx (*Lynx pardina*), bear (*Ursus arctos*), hare (*Lepus granatensis*), fox (*Vulpes vulpes*), gineta (*Genetta genetta*), stone martens (*Martes foina*), mouse (*Mus musculus*) badger (*Meles meles*), and human (*Homo sapiens*). The successful use in some forensic casework in our lab has shown satisfactory results in the identification and discrimination of mammalian species and is the main technique for detect the origin of the biologic samples.

Key Words: multispecies, genotyping, biodiversity, forensics

OP17 Analysis of the cross-reactivity of the canine ISAG 2.0 kit. M. L. López Díaz*, E. J. Santillana, and D. Parra, *Departamento de Medio Ambiente, Servicio de Criminalística, Dirección General de la Guardia Civil, Madrid, Madrid, Spain.*

The analysis of DNA in Forensic caseworks is an important tool when animals are involved. An increasing number of cases, about injuries or deaths caused by dogs, required the study of the biological traces to identify the guilty dog. The canine ISAG 2.0 microsatellite kit (Thermo Fisher) was evaluated in terms of cross-reactivity with human DNA. Some artifacts were observed in the electropherogram. Because of that we had difficulties to identify the genetic profile of the donor dog. An in vitro study of sensibility and reproducibility of the kit was performed in a mix of dog/human DNA to check the capability of discriminate them and was valuated in some forensic casework of the Criminalistic Service of the Guardia Civil from Spain.

Key Words: dog, forensics, genotyping

OP18 A robust and in-depth analysis and validation provides multiple metrics supporting use of the proposed Neogen canine parentage and profiling SNP set. O. Forman*[1], K. Lytle[2], R. Mouridsen[4], E. Laurell[3], and J. Donner[3], *[1]Wisdom Health, Waltham on the Wolds, Leicestershire, UK, [2]Wisdom Health, Vancouver, WA, USA, [3]Wisdom Health, Helsinki, Finland, [4]Eurofins, Galten, Denmark.*

Use of Single Nucleotide Polymorphism (SNP) markers in parentage testing provides an attractive alternative to the use of Short Tandem Repeats (STR) markers as they can be easily multiplexed, analysis is easy to automate and they are generally suited to high-throughput testing. SNP sites also have an extremely low mutation rate, and the chance of Mendelian inconsistencies due to formation of new alleles is far lower than for STR markers. With careful SNP selection, high power of exclusion values can be achieved and null alleles limited. Using a multi-analysis approach we demonstrate the suitability of the proposed Neogen SNP marker sets (A primary and secondary panel both of 100 SNPs – P1 and P2) for DNA profiling and parentage analyses by 1) comparing genotypes of the ISAG comparison test samples on 2 independent array platforms and a genotyping-by-sequencing (GBS) platform; 2) analysis of 50 trios with STRs and SNPs to demonstrate consistent Mendelian inheritance; 3) power of exclusion and power of discrimination (match probability) calculations across 60 breeds using at least 20 samples per breed; (Minimum values: Power of exclusion for 2 parents 1 offspring: P1 & P2 = 1.000; 1 parent, 1 offspring calculation: P1 & P2 = 0.996; Random match probability: P1 = 1 in 8.6×10^{23}); 4) manual raw genotyping data quality review in a 15,000 sample data set covering a diverse set of breed and mix breed dogs; 5) demonstration that parentage cases that could not be resolved using the current ISAG STRs, could be resolved using SNPs. We conclude that the proposed Neogen canine parentage and profiling SNP sets can be successfully used for such analysis purposes, equalling or surpassing STRs in performance. Use of a SNP-based genotyping system for DNA profiling is therefore justified.

Key Words: canine, parentage, profiling, identification, SNPs

OP19 Canine-specific STR typing of traces on the attacked cat. J. Zabavnik Piano*[1], M. Gombac[2], and M. Cotman[1], *[1]Institute of Preclinical Sciences, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia, [2]Institute of Pathology, Game, Fish and Bees, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia.*

According to legislation a dog that has bitten a man or an animal is qualified as a dangerous dog, therefore their owners must ensure the physical protection, in public places they should be on a leash and wearing a muzzle to avoid attacks. A corpse of a male pet cat with many lacerations was found on a street. The owner thought it was killed by a dog, therefore she reported this incident to the veterinary inspection and submitted the cadaver for the forensic necropsy. At the necropsy, the cat was severely anemic and numerous bite wounds, a comminute fracture of the sternum, pulmonary laceration and a duodenal perforation were discovered. The samples of the swabs around the bite wounds, presumably dog's hair caught in cat's claws and the cat's claws were taken for molecular investigation to determine whether these traces belong to a dog. Genomic DNA was extracted by QIAamp DNA Investigator Kit (Qiagen). The presence and identity of DNA in the samples was determined by PCR amplification of 18 short tandem repeats (STRs) and amelogenin gene using a set of Canine Genotypes, Panel 1.1 (Finnzymes Diagnostics, Espoo). The fluorescently labeled products were separated on an automatic ABI 3130 Genetic Analyzer (Applied Biosystems). The genetic profiles were determined by GeneMapper v 3.7. software. For all the examined samples canine specific STR profiles were obtained. The STR profile determined in the sample from claw belongs to more than one canine animal, STR profiles from the other samples were weaker with some peaks dropouts. On amelogenine locus only profile for X chromosome was obtained. The use of dinucleotide repeats in forensic genetics is not recommended, except for those markers that are already used widely in animal genetic studies. We were using commercially available canine genotyping kit with 17 dinucleotide markers and one tetranucleotide STR that are recommended for parentage testing and is routinely used in our laboratory. From the results we conclude that the attacker was a female dog and probably more than one dog was involved in the attack.

Key Words: animal forensics, dog attack, saliva stain, canine short tandem repeats profiling

OP20 Investigation of SNP marker combination for new Korean native chicken breeding stocks identification using high-density SNP chip data. S. H. Cho*[1], D. Seo[1], H. Kim[2], S. H. Lee[1], and J. H. Lee[1], *[1]Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea, [2]Insilicogen Inc, Yongin-si, Gyeonggi-do, Republic of Korea.*

Korean native chicken is a unique genetic resource, which has considered for the conservation of their genetic diversity and developing commercial native chicken breeds. The Korean government has launched a project to develop new chicken breeding stocks to meet the needs of consumers. On the other hand, researchers have started to develop genetic markers that can distinguish new commercial native chicken breeds from other breeds. In this project, high-density SNP array data were used to identify breeds and finding a minimum number of optimized markers for commercial use. A total number of 285 (15 Korean chicken lines and 5 commercial chicken lines) genomic DNA samples were extracted from chicken blood and genotyped by 600K high-density SNP array. A total number of 96 candidate markers for breed identification was selected by case-control association test using PLINK 1.9 software from 580,961 SNPs that have undergone a QC process. In this approach, the Korean chicken H and F lines, which are the essential lines for the production of new chicken breeding stocks, were assigned into case group and the other chicken lines were assigned to the control group. To organize optimized marker combinations based on the selected candidate markers, we generated 200 virtual offsprings from existing genotype data and selected 7 sets of markers through supervised machine learning models. Additional genotyping informa-

tion from 190 chickens was obtained and evaluate the accuracy of the marker combination by a blind test. Two kinds of optimal models were selected, which are Random Forest and AdaBoost among the 7 tested machine learning models. These 2 models suggested that the optimized marker combination were 40 and 36, respectively, and the accuracy of identification was 93.30% and 99.36%, respectively. These results can provide useful information for the identification of new chicken breeding stocks in the market with further verifications.

Key Words: Korean native chicken, SNP, new breeding stock, breed identification

Applied Genetics and Genomics in Other Species of Economic Importance

OP21 Invited Workshop Presentation: American mink genome sequencing project. Y. Miar*[1], G. Plastow[2], Z. Wang[2], and M. Sargolzaei[3,4], *[1]Dalhousie University, Truro, Nova Scotia, Canada, [2]University of Alberta, Edmonton, Alberta, Canada, [3]University of Guelph, Guelph, Ontario, Canada, [4]Select Sires Inc, Plain City, OH, USA.*

The fur industry is one of the oldest and the most historically significant industries in Canada. The industry has used American mink (*Neovison vison*) as the major source of fur for decades because of their high-quality fur and wide range of colors. This project will seek to (1) create the first accurate whole-genome sequence assembly of mink using next-generation sequencing technology to help understanding the biology and evolution of the order Carnivora, (2) design a robust and informative SNP assay (50–60K) for genomics discovery in mink, (3) discover genome structure and signature of selection as well as identify new genetic variants explaining variation in economically important traits, and (4) identify the genetic relationships among these economically important traits including feed efficiency, fur quality, reproductive performance, growth rate and pelt size. One hundred mink DNA samples from the Canadian Centre for Fur Animal Research (CCFAR) at Dalhousie Agriculture Campus (Truro, Nova Scotia), and 2 breeding populations (Blackstone Ridge Farm Inc., Berwick, Nova Scotia, and Parkinson Fur Farm, Rockwood, Ontario) have been sequenced using next generation whole-genome sequencing to high coverage (>30x) to create the first SNP assay for American mink. A DNA panel composed of 100 American mink from 7 to 10 breeds will be assembled to identify the most homozygous individual as the reference animal for whole-genome sequence assembly development. The phenotypic data and DNA samples from 1,606 animals were collected and these mink will be genotyped using the customized assay for designing a marker assisted selection (MAS) approach and assessing the potential of genomic selection (GS) in mink production systems. The ultimate objective is to develop the new tools for implementation of MAS or GS in mink breeding programs for development of superior, highly efficient, and healthy animals. This approach will help improve the overall performance of the Canadian mink industry, which is now in difficulty due to several economic factors such as declining fur prices, and high feed costs.

Key Words: American mink, genomics, feed efficiency, fur quality, growth and reproduction traits

OP22 Development of a new multiplex system for analysis of 16 microsatellite markers of reindeer (*Rangifer tarandus*). O. V. Babayan[1], Y. A. Stolpovsky[2], M. V. Kholodova[3], and V. A. Orekhov*[1], *[1]GORDIZ Ltd., Moscow, Russia, [2]Vavilov Institute of General Genetics Russian Academy of Sciences, Moscow, Russia, [3]A.N.Severtsov Institute of Ecology and Evolution Russian Academy of Sciences, Moscow, Russia.*

Reindeer breeding is essential part of life for the native ethnic groups of the North. The genetics structure of reindeer populations is not studied well. Published data is mainly based on different sets of markers and cannot be compared. There is no recommended standard marker panel for reindeer. We combined a set of previously published markers that can be amplified simultaneously in one reaction. We reviewed 41 known microsatellite markers to choose the most suitable marker set for the multiplex system. Fifteen markers were previously used in the study of reindeer. Twenty-six tetranucleotide markers were previously described in the study of California elk. We selected markers that meet the following requirements: high amplification efficiency and specificity in presence of primers of other markers in the same reaction, high level of polymorphism and allelic range suitable for combination with other markers in one multiplex to avoid overlapping. Eight markers appeared to be monomorphic for reindeer. After optimization stage we selected 16 markers including 10 dinucleotide (Rt6, BMS1788, Rt30, Rt1, Rt9, Rt7, Rt24, FCB193, BMS745, NVHRT16) and 6 tetranucleotide (OheQ, C217, C32, T40, C276, C143) markers. We used new developed 16-plex system for genotyping of 288 reindeers from 7 populations from different regions of Russia including 2 wild populations from Yamalo Nenets region and 5 domestic populations from Tuva, Yakutia, and Yamalo Nenets region. Seven most polymorphic loci (Rt6, BMS1788, Rt1, Rt9, Rt24, FCB193, OheQ) have PIC value > 0,800. Only one locus has PIC < 0,4 (C217 with PIC 0,195). Overall heterozygosity was quite high for all populations (>0,600) which indicates a high level of genetic diversity in studied populations. Private alleles were identified for each population. Obtained data allowed clearly differentiate the wild reindeer from domestic animals. There was significant difference (Fst) between domestic reindeers from geographically distant regions. Obtained results show that developed multiplex system can be used for genetic monitoring of breeds, studies of interbreed differentiation and paternity testing of reindeers.

Key Words: reindeer, microsatellite, genotyping

OP23 Association analysis of coat color in blue wildebeest. R. Van Deventer*[1,2], R. Roodt-Wilding[2], and C. Rhode[2], *[1]Unistel Medical Laboratories, Tygerberg, Western Cape, South Africa, [2]Stellenbosch University, Stellenbosch, Western Cape, South Africa.*

The blue wildebeest (*Connochaetes taurinus*) is an economically important African antelope species that is widely utilized in the South African wildlife industry. The golden color variant has become one of the most common variants that wildlife ranchers breed with. To facilitate breeding management, it has become important to determine the genetic underpinning of the golden coat color. Despite the economic importance of blue wildebeest, very few genomic resources are currently available for this species that can be used for management and research. In this study a genotyping-by-sequencing (GBS) approach was used to discover and genotype SNPs in blue wildebeest, which were then used in a case-control genome-wide association study (GWAS) to identify putative candidate genes involved in pigmentation. The study population consisted of 94 blue wildebeest, including 36 blue wildebeest with the golden coat color variation. The DArTseq genotyping platform was used for SNP discovery and a total of 20,563 SNPs were generated. The SNP marker sequences were mapped to the assembled *Bos taurus* genome to identify putative cattle orthologous genes. The GWAS identified 453 SNPs that were significantly associated with coat color in blue wildebeest (P-value ≤0.001). Of these, five of the SNP sequences mapped to *B. taurus* genes that could possibly be involved in pigmentation based on previous literature reports. An additional three SNPs, with an association P-value ≤0.05 that mapped to well-known pigmentation genes, were also considered. Based on the reported biological function, the *MYO5C*, *MYO7A*, *SLC6A3*, *SLC28A2*, *FZD4*, *TYR*, and *DRD2* genes are promising candidate genes for coat color determination in blue wildebeest. This is the first large genetic polymorphism discovery study performed in an African antelope species that is farmed commercially, and provides a foundation to further investigate

the causative mutations and biological pathways that contribute to coat color variation in this species.

Key Words: blue wildebeest (wild species), genome-wide association, SNP, coat color, management

OP24 Estimation of genomic breed composition of individual animals in composite beef cattle. Z. Li[1,2], X.-L. Wu*[1,3], W. Guo[2], J. He[1,4], H. Li[1,3], G. Rosa[3], D. Gianola[3], R. Tait Jr.[1], J. Parham[1], J. Genho[1], T. Schultz[1], and S. Bauck[1], *[1]Biostatistics and Bioinformatics, Neogen GeneSeek, Lincoln, NE, USA, [2]Department of Animal Science, University of Wyoming, Laramie, WY, USA, [3]Department of Animal Sciences, University of Wisconsin, Madison, WI, USA, [4]College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan, China.*

Three statistical models (admixture, linear regression, ridge regression BLUP) and 2 strategies for selecting SNP panels (maximum Euclidean distance and uniform spacing) were compared for estimating genomic breed composition (GBC) in 2 composite breeds: Brangus (62.5% Angus and 37.5% Brahman) and Santa Gertrudis (62.5% Milking Shorthorn and 37.5% Brahman), respectively. Each animal was genotyped with a GeneSeek Genomic Profiler bovine 50K SNP chip. Estimated GBC were consistent among uniformly spaced SNP panels of varying sizes and highly comparable between the 3 methods, but estimated GBC showed considerable differences between the 3 methods when less than 10,000 reference SNP panels were selected by maximizing Euclidean distance of allele frequencies between the ancestry breeds. Of the 3 methods, the admixture model performed most consistently among various SNP panel sizes, regardless of the 2 strategies for selecting these SNP panels. For the remaining 2 models, optimally spaced SNP panels needed to be 20,000 SNPs or more. Based on uniformly spaced 20K SNP panels, estimated genomic breed composition on average was 0.689 of Angus origin and 0.311 of Brahman origin for the Brangus cattle, and 0.612 of Shorthorn origin and 0.388 of Brahman origin for Santa Gertrudis cattle. Estimated genomic breed composition of ancestries for the Santa Gertrudis cattle roughly agreed with their pedigree estimated expected ratios, but estimated genomic breed composition for the Brangus cattle was 10.24% more Angus origin than the expected ratio. The latter could possibly be due to the selection of Brangus cattle for phenotypes where the Angus breed has an advantage.

Key Words: beef cattle, composite breeds, genomic breed composition, SNP, statistical models

Genetics of Immune Response and Disease Resistance

OP25 From phenotype to gene discovery: A case study of host genetics influencing porcine circovirus 2 susceptibility. L. Walker[1], H. Wijesena[1], K. Sutton[1], H. Vu[1], D. Nonneman[2], T. Smith[2], G. Plastow[3], S. Kachman[1], and D. Ciobanu*[1], *[1]University of Nebraska-Lincoln, Lincoln, NE, USA, [2]USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA, [3]University of Alberta, Edmonton, AB, Canada.*

Porcine Circovirus type 2 (PCV2) is the etiological agent of a group of associated diseases (PCVAD) that impact production efficiency and can lead to mortality. The objective of this research was to characterize the role of host genetics in susceptibility to PCV2. A genome-wide association of experimentally infected pigs (n = 974) genotyped with Porcine SNP60 BeadArray, provided evidence that host genetics has an important role in PCV2 susceptibility. The SNP genotypes combined explained 64% of the phenotypic variation for viral load with 2 major QTLs identified on SSC7 and SSC12. Gene annotation in the QTL regions was integrated with RNA/genome sequencing, high-density genotyping and in vitro siRNA/gene editing models to uncover genes and functional polymorphisms that could explain variation in susceptibility to PCV2. A missense polymorphism (*SYNGR2 p.Arg63Cys*) located in *SYNGR2*, was found to be responsible for the effect mapped on SSC12. The direct role of *SYNGR2* in PCV2 replication was demonstrated by in vitro siRNA and gene-editing models. The QTL mapped on SSC7 is located near the swine leukocyte antigen complex class II (SLAII) locus, a region involved in antigen recognition and immune response in a variety of infectious diseases and characterized by extended LD and highly polymorphic genes. Since the Porcine SNP60 BeadArray is relatively scarce in SNPs located in SLAII region, a novel Affymetrix Axiom myDesign SNP array was designed (SowPro90, 103,476 SNPs) which saturated the SLA locus with over 3,100 SNPs (70X fold increase). This information provided critical knowledge of the effective genetic diversity in pigs and the role of this locus in viral disease susceptibility. Together this research will aid in the development of genetic tests for early identification of genetic susceptibility to PCV2 and ability to monitor genetic diversity that could lead to improvement in the general health and welfare of pigs. USDA is an equal opportunity provider and employer.

Key Words: pig, circovirus, disease resistance, virus

OP26 Genetic markers for improved resilience to PRRSV-induced abortions in sows. R. N. Pena*[1], C. Fernández[2], M. Blasco-Felip[3], L. J. Fraile[1], and J. Estany[1], *[1]Departament de Ciència Animal, Universitat de Lleida, Lleida, Spain, [2]INZAR, S.L, Zaragoza, Spain, [3]Veterinarian, Zaragoza, Spain.*

The concept of resilience is used in production systems to define the capacity of animals of overcoming internal and external stressors. In pig production, responses to the porcine reproductive and respiratory syndrome virus (PRRSV) has been intensively studied as this infection cannot be efficiently controlled through vaccination. An epidemic PRRSV outbreak causes severe losses to the industry. In the reproductive case, there is an increase in the number of death and mummified piglets at delivery, and in the number of spontaneous abortions, particularly at the end of pregnancy. The individual response of the sow to the infection has a clear genetic component. Therefore, it is possible to select for females more resilient to the infection. In an effort to identify genetic markers associated with better ability to cope with PRRSV infection, we have tested a panel of 8 markers in 2 commercial pig populations with a high rate of abortions (about 30%) during a PRRSV outbreak. A subset of 180 (population 1) and 71 (population 2) sows were used for the analysis. The markers included 2 partially linked SNP markers (WUR1000125 in *GBP1* and rs340943904 in *GBP5*) in chromosome 4; 2 SNP markers in *CD163* (c.3534C > T and c.2494G > A); 2 in *HDAC6* (c.35G > A and c.2360C > T); a 275 bp insertion in the promoter of *MX1;* and a SNP substitution in the *USP18* promoter –1533G > A. A logistic regression was used to estimate the probability of abortion for each genotype. The *USP18* marker did not segregate in these populations. The rest of the markers segregated with a MAF ranging from 0.20 to 0.32. The abortion rate was significantly different (range 1.5 to 9 times) in the homozygous sows for the minor allele of *GBP1*, *GBP5*, *MX1*, CD163:c.2494G > A and HDAC6:c.35G > A markers. Heterozygous sows behaved like minor homozygous sows for GBP1, GBP5, CD163:c.2494G > A and HDAC6:c.35G > A but not for MX1. In addition, we also investigated the association of these markers with prolificacy traits using information of 592 farrowings from sows in population 1. Sows homozygous for the major allele of the *HDAC6*:c.35G > A marker had 0.88 piglets born dead less and 0.90 weaned piglets more ($P < 0.05$) than the other genotypes. These results are currently being validated in an independent and larger sow population surveyed for 6 years over PRRSV endemic and epidemic phases.

Key Words: pigs, resilience, reproduction, PRRS, abortion

OP27 Genomic factors affecting host resistance to highly pathogenic avian influenza. W. Drobik-Czwarno*[1,2], C. Donnelly[3], A. Wolc[2,4], J. E. Fulton[4], J. Arango[4], J. Smith[3], and J. Dekkers[2], *[1]Warsaw University of Life Sciences, Warsaw, Poland, [2]Iowa State University, Ames, IA, USA, [3]The Roslin Institute, Easter Bush Campus, Midlothian, UK, [4]Hy-Line International, Dallas Center, IA, USA.*

Highly pathogenic avian influenza (HPAI) has a profound effect both on the global poultry industry and public health. Although host resistance to HPAI is still not fully understood it is known to have a substantial genetic component. In this study, DNA samples from survivors and genetically matched controls from an H5N2 outbreak in 2015 in Iowa and an H7N3 outbreak in 2016 in Mexico were analyzed using full genome sequence information on 70 Iowa samples (15X) and 34 samples from Pedigreed individuals in Mexico (30X). Raw reads were mapped to the chicken reference genome, build 5. After marking duplicates, indel realignment, and base quality score recalibration, Single Nucleotide Polymorphisms (SNPs), along with short Insertions and Deletions (INDELs) were called using GATK 3.8. Variants obtained with joint genotyping were recalibrated based on 1 million known validated SNPs and filtered. For additional quality control, common SNPs were compared with variants genotyped with a 600k Affymetrix array for all individuals from the Iowa data set. Genotype concordance (GC) was above 90% for the majority of individuals, with mean GC equal to 92% and ranging from 76.7 to 97.3%. Imputation of missing genotypes was performed with Beagle 5 for the Iowa data set with 88% concordance for reference homozygotes, 86% for heterozygotes, and 77.7% for alternate homozygotes. Imputation to full sequence was then performed for an additional 85 Iowa samples genotyped on the 600k array. Files before and after imputation from both outbreaks were screened for allele frequency differences between controls and survivors. Additionally, predicted functional impact of variants, known biological information, and expression data were used to prioritize variants. Several variants and associated genes were identified that may be contributing to resistance mechanisms. Selected candidate genes and mutations are currently being functionally tested in vitro. The observed variation in resistance to HPAI can be further capitalized on through genomic selection or by providing targets for genome editing and improved vaccine design.

Key Words: highly pathogenic avian influenza, chicken, whole-genome sequencing

OP28 Differential H3K27ac peaks within bursa tissue of two inbred chicken lines under NDV infection and heat stress. G. Chanthavixay*[1], C. Kern[1], Y. Wing[1], P. Saelao[1], S. Lamont[2], R. Gallardo[3], N. Chubb[4], R. Gonzalo[4], and H. Zhou[1], *[1]Department of Animal Science, University of California, Davis, CA, USA, [2]Department of Animal Science, Iowa State University, Ames, IA, USA, [3]School of Veterinary Medicine, University of Califo, Davis, CA, USA, [4]Zoetis Inc, Kalamazoo, Mi, USA.*

Poultry within developing countries are greatly impacted by Newcastle Disease (ND), and additional abiotic stressors upon those birds are increasing due to global warming. B cells that develop within the avian-specific organ, bursa of Fabricius, aid in the clearance of pathogens and are crucial for future protection upon later infections of the same pathogen. Our project aims to study the genome-wide H3K27ac histone modifications within the bursa tissue to observe changes of activity in promoters and enhancers in response to treatment, and also to explore different H3K27ac profiles due to genetics. Therefore, we subjected 2 genetically distinct chicken lines, Leghorn and Fayoumi, at the age of 3 weeks to both infection with Newcastle Disease Virus and chronic heat stress, then performed ChIP-seq for H3K27ac on the bursa tissue collected at 6 d post infection (6DPI) for 2 individuals from each treatment-line group (4 groups). H3K27ac peaks were called using the MACS2 peak caller (q value < 0.01) for each sample, called peaks were confirmed between the 2 biological replicates for each group, and all sets of peaks were merged for one total set of 67,122 peaks. Read depth was quantified for each peak region in each sample and differential peaks were determined using DESeq2 (FDR < 0.05). There were many more differential peaks between treatment groups within Leghorn (n = 912) than within Fayoumi (n = 120). The majority of the peaks, 99% in Leghorn and 70% in Fayoumi, have decreased levels of H3K27ac in the non-treated group when compared with the treated group. Contrasting the lines, there were twice as many differential peaks between nontreated birds (n = 4,579) than treated birds (n = 2,113). The target genes of proximal H3K27ac peaks (within 2000 bp of transcription start site) were identified and used for GO term and KEGG pathway analysis. Additionally, all peaks from each differential analysis were used for transcription factor motif discovery using HOMER. Our data analysis suggests that H3K27ac changes due to treatment are affecting genes and pathways crucial to B cell differentiation and that the 2 lines have distinct bursa tissue response to treatment.

Key Words: poultry, epigenetics, Newcastle Disease, ChIP-seq, H3K27ac

OP29 ChHIB promotes ubiquitination and degradation of MyD88 to suppress innate immune response. F. Wang, Q. Li*, Q. Wang, and G. Zhao, *Institute of Animal Science of Chinese Academy of Agricultural Sciences, Beijing, China.*

Uncontrolled immune responses to bacterial infection have been shown to induce pathological damage. Homeostasis regulation of immune responses to bacteria is critical for avoiding excessive production of proinflammatory cytokines and type I interferon. Here, we report that the E3 ubiquitin ligase, ChHIB, was induced by bacterial lipopolysaccharides(LPS) infection in chicken macrophage cell line HD11. Knockdown of ChHIB augmented the interleukin-1β and interleukin-8 response to LPS. Biochemical analyses showed that ChHIB interacted with the central adaptor MyD88, which converts the signal from toll-like receptors to activate following pathways. Overexpression of ChHIB promoted the degradation of MyD88 via K48-linked ubiquitination at Lys118, Lys 124 and Lys 143 through a proteasome-dependent pathway. These findings indicate that E3 ligase ChHIB is an important negative-feedback regulator of innate immune responses to bacterial lipopolysaccharides by targeting MyD88.

Key Words: poultry and related species, innate immunity, animal health

OP30 MicroRNA expression in thymus from calves in a coinfection study challenged with bovine viral diarrhea virus and *Mycoplasma bovis*. E. Casas*, S. M. Falkenberg, R. P. Dassanayake, K. B. Register, and J. D. Neill, *USDA, ARS, National Animal Disease Center, Ames, IA, USA.*

T cells are an important component of the adaptive immune response and play a critical role in memory response. The thymus is the primary site for maturation of T cells, but is susceptible to pathogen-associated depletion. BVDV is known to cause reduction of the thymus. The objective of this study was to determine differences in microRNA (miRNA) expression in the thymus of calves challenged with bovine viral diarrhea virus (BVDV), *Mycoplasma bovis*, or both pathogens. Eleven calves approximately 2 mo of age were randomly assigned to one of 4 groups: Coinfection (CO; n = 3), bovine viral diarrhea virus (BV; n = 3), *Mycoplasma bovis* (MB; n = 3), and Control (CT; n = 2). On d 0, calves in groups CO and BV were intranasally challenged with BVDV and group MB with *Mycoplasma bovis*. On d 6, CO calves were challenged with *Mycoplasma bovis*. Calves were euthanized 17 d post-challenge and appropriate tissues were collected at necropsy. MiRNAs were extracted from the thymus and sequenced on the Illumina 3000 Hi-Seq sequencer. A total of 262,698,223 sequences were identified as miRNAs. A total of 313 miRNAs were analyzed. No differences in expression were observed between groups CT and MB. Calves in group CO had greater expression of miR-200b ($P = 0.0017$), miR-9–5p ($P = 0.0044$), and miR-147 ($P = 0.003$), and had the lowest expression of miR-2285p ($P = 0.0045$), when compared with the calves in groups BV, MB, CT. Calves in groups CO and BV had greater expression of

miR-141 (P = 0.0074), miR-29c (P = 0.0086), and miR-22–5p (P = 0.0051), and had lower expression of miR-154b (P = 0.0037), when compared with calves in groups CT and MB. Differential expression of miRNAs was detected in groups that were challenged with BVDV or in coinfection with *Mycoplasma bovis*. It is possible that BVDV could be responsible for the differential expression of these miRNAs in the thymus. MiR-2285p and miR-154b were downregulated in CO, but their function is unknown. These differences in miRNA expression could potentially be responses to pathogens in the thymus during colonization and production of respiratory disease.

Key Words: cattle, infectious disease, microRNA, non-coding RNA

OP31 Identification of ovine serum biomarkers during bacterial endotoxin challenge to characterize stress resilience. D. Naylor[1], A. Sharma*[1], A. Canovas[1], B. Mallard[2], and N. Karrow[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Pathobiology, University of Guelph, Guelph, ON, Canada.*

Livestock industries have become reliant on antimicrobials to help mitigate the effects of microbial stressors. Genetic selection of animals for enhanced stress resilience could be a possible strategy to reduce their usage. The cortisol response to the microbial stressor lipopolysaccharide (LPS) endotoxin is variable and moderately heritable (h2≅0.3) in sheep. The present study used a population of 112 Rideau Arcott sheep to identify High (>300 nmol/L, n = 5), Middle (150–280 nmol/L, n = 5) or Low (<100 nmol/L, n = 5) stress responders based on their peak cortisol response to systemic LPS challenge. A panel of serum cytokines (pro- and anti-inflammatory cytokines and chemokines), as well as microRNAs (*miR-132, miR-223, miR- 1246* and *miR-145*) and rectal temperature, were measured over time (0 (pre-challenge) and 2,4,6 post-challenge) to identify potential biomarkers that may be used to characterize stress resilience. Rectal temperatures coincided with cortisol levels and remained distinctive among the stress responding groups at all-time points. Pro-inflammatory cytokines levels (IL-6, IFN-γ, IP-10, TNF-α, chemokine CCL3) were comparatively higher 2 and 4 h post LPS challenge in the H stress responders, and the anti-inflammatory cytokines IL10 and IL1RA were also comparatively higher at 4h post challenge in H stress responders. IL1β, IL-8, CCL2 and IL-4 did not exhibit any changes among groups over time. The expression of *miR-132*, which targets the TLR-4 pathway, was 186-fold upregulated in H stress responders, and 75-fold upregulated in the M and L stress responders 2 h post LPS challenge. Similarly, *miR-223*, known to regulate granulopoiesis, was upregulated 2 h post LPS challenge, whereas the expression of *miR-1246*, an apoptosis regulator, was downregulated at both 2h and 4h post LPS challenge. Our results demonstrate that serum cytokines and miRNAs are differentially expressed in variable stress responding sheep, and these may serve as future biomarkers of stress resilience in sheep.

Key Words: serum, immune response, LPS challenge, microRNA expression, sheep

OP32 Whole-blood transcriptomic signatures after intramammary challenge with *Staphylococcus aureus*. U. Thom*[1], A. Heimes[1], J. Brodhagen[1], R. Weikard[1], W. Nolte[1], J. Günther[1], F. Hadlich[1], H. Zerbe[2], W. Petzl[2], M. M. Meyerholz[2], M. Hoedemaker[3], H.-J. Schuberth[4], S. Engelmann[5,6], and C. Kühn[1,7], *[1]Leibniz Institute for Farm Animal Biology (FBN), Institute of Genome Biology, Dummerstorf, Germany, [2]Clinic for Ruminants with Ambulatory and Herd Health Services, Centre for Clinical Veterinary Medicine, Ludwig-Maximilians-University Munich, Oberschleissheim, Germany, [3]Clinic for Cattle, University of Veterinary Medicine Foundation, Hannover, Germany, [4]Immunology Unit, University of Veterinary Medicine Foundation, Hannover, Germany, [5]Institute for Microbiology, Technical University Braunschweig, Braunschweig, Germany, [6]Microbial Proteomics, Helmholtz Centre for Infection Research, Braunschweig, Germany, [7]Agricultural and Environmental Faculty, University Rostock, Rostock, Germany.*

In cattle farming, mastitis is a major issue for animal health. It is also of high relevance to consumers due to potential zoonoses and impact on antimicrobial drug resistance promoted by frequent antibiotic treatment of intramammary infections. Although mastitis is the global term of mammary gland inflammation, the host response against distinct pathogens can differ significantly. *S. aureus* is considered a pathogen eliciting subclinical mastitis restricted to the mammary gland. However, in a previous study, we noted a specific hepatic response to *S. aureus* mammary infection including innate immune reactions as well as metabolic adaptations. For the current study, we took advantage of the same animal model with first lactation halfsib heifers having inherited an alternative paternal BTA18 subregion haplotype either favorable or unfavorable regarding somatic cell count, a surrogate trait for mastitis incidence. Five weeks after parturition, 2 udder quarters of 24 animals were challenged with *S. aureus*$_{1027}$. Blood samples were collected before and 96 h after challenge at the peak of inflammation. After RNA isolation, stranded mRNA libraries were prepared for paired-end sequencing. After alignment to the bovine genome assembly and read assembly to transcripts guided by the reference annotation, a custom annotation file was generated. Based on the newly established project specific annotation, read counts per gene were established and submitted to differential expression analyses. 28,955 loci were expressed in the whole blood transcriptome. At a significance threshold of $q < 0.05$, 170 loci showed differential expression with 142 loci upregulated and 28 loci downregulated 96 h after challenge. Our data indicate that *S. aureus* infection elicited a coordinated increase in defensin gene expression in circulating whole blood cells. S100A8, an important danger associated molecular pattern (DAMP) molecule, is one of the most upregulated genes in those cells. This demonstrates that at least in early lactation *S. aureus* infection elicits an immune reaction not restricted to the location of infection, which is in line with our data obtained for the liver transcriptomic response.

Key Words: cattle and related species, immunogenomics, RNAseq, adaptive immunity, animal health

OP33 MicroRNA alterations in circulating blood plasma, cerebrospinal fluid, and isolated exosomes of sheep naturally infected with classical scrapie. O. López-Pérez[1,2], D. Sanz-Rubio[1], S. Gómez-Redrado[1], A. Sanz[1], A. Hernaiz-Martorell[1], P. Zaragoza[1], J. Badiola[2], R. Bolea[2], JM Toivonen[1], and I. Martín-Burriel*[1,2], *[1]Laboratorio de Genética Bioquímica (LAGENBIO), Universidad de Zaragoza, IA2, IIS Aragón, Zaragoza, Spain, [2]Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Universidad de Zaragoza, IA2, IIS Aragón, Zaragoza, Spain.*

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are fatal neurodegenerative disorders that include scrapie in sheep and goats. As no simple diagnostic tests are yet available to detect TSEs in vivo, it is of crucial importance to identify an easily accessible source of biomarkers to provide useful alternatives for current diagnostic methodology. For this purpose, we analyzed by quantitative reverse transcription PCR a selected set of candidate microRNAs (miRNAs) in circulating blood plasma, cerebrospinal fluid (CSF) and exosomes derived from both biofluids of naturally scrapie-infected sheep that demonstrated clear classical scrapie symptoms and pathology. Significant scrapie-associated increase was repeatedly found for miR-342–3p and miR-21–5p in blood plasma, but only miR-21–5p was also increased in plasma-derived exosomes. Similarly, miR-342–3p, miR-146a-5p, miR-128–3p and miR-21–5p showed higher levels in CSF from scrapie-infected sheep, but none of them displayed detectable expression changes in exosomes derived from CSF. Although exosomes did not display any enrichment of miRNA levels, the analysis of overexpressed miRNAs in the total biofluid could help in scrapie diagnosis once the presence of the disease is suspected. This is the first demon-

stration, to our knowledge, of miRNA alterations in circulating blood plasma and CSF in any animal suffering from naturally occurring TSE.

Key Words: sheep, microRNA, qPCR, prion, biofluids

OP34 Single-cell RNA-seq (scRNA-seq) analysis of porcine peripheral blood mononuclear cells identifies replicating B, αβ-T, and γδ-T cells, as well as all major known circulating cell types. H. Liu[1], K. Byrne[2], C. Loving[2], and C. Tuggle*[1], *[1]Department of Animal Science, Iowa State University, Ames, IA, USA, [2]USDA-ARS-National Animal Disease Center, Ames, IA, USA.*

We are interested in the RNA expression profile and gene regulatory network controlling porcine immune responses. Recently developed scRNA-seq technologies provide unprecedented opportunities to study the diversity and dynamics of the pig immune system at single-cell resolution. As part of the FAANG project, we profiled the transcriptome of approximately 16,000 peripheral blood mononuclear cells (PBMCs) from 5 healthy pigs using the 10X Genomics Chromium and the Illumina Hiseq3000 platforms. From 84 to 161 million reads were obtained for each of the 5 libraries, of which ~90% were mapped to the Sscr 11.1 genome assembly. This resulted in 24–54,000 reads/cell. Applying graph-based clustering of scRNA-seq expression data imputed by using a low-rank approximation algorithm (ALRA), we identified 21 cell clusters that group together due to a similar expression pattern. These clusters were then annotated using genes known to have cell-type specific expression in human and other species. We identified clusters containing 5 major blood cell types (B, T, monocytes, natural killer, and dendritic cells) and multiple sub-populations of B, αβ-T, and γδ-T cells were recognized at the transcriptome level for the first time in pigs. Replicating B, αβ-T, and γδ-T cells were also identified. We then used the expression data in each cluster to identify lists of genes specifically/preferentially expressed in each cluster, with tens to hundreds of such genes identified. The identification of cell type-specific gene markers will be a useful resource to develop reagents for pig immunological studies. Our study sheds light on the pig peripheral immune system and provided important resources for further study of each subtype of circulating mononuclear immune cells.

Key Words: pig, immunity, sc-RNAseq, peripheral blood mononuclear cells, transcriptomics

OP36 Lightning Talk: Use of genetic and epigenetic tools to refine a genetic marker of host resilience to ovine lentivirus infection. A. T. Massa*[1], M. R. Mousel[2,3], B. M. Murdoch[4], J. B. Taylor[5], D. P. Knowles[1], and S. N. White[1,2], *[1]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, [2]Animal Disease Research Unit, Agricultural Research Service, USDA, Pullman, WA, USA, [3]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, USA, [4]Department of Animal and Veterinary Science, University of Idaho, Moscow, ID, USA, [5]Range Sheep Production Efficiency Research, Agricultural Research Service, USDA, Dubois, ID, USA.*

Ovine lentivirus (OvLV), a retrovirus related to human immunodeficiency virus, causes maedi-visna in sheep. Economic losses are insidious since the incurable, chronic infection has long incubation and no effective treatment yet surveys demonstrate up to 80.7% of open range flocks in the US contain seropositive sheep. The best strategy to combat morbidity and mortality from OvLV is prevention. Previously we discovered an insertion/deletion associated with 50% decreased proviral concentration in the blood of multiple breeds. Proviral concentration correlates with severity of histological pneumonia, arthritis, and mastitis so this validated marker predicts resilience to disease. The marker is located near 4 zinc finger transcriptional repressor genes. None of the 26 mutations predicted to create amino acid substitutions or possible splice site mutations in these 4 genes were significantly associated with resilience in Rambouillet sheep. Our hypothesis is that the resilient phenotype is explained by a mutation within a regulatory element that influences gene expression of these zinc finger genes. Chromatin immunoprecipitation with high throughput sequencing (ChIP-seq) for histone 3 lysine 27 acetylation (H3K27ac) was completed for alveolar macrophages to identify active enhancers in sheep on a genome-wide level. An active enhancer region 1500 base pairs in length was identified approximately 3000 base pairs upstream of *ZNF389*. The current insertion/deletion marker lies within this active enhancer element. Three single nucleotide polymorphisms were also identified within this enhancer that are significantly associated with resilience ($P < 0.002$) in 147 Rambouillet. This led to our current model that ovine lentivirus resilience is explained by mutations in enhancer elements that alter gene expression of zinc finger transcription factors. Zinc finger transcriptional repressor genes have been shown to co-evolve with retroviruses in other vertebrate species. Future work includes validating these SNPs in multiple breeds which may lead to a commercially viable genetic test and confirming the enhancer with additional ChIP-seq biological replicates.

Key Words: ovine lentivirus, marker-assisted selection, ChIP-seq, sheep

OP37 Lightning Talk: Integrative functional genomics of the bovine host response to infection with *Mycobacterium bovis*. T. Hall*[1], M. Mullen[2], C. Correia[1], G. McHugo[1], K. Killick[1], J. Browne[1], N. Nalpas[4], S. Gordon[2,5], and D. MacHugh[1,2], *[1]Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland, [2]Department of Life and Physical Sciences, Athlone Institute of Technology, Athlone, Ireland, [3]Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany, [4]UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland, [5]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Bovine TB (BTB), caused by infection with *Mycobacterium bovis*, is a major endemic disease affecting global cattle production. The key innate immune cell that first encounters the pathogen is the alveolar macrophage, which we have previously shown to be substantially reprogrammed during intracellular infection by *M. bovis*. In the current study we used differential expression with correlation- and interaction-based network approaches to analyze the macrophage transcriptional response to infection with *M. bovis* to identify core infection response pathways and gene modules. These outputs were then integrated with genome-wide association study (GWAS) data sets to enhance detection of genomic variants for susceptibility/resistance to *M. bovis* infection. The host gene expression data consisted of bovine RNA-seq data from alveolar macrophages infected with *M. bovis* at 24 and 48 h post-infection. These RNA-seq data were analyzed using 3 distinct analysis pipelines; novel response pathways and modules were further refined using cross-comparison and integration of the results. First, a differential expression analysis was carried out to determine the most significantly differentially expressed (DE) genes between conditions at each time point. Second, 2 networks were constructed at each time point using gene correlation patterns to determine changes in expression across conditions. Functional sub-modules within each correlation network were selected by statistical criteria for modularity. Third, a gene interaction base network of the mammalian host response to mycobacterial infection was generated using the GeneCards database (www.genecards.com) and InnateDB (www.innatedb.com). Differential gene expression data were superimposed on this base network to extract functional modules of interconnected DE genes. Bovine GWAS data was obtained from a published BTB susceptibility/resistance study. The results from the 3 parallel analyses were integrated with this data to determine which of the 3 approaches identified genes significantly enriched for SNPs associated with susceptibility/resistance to *M. bovis* infection. Our results indicate distinct and significant overlap in SNP discovery and demonstrate that network-based integration of relevant

transcriptomics data can leverage substantial additional information from GWAS data sets.

Key Words: tuberculosis, macrophage, bovine, GWAS, transcriptome

OP38 Lightning Talk: Whole genome re-sequence analysis reveals tick resistance and heat tolerance genes in Iraqi cattle breeds. A. Alshawi*[1,2], A. Essa[3], S. Al-Bayatti[3], A. Tijjani[1,4], S. Salman[3], and O. Hanotte[1,4], *[1]School of Life Sciences, Faculty of Medicine and Health Sciences, University of Nottingham, UK, [2]Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Baghdad, Iraqi Ministry of Higher Education and Scientific Research, Baghdad, Iraq, [3]Animal Genetics Resources Department, the Ministry of Iraqi Agriculture, Baghdad, Iraq, [4]International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.*

In spite of the historic global importance of cattle in Iraq, a country at the crossroad of the species domestication centers, their genomes have not yet been characterized. Here, we present the results of full genome sequence autosomal scan of 20 native Iraqi cattle from 2 breeds (Jenoubi, n = 10; and Rustaqi, n = 10) for candidate signatures of positive selection associated with environmental adaptation, including acquired and adaptive immunity (e.g., tick resistance) and thermotolerance. Candidate selected regions and genes were uncovered following 2 within population (*Hp* and *Tajima's D*) and 2 among populations (*XP-EHH* and *Fst*) analysis. Gene's functions ontology analysis at significant regions were performed using PANTHER, KEGG and DAVID tools. In Jenoubi, we identified T-cell and B cell activation genes (*CD3D, CD3E, CD3G* and *PTPN6*), while *B2M* and *LIPH* genes were found in Rustaqi. These genes may play significant role into the resistance to tick infestation, a major external parasitic burden in Iraq. Heat tolerance genes were also identified in Jenoubi (e.g., *DNAJB1*) and Rustaqi (e.g., *DNAJC4*). Furthermore, we noticed *HOX* genes (e.g., *HOXC11* and *HOXC12*) that are related to thermoregulation activity in Jenoubi. Genes involved in the animal physiological responses to external stimulus and stress are detected in both breeds. Our results provide, the first full genome insights on the genome adaptation of Iraqi cattle enlightening the importance to conserve and utilize these adaptations to control diseases and breeding improvement programs in the country.

Key Words: Iraqi cattle, genome sequencing, adaptive immunity, genomic selection

Horse Genetics and Genomics

OP40 The horse X chromosome: Old tricks, new insights. T. Raudsepp*[1], C. Castaneda[1], A. Hillhouse[1], A. Dubrow[1], M. Jevit[1], R. Juras[1], R. Bellone[2], and B. W. Davis[1], *[1]Texas A&M University, College Station, TX, USA, [2]University of California Davis, Davis, CA, USA.*

The most common chromosomal cause of mare sterility is X-monosomy, found in 25% of mares with reproductive problems. X-monosomy is also relatively common in humans, where 0.04% of all live born and 10% of spontaneous abortions carry the condition. In contrast, X-monosomy is rare among other species that have been investigated. Since the mammalian X chromosome is evolutionarily conserved and largely collinear between species, species differences in X-monosomy are likely related to differences in X chromosome regulation, and involve genes that escape X inactivation (XCI). These include pseudoautosomal (PAR) genes, functional X-Y gametologs, and a largely unknown species-specific and tissue-specific set of other escapees. Prior studies show that horse and human PARs are smaller than PARs in species where X-monosomy is rare. However, because XCI in mammalian females is random, identification of other XCI escapees requires evaluation on an individual cell level. Here, we accomplish that using single-cell (SC) RNaseq from lymphocytes and fibroblasts of one female horse and searched for biallelic variants. Among XCI escapees were known PAR genes, some but not all X-Y gametologs, and several novel genes. We also observed differences in XCI escape patterns between the 2 tissues/cell types. We expand SC RNaseq on additional horse tissues, but also to other mammalian taxa for comparison. Another intriguing feature of X-monosomy is a bias of X retention depending on parental origin. In human live-born X-monosomy, 75–90% cases carry maternal X, whereas paternal X is more prevalent among spontaneous abortions. Likewise, our initial survey in horses also indicates predominant retention of maternal X, suggesting either more errors in male meiosis, early embryonic imprinting events before XCI, or both. Finally, human studies claim that pure X-monosomy is rare and most cases are mosaics for an XX or XY cell line. Our preliminary survey by X-genotyping and FISH analysis in 63 X-monosomic horses does not support prevalent mosaicism. In conclusion, species-specific patterns of X-monosomy in mammals present an excellent opportunity to gain better knowledge of the molecular features and regulation of the X chromosome.

Key Words: horse, X-monosomy, single-cell RNAseq, parental origin, mosaicism

OP41 Whole-genome sequencing reveals the genetic mechanisms underlying the high-altitude adaptation in Tibetan horses. X. Liu*, Y. Ma, and L. Jiang, *Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.*

Chinese horse breeds had experienced a very long history of breeding, with good adaptation to extreme environments. We tried to detect genome-wide selective signatures toward the high-altitude adaptation of Tibetan horse. Tibetan horse populations have evolved mechanisms that allow them to survive and perform as the major transporter for local farmers at an altitude of 4500 m on the Qinghai-Tibetan Plateau. We performed whole genome resequencing of about 138 horses from all around China, including Tibetan horses (65), lowland horses (61), Przewalski (5) and Thoroughbreds (7). A composite of multiple signals from fixation index (Fst), ZHp (Heterozygosity), pi-ratio (nucleotide diversity), and Tajima's D-test revealed the top candidate region for the altitude adaptation reside the gene of *EPAS1*, which has been identified in many species, including human, goat, sheep and yak. We found 2 significant SNPs in EPAS1 (HIF2a) gene. Interestingly, the overexpression of these 2 EPAS1 mutants in A549 cells showed significantly increased activities than the wild type protein. In large horse populations (n = 948) these 2 EPAS1 SNPs showed even more remarkable genetic differentiation between Tibetan breeds and lowland horses. By measuring the blood samples on spot, we found significant physiological difference between Tibetan (n = 88) and lowland (n = 85) horses, including lower HMG level and HCT but higher MCHC in Tibetan horse. We also found the metabolic difference between Tibetan and lowland horses, including higher LDH and higher a-HBDH, suggesting a greater capacity for anaerobic lactate production in Tibetan horses. These results suggest that mutations in EPAS1 gene contribute to a hematology and metabolic basis in Tibetan horses, implying a quite similar adaptive mechanism of the Sherpa. Therefore, we tested the downstream target of EPAS1 gene, such as VEGFA, EDN, VHL, EGLN, and GLUT1 after the overexpression of 2 EPAS1 mutants separately in A549 cells. Both mutations lead to upregulation of all these 5 targets. Our study suggests that the 2 missense EPAS1 mutations represent key evolutionary changes underlying the adaptation to high-altitude hypoxia in Tibetan horses.

Key Words: Tibetan horse, hypoxia adaptation, *EPAS1*, genomic selection signatures, metabolic basis

OP42 Upstream regulators of gene and protein expression influencing glycogen repletion in horses. D. Vélez-Irizarry*[1], K. Aldrich[1], V. Mesquita[2], J. Pagan[2], and S. Valberg[1], *[1]Department of Large*

Animal Clinical Sciences, Michigan State University, East Lansing, MI, USA, [2]Kentucky Equine Research, Versailles, KY, USA.

Successful performance in horses depends on substrates such as glycogen for energy to power muscle contraction, yet replenishment of muscle glycogen following exercise occurs at a slow rate in horses compared with other mammals for unknown reasons. Our goal was to identify upstream genomic regulators (UpR) among differentially expressed genes (DEG) and proteins (DEP) identified in horses during glycogen depletion and repletion to elucidate factors limiting glycogen resynthesis. Five Thoroughbred horses fed an isocaloric diet of either high starch (HS: 36% NSC, 4% fat) or low starch, high fat (LS-HF: 12% NSC, 8% fat) performed 3 d of glycogen-depleting exercise, followed by 3 d to allow for glycogen repletion in a randomized crossover design. Total muscle RNA was extracted from gluteal muscle biopsies at pre-depletion, glycogen depletion, 24 and 72h post-depletion. Whole gene and protein expression were quantified with Illumina HiSeq 4000 and TMT LC-MS/MS, respectively, and mapped to EquCab3.0. Differential expression (DE) was determined with weighted least squares; time points were nested within horse and diet. Across time points there were 2,285 DEG on HS and 4,776 DEG on LS-HF compared with 129 DEP on HS and 160 on LS-HF (FDR ≤0.05). RcisTarget was used to find enriched UpR among the DEG on HS and LS-HF. A total of 349 UpR were enriched for the HS and 211 for LS-HF. Overlapping DEG and DEP were identified and focus given to enriched UpR. Among the 93 DEG that also had DEP, there were 2 genes enriched as UpR (AKR1A1, PGAM2), which involved the 72h repletion time point in both diets. KEGG pathways enriched for the target genes of AKR1A1/PGAM2 include cGMP-PKG, cAMP and PI3K-Akt signaling (FDR ≤0.05) which impact insulin sensitivity, glucose uptake, and thereby rapid glycogen resynthesis. Upregulation of DEGs involved in insulin-stimulated glucose uptake were not identified at any time point. There was regulation of DEG/DEPs involved in gluconeogenesis, particularly in horses on the LS-HF diet. Results suggest that the horses in this study rely more on the multiple steps required for gluconeogenesis than on rapid glucose-uptake for glycogen resynthesis.

Key Words: glycogen, transcriptome, proteome, upstream regulators, horse

OP43 Neocentromere formation and karyotype evolution in equids. F. M. Piras, E. Cappelletti, M. Corbo, S. Faravelli, E. Raimondi, S. G. Nergadze, and E. Giulotto*, *Department of Biology and Biotechnology, University of Pavia, Pavia, Italy.*

In the genus *Equus*, speciation occurred rapidly in an evolutionary time scale and was marked by exceptionally frequent centromere repositioning events and fusions involving centromeres. Consequently, the karyotypes of these species contain centromeres at different maturation stages, ranging from satellite-less evolutionarly "young" neocentromeres to completely "mature" satellite-based centromeres.We identified, by ChIP-seq with an antibody against CENP-A, the epigenetic mark of centromeric function, a total of 84 satellite-less centromeres in 8 equid species: horse, donkey, kiang, onager, Hartmann's zebra, Burchell's zebra and Grevy's zebra. A comparative genomic analysis of the ChIP-seq results coupled with cytogenetic approaches allowed us to trace the phylogenetic history of specific groups of orthologous chromosomes. In some cases, a neocentromere was observed in a single lineage. In other cases, the same neocentromere was found in separate lineages, suggesting the presence of hotspots for neocentromere formation. Interestingly, the phylogenetic history of some chromosomes allowed us to describe, at the DNA sequence level, different stages of centromere evolution from its birth to its complete maturation. In particular, at several neocentromeres, DNA sequence amplifications were observed suggesting that they may represent an intermediate stage toward the formation of new satellite DNA families. In many instances, centric fusion events led to the inactivation of the original centromeres and to neocentromere formation in a different position. These neocentromeres can be located very close to the fusion point or several megabases away. One of the fusion junctions was sequenced revealing that relics of pericentromeric satellite sequences were maintained while the major centromeric satellite was lost. Our discoveries proved that, in equids, neocentromere formation is a driving force of karyotype evolution and can be used as a marker to investigate the phylogeny of these species.

Key Words: horses and related species, evolutionary genomics, ChIP-seq, chromatin, chromosomal rearrangement

OP44 Methylome sequencing of tissues and cell-lines of Polish Coldblood horses. T. Zabek*[1], T. Szmatola[1], W. Witarski[1], E. Semik-Gurgul[1], A. Gurgul[1], and M. Bugno-Poniewierska[2], *[1]National Research Institute of Animal Production, Krakow, Poland, [2]Institute of Veterinary Sciences, University of Agriculture, Krakow, Poland.*

DNA methylation is responsible for cell and tissue identity across the development and adulthood. Because of this DNA methylation profiles are able to differentiate between particular cell-types and tissues. However the unclear matter are interrelations between DNA methylation and gene expression patterns which are of functional importance for the given biological target. The majority of loci showing tissue-related methylation profiles reveal great variability of tissue-specific transcription. The solid interrelation between differential methylation and gene expression patterns are the question of heterogeneity of studied biological target and its physiological stage. Relying on the results of RRBS methylome sequencing and locus specific bisulphite sequencing of horse tissues and cell lines we found a couple of loci which differential methylation is relevant for tissue- or cell-type specific gene expression. Six Polish Coldblood Horses of the same age were the donors of biological material. The target panel of tissues included left ventricle of heart (LVH), left lung (LU), left lateral lobe of the liver (LI) and articular cartilage from metacarpal joints which were also used to obtain chondrocyte cell lines in prolonged monolayer culture. We found methylome differences between tissues relevant more to their embryonic lineage than being individual-specific. A group of differentially methylated regions were associated with tissue specific transcription mainly in the liver tissue. We have also found CpG sites of differential methylation between differentiated and dedifferentiated chondrocytes. Observed methylation increase along with the growing number of passage of obtained cell-lines was associated with transcriptional decline being the sign of the loss of primary chondrocyte potential. Obtained results confirm that both tissue- and cell-type specific differences of DNA methylation might be linked to differential expression important for the functioning of particular biological system.

Key Words: RRBS, tissue- and cell-specific transcription

OP45 Identification of shared and species-specific k-mers in Equids and Caballines to characterize adaptive introgression events. K. de Silva*[1], E. Bailey[2], and T. S. Kalbfleisch[2], *[1]University of Louisville, Louisville, KY, USA, [2]University of Kentucky, Lexington, KY, USA.*

In a recent study, an allele was reported for the equine gene *CXCL16* that conferred susceptibility for persistent shedding of the Equine Arteritis Virus. It is comprised of non-synonymous variants at 4 positions in exon 1 of the gene producing 2 alternative proteins, one susceptible *CXCL16*S and the other resistant *CXCL16*R (1). The susceptibility allele contains a haplotype block that differs by between 15 to 18 SNPs from the resistant haplotype in the horse within the 1000bp window containing it. The susceptibility allele differed by far fewer SNPs (3 to 4) from corresponding locus in non-caballine equids. As such, this allele is likely the result of adaptive introgression from a non-caballine species. We are developing a method whose objective is the identification of the likely species of origin of this introgressed segment. Our method is a k-mer-based analysis which aims to provide new biological insight in our search for evidence of historical gene transfer events between species. The data consists of whole genome sequence from 5 female thoroughbreds including Twilight (the reference animal for the equine genome) and 8 non-caballines (Somali ass, Donkey, Ki-

ang, Grevyi, Onager, Zebra, Quagga and Boehmi). We used Jellyfish v2.2.6 to count 21 bp canonical k-mers in Illumina WGS data with no bloom filter or high or low cut-off. We counted all k-mers, including those with low frequency to ensure a high proportion of unique k-mers between sample groups. K-mers from 4 caballines including Twilight were compared against the k-mers of 8 non-caballines to identify the caballine specific k-mers and those shared among all equids. Of a total 8,308,928,880 k-mers analyzed from caballine whole genome we identified 4,101,987,596 k-mers (49.37%) specific to caballines and 4,206,941,284 k-mers (50.63%) shared between both groups. When we compare k-mers in EquCab3 chr11, out of 250,865,144 k-mers 172,167,721 k-mers (68.63%) specific to caballines and 78,697,423 k-mers (31.37%) shared between both groups. We will identify k-mers specific to each species and those shared between horse and only some of the other non-caballines species and finally the corresponding genes which are ancestrally shared. The data will guide us to a more complete understanding of the CXCL16 introgression event and provide a foundation to study other events that have contributed to the evolution of the modern horse.

OP46 An ancient mutation at *TBX3* enhancer contributes to the small body size in Chinese ponies. X. Liu, Y. Zhang, Y. Li, Y. Ma, and L. Jiang*, *Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.*

The domestic horse is one of the species exhibiting greatest body size variation. The genetic changes underlying body size variation have been recently identified in multiple domestic animals but remain unknown in Chinese native horses. Here, we combined the complete genome of 210 ponies (<107 cm, wither height) and 308 other horses (>130 cm) to uncover the genetic basis for the small body size selection in Chinese native ponies. We identified that *T-box 3* gene (*TBX3*) shows the strongest signature for positive selection in the pony genome. A regulatory mutation at the enhancer region of this locus appear strongly associated with average body size in a large panel of 908 Chinese native horses. Intriguingly, we found that this mutation is the same as the ancestral allele of Przewalski's horses but different from that of Shetland miniature horses, indicating the origination of the mutation is likely ancestral and independent with the foreign ponies. Functional validation through nucleotide mutagenesis shows that this mutation abrogates the binding of unknown transcription activators and thus decreases the transcription of TBX3 gene. Our study demonstrates that the TBX3 regulatory mutation provided key evolutionary molecular variation to the small body size of Chinese ponies. It reveals possible targets for genomic selection programs aimed at increasing body size in livestock and provide new insights to the regulation of TBX3 gene.

Key Words: Chinese pony, T-box3, WGS, regulatory mutation, positive selection

OP47 Genetic diversity and relationships among native Japanese horse breeds and horses outside of Japan using genome-wide SNP data. T. Tozaki*[1,3,6], M. Kikuchi[1], H. Kakoi[1], K.-I. Hirota[1], S.-I. Nagata[1], D. Yamashita[2], T. Ohnuma[2], M. Takasu[3], I. Kobayashi[4], S. Hobo[5], D. Manglai[6], and J. Petersen[7], *[1]Genetic Analysis Department, Utsunomiya, Tochigi, Japan, [2]Japan Equine Affairs Association, Chuo-ku, Tokyo, Japan, [3]Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Gifu, Japan, [4]Sumiyoshi Livestock Science Station, Field Science Center, University of Miyazaki, Miyazaki, Miyazaki, Japan, [5]Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Kagoshima, japan, [6]College of Animal Science, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China, [7]Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE, USA.*

Eight horse breeds, Hokkaido (HK), Kiso (KS), Misaki (MS), Noma (NM), Taishu (TS), Tokara (TK), Miyako (MY), and Yonaguni (YN), are native to Japan and relatively free of artificial selection. Although Japanese native breeds are believed to have originated from ancient Mongolian horses, the phylogenetic relationships among these breeds are not well elucidated. In this study, we compared genetic diversity among 32 international horse breeds previously evaluated in the Equine Genetic Diversity Consortium (733 horses) and the 8 Japanese native breeds (HK: 42, KS: 23, MS: 21, NM: 24, TS: 25, TK: 24, MY: 35, YN: 24) using genome-wide SNP genotype data (20,287 SNPs pruned by LD from the EquineSNP70 BeadChip). The expected heterozygosity showed the 8 Japanese breeds (0.158–0.267) have low diversity compared with the other international breeds (all breeds: 0.277). The polymorphic proportion showed the 7 Japanese breeds (0.437–0.792) except for HK (0.864) have relatively low diversity compared with the other international breeds (all breeds: 0.833). Phylogenetic and cluster analyses demonstrated relationships among the breeds that largely reflect their geographic distribution in Japan. These data support historic records, suggesting a Mongolian origin of native Japanese horses, and refute the prior assumption that the northern and southern lineages were derived from separate founder populations. These data help to explain the history of the Japanese native horses, identify uniqueness within the breeds, and will also serve as a baseline from which to monitor future breed diversity and make conservation decisions for endangered populations.

Key Words: horse, equine, Hokkaido, SNP

OP48 Genomic studies of stallion fertility: comparing fertility records with *FKBP6* genotype and copy numbers of Y multi-copy genes. C. Castañeda*[1], A. Hillhouse[2], S. Teague[3], C. Love[3], D. Varner[3], and T. Raudsepp[1], *[1]Texas A&M Department of Veterinary Integrative Biosciences, College Station, Tx, USA, [2]Texas A&M Institute for Genome Sciences and Society, College Station, TX, USA, [3]Texas A&M College of Veterinary Medicine, Large Animal Hospital, College Station, TX, USA.*

Stallion fertility is an economically vital element of the equine industry. However, despite the recently improved horse reference sequence and continuing progress of equine genomics, molecular tools for evaluating stallion fertility remain sparse. The FK506 binding protein 6 (*FKBP6*) gene in chr13 is among the few genes that have been associated with stallion fertility. A double homozygous AA-AA genotype of 2 SNPs in *FKBP6* exon4 is a susceptibility genotype for subfertility due to impaired acrosome reaction (IAR). However, molecular mechanisms between *FKBP6* and IAR remain enigmatic. Here we studied 168 Thoroughbred breeding stallions to determine the prevalence of the AA-AA genotype and correlate this with breeding record data, such as the number of mares bred per season, per-cycle pregnancy rate (PR) and seasonal PR. We developed Taqman allelic discrimination assays for the 2 SNPs and proved those as reliable, cost- and time-efficient alternatives to genotyping by Sanger sequencing. We found that 3 of the 167 stallions carried the susceptibility genotype, which agrees with the reported 2% prevalence among Thoroughbred males. Notably, all 3 stallions had poor fertility (per-cycle pregnancy rate of 25 + 17%), while 144 stallions with fertility data and without the double homozygous AA-AA genotype had a higher per-cycle pregnancy rate (62 + 10%). Another putative candidate region for stallion fertility is the Y chromosome, which carries several multi-copy and testis-specific genes. Here we investigated copy number (CN) variation of selected Y genes in relation to stallion fertility. We quantified CN of 4 multi-copy genes (*TSPY*, *ETSTY1*, *ETSTY2*, and *ETSTY5*) by digital droplet PCR in 22 of the 168 stallions, representing 3 fertility groups: per-cycle PR below 40%, between 40% and 70%, and over 70%. Preliminary analysis does not suggest correlation between Y gene CNs and per-cycle PR in this study cohort. Both studies underscore that any progress in the genomics of stallion reproduction requires systematic collection of fertility data combined with advanced genomics analyses.

Key Words: stallion, fertility, genotyping, subfertility

OP49 Population structure analysis of the Persian horse breeds and their comparison to worldwide populations using genome-wide SNP genotypes. N. YousefiMashouf[1,3], J. L. Petersen[2], H. Mehrabani Yeganeh[3], A. Nejati Javaremi[3], T. S. Kalbfleisch[1], M.

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Horse domestication may have occurred in or near the region of Persia. Investigation of Persian horse breeds may provide insights regarding the genetic diversity among horses and the early domestication events. Four Persian breeds are subject of this study: Kurdish, Persian Arabian, Caspian and Turkoman. At the first phase of the study, DNA samples from 58 Kurdish horses, 38 Persian Arabians and 84 American Thoroughbred horses were genotyped across 670,796 SNP markers (Affymetrix). The data set was pruned resulting in 9,170 SNPs in 50 Kurdish, 24 Persian Arab and 59 Thoroughbred horses. The data were analyzed using principal component analysis, F statistics, cluster analyses, analysis of molecular variance and genetic distance. The first eigenvector explained 6.84% of the variance, discriminating Thoroughbred from Persian breeds, and the second explained 2.06% distinguished between Kurdish and Persian Arabian populations. Pairwise F_{st} between the 2 Persian breeds was calculated as 0.014, several fold less than that between the Thoroughbred and either of Kurdish or Persian Arab populations (0.054 and 0.063 respectively), reflecting a close genetic relationship between the Persian Arabian and Kurdish and distance from the Thoroughbred. Cluster analysis assigned Kurdish and Thoroughbred horses to distinct clusters (0.95 and 0.95 respectively), but the Persian Arab's genome formed a partially (0.53) distinct cluster with several individuals overlapping those in the Kurdish population. The results imply a gene flow between the Persian Arab and Kurdish horses. The expected heterozygosity was 0.328, 0.324 and 0.341 for Thoroughbred, Persian Arab and Kurdish populations, respectively. Treemix software, employed to infer migration events, found no evidence of migration between the Kurdish and Persian Arabian, contradicting our expectation based on cluster analysis. These results have implications for developing conservation strategies to achieve sound breeding goals while maintaining genetic diversity. At the next phase, we will extend our analyses to another SNP data set on the 2 other Persian breeds (Turkoman and Caspian) genotyped with Illumina SNP70 array, together with a select of divergent breeds world-wide which have been genotyped with Illumina SNP50.

Key Words: horse, population genomics, genotyping, breed diversity, conservation

Applied Genetics of Companion Animals

OP50 An evaluation of a 118-SNP marker panel for feline identification and parentage verification. M. de Groot*, T. Ras, and W. van Haeringen, *VHLGenetics, Wageningen, the Netherlands.*

Cat parentage verification based on STRs is widely applied in parentage verification and forensic casework, as several cat STR panels have been developed and validated, including a 15 STR panel approved by ISAG. With an increasing focus on genetic health of pedigree cats, the opportunity arises to include a SNP panel for parentage verification and identification to a genetic trait and health panel. However, no international standard for a feline SNP panel for parentage verification and identification is formally acknowledged by a body like ISAG today. However, a trial SNP (n = 118) panel was distributed earlier for comparison testing. The aim of this study is to evaluate the performance of the SNP panel for identification and parentage verification. Feline samples from over 40 different breeds were collected over a course of one year and DNA was extracted using LGCs sbeadex chemistry according to manufacturer's protocol. Sample were genotyped on the 118 SNP markers using Thermo Fisher's Ion S5 Target GBS technology. Samples with a minimum sample call rate of 0.85 (n = 3748) were included in the analysis. A coverage of 20X was accepted. Performance characteristics were determined with the program CERVUS v3.1.3 (Marshall et al., 1998; Kalinowski et al., 2007). Markers with a marker call rate lower than 0.9 and an HO (observed heterozygosity) lower than 0.250 were excluded from the final analysis. From the first analysis, 113 markers were selected meeting the test criteria. Genotyping the remaining 113 markers across breeds, a mean expected heterozygosity of 0.4516, a NE-1P (combined non-exclusion probability, first parent) of >0.9999 and a NE-I (Combined non-exclusion probability, identity) of >0.9999 was observed. Testing 113 SNP markers results in a highly usable panel for identification and parentage verification for felines. Combining markers with high heterozygosity creates a panel that is widely applicable among multiple breeds.

Key Words: cats and related species, genetic identification, SNP, genetic marker, parentage

OP51 A recommendation for a SNP marker panel for canine identification and parentage verification. M. de Groot*, T. Ras, and W. van Haeringen, *VHLGenetics, Wageningen, the Netherlands.*

Kennel clubs play an important role keeping track of canine pedigrees and issuing pedigree certificates. Some kennel clubs, like the Dutch and Belgian kennel club, require mandatory parentage verification for a puppy before a pedigree certificate is issued. So far, kennel clubs accept only STR profiles for parentage verification. However, the advantage to combined SNP markers for parentage verification with genetic trait and disease markers and thereby decreasing the cost price, is an argument for kennel clubs to switch from STRs to SNPs for identification and parentage verification. Lacking an internationally recognized standard however, is an argument that keeps kennel clubs from switching. The aim of this study is to make a recommendation for a primary panel based on 200 SNPs and a secondary panel of 100 additional SNPs. In this research, 3 previously defined marker panels, referred to as the Orivet (n = 125), NeoGen (n = 197) and AKC (n = 88) panel, were evaluated. Canine samples from over a hundred different breeds were collected over a course of one year and DNA was extracted using LGCs sbeadex chemistry according to manufacturer's protocol. Sample were genotyped on the combined 410 SNP markers using Thermo Fisher's Ion S5 Target GBS technology. Samples with a minimum sample call rate of 0.85 (n = 2145) were included in the analysis. Performance characteristics were determined with the program CERVUS v3.1.3. Markers with a marker call rate lower than 0.9 and an HO (observed heterozygosity) lower than 0.250 were excluded from the final analysis. From the first analysis, 350 markers were selected meeting the test criteria. Genotyping the remaining 350 markers, a mean expected heterozygosity of 0.4261, a NE-1P (combined non-exclusion probability, first parent) of > 0.9999 and a NE-I (Combined non-exclusion probability, identity) of > 0.9999 was observed. Combining the different marker panels results in a highly usable panel for identification and parentage verification for canines. Combining markers with high heterozygosity creates a panel that is widely applicable among multiple breeds.

Key Words: dogs and related species, genetic identification, SNP, genetic marker, parentage

OP52 Development of targeted GBS panels for breeding and parentage applications in cats. A. Burrell*, P. Siddavatam, M. Swimley, C. Willis, H. Suren, K. Gujjula, and R. Conrad, *Thermo Fisher Scientific, Austin, TX, USA.*

Parentage testing and genomics-assisted breeding are critical aspects of successful veterinary management. Due to its highly accurate and reproducible results, targeted GBS is becoming an increasingly favored technology for SNP genotyping. With the utilization of next-generation sequencing, labs can test hundreds of samples across thousands of SNPs simultaneously in a simple high throughput workflow starting from either extracted nucleic acid or crude lysis samples. We devel-

oped a targeted sequencing panel, one for the combined detection of feline genetic defect/trait detection and parentage verification. Utilizing the AgriSeq™ HTS Library Kit, a high-throughput targeted amplification and re-sequencing workflow, the panel's performance was tested on >96 diverse DNA samples. Libraries were sequenced on the Ion S5 using an Ion 540 chip with genotyping calling generated using the Torrent Variant Caller (TVC) plugin. The mean genotype call rate of markers across the samples was >95%. Concordance across replicate library preparations and independent sequencing runs was >99% for both panels. Panel results also were compared with genotyping results from qPCR, and/or CE sequencing for orthogonal confirmation of accuracy and the genotype calls were 100% concordant with the AgriSeq workflows. The data demonstrates the utility of the AgriSeq targeted GBS approach for feline SNP genotyping applications. For Research Use Only. Not for use in diagnostic procedures.

Key Words: feline, AgriSeq, parentage, GBS, NGS

OP53 Development of targeted GBS panels for breeding and parentage applications in dogs. A. Burrell*, P. Siddavatam, M. Swimley, C. Willis, H. Suren, K. Gujjula, and R. Conrad, *Thermo Fisher Scientific, Austin, TX, USA.*

Parentage testing and genomics-assisted breeding are critical aspects of successful veterinary management. Due to its highly accurate and reproducible results, targeted GBS is becoming an increasingly favored technology for SNP genotyping. With the utilization of next-generation sequencing, labs can test hundreds of samples across thousands of SNPs simultaneously in a simple high throughput workflow starting from either extracted nucleic acid or crude lysis samples. We developed 2 targeted sequencing panels, one for canine parentage verification and one for canine genetic defect/trait identification. Utilizing the AgriSeq™ HTS Library Kit, a high-throughput targeted amplification and re-sequencing workflow, each panel's performance was tested on >96 diverse DNA samples. Libraries were sequenced on the Ion S5 using an Ion 540 chip with genotyping calling generated using the Torrent Variant Caller (TVC) plugin. The mean genotype call rate of markers across the samples was >95% for both panels. Concordance across replicate library preparations and independent sequencing runs was >99% for both panels. Each panel's results were compared with results from an Axiom Canine HD array, qPCR, and/or CE sequencing for orthogonal confirmation of genotype accuracy and the genotype calls were >99% concordant with the AgriSeq workflows. The data demonstrates the utility of the AgriSeq targeted GBS approach for canine SNP genotyping applications. For Research Use Only. Not for use in diagnostic procedures.

Key Words: canine, breeding, AgriSeq, ISAG, parentage

OP54 Can-ID: A SNP-based genetic Identification system to evaluate canine samples on two platforms: Open Array and AgriSeq targeted GBS. O. Ramirez*[1], K. R. Gujjula[2], A. Sánchez[1,3], H. Suren[2], O. Francino[1,3], R. Ramadhar[2], and L. Altet[1], *[1]Vetgenomics, Barcelona, Spain, [2]Thermo Fisher Scientific, Austin, TX, USA, [3]Molecular Genetics Veterinary Service (SVGM), Veterinary School, Universitat Autònoma de Barcelona, Barcelona, Spain.*

Genetic identification establishes a secure and permanent DNA profile that is very useful in cases of lost or stolen dogs or to prove parentage. SNP-based genetic data overcome STR limitations which can negatively influence the downstream analysis (higher rate of errors in genotyping due to presence of artifacts and scarce reproducibility between laboratories or over time). These limitations increase in paternity testing and in the analysis of forensic samples due to high mutation rate and the use of long amplicons. To overcome all these limitations, we have developed Can-ID for use on 2 genotyping platforms AppliedBioSystems Open Array and AppliedBioSystems AgriSeq targeted GBS. Can-ID panel contains 3 types of SNPs. First, contains 92 highly polymorphic SNPs to obtain a unique DNA profile for each dog. Second, 15 mitochondrial highly polymorphic SNPs that allow Can-ID discard biological samples that contains DNA from more than one animal (e.g., feces contaminated with the urine of another dog), and so avoid false positives. And third, 13 SNPs associated with phenotype traits allow obtaining for a "composite picture" of the individual from forensic samples. Can-ID have been validated in more than 5 thousand of dog samples (invasive and non invasive). Also we have performed a comparative study between 2 platforms: AppliedBioSystems Open Array and AppliedBiosytems AgriSeq targeted GBS. The probability of identity, calculated under the assumption that individuals may be related, of Can-ID panel was 1.3×10^{-23}. Can-ID is the method used in 10 villages from Catalonia (Spain) aimed at reducing the problems of canine excrement in public places. Of 603 feces samples analyzed, 68 (11.27%) were discarded because it contains DNA from more than one individual and in 176 (29.51%) the dog, and therefore the in-civic owner, was identified. Results herein support the use of Can-ID panel for identify testing for forensic casework and parentage testing following the National Research Council's recommendations. Notably, Can-ID panel is also capable of genotyping wolf (*C. lupus*) samples, making it useful in conservation studies as well.

Key Words: dog, identification system, Open Array, AgriSeq

OP55 End-to-end AgriSeq targeted GBS long indel solution. H. Suren[1], C. Willis*[1], K. Reddy Gujjula[1], P. Siddavatam[1], J. Wall[1], C. Carrasco[1], R. Conrad[1], and J. Schmidt[2], *[1]Thermo Fisher Scientific, Austin, TX, USA, [2]Thermo Fisher Scientific, Santa Clara, CA, USA.*

AgriSeq targeted genotyping-by-sequencing (GBS) is successfully being used as a high throughput, customizable and cost effective genotyping solution in animal and plant breeding studies, parentage testing and genetic purity. One of the powers of this technology is its capability to support different types of markers including single nucleotide polymorphisms (SNPs), multiple nucleotide polymorphisms (MNPs), insertions and deletions (InDels), and other structural variants (e.g., inversions, duplications). InDels markers longer than 50bp can be a challenge for amplicon based GBS. The inclusion of these marker type require a different strategy during the panel design and genotype calling. We employed a 3 amplicon strategy to facilitate genotype calls from both the alleles and developed a new pipeline to automate the generation of present/absent calls. We successfully developed a custom canine SNP genotyping panel with 16 long InDels and evaluated the performance with known true genotype samples. The robustness of this technology has been demonstrated across 384 samples using 20 canine long indel markers whose length ranges from 62bp to 6Mbp. Overall, 90% call rate across samples and 100% concordance calls with true genotypes were observed. We found that primer design and down-stream analysis were not impacted by the indel size. High call rate across multiple samples with varying indel size indicates the reproducibility and flexibility of the method. AgriSeq targeted GBS offers customers end to end solution for genotyping diverse marker types simultaneously using same workflow. For Research Use Only. Not for use in diagnostic procedures.

Key Words: AgriSeq, targeted GBS, long indel, data analysis, bioinformatics

OP56 High-resolution melt analysis for detecting the causative point mutation for the prcd-PRA in the Bolognese dog breed. C. Previtali*, S. Arabi, G. Bongioni, R. Capoferri, A. Pozzi, and M. Montedoro, *Istituto Spallanzani, Rivolta d'Adda, Cremona, Italy.*

The Bolognese is an ancient breed originating from the North of Italy belonging to the Bichon family group. In 2017, the Italian Bolognese population counted 3291 males and 3305 females for a total of 6596 live animals, while the subpopulation that generated offspring was composed of 1022 live animals. The Bolognese is a rather healthy breed with very few genetic health issues. Despite this reassuring aspect, great attention has been devoted to a specific type of oculopathy, the prcd-PRA (progressive rod-cone degeneration-PRA). This form of PRA described in more than 30 canine breeds is an autosomal recessive trait

caused by a point mutation located on Chromosome 9. The objectives of this work were the development of an alternative molecular assay for detecting the point mutation and the evaluation of the incidence of the disease in the Italian Bolognese population. To this aim, the High Resolution Melt Analysis (HRMA) was used to identify carrier (GA), clear (GG) and affected (AA) animals. Several 110 blood samples was collected from 42 males and 68 females, and the DNA was extracted. The samples represented about 2% of the living animals corresponding to 11% of the breeding subpopulation. A PCR was conducted in the presence of a dsDNA-binding dye followed by a melting step. The analysis is based on the detection of fluorescence changes associated with the release of dye following dissociation of the dsDNA into single strands. The output is a melt curve profile, which is characteristic of a specific genotype. The results showed that the causative mutation was present for 30% in heterozygosity (carrier) and for 1% in homozygosity (affected). The samples were further submitted to Sanger sequencing analysis. The results confirmed the totality of genotypes detected by HRMA thus providing for the validation of the HRMA protocol as a valid method for the detection of the mutation responsible for prcd-PRA. Moreover, given the number of animals sampled in the present work with respect to the current population, it is possible to conclude that the results are representative of the real incidence of the disease in the Italian Bolognese population.

Key Words: oculopathy, Bolognese, dog, prcd-PRA, HRMA

OP57 Analysis of clinical samples from Doberman and Toy Poodle dogs with a targeted next-generation genotyping system.
A. Arizmendi[1,2], L. S. Barrientos[1], J. A. Crespi[1], G. R. Garces[1], G. Giovambattista[1], and P. P. García*[1], *[1]Instituto de Genética Veterinaria (IGEVET), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (UNLP), La Plata, Buenos Aires, Argentina, [2]Sevicio de Cardiología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (UNLP), La Plata, Buenos Aires, Argentina.*

Next-generation sequencing (NGS) is a powerful tool to study DNA or RNA samples. New methods and protocols based on NGS have been developed to carry out the analysis of genetic variation for animal parentage testing, disease screening and trait detection. Targeted NGS is aimed at achieving 'targeted enrichment' of genome subregions to reduced significantly the sequencing of genomic loci of interest, as well as costs and efforts, compared with whole-genome sequencing (WGS). We generated genotyping information of 387 targets from 95 clinical canine samples (76 Doberman and 19 Toy Poodle dogs) and 3 control samples using AgriSeq Targeted GBS. Based on these data, we calculated the exclusion power of 228 parentage markers with Cervus 3.0 software. Furthermore, we detected disease/trait markers presenting polymorphism and calculated their allele frequencies within each breed. In the case of parentage markers, the assigned parents showed a higher LOD score (>1.22 x10^{16}), and the available pedigree data of offspring agreed with the assigned parent information. Interestingly, full siblings were also assigned like parents. On the other hand, we found 19 polymorphic disease/trait markers in the total sample, 3 of which (progressive rod-cone degeneration, von Willebrand disease 1 and dilated cardiomyopathy) were validated by pyrosequencing with 100% concordance. The mutant allele for cone-rod dystrophy3 (CRD3) was found in both groups, a variant which had not been reported in either breed to date. Sequencing of genomic loci of interest, costs and efforts can be reduced significantly with targeted NGS as compared with WGS. The AgriSeq Targeted GBS is a very good alternative for the massive genetic evaluation of animal populations.

Key Words: dogs and related species, animal breeding, high-throughput sequencing (HTS), polymorphism, genomic selection

OP58 First steps in animal genetic testing in Bulgaria.
S. Tincheva*[1], S. Atemin[1,2], R. Toshkov[3], T. Todorov[1], and A. Todorova[1,2], *[1]Genetic Medico-Diagnostic Laboratory "Genica," Sofia, Bulgaria, [2]Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria, [3]Veterinary clinic "Kakadu," Sofia, Bulgaria.*

Molecular genetic testing can be a powerful tool for identification of autosomal recessive mutations to avoid crossbreeding between carriers, inborn predispositions aiming to prevent or at least delay the development of a disease or eventually for determination of the sex of an individual. Here we resume our work representing as far as we know the first genetic testing experience of animals, more precisely companion animals and birds, in Bulgaria. Having a solid background in human molecular genetics, we decided to expand our interests and enter the field of animal genetics. So far, we have implemented basic genetic tests for variations with well-known correlation to a pathological condition and thus with a high practical value. The Multi-Drug Resistance Gene (*MDR1*, *ABCB1*) has been our first target. We successfully performed genetic testing for identification of the c.227_230delATAG mutation in a control group and identified the ABCB1–1? genotype in a pair of Australian Shepherd dogs – the one being a heterozygous and the other a homozygous carrier. Concerning the feline genetics, so far, we have focused our work on the Polycystic Kidney Disease (PKD). Optimization of the molecular genetics assay and application of the protocol to a control group of cats of different breeds has been executed. The c.10063C > A variant in the *PKD1* gene was as expected detected in Persian cats. Using a widely known primer set (Lyons et al., 2004) for PCR amplification and Sanger sequencing, we even observed an allele drop-out of the wild type allele reminding us of the necessity to be careful when implementing new protocols. Lastly but not least, we determined the sex of different species of birds: domestic chicken (*Gallus gallus domesticus*) and parrots (*Serinus canaria*, *Nymphicus hollandicus*, *Melopsittacus undulatus*). For the future, we plan expanding our activities in canine and feline genetics by identifying typical for certain breeds mutations. A test for Lagotto Storage Disease (LSD, Lysosomal Storage Disease) is currently in a process of introduction.

Key Words: cats, dogs, genetic disorder, avian, Bulgaria

Avian Genetics and Genomics

OP59 Initiative for Global Chicken Genome Project (GCGP).
M.-S. Peng*[1], J. Han[2,3], O. Hanotte[4,5], D.-D. Wu[1], and Y.-P. Zhang[1], *[1]Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China, [2]International livestock Research Institute, Nairobi, Kenya, [3]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [4]International livestock Research Institute, Addis Ababa, Ethiopia, [5]University of Nottingham, University Park, Nottingham, UK.*

As the most abundant and widespread domesticates, the characterization of genomic diversity of chickens at a global scale are indeed required for the sustainable conservation and utilization of these valuable resources. The Kunming Institute of Zoology - Chinese Academy of Sciences (KIZ, CAS) has been leading a 2-phase international collaborative project to improve our current knowledge on chicken genomics. Through collaboration with the International Livestock Research Institute (ILRI), Zoological Survey of India, and the University of Oxford among many others, we have completed the phase one project in generating around 1000 genomes of chickens and wild junglefowls sampled from East Eurasia where the putative center of chicken domestication is located. The population genomic analyses revealed a remarkably complex picture of the early domestication and subsequent dispersal of chickens in this region. In the phase 2 project, the topic will be the genomic changes of chickens during their global dispersal out of the East Eurasia. We plan to generate at least 2000 genomes of different indigenous populations/ecotypes, improved lines, and fancy chickens from Asia, Africa and Europe. The genomic data will be important resourc-

es for depth studies on their demographic trajectory, dating and wide geographic spread, as well as the genetic mechanisms underlying their breeding and response to natural/artificial selection for rapid adaptation to diversified environmental, climatic and management conditions. The development of genomic technology, multi-function database, and bioinformatic tools will be expected to facilitate data sharing and mining. We have been therefore trying to establish a world-wide collaborative network of scientists from multidisciplinary fields, to work together and contribute to this important initiative.

Key Words: chicken, genome, diversity, adaptation, history

OP60 A new 55K SNP genotyping array for the chicken. R. Liu*[1], S. Xing[1,2], R. P. M. A. Crooijmans[2], G. Zhao[1], and J. Wen[1], *[1]Chinese Academy of Agricultural Sciences Institute of Animal Science, Beijing China, [2]Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands.*

Background: China has the richest local chicken breeding resources in the world, and is the world's second largest producer of meat-type chickens. Development of a moderate-density SNP array for genetic analysis of indigenous chickens and breeding of meat-type chickens is urgently needed for conventional farms, the breeding industry and research areas. Results: Eight representative local breeds and 2 commercial broiler lines with 3 pools of 48 individuals within each line were sequenced and supplied the major resource of SNPs. There were 7.09 million - 9.41 million SNPs detected in each breed/line. After filtering using multiple criteria such as preferred incorporation of trait-related SNPs and uniformity of distribution across the genome, 52.18 K SNPs were involved in the final array. It consists of: (i) 19.22 K SNPs from the genomes of white-feathered, yellowfeathered,and cyan-shank partridge chickens; (ii) 5.98 K SNPs related to economic traits from the Illumina 60 K SNP Bead Chip, which were found as significant associated SNPs with 15 traits in an F_2 resource population, Beijing-You-crossed Cobb, by genome-wide association study (GWAS) analysis; (iii) 7.63 K SNPs from 861 candidate genes of economic traits; (iv) the 0.94 K SNPs related to residual feed intake; and (v) 18.41 K from chicken SNPdb. The economic traits mentioned in the categories (ii) and (iii) included growth, feed efficiency, meat quality, immune traits, etc.The polymorphisms of 9 extra local breeds and 3 commercial lines were examined with this array, and 40–47 K SNPs were polymorphic (with minor allele frequency >0.05) in those breeds. The multidimensional scaling result showed that those breeds can be clearly distinguished by this newly developed genotyping array. Conclusions: We successfully developed a 55 K genotyping array designed using SNPs segregated in typical local breeds and commercial broiler lines, and 14.55 K SNPs associated with economic traits were incorporated. The array has a wide range of application potentials such as breeding with genomic selection, genome-wide association studies of traits of interest, and investigation of diversity of different chicken breeds.

Key Words: genotyping array, SNP, indigenous chicken, genome-wide

OP61 An open chromatin region on GGA1 has an important effect on regulating chicken growth. X. Cao*[1,2], Y. Wang[2,3], and X. Hu[1,2], *[1]College of Biological Sciences, China Agricultural University, Beijing, China, [2]State Key Laboratory of Agro-biotechnology, China Agricultural University, Beijing, China, [3]College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Body weight is one of the most important economic traits of chickens. Exploring the genetic mechanism of body weight has vital significance for chicken meat industry. In our previous study, a 1.2 Mb QTL and a 12 Kb haplotype in the QTL interval associated with body weight were detected on the chicken (Gallus gallus) chromosome (GGA) 1 using an advanced intercross population constructed by Huiyang Beard Chicken (a slow-growing domestic breed) and High Quality chicken Line A (a fast-growing broiler). In current study, we explored the 12 Kb haplotype block and its effect on regulating gene expression and chicken growth in duodenum at 7 weeks, as the digestion and absorption of food play an important role in gaining weight. The favorable allele for high body weight was defined as H haplotype utilizing 6 tag SNPs, in contrast to L haplotype for low body weight. Progeny test demonstrated that HH genotype chickens had a significantly higher body weight comparing to LL genotype chickens. In the 1.2 Mb QTL region, 3 genes presented different expression in duodenum between HH and LL individuals, which have been proved involving in gastrointestinal motility or energy metabolism. Through ATAC-Seq, we detected an open chromatin region containing 2 tag SNPs in the haplotype block and it suggested that 1) the open chromatin region might affect the expression of genes in the QTL region, and 2) the SNPs in the open chromatin region lead to the change of gene expression. Considering the effect of energy metabolism on growth, we measured protein levels of AMP-activated protein kinase (AMPK), the key regulator in energy regulation,through Western Blot. The increasing expression of both AMPK and phosphorylated AMPK in LL chickens comparing to HH chickens, suggested that the open chromatin region in the 12 Kb haplotype block might affect the body weight of chicken in a way of regulating energy metabolism.

Key Words: chicken, animal breeding, duodenum, genotyping, ATAC-Seq

OP64 Transcriptome sequencing reveals key potential long non-coding RNAs related to duration of fertility trait in the uterovaginal junction of egg-laying hens. A. Adetula*[1], L. Gu[1], C. Nwafor[2], X. Du[3], S. Zhao[1], and S. Li[1], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction, Ministry of Education, Key Laboratory of Poultry Genetics and Breeding of the Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei Province, China, [2]Faculty of Agriculture, Benson Idahosa University, Benin, Edo State, Nigeria, [3]College of Informatics, Huazhong Agricultural University, Wuhan, Hubei Province, China.*

Duration of fertility (DF) is an important functional trait in poultry production and lncRNAs have emerged as important regulators of various process including fertility. In this study we applied a genome-guided strategy to reconstruct the uterovaginal junction (UVJ) transcriptome of 14 egg-laying birds with long- and short-DF (n = 7); and sought to uncover key lncRNAs related to duration of fertility traits by RNA-sequencing technology. Examination of RNA-seq data revealed a total of 9,977 lncRNAs including 2,576 novel lncRNAs. Differential expression (DE) analysis of lncRNA identified 223 lncRNAs differentially expressed between the long- and short-DF groups, with 81 upregulated and 142 downregulated. DE-lncRNA target genes prediction uncovered over 200 lncRNA target genes and functional enrichment tests predict a potential function of DE-lncRNAs. Gene ontology classification and pathway analysis revealed 8 DE-lncRNAs, with the majority of their target genes enriched in biological functions such as cellular response to cytokine, response to protein homodimerization, reproductive structure development, developmental process involved in reproduction, regulation of protein modification, osteoblast differentiation and ossification, in utero embryonic development, response to cytokine, carbohydrate binding, chromatin organization, response to growth factors, and immune pathways. Differential expression of lncRNAs and target genes were confirmed by qPCR. The discovery of these 2,576 novel lncRNAs in this study significantly expands the utility of the UJV transcriptome and our analysis identification of key lncRNAs and their target genes regulating DF will form the baseline for understanding the molecular functions of lncRNAs regulating DF and extend the knowledge of the molecular mechanisms underlying fertility.

Key Words: duration of fertility, long non-coding RNAs, egg-laying hens, uterovaginal junction, RNA-seq

OP65 Sauropsida ribosomal repeat: Deciphering of the intergenic spacer in chicken and terrapin. A. Dyomin[1,2], S. Galkina*[1], V. Fillon[3], S. Cauet[4], C. Lopez-Roques[5], N. Rodde[4], C. Klopp[6], A. Vignal[3], A. Sokolovskaya[1], A. Saifitdinova[1,7], and E. Gaginskaya[1],

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Each ribosomal repeat (rDNA unit) consists of a pre-*rRNA* gene cluster (*5'ETS*, *18S rRNA*, *ITS1*, *5.8S rRNA*, *ITS2*, *28S rRNA*, *3'ETS*) transcribing in a single molecule and intergenic spacer (*IGS*) containing regulatory elements. In chicken *Gallus gallus* rDNA units are situated in the only Nucleolar organizer region (NOR) on chromosome 16. As in many other eukaryotes, chicken rDNA repetitive units are missing in the current version of assembled genome (GRCg6a). Our previous attempt to assemble a full rDNA unit using Illumina data was not successful due to the complex structure of repeats in *IGS*, only transcribed rRNA gene cluster has been deciphered (NCBI Nucleotide: KT445934). Here, we describe a chicken *IGS* structure basing on PacBio single-molecule sequencing of a BAC clone WAG137G04 containing chicken NOR fragment with 3 complete *IGS*s from one White Leghorn individual. Also, Red jungle fowl contig NT_456123.1 was included into analysis. We identified several novel tandem repeats, which form regular highly organized structures. Most of repeats are highly GC-rich (65–81%) contributing to the total IGS high GC composition (68% vs. 52% in human). *IGS* is heterogeneous in length due to a copy number variation of some repeats. The alignment of transcriptome reads from different chicken tissues against the most complete rDNA sequence from WAG137G4_utg0 contig has revealed a weak transcriptional activity at certain central sites of *IGS* We have compared the *IGS* organization between chicken and terrapin (*Malaclemys terrapin)*; the complete ribosomal repeat sequence of terrapin was assembled using the raw data of sequencing. In both species, the *IGS* contains very long conservative GC rich tandem repeats and lack of the inverted sequence copies capable to form hairpins. It turned out that on contrast to *IGS* of mammals, amphibians and fish, the *IGS* in chicken and terrapin are GC-enriched and contain many putative CpG islands. These common features in the *IGS* structure appear to be significant when considering the genome evolution in the Sauropsida group. Financial and technical support: RFBR (#18–04–01276), "Chromas" and "Molecular and Cell Technologies" Resource Centres of Saint Petersburg State University, SPbU project 1.40.1625.2017.

Key Words: *Gallus gallus*, *Malaclemys terrapin*, nucleolus organizer region (NOR), rRNA genes, tandem DNA repeats

OP66 Genome-wide association study of dwarf phenotypes in Dutch chicken breeds. Z. Wu*, C. Bortoluzzi, M. F. L. Derks, M. A. M. Groenen, and R. P. M. A. Crooijmans, *Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands.*

Body size is a phenotypic trait studied in many species. One of the extreme forms of this phenotype is dwarfism, which occurs in many forms and many species. In chicken, dwarfism is known in different forms, including autosomal dwarfism, sex-linked dwarfism and, bantam. Of these, the bantam phenotype is the most common form. In the last decades, fancy breeders in the Netherlands have utilized traditional bantams to "bantamize" the Dutch large fowls to produce dwarf versions of the original native breeds. Hence, for every large form of the traditional breed there is now also a bantam form with the same appearance as the large fowl. The resources of the historical Dutch large fowls and their small-sized counterparts offer a powerful opportunity to study the complexity of bantam phenotype and human-mediated selection. Using whole-genome sequence data we conducted Genome-Wide Association Study (GWAS) on 136 chickens from 38 breeds, including traditional bantams, like Dutch bantam. Based on the bantamization breeding history, the chickens were grouped into 3 groups according to the bantams used for the bantamization procedure. Here, each GWAS were performed on the group of chickens from the traditional large breeds, their bantam counterparts, and the true bantam breeds used as the bantam origin. Results of GWAS did not show any commonly shared genomic region between these 3 groups potentially associated with dwarfism, which suggests that the bantam trait within Dutch population is not a simple trait caused by a single underlying gene. Despite that, 2 of the studies revealed a shared significant locus, potentially regulates body size. Further validation of the associated locus is ongoing. Our findings reveal the novel genetic nature underlying the bantam phenotype in traditional Dutch breeds, providing significance in the breeding of dwarf chickens.

Key Words: chicken, body size, bantam, GWAS

OP67 Runting and stunting syndrome in sex-linked dwarf chicken is associated with mitochondrial DNA depletion. H. Li*, Q. Nie, Q. Luo, W. Luo, and X. Zhang, *Department of Animal Genetics, Breeding and Reproduction, College of Animal Science, South China Agricultural University, Guangzhou, Guangdong, China.*

Runting and stunting syndrome (RSS), which is characterized by low body weight, generally occurs early in life and leads to considerable economic losses in the commercial broiler industry. Our previous study has reported that RSS is a kind of mitochondrial disease with mtDNA depletion in poultry. However, the molecular mechanism of RSS remains unknown. In this study, we identified a homogeneous mutation c.409G > A (p. Ala137Thr) of *TWNK* gene in RSS chickens from strain N301. Bioinformatics investigations supported the pathogenicity of the *TWNK* mutation, which is located on the linker region of Twinkle primase domain and might further lead to mtDNA depletion in chicken. In addition, we also found that overexpression of the *TWNK* wild-type can increase mtDNA content in LMH and DF-1 cells, whereas overexpression of the *TWNK* A137T can cause mtDNA depletion in LMH and DF-1 cells. In conclusion, we demonstrated for the first time that the homogeneous *TWNK* c. 409G > A (p. Ala137Thr) mutation is associated with Runting and Stunting Syndrome and mtDNA depletion in chicken.

Key Words: mitochondrial DNA, mitochondrial DNA depletion syndrome, runting and stunting syndrome, SLD chicken, TWNK mutation

OP68 Discovering lethal alleles across the turkey genome using transmission ratio distortion approach. E. A. Abdalla*[1], S. Id-Lahoucine[1,2], B. J. Wood[1,3], A. Cánovas[1], J. Casellas[2], and F. C. Baes[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain,* [3]*Hybrid Turkeys, Kitchener, ON, Canada.*

Reproductive efficiency is one of the most important challenges in livestock production and with the availability of large genomic data sets there are research opportunities available to discover lethal alleles that have an impact on reproduction. For instance, it is possible to trace the inheritance of both alleles and allelic combinations inherited from parent to offspring using genotype trios. A trio is the information of sire, dam and offspring genotypes together. Tracing lethal alleles can be achieved using transmission ratio distortion (TRD) methodologies which are observable difference in the expected inheritance pattern. The objective of this study was to assess TRD in a turkey population to define potential lethal haplotypes, followed by finding the functional evidence that may support the impact of those haplotypes on reproduction. Genomic data of 56,393 single nucleotide polymorphisms (SNP) from 23,242 turkeys were used (11,047 offspring-trios, 500 sires and 2,013 dams). Sliding windows of 4, 10 and 20 SNP haplotypes were used to scan autosomal chromosomes. Classical recessive inheritance patterns, those lethal only in the homozygous state, were found for 14 haplotypes displaying additive and dominance TRD effects. There were 12 haplotypes identified showing a complete or near-complete absence (lethality) for homozygous individuals, but also an under-representation for carrier (heterozygous) offspring. Within these regions, only one showed parent-specific TRD where dam penetrance was high and sire penetrance was low. The functional and positional analyses showed potential candidate genes involved in fertility and embryo development. The results of this study revealed novel candidate lethal haplotypes and

candidate genes that could be managed within a turkey breeding program.

Key Words: lethal haplotypes, reproduction, transmission ratio distortion, turkey

OP69 Effect of gga-miR-1612 and gga-miR-6701-3p on ALV induced lymphoma cell line DT40 by targeting BCL11B. L. Qiu*[1], L. Zhao[1], X. Liu[2], and G. Chang[1], *[1]Yangzhou University, Yangzhou, Jiangsu, China, [2]Poultry Institute, Chinese Academy of Agricultural Science, Yangzhou, Jiangsu, China.*

Avian leucosis viruses (ALVs) are retroviruses that induce malignant neoplasms in poultry, which results in tumor formation, immunosuppression, with ensuing high mortality rates and seriously threatened the prosperity of Chinese poultry industry. Previous transcriptome analysis showed regulating networks between differentially expressed (DE) noncoding RNAs (ncRNAs) and mRNAs involved during ALV-J-induced tumorigenesis, in which B-cell chronic lymphocytic /lymphoma 11B (BCL11B) gene was significantly downregulated in ALV-J-infected chickens, and gga-miR-1612 and gga-miR-6701–3p were predicted to be the potential targets. Many investigations have focused on the role of Bcl11b in the regulation of immunology and tumorigenesis in human. However, the mechanisms of Bcl11b underlying the differences in ALV-J-infected and uninfected chickens are not well known. In this study, we further investigated the relationship between miR-1612 and miR-6701–3p and Bcl11b and the function of miR-1612 and miR-6701–3p. The cloning assay showed 4 kinds of alternative splicing of Bcl11b in ALV-J-infected chickens and 3′UTR of Bcl11b was cloned by 3′ rapid amplification of cDNA ends assay (RACE) based on primers designed from CDS sequences, then the vectors of Luciferase expression and mutation was constructed according the prospective miRNAs targeting sites analyzed by Targetscan and MiRDB bioinformatics database. The dual luciferase reporter assay system showed miR-1612 and miR-6701–3p can bind to Bcl11b in chickens. The gain/loss assay showed miR-1612 and miR-6701–3p inhibited cell proliferation by targeting Bcl11b in DT40 cells which is an ALV induced bursal lymphoma cell line. As expected, the effects triggered by BCL11B overexpression were in accordance with that triggered by miR-1612 and miR-6701–3p inhibitors, suggesting that Bcl11b was a regulator of ALV-J-induced tumorigenesis and miR-1612 and miR-6701–3p played roles in regulating Bcl11b.

Key Words: subgroup J avian leukosis virus, Bcl11b, microRNA, tumorigenesis, chicken

OP71 Integrated metabolomic and transcriptomic analysis evaluating heat and feed stress in layer chickens. D. Laloë[1], F. Jehl[2], C. Desert[2], M. Boutin[2], S. Leroux[3], D. Esquerre[4], C. Klopp[5], D. Gourichon[6], F. Pitel[3], S. Lagarrigue[2], and T. Zerjal*[1], *[1]INRA, GABI, Jouy-en-Josas, France, [2]INRA-AGROCAMPUS OUEST, Saint Gilles, France, [3]INRA, GenPhySE, Castanet Tolosan, France, [4]INRA, Plateforme GENOTOUL, Castanet-Tolosan, France, [5]INRA, SIGENAE, Castanet-Tolosan, France, [6]INRA, PEAT, $Nouzilly, France.*

Highly productive animals are sensitive to ambient temperature and feed quality changes. A better understanding of the mechanisms underlying animal adaptation to suboptimal production environments is needed to promote innovation in farm animal production. We used liver metabolomic and transcriptomic approaches to identify discriminatory metabolites and genes between layer chickens fed, for 14 weeks, a 15% energy-depleted diet versus a commercial diet and between layer chickens reared, for 4 weeks, at high ambient temperatures (32°C) versus thermos-neutrality. A total of 48 Rhode Island Red hens from 2 lines divergently selected for residual feed intake were used in this study. Liver samples were collected from animals of 31 weeks of age at the end of the stress period. The samples were analyzed by ^{1}H-NMR spectroscopy and NMR spectra were reduced to 142 buckets. The same samples were sequenced using Illumina HiSeq3000, yielding an average per sample of 90 million paired-end reads. The reads were mapped to the *Gallus gallus*-5 reference genome by STAR software and counted by RSEM software using the Ensembl V87 GTF annotation. A multivariate analysis know as redundancy analysis (RDA) was used to model the impact of the line and stress on the metabolomics and transcriptomics profiles. Both the line and the stress significantly affected the metabolomics and transcriptomics but the impact was, in large part, stress dependent. Co-inertia multivariate method analysis (CoIA) was used to identify significant covariations existing between metabolomic and transcriptomic data. Covariation was observed, in both stresses, in genes and metabolites involved in lipid and amino acids metabolism. Under heat stress, covariation was also observed among genes and metabolites with activity in carbohydrate metabolism and energy production. Under feed stress it was observed for genes and metabolites involved in cell cycle and growth.

Key Words: chicken, metabolomic, transcriptomic, adaptation, abiotic stress

OP72 Candidate signatures of positive selection in Ethiopian chicken. A. Kebede[1,2], K. Tesfaye[1], G. Belay[1], A. Vallejo*[5], T. Dessie[3], N. Sparks[4], O. Hanotte[3,5], L. Raman[5], and A. Gheyas[4,5], *[1]Addis Ababa University (AAU), Addis Ababa, Ethiopia, [2]Amhara Regional Agricultural Research Institute (ARARI), Bahir Dar, Ethiopia, [3]International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [4]Center for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Edinburgh, UK, [5]School of Life Sciences, University of Nottingham, Nottingham, UK.*

Selective breeding for genetic improvement is expected to leave distinctive selection signatures within genomes. The identification of selection signatures can help elucidate the mechanisms of selection and accelerate genetic improvement. Ethiopia has several chicken ecotypes, which have evolved in different ago-ecologies. Here, we assess the footprints of candidate signatures of positive selection from whole genome autosomal sequences comprising 14,857,039 SNPs genotyped in Improved Horro, Local Horro, Hugub, Arabo and Jarso chicken populations of Ethiopia. We identified selection signals in 20 kb windows, with sliding steps of 10 kb based on estimators of pooled heterozygosity (*Hp*) and F-statistics (*Fst*). Selective sweep analyses using *Hp* and *Fst* identified genomic regions associated with production and reproduction. A total of 595 candidate genes showed high evidence of positive selection in indigenous chicken populations, including genes that were related to traits such as growth and egg production. Gene ontology analysis displayed several biological processes and KEGG pathways involved in estrogen biosynthesis, nervous system development processes, calcium signaling, and biosynthesis of unsaturated fatty acids. The regions identified in this study are expected to provide genomic landmarks to enhance the ongoing breed improvement operations in improved Horro and for the other 4 chicken populations.

Key Words: indigenous chicken, Improved Horro, signature of selection, SNP

OP73 Invited Workshop Presentation: Characterization of the putative host-parasite interactome in the salmon-louse relationship. L. Braden*[1,2], J. Poley[3], W. Cai[2], and M. Fast[2], *[1]AquaBounty Canada, Souris, PE, Canada, [2]Hoplite Lab, Department of Pathology and Microbiology, Atlantic Veterinary College-UPEI, Charlottetown, PE, Canada, [3]Centre for Aquaculture Technologies, Souris, PE, Canada.*

The host-parasite relationship between the salmon louse *Lepeophtheirus salmonis* (*Lsal*) and its host involves the skin-attachment site where there is an attack response by the parasite and a defense response by the host. *Lsal*-derived proteins secreted at the attachment site have been predicted to be immunosuppressive, however, little is known about the impact they have on consequent host responses. Furthermore, there is no information as to the relative contribution of louse virulence to variability in host susceptibility, or the host proteins involved in this interaction. Host-parasite protein interactions (HPPIs) play a critical role in initiating pathogenesis and maintaining infection. Thus, both a characterization of putative *Lsal*virulence factors in addition to identifying HPPIs would greatly enhance our understanding of this host-parasite relationship and facilitate discovering novel pharmacological/vaccine targets or identifying biomarkers of resistance. Here we use a combination of transcriptomics and proteomics to predict the interactome of *Lsal*-salmon. We identified a cluster of *Lsal*genes associated with feeding using consensus lists across 6 different studies and applying a correlational analysis to the parasite transcriptomes. These genes were annotated by predicted ORFs, adding UniProt, GenBank and LiceBase accessions, identifying conserved protein domains and signal peptides, and determining orthologs to known virulence factors in *Ixodes* spp. Using LC-MS/MS, we identified a suite of proteins in *Lsal*secretory/excretory products to produce a list of interacting louse proteins that were concordant with the transcriptome. The genome of the susceptible host, Atlantic salmon, was mined for predicted interacting proteins that would be involved in the host-parasite interaction. Using interolog and domain-based approaches, interactions between louse and salmon proteins were inferred. Expression patterns of key *Lsal* interacting proteinsprofiled after parasitizing susceptible or resistant hosts, in situ localization, and RNA interference experiments support the importance of these virulence factors as mediators of the host-parasite interactions.

Key Words: salmon lice, interactome, protein:protein interactions, immunomodulation, transcriptomics

OP74 The molecular basis of salmon sexual maturation: An integrative multi-omics approach. A. Mohamed*[1], M. Sanchez[1], M. Menzies[1], A. Reverter[1], B. Evans[2], H. King[3], and J. Kijas[1], *[1]CSIRO Agriculture, St Lucia, Queensland, Australia, [2]Tassal Group Ltd., Hobart, Tasmania, Australia, [3]CSIRO Agriculture, Hobart, Tasmania, Australia.*

Early onset of sexual maturation in Atlantic salmon presents an ongoing challenge for the salmon aquaculture industry due to negative impacts on growth rate and product quality. Consequently, a significant amount of research aims to understand the biological mechanisms driving early salmon maturation. We performed a dedicated animal experiment to elucidate genes and regulatory networks associated with the apparent "decision" of animals to commence sexual maturation. Samples from 3 tissues (pituitary gland, ovary and liver) were collected before and after application of a photoperiod trigger designed to initiate maturation. Deep RNA sequencing (RNA-seq), whole genome bisulfite sequencing (WGBS) and Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) were performed to profile genome-wide gene expression, DNA methylation and open chromatin during this time-course experiment. Transcriptomic analyses identified clusters of differentially expressed genes (DEGs) in the analyzed tissues, which described their physiological roles. The identity of the DEGs, in response to the onset of maturation, revealed the key players involved in the earliest triggers into maturation such as gonadotropins genes in the pituitary along with genes implicated in follicular development and energy homeostasis in ovary and liver, respectively. Analyzing the status of the methylome and accessible chromatin elucidated the epigenetic mechanisms underlying the observed transcriptomic remodeling in these tissues. Gene regulatory networks were constructed by combining the results from these multi-tissue/omics analyses along with results from GWAS and predicted transcription factors in salmon. These networks revealed key genes and regulators that will improve our understanding of the mechanisms of salmon sex maturation and potentially pave the way to new strategies to control the timing of maturation in Atlantic salmon.

Key Words: transcriptome, methylome, ATAC-seq, salmon, puberty

OP75 Different transcriptional response of susceptible and resistant fish hints at the mechanism of KHV disease resistance in carp. R. Tadmor-Levi[1], A. Doron-Faigenbaum[2], G. Hulata[2], and L. David*[1], *[1]Department of Animal Sciences, R.H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel, [2]Agricultural Research Organization, Bet Dagan, Israel.*

Infectious diseases challenge production and further development of the aquaculture sector. Breeding of disease resistant strains is a sustainable solution to this problem. Identifying the mechanism confering resistance will enhance breeding of resistant strains and understanding bony-fishes immunity. Cyprinid herpes virus-3 (CyHV-3or KHV) is a dsDNA virus damaging production of common carp, one of the top produced fish worldwide. Applying family selection, we have been breeding resistant carp strains. By analyzing viral loads in host tissues during infection, we found that resistant fish restrain the viral spread in their bodies better than susceptible fish, suggesting that in this case, resistance reflects a difference in their immune response. To further understand the mechanism, RNaseq was applied to characterize the transcriptomic response to infection in the spleen of susceptible and resistant fish. Distinct profiles were found for susceptible and resistant fish at d 0 before infection, reflecting genetic background differences. More importantly, a distinct transcriptional response to infection was elicited between these fish types. In susceptible fish, over 4 times more differentially expressed genes were upregulated between d 0 and 4 to infection compared with resistant fish. Upregulated genes in susceptible fish were significantly enriched for interferon and interferon induced genes. Upregulated genes in both susceptible and resistant fish were enriched for leukocyte migration function, but different chemokines were elicited by each fish type. Chemokines are a large gene family with multiple homologs in bony-fishes and with specific paralogs in the duplicated genome of the common carp. Interestingly, some degree of functional divergence in expression levels due to infection was observed between homologs of these chemokines and also between carp-specific paralogs. Analyses of further time points for specific genes from these pathways indicated that resistant fish mount a neutrophil-assisted response that susceptible fish were lacking. The transcriptomic response is concordant with the differences between these fish types in tissue viral loads and mortality. Our results hinted at some of the differences between susceptible and resistant fish and highlighted some genes and pathways taking part in the disease resistance mechanism.

Key Words: fish, immunogenomics, RNA-seq, infectious disease, aquaculture

OP76 Analysis of adaptive plasticity of pikeperch (*Sander lucioperca* L., 1758) after temperature change based on gene expression data. T. Goldammer*[1], F. Swirplies[1], S. Wuertz[2], B. Baßmann[3], A. Orban[2,4], N. Schäfer[1], R. M. Brunner[1], F. Hadlich[1], A. Rebl[1], and M. Verleih[1], *[1]Fish Genetics Unit, Institute of Genome Biology, Leibniz*

Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Department of Ecophysiology and Aquaculture, Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany, [3]Aquaculture & Sea-Ranching, Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany, [4]Food Chemistry and Food Biotechnology, Justus Liebig University Giessen, Gießen, Germany.

The pikeperch is an uncommon aquaculture species so far (900t; FAO 2018) that is characterized as fast growing fish and by its excellent flesh quality. However, pikeperch is also considered a highly stress-susceptible domestic species. Therefore, the success of pikeperch breeding in aquaculture depends on optimal rearing conditions. Until now, only little information has been published yet on the adaptive plasticity and resilience of pikeperch, specifically on temperature change. Water temperature is doubtlessly one of the most important factors affecting the well-being of fish. An ambient water temperature of significantly more than 20°C has been suggested as optimal for the growth of pikeperch in aquaculture farms, although natural habitats of pikeperch seldom reach such temperatures. We profiled the response of juvenile pikeperch exposed to gradually increasing water temperatures between 15°C and 25°C within 11 d. Multiplex-qPCR assays with 38 genes and liquid chromatography/mass spectrometry were used for recording a broad spectrum of potential stress-marker indicators. The analyzed genes characterizing heat shock and hypoxia response, immune activation, energy supply and development. In addition, the suitability of 8 potential reference genes for data normalization has been assessed. The data revealed that pikeperch do not develop severe responses to ambient temperatures between 15 and 25°C, although particular parameters indicated a phased restriction of optimal conditions, above all the heat-shock genes SERPINH1, HSP90AA1, HSPA8b; the acute-phase genes HP, C3, SAA; and the transcription factor-encoding gene HMX1. This report paves the ground for future assessment studies on the imbalanced homeostasis of pikeperch by means of molecular tools. (Project coordination: Campus bioFISCH-MV; funding: European Maritime and Fisheries Fund (EMFF) and the Ministry of Agriculture and the Environment in Mecklenburg-Western Pomerania; grant: MV-II.1-RN-001).

Key Words: fish, functional genomics, functional assay, biomarker, animal health

OP77 Optimising genotype imputation strategies for genomic selection in farmed Atlantic salmon. S. Tsairidou*[1], A. Hamilton[2], D. Robledo[1], J. Bron[3], and R. Houston[1], *[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK, [2]Hendrix Genetics Aquaculture BV/ Netherlands Villa 'de Körver', Spoorstraat 695831 CK Boxmeer, The Netherlands, [3]Institute of Aquaculture, University of Stirling, Stirling, UK.*

Genomic selection is increasingly applied in aquaculture breeding to expedite genetic gain for key production and disease resistance traits. However, effective application in breeding programmes depends on large training data sets of phenotypes and genotypes. In livestock breeding, genomic information on large populations can be achieved in a cost-effective manner through genotype imputation. In typical aquaculture breeding programmes where large full-sibling families are available, the value of imputation is yet to be fully assessed. The aim of this study was to evaluate strategies for genotype imputation in Atlantic salmon breeding programmes, by assessing the trade-off between the cost of genotyping at a given density and the impact on genomic prediction accuracy. Optimal high density (HD) SNP panels were identified and imputation accuracies for a range of low density (LD) SNP panels were systematically assessed. Then, genomic prediction accuracies were compared. Analyses were performed via a software pipeline using: (a) PLINK and in-house built software in R and Shell for selection of HD and LD SNP panels and calculation of genomic relationship matrices; (b) FImpute for genotype imputation; and, (c) ASReml for estimating breeding values and cross-validated prediction accuracies. The study focused on (i) a Scottish Atlantic salmon breeding program population challenged with *L. salmonis*, and (ii) a Chilean population challenged with *C. rogercresseyi* (both Landcatch). Genomic prediction was assessed for sea lice resistance and growth traits, known to have polygenic genetic architecture. Relatively low density panels are sufficient to give near-maximal prediction accuracy in the absence of imputation, due to close relationships between training and validation populations. Although imputation to HD is likely to benefit the discovery of quantitative trait loci and fine-mapping of regions identified by genome-wide association analyses, genomic prediction appeared to reach near-maximum accuracy with less than 1,000 SNPs. However, the value of genotype imputation in genomic selection varies depending on the population and the trait.

Key Words: fish, animal breeding, bioinformatics, aquaculture

OP78 Genomic tools for an efficient management of genetic resources in Atlantic salmon. M. Saura*[1], A. Chtioui[1,5], M. Gabián[2], P. Morán[2], A. Caballero[2], E. Santiago[3], AI Fernández[1,6], M. P. Kent[4], L. Covelo-Soto[2], A. Fernández[1], and B. Villanueva[1], *[1]INIA, Madrid, Spain, [2]Universidad de Vigo, Vigo, Spain, [3]Universidad de Oviedo, Oviedo, Spain, [4]Center for Integrative Genetics, Norwegian University of Life Sciences, Ås, Norway, [5]Georg-August-Universität Göttingen, Göttingen, Germany, [6]Red CIBER, Madrid, Spain.*

Human activity and environmental factors have caused a considerable depletion in wild populations of Atlantic salmon during the last century. These populations are at increased risk because of reduction in genetic variability and increased rates of inbreeding depression. An efficient management of genetic resources requires controlling factors that reduce effective population size, such as unequal sex ratio. However, sex determination in fish is not straightforward at early stages of development. In this study, we have used high-dense genotyping information to (1) evaluate the genetic status of wild Atlantic salmon populations from 6 Spanish rivers (Miño, Ulla, Eo, Sella, Urumea, Bidasoa), and to (2) investigate the genetic regulation of sex determination in this species, with the last aim of optimising the conservation programmes of these populations. For the first objective, we inferred current and historical series of effective population size from the analysis of the spectrum of linkage disequilibrium. Current estimates for the different populations were between 100 and 600. Historical estimates evidenced reductions in effective population time. The combined results from regional heritability mapping and genome-wide association analyses showed that chromosomes Ssa02 and Ssa21 explained the highest proportion of heritability for sex, and harbored important genes related to sex in other species of the class Actinopterygii. In summary, our results suggest that the genetic status of these populations, although safe for avoiding inbreeding depression in the short term, may be critical in the long term, particularly in this area that represents the South distribution limit of the species in Europe, thus conservation strategies are recommended. Our findings also support previous studies indicating that Ssa02 is the predominant sex-determining chromosome in Atlantic salmon, but also provide new knowledge on how sex determination operates in this species, highlighting the potential of genomic tools for managing genetic resources in fish.

Key Words: fish, conservation genomics, genome-wide association, biodiversity

OP79 Determination of genetic structure and selection signatures in Coho salmon (*Oncorhynchus kisutch*) populations by genome-wide SNP analyses. M. E. López*[1,2], A. Barría[2], E. Rondeau[3], B. Koop[3], and J. M. Yáñez[2], *[1]Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Universidad de Chile, Santiago, RM, Chile, [3]University of Victoria, Victoria, BC, Canada.*

Coho salmon (*Oncorhynchus kisutch*) is one of the 3 most important species of farmed salmon in the world, and Chile is the main producer of this species. Phenotypes of farmed population have evolved due to the combined influence of domestication and selection through reproduction in captivity and human directional selective breeding. These events might have shaped the genetic diversity of these species

throughout history, and their present genomes may contain traceable signatures of selection. Detecting genomic selection signatures is a main goal of modern population genetics as it enhances our knowledge of the molecular mechanisms shaping the genome as well as providing functional information on specific genomic regions that might have biological or productive interest. In this study we evaluate genetic diversity and structure and identify signatures of selection among the most important domestic strains of coho salmon in Chile using a medium density SNP array (~130K). Population structure and signatures of selection were examined using principal component analysis (PCA), admixture analysis, pairwise distances (FST), integrated haplotype score (iHS) and runs of homozygosity (ROH). Based on the results of our study, we discuss the ability to detect genomic regions that may underlie important traits of practical interest for aquaculture and to better understand the effect of domestication in Coho salmon.

Key Words: fish, genotyping, animal domestication

OP80 Identification of a major locus determining a coloration defect in gilthead seabream (*Sparus aurata*). F. Bertolini[1,2], A. Ribani[3], F. Capoccioni[4], L. Buttazzoni[4], V. J. Utzeri[3], S. Bovo[3], M. Caggiano[4], L. Fontanesi*[3], and M. F. Rothschild[2], *[1]National Institute of Aquatic Resources, Technical University of Denmark, Lyngby, Denmark, [2]Department of Animal Science, Iowa State University, Ames, IA, USA, [3]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [4]Centro di ricerca di Zootecnia e Acquacoltura, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Roma, Italy, [5]Panittica Italia Società Agricola Srl, Brindisi, Italy.*

Cultivated gilthead seabream (*Sparus aurata*) are affected by several phenotypic abnormalities that span from skeletal malformations, lack of operculum and coloration defects. Fry that do not fulfill specific standards are discarded causing major economic losses. Their frequency seems to increase in inbred populations supporting that genetic factors might be causative, at least in part and for some of these defects. The *S. aurata* genome has been recently sequenced and a preliminary assembled genome version for this species, which is publicly available, could help the identification of the genetic factors associated with these abnormalities. In this study, a whole genome resequencing approach was used to identify a major locus causing a coloration defect that has been recently observed among cultivated fry of this aquaculture species. Two equimolar DNA pools were constructed using DNA extracted from 30 normally colored and 30 defective fry. Fish were sampled from the same commercial hatchery. Then whole-genome re-sequencing was carried out from the 2 DNA pools using Illumina paired end technology at about 90X. Variant calling and allele frequencies were obtained using the CRISP software. Fst was calculated on sliding windows of different sizes (ranging from 100 kb to 2 Mbp). Only one sharp Fst peak, located on chromosome 6, was identified from this genome scan, suggesting the presence of a major locus in this region that may affect fish color. Comparative functional analyses across fish species of the identified genome region did not reveal any obvious candidate genes, suggesting that the causative mutation(s) might involve novel mechanisms not yet described or that the annotation of this region should be refined. A detailed analysis of this region is underway with the aim to identify markers that could be used to eradicate the defect in the analyzed gilthead seabream population. This study was supported by the Ensminger fund, State of Iowa funds and University of Bologna RFO funds.

Key Words: fish, genome sequencing, functional genomics, coat color, aquaculture

OP81 From sea to plate: Genomically enabling the Australasian snapper (*Chrysophrys auratus*) for aquaculture. M. Wellenreuther*[1,2], D. Ashton[1], E. Hilario[3], R. Crowhurst[3], P. M. Whittle[1], A. Catanach[4], J. Le Luyer[5], C. Deng[4], C. David[4], P. Ritchie[6], and L. Bernatchez[7], *[1]The New Zealand Institute for Plant & Food Research Ltd., Nelson, New Zealand, [2]School of Biological Sciences, University of Auckland, Auckland, New Zealand, [3]The New Zealand Institute for Plant & Food Research Ltd., Auckland, New Zealand, [4]The New Zealand Institute for Plant & Food Research Ltd., Lincoln, New Zealand, [5]Ifremer, UMR 241 Ecosystèmes Insulaires Océaniens, Centre Ifremer, Tahiti, French Polynesia, [6]School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand, [7]Institut de Biologie Intégrative et des Systèmes (IBIS), Pavillon Charles-Eugène Marchand, Université Laval, Québec, Canada.*

The Australasian snapper (Sparidae: *Chrysophrys auratus*) has been identified as a promising candidate for aquaculture, and a breeding program was initiated in 2016 to select for faster growth. Here we report on recent developments to genomically enable this species for aquaculture breeding by 1) presenting a high-quality genome assembly – which integrates short-insert and mate-pair sequencing reads along with optical mapping and a linkage map - that represents a valuable resource to aquaculture programmes around the world using closely related bream species. We further present 2) a catalog of SNPs and structural variants across the genome from 12 whole-genome re-sequenced wild snapper to both characterize the extent of genetic variation in this species and to quantify the extent of variant overlap with coding regions as well as those under putative selection. We then 3) use a GBS data set of a hatchery reared and phenotyped pedigree to describe the first QTLs for growth in this species, and highlight some of the key findings from a growth-temperature rna-seq experiment on wild and domesticated snapper to elucidate the genes responsible for growth under different temperatures regimens. Phenotyping in this species was conducted with automated image-based software that can both identify and track individuals over time and extract trait parameters with high precision. Finally, we will discuss how these genomic findings can be used to inform the future research directions of the breeding program in this commercially and culturally important species.

Key Words: genome assembly, SNPs, structural variation, gene expression, growth

OP82 Challenges and opportunities for genomics in selective breeding programs for marine mass-spawning fish species. J. M. Yáñez*[1], M. E. López[2], P. Cáceres[1], and G. M. Yoshida[1], *[1]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile, [2]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Marine mass-spawning fish species represent an important source for aquaculture diversification worldwide. The reproductive biology of these species represents considerable challenges for the design and establishment of selective breeding programs. For instance, the impossibility of carrying out individual crosses (i.e., using artificial strip spawning of broodstock) makes pedigree traceability and control over the number and size of families in the progeny cohorts difficult. Genomic information can be used to overcome these issues and assist the establishment of breeding programs in marine mass-spawning fish species. Here we present a case study in which we develop and implement genotyping-by-sequencing tools and low-density single nucleotide polymorphisms (192 SNP) panel for the evaluation of broodstock genetic variability, diversity and structure, pedigree reconstruction, genetic parameter estimation and genomic predictions for growth in cobia (*Rachycentron canadum*). Low-density SNP genotypes were obtained from one thousand fish, including broodstock and production animals. This information was used for family inference and estimation of heritability for growth ($h^2 = 0.31$). A linkage map spanning 599 cM across 22 linkage groups was also built. In addition, genomic prediction models are compared with traditional predigree-based approaches for genetic evaluation of growth. Finally, we discuss about the potential use of genomic information to assist the practical implementation of a genetic improvement program and their implications on the design of the breeding plan.

Key Words: genotyping-by-sequencing, genomic selection, heritability, genetic correlation, growth

OP83 ***Suina* genomes and phylogenomics.** L. Eory[1], A. Warr[1], H. A. Finlayson[1], S. J. Girling[3], K. Gharbi[2], T. Watson[1], T. Burdon[1], A. A. MacDonald[1], E. Okoth[5], A. Djikeng[4,1], M. Watson[1], and A. L. Archibald*[1], *[1]The Roslin Institute and R(D)SVS, University of Edinburgh, Edinburgh, UK, [2]Edinburgh Genomics, University of Edinburgh, Edinburgh, UK, [3]The Royal Zoological Society of Scotland, Edinburgh, UK, [4]Centre for Tropical Livestock Genetics and Health, Nairobi, Kenya, [5]International Livestock Research Institute, Nairobi, Kenya.*

The pig family of animals (*Suidae*) and the related peccary family (*Tayassuidae*) together constitute the *Suina* suborder also known as *Suiformes*. The wild Suina species are found in Europe, Asia, Africa and South America. Several Suina species are endangered and others represent potential reservoirs of pathogens that pose serious risks to domestic pigs (e.g., African Swine Fever virus). For domestic pigs (*Sus scrofa*) genome sequence resources included: 2 highly contiguous annotated genome sequences (Sscrofa11.1; USMARCv1.0) as well as several other genomes assembled to contig or scaffold level plus multiple re-sequenced genomes. There are also some whole genome shotgun sequence data available for *Sus barbatus*, *Sus cebifrons*, *Sus celebensis*, and *Sus verrucosus* and *Phacochoerus africanus.* We have sequenced and assembled the genome of 3 African Suidae species at contig level. For the Common Warthog (*Phacochoerus africanus*) we generated ~60x genome coverage in long reads using the Pacific Biosciences Sequel platform from a single female individual from whom we also generated 60x genome coverage in Illumina short reads and RNA-seq data from 15 tissue samples. The long read data were assembled with Falcon-unzip which yielded a phased draft genome of 2.42 Gbp in 1,454 contigs with a contig N50 of 4.1 Mbp and a longest contig of 26.5 Mbp. We are currently scaffolding these contigs using Dovetail's Hi-C and Hi-Rise approach. We have also generated Illumina short whole genome sequence data from several other warthogs to characterize genetic diversity in this species. For the Red River Hog (*Potamochoerus porcus*) and Bushpig (*Potamochoerus larvatus*) we used the DISCOVAR approach based on 97x and 84x genome coverage respectively in Illumina 250 bp paired end reads. The assembly statistics are: Red River Hog 2.51 Gbp genome, 124,126 contigs, contig N50 160,454 bp, longest contig 1.69 Mbp; Bushpig 2.55 Gbp genome, 182,846 contigs, contig N50 108,171 bp, longest contig 1.31 Mbp. We are generating long reads for the Red River Hog genome using Oxford Nanopore Technology to establish a more contiguous assembly for this species. Finally, we have established a phylogenetic tree for these and other Suina species based on our own sequence data together with sequence data in the public databases.

Key Words: genome, suina, phylogenomics

OP84 **Loss of function mutations in the pig causing embryonic lethality.** M. F. L. Derks[1], A. B. Gjuvsland[2], M. Bosse[1], M. S. Lopes[3,4], M. van Son[2], B. Harlizius[3], E. Grindflek[2], H. J. Megens[1], and M. A. M. Groenen*[1], *[1]Wageningen University & Research, Animal Breeding and Genomics, Wageningen University & Research, Animal BreedingWageningen, the Netherlands, [2]Norsvin, Hamar, Norway, [3]Topigs Norsvin Research Center, Beuningen, the Netherlands, [4]Topigs Norsvin, Curitiba, Brazil.*

Lethal recessive alleles can result in pre- or postnatal death in homozygous affected individuals and in reduced fertility in the population. Especially in small size domestic and wild populations, such alleles might be exposed by inbreeding, caused by matings between related parents that inherited the same recessive lethal allele from a common ancestor. We have screened commercial pig populations for recessive lethal mutations and have identified 6 recessive lethal mutations that occur at relatively high minor allele frequencies (up to 7%) in commercial pig populations. While these lethal mutations have a large effect on carrier-by-carrier matings and decrease litter sizes by 15 to 22%, the mutations are maintained at stable frequencies within the population. These lethal mutations include 2 splice-site variants (*POLR1B*, *TADA2A*), one large deletion (*BBS9*), one frameshift (*URB1*), and one missense (*PNKP*) variant, resulting in a complete loss-of-function of these essential genes. Moreover, we show that the deletion within the *BBS9* gene is maintained in the population because of a strong positive effect on growth in heterozygotes (i.e., balancing selection), *while the other lethals are likely the* result of genetic drift. Together, the recessive lethal alleles affect up to 3% of the litters within a single population and are responsible for the death of 0.5% of the total population embryos. Moreover, we provide compelling evidence that the identified embryonic lethal alleles contribute to the observed heterosis effect for fertility (i.e., larger litters in crossbred offspring). All mutations identified are population specific and therefore, no embryonic losses are expected in the crossbred offspring from these breeding lines. These observations provide a unique model to better understand fertility and heterosis in livestock.

Key Words: pig, genome sequence, lethal variation, heterosis, SNP

OP85 **Pig transcriptome analysis suggests a global regulation mechanism enabling temporary bursts of circular RNAs.** A. Robic*[1], K. Feve[1], T. Faraut[1], S. Djebali[1], R. Weikard[2], and C. Kuehn[2,3], *[1]GenPhySE, University of Toulouse, INRA, ENVT, Castanet Tolosan, France, [2]Institute Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [3]Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany.*

Although the functions of most of circular RNAs (circRNAs) are not characterized, they likely impact many biological processes. Indeed, in addition to generating a linear transcript many protein-coding genes produce circRNAs that are tightly regulated.By analyzing Total-RNaseq from 7 pubertal testis samples we detected 126 introns in 114 genes able to produce intronic circRNAs and 5,236 exonic circRNAs produced by 2,516 genes. Comparison of Total-RNaseq data sets from porcine testis, embryonic cortex and postnatal muscle stages revealed a high abundance of intronic and exonic circRNAs for 2 samples from pubertal testis and embryonic cortex, respectively. In pubertal testis with circRNAs in abundance, 24% of protein-coding genes produced linear and circular transcripts. This abundance was due to higher production of circRNA by the same genes than in other testis samples rather than to the recruitment of new genes. No global relationship between exonic circRNA and mRNA productions was found in pubertal testis. We showed that exonic circRNAs are typically produced by large genes that are also able to produce mRNAs. We suggest ExoCirc-9244 (origin: SMARCA5) to be a marker of circRNA abundance in testis. Among 76 pubertal testes analyzed by RT-PCR, we identified 12 transcriptomes containing a large quantity of ExoCirc-9244 and which are therefore suspected to have an overall high circRNAs abundance. By qRT-PCR, we confirmed results concerning ExoCirc-9244 and showed that samples expressing ExoCirc-9244 had low steroid synthesis potential (confirmed by plasma estradiol level).Even though our data indicate that this massive production of circRNAs is much more related to the structure of genes generating circRNAs than to their function, we highlighted with ExoCirc-9244, a particular stage of pubertal testis development characterized by a low steroid production and an abundance of circRNA. We suggest that bursts of circRNAs might be linked to an abrupt switch of the cellular metabolism: a distinct stage in developmental processes of embryonic cortex or pubertal testis may require circRNAs. In these circumstances the number of circRNAs would be more important than their individual characteristics, which would be a unique feature in the transcripts world.

Key Words: circular RNAs, pig, RNAseq, physiological genomics, non-coding RNA

OP86 Characterization of A-to-I editing events in fetal pig tissues. R. J. Corbett*[1], S. A. Funkhouser[1], D. J. Nonneman[2], T. P. L. Smith[2], and C. W. Ernst[1], *[1]Michigan State University, East Lansing, MI, USA, [2]U.S. Meat Animal Research Center, Clay Center, NE, USA.*

RNA editing results in alterations to RNA molecules, producing transcript sequences that are inherently different from the parent DNA molecule. Adenosine-to-Inosine (A-to-I) editing is widespread in metazoans, and is carried out by adenosine deaminase acting on RNA (ADAR) enzymes. The extent to which A-to-I editing influences transcript diversity in the pig has been evaluated; however current studies have been conducted primarily in adult tissues, limiting understanding of how editing processes or patterns change during pig development. The objective of this study was to assess A-to-I editing dynamics in 4 pig fetal tissues (whole brain, liver, skeletal muscle, and placenta) at 30 and 70 d gestation (dg). RNA-sequencing data from each tissue as well as whole-genome sequencing data for a single 70dg White-Composite × Meishan pig were used to identify A-to-G DNA-to-RNA mismatches—indicative of putative A-to-I edits—using the editTools R package. High-confidence sites were subsequently scanned in RNA-seq data from tissues of 3 additional pigs (1 70dg, 2 30dg) to identify conserved mismatch sites. We identified a total of 618 putative shared A-to-I editing sites, with 249, 60, 110, 199 sites identified in brain, liver, muscle, and placenta, respectively. We observed a significant increase in mean editing level across sites in brain ($P = 0.011$), and a significant decrease in mean editing level of sites in muscle ($P = 0.045$) from 30dg to 70dg. Consistent with changes in mean editing level, brain tissue exhibited an increase in *ADAR2* transcript abundance at 70dg (log2FC = 0.82, $P = 0.06$), while muscle exhibited a decrease in *ADAR1* abundance (log2FC = −0.377, $P = 0.03$). We identified 22 differentially edited sites between stages, the majority of which were brain-specific and exhibited increased editing at 70dg, and resulted in variants in intronic, 3′ UTR, and coding sequences. Our results suggest dynamic global and site-specific editing patterns in tissues with differential *ADAR* expression throughout development. Future work will assess editing patterns at high-confidence sites identified in adult pig tissues in the fetal transcriptome. USDA is an equal opportunity provider and employer.

Key Words: pig, RNA editing, development

OP87 Whole-genome sequencing reveals population structure and diversity of Nigerian indigenous pigs. A. C. Adeola*[1,2], S. Khederzadeh[1,3], D. H. Mauki[1,2], C.-P. Huang[1,3], C. Yan[1,3], O. O. Oluwole[4], S. C. Olaogun[5], L. M. Nneji[1,2], P. M. Dawuda[6], O. G. Omitogun[7], L. Frantz[8], R. W. Murphy[9], M.-S. Peng[1,2], H.-B. Xie[1,2], Y.-P. Zhang[1,10], *[1]State Key Laboratory of Genetic Resources and Evolution & Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China, [2]Sino-Africa Joint Research Center, Chinese Academy of Sciences, Nairobi, Kenya, [3]Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan, China, [4]Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Oyo State, Nigeria, [5]Department of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria, [6]Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine, University of Agriculture Makurdi, Makurdi, Benue State, Nigeria, [7]Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, [8]The Palaeogenomics and Bio-Archaeology Research Network, Research Laboratory for Archaeology, University of Oxford, Oxford, UK, [9]Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, Toronto, Canada, [10]State Key Laboratory for Conservation and Utilization of Bio-Resources, Yunnan University, Kunming, Yunnan, China.*

The population structure and evolutionary history of African indigenous pig is of particular interest and fundamental for understanding its genetic diversity. Herein, we analyze whole-genome sequences of 51 Nigerian Indigenous pigs (NIP) from 3 different geographic regions plus 210 wild and domestic pigs from Far East, Near East and Europe. We found that the NIPs clustered into 3 distinct subpopulations with varying levels of European and East Asian pig ancestry. Evidences from phylogenetic, PCA and ADMIXTURE analyses revealed that 2 subpopulations clustered closer to European Wild boar and domestic pigs, and the remaining one to East Asian wild and domestic pigs. Significant gene flow was detected from European wild boar into the NIPs, indicating that majority of NIP are of European ancestry. The 2 NIP subpopulations with substantial European ancestry showed a high level of genetic diversity and rapid LD decay, while the subpopulation with East Asian ancestry showed a lower genetic diversity and slower LD decay. It indicates that the NIP with East Asian ancestry might have a smaller effective population size, consistent with our observation of its contribution to the small fraction in the sampled NIPs. And the related gene flow occurred recently. Our study provides insights into NIP population structure and diversity, and would aid in future conservation efforts

Key Words: Nigerian indigenous pigs, population genomics, population structure, admixture, conservation

OP88 Genetic heterogeneity of wattles in sheep and pigs. J. Paris*[1], A. Grahofer[2], A. Letko[1], I. Haefliger[1], N. Hirter[1], P. Ciaramella[3], G. Leuhken[4], and C. Droegemueller[1], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Clinic for Swine, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [3]University of Naples Federico II, Department of Veterinary Medicine and Animal Production Veterinary, Napoli, Italy, [4]Institute of Animal Breeding and Genetics, Justus Liebig University, Gießen, Germany.*

Wattles are dominantly inherited thumb-shaped appendages on the ventral throat and has been known for a long time in different species as goat, sheep or pig. Even if wattles in sheep and pig are not as common as in goats, there are some rare domestic breeds known which show this phenotype. In goat, the responsible locus was mapped to a region on chromosome 10 containing a functional candidate gene for limb development and outgrowth. In this study, we investigated the genetic background of the wattles in German East Friesian sheep and Ouessant sheep originating from France, and Italian Casertana pigs and Kune Kune pigs originating from New Zealand. Ovine 600k array genotype data of 94 sheep was used for the investigation in sheep. This data contained 48 East Friesian sheep (24 with and 24 without wattles) and 46 Ouessant sheep (23 with and 23 without wattles). Within-breed and multi-breed GWAS detected a single highly associated genome region on chromosome 18. Furthermore, WGS of 4 sheep, a case and a control of each breed, was performed. Filtering for private variants in the 2 cases showed no evidence for shared protein-changing SNV, but 7 non-coding SNVs and a 4.5 kb tandem duplication. In pigs, porcine 51k array genotypes of 35 animals were available, including 10 Casertana pigs (5 with and 5 without wattles) as well as 25 Kune Kune pigs (18 with and 7 without wattles). Similar analysis was carried out as in sheep. GWAS revealed a significantly associated region on chromosome 1 and a 2.5 Mb-sized haplotype present in all cases of both breeds. WGS of 4 pigs, a case and a control of each breed, was carried out. Filtering for private variants revealed a list of non-coding SNVs including a single protein-changing SNV present in both cases. In conclusion, this study revealed different genomic loci harboring variants causing wattles in 2 different species. We report 2 variants that are likely to cause this heritable phenotype. Interestingly, the affected genes differ from the gene associated with wattles in goats. This indicates an unexpected genetic heterogeneity causing this unique morphological trait across domestic animal species.

Key Words: pigs and related species, sheep and related species, genome-wide association, SNP, genome sequencing

OP89 The little Pygmy hog (*Porcula salvania*), a big piece in resolving the fascinating history of the complex speciation of the *Suidae*. L. Liu*[1], M. Bosse[1], H-J. Megens[1], L. Frantz[2,3], Y. Lee[1], E. Irving-Pease[3], G. Narayan[4,5], M. Groenen[1], and O. Madsen[1], *[1]Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands, [2]School of Biological and Chemical Sciences, Queen Mary University of London, London, UK, [3]Palaeogenomics and*

Bioarcheology Research Network, Research Laboratory for Archeology and History of Art, University of Oxford, Oxford, UK, [4]Durrell Wildlife Conservation Trust, Jersey, Channel Islands, UK, [5]Pygmy Hog Conservation Programme, Guwahati, Assam, India.

Wild boar (*Sus scrofa*) colonized mainland Eurasia and North Africa, most likely from East Asia during the Plio-Pleistocene (2–1 Mya). Extensive fossil evidence from Eurasia suggests that this expansion resulted in the replacement of multiple suids species. Recent reports, based on genome-wide information, however, suggested that wild boar did not replace the species it encountered, but instead exchanged genetic materials with them through admixture. The incorporation of these lineages into the gene-pool of wild boar has been hypothesized to be one of the keys to its successful expansion. The pygmy hog (*Porcula salvania)* is the only suid species, in mainland Eurasia, known to have outlived this wave of expansion. This highly endangered species thus provides a unique opportunity to test the hypothesis that wild boar interbred with local suid species as it expanded across Eurasia. Analyses of 6 pygmy hog genomes, combined with additional suid genomes, indicate that despite large phylogenetic divergence (~2 My), wild boar and pygmy hog did indeed interbreed as the former expanded across Eurasia. Moreover, it sheds further light on a genealogical discordance observed on the X chromosome of pigs, which is caused by post-speciation admixture. The donor of this admixture pertains to a now-extinct species with a deep phylogenetic placement between pygmy hog and sub-Saharan suids. This suggests that, the rapid spread of S. scrofa across the old world might have been facilitated through inter-specific admixture. Overall, our analysis demonstrates the importance of inter-specific admixture during rapid range expansion.

Key Words: phylogenetics, speciation, introgression, population demography

OP90 Sexing in pigs by using gene editing. S. Kurtz*, A. Frenzel, A. Lucas-Hahn, P. Hassel, R. Becker, H. Niemann, and B. Petersen, *Institute of Farm Animal Genetics, Friedrich-Loeffler-Institute, Mariensee, Neustadt am Rübenberge, Germany.*

Sexing by gene editing in pigs is an alluring alternative to the surgical castration of piglets. Current alternatives like boar fattening, immunocastration and sperm sexing are often economically not well applicable or lack consumer acceptance. In mice, the SRY-gene was first described as a genetic developmental switch for the male phenotype. The knockout of the murine SRY-gene by TALEN suppressed testis development in the fetal gonadal ridges and generated a female phenotype. In addition, the knockout of the 5′ flanking region of rabbit SRY gene results in an equal phenotype as in mice. In our study, we aim to generate a knockout of the porcine SRY-gene to investigate its function in pigs. Therefore, we constructed a CRISPR/Cas9 vector causing a deletion within the SRY locus upstream of the HMG-box region. Mutated cells served as donor cells in SCNT. Alternatively, the same CRISPR/Cas vectors targeting the SRY locus were microinjected into porcine zygotes. The production of SRY-knockout pigs by cytoplasmic microinjection resulted in offspring with a deletion of ~72bp. However, the expected sex reversal did not occur. In ongoing experiments, we want to induce a larger deletion, which targets the HMG-box region of the SRY gene. This could reveal the potential role of the HMG-box for the functionality of the porcine SRY protein and its sex-determining function.

Key Words: SRY gene, CRISPR/Cas9, sex determination, pig

OP91 Integrative genomics analysis of blood and microbiota data suggests a NAFLD-related disorder in SLAdd minipigs. M. Moroldo*[1], P. Munyaka[2], J. Lecardonnel[1], G. Lemonnier[1], E. Venturi[3], C. Chevaleyre[3], J. Estellé[1], and C. Rogel-Gaillard[1], *[1]GABI, INRA, Jouy-en-Josas, France, [2]University of Alberta, Edmonton, AB, Canada, [3]ISP, INRA, Nouzilly, France.*

Minipigs are a group of small-sized swine lines (i.e., 20 to 90 kg) with major physiological differences compared with conventional pigs, and most of them tend to be obese. The inbred SLAdd (DD) minipig line is homozygous at the MHC locus (Hp4.4 haplotype) and is especially susceptible to obesity. Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease in Western countries that is linked to obesity, type 2 diabetes mellitus and cardiac pathologies. Minipig lines could be useful animal models to study this disease. To characterize the metabolic disorders that affect DD pigs, we measured intermediate phenotypes from blood and feces and used Large White (LW) pigs as controls. Three data sets were produced, i.e., complete blood counts (CBCs), microarray-based blood transcriptome, and fecal microbiota characterized by 16S rRNA sequencing. Compared with LW pigs, several NAFLD-related blood parameters such as hematocrit, hemoglobin, and platelet distribution were higher in DD pigs. Transcriptome profiling identified 3,046 differentially expressed genes (DEGs), many of which were found in NAFLD-related pathways, such as 'adipogenesis', 'insulin resistance', 'sirtuin signaling', 'hepatic fibrosis', 'mTOR signaling' and 'endoplasmic reticulum stress'. 16S rRNA sequencing confirmed these findings, but highlighted obesity more clearly. The Firmicutes to Bacteroidetes ratio was higher in the DD (2.7) than in the LW group (1.6). The Clostridiaceae and Prevotellaceae families and the *Clostridium* and *Streptococcus* genera were more abundant in DD pigs, while *Prevotella* was less abundant. To refine this analysis, we used integration methods. A first sPLS analysis highlighted associations between CBCs and DEGs such as *RAB40B* and *VNN2*. A second sPLS identified links between genera such as *Prevotella* and DEGs such as *FUT1*, *STX12* and *DIRAS2*. Lastly, an MFA analysis showed that the data structure was mainly determined by the breed. Our results show that DD pigs present symptoms of a NAFLD-related disease. Thus, exploring intermediate phenotypes of minipig lines could identify new models to study metabolic diseases.

Key Words: minipig, NAFLD, transcriptome, microbiota, integration

OP93 A first insight into the boar sperm microbiome. M. Gòdia[1], S. Lopez[5], J. E. Rodríguez-Gil[2], S. Balasch[3], C. Lewis[4], A. Castelló[1], A. Clop*[1,6], and A. Sanchez[1,2], *[1]Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Cerdanyola del Valles, Catalonia, Spain, [2]Universitat Autònoma de Barcelona, Cerdanyola del Valles, Catalonia, Spain, [3]Grup Gepork S.A, Masies de Roda, Catalonia, Spain, [4]PIC Europe, Sant Cugat del Vallés, Catalonia, Spain, [5]University of Barcelona, Barcelona, Catalonia, Spain, [6]Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Catalonia, Spain.*

The microbiome has emerged as a major contributor to many phenotypes including semen quality and male fertility in animals. The objective of this study was to obtain a preliminary characterization of the boar sperm microbiome and the correlation between bacterial abundance and semen quality traits (viability, motility, acrosome, osmotic resistance, measured at 5 and 90 min after incubation at 37C, and morphological abnormalities). We obtained 40 ejaculates, each from a different boar. Samples were diluted in extender with antibiotics and purified by gradient centrifugation with Bovipure™. RNAs were extracted and sequenced in Illumina HiSeq2000 systems to generate 75 base pair long paired end reads. After mapping the reads to the swine genome (Sscrofa11.1) using HISAT, 19% of the reads remained unmapped. The unmapped reads were filtered by removing these that matched to repeat elements annotated in RepBase. The remaining reads were aligned to the Kraken microbial genomes using the devoted Kraken software. Despite the facts that the ejaculates contained antibiotics and that bacteria had been filtered out by gradient centrifugation, we still identified a bacterial population in all the samples. The most abundant phyla were Proteobacteria (39.1% of bacterial reads aligned to the genomes of this phyla), Firmicutes (27.5%), Actinobacteria (14.9%) and Bacteroidetes (5.7%). The most abundant bacteria were from environmental or intestinal origin, indicating that these contaminated sperm after ejaculation. Neither obvious pathogens nor antimicrobial resistance genes were identified. We also evaluated the correlation between bacterial abundance and semen quality traits and several connexions

were identified. Most effects involved traits measured after incubation of the samples at 37C during 90 min, while few correlations were found for traits measured after only 5 min incubation, thus suggesting that incubation favored the proliferation of bacteria and hence their impact on phenotypes. Some correlations involved bacteria already related to semen quality or fertility in humans (e.g., *Lactobacillus crispatus*) and pigs (e.g., *Pseudomonas aeruginosa*).

Key Words: pig, sperm, microbiome, semen quality

OP94 Gene editing for resistance against diseases in pigs—What are the consequences of alternative strategies? E. Jonas*, *Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Classic selection depends on the presence of variants relevant for the breeding goal in the population. In some cases crossbreeding can be applied to introduce advantageous traits from other populations of the same species. Alternatively changes can be artificially introduced into the genome by methods of genetic engineering. While classical genetic modifications (GM) have been applied in plant breeding, they were less successful in animal breeding. The more recent suggested method of gene editing however, has now opened new possibility also in livestock populations. But several issues are yet to be explored such as the legislation on gene editing, traceability of gene edited animals, improvement of technical details as well as ethical validity. Traits with large hope for improvement using gene editing are related to diseases resistance. Infectious diseases cause economic and welfare problems in livestock production. Even though genetic factors (e.g., certain levels of heritability) have been identified for resistance to some diseases, breeding for improved resistance is not straightforward. African swine fever (ASF) is currently spreading into Europe causing large losses in pig production. The project presented here aims to simulate backcrossing and gene editing to introduce resistance to ASF in Swedish pig herds. We will later also simulate the spread of ASF when different scenarios, including resistant animals, culling or vaccination, apply. Input and output from the simulations will be discussed with an ethicist specialized in questions of animal production. As learned from hurdles using classical GM in plant breeding, it is relevant to be open and inform the public about applications of gene editing in livestock and to ethically discuss consequences of gene editing but also of the alternatives.

Ruminant Genetics and Genomics

OP95 Local ancestry and functional genomics of trypanotolerant and trypanosusceptible admixed African cattle breeds. G. P. McHugo[1], G. M. O'Gorman[2], E. W. Hill[1], and D. E. MacHugh*[1,3], *[1]UCD School of Agriculture and Food Science, University College Dublin, Belfield, Ireland, [2]National Office of Animal Health Ltd., Enfield, UK, [3]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Ireland.*

African cattle represent a complex mosaic of *Bos taurus* (taurine) and *Bos indicus* (zebu) ancestry with most breeds containing varying levels of taurine-zebu admixture. The 2 types of cattle diverged at least 500,000 years ago and significant genomic differences have accumulated since that time. One important evolutionary adaptation in certain African taurine populations is a genetically determined tolerance to infection by protozoan trypanosome parasites (*Trypanosoma* spp.), which are transmitted by infected tsetse flies (*Glossina* spp.) and cause African animal trypanosomiasis (AAT) disease. The annual financial burden of AAT is approximately $4.5 billion, and AAT is one of the largest constraints to livestock production in the areas of sub-Saharan Africa with significant tsetse densities. The West African taurine N'Dama breed is trypanotolerant; they have a capacity to control parasite loads and to limit disease pathology compared with trypanosusceptible zebu breeds. However, zebu or zebu-taurine hybrid animals are generally larger, produce higher milk yields and are therefore favored by many farmers. Using local ancestry analysis of genome-wide high-density SNP data, we have examined hybrid West African cattle populations to study sub-chromosomal admixture. A sliding window approach was used and genes within 1 Mb up- and downstream from the top ancestry windows were used in gene set enrichment analyses. Results from this study demonstrate that the top physiological system development and function pathways for genes located within taurine local ancestry windows include immunobiology pathways, while the top zebu pathways are related to growth and development. These local ancestry results were also integrated with gene expression data from bovine trypanosome infection studies to further explore the functional biology of differentially introgressed genomic regions in African hybrid cattle.

Key Words: cattle and related species, integrative genomics, admixture, disease resilience

OP96 Resequencing the most Northern cattle breeds provides insight into their history and adaptation to cold. L. Buggiotti[1], A. Yurchenko[2], H. D. Daetwyler[3,4], C. J. Vander Jagt[3], and D. M. Larkin*[1,2], *[1]Royal Veterinary College, London, UK, [2]Institute of Cytology and Genetics, Novosibirsk, Russia, [3]Agriculture Victoria, Bundoora, Victoria, Australia, [4]La Trobe University, Bundoora, Victoria, Australia.*

Studying genetics of native animal populations formed in extreme environments is important to understand mechanisms of adaptation and to help designing productive livestock breeds adapted to severe climates. Here we report whole genome resequencing of 2 cattle populations adapted to cold climates of Russia. These breeds have different histories. While the Kholmogory cattle was formed in the European part of Russia by admixing local landraces with the dairy cattle brought from Europe starting in the 17th century, the origin of the Yakut cattle is still unknown. Our earlier data show that the Yakut cattle shares common origins with the taurine breeds from Asia, such as the Korean Hanwoo and Japanese Wagyu. Here we used 40 resequenced Kholmogory and Yakut cattle combined with published genomes of Holstein and Hanwoo breeds and additional native breeds from China and Africa to: a) better understand origins of the Yakut cattle and the genetic mechanisms of its adaptation to extremely cold climates of Siberia where occasionally winter temperatures fall below −50°C, b) identify common adaptations of the Kholmogory and Yakut cattle which made them adapted to the climates of Russia, c) identify candidate genetic variants which could be used to make future generations of productive breeds adapted to cold climates. We identified over 20 million high quality SNPs in our sequencing data set. Based on the historical demography analysis (MSMC) the Yakut cattle indeed diverged more recently from Hanwoo than from Holstein while the Kholmogory shared a more recent ancestor with Holstein suggesting that the Asian and European taurine breeds could represent genetically different populations. The Treemix analysis implied admixture between the Yakut and some of the Chinese native breeds suggesting that the ancestors of these breeds could form at the same territory. Strong signatures of selection in the Yakut cattle was found near the gene *GRIA1* involved in thermoregulation and in the region of *RETREG1* in Yakut and Hanwoo breeds known to be related to inability to feel cold temperatures in humans providing insights into the mechanisms of extreme cold adaptation. This work was supported by the Russian Foundation for Basic Research (RFBR) grant 18–016–00185.

Key Words: local breeds, resequencing, adaptation to cold, cattle, signatures of selection

OP97 Trends of CNV research in ruminants. G. Liu*, *Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD, USA.*

Abstract not available.

OP98 Multi-level conservation of chromosome conformation across livestock species reveals evolutionary links between genome structure and function. S. Foissac*[1], S. Djebali[1], N. Vialaneix[2], M. Zytnicki[2], A. Rau[4], S. Lagarrigue[3], H. Acloque[4], and E. Giuffra[4], *[1]GenPhySE, INRA, Toulouse Auzeville, France, [2]MIAT, INRA, Toulouse Auzeville, France, [3]PEGASE, INRA, Rennes, France, [4]GABI, INRA, Paris Jouy-en-Josas, France.*

The spatial conformation of the chromatin in the nucleus of animal cells is highly organized at several levels. Little is known, however, about its evolution, mainly because of the limited number of species in which chromosome conformation experiments have been performed. In the context of the FAANG pilot project FR-AgENCODE, we realized Hi-C experiments on tissue samples from 3 livestock species (pig, goat and chicken livers) and integrated the resulting data with those from other assays on the same samples (RNA-seq and ATAC-seq). Within each species, general features of the genome organization -like Topologycally Associating Domains (TADs) and genome A/B compartments- could be identified. They display functional properties in line with previous reports on other model organisms like human and mouse. By comparing these structural features across species we further investigated the connections between genome conformation and function. In particular, we found a significant conservation of the gene distribution among active and inactive compartments of the chromatin throughout evolution. In addition, comparing 3D interaction profiles of orthologous regions showed that conserved TAD boundaries have stronger insulation properties than recent ones, supporting a specific selective pressure on structural components of the genome. Accordingly, various blocks of synteny contain conserved elements from several levels of organization that contribute to the gene regulatory network: sites of chromatin accessibility, orthologous genes, TADs and A/B compartments. Altogether, these results shed new light on the conservation of genome conformation from birds to mammals and illustrate the various degrees of connection between genome structure and function.

Key Words: 3D genome architecture, multispecies, Functional Annotation of Animal Genomes (FAANG), comparative genomics, Hi-C

OP99 Epigenetic factors to face environmental variations in small ruminants. L. Denoyelle*[1,3], P. de Villemereuil[1], F. Boyer[1], M. Khelifi[1], C. Gaffe[1], F. Alberto[1], B. Benjelloun[2,1], and F. Pompanon[1], *[1]Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, Grenoble, France, [2]Institut National de la Recherche Agronomique Maroc (INRA-Maroc), Centre Régional de Beni Mellal, Beni Mellal, Morocco, [3]GenPhySE, INRA, INPT, ENVT, Université de Toulouse, Castanet-Tolosan, France.*

Species can optimize their fit to environmental conditions. Individuals can move to places with better conditions, or populations can adapt by natural selection of the best suited traits. Adaptation is a long-term evolutionary mechanism requiring several dozens to hundreds generations to increase the frequency of the adapted alleles. Besides this genetic-based response short-term mechanisms based on phenotypic plasticity may mediate the response to environmental stresses. Phenotypic plasticity, which is the ability of a same genotype to express different phenotypes in different environments, may rely on the presence of epigenetic marks (such as DNA methylation) in the genomes, that regulate gene expression. This presentation focuses on the environment-related variation of DNA methylation patterns along the genome in goat (*Capra hircus*) and sheep (*Ovis aries*), living in field conditions in Morocco. For each species, we studied 2 groups of animals from environments with contrasted ambient temperatures (desert vs. Mediterranean climates). For this purpose, individuals methylomes were generated by sequencing of DNA methylated fragments, previously retrieved by immunoprecipitation. Then, we identified 5 and 2 differentially methylated genomic regions between the 2 groups for goat and sheep, respectively. We didn't find any homologous regions that are differentially methylated between the 2 species. Finally, we identified 4 genes for goats and 2 genes for sheeps that could be differentially expressed in relation to the variation of ambient temperatures.

Key Words: DNA methylation, *Ovis aries*, *Capra hircus*, local acclimatation

OP100 A scalable pipeline to scan for selective sweeps in whole-genome sequences: Application to bovine. D. F. Cardoso*[1,2], L. R. Porto-Neto[2], M. Nával-Sánches[2], H. Tonhati[1], and A. Reverter[2], *[1]Department of Animal Science, School of Agricultural and Veterinarian Sciences, Sao Paulo State University (UNESP), Jaboticabal, SP, Brazil, [2]CSIRO Agriculture & Food, St. Lucia, Brisbane, Australia.*

Past and ongoing selection creates selective sweeps in the genome and a variety of methodologies exists to detect them. It is postulated that a composite method combining distinct approaches into a single metric may improve the resolution in detecting selective sweeps. Here, we introduce an early version of a tool directed toward scan for signatures of selection at the sequence level, which runs in Linux operating system. This pipeline performs 8 methodologies for the detection of selective sweeps using whole-genome sequences as input. Five of the methods are performed within-population (Tajima's D, iHS, CLR, Fu and Li's D* and F*), while 3 are based on across population differentiation (F_{ST}, XP-EHH and XP-CLR). Additionally, results from the 8 analyses are combined into a single composite metric (DCMS). A quality control in the first step checks the adequacy of the input files and generates a default parameter's file that can be then modified if not all methods are to be ran, or default parameters are to be changed. Then, a further executable invokes the methods set in the parameters' file, by systematically calling a collection of R and shell scripts handling some available tools/software (e.g., Selscan, Sweepfinder, vcf-kit). We applied the described pipeline to a bovine data set of 30 taurine (*Bos primigenius taurus*) and 20 indicine (*Bos primigenius indicus*) samples, and 34,671,856 autosomal SNVs. This process spent 24h14m, running in parallel each metric per chromosome on a 5-core and 1Gb of memory in the Intel® Xeon® E5–2600 processors. The CLR tests limited the runtime by spending ~23h for large chromosomes, such as BTA1 and BTA2. We detected, with DCMS, known regions to be under selection in beef cattle e.g., vicinities of *PLAG1* and *CRH* genes. The absolute pairwise correlation of individual metrics ranged from 0.04 (XP-CLR and iHS) to 0.89 (Fu and Li's F* and Fu and Li's D*), whereas the pairwise correlation involving DCMS ranged from 0.34 (CLR) to 0.66 (Fu and Li's F*), suggesting a composite score well-weighed by the distinct individual metrics. Further efforts to optimize the runtime are in progress.

Key Words: cattle, population genomics, computational pipeline, selection

OP101 Identification of novel haplotypes and recessive lethal alleles affecting reproduction and its pleiotropic effects in cattle. A. Cánovas*[1], S. Id-Lahoucine[1], A. Suarez-Vega[1], P. Fonseca[1], S. P. Miller[2], M. Lohuis[3], F. Schenkel[1], M. Sargolzaei[1,4], F. Miglior[1], J. F. Medrano[5], and J. Casellas[6], *[1]University of Guelph, Department of Animal Biosciences, Centre for Genetic Improvement of Livestock, Guelph, ON, Canada, [2]Angus Genetics Inc, Saint Joseph, MO, USA, [3]Semex Alliance, Guelph, ON, Canada, [4]Select Sires Inc, Plain City, Ohio, USA, [5]University of California-Davis, Department of Animal Science, Davis, CA, USA, [6]Universitat Autonoma de Barcelona, Bellaterra, Spain.*

The availability of genomic data has enabled the screening of homozygous haplotypes and recessive lethal alleles that could affect reproductive performance in cattle. Here, we propose an alternative approach based on tracing the inheritance of alleles from heterozygous parents to offspring to identify genomic regions with transmission ratio distortion (TRD). The TRD refers to the significant departure from the expected Mendelian inheritance of alleles from heterozygous parents to offspring. This phenomenon has been attributed to various biological mechanisms, which are related to a decline in fertility and reproductive success. The newly developed TRDscan software was used to identi-

fy genomic regions with TRD using 436,651 genotyped Holstein dairy cattle. SNP-by-SNP analysis was performed using 132,990 SNPs. The TRD haplotypes were identified using a sliding windows approach of 2-,4-,7-,10- and 20-SNP from 373,793 trios of genotypes of Holstein cattle (including sire, dam and offspring). A total of 109 SNPs and 495 haplotypes were identified with very significant TRD (Bayes factor (BF ≥100)). Genomic regions with strong evidence of TRD were located in previously known regions with recessive lethal alleles (e.g., HH0, HH1, HH3, HH5). Novel genomic regions with significant TRD were also identified. A total of 302 genes within significant TRD were functionally clustered into specific phenotypes related to male and female infertility and postnatal lethality. Among them, the 5 highest enriched gene ontology/biological processes where embryo development, circulatory system development, heart development, positive regulation of gene expression and chromosome organization. Approximately 18% of all QTLs mapped around the TRD regions were fertility related QTLs, such as calving ease, scrotal circumference, fertility index, non-return rate and number of inseminations per conception. These results reinforce the usefulness of combining the TRD approach with functional genomics to precisely target genomic regions associated with fertility, embryonic development processes, gestation losses and post-natal lethality in cattle.

Key Words: cattle and related species, comparative genomics, functional genomics, genotyping, complex trait

OP102 Involvement of *PRAMEY* in the bovine sperm development and maturation. W. Liu*, C. Kern, and W. Feotosa, *Department of Animal Science, Center for Reproductive Biology and Health (CRBH), College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, USA.*

The preferentially expressed antigen in melanoma, Y-linked (*PRAMEY*) gene subfamily is a cancer/testis antigen (CTA) and is expressed predominantly in spermatogenic cells, playing an important role during bovine spermatogenesis. The objective of this study is to investigate PRAMEY dynamic in spermatozoa, fluid and tissues from testis, caput and cauda epididymis, and its subcellular localization during sperm maturation. Four different isoforms of the PRAMEY protein including the 58, 30, 26, and 13 kDa isoforms were identified by Western blot with a PRAMEY-specific antibody. The 58 kDa protein, which is the predicted molecular weight for the intact PRAMEY, was weakly expressed in different age of testis (20d, 4m, 8m and 2y) and in adult testicular sperm and fluid, while the 30 kDa isoform was highly expressed only in 8m and 2y testis as well as in adult testicular sperm and fluid, suggesting their involvement in spermatogenesis. In contrast, the highly expressed 26 kDa was an epididymal sperm-specific isoform and the 13 kDa isoform was marked in sperm, fluid and tissue from the cauda segment of the epididymis, suggesting their involvement in sperm maturation. When head and tail of epididymal sperm were analyzed separately for the 30, 26, and 13 kDa isoforms, we found a remarkable decrease of 10.9, 5.4, and 3.8 fold, respectively, in protein expression from the caput to cauda epididymis in sperm heads, but there was a small decrease in the expression for both the 30 and 26 kDa isoforms in sperm tail (1.9 and 1.2 fold respectively). In contrast, the 13 kDa isoform increased 4 fold in sperm tails from caput to cauda, suggesting this isoform may have a significant role in tail function. Corroborating with that, evaluation of the mitochondria isolated from caput and cauda sperm revealed that the 30 and 26 kDa isoforms decreased in sperm mitochondria from caput to cauda, whereas the 13 kDa isoform increased. Taken together, our data suggest the involvement of the 58 and 30 kDa isoforms in spermatogenesis and of the 26 and 13 kDa in sperm maturation, especially the 13 kDa isoform, in sperm motility.

Key Words: PRAMEY, spermatogenesis, testis, epididymis, cattle

OP103 Investigating emerging inherited diseases in Australian livestock: A snapshot. S. A. Woolley*[1], E. R. Tsimnadis[1], R. L. Tulloch[1], P. Hughes[1], B. Hopkins[1], S. E. Hayes[1], M. R. Shariflou[1], A. Bauer[2], V. Jagannathan[2], C. Drögemüller[2], T. Leeb[2], M. S. Khatkar[1], C. E. Willet[3], B. A. O'Rourke[4], I. Tammen[1], *[1]The University of Sydney, Faculty of Science, Sydney School of Veterinary Science, Camden, NSW, Australia, [2]The University of Bern, Institute of Genetics, Vetsuisse Faculty, Bern, Switzerland, [3]The University of Sydney, Sydney Informatics Hub, Core Research Facilities, Sydney, NSW, Australia, [4]The Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia.*

The advancement of animal breeding in the last few decades has enabled desirable traits and elite genetics to be disseminated throughout livestock populations within relatively short periods of time. Small effective population sizes and inbreeding poses a risk for the inheritance of deleterious alleles and can lead to the emergence of recessive inherited diseases. Emerging inherited diseases not only impact production efficiency and profitability, but can also cause numerous animal welfare concerns. This study investigated several emerging inherited diseases with a predicted recessive mode of inheritance in Australian cattle and sheep for which the causative mutation had not been characterized. This study aimed to characterize disease-causing mutations and to develop diagnostic DNA tests to enable improved management decisions to be made for at-risk populations. Regions of interest and novel variants were identified through SNP-based homozygosity mapping using an 80K SNP chip for affected and obligate carrier animals as well as positional candidate gene sequencing approaches. Inherited diseases with no defined region of interest or novel variants identified within candidate genes were re-sequenced via whole-genome sequencing at 30X coverage with 150bp paired-end reads. Standard bioinformatics pipelines were used to identify novel variants across the genome and within other candidate genes. Candidate disease-causing mutations were identified for congenital mandibular prognathia in Droughtmaster cattle, ichthyosis fetalis in Shorthorn cattle, Niemann-Pick type C in Angus cattle, brachygnathia, cardiomegaly and renal hypoplasia syndrome in Merino sheep, ovine dermatosparaxis in Merino sheep and pulmonary hypoplasia with anasarca in Persian sheep. These candidate disease-causing mutations are currently being validated. The outcomes from this study showcase the ability to identify rare candidate disease-causing mutations with limited sample size and pedigree information. The communication of these results will help provide awareness of emerging inherited diseases in livestock populations.

Key Words: genetic disorder, genome sequencing, cattle and related species, sheep and related species

OP104 A de novo mutation causes polledness and a modified shape of the skull in Fleckvieh cattle. L. Gehrke[1], M. Upadhyay*[2], K. Heidrich[2], E. Kunz[2], D. Seichter[3], A. Graf[2], S. Krebs[2], A. Capitan[4], G. Thaller[1], and I. Medugorac[2], *[1]Christian-Albrechts-University Kiel, Kiel, Schleswig-Holstein, Germany, [2]Ludwig Maximillians University Munich, Munich, Bavaria, Germany, [3]Tierzuchtforschung e.V. München, Grub, Bavaria, Germany, [4]GABI, INRA, AgroParisTech, Paris, France.*

Genetic heterogeneity refers to the phenomenon where mutations in different loci (locus heterogeneity) or within the same locus (allelic heterogeneity) lead to a similar phenotype. In cattle, allelic heterogeneity is observed for the polled condition. In fact, at least 3 different alleles at the *polled* locus have been identified in cattle. In this study, we describe a case of a polled Fleckvieh bull born to horned parents that also implies locus heterogeneity of polledness. Genotyping of the case bull, its sire, grandsires and its polled and horned offspring was carried out using the bovine50K SNP array to determine the genetic basis of the de novo polledness condition. Additionally, Illumina paired-end and Oxford Nanopore sequencing technologies were employed to identify the exact candidate mutation for the polledness. Later, sanger sequencing technology was also used to validate the candidate mutation. The approach identified an 11-bp de novo deletion as the candidate mutation for the polled condition that first arose in a Fleckvieh bull and later passed onto its offspring. The 11-bp deletion event encompassed the second exon of the *ZEB2* gene and led to a translational frameshift. The

frameshift caused a premature termination of translation, leading to a truncated protein. Compared with the wild type, the truncated *ZEB2* protein is predicted to be shortened by about 91%. Mutations in the *ZEB2* gene cause multiple congenital anomalies in humans as well as in cattle. However, apart from displaying polledness, a modified shape of the skull and presumably a short stature, the individuals carrying the 11-bp deletion in *ZEB2* gene did not display any other clinical symptoms. Because the *ZEB2* gene encodes a Smad Interacting Protein 1 (SIP1) that plays a vital role in epithelial-mesenchymal transition, it can be hypothesized that the truncated *ZEB2* protein might have lacked essential domains associated with the differentiation of horn buds. To conclude, the results of this study point toward a complex genetic pathway involved in bovine polledness that requires further investigation.

Key Words: cattle, polledness, ZEB2 gene, de novo deletion, frameshift

OP105 Graph-based variant genotyping reveals natural sequence variants (NSVs) for targeted genome editing to enhance genetic resistance to *Mycobacterium bovis* infection in cattle. C. N. Correia*[1], D. Crysnanto[2], T. S. Sonstegard[3], S. V. Gordon[4,5], T. J. Hall[1], J. Bostrom[3], D. F. Carlson[3], D. A. Simpson[6], A. Margariti[6], S. Kelaini[6], H. Pausch[2], and D. E. MacHugh[1,5], *[1]UCD School of Agriculture and Food Science, University College Dublin, Dublin, Ireland, [2]Animal Genomics, Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland, [3]Recombinetics Inc, St Paul, MN, USA, [4]UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland, [5]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland, [6]Centre for Experimental Medicine, Queen's University Belfast, Belfast, UK.*

Mycobacterium bovis infection, the cause of bovine tuberculosis (BTB), costs more than $3 billion to global agriculture annually and the primary financial burden of BTB in developed countries is the control of infection. Our group has previously described the impact of *M. bovis* infection on gene expression in the mammalian alveolar macrophage, the key innate immune cell that encounters the pathogen in the lung. In this regard, we have demonstrated that the bovine alveolar macrophage transcriptome is substantially reprogrammed as a consequence of both host-driven defense responses and mycobacterial-induced perturbation and manipulation of cellular processes. The overall goal of this project is to identify natural sequence variants (NSVs) in key genes and genomic regulatory elements associated with the bovine host macrophage response to infection with *M. bovis*. In this context, graph-based variant genotyping offers a robust framework to identify NSVs that segregate in cattle breeds and populations that have been shown to be resilient to BTB; in particular, *indicine* cattle and *taurine* × *indicine* hybrids. Graph-based methods for NSV identification facilitate systematic comparison of sequencing reads to a population variation-aware genome graph for a particular species or subspecies. Here we report application of this approach to whole-genome sequence (WGS) data from Nelore (*Bos taurus indicus*) and Holstein-Friesian (*Bos taurus taurus*) cattle. Our analyses have identified promising candidate NSVs for BTB resilience in a range of genes associated with the macrophage response to mycobacterial pathogens, including *M. bovis* and *M. tuberculosis*, the primary cause of human TB. These NSVs will be used for genome editing of bovine induced pluripotent stem cell (iPSC)-derived macrophages (iPSCDM), which can be used to evaluate the impact of the edits on the transcriptional activity of the mammalian macrophage in response to in vitro challenge with *M. bovis*.

Key Words: WGS, graph-genotyping, cattle, tuberculosis, genome editing

OP106 Variants in the bovine X chromosome are relevant to predict bull fertility. M. R. S. Fortes*[1,2], L. R. Porto-Neto[3], N. Satake[4], L. T. Nguyen[2], A. C. Freitas[5], T. P. Melo[5], B. Hayes[2], F. S. Raidan[3], A. Reverter[3], and G. B. Boe-Hansen[4], *[1]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Qld, Australia, [2]Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Brisbane, Qld, Australia, [3]CSIRO Agriculture and Food, Brisbane, Qld, Australia, [4]School of Veterinary Science, The University of Queensland, Gatton, Qld, Australia, [5]Universidade Estadual de Sao Paulo, Jaboticabal, SP, Brazil.*

Bull breeding soundness evaluations are standard industry practice to assure that bulls are fertile before the mating season. Recent work by our group and others confirmed that at least some bull fertility traits are moderate to highly heritable (approx. Twenty to 50%) and correlated with female fertility traits. Genomic selection for bull fertility traits might benefit from strategies that consider their genetic architecture. However, the identification of mutations underlying the variation in these traits has been complicated by the fact that many suggestive QTL mapped to the X chromosome and its assembly was not ideal until recently. The objective of this study was to explore the genetics underpinning bull fertility, through identifying QTL across the genome, including the X chromosome. To this aim, 25 fertility-related phenotypes were measured in 1,099 Brahman and 1,719 Tropical Composite bulls. Measurements included standard bull breeding soundness indicators, as well as sperm morphology, sperm DNA fragmentation, and protamine deficiency. We performed association analyses and identified thousands of significant polymorphisms ($P < 10^{-8}$) for scrotal circumference (SC) and a range of sperm morphology traits. Confirmed across breeds, the significant variants suggest a polygenic architecture for bull fertility, but one that is biased toward at least 3 distinct QTLs on the X chromosome. We identified evidence for pleiotropy, as some QTL were associated with multiple phenotypes, which could be expected as various phenotypes were genetically correlated (r^2 ~0.30–0.50). Genetic correlations and shared QTL indicate that these phenotypes arise due to variants that affect the underlying developmental physiology: the process of puberty and the consequential sperm production. Annotation of the discovered QTL, in terms of genes and causal mutations, will expand our knowledge of bull fertility biology, with potential insights for other mammals. Mutations associated with male fertility accumulate in the bovine X chromosome, as they do in humans and mice suggesting an evolutionary conversion through specialization of this chromosome.

Key Words: X chromosome, quantitative trait loci, male fertility, beef cattle, genomics

Cattle Molecular Markers and Parentage Testing

OP107 Development of targeted GBS panels for breeding and parentage applications in cattle and swine. A. Burrell[1], P. Siddavatam[1], M. Swimley[1], C. Willis*[1], M. de Groot[2], R. Ferretti[3], and R. Conrad[1], *[1]Thermo Fisher Scientific, Austin, TX, USA, [2]VHL Genetics, Wageningen, Netherlands, [3]Neogen GeneSeek, Lincoln, NE, USA.*

Parentage testing and genomics-assisted breeding are critical aspects of successful herd management. Due to its highly accurate and reproducible results, targeted GBS is becoming an increasingly favored technology for SNP genotyping. With the utilization of next-generation sequencing, labs can test hundreds of samples across thousands of SNPs simultaneously in a simple high throughput workflow starting from either extracted nucleic acid or crude lysis samples. We developed targeted sequencing panels for both cattle parentage, based on 200 SNP markers selected by the International Society of Animal Genetics (ISAG), and swine breeding using a 1500 SNP imputation panel. Utilizing the AgriSeq™ HTS Library Kit, a high-throughput targeted amplification and re-sequencing workflow, each panel's performance was tested on >96 diverse cattle and swine DNA samples. Libraries were

sequenced on the Ion S5 using an Ion 540 chip with genotyping calling generated using the Torrent Variant Caller (TVC) plugin. The mean genotype call rate of markers across the samples was >98% for the cattle panel and >96% for the swine panel. Concordance across replicate library preparations and independent sequencing runs was >99.9% for both panels. Panel results were compared with results from a DNA array and the genotype call concordance was >99% with the AgriSeq workflows. The cattle panel was also used on field samples by a Netherland service lab to successfully determine the parentage relationships of 45 calves with 48 potential mother cows. The data demonstrates the utility of the AgriSeq targeted GBS approach for cattle and swine SNP genotyping applications. For Research Use Only. Not for use in diagnostic procedures.

Key Words: cattle, swine, breeding, AgriSeq, ISAG

OP108 Poll diagnostics, scur genetics, and production concurrence in naturally hornless cattle. I. A. S. Randhawa*[1], M. R. McGowan[1], L. R. Porto-Neto[2], B. J. Hayes[3], and R. E. Lyons[1], *[1]School of Veterinary Science, University of Queensland, Gatton, QLD, Australia, [2]Agriculture and Food, CSIRO, St Lucia, QLD, Australia, [3]Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, St Lucia, QLD, Australia.*

Horned cattle are equipped for self-defense and to compete for feeding and mating opportunities. In modern commercial production systems, horned animals are potential hazards for other animals, during transport, in feedlot, and to farm workers. To manage the problem, cattle can undergo various procedures to surgically or chemically remove the horns, or breeding strategies can be developed to enable selection for naturally hornless (polled) cattle. Physical dehorning is a welfare issue due to the distress it causes to the animals and workers. Disbudding and dehorning procedures have negative effects on growth rate, increased risks of infection and mortality, and there are risks to workers and increased labor costs. The naturally polled status is a qualitative trait mapped to bovine autosome 1, with 4 sequences variants identified and linked to the hornless status, i.e., the *Celtic*, *Friesian*, *Mongolian* and *Guarani* mutations. The underlying genes and causal mutations for horns and polledness are unknown. While the polled status is dominantly inherited, heterozygous animals may grow horns of varying size which are not attached to the frontal region of the skull; these are termed scurs. To date, the genetic basis of scurs also remains unknown. Selective breeding for genetically polled animals is an alternative approach to dehorning that can be assisted by a genetic test for polled status. However, the current commercial test needs to be improved given their ascertainment bias toward several breeds. We explored the genetic variation across several cattle breeds of European (*Bos taurus*) and Zebu (*Bos indicus*) ancestry, and their composites, matching with the genetic test for polled, and correlated with some production traits. Our investigations provide an optimized poll gene test with high efficiency across multiple breeds, given its predictions relying on the genetic linkage between the contiguous markers harbouring *Celtic* and *Friesian* mutations. Furthermore, we present the genetic mapping and inheritance of scurs, pliancy of polled and scurs with sex hormones and genetic correlations between production and reproductive traits in beef cattle. These findings will provide better insights for making progress toward efficient selection for naturally polled cattle.

Key Words: poll gene testing, scur inheritance, animal welfare, beef production, reproductive efficiency

OP109 Validation of the OpenArray SNP assays for cattle parentage control. A. Piestrzynska-Kajtoch*, D. Rubis, A. Fornal, A. Gurgul, I. Jasielczuk, and A. Radko, *National Research Institute of Animal Production, Balice, Poland.*

The cattle SNP genomic evaluation has become a global standard. It led to designing the cattle SNP panel for parentage verification. There are many instruments which could be used for SNP genotyping. In this study, we used QuantStudio 12K Flex Real-time PCR System to develop the SNP parentage verification method for those individuals, which do not undergo genomic evaluation and as a supporting, alternative tool for difficult parentage cases. In addition, we attempted to validate this method with results obtained from the Illumina genotyping assay. TaqMan MGB genotyping assays (FAM and VIC fluorescent probes with primer pair) were prepared for ISAG core and ISAG additional bovine SNP panels. Some additional assays were designed for sex chromosomes and for ISAG panel SNPs with close, neighboring, polymorphic sites. There were 246 assays in total. DNA was isolated from blood or tissue of 285 cattle individuals (Holstein) and normalized (±50 ng/µl). 167 samples were analyzed on both BovineSNP50 assay on HiScanSQ (Illumina) and OpenArray plates on Quant Studio 12K Flex Real-time PCR System (Applied Biosystems), according to manufacturer protocols. 118 samples were run on the OA plates in duplicate. The primary analysis of the OA data files was performed with Genotyping qPCR module (Thermo Fisher Scientific Cloud Environment). Thirteen assays (mostly additional) have failed due to low amplification or serious clustering problems and have been omitted in further analysis. 86 data points were lost because of AccuFill mistakes (robot's pipetting). Two assays for SNP core panel need to be redesigned. The remaining assays had a call rate above 95%, which is acceptable according to the literature. We have also calculated OA assay's accuracy on the basis of the comparison with results from the Illumina platform – separately for each assay and for both ISAG SNP panels. Five SNPs were monomorphic in the studied group, but we have investigated only Holstein cattle. Our OpenArray based method for bovine SNP parentage control is encouraging, but it still needs few assays redesigning to become fully applicative. The study was financed by the Ministry of Agriculture and Rural Development Multiannual Programme no. 03–17–27–90.

Key Words: cattle and related species, SNP, genetic marker, parentage

Comparative MHC Genetics: Populations and Polymorphism

OP110 Invited Workshop Presentation: IPD-MHC Database: Improving analysis tools to promote MHC research. G. Maccari[1,2], J. Robinson[2,3], S. G. E. Marsh[2,3], and J. A. Hammond*[1], *[1]The Pirbright Institute, Pirbright, Woking, UK, [2]Anthony Nolan Research Institute (ANRI), Royal Free Hospital, London, UK, [3]UCL Cancer Institute, Royal Free Campus, London, UK.*

The IPD-MHC Database and website (www.ebi.ac.uk/ipd/mhc/) provides a centralized repository of manually curated sequences from the major histocompatibility complex (MHC) of non-human species, currently hosting over 9,000 alleles from 78 species. It also acts as the official source of information and guidelines from the Comparative MHC Nomenclature Committee. A new version of IPD-MHC was released in 2016, which provided a universal cross-species data submission and display tool to address the increasing amount and complexity of submitted data. This version also improved data presentation and enabled better tools to interactively explore the large volume of data available. These include a fast and intuitive alignment tool that allows inter- and intra- locus comparison at different levels, presenting only the alignment region of interest. The integrated BLAST tool allows users to find regions of sequence similarity within the entire IPD-MHC data set and now includes human data from the IPD-IMGT/HLA Database. As IPD-MHC continues to develop, it is essential that submission and analysis tools continue to align with user requirements and promote MHC research, while also maintaining MHC Committee requirements. Based on user feedback, the IPD-MHC website is soon to undergo a feature rich release, introducing new ways to visualize and explore the data. This includes incremental inter- and intra- locus

alignment of human and non-human alleles to allow efficient visualization of alignments composed of thousands of sequences in real-time. The incremental alignments will integrate all the current tools, plus the ability to save and share the user's bespoke visualization to facilitate collaboration. Where available, curated haplotype information will be linked to allele sequences and integrated with genomic browsers. This will allow the overlap of IPD-MHC data with the currently available EMBL genomic data. We are also developing a tool to visualize and compare polymorphisms displayed on tertiary structure predictions for all alleles, alongside classifications based on predicted peptide binding as well as primary sequence. Feedback and suggestions from the research community are encouraged and welcome.

OP111 Nomenclature for factors of the Swine Leukocyte Antigen (SLA) system: Update 2019. S. E. Hammer*[1], J. K. Lunney[2], A. Ando[3], C. Rogel-Gaillard[4], J.-H. Lee[5], L. B. Schook[6], and S. Ho[7], *[1]Institute of Immunology, University of Veterinary Medicine Vienna, Vienna, Austria, [2]USDA, Beltsville, MD, USA, [3]Tokai University School of Medicine, Isehara, Japan, [4]GABI, INRA, Jouy-en-Josas, France, [5]Chungnam National University, Daejeon, Republic of Korea, [6]University of Illinois, Urbana, IL, USA, [7]Gift of Life Michigan, Ann Arbor, MI, USA.*

The SLA system is among the most well characterized MHC systems in non-human animal species. A systematic nomenclature for the genes, alleles and haplotypes of the swine MHC is critical to the research in swine genetic diversity, immunology, health, vaccinology, and organ or cell transplantation. The SLA Nomenclature Committee was formed in 2002 as a joint ISAG/IUIS-VIC committee, with the primary objectives to validate newly identified SLA sequences according to the guidelines established for maintaining high quality standards of the accepted sequences; to assign appropriate nomenclatures for new alleles as they are validated; and to serve as a curator of the IPD-MHC SLA sequence database (https://www.ebi.ac.uk/ipd/mhc/group/SLA), the repository for all recognized SLA genes, their allelic sequences and haplotypes. In 2016, the Committee began re-designating each allele an official number, adopting the HLA Nomenclature System with colons as field separators (e.g., SLA-1*01rh28 [a] SLA-1*01:03). Phylogeny will remain the primary approach for assigning SLA-1, −2, −3, DRA, DRB1, DQA and DQB1 alleles into allele groups with similar sequence motifs, while alleles of the other loci including SLA-4, −5, −6, −7, −8, −9, −11, −12, DMA, DMB, DOA, DOB1, DOB2, DQB2, DRB2, DRB3, DRB4, DRB5, DYB, MIC1, MIC2, TAP1 and TAP2, are designated sequentially as they are discovered. There are currently 266 class I (incl. SLA-9, −11, −12), 227 class II (incl. DRA, DRB1, DQA, DQB1, DMA, DMB, DOA, DOB1, DRB2–5, DQB2, DOB2, DYB), 2 SLA-related (MIC-1, MIC-2) and 2 non-SLA alleles (TAP1, TAP2) officially designated. There are also 73 class I (SLA-1–3–2) and 52 class II (DRB1-DQB1) haplotypes designated at allele level resolution. Recent evidence has suggested certain loci in the SLA system, previously recognized as pseudogenes (e.g., SLA-9, SLA-11, DQB2, DOB2), may be expressed at the transcript level. The committee will consider reclassifying some of these loci as putative functional genes as additional evidence accumulates. Continuous efforts on characterizing SLA alleles and haplotypes and their diversity in various pig populations will further deepen our understanding of the architecture and polymorphism of the SLA system and their role in disease, vaccine and transplant responses.

Key Words: pigs, immunogenomics, databases/repositories or genotyping, adaptive immunity, animal health or biomedical model

OP112 Target resequencing for bovine major histocompatibility complex region. S.-N. Takeshima*[1,2], A. Kawamura[1], A. Ishida[1], Y. Murakawa[1], G. Giovambattista[3], and Y. Aida[2], *[1]Jumonji university, Niiza, Saitama, Japan, [2]Nakamura Laboratory, Baton Zone Program, RIKEN Cluster for Science, Technology and Innovation Hub, Wako, Saitama, Japan, [3]UNLP, La Plata, Argentina.*

Major histocompatibility complex (MHC) is one of most polymorphic regions in vertebrate genome. Bovine MHC (BoLA) region, embedded at chromosome 23, and constructed by BoLA class IIb, BoLA class IIa, BoLA class III and BoLA class I region. BoLA region contain the genes in high density. Additionally, each quite polymorphic BoLA genes frequently shows copy number variations. Therefore, it is difficult to genotyping BoLA region by genome-wide re-sequencing. For long time, UMD3.1 cattle genome reference was used in worldwide for bovine whole genome study, but recently, newer bovine genome reference called ARS-UCD1.2 was published. In this study, we tried to perform re-sequencing of BoLA region using 2 set of probes designed based on UMD3.1 and ARS-UCD1.2 and compared the result. Genomic DNA were collected from 9 heads of Japanese black cow and 1 Holstein cow. BoLA-DRB3 genotyping were performed by PCR sequence-based typing (SBT). DNA libraries were constructed from each DNA using KAPA HyperPlus Kit, and selected the libraries using SeqCap EZ and custom-made probes. Ten cows using for this study were subjected to BoLA-DRB3 typing and the genotypes were DRB3*012:01/014:01, *014:01/*016:01, *016:01/*016:01, *005:03/*012:01, *002:01/*015:01, *007:01/*010:01, *007:01/*009:02, *007:01/*014:01, *011:01/*012:01 and *010:01/*011:01. Next, we constructed 4 kind of probes based on 2 bovine genome references. The target regions were as follows; set 1: BoLA region excluded intron sequences using UMD3.1, set 4: Whole BoLA region using UMD3.1, BoLA1: BoLA region excluded intron sequences using ARS-UCD1.2, and BoLA2; Whole BoLA region using ARS-UCD1.2. All of 4 result were compared with BoLA-DRB3 PCR-SBT results and there are no conflicts between the result of PCR-SBT and target resequencing result. The variant call result containing a lot of SNPs which difficult to validated in single position. Therefore, we compared the rate of the succession SNPs and average of sequencing depth in each cow among 4 compared probes. Finally, we determined that BoLA2 probe were most suitable for re-sequencing whole BoLA region, and this result may be useful for study about the association between various economical and disease and of BoLA region.

Key Words: bovine leukocyte antigen (BoLA), target re-sequencing, cow, Japanese black (Wagyu), Holstein

OP113 Bovine leukemia virus proviral load were associated with bovine MHC DRB3 and DQA1 alleles in Japanese Holstein population from 2011 to 2014. Y. Aida*[1,2], S.-N. Takeshima[1,3], A. Ohno[2], and L. Borjigin[1], *[1]Nakamura Laboratory, Baton Zone Program, RIKEN Cluster for Science, Technology and Innovation Hub, Wako, Saitama, Japan, [2]Viral Infectious Diseases Unit, RIKEN, Wako, Saitama, Japan, [3]Department of Food and Nutrition, Faculty of Human Life, Jumonji University, Niiza, Saitama, Japan.*

Bovine leukocyte antigens (BoLAs) are used extensively as markers of disease and immunological traits in cattle. BoLA-DRB3 gene is most polymorphic gene among class II genes, and it is well known that the polymorphism were associated with bovine leukemia virus (BLV) infection. The association of BoLA-DRB3*009:02 allele with low proviral load were predicted using low-resolution typing method, such as PCR-RFLP, for Holstein cow. However, there is few association studies using high-resolution genotyping method, such as PCR-sequence based typing (SBT), for Holstein cow. Additionally, Yuan et al. suggested that the cow which showed the proviral load over 14,000 copies/10^5 cells secrete BLV provirus into nasal sample and it suggested that these cows were high-risk transmitter. Therefore, we here categorized the cow which shows the proviral load over 10,000 as the high-risk BLV spreader cow, and the cow which shows the proviral load under 10,000 as the low-risk BLV spreader cow. DNA samples were collected from 1290 Holstein cows belonging to BLV-positive commercial dairy farms located in the 23 prefectures of Japan, from 2011 to 2014. BoLA-DRB3 genotyping were performed by PCR-SBT. BLV proviral load were calculated by a quantitative real-time PCR method, BLV-CoCoMo-qP-

CR-2. We collected 1,290 blood samples from 1,290 heads of cow and there are 910 cows determined as BLV positive cow. The 910 cows were separated into 341 heads of "low-risk spreader" and 569 heads of "high-risk spreader." BoLA-DRB3 allele frequencies of these 2 groups were calculated and estimated p-values compared with each BoLA-DRB3 alleles and 2 spreader groups. From these 23 BoLA-DRB3 alleles, DRB3*002:01, *009:02, *012:01, *014:01 and *015:01 were determined as BLV provirus associated alleles. BoLA-DRB3*002:01, *009:02 and *014:01 were determined as resistant alleles (O.R. > 1), and BoLA-DRB3*012:01 and *015:01 were determined as susceptible alleles (O.R. < 1). In this study, we confirmed that BoLA-DRB3 was good marker for determine which cow spread the BLV and the result may be useful for eliminate BLV from farm without separate the cow into several cowshed.

Key Words: BoLA-DRB3, BoLA-DQA1, bovine leukemia virus, proviral load, Japanese Holstein

OP114 Assessing the genetic diversity of MHC-linked and non-MHC linked microsatellite markers in local chickens from four geographical regions. P. Manjula*, S. H. Cho, D. Seo, and J. H. Lee, *Division of Animal and Dairy Science, Chungnam National University, Daejeon, Republic of Korea.*

In poultry, microsatellite (MS) markers became a favorable genetic marker, which largely used in genetic mapping, diversity analysis, and population genetic inference. MS markers linked to the major histocompatibility complex (MHC) have shown a direct relationship with MHC-B haplotype in chicken. Especially, the LEI0258 marker could provide a good indication of the variability of MHC-B haplotype in different chicken populations. This study investigated the molecular marker diversity of different ecotypes of indigenous chickens sampled from 4 different countries based on the MHC linked and non-MHC linked MS markers. The study population consisted of 381 DNA samples. Including local chickens from Sri Lanka, Bangladesh, Eastern part of Nigeria, and South Korea. Five commercial chicken lines, sampled from Korea were included for the comparison. Fifteen polymorphic MS markers; including 14 non-MHC linked markers and one MHC-linked marker were genotyped. All MS markers were polymorphic with 234 alleles, of which 38 alleles (193 bp ~489 bp) were identified for LEI0258 marker. Mean number of alleles, effective allele size, private alleles, heterozygosity, and polymorphic information content (PIC) was higher for LEI0258 marker compared with other MS markers. Mean observed heterozygosity (Ho) values per locus were ranged from 0.503 (LEI0141) to 0.793 (MCW104). Average Ho values for the population was given between 0.632 (Hilly chicken) and 0.850 (Hy-line Layer chicken). PIC values for all indigenous chicken groups were higher (48.41% - 78.0%) than that of commercial chickens (41.5% - 52.8%). Considerably high genetic diversity was observed within each indigenous population. Lowest gene diversity value of 0.621 and the highest value of 0.807 were observed in commercial Lohmann brown chicken(LO) and Tabbowa group1(T), respectively. Wright's inbreeding coefficient (F_{IT}) values for all loci were observed between 0.072 ± 0.034 and 0.375 ± 0.051. High genetic distance was observed between commercial and local chicken breeds. This information may support the future studies of these breeds and conservation of rare breeds considering their contribution to the total genetic diversity. Particularly, high MHC-linked LEI0258 allele diversity and heterozygous nature indicated the presence of diverse MHC haplotypes and their importance in disease resistance in local breeds.

Key Words: local chicken, genetic diversity, microsatellite, MHC

OP115 Effectiveness of bovine leukemia virus (BLV) infection control strategies using cattle carrying resistant and susceptible bovine MHC DRB3 alleles. L. Borjigin*[1], L. Bai[2], T. Hirose[1], H. Sato[1], S. Watanuki[1], S. Yoneyama[3], M. Inokuma[4], K. Fujita[4], Y. Shinozaki[5], R. Yamanaka[6], A. Yasui[6], Y. Sohei[6], M. Baba[6], S.-N. Takeshima[1,7], Y. Aida[1], *[1]Nakamura Laboratory, Baton Zone Program, RIKEN Cluster for Science, Technology and Innovation Hub, Wako, Saitama, Japan, [2]Photonics Control Technology Team, RIKEN Center for Advanced Photonics, Wako, Saitama, Japan, [3]Kenou Livestock Hygiene Service Center, Utsunomiya, Tochigi, Japan, [4]Chuo Livestock Hygiene Service Center, Chiba, Japan, [5]Nanbu Livestock Hygiene Service Center, Chiba, Kamogawa, Japan, [6]Kumagaya Livestock Hygiene Service Center, Kumagaya, Saitama, Japan, [7]Department of Food and Nutrition, Jumonji University, Niiza, Saitama, Japan.*

Bovine Leukemia Virus (BLV) infects cattle worldwide and is causing serious economic damage. BLV mainly infects by horizontal transmission. The cattle with high proviral loads (PVL) are considered to be a major infectious factor in a population, while the cattle with low PVL are difficult to transmit BLV to other cattle. Previously, we identified "resistant (R) cattle" carrying bovine leukocyte antigen (BoLA)-DRB3*009:02 and *014:01:01 alleles which associated with a low PVL, and "susceptible (S) cattle" carrying BoLA-DRB3*015:01 and *012:01 alleles which associated with a high PVL in Holstein cattle. In this study, we investigated the distribution and actual PVL of these cattle at 5 farms in the Kanto region of Japan, and then we conducted the BLV control strategies using the R and S cattle. PVL of a total of about 350 cattle from 5 farms were estimated by an accurate PVL measuring system, CoCoMo-BLV-qPCR method, and BoLA-DRB3 alleles were identified by PCR-sequence-based typing method. Among 5 farms, the population of cattle carrying R and S alleles were 20.5% and 41.6%, respectively. Interestingly, PVL of the R cattle (mean 3,281 copies/10^5 cells) were significantly lower than that of the S cattle (mean 28,439 copies/10^5 cells). Next, we repeated the BLV control strategies twice within 2 years under the field condition. We first investigated whether R cattle are useful as a biological barrier to prevent new infection or not. Interestingly, no new BLV-infected cattle were detected in 2 farms for 3 or 5 mo after we placed the R cattle between the other infected and uninfected cattle. Second, we actively eliminated the S cattle with high PVL from the farms. As a result, the average of PVL were significantly reduced at all 5 farms and the BLV negative rate increased at the 4 farms. These results strongly suggest that concurrent approach of the effective utilization of the R cattle as a biological barrier to prevent new infection and the preferential elimination of the S cattle to decrease PVL is useful for BLV infection control and development of effective BLV eradication program.

Key Words: bovine leukemia virus, proviral loads, BoLA-DRB3, resistant cattle, susceptible cattle

OP116 Characterization of bovine MHC DRB3 diversity in worldwide cattle breeds focusing on Myanmar cattle. G. Giovambattista*[1,2], K. K. Moe[1,3], M. Palati[1], L. Borjigin[1], H. H. Moe[3], S.-N. Takeshima[4], and Y. Aida[1], *[1]Nakamura Laboratory, RIKEN Baton Zone Program, RIKEN Industrial Co-creation Program, Wako, Saitama, Japan, [2]IGEVET (UNLP-CONICET LA PLATA), Facultad de Ciencias Veterinarias UNLP, La Plata, Buenos Aires, Argentina, [3]University of Veterinary Science, Yezin, Nay Pyi Taw, Myanmar, [4]Department of Food and Nutrition, Faculty of Human Life, Jumonji University, Niiza-shi, Saitama, Japan.*

Myanmar cattle population is mainly comprised by native breeds and Holstein crossbreeds, which are composed of bovines highly adapted to the harsh tropical climates of this country. Myanmar native cattle included different breeds that are defined by their geographical location and coat color. The main aim of this work was to assess the genetic diversity and genetic structure of the BoLa-DRB3 gene in the Myanmar cattle population at allele and molecular levels, a locus that play a role in the immune response and resistence to infectious diseases. Blood samples were obtained from 294 cattle from 6 regions of Myanmar (Bago, n = 38; Sagaing, n = 77; Mandalay, n = 46; Magway, n = 46; Kayin, n = 43; and Yangon, n = 44). Purified DNAs were genotyping by the SBT method and DNA electropherograms were analyzed using Assign 400ATF software. 71 alleles were detected in Myanmar cattle, including 4 new variants. AMOVA evidenced that intra- and inter-breed genetic diversity in the BoLA-DRB3 gene of Holstein and Native groups and inter-groups account for the 0.4% and 2%, respectively of the genetic variances at BoLA-DRB3 gene, respectively, (F_{ST}

range between 0 - 0.054). The gene diversity within population varied between 0.976 in Kayin Native and 0.916 in Kayin Holstein, respectively, while, the number of alleles ranged from 12 in Bago Holstein to 40 in Sagaing native. PCA and tree analyzes showed that Myanmar native populations grouped in a narrow cluster that diverges clearly from the Myanmar crossbreed Holstein crossbred populations. Furthermore, some Myanmar native populations (Bago, Mandalay and Yangon) seem to be closer to Zebu breeds (Nellore, Gir, and Brahman), while others (Kayin, Magway and Sagaing) are more related to Philippine native in that BoLA-DRB3 gene. On the contrary, the Holstein population showed a large dispersion, probably due to the different degree of mixing with native populations. The results presented in the current work will contribute to increase our knowledge about the genetic diversity of BoLa-DRB3, and how this diversity is distributed among Myanmar regions.

Key Words: cattle and related species, immunogenomics, genotyping, breed diversity, breed/population identification

OP117 Determination of MHC haplotypes in pure-breed Spanish horses using microsatellites. L. Barrachina*[1,2], A. Cequier[1], A. Vitoria[1,2], C. Cons[1], A. Sanz[1], P. Zaragoza[1], A. Romero[1,2], FJ Vázquez[1,2], and C. Rodellar[1], *[1]Laboratorio de Genética Bioquímica LAGENBIO (Universidad de Zaragoza), Instituto Agroalimentario de Aragón (Universidad de Zaragoza-CITA), Instituto de Investigación Sanitaria de Aragón (IIS), Zaragoza, Spain, [2]Servicio de Cirugía y Medicina Equina, Hospital Veterinario, Universidad de Zaragoza, Zaragoza, Spain.*

In horses, donor-receptor matching for major histocompatibility complex (MHC) affects the immune response when transplanting allogeneic cells for therapy. MHC haplotype determination by serology has limitations, but shows high correlation with polymorphic intra-MHC microsatellites analysis. Besides, MHC genes are related with immune functions and can reflect genetic diversity, making these regions worth of investigation. This study aimed to analyze MHC diversity in pure breed spanish (PRE) horses using a validated 10 markers-microsatellite panel to gain insight into MHC variability in this breed. 44 PRE horses (29 related, 15 unrelated) were analyzed. We identified 21 MHC haplotypes in related animals (parent-offspring pairs/trios or half-siblings), 8 of which were found in 13 unrelated horses, provisionally assigning 6 haplotypes to remaining constellations. Two unrelated horses could not be assigned with any identified or previously known haplotype. None of the identified 27 haplotypes was previously reported. Haplotype frequencies in unrelated horses (n = 25, including animals from related-group but unrelated among them) showed 12% frequency for the 2 most frequent haplotypes, followed by 2 haplotypes with 10% and 8% frequency. The other haplotypes only appeared 1–3 times (2–6%). Eleven new alleles were found in 6 of the 10 markers, 4 of them in COR112 region. Allelic frequencies showed 5–8 alleles per region with no marked predominance except for region UMNJH-38, where only 3 alleles were identified and 84% animals shared the same one. Since recombination events are described, 2-subhaplotypes system was proposed by grouping the 4 markers for MHC-I and III and the 6 markers for MHC-II. Among the 27 haplotypes some subhaplotypes were observed: 6 I-III subhaplotypes and 8 II-subhaplotypes, plus 11 I-III subhaplotypes and 8 II-subhaplotypes that seemed to be combinations of the original ones. Larger sample needs to be examined, but this study provides preliminary information to gain insight into the extent of MHC-variability in PRE horses, which is relevant for genetic diversity and future transplant-based therapies requiring donor-receptor MHC matching in PRE and other horse breeds.

Key Words: major histocompatibility complex, equine leukocyte antigen, polymorphism, haplotype, microsatellite panel

Domestic Animal Sequencing and Annotation

OP118 Invited Workshop Presentation: Navigating the genome with epigenome maps: Profiling *cis* regulatory elements at high-throughput and single-cell resolution. J. Chiou*[1], J. Y. Han[2], C. Zheng[3], F. Cheng[2], M. Schlichting[3,4], S. Huang[3,4], J. Wang[3,4], Y. Sui[3,4], A. Deogaygay[3], M.-L. Okino[3], Y. S. Sun[3], P. Kudtarkar[3], R. Fan[3], M. Sander[3,4], K. Galton[3,5], S. Preissl[2,4], and D. Gorkin[2,4], *[1]Biomedical Graduate Studies Program, University of California San Diego, La Jolla, CA, [2]Center for Epigenomics, University of California San Diego, La Jolla, CA, [3]Department of Pediatrics, University of California San Diego, La Jolla, CA, [4]Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA, [5]Institute for Genomic Medicine, University of California San Diego, La Jolla, CA.*

Genetic variants associated with complex phenotypes are enriched in cis regulatory sequences, which can be mapped genome-wide with epigenomic assays like ATAC-seq and ChIP-seq. Recent developments in single cell epigenomic technologies create exciting new opportunities to dissect cell type-specific regulatory landscapes from heterogeneous tissues, and provide new insights into the genetic architecture of complex phenotypes and disease. I will discuss recent advances in our ability to map cis regulatory sequences at high throughput and single-cell resolution, with a focus on our recent efforts to use single-cell ATAC-seq in human pancreatic islets to better understand islet biology and the genetic architecture of Type II diabetes.

OP119 Update on the functional annotation of the equine genome project with a focus on histone modifications across tissues. R. R. Bellone*[1,2], J. L. Petersen[3], N. B. Kingsley[2], C. Creppe[4], S. Peng[1], E. N. Burns[1], T. Kalbfleisch[5], C. Kern[6], H. Zhou[6], J. N. MacLeod[5], and C. J. Finno[1], *[1]University of California-Davis, School of Veterinary Medicine, Department of Population Health and Reproduction, Davis, CA, USA, [2]University of California-Davis, School of Veterinary Medicine, Veterinary Genetics Laboratory, Davis, CA, USA, [3]University of Nebraska-Lincoln, Department of Animal Science, Lincoln, NE, USA, [4]Diagneode, Liège, Belgium, [5]University of Kentucky, Gluck Equine Research Center, Lexington, KY, USA, [6]University of California-Davis, Department of Animal Science, Davis, CA, USA.*

A primary aim of the Functional Annotation of ANimal Genomes (FAANG) initiative is to characterize tissue-specific regulatory elements across the genomes of several agricultural species including the horse. Toward this goal, the equine FAANG community has developed a biobank of over 80 tissues, 4 fluid types and 9 microbiome samples collected from 2 adult Thoroughbred mares. This biobank has served as a resource for the community and has enabled transcriptome profiling of over 50 tissues, which aided in annotating the newest assembly of the horse genome. Adipose, parietal cortex, heart, liver, lung, ovary, and skeletal muscle were prioritized for functional annotation efforts to facilitate cross species comparisons. Lamina was also prioritized because of its important role in equine biology and health. Four histone modifications were evaluated in both horses by ChIP-seq to characterize active promoter and enhancer regions (H3K4me3, H3K4me1, and H3K27ac), or inactive regulatory regions (H3K27me3). Chromatin shearing, immunoprecipitation, and Illumina sequencing were performed by Diagenode's Epigenomic Services. Single-end 50 bp reads of Illumina Hiseq data were evaluated using the FAANG ChIP-seq Pipeline. Peaks were called by MACS2 software for the activating marks and Sicer for H3K27me3. Peaks enriched in both replicates were considered in downstream analyses. When evaluating histone marks unique to each of the 8 tissues, we identified 87K tissue-specific active enhancers and 19K tissue-specific active promoters. Lamina had the second highest number of tissue-specific enhancer marks (H3K4me1 and H3K27ac), after liver, with over 16.6K unique peaks found across the genome.

These data highlight the specialized function of this equine-specific tissue and combined with further analyses may assist in our understanding of the pathogenesis of laminitis and other connective tissue phenotypes. CTCF ChIP-seq is underway to identify genomic insulators in the 8 prioritized tissues. Optimization of ATAC-seq, a method to characterize open chromatin, is ongoing in 2 select tissues. These data provide a valuable baseline for understanding genome function in the healthy, adult Thoroughbred mare and will contribute new insights into tissue specific genome regulation.

Key Words: functional annotation, ChIP-seq, histone marks, equine

OP120 The Ovine FAANG Project: A high-resolution atlas of transcription start sites in the new Rambouillet sheep genome. E. L. Clark*[1], M. Salavati[1], I. Gazova[1], R. Clark[2], T. P. Smith[3], K. C. Worley[4], N. E. Cockett[5], A. L. Archibald[1], and B. Murdoch[6], *[1]The Roslin Institute, University of Edinburgh, Edinburgh, UK, [2]Clinical Research Facility, University of Edinburgh, Edinburgh, UK, [3]USDA, ARS, USMARC, Clay Center, NE, USA, [4]Baylor College of Medicine, Houston, TX, USA, [5]Utah State University, Logan, UT, USA, [6]University of Idaho, Moscow, ID, USA*.

The overall aim of the Ovine FAANG project is to provide a comprehensive annotation of the new highly contiguous reference genome for sheep Oar_rambouillet_v1.0. The project has 3 objectives: 1) to develop a deep and robust data set of transcribed elements and regulatory features in the sheep genome; 2) to provide a high quality annotation of the new ovine reference assembly; 3) to provide ovine FAANG data and support to the livestock genomics community. To contribute to these objectives we have generated a high-resolution atlas of transcription start sites (TSS) for sheep. Mapping of TSS is a key first step in understanding transcript regulation and diversity. Using 56 tissue samples collected from the reference rambouillet ewe Benz2616 we have performed a global analysis of TSS using Cap Analysis Gene Expression (CAGE) sequencing. CAGE measures RNA expression by 5′ cap-trapping, and identifies the 5′ ends of non-polyadenylated RNAs including lncRNAs and miRNAs and has been specifically designed to allow the characterization of TSS within promoters to the single-nucleotide resolution. This level of sensitivity allows investigation of the regulatory inputs driving transcript expression, and construction of transcriptional networks. We have adapted an analysis pipeline for CAGE which uses TagDust2 for clean-up and trimming, Bowtie2 for mapping, CAGEr for normalization and clustering and IGV for visualization. Using this pipeline we have been able to map genome-wide TSS for the 56 tissues from Benz2616. Mapping of CAGE tags indicated that, for numerous transcripts, TSS vary across tissues. The majority (>80%) of these TSS were novel and did not overlap the current annotated 5′ end of transcripts. As such this comprehensive global annotation of TSS in sheep will significantly enhance the annotation of gene models in the new ovine reference assembly. The CAGE data set will also be used to validate and enhance gene expression data from Ilumina RNASeq, miRNASeq and IsoSeq libraries that we have generated through the Ovine FAANG project. These data sets will be analyzed in parallel to confirm TSS and for novel isoform validation. This will provide one of the highest resolution annotations of transcript regulation and diversity in a livestock species to date.

Key Words: sheep and related species, Functional Annotation of Animal Genomes (FAANG), CAGE, functional assay, genome annotation

OP121 Genome-wide identification of functional DNA elements in the pig genome. Z. Yunxia[1], H. Mingyang[1], H. Ye[1], X. Yueyuan[1], Z. Huanhuan[1], F. Yuhua[1], Y. Hongbo[2], Y. Feng[2], L. Xinyun[1], and Z. Shuhong*[1], *[1]Key Lab of Agricultural Animal Genetics, Breeding, and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan, China, [2]Department of Biochemistry and Molecular Biology, College of Medicine, The Pennsylvania State University, University Park, PA, USA.*

The swine genome sequencing project provides a valuable resource for further improvement of this important livestock specie as meat producer and biomedical model. While the linear DNA sequence is revealed and widely available, the cis-regulatory elements embedded in the pig genome and their regulatory functions are largely uncharacterized. To fill this gap, we adopted a strategy similar to ENCODE and Roadmap Epigenomics projects to identify the cis-regulatory elements in the pig genome. RNaseq, ATAC-seq, and ChIP-seq for histone markers, H3K27ac and H3K4me3, were performed to generate a comprehensive map of transcriptomes, open chromatin regions, and regulatory elements in a variety of pig tissues including skeletal muscle, spleen, heart, kidney, liver, backfat, lung, thymus, small intestine (duodenum), cerebrum and cerebellum isolated from 2 lean type breeds (Large White and Duroc) and 2 fatty breeds (Meishan and Enshi Black). We identified more than 15,000 cis-regulatory elements, representing the most comprehensive functional annotation so far in the pig genome. Among these cis-regulatory elements, over 53% of them were functional conserved with human cis-regulatory elements based on comparison of our data to the data generated by human ENCODE and Roadmap Epigenomics projects. To further explore the 3 dimension (3D) structure of pig genome, we performed high-throughput chromosome conformation capture (Hi-C) experiment. A total of 2,393 and 2,305 boundaries and topologically-associating domains (TAD) were identified in muscle tissue respectively. Here, 19,736 significant long range interactions were identified between distal cis-regulatory elements by using loop of HiC matrix and cis-regulatory elements correlation analysis, which including 15,844 enhancer-enhancer, 3,637 enhancer-gene (enhancer-promoter) and 255 gene-gene (promoter-promoter) long rang interactions. By comparing our Hi-C matrix to human 3D genomic data, we found that over 79% of the pig boundaries were also conserved in human, indicating the TAD were highly conserved. It is worth noting that 28 of above conserved inter boundaries were rearranged as 14 TADs in human genome. Moreover, SNP distribution at the boundary and TAD regions was mapped using a population of 490 pigs with available whole-genome sequencing data. Interestingly, the results revealed a significantly higher SNP density in the boundary regions compared with that in TAD regions.

Key Words: pig, cis-regulatory elements, Hi-C, topologically associating domain

OP122 The impact of the 1000 Bull Genomes Project and its future. A. J. Chamberlain*[1], C. J. Vander Jagt[1], R. Xiang[2], M. E. Goddard[1,2], I. M. MacLeod[1], R. D. Schnabel[3], B. J. Hayes[4], and H. D. Daetwyler[1,5], *[1]Agriculture Victoria, Centre for AgriBiosciences, Bundoora, Victoria, Australia, [2]Faculty of Veterinary & Agricultural Science, The University of Melbourne, Parkville, Victoria, Australia, [3]Division of Animal Sciences, Informatics Institute, University of Missouri, Columbia, MO, USA, [4]Centre for Animal Science, The University of Queensland, St Lucia, Queensland, Australia, [5]School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

The 1000 Bull Genomes Project has provided a platform for detailing the variation in the bovine genome. Starting with 133 animals from 2 breeds in 2012 the project grew to 2703 animals from 121 breeds in 2018. The project has had lasting impact on the identification of deleterious mutations, such as genetic recessive diseases and embryonic lethals, enabling the screening of 100s of thousands of cattle. It has also impacted the accuracy of genomic selection. At Agriculture Victoria we have utilized various evolutionary and regulatory data, including but not limited to expression and metabolite QTL, histone modification marks, selection signatures and variant annotations, to determine the contribution variants in these classes have toward the genetic variance of 34 traits important to dairy cattle. Seventeen million variants from run6 were ranked according to their contribution to these traits and a 5Mb sliding-window clustering analysis used to select 300K informative variants. These were used for Bayesian genome mapping in multiple traits, reducing the set to 80K informative markers. After checking designability, 40K have now been included on an InfiniumXT beadchip for validation in the Australian dairy cattle herd. Preliminary tests with imputed genotypes show these variants generally increased the accuracy of genomic prediction for milk traits compared with stan-

dard 50K chip genotypes, particularly for animals less represented in the reference population. Run 7 of the 1000 bull genomes project is a major revision. First, the project has utilized the new bovine reference ARS-UCD1.2. This has required that all animals previously submitted to the project be realigned. Second, the project has switched to using the Genome Analysis Tool Kit (GATK) variant calling pipeline that utilizes Haplotype Caller and Variant Recalibration based on true variants for filtration. This pipeline has required the development of a new fastq to bam file processing pipeline that follows GATK best practises. Alignment is still performed using BWA. Run 7 included 3998 animals, 3067 Taurus, 838 Indicus, and 93 out-species. A total of 182 breeds. These animals have been contributed from 43 participating institutions from 22 countries.

Key Words: cattle, genome sequence, deleterious mutations, variant detection, genomic selection

OP123 Analysis of alternative splicing events across cattle tissues by genome-wide integration of PacBio Iso-seq and RNA-Seq data. H. Beiki, J. Koltes, Z.-L. Hu, and J. Reecy*, *Iowa State University, Ames, IA, USA.*

Alternative splicing (AS) is a primary mechanism of functional regulation and protein diversity in the eukaryotic genomes. Long-read transcript isoform sequencing analysis of the human and pig has revealed that more than 80 percent of multi-exonic genes are alternatively spliced. Despite the importance of cattle as a significant source of nutrition to nearly 6.6 billion humans, our current knowledge of cattle transcriptome is limited. To identify a more complete catalog of transcript isoforms across cattle tissues, we processed 1197 high quality (minimum of 30 million high-quality reads with a median length >30 nucleotides) Illumina short-read RNA-seq samples from 43 cattle tissues. Tissue-specific transcriptomes were constructed based on this data and integrated with error-corrected PacBio Iso-seq based transcriptomes. Comparison of transcript structures across of major cattle tissues/organs and analysis of tissue specific transcript/gene will be performed. To get a comprehensive view of transcriptome-based phylogeny of livestock species, we will eventually add other species to this analysis. To provide public access to the processed data, pipelines and results, we have launched a new version of EpiDB database. The present study will provide a unique resource for comparative genomics and will facilitate the analysis of tissue specificity and cross-species conservation in higher organisms.

Key Words: cattle, gene expression, network analysis, genome annotation

OP124 BovReg: An international consortium for functional annotation of the bovine genome. C. Kühn*[1], J. Vanselow[1], C. Notredame[2], D. Rocha[3], D. Boichard[3], D. Allaer[4], C. Charlier[5], H. Pausch[6], Y. deHaas[7], M. Lund[8], J. Vilkki[9], H. Taniguchi[10], F. Meijboom[11], D. Zerbino[12], A. Rosati[13], G. Plastow[14], E. Clark[15], J. Prendergast[15], A. Bruce[15], M. Schmicke[16], A. Chamberlain[17], H. Daetwyler[17], V. Blanquet[18], A. J. Amaral[19], and D. Bruce[20], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Centre for Genomic Regulation (CRG), Barcelona, Spain, [3]Institut National de Recherche Agronomique (INRA), Jouy-en-Josas, France, [4]DIAGENODE, Liege, Belgium, [5]GIGA, Université de Liège, Liege, Belgium, [6]Eidgenoessische Technische Hochschule, Zuerich, Switzerland, [7]Stichting Wageningen Research, Wageningen, Netherlands, [8]Aarhus University, Foulum, Denmark, [9]Natural Resources Institute Finland (LUKE), Jokioinen, Finland, [10]Institute of Genetics and Breeding, Jastrebiec, Poland, [11]University Utrecht, Utrecht, Netherlands, [12]European Molecular Biology Laboratory (EMBL-EBI), Hinxton, UK, [13]European Association for Animal Production, Rome, Italy, [14]University of Alberta, Edmonton, Canada, [15]The University of Edinburgh, Edinburgh, UK, [16]Stiftung Tieraerztliche Hochschule Hannover, Hannover, Germany, [17]Agriculture Victoria, Centre for Agribiosciences, Bundoora, Australia, [18]University of Limoges, Limoges, France, [19]Centre for Interdisciplinary in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, [20]Edinethics Ltd., Edinburgh, UK.*

Despite the revolution in functional genome analysis a wide gap in understanding associations between the (epi)genome and complex phenotypes of interest currently remains and impedes efficient use of annotated genomes for precision breeding. As part of the global FAANG initiative and funded by a recent EU H2020 project call, the BovReg consortium is set up to provide a comprehensive map of functionally active genomic features in cattle and how their (epi)genetic variation in beef and dairy breeds translates into phenotypes. This constitutes key knowledge for biology-driven genomic prediction needed by scientific and industry livestock communities. BovReg brings together a critical mass of experts in ruminant research and beyond encompassing bioinformatics, molecular and quantitative genetics, animal breeding, reproductive physiology, ethics and social science. A total of 20 partners from the EU, UK, Switzerland, Canada and Australia form a global interdisciplinary team, which builds upon previous and ongoing national and EU-funded research and collaborations with industry partners. BovReg is set up to generate functional genome data based on FAANG core assays from representative bovine tissues and newly established cell lines covering different ontological stages and phenotypes applying novel bioinformatic pipelines in a standardized and reproducible fashion. Key traits for BovReg are phenotypes related to robustness, health and biological efficiency. Data, metadata, knowledge and protocols will be deposited in European biological archives, aiming to set up and maintain a knowledge hub and establish gold standards. Long-term availability of data, methods, targeted dissemination and communication activities are guaranteed by EMBL-EBI, FAANG and EAAP and will follow ELIXIR guidelines. BovReg will develop biology-driven genomic prediction tools by integrating biological knowledge of regulatory genomic variation and genomic selection methods for local and global cattle populations. The results will enable more environmentally sustainable cattle production, while respecting animal-welfare.

Key Words: annotation, genome, bovine, regulatory elements, FAANG

OP125 Evolution of gene regulation in ruminants differs between evolutionary breakpoint regions and homologous synteny blocks. M. Farré*[1], J. Kim[2], A. A. Proskuryakova[3,4], Y. Zhang[5], A. I. Kulemzina[3], Q. Li[6], Y. Zhou[6], Y. Xiong[6], J. L. Johnson[7], P. L. Perelman[3,4], W. E. Johnson[8,9], W. Warren[10], A. V. Kukekova[7], G. Zhang[6,11,12], S. J. O'Brien[13], O. A. Ryder[14], A. S. Graphodatsky[3,4], J. Ma[5], H. A. Lewin[15], D. M. Larkin*[1,16], *[1]Royal Veterinary College, University of London, London, UK, [2]Konkuk University, Seoul, Korea, [3]Institute of Molecular and Cellular Biology, Novosibirsk, Russia, [4]Novosibirsk State University, Novosibirsk, Russia, [5]Carnegie Mellon University, Pittsburgh, PA, USA, [6]BGI-Shenzhen, Shenzhen, China, [7]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [8]Smithsonian Conservation Biology Institute, Front Royal, VA, USA, [9]Smithsonian Institution, Suitland, MD, USA, [10]Washington University School of Medicine, St. Louis, MO, USA, [11]Kunming Institute of Zoology, Kunming, China, [12]University of Copenhagen, Copenhagen, Denmark, [13]St. Petersburg State University, St. Petersburg, Russian Federation, [14]San Diego Zoo, Escondido, CA, USA, [15]University of California, Davis, Davis, CA, USA, [16]The Federal Research Center Institute of Cytology and Genetics, Novosibirsk, Russia.*

The role of chromosome rearrangements in driving evolution has been a long-standing question of evolutionary biology. Here we focused on ruminants as a model to assess how rearrangements may have contributed to the evolution of gene regulation. Using reconstructed ancestral karyotypes of Cetartiodactyls, Ruminants, Pecorans, and Bovids, we traced patterns of gross chromosome changes. We found that the lineage leading to the ruminant ancestor after the split from other cetartiodactyls, was characterized by mostly intrachromosomal changes while the lineage leading to the pecoran ancestor (including all livestock ruminants) included multiple interchromosomal changes. We observed that the liver cell putative enhancers in the ruminant evolutionary breakpoint regions are highly enriched for DNA sequences

under selective constraint acting on lineage-specific transposable elements (TEs) and a set of 25 specific transcription factor (TF) binding motifs associated with recently active TEs. Coupled with gene expression data, we found that genes near ruminant breakpoint regions exhibit more divergent expression profiles among species, particularly in cattle, which is consistent with the phylogenetic origin of these breakpoint regions. Notably, this divergence was significantly greater in genes with enhancers that contain at least one of the 25 specific TF binding motifs and located near bovidae-to-cattle lineage breakpoint regions. Taken together, by combining ancestral karyotype reconstructions with analysis of *cis* regulatory element and gene expression evolution, our work demonstrated that lineage-specific regulatory elements co-localized with gross chromosome rearrangements may have provided valuable functional modifications that helped to shape ruminant evolution.

Key Words: genome evolution, gene regulation, rearrangements, enhancers, chromosomes

OP126 eMIRNA: A comprehensive pipeline for discovery and annotation of microRNAs in multiple species. E. Mármol-Sánchez*[1], S. Cirera[2], R. Quintanilla[3], A. Pla[4], and M. Amills[1,5], *[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [2]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark, [3]Animal Breeding and Genetics Program, Institute for Research and Technology in Food and Agriculture (IRTA), Torre Marimon, Caldes de Montbui, Spain, [4]Department of Medical Genetics, University of Oslo, Oslo, Norway, [5]Departament de Ciència Animal I dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

MicroRNAs (miRNAs) are a group of small non-coding RNAs widely recognized as key post-transcriptional regulators in many relevant biological processes. Although the functional role of several miRNAs has been thoroughly studied in reference species such as humans and rodents, these and other relevant species still lack an accurate and complete miRNA annotation. The computational prediction of novel miRNA candidates by applying Machine Learning algorithms constitutes an emerging and active research field for improving miRNA annotation and several methods have been published over the past few years. Despite these efforts, many tools for miRNA prediction suffer from difficult implementation, cumbersome interpretation or are no longer available to be used for straightforward novel miRNA detection. We present eMIRNA, a new comprehensive pipeline for de novo miRNA recognition and homology-based comparison between species that allows users to predict novel and already annotated miRNAs from scratch. This user-friendly end-to-end pipeline allows the use of small RNA-seq data or homology-based search in the species of interest. We show the superiority of the eMIRNA pipeline compared with other state-of-the-art similar tools. We further demonstrate its inter-species exchangeability and applicability in a real case scenario by successfully profiling novel miRNAs in the porcine genome.

Key Words: microRNAs, bioinformatics tools, machine learning, multispecies

Genome Edited Animals

OP128 A genome-wide CRISPR library for high-throughput genetic screening in pig cells. S. Xie*, C. Zhao, H. Liu, T. Xiao, X. Cheng, X. Nie, X. Han, C. Li, X. Li, D. Zhang, and S. Zhao, *Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education & Key Lab of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, China.*

The simplicity of the CRISPR/Cas9-mediated genome editing has opened up the possibility of performing genome-wide targeted mutagenesis in cell lines, enabling screening for cellular phenotypes caused by genetic aberrations. Recently, the GeCKO (genome-wide CRISPR knockout) screening strategy has been utilized to investigate virus-host interactions. Until now, there have been no reports on using genome-wide CRISPR/Cas9 knockout library to screening genes in pig cells. Here, we designed a sgRNA library with high single guide RNAs (sgRNAs) activity and specificity, we first evaluated the effects of the 5′-end length of sgRNA and microsatellite on sgRNAs activity and off-target effects. The results indicated that although the sequence length is associated with on-target activity of sgRNA in site-dependent manner, it did not affect the specificity of sgRNAs. The activity and specificity of sgRNAs can be sensitively detected by Target capture sequencing in combination with *in silico* prediction, However, when analyzing CRISPR high-throughput sequencing data, it is necessary to rule out the interference of microsatellite DNA when evaluating the off-target effects of sgRNA. Second, CRISPR-lib software was developed, which is a high-throughput local tool for generation of genome-wide sgRNAs. Subsequently, a pig genome-wide CRISPR library (PigGeCKO) was designed by CRISPR-lib, which consisting of more than 85,000 sgRNAs. Further, PK-15 cells are transduced with Cas9-expressing lentivirus and selected to optimal Cas9 expression levels. The pooled PigGeCKO library was then transduced into cells so that most the cells receives only one sgRNA construct. Next, High-throughput sequencing reads were first mapped to the PigGeCKO library sgRNA sequences with the SeqMap program. Enriched genes were then identified via MAGeCK, a Model-based analysis of Genome-wide CRISPR/Cas9 Knockout method for prioritizing sgRNAs and genes. Finally, using knockout library screens, we successfully identified the host genes essential for the survival of cells by Japanese encephalitis virus (JEV) infection, which were confirmed by functional validation. To sum up, the broad application of this powerful genetic screening strategy in pig will not only facilitate the rapid identification of genes important for virus infection but will also enable the discovery of genes that participate in other biological processes.

Key Words: pig, genome-wide CRISPR knockout, Japanese encephalitis virus, bioinformatics tools

OP129 Precision editing for IAV resistance in pig cells. Y. Du*[1,2], S. Lillico[1], C. Proudfoot[1], and B. Whitelaw[1], *[1]The Roslin Institute, University of Edinburgh, Edinburgh, UK, [2]Radcliffe Department of Medicine, University of Oxford, Oxford, UK.*

Influenza A virus (IAV) is a highly infectious pathogen circulating in avian, porcine and humans. Pigs are susceptible to both human and avian IAVs, owing to the existence of 2 types of sialic acid (SA)-containing host cell virus receptors, SA α2,6-gal and SA α2,3-gal. This enables pigs to serve as intermediate hosts in the production of reassortant IAV strains, which facilitates cross-species transmission, and can lead to epidemics and pandemics. Current vaccines and antiviral drugs have limited efficacy against newly emerging and re-emerging IAV strains. With the aim of developing novel genetic approaches to combat IAV, we are exploring strategies for host resistance by reducing the expression of α2,6-SA-containing IAV receptors in pigs, such that they are less susceptible to human IAV infections. β-galactoside α2,6-sialyltransferase 1 (ST6Gal1) mediates N-linked α2,6-sialylation on cell surfaces by catalyzing the addition of α2,6-SA to the terminal N-glycans. ST6Gal1 is involved in a wide range of biological events, especially viral recognition and immune regulation. ST6Gal1 is encoded by the ST6GAL1 gene, which displays a tissue-specific expression pattern through the predicted multiple promoter regions of the ST6GAL1 gene. Recognizing that inactivating the ST6GAL1 gene could have adverse phenotypic

effects given the widespread expression profile of ST6GAL1 and the diversity of its biological functions, we pursued a more subtle approach to engineering the ST6GAL1 gene — that of removing a single promoter region to alter the expression profile using CRISPR/Cas9 genome editing. The resulting edited pig cells (ST6GAL1ΔP) were viable and exhibited reduced α2,6-SA expression levels on the cell surfaces. Human IAV was less to infect ST6GAL1ΔP compared with non-edited cells, suggesting that a strategy to reduce the biosynthesis of α2,6-sialylated glycan structure exclusively on the airway could offer an antiviral strategy, independent of inducing a humoral immune response. We propose this work lays the foundation for generating engineered pigs for IAV host resistance as a strategy to achieve improvement in pig herds.

Key Words: influenza, sialic acid, ST6GAL1, CRISPR/Cas9, pig

OP130 Programmable base editing of the goat and sheep genomes for genetic improvement. S. Zhou[1], G. Li[1], B. Cai[1], C. Li[1], B. Ma[1], H. Yu[2], B. Petersen[3], T. Sonstegard[4], X. Huang[5], Y. Chen[1], and X. Wang*[1], *[1]Northwest A&F University, Yangling, Xianyang, China, [2]Guilin Medical University, Guilin, China, [3]Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt, Germany, [4]Recombinetics, Saint Paul, MN, USA, [5]School of Life Science and Technology, ShanghaiTech University, Shanghai, China.*

The ability to alter single bases without homology directed repair (HDR) of double-strand breaks provides a potential solution for editing livestock genomes for economic traits, which are often multigenic. Progress toward multiplex editing in large animals has been hampered by the costly inefficiencies of HDR via microinjection of in vitro manipulated embryos. Herein, we report our success in inducing base pair changes in the genomes of 2 important livestock species, goats and sheep. We designed sgRNAs to induce nonsense codons (C-to-T transitions) in the goat *FGF5* gene, to produce animals with longer hair fibers. Five goat kids produced from Base Editor 3 (BE3) microinjected single-cell embryos had alleles with a targeted nonsense mutation. The phenotypic effects on hair fiber were characterized by H&E and immunofluorescence staining, and Western blotting. Differences in morphology were detectable, even though mosaicism was probably affecting the levels of FGF5 expression. PCR amplicon and whole genome resequencing analyses for off-target changes caused by BE3 were low at a genome-wide scale. In sheep, we targeted the p.96R > C variant in the *SOCS2* gene, which has profound effects on body weight, body size, and milk production in sheep. We obtained lambs with defined point mutations by the co-injection of BE3 mRNA and a single guide RNA (sgRNA) into zygotes. Observations of body size and body weight in the edited group showed enhanced growth. Moreover, targeted deep sequencing and unbiased family trio-based whole-genome sequencing revealed undetectable off-target mutations in edited animals. This is the first evidence of base editing in large mammals produced from microinjected single cell embryos. Our results support further optimization of BEs for introgressing complex human disease alleles into large animal models, and to evaluate potential genetic improvement of complex health and production traits in livestock.

Key Words: base editing, genome editing, SNP, off-target, whole-genome sequencing

OP131 Trio-based deep sequencing reveals rare off-target mutations in Cas9-edited animals and their offspring. C. Li[1], S. Zhou[1], Y. Jiang[1], B. Ma[1], T. Sonstegard[2], X. Huang[3], B. Petersen[4], Y. Chen[1], and X. Wang*[1], *[1]Northwest A&F University, Yangling, China, [2]Recombinetics, Saint Paul, MN, USA, [3]School of Life Science and Technology, ShanghaiTech University, Shanghai, China, [4]Institute of Farm Animal Genetics, FLI, Neustadt, Germany.*

Unintended off-target mutations induced by CRISPR/Cas9 nucleases may result in unwanted consequences, and could possibly raise regulatory concerns over the use of this technology for genetic improvement. Here we took advantage of our previously generated gene-edited sheep and performed family trio-based whole-genome sequencing (WGS) which is capable of discriminating variants in the edited progenies that are inherited, naturally generated, or induced by genetic modification. Three family trios were re-sequenced at a high average depth of genomic coverage (~25.8 ×). After developing a pipeline to comprehensively analyze the sequence data for de novo single nucleotide variants, indels and structural variations from the genome; we only found a single unintended event in the form of a 2.4 kb inversion induced by site-specific double-strand breaks between 2 sgRNA targeting sites at the *MSTN* locus with a low incidence. Moreover, we generated offspring from edited goats we obtained previously, and sequenced the members of 4 family trios (gene-edited goats and their offspring) to ~36.8 × coverage. Our results revealed that the incidence of de novo mutations in the offspring was equivalent to normal populations. We further conducted RNA sequencing using muscle and skin tissues from the offspring and control animals, the differentially expressed genes (DEGs) were related to muscle fiber development in muscles of *MSTN*-edited animals, skin development and immune responses in skin tissues of *FGF5*-edited animals. Taken together, the result of prediction and verification of off-targets sites in edited animals and their offspring revealed almost negligible risk for off-target mutations. Our work provides adequate molecular evidence to support the reliability of conducting Cas9-mediated genome editing in large animal models for biomedicine and agriculture.

Key Words: genome editing, CRISPR/Cas9, whole-genome sequencing, off-target, de novo mutation

OP132 Efforts to produce genetically edited goats that carry the ovine callipyge mutation. T. Hadfield*[1], M. Regouski[1], C. Reichhardt[1], C. Bidwell[3], K. Thornton[1], S. Fahrenkrug[2], D. Webster[2], D. F. Carlson[2], I. A. Polejaeva[1], and N. Cockett[1], *[1]Utah State University, Logan, UT, USA, [2]Recombinetics Inc, St. Paul, MN, USA, [3]Purdue University, West Lafayette, IN, USA.*

Goat meat is a major source of protein for people in developing countries. Given that goats have a large impact on the socio-economic status of people in these countries, goats with enhanced meat production would be beneficial. The callipyge (CLPG) mutation in sheep results in muscle hypertrophy of fast twitch glycolytic muscle fibers without requiring any additional feed or care. The goal of this project is to introduce the CLPG mutation into goats using gene editing. The CLPG mutation is a single A-to G nucleotide substitution located in the intergenic region of 2 imprinted genes, DLK1 and MEG3. It is well established that sheep inheriting the CLPG mutation from their sire and the wildtype allele from their dam have 30% more muscling in the longissumus dorsi and gluteus medius muscles. Using the TALEN gene editing approach, cell colonies that were positive for the ovine CLPG mutation were derived from the fetal cells of a male Spanish goat. Two cloned males were produced from the positive cells in 2016 and bred to wild type (wt) female Spanish goats. PCR-RFLP testing revealed only one of the resulting 8 offspring inherited the CLPG allele and none of the offspring exhibited the expected phenotype. Because all 8 offspring were expected to carry the mutation, 10.9 kb of sequence surrounding the CLPG allele in the 2 male clones were analyzed. One chromosome contained the CLPG mutation but the other chromosome had a 1500-bp deletion flanking the CLPG mutation. Using PCR with primers that flank the deletion and PCR-RFLP that differentiates between the wild-type (+) and CLPG alleles, we determined that 7 of the 8 offspring were del/+ and one was CLPG/+ (the offspring previously identified). In 2018, we bred the original 2 male clones to wt female Spanish goats which produced 11 CLPG/+ and 16 del/+, but none developed pronounced muscling. Breeding across all genotypes was conducted in Fall 2018 with anticipated births in Spring 2019. Characterization of the muscle fiber type properties in the wt goat will provide essential information needed to better understand the mechanism of the muscle hypertrophy in goats.

Key Words: goats, callipyge, gene editing

OP133 Generation of pigs with a Belgian Blue mutation in *MSTN* using CRISPR/Cpf1-assisted ssODN-mediated homologous recombination. Y. Zou[1,2], Z. Li[2], Y. Zou[3], H. Hao[2], J. Hu[2], N. Li[2], and Q. Li*[2], *[1]State Key Laboratory of Silkworm Genome Biology, Key Laboratory of Sericultural Biology and Genetic Breeding, Ministry of Agriculture, College of Biotechnology, Southwest University, Chongqing China, [2]State Key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing China, [3]College of Veterinary Medicine, China Agricultural University, Beijing China.*

CRISPR/Cpf1 has emerged recently as an effective tool for genome editing in many organisms, but its use in pigs to generate precise genetic modifications has seldom been described. Myostatin (MSTN) is a well-characterized negative regulator of muscle development, and natural mutations in this gene cause a double-muscled phenotype in many species. However, to the best of our knowledge, no naturally occurring mutation in *MSTN* has been found in pigs. In addition, no living pig models with sophisticated modifications orthologous to natural mutations in *MSTN* have yet been reported. In this study, we exploited the CRISPR/Cpf1 system to introduce a predefined modification orthologous to the natural *MSTN* mutation found in Belgian Blue cattle (thus known as the Belgian Blue mutation). We found that the cutting efficiency of CRISPR/Cpf1 was 12.3% in mixed porcine fetal fibroblasts in drug free medium, and 41.7% in clonal colonies obtained using G418 selection. We co-transfected the Cpf1-sgRNA vector, ssODN template, and a self-excision cassette into porcine fetal fibroblasts. After G418 selection, we examined 8 clonal colonies and found that 5 were genetically modified. Of these 5, 2 harbored the precise 11 bp deletion. Using one heterozygous clonal colony, we successfully generated 2 cloned Duroc piglets that were heterozygous for the Belgian Blue mutation. In summary, our results demonstrate that CRISPR/Cpf1 system can be used efficiently to generate double-stranded breaks, and also to mediate homologous recombination to introduce precise genomic modifications in pigs.

Key Words: *MSTN*, CRISPR/Cpf1, Belgian Blue mutation, genetically modified pig, single stranded oligodeoxynucleotide

OP134 Transgene insertion site mapping in a goat TGF-β biomedical model of atrial fibrillation. A. J. Thomas[1,2], K. P. Morgado[1,2], M. Regouski[2], R. Ranjan[3], I. A. Polejaeva[2], and C. J. Davies*[1,2], *[1]Center for Integrated BioSystems, Utah State University, Logan, UT, USA, [2]Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA, [3]Department of Internal Medicine, Division of Cardiovascular Medicine, University of Utah, Salt Lake City, UT, USA.*

A transgenic goat model of atrial fibrillation, the most common sustained human cardiac arrhythmia, was created by random insertion of a construct containing either a mouse or goat α-Myosin Heavy Chain promoter driving cardiac expression of a constitutively active human TGF-β1 gene. Breeding of founder goats was used to produce progeny carrying the transgene. Transgenic goats produced by random insertion can have more than one integration site. Consequently, it is important to identify the transgene integration site(s) in founder goats and track segregation of the transgene in their offspring. To identify the transgene insertion sites: sequencing libraries were constructed from genomic DNA of 6 somatic cell nuclear transfer founder goats and 7 of their offspring, subjected to target selection using RNA baits made from the transgene construct, and sequenced on an Illumina MiSeq sequencer. Sequence reads were aligned to the goat genome using the viral integration module of the DNAStar SeqMan NGen alignment program. Alignment files were visually scanned to identify transgene integration sites. True integration sites were identified by alignment of multiple sequences from the library comprised of both goat genomic sequence and plasmid sequence. Four lines of TGF-β1 goats were produced (TGFB:12, 16/18, F1 & G1/G2). Two of these lines (TGFB:16/18 & G1/G2) had multiple founder animals, derived from the same fibroblast colony, with identical insertion sites. The TGFB:G1/G2 line had 6 founder goats with the same TGF-β insertion sites: 2 on chromosome 4 (Chr4) and one in a repeat element on Chr21. The Chr4 and Chr21 insertions segregate independently in the offspring from this line. Polymerase chain reaction (PCR) primers flanking each integration site were designed and validated. These primers were used to track segregation of the transgenes in 67 descendants of the founder goats. One descendant was not transgenic while the other 66 inherited one or 2 chromosomes with transgene integration sites. This study demonstrates an effective method for mapping transgenes inserted by random insertion or finding off-target insertion events when targeted insertion methods are used.

Key Words: goats and related species, transgenics, sequence capture, high-throughput sequencing, biomedical model

OP135 Loss of GHR in GHR knockout pig model leads to hepatic steatosis via PPARa signaling. Q. Han, H. Chen, and R. Zhang*, *China Agricultural University, Beijing, China.*

Laron syndrome is a rare genetic disease inherited in an autosomal recessive manner and featured with insensitivity to GH. The disorder, which is characterized by obesity, dwarf, possibly hypolipidemia, fatty liver and cardiovascular disease,is caused by mutations of the gene encoding the corresponding growth hormone receptor (GHR). Previous studies of Laron syndrome are conducted using GHR mutant mouse model that showed some differences in lipid metabolism from humans. Here we used pigs that are genetically, anatomical, and physiological more relevant to humans compared with mice to study the relationship between GHR mutations and lipid metabolism. We found that GHRKO pigs have obvious dwarfism phenotypes, dyslipidemia, increased triglyceride content in liver and hepatic steatosis. We then performed RNA-seq analysis, followd by experimental verification to explain the observed phenotypes in GHRKO pigs. We found that GHRKO pigs exhibited downregulation in hepatic fatty acid β-oxidation, PPARA signaling pathway, and fatty acid degradation pathway. Therefore, we conclude that GHR mutations lead to reduced fatty acid oxidation in the liver and increased lipid accumulation by downregulating the PPARA signaling pathway, ultimately leading to hepatic steatosis. This study suggested a new therapeutic target for the treatment of lipid metabolism diseases caused by GHR mutation.

Key Words: GHR, hepatic steatosis, knockout pig model, PPARA

OP136 Transgenic chickens generating through targeting PGC with antibody-directed lentiviral. Z. Jiang*[1,2], H. Wu[1,2], J. Tian[1,2], and X. Hu[1,2], *[1]College of Biological Sciences, China Agricultural University, Beijing, China, [2]State Key Laboratory of Agro-biotechnology, China Agricultural University, Beijing, China.*

Recent advances in avian transgenesis studies highlight the possibility of utilizing the lentiviral vectors as a gene transduction tool in both experimental and clinical settings that require long-term transgene expression. However, both high gonadal chimerism rate and passage efficiency are necessary for transgenic research, which stand in the way for the broad usage of lentiviral vectors. In this work, we implemented a simple strategy retargeting lentiviral vectors to primordial germ cells (PGCs) through in vitro covalent modification of the virions using specific cell targeting proteins. The lentiviral vectors pseudotyped with a modified chimeric Sindbis virus envelope (termed m168), which conjugated with antibody specific for PGCs membrane protein, were used in our study. Conditions to optimize the efficiency of gene delivery were established using the different virus packaging system and SSEA4 antibody as the cell surface target, which was associated with primordial germ cell phenotypes. We found that the m168 pseudotyped lentiviral of 3-vectors system have higher p24 and better transduction levels than the m168 pseudotyped lentiviral of 4-vectors system. By varying the proportion of antibody, conjugation of p24 (1μg) viral particles with 1μg anti-HLA or anti-SSEA4 would result in targeted gene transfer into 293T or PGCs. Furthermore, SSEA4 mediated M168 pseudotyped lentiviral vectors have higher targeting and infectious than vesicular stomatitis virus G (VSV-G) pseudotyped for the PGCs infection. Here,

we reported successful targeting in chicken gonads through sub-embryonic injection of a lentiviral vector pseudotyped with m168 and it may provide a potentional strategy to improve the gonad chimeric and passage efficiency. Further improvement of this targeting technology will promote applications not only in transgenic but also genetic and medical fields.

Key Words: M168 pseudotyped lentiviral, primordial germ cell, targeted infection, transgenic chickens, SSEA4

OP137 In vitro transfection of chicken primordial germ cells and generation of cell-chimeric chicken using the Sleeping Beauty transposon system. S. Altgilbers*, S. Klein, W. A. Kues, and S. Weigend, *Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics, Mariensee, Neustadt, Germany.*

Primordial germ cells (PGCs) are the precursors of the gametes and pass genetic information from one generation to the next. In this study, we established a stable transfected PGC cell line using the Sleeping Beauty transposase in combination with a ubiquitously expressed Venus reporter transposon plasmid. After transfection, the PGCs were enriched using fluorescence activated cell sorting of Venus expressing cells. We performed microinjection of the modified PGCs into eggs at 2.5 d of development. 3000 PGCs per embryo were injected into the blood vessel and 76 treated recipients were incubated until hatching. We were able to hatch 14 potential cell chimeric male chicks, which are raised to maturity for sperm testing. A total of 16 female chicks and the embryos, which died during the experiment, were used to estimate the gonadal transfection efficiency. The gonads were isolated and the fluorescence signal of the Venus reporter was detected in 81% of the 16 hatched chicks. Overall, transfected primordial germ cells were found in 89% of 46 investigated gonads. The fluorescence microscopy data were verified by Venus-specific PCR. The maintained primordial germ cell status of the Venus-positive cells in the gonads was verified by co-staining with the SSEA1 surface marker. The results demonstrate that the in vitro transfected primordial germ cells were able to colonize the gonads. The male chicks will be grown up to maturity, and the proportion of spermatozoa derived from the injected PGCs will be determined by FACS and PCR. Based on the preliminary gonad colonization data, we believe that the Sleeping Beauty transposon system is an efficient method for the production of cell-chimeric chicken, and that it will allow germline transmission of desired genetic modifications.

Key Words: poultry and related species, cell culture, genetic engineering

OP138 Genome modification in chicken for therapeutic protein production. Y. M. Kim*, Y. H. Park, J. M. Kim, J. S. Park, H. J. Lee, K. Y. Lee, and J. Y. Han, *Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea.*

Genome modification technology has been provided numerous opportunities for basic research and industry. Recent progress in genome modification technology such as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) system combined with primordial germ cell (PGC)-mediated germline transmission system allows more potential applications in genome modified poultry for diverse purposes. Especially, the practical use of these techniques was successfully adopted in producing genome edited chickens for application of industrial field including bioreactor system. Since the relatively short generation time, sufficient reproductive capacity and high egg production characteristics, chicken has been considered as the most efficient animal bioreactor for recombinant protein production. In addition, a half of the chicken egg white proteins was translated from the single egg white gene, *Ovalbumin*, the mass production of functional proteins in eggs has been highly expected. In this regards, we have applied the modified egg white promoter for development of an efficient chicken bioreactor system. As results, the transgene was successfully integrated chicken genome, and the products derived from genome modified chicken showed consistent expression level and higher efficacy compared with commercial counterpart. The results suggest that chicken bioreactor developed by genome modification technologies could be an alternative system for producing human therapeutic proteins.

Key Words: poultry and related species, genetic engineering, transgenics, biomedical model

ISAG-FAO Genetic Diversity

OP139 Invited Workshop Presentation: Towards a sustainable management of farm animal genetic diversity in the era of whole-genome data: The case of sheep and goats. F. Pampanon*[1] and B. Benjelloun[2], *[1]Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, Grenoble, France, [2]Institut National de la Recherche Agronomique Maroc (INRA-Maroc), Morocco.*

Characterizing farm animal genetic diversity is a major challenge for developing sustainable breeding strategies and conservation actions in the context of global change. As the ability of farm animals to face environmental changes relies on their diversity and phenotypic plasticity, we need to understand the genetic and epigenetic bases of adaptation to climatic extremes to predict livestock resilience and conserve genomic resources. By developing population genomics and landscape genomics approaches based on whole genome analyses of wild and domestic sheep and goats, our research aims at deciphering the evolutionary mechanisms responsible for the emergence of domestic breeds and their local adaptation to extreme environments. Based on recent results that we obtained, we will illustrate: (i) the discovery of genes involved in shaping domestic breeds during early domestication and further improvements; (ii) the variation of adaptive genetic diversity along environmental gradients in indigenous breeds from Northern Africa; (iii) the possible role of gene regulation through epigenetic marks to face environmental stresses; (iv) the evaluation of whole genome genotyping strategies to assess the genome diversity of livestock (e.g., SNP arrays vs whole genome sequences at various sequencing depth). We will discuss the impact of such results for the definition, conservation and valorisation of operational farm animal genomic resources.

OP140 Genetic characterization of cattle populations for optimized performance in African ecosystems. R. Crooijmans*[1], N. Ghanem[2], C. Ginja[3], D. Kugonza[4], L. Makgahlela[5], and J. Kantanen[6], *[1]Animal Breeding and Genomics, Wageningen University and Research, Wageningen, the Netherlands, [2]Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt, [3]Centro de Investigacão em Biodiversidade e Recursos Genéticos, University of Porto, Porto, Portugal, [4]Animal breeding and Genetics, Makerere University, Kampala, Uganda, [5]Animal breeding and Genetics, Agricultural Research Council, Pretoria, South Africa, [6]Natural Resources Institute Finland, Jokioinen, Finland.*

Traditional local cattle breeds are under severe pressure of extinction worldwide due to their low production performance compared with commercial breeds, as well as new legislations. Breed replacement or crossbreeding with commercial transboundary cattle is a threat to these native breeds. One very big advantage of native cattle breeds is the high adaptation to the ecosystems they have lived in. But how did such adaptation shape the genome of these animals? Adaptation to an ecosystem is already a collection of traits which result in resilient animals that survived in time. There are several stressors that have influenced adaptation of ecotypes such as climate (change), heat, diseases,

availability of feed and water. Within this project we defined standardized protocols (SOPs) for phenotype collection in 6 different countries (Finland, The Netherlands, Portugal, Egypt, Uganda and South Africa) on traditional cattle breeds. The derived phenotypes are used in combination with whole genome sequence data of the same animals to try to find association to different ecotypes. Over 700 animals will be re-sequenced. Meanwhile, to promote communication, information exchange and outreach, an App has been developed where farmers can submit animal records and also access the database to get information/reports on their animals and/or breed. In addition to selective sweep analysis, an inventory on diversity will be obtained, in particular concerning patterns of admixture and the degree of inbreeding.

Key Words: cattle, traditional, ecosystem, adaptation, WGS

OP141 Towards a complete genomic characterization of African indigenous cattle. A. Tijjani*[1,3], K. Marshal[2,3], H. Kim[4,5], H. Jianlin[2,6], and O. Hanotte[7,8], *[1]International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [2]International livestock Research Institute (ILRI), Nairobi, Kenya, [3]Center for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Edinburgh, UK, [4]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea, [5]C&K genomics, Seoul National University Research Park, Seoul, Republic of Korea, [6]ILRI-CAAS Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, [7]LiveGene–CTLGH, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [8]Cells, Organism and Molecular Genetics, School of Life Sciences, University of Nottingham, Nottingham, UK.*

Genetic and genomic variations in African indigenous livestock populations remain huge resources, yet to be tapped. These resources are prerequisite for a complete characterization of local breeds toward genetic improvement contributing to food security and poverty reduction on the continent. The International Livestock Research Institute (ILRI) is leading an unprecedented research effort aiming at harnessing the genetic resources of indigenous cattle breeds across Africa. This is being achieved through an extensive collaboration of African national partners together with ILRI Livestock Genetics program, supported by the University of Nottingham (UK), the ILRI - CAAS Joint Laboratory on Livestock and Forage Genetic Resources (China), Seoul National University (South Korea), and the Centre for Tropical Livestock Genetics and Health (CTLGH) program based at the University of Edinburgh and SRUC (Roslin Institute, UK). So far, samples have been obtained from around 45 cattle breeds of zebu, taurine and admixed origins and comprising of over 1,500 individuals. High coverage (up to 30x) of whole genome re-sequencing of more than 25 breeds consisting of over 250 samples have now been completed. We aim to catalog the entire functional diversity, including SNPs and structural variations, of African cattle. Our current objectives include the identifications of the most informative SNPs for designing the African cattle reference SNP genotyping arrays to be applied to breeding improvement programs and the genomic regions underpinning adaptation and productivity, as well as the capacity building among African collaborating scientists through training in bioinformatics and genomics. Future activities may include de novo genome sequencing and assembly of African cattle breeds and a transcriptome catalog of gene expression, paving the way to the pan-genome analyses of African cattle.

Key Words: African cattle, functional diversity, adaptation, genomic characterization, SNPs

OP142 Genomic diversity and differentiation of Iberian native cattle. R. da Fonseca[1,2], I. Ureña[3], S. Afonso[3], A. E. Pires[3,4], E. Jørsboe[2], L. Chikhi[5,6], and C. Ginja*[3], *[1]Center for Macroecology, Evolution and Climate, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark, [2]The Bioinformatics Centre, Department of Biology, University of Copenhagen, Copenhagen, Denmark, [3]CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Vairão, Portugal, [4]LARC, Laboratório de Arqueociências, Direcção Geral do Património Cultural, Lisboa, Portugal, [5]Laboratoire Évolution et Diversité Biologique, Université de Toulouse Midi-Pyrénées, CNRS, Toulouse, France, [6]Instituto Gulbenkian de Ciência, Oeiras, Portugal.*

The complex origin of Iberian primitive breeds is reflected in their high genetic diversity relative to their European counterparts despite the geographic distance of this territory from the presumed Near-Eastern domestication center. This renders Iberian livestock a great example for investigating the genomic impact of the intricate processes of diversification regarding the last 200 years of specific breed formation and earlier admixture events. We sequenced 48 genomes, to between 1.4X and 2.3X depth of coverage, belonging to 8 breeds of Iberian native cattle, namely the Portuguese breeds Alentejana, Arouquesa, Barrosã, Brava de Lide, Maronesa, Mertolenga, Mirandesa and Preta. We described genome-wide diversity and introgression in Iberian breeds in relation to 60 previously published taurine (*Bos taurus*) and zebu (*B. indicus*) cattle genomes from Europe, Africa and Asia, and sequence data from one European aurochs (*B. primigenius*). We confirm that there is a clear genetic distinction among Iberian cattle breeds, with 8 well-defined clusters using NGSadmix, and observed high levels of breed differentiation (average F_{ST} 9%). The principal components analyses showed the high differentiation of Mirandesa and Brava due to genetic drift as a result of drastic demographic changes, i.e., a significant reduction in population size and reproductive isolation related to semi-feral breeding conditions, respectively. *D*-statistics suggest significant gene flow or admixture from African taurine cattle in all 8 breeds. Also, these Iberian cattle included distinct mtDNA and Y-chromosome haplotypes from multiple origins. We found no evidence of indicine introgression or recent admixture with transboundary commercial breeds in Iberian native cattle. We demonstrate that breed management and associated demographic processes had profound effects on genomic diversity and resulted in unusual patterns of genetic differentiation for autosomes versus sex chromosomes. Our results indicate that genetic differentiation measured using chromosome X might be more representative of the native populations of domesticated cattle, and that comparisons between breeds using autosomal data might be misleading without an appropriate demographic model.

Key Words: Iberian primitive cattle, genome sequencing, breed diversity, population genomics, introgression

OP143 Integrating in situ and ex situ genomic data of domestic chicken breeds for conservation in China. M. Zhang* and K. Wu, *China Agricultural University, Beijing, China.*

The effective conservation and use of farm animal especially for chicken are vital for creating and maintaining sustainable increases in the productivity of healthy food for mankind. In situ and ex-situ conservation are 2 main protection strategies for Chinese domestic chicken breeds. However, few studies have compared the genomic diversity and population structure of conserved chicken between ex-situ and in situ populations in China. In the present study, a total of 361 individuals including 3 Chinese domestic chicken breeds collected from in situ and ex-situ populations were genotyped using genotyping-by-sequencing (GBS) to compare the genetic diversity and population structure. After sequence alignment, variant calling and quality control (QC), 1539911 high-quality SNPs were obtained for subsequent analysis. No matter in situ or ex-situ population, all 3 chicken breeds maintained relatively high genomic diversity in terms of heterozygosity (Ho, He), the proportion of polymorphic markers (P_N), allelic richness (A_R) and inbreeding coefficient based on runs of homozygosity (F_{ROH}). Population structure of the 3 native chicken breeds was analyzed using principal component analysis (PCA), neighbor-joining (NJ) tree, and STRUCTURE analysis, the results suggested that population stratification had occurred in different conserved populations. All F_{ST} values between in situ and ex-situ within a breed were higher than 0.05 (from 0.05 to 0.1), indicating that moderate genetic differentiation appeared. The Ne of the in situ population was higher than the ex-situ, for instance, the average Ne (for

gga1–5) was 129.44 in YBEC (in situ), while only 50.34 was calculated in BEC15 (ex-situ). We concluded that being small and maintained in controlled environments, ex-situ conserved chicken populations would retain less genetic diversity than in situ. This study brought new insight into conservation genomics used in the actual conserved chicken population and provided the scientific basis for further optimizing the conservation programs in in situ and ex-situ population of Chinese domestic chicken breeds.

Key Words: Chinese domestic chicken, genomic diversity, conservation, in situ, ex situ

OP144 Investigating introgression of river-buffalo loci in the genome of Brazilian Carabao swamp buffaloes. M. Barbato[1], L. Colli[1], M. Milanesi[2,3], Y. T. Utsunomiya[2,3], J. R. V. Herrera[4], L. Cruz[4], P. Baruselli[5], M. M. J. Amaral[6], M. G. Drummond[7], J. F. Garcia[2,3], J. L. W. Williams[8], International Buffalo Consortium[8], and P. Ajmone-Marsan*[1], *[1]Department of Animal Science, Food and Nutrition - DIANA, Università Cattolica del S. Cuore, Piacenza, Italy, [2]Universidade Estadual Paulista "Júlio de Mesquita Filho," Faculdade de Medicina Veterinária de Araçatuba, Araçatuba, Brazil, [3]International Atomic Energy Agency (IAEA), Colaborating Centre on Animal Genomics and Bioinformatics, Araçatuba, Brazil, [4]Philippine Carabao Centre, Nueva Ecija, Philippines, [5]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil, [6]Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, São José do Rio Preto, Brazil, [7]R&D Department, Myleus, Belo Horizonte, Brazil, [8]The Davies Research Centre, School of Animal and Veterinary Science, University of Adelaide, Roseworthy, SA, Australia.*

Water buffalo was domesticated about 5,000–6,000 years ago and since then has become one of the most important livestock species in tropical and sub-tropical environments providing labor, meat and dairy products. There are 2 water buffalo species, swamp and river buffalo, which differ in morphology, production attitudes, geographic range and chromosome number. Despite the difference in chromosome number, these 2 subspecies are inter-fertile and the river type is sometimes used to upgrade milk production of the swamp type. Here we investigated admixture and local ancestry of 2 populations: Brazilian Carabao, which were established hundreds of years ago in Brazil, and the recently created Philippine cross bred population. Data were produced using the 90K buffalo SNPs array (Axiom Buffalo Genotyping Array – ThermoFisher scientific). In addition, worldwide pure river and swamp breeds were genotyped and used as reference. We identified shorter river type-derived haplotypes in Carabao compared with those found in the Philippine population, confirming an older event of admixture. Several genomic regions appeared to be conserved among Carabao individuals, which are putatively associated with adaptation and production traits. Local ancestry analysis revealed preferential introgression of river loci into the swamp genome in regions carrying genes related to dairy production and fertility.

Key Words: river buffalo, swamp buffalo, introgression, admixture

OP145 Y-chromosomal haplotyping in horses—New achievements and future potentials. S. Felkel, V. Dobretsberger, D. Rigler, C. Vogl, G. Brem, and B. Wallner*, *University of Veterinary Medicine Vienna, Vienna, Austria.*

Polymorphic markers on the male-specific region of the Y chromosome (MSY) are widely used to infer historic demography. Because of its lack of recombination and the strictly male specific inheritance, the MSY perfectly mirrors male genealogies. However, the low sequence variation on the horse MSY impeded fine-grained haplotyping in this species. We recently established a workflow to predict the regions on the highly repetitive Y-chromosome that are suitable for unambiguous variant calling from NGS data. We generated a MSY haplotype tree based on biallelic variants ascertained from more than 200 horses on about 6 MB of the MSY. This robust MSY topology served as a backbone for a haplotype determination in a comprehensive data set. By linking MSY haplotypes and pedigree information, we nailed down the MSY haplotypes of several influential founder stallions – ranging from the Thoroughbreds, to Arabian, Spanish and some coldblooded sires. We show the potential of MSY haplotyping to trace the influence of particular sires within and across populations and give examples how MSY haplotyping can be revealing to illuminate the development and origin of horses by studying rural populations and historic/ancient remains. Our effort brings the accuracy of Y-chromosomal lineage tracing in horses similar to that in humans and therefore sire line screening could be implemented for forensic applications.

Key Words: horses and related species, phylogeny, sequence variation, Y chromosome, forensics

OP146 Introgression with domestic goats has expanded the genetic variability of the Spanish ibex. T. Figueiredo-Cardoso[1], R. Tonda[2], M. G. Luigi-Sierra[1], A. Castelló[1,3], B. Cabrera[1,3], A. Noce[1], S. Beltrán[2], R. García-González[4], A. Fernández-Arias[5], J. Folch[6], A. Sánchez[1,3], A. Clop[1], and M. Amills*[1,3], *[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [2]Centre Nacional d'Anàlisi Genòmica-Centre for Genomic Regulation (CRG), Barcelona, Barcelona, Spain, [3]Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [4]Instituto Pirenaico de Ecología (IPE-CSIC), Spain, [5]Servicio de Investigación Agroalimentaria, Spain, [6]Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Zaragoza, Spain.*

The Spanish ibex (*Capra pyrenaica*) is a wild goat species distributed in the Iberian Peninsula. Based on phenotypic criteria, 4 subspecies have been defined: *C. p. hispanica* (CPH, south and east of the Iberian Peninsula), *C. p. victoriae* (CPV, center and northwest of the Iberian Peninsula), *C. p. lusitanica* (CPL, Galicia and north of Portugal) and *C. p. pyrenaica* (CPP, Pyrenees mountains). Hunting, epidemics and habitat loss caused the extinction of CPL (disappeared in the 19th century) and CPP (extinct in the year 2000) as well as severe population bottlenecks decreasing the diversity of CPV and CPH. By using a high throughput genotyping approach, we have demonstrated that interspecific hybridization with domestic goats has been an important source of novel variability for Spanish ibexes living in Tortosa-Beceite. Individual sequencing of one of the last CPP representatives (× 16.6 coverage) and Pool-sequencing (× 39 coverage) of 30 CPH and 23 CPV individuals revealed an extensive sharing of SNPs (96%) between the CPP individual and the extant CPV and CPH subspecies, thus suggesting that the extinction of CPP did not cause a major loss of diversity in *Capra pyrenaica*. Sequencing experiments also revealed that the genome of one of the last CPP representatives contains stop-gained mutations, with heterozygous genotypes, in the WASF2, RBM17 and SERPINB10 genes. The inactivation of WASF2 and RBM17 causes embryonic lethality, while SERPINB10 belongs to a family of serin proteases with key roles in immunity and other biological processes. Our results suggest that the dramatic reduction of the CPP population during the19th-20th centuries led to the progressive accumulation of mutations with harmful effects (genomic meltdown) that probably contributed to its extinction by limiting fitness and reproductive success.

Key Words: conservation, hybridization, goats and related species

OP147 Unraveling the genomic diversity and population structure of 20 autochthonous European pig breeds. M. Muñoz*[1], R. Bozzi[2], J. M. García-Casco[1], Y. Núñez[1], A. Ribani[3], M. Škrlep[4], R. Quintanilla[5], M. J. Mercat[6], J. Riquet[7], J. Estellé[8], M. Candek-Potokar[4], A. I. Fernández[1], L. Fontanesi[3], and C. Óvilo[1], *[1]Departamento Mejora Genética Animal, INIA, Madrid, Spain, [2]DAGRI, Animal Science Section, Università degli Studi di Firenze, Firenze, Italy, [3]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [4]Kmetijski inštitut Slovenije, Ljubljana, Slovenia, [5]IRTA, Programa de Genética y Mejora Animal, Barcelona, Spain, [6]IFIP – Institut du Porc, Le Rheu, France, [7]INRA, Génétique Physiologie et Système*

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European autochthonous pig breeds are very relevant as a local resource. They have common characteristics as good environmental adaptation, high adipogenic potential, rusticity and excellent meat quality products. However, some of them are in great danger of becoming extinct and development of conservation policies and promotion of their sustainable use are needed. One key factor for the conservation of these breeds consists in their genetic characterization. In the current study, we assessed the genomic diversity of 20 European local pig breeds (Alentejana, Apulo-Calabrese, Basque, Bísara, Majorcan Black, Black Slavonian, Casertana, Cinta Senese, Gascon, Iberian, Krs?kopolje, Lithuanian indigenous wattle, Lithuanian White Old Type, Mora Romagnola, Moravka, Nero Siciliano, Sarda, Schwäbisch-Hällisches Schwein, Swallow-Bellied Mangalitsa and Turopolje) from 7 countries (Croatia, France, Germany, Italy, Lithuania, Portugal, Servia, Slovenia, Spain) and wild pigs sampled in the Iberian Peninsula. A total of 992 DNA samples were genotyped with GGP Porcine HD Genomic Profiler v1 chip (68,528 SNPs). Different SNP sets were used according to the analyses performed to compute: genetic diversity parameters, genetic distances, population structure, linkage disequilibrium and effective sample size. The results revealed that several breeds as Apulo Calabrese, Casertana, Mora Romagnola and Turopolje have low genetic diversity exhibiting low heterozygosity and very small population effective size, therefore, strategies of conservation should be applied in these breeds. Principal component analyses showed individuals belonging to the same breeds cluster together with overlapping between several breeds, particularly marked for Iberian and Alentejana breeds, which suggests genetic closeness. In addition, few breeds were clearly isolated from the rest. These findings provide relevant information for the implementation of further conservation and selection strategies. This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 634476. The content of this abstract reflects only the author's view and the European Union Agency is not responsible for any use that may be made of the information it contains.

Key Words: genomic diversity, pig breeds, SNP chip, conservation

OP148 IMAGE: Innovative Management of Animal Genetic Resources. O. Cortes*[1], L. T. Gama[2], S. Dunner[1], IMAGE Consortium[3], and M. Tixier-Boichard[3], *[1]Veterinary Faculty. University Complutense of Madrid, Madrid. Spain, [2]CIISA, Faculdade de Medicina Veterinaria, Universidade de Lisboa, Lisboa. Portugal, [3]INRA, France.*

IMAGE is a project funded by the Horizon 2020 Research and Innovation Programme of the European Union. The ultimate goal of the project is to demonstrate the benefits brought by gene banks to the development of more sustainable livestock farming systems. Thirteen EU countries are involved, together with Switzerland and 4 non-European countries (Argentina, Colombia, Egypt and Morocco), in a project that unites 28 partners including 3 SMEs, 3 NGOs, FAO, 9 research institutions, 11 higher education and research, and INRA Transfert. Image has still one year to go (2016–2020) and significant progress has been achieved on innovative approaches for animal gene banks. The IMAGE Dialogue Forum, a platform designed to involve all stakeholders in the discussion about the future of genetic management of animal gene banks, has covered important topics such as sanitary regulations, data/material sharing, ethics and standardization of gene bank management in the 3 sessions celebrated so far, encompassing more than 50 participants from different origins. IMAGE surveys have obtained a detailed information about the diversity of germplasm and genomic collections across Europe from 61 organizations representing 20 different countries. Improvement of cryopreservation of germplasm, strategies toward breed conservation using genomic tools, genomic selection or introgression of specific characteristics into another breed are some of the topics of the papers already published with 15 articles in peer-review journals and 2 book chapters based on avian, horse, pigs, goats and cows species. New sequencing data are being produced that will significantly improve the knowledge and attractiveness of gene bank collections for research and breeding. Three post-graduate courses were organized in Argentina, Colombia and Netherlands which gathered students from Europe, America and Africa The presence of the IMAGE project in social-media is wide and diverse. It has a youtube channel, and is represented in twitter (@imageh2020) and periodically the projects news are disseminated through Newsletters fully available, as all the documentation of the project, in the webpage (www.imageh2020.eu).

Key Words: H2020, gene banks, genomic, livestock

OP149 Sequencing of reindeer (*Rangifer tarandus*) genomes: Insights into evolution, domestication, and adaptation. M. Weldenegodguad[1,2], K. Pokharel[1], Y. Ming[3], M. Honkatukia[1,4], J. Peippo[1], T. Reilas[1], K. H. Røed[5], and J. Kantanen*[1], *[1]Natural Resources Institute Finland, Jokioinen, Finland, [2]Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland, [3]BGI-Tech, Guangdong, China, [4]NordGen Nordic Genetic Resources Centre, Ås, Norway, [5]Norwegian University of Life Sciences, Oslo, Norway.*

Semi-domesticated reindeer (*Rangifer tarandus*) have pivotal economic, societal and cultural value for indigenous people and pastoralists in northern and subarctic regions in Eurasian. Currently, there exist several semi-domesticated and wild *Rangifer*-populations, the taxonomic status of which has been actively debated. To examine genetic diversity, domestication history, taxonomy and adaptation, we deep-sequenced and de novo assembled one reindeer genome, resequenced 23 other *Rangifer* sp. samples. Genomic DNA of a 1-year-old Finnish male reindeer was sequenced at high coverage (100X) on the Illumina HiSeq2500 and 4000 platforms. Seven paired-end DNA libraries with insert sizes ranging from 170bp to 20kb were constructed. The genome assembly, annotation, and orthology analysis were conducted using a robust bioinformatics pipeline. In the resequencing approach, 23 semi-domesticated and wild reindeer and caribou were sequenced (10X) using Illumina HiSeq2500 platform. The data were subjected e.g., for the principal component analysis. A total of 300.5 Gb of clean data was assembled using SOAP denovo resulting into 256,454 scaffolds (N50 = 502 Kb) with cumulative scaffold length of 2.66 Gb and spanning 90% of the estimated (2.9 Gb) genome size of reindeer. Using a homology based approach the reindeer genome was predicted to harbor 27,332 protein coding genes, 98% of which were functionally annotated. The resequenced animals grouped into 2 main clusters: northern European and northern Russian/northern American. The draft quality of the reference genome along with the annotations will provide important insights into the evolution and demographic history of the reindeer and taxonomy of *Rangifer* sp. Our findings suggest that there have been at least 2 domestication events in the history of reindeer.

Key Words: *de novo* sequencing, domestication, reindeer, resequencing, taxonomy

Plenary Session II

OP150 EMBL-EBI, changes in the reference genomes sequence and archive. A. Frankish*, *EMBL-EBI, United Kingdom.*

Abstract not provided.

OP151 Evolutionary changes in sequence, regulation, and gene expression between *Bos taurus* and *Bos indicus*. M. Naval-Sánchez*[1], M. Menzies[1], L. R. Porto-Neto[1], D. F. Cardoso[1,2], C. Kern[3], M. Halstead[3], M. R. S. Fortes[4], A. Cánovas[5], B. J. Hayes[6], H. D. Daetwyler[7,8], P. J. Ross[3], H. Zhou[3], J. Kijas[1], and A. Reverter[1], *[1]CSIRO Agriculture & Food, St. Lucia, QLD, Australia, [2]Department of Animal Science, School of Agricultural and Veterinarian Sciences, Sao Paulo State University (UNESP), Jaboticabal, SP, Brazil, [3]3Department of Animal Science, University of California Davis, Davis, CA, USA, [4]School of Chemistry and Molecular Biosciences, The University of Queensland, Queensland, Australia, [5]Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [6]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, Queensland, Australia, [7]Agriculture Victoria, AgriBio, Centre for AgriBioscience, Centre for, Bundoora, Victoria, Australia, [8]School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

The majority of world cattle population can be divided as Taurine (*Bos taurus*) or Indicine (*Bos indicus*). Taurine cattle are mostly based in temperate environments, while Indicine breeds are highly adapted to environments where heat is a constant. Besides temperature, other adaptation traits such as disease and parasite resistance, as well as differences in human herd management and selection processes have shaped separate patterns of genetic variation in both cattle lineages. With the aim to explore how selection and genomic differences impact the function of regulatory elements and gene expression, we performed a comparative analysis at the level of sequence, open-chromatin and gene expression between these 2 cattle lineages. In particular, the genome sequences of 185 Taurine and 103 Indicine individuals were analyzed. Genome-wide detection of candidate selective sweeps based on site frequency measures (Fst) and nucleotide diversity (π) resulted in 657 and 242 20Kb bins in the Taurine and Indicine genomes, respectively. A major finding is a large selective sweep in the Indicine genome located on chr5: 47,670,001–48,100,000 and spanning several genes including *HELB*, *IRAK3*, *GRIP1* and part of *HMGA2*. In our quest to characterize the functional impact of selective sweeps, as well as to improve the annotation of regulatory elements in the cattle genome, we performed ATAC-seq for 3 biological replicates of Brahman (*Bos indicus*) and 2 replicates of Hereford (*Bos Taurus*) breeds, and 4 tissues: muscle, adipose, liver and hypothalamus. The comparative analysis of regulatory elements provides insight on their evolutionary turnover and increases our understanding of non-coding variation and its impact on regulatory elements. Next, we assess the enrichment of previously identified selective sweeps for regulatory features, and confirm that differences between Taurine and Indicine breeds involve mostly coding and proximal regulatory elements. Finally, we explore the impact of selection at the gene expression level by incorporating RNA-Seq information for the same individuals and tissues as the ATAC-seq data was generated.

Key Words: cattle, selection, evolution, ATAC-seq, functional annotation

OP152 Integrative genomics and network biology in livestock species: New knowledge from existing (and elderly!) data sets. D. E. MacHugh*, *UCD School of Agriculture and Food Science, University College Dublin, Belfield, Ireland.*

High-throughput genomics technologies are currently driving significant change in animal biosciences research and how genetic improvement is implemented in domestic species. During the past decade, large single-nucleotide polymorphism (SNP) and whole-genome sequence (WGS) data sets have been generated for many livestock populations. In recent years, these high-density genome-wide genetic data have also been complemented with high-resolution transcriptomics and epigenomics data sets. We have been developing methods to integrate these different types of genomics data with phenotypic data and leverage new knowledge from existing and archival data sets. Here I provide some illustrative examples, highlighting integrative genomics studies on 1) host-pathogen interactions for mycobacterial infections in cattle and humans; 2) systems genetics of exercise physiology in Thoroughbred racehorses; and 3) local ancestry and functional genomics of trypanotolerance and bioenergetics in African cattle populations.

Plenary Session III

OP153 Transgenerational epigenetic inheritance: How strong is the evidence? H. Khatib*, *Department of Animal Sciences, University of Wisconsin-Madison, Madison, WI, USA.*

Transgenerational epigenetic inheritance is the transfer of epigenetic marks such as DNA methylation, histone modifications, and non-coding RNAs across subsequent generations via the germline. Although this phenomenon is well documented in different species including plants, nematodes, flies, fish, and rodents, it is not clear if it occurs in mammals. In contrast, intergenerational epigenetic inheritance involves the transmission of epigenetic marks from the gametes to the embryo for one or 2 generations. Many studies have reported that epigenetic marks are plastic and can be altered in response to environmental exposures such as nutrition, stress, smoking, and air pollution. These epigenetic alterations can be inherited either intergenerationally or transgenerationally. However, the mechanisms of the inheritance of these epigenetic marks remain unclear. In several studies in sheep, beef cattle, and dairy cattle we demonstrated that different maternal diets supplemented before or during pregnancy altered the transcriptome and the epigenome of the offspring tissues. Importantly, we found that maternal nutrition during pregnancy in sheep induces epigenetic changes in the fetus, which in turn lead to alternations in gene expression of imprinted genes and DNA methyltransferases. The response of imprinted genes to different maternal energy sources in fetal tissues suggests important roles in the adaptation to energy balance. The fact that some imprinted genes have regulatory functions in both embryonic growth and metabolism suggests important roles in fetal programming of these genes. Furthermore, integrative whole-genome DNA methylation and transcriptome analysis revealed an association between gene expression and inter-/intragenic methylated regions in fetal tissues in response to maternal diet during pregnancy. The findings of these studies imply that maternal diet before and during gestation can shape the epigenome and the transcriptome of fetal tissues, and putatively affect phenotypes of the next generation. Therefore, future studies should focus on the long-term effects of these epigenetic changes on production and reproduction traits in livestock.

OP154 Whole-genome sequencing of 722 canids reveals novel genomic regions under selection and variants influencing morphology and longevity. J. Plassais[1], J. Kim[1], B. W. Davis[1,2], D. M. Karyadi[1], A. N. Hogan[1], A. C. Harris[1], B. Decker[1,2], H. G. Parker[1], and E. A. Ostrander*[1], *[1]National Institutes of Health, Bethesda, MD, USA,*

[2]Texas A and M University, College Station, TX, USA, [3]Brigham and Women's Hospital, Boston, MA, USA.

Domestic dog breeds present an extraordinary diversity of morphologic traits and breed-associated behaviors resulting from human selective pressures. Most breeds were created within the last 200 years by either reproductively isolating small numbers of founder animals with specific characteristics or by combining founders from multiple breeds of desired phenotypes. As a result, canines represent a remarkable system for understanding and identifying genetic variants controlling mammalian traits, especially those associated with morphologic variation. To identify the genetic underpinnings of such traits, we analyzed 722 canine whole genome sequences (WGS), documenting over 91 million single nucleotide variants and small indels, thus creating the largest public catalog of genomic variation for a companion animal species to date. We undertook both selective sweep analyses and genome-wide association studies (GWAS) inclusive of over 144 modern breeds, 54 wild canids and 100 village dogs. Our results identify new sequence variants of strong impact associated with several phenotypes, including longevity, ear and tail position, leg length and size and body weight variation. We show that genes found to date account for greater than 90% of breed body-size variation. We also describe the genetic relationship between regulatory mutations, body size and longevity, demonstrating that only 4 body size genes found to date, including *LCORL*, *HMGA2, IGF1* and an unmapped locus on CFA26 contribute to lifespan. Finally, we used WGS from wild canids and indigenous dogs to refine our GWAS results, demonstrating that alleles which distinguish common breed-associated traits have been under selection since early breed formation. We thus show that GWAS and selection scans performed with WGS are powerful complementary methods for expanding the utility of the dog for studies of mammalian growth and biology. Almost 400 modern domestic dog breeds exist, each with a unique history and genetic profile. Our laboratory at NIH has assembled one of the largest and most diverse data sets of domestic dog breeds, reflecting their extensive phenotypic variation and heritage, to identify breed-defining loci. Our laboratory has used genetic distance, introgression, and genome-wide haplotype sharing analyses to uncover geographic patterns of development and independent origins of common traits, revealing the hybrid history of breeds. Most recently, we have assembled a large database of canine whole genome sequence and demonstrated its utility in genome-wide association studies for disentangling the genetics underlying breed-specific morphologic, behavior, and disease susceptibility traits. These analyses characterize the complexities of breed development, resolving long-standing questions regarding individual breed origination, the effect of migration on geographically distinct breeds, and by inference, transfer of trait and disease alleles among dog breeds.

Key Words: genetics, GWAS, dog WGS, body size

OP155 Update on the analysis and applications of microbiome information. C. Rogel-Gaillard*, *GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France.*

Microbiomes interplay with their hosts, and more and more studies aim at studying the reciprocal links between their composition and functionalities, and the host phenotype variability. A new biological scale has emerged with the concept of holobiont defined as a living organism surrounded by its inner and outer symbiotic microbial communities. Thus, holobionts are complex ecosystems that interact dynamically with the environment. Host and microbiome genomes have likely coevolved resulting in species-specific microbiomes as revealed by the information provided by the gene catalogs of gut microbiomes for humans, mice, pigs and for an increasing number of other species. There is increasing evidence that gut microbiomes contribute to animal health and performance, including the metabolism of nutrients, stimulation of epithelium cell proliferation, maturation of immune system, protection from pathogens, signaling to the brain. Indeed, microbiomes stand at the crossroad of multiple functions that are potentially related to feed efficiency, immune capacity, welfare, environmental footprint, etc. and that are of upmost interest for sustainable animal breeding. Identifying microbiomes that positively contribute to health and production is a first big issue. Understanding how to modulate microbiomes is a second big issue that requires deciphering the respective roles of the host genetics, environment and feed diets in shaping individual microbiomes. Developing standardized methods for producing and analyzing microbiome data is a third big issue. We will illustrate these questions by presenting results obtained mostly in pig. We will further discuss how research on microbiomes contribute to advances in animal biology and provide opportunities for animal breeding systems.

Key Words: microbiome, genetics, pig

OP156 Making the best of large datasets when sequence analysis methods do not scale. C. Notredame*, *Centre for Genomic Regulation (CRG), Barcelona Institute for Science and Technology, Barcelona, Spain.*

The evolutionary angle has probably been one of the most productive way of looking at animal data. Because it reflects the constraints that are to be fulfilled for survival, evolution provides an extremely powerful entry point in the understanding of complex living systems. The same rational applies to any organism exposed to strong pressure of selection, such as domestic crops and animals selected for specific types of productivity. While the theory behind this approach has been carefully crafted over more than a century, it is only over the last decade that sufficient data has been made available to start exploring the most daring predictions of evolution, and more importantly to make the best of this powerful conceptual framework to better understand a wide variety of highly relevant issues such as improved breeding strategies, parasite resistance and even cancer treatment. Unfortunately, the methods currently available do not scale with the current data output and almost every link in the complex chain of analysis currently available for data processing is under some kind of pressure to be improved. In this seminar, I will focus on homology based modeling methods such as multiple sequence alignment and I will expose their limitations, recent progresses and the challenges that are to be overcome if we want to make the best of current data. This issue is critical at a time when 66.000 new animal genomes have been announced. I will take this occasion to introduce the most recent developments of the T-Coffee multiple sequence alignment package (www.tcoffee.org) and present novel large-scale strategies allowing unprecedented levels of data integration. Large scale computation comes along with important reproducibility challenges and I will introduce the Nextflow (www.nextflow.io) method developed in our group that allows complex and intensive computations to be deployed efficiently and reproduced at any relevant level.

Applied Sheep and Goat Genetics

OP157 Invited Workshop Presentation: AdaptMap project: Exploring worldwide goat diversity and adaptation. L. Colli*[1], E. L. Nicolazzi[1], F. Bertolini[3,4], C. P. Van Tassell[5], M. F. Rothschild[3], B. D. Rosen[5], T. S. Sonstegard[6], B. Sayre[7], P. Ajmone-Marsan[1], P. Crepaldi[8], G. Tosser-Klopp[9], S. Joost[10], A. Stella[11], and AdaptMap Consortium[12], [1]*DIANA Department of Animal Science, Food and Nutrition & BioDNA Research Centre on Biodiversity an Ancient DNA, Università Cattolica del S. Cuore, Piacenza, Italy,* [2]*Fondazione Parco Tecnologico Padano, Lodi, Italy,* [3]*Department of Animal Science, Iowa State University, Ames, IA, USA,* [4]*National center of aquatic resources, Technical University of Denmark, Lyngby, Denmark,* [5]*Animal Genomics and Improvement Laboratory, USDA, Agricultural Research Service, Beltsville, MD, USA,* [6]*Acceligen Inc, St. Paul, MN, USA,* [7]*Department of Biology, Virginia State University, Petersburg,*

VA, USA, [8]Dipartimento di Medicina Veterinaria, University of Milan, Milan, Italy, [9]GenPhySE, INRA, Université de Toulouse, INPT, ENVT, Castanet Tolosan, France, [10]Laboratory of Geographic Information Systems (LASIG), School of Architecture, Civil and Environmental Engineering (ENAC), École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, [11]Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, Milan, Italy, [12]www.goatadaptmap.org.

The AdaptMap project investigates worldwide goat diversity with a focus on domestication, adaptation associated with local environmental conditions, and adaptation in response to selection for production systems. The ultimate goal of the project is to enable sustainable goat breeding by leveraging the use of genomic information. During the first phase of AdaptMap, several analyses were performed to characterize population genetics and history, to identify selection signatures and signals of environmental adaptation through landscape genomics, and to devise a panel of SNPs for parentage assessment. Overall, our results showed a close association between the distribution of diversity and geography, with a clear partitioning of the populations into groups according to the continental origin. The 3 major gene pools corresponded to goats from Europe, Africa and western Asia, a pattern consistent with the recognized paths of human migration out of the Fertile Crescent. Within these major pools, further patterns of variation mirrored geographical sub-structuring, human history and animal husbandry practices. Comparing the European and African gene pools, European breeds tended to be more discreet, in line with the practice of creating breeds through active human-controlled genetic isolation, while greater gene flow was observed in Africa. Among the observed "hotspots" of runs of homozygosity, some were unique across continental groups. The results of selection signature analysis were generally consistent with previous evidence from independent studies. Signals of adaptation to the environment were also identified, showing the strongest association with differences in mean annual temperature. Finally, a highly informative marker panel for parentage assessment was developed to assist breeding in goat populations worldwide. Taken together, our evidence outlines a remarkable diversity occurring at the global scale, locally partitioned and often affected by introgression from cosmopolitan breeds. This provides a useful framework to direct improvement and conservation actions, and to select breeding targets.

OP158 Flock54: A new targeted marker panel for the sheep industry. L. Eidman[1], J. Thorne[2,3], M. Duan[2,4], S. Hunter[5], K. Davenport[2], G. Becker[2], and B. Murdoch*[2], *[1]Superior Farms, Dixon, CA, USA, [2]Animal and Veternary Science, University of Idaho, Moscow, ID, USA, [3]Texas A&M, ArgiLife Research and Extension Center, San Angelo, TX, USA, [4]School of Molecular Biosciences, washington Statew University, Pullman, WA, USA, [5]IBEST Genomics Resource Core, University of Idaho, Moscow, ID, USA.*

Genomic tools have enabled the acquisition of information to aid and accelerate the rate of genetic improvement of agricultural livestock species. With price still a limiting factor to widespread utilization in the US sheep industry, there is a need for a lower density panel that reports markers for Mendelian inherited traits and other well-known traits relevant to sheep production. The objective of this project was to design a genetic marker panel using amplicon targeted Genotyping By Sequencing (GBS). A 1000 marker DNA panel was designed to provide genotypic data for known single gene causative mutations, pedigree assignment and markers associated with quantitative trait loci common to the Applied Biosystems Axiom Ovine Genotyping array (50K). These markers are evenly spaced throughout the genome and located in either a transcript or quantitative trait locus. The flanking sequences of each of the markers were mapped to sheep reference genomes Oar_v3.1 and Oar_rambouillet_v1.0 to ensure the unique mapping of sequencing reads for data analyses. Sheep tissue or blood samples were collected by Superior Farms producer members, DNA was extracted from 384 samples and sent to Thermo Fisher Scientific for next-generation sequencing using Ion 540 chip. Both targeted and novel mutations within the sequenced region were called using Ion Torrent Suite 5.10. Only the markers with a minimum of 50X sequence coverage were retained. After excluding under-performing samples and markers, 970 targeted markers with 50X sequence coverage or greater and an overall average call rate of 98% were included in the data set. Furthermore, novel genetic mutations identified in these important targeted regions were characterized. Data from this panel can be used to identify known causative mutations, improve pedigree accuracy and assist in genetic selection for the sheep industry at a cost of US$15. The application of this cost-effective sheep genomics marker panel can be used to promote improved production, animal health and profitability of the US sheep industry.

Key Words: sheep, DNA-sequencing, genotyping, genetic improvement, genomic selection

OP159 Effect of genotyping strategies in the sustained genetic gain across multiple generations of selection using ssGBLUP. M. Sánchez-Mayor*[1], V. Riggio[2], L. F. de la Fuente[1], B. Gutiérrez-Gil[1], J. J. Arranz[1], and R. Pong-Wong[2], *[1]Dpto. Producción Animal, Universidad de León, León, Spain, [2]The Roslin Institute and R(D)SVS, University of Edinburgh, Roslin, Midlothian, UK.*

Single-step GBLUP (ssGBLUP) is a BLUP-type genomic evaluation method, which uses a relationship matrix calculated using genotype and pedigree information, allowing to include both genotyped and ungenotyped individuals into a single evaluation. The practical implication of this method is that the genotype information propagates across all animals, improving the accuracy of the breeding values of ungenotyped candidates. A simulation study was carried out to quantify the impact of the genotyping strategy on the sustained genetic gain across multiple generations. To mimic a sheep population, the population structure was simulated with large paternal half sib and small maternal half sib families, but discrete generations were also assumed to avoid changes in generation interval affecting the results. Each generation was composed of 900 individuals (300 males, 600 females) from which 30 males were selected and, each mated to 10 selected females (300 females in total). To create an initial reference population, the first 2 generations were unselected with all animals being genotyped (i.e., initial population of size 1800). Thereafter, a proportion of the candidate offspring were genotyped and the genetic evaluation carried out using ssGBLUP including all candidates plus all ancestor animals since the first generation. The scenarios compared were standard BLUP, GBLUP with all genotyped animals and 5 ssGBLUP with the number of candidates genotyped being 10%, 20%, 30%, 40% and 50%, respectively. The strategy to select the proportion of genotyped animals were: random and the top best animals based on their phenotypic record. The selection scheme was carried out for 9 generations (11 generations simulated). Results showed that the genetic gain of ssGBLUP increases almost linearly with the proportion of individuals genotyped. The comparison between genotyping strategies showed that the selection at random yielded greater genetic gain than when genotyping the best animals in the first generation. However, in the following generations, the scheme where the best animals are genotyped yielded grater cumulative response.

Key Words: ssGBLUP, genotyping, genetic gain

OP160 Genome-wide association with footrot in hair and wool sheep. M. U. Cinar[1,2], R. D. Oliveira[1], T. S. Hadfield[3], A. Lichtenwalner[4,5], R. J. Brzozowski[5], C. T. Settlemire[6], S. B. Schoenian[7], C. Parker[8], H. L. Neibergs[9,10], N. Cockett[3], and S. N. White*[11,1], *[1]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, [2]Faculty of Agriculture, Erciyes University, Kayseri, Turkey, [3]Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA, [4]Department of Animal and Veterinary Science, University of Maine, Orono, ME, USA, [5]Cooperative Extension, University of Maine, Falmouth, ME, USA, [6]Departments of Biology and Chemistry, Bowdoin College, Brunswick, ME, USA, [7]Western Maryland Research & Education Center, University of Maryland, Keedysville, MD, USA, [8]Retired, Ohio, USA, [9]Department of Animal Science, Washington State Universit, Pullman, WA, USA, [10]Center for Reproductive Biology, Washington State University,*

Pullman, WA, USA, [11]USDA-ARS Animal Disease Research, Pullman, WA, USA.

Footrot is a polymicrobial disease of sheep in which *Dichelobacter nodosus* and *Fusobacterium necrophorum* often play major roles, and it results in severe lameness with hoof separation from the underlying tissue. Economic losses have been estimated at more than $20 million per year in the UK, which is the country with the best analysis; globally, footrot in the 975 million sheep outside the UK certainly has much higher costs. There are a variety of treatment and prevention methods, but many are labor-intensive and expensive. Genetic footrot resistance and/or resilience would be a valuable tool to advance control especially because it could help in prevention without the need for exposure. A sample of 251 US sheep including Katahdin, Blackbelly, and various European-influenced crossbred sheep from 9 farms with footrot issues were scored for hoof condition. A large subset (200) were genotyped with the OvineHD array (>600,000 SNP per animal), and 51 others were genotyped with the Ovine50K array (>50,000 SNP) and then imputed up to create a uniform genotype data set. Mixed models were used for genome-wide association, and they accounted for farm and genotype-derived principal components in addition to a random term for the genomic relationship matrix. Genome-wide significant loci were observed on ovine chromosomes 1, 3, 16, 17, and 18 including loci near genes involved in the immune response and in hoof development. This is the first study to report multiple genome-wide significant loci associated with ovine footrot. These results provide a strong foundation for future work toward discovering functional mutations underlying footrot susceptibility. With additional research, these data will improve tools for selectively breeding healthy sheep.

Key Words: footrot, sheep, resistance, susceptibility, genome-wide association

OP161 Genomic background of heat stress in Assaf sheep. M. J. Carabaño*[1], I. Ureña[1], J. H. Calvo[2], M. A. Jiménez[3], M. Ramon[4], C. Díaz[1], F. Freire[3], and M. Serrano[1], *[1]INIA, Madrid, Spain, [2]CITA-ARA-ID, Zaragoza, Spain, [3]ASSAF.E, Toro, Spain, [4]IRIAF, Valdepeñas, Spain.*

Extreme weather events pose a challenge for animal production in the Mediterranean area. Highly selected breeds are expected to be more susceptible than local breeds to environmental challenges. This study aimed at researching the genetic background of heat stress response in the Spanish Assaf population using milk recording information and meteorological data from weather stations. A total of 1.1 million of milk, protein and fat test day records from 150,000 ewes were used to generate pseudo-phenotypes that characterize individual heat stress response. Pseudo-phenotypes were obtained by fitting a random regression model, which included environmental effects (herd-year and season of lambing, parity and days in milk combination and number of lambs born) and a random regression on heat load for each ewe. Heat load was the average of the test day and the 3 previous days for daily average values of a temperature-humidity index (THI). A quadratic Legendre polynomial regression was used to describe individual responses. For rams, pseudo-phenotypes were obtained from a weighted average of their daughters pseudo-phenotypes. Genotypes of a custom 50K SNP Affymetrix microarray for 1,091 rams and 766 ewes were used in the genome-wide association study (GWAS) with the GCTA software. For milk, fat and protein yields, associations at the genome level ($p_{Bonferroni} < 0.01$) were found in chromosomes 5, 13, 17, 26 and X. Three known genes were annotated in these regions. *ADRB3* β 3-adrenergic-receptor (OAR26) is an obesity gene that is involved in the regulation of energy balance and a variety of physiological functions by increasing lipolysis and thermogenesis. *ZNF182* Zinc Finger Protein 182 gene (OARX) has roles in various cellular functions, including cell proliferation, differentiation and apoptosis, being also, a high regulator of transcription. In mouse, variation at this gene has been related with decreased food intake. *PPEF1* protein phosphatase with EF-hand domain 1 gene (OARX) suppresses genotoxic stress response via de-phosphorylation of *PDCD5*. In *Arabidopsis thaliana*, this gene is involved in heat stress tolerance.

Key Words: heat stress, GWAS, sheep

OP162 Functional fertility genomics in sheep (*Ovis aries*). K. Pokharel*[1], J. Peippo[1], M. Honkatukia[2], M.-H. Li[3], and J. Kantanen[1], *[1]Natural Resources Institute Finland, Jokioinen, Finland, [2]Nordgen – The Nordic Genetic Resources Center, Ås, Norway, [3]Chinese Academy of Sciences (CAS), Beijing, China.*

In sheep, ovulation rate and litter size are complex, economically important traits affected by endocrinological, genetic and environmental conditions. We have analyzed factors affecting fecundity of the highly prolific native Finnsheep and less prolific cosmopolitan Texel breeds and searched for related structural and functional changes in their genomes. Total of 31 ewes representing the breeds and their F1-crossbreds were included in the study. Experiments were focused on 2 different time points during the establishment of pregnancy: follicular growth phase (*first phase*) and early pregnancy before implantation (*second phase*). In the first phase, one ovary from each ewe was surgically removed during follicular growth phase of the estrus cycle for transcriptomic study. The second set of tissues representing second phase was collected from pregnant ewes in the slaughter house. Approximately 21000 genes and 531 miRNAs were expressed in the data set with few miRNAs differentially expressed between the breed groups. We observed a cluster of miRNAs on ovine chromosome 18 that is homologous to the one present on Human chromosome 14. We identified several putative markers (e.g., GDF9 V371M mutation) and genes (e.g., CST6, MEPE, and HBB) with expression level significantly different between the breed groups. Meanwhile several genes including those that were differentially expressed between the breeds lacked annotations. We observed that CA5A was found to be always upregulated in F1 cross-bred ewes compared with both pure-breeds. Our data suggested a role of imprinting in the fertility performance in F1-ewes. Taken together; our results will improve the knowledge of important fertility traits as well as genomics of sheep reproduction in general.

Key Words: RNA-Seq, ovary, corpus luteum, endometrium

OP163 Genetic diagnosis of sex chromosome aberrations in sheep based on parentage test by microsatellite DNA and analysis of X- and Y-linked markers. J. A. Bouzada*, J. M. Lozano, M. R. Maya, A. Trigo, L. B. Pitarch, T. Mayoral, and E. Anadón, *Laboratorio Central de Veterinaria, Algete (Madrid) Spain.*

Autosomal chromosome aberrations have little impact on animal breeding since the carriers usually show abnormal body conformation and are thus promptly eliminated by breeders. In contrast, anomalies involving sex chromosomes are more tolerated by species due to random X chromosome inactivation of one of the 2 X chromosomes in all somatic cells for gene dosage compensation and the fact that Y chromosome carries few genes. Hence most carriers often show a regular body conformation but abnormal sex development, mainly in females, causing sterility (most of cases) or subfertility. A reliable survey at an early stage is therefore required because detection and characterization of sex chromosome aberrations in newborn has important economic effects. It is possible to identify profiles that are indicative of chromosome abnormalities, including additional X-linked markers in usual panels for pedigree and parentage. Abnormal profiles of genetic markers located on sex chromosomes can help identify animals with chromosomal defects. Markers panel used for sheep DNA testing by Laboratorio Central de Veterinaria (Madrid) consisting of 17 autosomal microsatellite markers (CD5, CSRD247, ETH152, HSC, ILSTS005, ILSTS011, INRA005, INRA006, INRA023, INRA049, INRA063, INRA172, MAF65, McM42, McM527, OarFCB20 and SPS115), 2 microsatellite markers linked to sex chromosomes (ILSTS017 and OarAE133) and the Amelogenin marker, a gene with distinct X and Y alleles, has been proved as a very useful tool for genealogical control and detection of chromosomal abnormalities in sheep. A new markers panel with additional sex-linked markers (OarAE25, OarCP131, INRA030 and SRYM18) was imple-

mented for extended analyses of suspect cases. Detection at an early age and understanding of the prevalence of sex chromosome aberrations should assist in the diagnosis and management of sheep kept for breeding.

164 Selection signatures in goat breeds reveal the molecular basis for six different coat color phenotypes. J. Henkel*[1], R. Saif[1,2], V. Jagannathan[1], C. Drögemüller[1], C. Flury[3], and T. Leeb[1], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Institute of Biotechnology, Gulab Devi Educational Complex, Lahore, Pakistan, [3]School of Agricultural, Forest and Food Sciences HAFL, Bern University of Applied Sciences, Zollikofen, Switzerland.*

Domestication and human selection have formed diverse goat breeds with characteristic phenotypes. This process correlated with the fixation of causative genetic variants controlling breed-specific traits within regions of reduced genetic diversity, so called selection signatures or selective sweeps. We performed a comprehensive screen for selection signatures in 20 genetically diverse modern goat breeds and Bezoar goats, the wild ancestor of domesticated goats. We pooled DNA from 12 animals of each breed and sequenced the obtained breed pools to ~30x coverage. The sequence reads were mapped to the goat reference genome (ARS1) and single nucleotide variants were called. For each pool, heterozygosity scores within a sliding window of 150 kb were calculated and negative Z-transformed H_p scores ($-ZH_p$) were plotted. This approach revealed on average 111 selection signatures per breed with $-ZH_p > 4$. In 2 Pakistani goat breeds, the Pak-Angora and Barbari, we found a strong selection signature in a region harboring the *KIT* gene. *KIT* is a well known depigmentation gene and several previously discovered *KIT* variants result in diverse depigmentation phenotypes. The selection signature in the Pak-Angora goat breed revealed a copy number variant (CNV) downstream of *KIT*. The same locus in the Barbari goat breed harbored an alternative variant of the CNV. These CNVs could explain the white spotted phenotype of Barbari and the completely white phenotype of Pak-Angora. Another selection signature in Swiss goat breeds with white markings or specific symmetric color patterns was found at the *ASIP* locus encoding the agouti signaling protein. This selection signature revealed 4 different CNV alleles most likely affecting region-specific expression levels of *ASIP*. One of the CNV alleles corresponded to a previously published *ASIP* allele in Saanen goats, while the 3 other *ASIP* alleles have not been reported before. In conclusion, this study revealed new loci under selection in 20 different goat breeds. We report 6 structural variants at the *KIT* and *ASIP* loci that are likely to cause differences in caprine coat color phenotypes.

Key Words: goats and related species, evolutionary genomics, genome sequencing, selection scan, coat color

OP165 Polled intersex syndrome (PIS) in goats—Nanopore sequencing revealed a complex structural variant and made it possible to devise a simple genetic test for identification of intersexual goats. R. Simon*[1], H. Tschanz-Lischer[2], I. Keller[2], I. Häfliger[3], A. Pienkowska-Schelling[3], C. Schelling[4], C. Drögemüller[3], and G. Lühken[1], *[1]Institute of Animal Breeding and Genetics, Justus Liebig Univerity, Giessen, Germany, [2]Interfaculty Bioninformatics Unit, University of Bern, Bern, Switzerland, [3]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [4]Clinic for Reproductive Medicine, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland.*

In goats the trait of polledness is, in contrast to other bovine species, connected with disorders in sexual development. The so-called polled intersex syndrome (PIS) is characterized by the fact that homozygous polled and genetically female (XX) individuals are infertile due to phenotypically diverse intersexuality, making the identification of such cases difficult. In 2001, a ~11.7 kb deletion was detected as PIS-associated genomic variant. After trials to establish genetic testing for PIS in goats with the existing molecular genetic information had failed, it was decided to take a closer look at the known variant using Oxford Nanopore sequencing, an emerging long read sequencing technology. We sequenced the whole genome of 2 goats, a PIS-affected polled and a horned goat as control. A genome coverage of 9x and 14x for the homozygous polled and the horned goat, respectively was reached. Mapping against the current goat reference genome (ARS1) was performed with Minimap2. Sequence data confirmed the presence of the published deletion on chromosome 1 in the homozygous polled goat but also indicated the presence of a more complex structural variant. Some of the around 4 kb long sequence reads map to the regions flanking the PIS-deletion however not continuously. Significant parts of the reads map to a region located about 20 Mb downstream on chromosome 1. In addition, within this particular region a copy number variant was observed in the polled goat. A closer look revealed that a duplicated copy of a segment (~500 kb size) is inversely inserted into the region of the known PIS-associated deletion. We succeeded to validate this structural variant by fluorescence in situ hybridization using BAC clones. Sanger sequencing confirmed the complex variant with PCR primers flanking the chromosomal breakpoints. Finally, a practicable proof of genetic horn status and sex of goats was established applying an easy to use and robust multiplex PCR. Meanwhile, more than 1000 goats of different known breeds, sex and horn status have successfully been tested. In conclusion, this study revealed a more complex structural variant causing PIS in goats based on whole genome long read sequencing. We report a simple genetic test that allows the determination of the polled genotype in goats for the first time.

Key Words: goats and related species, animal breeding, genome sequencing, fertility

Companion Animal Genetics and Genomics

OP166 A comparative review of the deleterious variants burden in the domestic cat. W. C. Warren*[1], R. Buckley[1], F. H. G. Farias[2], R. Middelton[3], W. J. Murphy[4], and L. A. Lyons[1], *[1]University of Missouri, Columbia, MO, USA, [2]Washington University School of Medicine, St Louis, MO USA, [3]4Nestlé Purina Research, St Louis, MO, USA, [4]Texas A&M University, College Station, TX, USA.*

Substantial progress in the association of human sequence variation to detrimental health consequences has been made, but we still have much to learn. A benign versus pathogenic variant identification conundrum persists. In companion animals, our understanding of the putative deleterious variant similarities to human gene orthologs is missing, especially true for domestic cats. We have sequenced in-depth (30x) > 200 cats, including fancy breeds, crosses, wild subspecies and other felid species. Single nucleotide variants (SNVs) and indels were called against the *Felis catus* 9.0 reference to classify their frequency, orthology to human and putative pathogenic impact. In a subset of unrelated cats (n = 60) we estimated an average of 9.7 million SNVs per cat. In total, we identified 85,350 missense mutations, of which 20,775 were considered rare (allele frequency <1%). Also, within coding regions we identified 8,648 indels, with 1,640 considered rare. For loss-of-function (LOF) SNVs, our sequenced cats carried 1,310, of which the stop gain classification was highest. Importantly, we identified that 26% of these LOF SNVs were rare (<1%) and thus valuable for feline disease research in the future. Overall, we found 701 LOF SNVs in human gene orthologs with 434 of these predicted to be in genes where LOF isn't tolerated. At allele frequencies < 10%, we found 46 SNVs in our human LOF intolerant gene set displayed LOF homozygosity, suggesting these cats should have some health consequence if these genes are intolerant to LOF in the cat. This first whole genome study of the segregating SNVs among cat populations represents a significant step toward using this data to more confidently assign an allele disease causality, be it cancer, common or rare designations.

Key Words: feline, disease, genomics

OP168 Mining the 99 Lives cat genome sequencing initiative database. L. A. Lyons*, R. M. Buckley, and 99 Lives Consortium, *College of Veterinary Medicine, University of Missouri, Columbia, MO, USA.*

The 99 Lives Project includes over 200 cat genomes. All the whole-genome sequencing data has been produced by Illumina technology with PCR-free libraries of 350 – 550 bp and 100 – 150 bp paired-end reads. Genome coverages range from >15x to > 50x coverage, most genomes are 30x coverage. Thirty-three cat breeds are represented and >50 moggies from different world regions. A goal for 2019 is to incorporate an additional 24 unrepresented cat breeds. Fifteen different wild felid species are included with intentions to have at least one representative from all the 37 cat species. Approximately 40 different research institutions, zoological parks, pet food companies and animal health companies are contributing and form the consortium. The largest contributions have come from investigators at MU (~52%), Cornell University and the University of California, Davis. Variant calling has been completed by MU using the new long-read cat genome assembly Version 9.0. The variant call file and sequence data is available to all consortium members. The genomes submitted by MU incorporate over 59 different cat diseases and traits. Five projects have been published and several addition studies are in preparation. Most recently, the DNA variants causal for dwarfism, hydrocephalus, Chediak-Higashi Syndrome and Ehlers-Danlos Syndrome have been identified. Mining the 99 Lives data set supports genetic testing services by identifying variants flanking known casual variants for traits and diseases. These variants may disrupt primers for PCR- and sequencing-based assays and cause allelic dropout or poor amplification of a specific allele, particularly in hybrid cat breeds (Bengals, Savannahs, Chausie). Potential new causal variants may be identified as well. Additional variants are suspected to cause white spotting phenotypes in cats, beyond the known *KIT* variants. The 99 Lives data set contains 54 *KIT*, 5 *KITLG*, 22 *EDNRB*, and 9 *MITF* that could influence melanocyte migration. *MLPH* has 23 missense variants besides the common dilution mutation, which could alter tone of coloration. *FGF5*, which affects long length, has 32 variants including several LOF variants. Allele frequencies can also be estimated, importantly for diseases. 99 Lives is a diverse resource for the genetics community.

Key Words: domestic cat, genome, disease, *Felis*, companion animal

OP214 Precision medicine in dogs: Some new causative variants for inherited diseases. Tosso Leeb*[1] and Dog Biomedical Variant Database Consortium (DBVDC)[2], [1]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Switzerland,* [2]*DBVDC (Gus Aguirre, Catherine André, Danika Bannasch, Doreen Becker, Brian Davis, Cord Drögemüller, Kari Ekenstedt, Kiterie Faller, Oliver Forman, Steve Friedenberg, Eva Furrow, Urs Giger, Christophe Hitte, Marjo Hytönen, Vidhya Jagannathan, Tosso Leeb, Hannes Lohi, Cathryn Mellersh, Jim Mickelson, Leonardo Murgiano, Anita Oberbauer, Sheila Schmutz, Jeffrey Schoenebeck, Kim Summers, Frank van Steenbeek, Claire Wade).*

Abstract not available.

OP170 An early onset retinopathy in Golden retriever dogs. S. Mäkeläinen*[1], K. Narfström[2], B. Ekesten[3], G. Andersson[1], and TF Bergström[1], [1]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden,* [2]*Section for Comparative Ophthalmology, College of Veterinary Medicine, University of Missouri-Columbia, Columbia, Missouri, USA,* [3]*Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Progressive retinal atrophy (PRA) and other inherited retinopathies result in visual impairment and blindness in dogs and humans. In Golden retriever dogs, 3 different forms of PRA and the genetics behind them have been identified. We have recently observed a fourth form of retinopathy that affects the photoreceptors; more severely rods than cones. In comparison to classical PRA caused by variants in the *PRCD*, *SLC4A3* (GR PRA I) and *TTC8* genes (GR PRA II), this previously undiscribed retinopathy appears to have an earlier onset. Further, there is mainly a normal fundus appearance while clinical signs such as pupillary light reflexes and night vision are abnormal. Here, we used a whole-genome sequencing (WGS) approach of an affected dog, its unaffected sibling and their unaffected parents using Illumina paired-end sequencing with 150bp read length on a NextSeq500 platform. This resulted in an average coverage of 16.9X and identification of 4,764 exonic indels and 48,366 exonic single nucleotide variants (SNVs). Assuming an autosomal recessive mode of inheritance we used conditional filtering of the variants and identified 45 exonic indels and 842 SNVs. We then filtered these variants against 33 additional whole-genome sequenced dogs respresenting 8 different breeds and found 9 indels and 113 SNVs private to the affected individual. Eight of the indels resulted in frameshift insertions or deletions and one was a nonframeshift insertion. 46 SNVs were nonsynonymous and 5 of these were considered deletorious based on Polyphen-2 and Provean scores. These variants are currently being functionally evaluated bioinformatically and by Sanger sequencing of additional dogs. One of the nonsynonymous substitutions is a particularly strong candidate as a causative variant for this novel retinal disease.

Key Words: dog, monogenic disease, whole-genome sequencing, retinopathy, animal health

OP171 Multi-breed comparison of canine lymphoma susceptibility. S. A. Mortlock, M. M.-J. Chen, P. Soh, V. W. T. Hsu, M.-S. Khatkar, P. F. Bennett, R. M. Taylor, and P. Williamson*, *The University of Sydney, Sydney, NSW, Australia.*

Lymphoma is a common malignancy seen in dogs with variable incidence rates observed across different breeds. The identification of breed as an epidemiological factor in lymphoma along with reports of familial clustering of cases suggests a genetic factor is contributing the etiology of the disease. Numerous breeds have been classified as having a higher risk of lymphoma including the mastiff breeds, Bernese mountain dogs, Doberman pinscher and Cocker spaniels, while several other breeds have been reported as having a reduced risk. Using the Illumina CanineHD Beadchip and GCTA, GWAS analysis of genotypes from Bullmastiff cases (n = 44) and healthy controls (n = 88) revealed 2 regions on CFA13 and CFA33 were identified within the top ranked SNP (FDR < 0.05). Five putative haplotypes on CFA13 were each associated with increased risk of early onset disease in Bullmastiff dogs. Approximately 65% of cases were homozygous for all risk haplotypes compared with 10% of control dogs. Strong linkage disequilibrium and homozygosity across the region made fine mapping of the region difficult. However, the region covers an interesting candidate complex containing the Myc-PVT1 syntenic region and overlapping coding regions for *miR-1204, miR-1205 and miR-1206*. This region has been recognized for its involvement in a range of human cancers, including lymphoma. The frequency of the CFA13 risk haplotypes was examined in a further sub-population of Bullmastiff dogs and compared with 15 breeds with varied risk of lymphoma. Based just on the risk haplotypes, Bullmastiffs clustered separately to other breeds. The frequency of risk haplotypes was calculated for multiple breeds along with the proportion of dogs homozygous for each haplotype and SNP-wise ROH frequency across the genome. A difference in haplotype frequency and homozygosity across risk haplotypes 2 and 4 was identified between high and low risk breeds, highlighting these 2 regions as lymphomagenesis candidates.

Key Words: dog, cancer, GWAS, breed, lymphoma

OP172 Assessing the genetic contribution to complex behavioural traits in German Shepherd dogs. J. Friedrich*[1], P. Arvelius[2], E. Strandberg[3], A. Talenti[1], E. Sánchez-Molano[1], R. Pong-Wong[1], J. Hickey[1], M. Haskell[4], and P. Wiener[1], [1]*Division of Genetics and Genomics, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK,* [2]*Swedish Armed Forces Dog Training Center, MÄRSTA, Sweden,* [3]*Department of An-*

imal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden, [4]Scotland's Rural College, Edinburgh, UK.

The close co-existence with humans, favorable genetic structure and intense selection for behavior characteristics resulting in a high diversity of behavioral features highlights the potential of dogs for studying the genetic contribution to behavior variance. Results from these studies can have implications for animal welfare and human conditions. However, behaviors are complex traits, which have been shown to be influenced by numerous genetic and non-genetic factors, complicating their analysis and demanding innovative approaches. In this study, we examined the genetic contribution to behavior variance by combining quantitative and population genetics approaches. First, the association between behavioral traits of German Shepherd dogs (GSDs) and genetic variants was analyzed, while accounting for various environmental factors. Several behavioral traits that were defined based on dog owner responses to questions of the established Canine Behavioral Assessment and Research Questionnaire (C-BARQ) exhibited moderate heritabilities, with the highest estimates identified for Human-directed playfulness and Non-social Fear. Furthermore, we identified genomic regions distributed over 17 autosomes that showed at least chromosome-wide significant association with the analyzed traits; for several traits, multiple regions were identified, supporting the hypothesis that behavior traits are influenced by multiple genes. Second, we exploited the different breeding backgrounds of the analyzed GSD cohorts to identify signatures of recent selection for behavior. Several statistics, e.g., the integrated haplotype score, the fixation index and the cross-population extended haplotype homozygosity, were calculated to detect these signatures within and between cohorts. Putative selection signatures within cohorts were found in regions on CFA4 and CFA19, both of which harbour behavior-related candidate genes. Target regions for divergent selection between cohorts were found on CFA12 and CFA32, both of which have been described previously to be associated with morphological and behavioral variation in different dog breeds.

Key Words: GWAS, regional heritability mapping, C-BARQ, selection signatures, human-directed playfulness

OP173 An haplotype view of cystinuria in dog. S. Frattini*[1], M. Cortellari[1], A. Talenti[2], A. Negro[1], C. Biagini[1], M. Polli[1], and P. Crepaldi[1], *[1]Department of Veterinary Medicine, University of Milan, Milan, Italy, [2]The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, UK.*

Canine cystinuria is an autosomal recessive disorder that affects a dog's ability to filter cystine out of urine. The Solute Carrier Family 3 Member 1 (*SLC3A1*) is a gene, coding for a portion of the membrane protein that controls the reabsorption of dibasic amino acids at renal level, pivotal in the pathology onset. Aim of the work is to deepen the haplotype variability associated with cystinuria in English Bulldog (EB), French Bulldog (FB), and some other afflicted breeds. Our work started from a sample of 19 dog, 9 FB and 10 EB, genotyped at the locus c.A2092G on the exon 10 of the gene SLC3A1 on chromosome 10. Samples were then genotyped with 230k SNPChip (Illumina) and haplotypes, built around gene SLC3A1 (±1.0 Mb), were explored. We investigated differences in haplotypes among 3 group of animals: dogs homozygous for the mutation at the SLC3A1 *locus* (classified as PP), heterozygous (NP) and wild type (NN). The 6 samples classified as PP showed a unique and identical haplotype of 44 SNPs at ± 1.0 Mb around the gene, with a core haplotype of 4 SNPs within the gene. The analysis was expanded to a partially public data set comprising some of the most susceptible breeds for the pathology: Newfoundland, Labrador Retriever, Australian Cattle Dog, Miniature Pinscher, Rottweiler and Dachshund, in addition to further EB and FB. Of the 93 analyzed animals, only 4 (3 EB and 1 FB) showed the same haplotype. Considering the core haplotype, 4 Newfoundland out of 10 in the data set presented this haplotype. These results suggest that the severity of this pathology could be evaluated not only at the known causal mutation but as a conserved haplotype. The present work showed the applicability of dense SNP panels in the identification of haplotypes linked to causal mutations for canine cystinuria. The detection of target haplotypes associated with major known pathologies afflicting canine species, for each breed, could make possible the use of this versatile tool as a first general health check control in dog. Aknoledgments: Authors thank Vetogene Lab for the supply of the 19 samples genotyped

Key Words: dogs and related species, genetic identification, diagnostics, breed diversity, animal health

Comparative and Functional Genomics

OP174 FAANGMine: A genomic data-mining warehouse for domesticated animal species. C. G. Elsik*, M. Shamimuzzaman, D. A. Triant, J. J. Le Tourneau, and A. T. Walsh, *University of Missouri, Columbia, MO, USA.*

We are developing FAANGMine (http://faangmine.org), a genomic data mining warehouse for domesticated animal species, including species of interest to the Functional Annotation of Animal Genomes (FAANG) Consortium. The first FAANGMine release contains genomes of cat, chicken, cow, dog, goat, horse, pig, sheep and water buffalo. Gene annotations of human, mouse and rat are also included to facilitate comparison to model organisms. FAANGMine uses the InterMine platform to integrate data from a variety of sources, including reference genome assemblies, genes (NCBI, Ensembl), proteins (UniProt), protein families and domains (InterPro), orthologs and paralogs (EnsemblCompara, OrthoDB, TreeFam), pathways (KEGG, Reactome), interactions (BioGRID, IntAct), Gene Ontology (GO), QTL (AnimalQTLdb), variation (Ensembl) and publications (PubMed). Simple and sophisticated search tools enable researchers without scripting skills to create and export customized annotation data sets merged with their own research data for use in downstream analyses. Built-in query templates provide starting points for data exploration, while the QueryBuilder tool supports construction of complex queries. The List Analysis and Genomic Regions search tools execute queries based on uploaded lists of identifiers and genome coordinates, respectively. Data can be exported in a variety of formats, including gff, fasta, json and tab-delimited files. We plan to expand FAANGMine by incorporating data generated by the FAANG Consortium to enable fine-grained data mining of functional elements in combination with gene annotations and additional biological data.

Key Words: multispecies, functional genomics, genome annotation, bioinformatics tools, data mining

OP175 High-order gene-by-gene and gene-by-environment interactions: Is there a need to consider them when dissecting the genetic basis of complex traits? Ö. Carlborg* and Y. Zan, *Department of Medical Biochemistry and Microbiology, Uppsala, Sweden.*

Most biological traits are the result of the actions and interactions of multiple genes and environmental factors. Understanding the genetic architecture of such complex traits is a grand challenge in biology. Over the past 20 years, thousands of loci affecting e.g., complex human diseases and agricultural production traits have been identified. The standard way to model the genetic contributions by these loci is to assume that i) they are bi-allelic and ii) the total genetic contribution is the sum of the allelic effects. This additive modeling of genetic effects within and across loci is statistically powerful and useful to, for example, estimate how much of the trait variation in a population is due to genetics, map individual genes contributing to this variation and predict the immediate response of a population to selection. Molecu-

lar studies, however, find that the biological mechanisms contributing to these traits are often complex and it has been shown that this complexity is not reflected in the results of statistical quantitative genetics analyses of experimental data. We have explored the polygenetic basis of complex traits in animals, plants and microbes. The focus has been to understand how non-additive genetic mechanisms contribute to the phenotypic variation for complex traits in both experimental and natural populations. Using the findings from these studies as a basis, it is shown how the genetic effects of multi-locus genotype-phenotype maps associated with networks of interacting (epistatic) loci emerge into different statistical model parameters in quantitative genetics analyses. Using empirical findings as a basis, it is illustrated which insights can, and which cannot, be confidently inferred from such studies.

Key Words: quantitative genetics, population genetics, epistasis, genotype by environment interactions, high-order interactions

OP176 Evaluation of RNA-Sequencing pipelines for optimized power and accuracy of SNP and INDEL identification. S. Lam*[1], F. Miglior[1], J. Zeidan[1], I. Gómez-Redondo[1,2], A. Suárez-Vega[1], P. A. S. Fonseca[1], F. Schenkel[1], and A. Cánovas[1], *[1]Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Spanish National Institute for Agriculture and Food Research and Technology, Madrid, Spain.*

Evaluation of RNA-Sequencing (RNA-Seq) pipelines is critical to optimize power and accuracy of analysis for identifying genetic variants (SNPs and INDELs) in coding regions. Variant calling pipelines were compared with determine more optimized and accurate approaches for variant detection using RNA-Seq data. The new developed pipeline was used to identify unique variants associated with feed efficient beef steers. The RNA-Seq data (NCBI accession numbers: PRJEB15314 and PRJEB15314) used were from muscle and liver tissues from 12 Nellore beef steers (n = 6 low-RFI, n = 6 high-RFI), selected from 585 steers that had their residual feed intake (RFI) determined. Three multi-sample calling pipelines were compared including i) non-merged samples; ii) merged samples for low-RFI and for high-RFI for each tissue, and iii) merged samples for low- and high-RFI for both tissues. The data was aligned against UMD3.1 (release 94) assembly using STAR. Variants were called using BCFtools and variant annotation was performed using VeP and ToppGene. The approaches were compared by assessing filters (Phred score quality, location of variants, minimum read depth) and presence of variants unique to each approach using BCFtools. The use of approach i), i.e., non-merged calling, detected individual genotypes (GT) for each biological sample, revealing variability between individuals. The use of approaches ii) and iii) revealed greater read depth and power for GT calling and detection. In further analyses, approach iii), which had the greatest read depth and power, was used to detect variants within low- (SNPs: 13,145, INDELs: 371) and high-RFI (SNPs: 14,663, INDELs: 429) groups. Positional candidate genes located within variants (2,828 in low-RFI, 2,979 in high-RFI) were significantly ($P < 0.05$) associated with immune and metabolism pathways. RNA-Seq pipelines applied to detect genetic markers to be used for selecting for desirable traits in livestock should be chosen based on an experimental strategy that would maximize power of detection and accuracy of GT calling to increase confidence of variant detection.

Key Words: feed efficiency, RNA-Seq, SNP, transcriptome, cattle and related species

OP177 Genetic control of temperament traits across species: association of autism spectrum disorder genes with cattle temperament. R. Costilla*[1,2], K. Kemper[1], E. Byrne[1], L. Porto-Neto[4], R. Carvalheiro[5], D. Berry[6], D. Purfield[6], J. Doyle[6], S. Moore[2], N. Wray[1], and B. Hayes[2], *[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia, [2]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Australia, [3]Queensland Brain Institute, The University of Queensland, Brisbane, Australia, [4]Commonwealth Scientific and Industrial Research Organization (CSIRO), Agriculture and Food, Brisbane, Australia, [5]School of Agricultural and Veterinarian Sciences, Sao Paulo State University, Sao Paolo. Brazil, [6]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland.*

Temperament (personality) traits are relevant to both human and cattle well-being. In humans, they correlate with psychological and psychiatric disorders. In cattle, they impact on animal welfare and product quality and hence are of direct commercial importance. We hypothesized that genetic factors contributing to variation in temperament among individuals within a species will be shared across humans and cattle and indeed mammals. We conducted a series of whole genome sequence based genome-wide association studies on cattle flight time, a temperament phenotype defined as the time taken for an animal to cover a short fixed distance after being released from an enclosure, with a total sample of 9223 animals. Significantly associated regions were located on 4 bovine chromosomes and enriched in pathways related to synaptic transmission, regulation of the circadian rhythm, and neuron development functions. Given that human studies are better powered than those in cattle, we investigated the association of cattle temperament with polymorphisms in bovine orthologous genes reported to be associated with neuroticism, schizophrenia, autism spectrum disorders (ASD), and developmental delay disorders. We found enrichment of variants in, or close to, ASD susceptibility genes. These variants explained 7% of the total additive genetic variance in the biggest cattle cohort (randomized permutation test genome-wide P-value <0.02). ASD genes with the most significant associations were *GABRB3, CUL3,* and *INTS6*. We also confirmed these genes were highly expressed in bovine brain cerebellum and caudal lobe. These findings provide quantitative molecular evidence that genetic control of temperament traits might be shared across humans and cattle and highlight the potential for future analyses to leverage results between species.

Key Words: multispecies, genome-wide association, gene set enrichment analysis, complex trait, biomedical model

OP178 Copy number variants reveal traces of recent selection in two dairy cattle breeds. Y. L. Lee*[1], A. Bouwman[1], M. A. M. Groenen[1], E. Mullaart[2], R. F. Veerkamp[1], and M. Bosse[1], *[1]Wageningen University & Research, Animal Breeding and Genomics, Wageningen, the Netherlands, [2]CRV B.V, Arnhem, the Netherlands.*

Copy Number Variations (CNVs − gain or loss of DNA segments) are genetic variants that are known to play a role in shaping a wide range of phenotypes. Using Illumina BovineHD Genotyping BeadChip (770K) data from 2 major dairy cattle breeds, Holstein Friesian (HF, n = 315) and Jersey (JER, n = 107), we aimed at exploring the functional impact of CNVs, and to detect traces of selection using CNVs. Using the ARS-UCD1.2 genome assembly, we identified 14,272 CNVs, consisting of 9,171 deletions (mean length = 44.2 kb) and 5,101 duplications (mean length = 74.6 kb). These CNVs were aggregated into 1,755 CNV regions (CNVR) based on 1 bp overlap. We found that the bovine CNVRs overlapped with 1,739 out of 27,570 Ensembl autosomal genes (6.3%). The number of genes overlapping with CNVRs was significantly higher than expected by chance (permutation test, $P < 0.001$). Gene ontology enrichment analyses showed that these overlapping genes were highly enriched for immune response (FDR = 0.001) and response to stimulus (FDR <0.001). Further, we calculated population differentiation index (Fst), on 1,471 bi-allelic CNVRs to detect highly differentiated regions between HF and JER populations. There were 42 highly differentiated CNVRs with Fst ranging between 0.1 and 0.43 (16 genic and 26 intergenic CNVRs). Among the 16 genic CNVRs with high Fst, CNVR 380 (Fst = 0.21) and CNVR 1458 (Fst = 0.15), contain the promising candidate genes under selection, *MGAM* and *ADAMTS17*. The *MGAM* gene codes for a starch digestion enzyme, while *ADAMTS17* is associated with stature in various species. These 2 genes can explain the recent selection contributing to the breed for-

mation of HF and JER. Our findings highlight the potential functional impact of CNVs, and their role in recent selection.

Key Words: copy number variations, population divergence, dairy cattle

OP179 Chromatin accessibility conservation across four livestock species. S. Djebali*[1], S. Foissac[1], N. Vialaneix[2], K. Munyard[3], A. Rau[4], T. Faraut[1], S. Lagarrigue[5], H. Acloque[4], E. Giuffra[4], and FR-AgENCODE Consortium[1,4], *[1]GenPhySE, University of Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France, [2]MIAT, INRA, Castanet Tolosan, France, [3]Curtin University, School of Biomedical Sciences, CHIRI Biosciences, Perth, Australia, [4]GABI, AgroParisTech, INRA, Université Paris Saclay, Jouy-en-Josas, France, [5]UMR PEGASE, INRA, Rennes, France and UMR PEGASE, Agrocampus Ouest, Rennes, France.*

Within the FAANG consortium, the FR-AgENCODE pilot project has generated transcriptome (RNA-seq) and chromatin accessibility (ATAC-seq) data in the liver and immune cells of 2 males and 2 females of 4 vertebrate species (cattle, goat, chicken and pig), in addition to Hi-C data (see abstract #79896 by S. Foissac et al.). The first single-assay and integrative analyses performed on these data were mostly done on each species separately. Although these analyses provided several interesting insights (https://www.biorxiv.org/content/10.1101/316091v1), they did not take full advantage of our data richness, namely the availability of the same kind of functional data on species belonging to different parts of the phylogenetic tree. Here we have investigated the relationship between chromatin accessibility conservation across vertebrates and functionality. We first defined orthologous accessible regions by projecting accessible regions from our 4 species to the human genome. Two accessible regions are called orthologous if their projection on the human genome overlap. In doing so we identified 19,982, 7,877 and 1,083 regions shared between 2, 3 and 4 species, respectively. Our main results are as follows: The level of accessibility of a region, measured by ATAC-seq, increases with the number of shared accessibility-orthologs. Regions with more shared accessibility-orthologs are more likely to be close to gene promoters. The functional conservation of an accessible region, measured by the number of species exhibiting a tissue-differential accessibility, is correlated with sequence conservation, measured by phastCons. This confirms and reinforces previous findings in human and mouse. A hierarchical clustering of ATAC-seq samples based on the correlation between the accessibility of the 1083 orthologous regions shared between 4 species shows that clustering occurs first by species and then by tissue. This is different from what we found for RNA-seq (i.e., all liver samples clustering together). Based on these findings, we are currently working to define more precisely the functional regulatory relationships in liver and immune cells in light of their conservation during evolution. We anticipate that the comparison of these relationships to known QTLs should provide interesting results.

Key Words: ATAC-seq, comparative genomics, bioinformatics, vertebrate livestock species, FAANG

OP180 Meta-analysis of differentially co-expressed gene modules for high- and sub-fertile beef cows. P. A. de Souza Fonseca*, A. Suárez-Vega, S. Lam, F. S. Schenkel, S. Id-Lahoucine, and A. Canovas, *University of Guelph, Guelph, ON, Canada.*

Improved reproductive efficiency may lead to economic benefits for livestock. However, several factors limit our understanding of fertility traits, including genetic differences between populations and statistical limitations. This study identified differentially co-expressed (DcoEx) gene modules between 10 high- (HF) and 10 sub-fertile (SF) beef cows (predominantly Angus heifers). The fertility status was based on the pregnancy outcome ratio after successive high-quality embryo transfers protocol of estrus synchronization (PG-6d-CIDR and GnRH) where heifers that did not exhibit standing estrus received GnRH injection on d 0. RNA-sequencing (RNA-Seq) data from endometrium of HF and SF cows were retrieved from Gene Expression Omnibus (ID: GSE81449, GSE107891). RNA-Seq data was aligned using STAR software, read counts were obtained using RSEM software and DESeq2 package was used to normalize and calculate the Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values. The identification of DcoEx modules was performed by the R package WGCNA using shared genes between HF and SF with an FPKM >0.2 in each group (16,439 genes). The R package km2gcn was used to reallocate the genes within modules using a k-means clustering approach. Three and 9 DcoExp modules were identified with a unique co-expression pattern in HF and SF cows, respectively. The genes within these modules are associated with fertility-related processes, including fertilization, decidualization, and steroid biosynthesis. In addition, SNPs and INDELs exclusive to HF or SF groups were called and annotated (20,076 and 18,764, respectively). The top 10 hub-genes of each module were scrutinized for variants identified exclusively in one of the groups and the co-localization with previously reported fertility-related female QTLs. Four and 21 top-hub genes in the HF and SF DcoExp modules emerged as functional candidate genes, respectively. The identification of hub-genes in DcoExp modules for contrasting the fertility based groups analyzed here may help to identify functional candidate genes and biological processes associated with implantation and retention of the embryos in beef cattle.

Key Words: fertility, bioinformatics, WGCNA, RNA-seq, systems biology

OP181 Broadening the miRNA catalogue in livestock species: A contribution to the functional annotation of animal genomes. A. J. Amaral*[1], C. Anthon[2], G. Corsi[2], A. Vasconcelos[1], S. Marthey[3], A. Hoffman[4], J. Lagnel[5], F. Haack[6], K. Pokharel[7], O. Palasca[2], S. Seemann[2], L. T. Gama[1], M. A. M. Groenen[8], J. Kantanen[7], R. P. M. A. Crooijmans[8], M. Rijnkels[9], T. Kalbfleisch[10], E. Giuffra[3], P. F. Stadler[4], O. Madsen[8], and J. Gorodkin[2], *[1]Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, [2]Center for noncoding RNA in Technology and Health, Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark, [3]GABI, AgroParisTech, INRA, Université Paris Saclay, Jouy-en-Josas, France, [4]Bioinformatics Group, Department of Computer Science University of Leipzig, Leipzig, Germany, [5]INRA PACA, Montfavet Cedex, France, [6]Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany, [7]Natural Resources Institute Finland, Jokioinen, Finland, [8]Wageningen University, Wageningen, Netherlands, [9]Veterinary Integrative Biosciences,College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, [10]Department of Biochemistry and Molecular Genetics, School of Medicine, University of Louisville, Louisville, KY, USA.*

MicroRNAs play a crucial role in the regulation of gene expression. Their action is crucial in many biological processes and functions, such as cell development and differentiation, and in response to disease. Moreover, it has been shown that polymorphisms in miRNAs can be linked to diseases and complex traits. An improved annotation of miRNAs in domestic animals is therefore required to acquire a comprehensive understanding of their impact on livestock traits. There are a large number of published studies with public data sets across most of livestock species and covering a wide range of tissues. However there have been few resources to capitalize on these data to better understand these features, distribution and biogenesis in these genomes. Therefore, a working group was established for the development of analysis pipelines and methods of data analysis of small-RNA-seq data in the framework of COST-Action FAANG-Europe. A total of 846 quality approved small-RNA-seq data sets available from public repositories for 6 livestock species (*Gallus gallus, Sus scrofa, Equus caballus, Ovis aries, Capra hircus and Bos taurus*) were used to quantify miRNA expression in different tissues as well as to identify putative novel miRNA candidates. Our analyses has identified across the 6 species a total of 1,404 novel pre-miRNAs, with a larger impact for *Bos taurus and Sus scrofa,* in which these represent an increase of 50% for these species in comparison with miRBase v22. We will use these to perform large-scale

analysis of miRNA function and biogenesis. These analyses will include global expression comparison, co-expression of miRNA clusters. Additionally we will report for the first time in these tissues a global analysis of miRNA epi-transcriptomic modifications and access their prevalence across species and tissues. We believe these findings will further contribute to the understanding of the functional genome of the studied species. AJA is supported by a post-doctoral fellowship within IMAGE H2020 project (REF 677353–2). This abstract is based upon the work from COST Action FAANG-Europe (CA15112) supported by COST (European Cooperation in Science and technology) and co-funded by FCT grant UID/CVT/00276/2013.

Key Words: FAANG, miRNAs, livestock, regulation of gene expression, bioinformatics

OP182 Detection of long non-coding RNAs from the differential transcriptomic analysis of abomasal lymph node from resistant and susceptible sheep to the infection by *Teladorsagia circumcincta*. P. K. Chitneedi[1], C. Kühn[2], R. Weikard[2], J. J. Arranz[1], M. Martínez-Valladares[3,4], and B. Gutiérrez-Gil*[1], *[1]Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, León, Spain, [2]Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [3]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, León, Spain, [4]Instituto de Ganadería de Montaña, CSIC-Universidad de León, Grulleros (León), Spain.*

Here we detected long noncoding RNAs (lncRNAs) in the transcriptome of abomasal lymph node tissue extracted from adult sheep classified as resistant or susceptible to the infection by the gastrointestinal nematode (GIN) *Teladorsagia circumcincta*. A group of 18 ewes naturally infected by GIN showing extreme high and low fecal egg count (FEC) were dewormed and infected with *T. circumcincta* larvae. Based on their accumulative FEC at d 31 post-infection, 6 ewes were classified as 'Susceptible' and 6 ewes as 'Resistant'. After a second deworming, the 12 selected ewes were infected again with *T. circumcincta* larvae and sacrificed at d 7 post-infection to collect individual abomasal lymph node tissue samples. The total polyA RNA extracted from these samples was sequenced using an Illumina Hi-SEq 2000 sequencer by generating paired-end reads of 75 bp, with a depth of 30M reads per sample. Using a standard bioinformatic pipeline, and after quality assessment of the raw sequence data and trimming of adapter and low-quality sequences, the obtained reads were aligned and sorted against the sheep reference genome (Oar_v.3.1 ensembl release 95). Then we detected potential transcripts by reference-guided transcript assembly, merging and transcript annotation and finally counted the number of transcripts in each sample in the annotated feature file. On average, the data contained 44,203 genes with 77,039 transcripts. This annotated feature file was used to detect the lncRNA with the FEELnc package. In total 9,105 lncRNA were detected. Among them 2,092 were classified as novel with the gffcompare tool. After differential gene expression with DESeq2 between susceptible and resistant ewes, we found 440 genes differentially expressed with 158 upregulated and 282 downregulated in resistant sheep. Out of the 440 differentially expressed genes, 44 contain 63 lncRNA transcripts out of these 30 were novel. Using BLASTN to perform a sequence similarity search against the sheep reference genome, no overlapping genes were detected in the regions of the 30 novel putative lncRNA transcripts.

Key Words: sheep and related species, non-coding RNA, bioinformatics tools, RNA-seq

OP183 Circular RNA expression in turkey skeletal muscle and response to thermal challenge. K. M. Reed*[1], K. M. Mendoza[1], and J. E. Abrahante[2], *[1]Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, USA, [2]University of Minnesota Informatics Institute, University of Minnesota, Minneapolis, MN, USA.*

An emerging area in the regulation of gene expression is the discovery of circular RNAs (circRNAs) which are novel, single-stranded RNAs generated from exonic/intronic sequences joined head to tail. Being circular, they lack the poly-A tail of traditionally processed mRNAs and are resistant to RNAase R digestion. Although their functions are poorly understood, these RNAs can function as microRNA sinks and regulators of splicing and transcription, and are thus potential modifiers of gene expression. The abundance of circRNAs has been unappreciated in conventional RNA-seq analyses. Analysis of this data with new algorithms has found circRNAs to be widely expressed with modest sequence conservation. In this study, we mined RNA-seq data and tested for the presence of circRNA species in sequences obtained from breast muscle of 2 lines of turkey. Sequencing data were generated from muscle tissues of turkey poults of the comparatively slower-growing (randombred control 2, RBC2) line, and the faster-growing body-weight selected (F) line, exposed to thermal challenge (31, 35 or 39? C) for 3 d post-hatch. RNA-seq reads from 28 paired-end libraries (average 18,788,823 reads/library) were mapped to the turkey genome (UMD5.0) and circRNAs were predicted using CIRI software. Data mining of the RNA-seq data sets identified over a thousand potential circRNAs. Differential expression analysis suggests that studies on the presence, abundance and relative expression of these non-coding RNA molecules will enhance our understanding of their function and potential role in the biological response to thermal challenge in muscle.

Key Words: poultry, functional genomics, RNA-seq, muscle, meat production

OP184 Functional analysis of G-protein-coupled receptors during porcine subcutaneous preadipocytes differentiation. M. Taniguchi*[1], A. Arakawa[1], I. Nakajima[1], H. Uenishi[2], and S. Mikawa[1], *[1]Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan, [2]Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan.*

G-protein-coupled receptors (GPCRs) are membrane proteins that enable cells to sense molecular signals and to respond in a broad range of biological processes according to the external circumstances of cells. Recent studies elucidated that short-medium chain fatty acids can stimulate GPCRs to activate signaling pathways for adipocyte differentiation. In order for the process of differentiation of Porcine Subcutaneous Pre-Adipocytes (PSPA), it has been demonstrated that octanoate (C8:0) supplementation in the differentiation medium is essential for growth arrest which triggers the differentiation initiation of PSPA followed by lipid synthesis and accumulation to develop into fully differentiated adipocyte. We, therefore, aimed to elucidate GPCR genes expressed at a semi-confluent phase of the PSPA using the Genome Sequencer FLX to obtain 5′ end of messenger RNA sequence. Consequently, we detected 11 candidate GPCR genes considered to be expressed before the PSPA differentiation induction. Of 11 GPCRs, *leucine rich repeat containing G protein-coupled receptor 4* (*LGR4*) gene appeared to be associated with preadipocytes differentiation by functional annotation of those GPCRs. So, we intended to analyze gene knockdown effect using transfection of small interfering RNA (siRNA) for *LGR4* gene into the PSPA followed by differentiation induction. The result indicated that the knockdown of *LGR4* gene obviously retarded the PSPA differentiation and decreased lipid synthesis in the cells. Therefore, we further investigated gene expression profiles of PSPA transfected with *LGR4* siRNA using a porcine custom 4 × 44K microarray. Our result of the gene expression microarray indicated that larger numbers of downregulated genes were detected more than upregulated genes by the siRNA transfection. Gene ontology analysis elucidated that upregulated genes by *LGR4* gene knockdown were categorized in biological processes of cell adhesion. Interestingly, downregulated genes, on the other hand, were categorized in PPAR signaling pathway which is known for the key pathway of adipogenesis and lipid synthesis. The adipocytes differentiation of PSPA might be attributable to the alteration of gene expression profiles regulated by *LGR4* identified in this study.

Key Words: adipocytes, differentiation, G-protein-coupled receptor, porcine

OP185 Development of an AgriSeq targeted GBS panel for equine SNP parentage verification and sire/dam allocation. P. Flynn*[1,3], R. Morrin-O'Donnell[1], J. Carlsson[3], P. Siddavatam[2], S. Chadaram[2], H. Suren[2], C. Carrasco[2], and R. Conrad[2], *[1]Weatherbys Scientific, Naas, Ireland, [2]Thermo Fisher Scientific, Austin, TX, USA, [3]University College Dublin, School of Biology & Environmental Science, UCD, Belfield, Dublin, Ireland.*

Transitioning from STR to SNP molecular markers to perform parentage verification is becoming an ever more discussed topic within the global Equine community. A key area that requires exploration is ensuring maintenance of test integrity when moving to SNP based parentage. Due to its highly accurate and reproducible results, targeted GBS is becoming an increasingly favored technology for SNP genotyping. AgriSeq™ GBS with Ion Torrent NGS technology offers a fast, flexible, multiplexing, customizable, cost-effective solution to study hundreds of samples across 50 to 5 thousand markers within each genotyping run using either extracted nucleic acid or crude lysis. We developed a targeted ~500 SNP Equine GBS panel consisting of ISAG parentage verification SNPs and an additional SNP panel for Sire/Dam allocation. The panel design utilized latest reference genome build - EquCab3.0, with corrections for markers mapping to previous reference genomes EquCab1 and EquCab2. Utilizing AgriSeq HTS Library Kit, a high-throughput targeted amplification and re-sequencing workflow, the panel's performance was tested on a diverse set of Equine Thoroughbred DNA Sire/Dam/Offspring Trios. Libraries were sequenced on Ion S5 using an Ion chip with genotype calls generated using Torrent Variant Caller (TVC) plugin. All Equine animals were also genotyped for 16 STR markers (inclusive of 12 ISAG STRs) which allowed for comparative assessment of parentage efficacy and Sire/Dam allocation accuracy between SNP and STR markers. Furthermore, previously demonstrated flexibility of AgriSeq GBS panel design also facilitates considerations for additional SNPs for diagnostic traits and STR imputations to future panel versions. Results of the panel performance including mean genotype call rate of markers across samples, concordance across replicate library preparations and independent sequencing runs, parentage efficacy and Sire/Dam allocation accuracy results are reported. The data demonstrates Equine SNP parentage in practice and the utility of the AgriSeq targeted GBS as a platform to support Equine SNP parentage verification genotyping needs.

Key Words: equine, parentage, GBS, AgriSeq, IonS5

OP186 Development of an equine SNP parentage panel which complements historic and current high-density genotyping resources. R. G. Tait Jr.*[1], D. J. G. Arts[2], R. Ferretti[1], H. Hofeneder-Barclay[3], B. Simpson[1], L. Kock[1], and J. Qiu[1], *[1]Neogen GeneSeek Operations, Lincoln, NE, USA, [2]KWPN Royal Dutch Sport Horse Studbook, Ermelo, Netherlands, [3]Neogen Europe, Ayr, UK.*

In the era of routine parentage verification programs and genomic enhanced estimated breeding value (GE-EBV) genetic evaluations it is important to have SNPs in common for both of these objectives such that the SNP panel used for GE-EBVs is wholly informative for the parentage testing process. Historically, relatively small SNP panels (typically 100 primary and 100 supplemental SNPs) have been an economically efficient way to capture SNP genotype information for parentage verification. With transitions of commercially available high density SNP genotyping arrays from Illumina EquineSNP50 to the GGP Equine platform and limited distribution of previously proposed equine SNP parentage panels, we found limited presence of the previously proposed SNPs included on the GGP Equine (80 SNPs). We have expanded those 80 SNPs present on both EquineSNP50 and GGP Equine to develop a panel of 200 SNPs present on both platforms to be used for parentage verification. This 200 SNP panel has 3 to 17 SNPs per chromosome. SNPs were identified to extend the coverage of each chromosome and spacing between previously proposed markers as well as achieve high minor allele frequencies. Distances between SNPs ranged from 859,335 to 17,877,698 bp with average of 12,131,313 bp and median of 11,942,131 bp (EquCab2.0). The small 859,335 bp distance was between 2 of the previously proposed parentage SNPs. In a large sample of warmblood horses (n = 2,889) the minor allele frequencies of the selected SNPs ranged from 0.293 to 0.500 with mean of 0.452 and median of 0.463. The power to exclude a random one parent one offspring relationship for the 80 previously proposed SNPs with data across genotyping platforms is 0.999955744 (44 in 1 million allowed). In comparison, the power to exclude the same relationship for this 200 SNP panel is 0.999999999993790 (42 in 10 billion allowed), a 10,000-fold increase in power. This SNP panel has now been used for almost 16,000 parent-offspring comparisons in a commercial warmblood sporthorse population. This 200 SNP standalone panel is a very powerful tool for parentage verification that leverages historical high density genotyping on key animals within populations and facilitates robust pedigree verification for genetic evaluations.

Key Words: horse, parentage, SNP

OP187 Potential methods of detecting indiscriminate genetic manipulation in thoroughbred racehorses. T. Tozaki*[1], A. Ohnuma[1], M. Kikuchi[1], H. Kakoi[1], K.-I. Hirota[1], K. Kusano[2], and S.-I. Nagata[1], *[1]Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan, [2]Ritto Training Center Racehorse Hospital, Japan Racing Association, Ritto, Shiga, Japan.*

Indiscriminate genetic manipulation for improvement of athletic ability is evolving as a major threat to human sports and the horse racing industry. Gene doping (i.e., abuse/misuse of gene therapies) and genetically modified racehorses should be prohibited to ensure fair horse racing. Therefore, the development of methods for detecting such indiscriminate genetic manipulations is urgently needed. In this study we aimed to develop a highly sensitive method for the detection of horse erythropoietin (*EPO*) transgenes using droplet digital PCR (ddPCR). Two TaqMan probe-primer sets were designed at the exon/exon junction and in different exons, respectively for detection of *EPO* transgenes. An *EPO* transgene cloned into a plasmid was used as a model of gene doping material. The spiked *EPO* transgene was extracted from horse plasma and urine by magnetic bead DNA isolation, followed by ddPCR amplification for absolute quantification of detected transgenes. The validated method produced a high recovery rate (at least 50%) of spike transgenes. These results indicated that the spiked transgene could be quantitatively detected at concentrations greater than 160 copies ml^{-1} of plasma and urine. In addition, we succeeded in detecting the *EPO* transgene from a horse injected intramuscularly with 20 mg of *EPO* transgene. This is the first study to demonstrate detection of *EPO* transgenes from plasma and urine of a horse administered *EPO* transgenes as a gene doping control. As another strategy for the detection of genetically modified racehorses, we first collected the whole genome sequence SNP and INDEL databases from 33 thoroughbred horses, before ultimately collecting data on 100 horses. The average numbers of detected filtered SNPs and INDELs were 4,975,186 and 694,386, respectively, based on a reference genome sequence (Equcab2.0). The SNP and INDEL information will be mined for information regarding regions in which normal variations exist in genomes of thoroughbred horses. Although this study is on-going, we are currently attempting to analyze parentage using ultra deep sequencing data to detect mismatch and de novo mutation.

Key Words: horse, equine, gene doping, genome editing, Thoroughbred

OP188 Investigating large structural variants in African cattle using long-read sequencing and optical mapping. A. Talenti*[1], H. Hemmink[2], E. A. J. Cook[2], D. Wragg[1], J. Powell[1], C. Ezeasor[3], E. Obishakin[4], A. Fisch[5], R. Kelly[6], I. K. Silwamba[7], W. Amanyire[8], D. Muhanguzi[8], M. Watson[1], P. Wiener[1], P. Toye[2], L. Morrison[1], T. Connelley[1], and J. Prendergast[1] *[1]The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, UK, [2]The International Livestock Research Institute, Nairobi, Kenya, [3]University of Nigeria, Nsukka, Enugu State, Nigeria, [4]National Veterinary Research Institute, Vom-Jos, Nigeria, [5]University of São Paulo, São Paulo, Brazil, [6]Royal (Dick) School of Veterinary Medicine, University of Edinburgh, Edinburgh, UK, [7]School of Veterinary Medicine, University of Zambia, Lusaka, Zambia, [8]College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda.*

Infectious diseases, environmental conditions and the limited productivity of indigenous cattle pose challenges to African cattle farmers. Despite this, several indigenous cattle breeds are adapted to different environments and are naturally resistant to several lethal endemic diseases. The genetic changes underlying these phenotypes are though generally unknown. In this context, large structural variants (SVs) are of particular interest because they are more likely to show a large effect on phenotypes than the more commonly studied single nucleotide variants. However, compared with short variants, SVs are more challenging to detect using the most common sequencing technologies. In this study, we detected novel SVs in African cattle breeds using 2 approaches: i) assembly of a high-quality reference genome of an African taurine breed; ii) detection of SVs across breeds using Bionano optical mapping. We assembled the genome of an N'Dama bull, an indigenous African taurine breed resistant to trypanosomiasis, using 40X PacBio long reads and the FALCON-unzip assembler. Following polishing of the genome with 80X of Illumina short read data, the genome was scaffolded using a reference-assisted approach. Ultra-long optical mapping molecules for 18 cattle, spanning both African and European breeds, were obtained using the Bionano Saphyr platform to map large SVs. The assembly produced a contig N50 = 3.3Mb and L50 = 227, respectively. Genome completeness was 85% based on complete universal orthologs (BUSCO), providing a high-quality reference genome for African taurine breeds. A total of 12Mb that does not align to the current *Bos taurus* reference genome (Hereford, UCD-ARS1.2) represents novel sequence from the N'Dama. Finally, the analysis with the Bionano solve pipeline allowed us to detect an average of ~4,000 SVs per sample. These new resources will help us to exploit the genetic resource of indigenous cattle breeds, and provide specific solutions to the challenges of African livestock production. Acknowledgments: Authors are grateful to BBSRC for project funding.

Key Words: cattle and related species, genome assembly, genome sequencing, chromosomal rearrangement, animal health

OP189 The effect of allele ancestry on production traits of tropical composite cattle. L. R. Porto-Neto*[1], S. M. McWilliam[1], M. Naval-Sanchez[1], B. J. Hayes[2], and A. Reverter[1], *[1]Commonwealth Scientific and Industrial Research Organization (CSIRO), St Lucia, QLD, Australia, [2]Queensland Alliance for Agriculture and Food Innovation (QAAFI), St Lucia, QLD, Australia.*

Crossbreeding between *Bos taurus* and *Bos indicus* cattle is a common strategy applied worldwide to make beef and milk production more efficient in the tropics. The expectation is that taurine alleles assist improvements in beef quality, milk yield, and fertility, while zebu alleles contribute to heat and disease resistance and tolerance to poor quality fodder, with the additional benefit of hybrid vigor. There are several examples of crossbred cattle outperforming pure-bred cattle for many production traits under tropical conditions — however, a fine map of the effects of allele ancestry affecting production traits is yet to be refined. Here we used data from the 1000 Bulls Genome Project (run6) as a reference to impute, using Minimac3, SNP-array genotypes of 3,318 tropical composite cattle up to whole genome sequence (~22.6M SNP). We then used ChromoPainter v2 to estimate the likelihood of the alleles' ancestry (taurine or zebu) and test the association between this taurine likelihood and 36 production traits including measurements of parasite load, growth, fertility, and conformation traits. Different traits were affected differently by ancestry, some traits, e.g., coat length, sheath score, tick load, and color had significant correlations, while others, e.g., rectal temperature and body condition score seems not to be affected by ancestry. The definition of genomic regions that are adversely affected by zebu or taurine ancestry, in the future, could guide crossbreeding to maximize its benefit, effectively breeding toward more productive tropical cattle.

Key Words: adaptation, environment, harsh, disease, parasite

OP190 Autosomal genome evidence for introgression from other *Gallus* species into African and Middle East indigenous chicken. A. S. Al-Jumaili*[1,2], A. Gheyas[3,4], A. Kebede[5,6], J. Smith[3,4], and O. Hanotte[1,5], *[1]School of Life Sciences, the University of Nottingham, University Park, Nottingham, United Kingdom, [2]University of Anbar, Anbar, Iraq, [3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK, [4]Centre for Tropical Livestock Genetics and Health, The Roslin Institute, Edinburgh, UK, [5]LiveGene, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [6]Addis Ababa University (AAU), Addis Ababa, Ethiopia.*

Across species, gene flow is increasingly being recognized as playing an important role in the historical genetic background of a species. In particular, adaptive introgression may be related to environmental adaptation. Also, considering the geographic distribution of related wild species, it could help to trace the history of dispersion of a species. Here we investigate if introgression can be used to trace dispersion routes of indigenous chicken outside its putative centers of origins. Domestic chicken from 3 countries (Iraq n = 27, Ethiopia n = 283 and Saudi Arabia n = 5 NCBI accession no. SRP142580) beside the 4 *Gallus* species (Ceylon = 8, Green n = 12, Grey n = 3 and Red n = 6) were analyzed to assess their overall genetic background and frequency of introgressed haplotypes using the 4 taxons ABBA – BABA approach. All samples were analyzed using an Illumina HiSeqX sequencer and SNPs data were generated following the GATK best practices protocol restricted to bi-allelic sites. Haplotype maximum likelihood trees at 6 candidate introgressed regions were constructed allowing counting haplotype frequency in the studied population. The results have found no gene flow from Ceylon and Green junglefowl into the gene pool of Iraq, Ethiopia and Saudi village chicken at the opposite of Grey junglefowl introgression. Overall, the frequencies of the latter were low in Iraq and Saudi Arabia, South Ethiopian populations, compared with the North, Central-East and West Ethiopian populations. The results are adding further support for an Indian subcontinent center of terrestrial dispersion for the Iraqi and Saudi village chicken. For Ethiopia, the situation is likely more complex and, with the country having likely witnessed both a terrestrial and maritime arrival of the domestic chicken, in agreement with the today geographic dispersion of Grey junglefowl.

Key Words: introgression, dispersion routes, indigenous chicken

OP191 Single nucleotide polymorphism selection methods to optimize imputation accuracy for South African Drakensberger beef cattle. S. F. Lashmar[1,2], D. P. Berry[3,1], R. Pierneef[2], F. C. Muchadeyi[2], and C. Visser*[1], *[1]University of Pretoria, Department of Animal & Wildlife Science, Pretoria, Gauteng, South Africa, [2]Agricultural Research Council, Biotechnology Platform, Pretoria, Gauteng, South*

Africa, [3]Teagasc, Animal and Grassland Research and Innovation Center, Fermoy, Cork, Ireland.

Incorporating genomic breeding values (GEBVs) into selection decisions has become a key focus in the South African (SA) beef industry especially for local, commercial breeds. Using commercially available high-density single nucleotide polymorphism (SNP) panels for genomic selection (GS) is financially unfeasible, while many lower-density panels are not optimized for SA breeds suffering from ascertainment bias. The development of a low-density panel (LDP) optimized for SA breeds hence is necessary. The aim of this study was to determine the most appropriate method of selecting SNPs for inclusion on a LDP by establishing the minimal number and ideal SNP characteristics required to achieve acceptable imputation accuracy to higher density. Selection methods were based on 1) random sampling (RAN), 2) even dispersal across the genome (EVE), 3) maximizing minor allele frequency (MAF) and 4) a combination of MAF and linkage disequilibrium (MAFxLD) to construct 2.5K, 5K, 10K, 20K and 50K panels. Unselected SNPs were masked and imputed for a 235-animal validation population (youngest animals with no more than 3 paternal sibs) using 120 608 SNPs of 900 animals as reference. Across all SNP densities, MAFxLD outperformed other methods. Mean ± SD correlation-based imputation accuracy per animal (COR_{ANIM}) ranged from 0.872 ± 0.075 (2.5K) to 0.985 ± 0.014 (50K) for the RAN method and from 0.908 ± 0.063 (2.5K) to 0.988 ± 0.011 (50K) for the MAFxLD method. Using the MAFxLD method, mean ± SD allele concordance per animal (ACR_{ANIM}) exceeded 97% (0.970 ± 0.022), 98% (0.982 ± 0.014) and 99% (0.992 ± 0.007) when 5K, 10K and 50K SNPs were used. A strong relationship (r = 0.712; P < 0.001) was observed between autosomal length and mean SNP-based allele concordance (ACR_{SNP}). High-MAF SNPs (0.4 < MAF ≤0.5) displayed a 0.071 unit stronger SNP-based correlation (COR_{SNP})and 0.039 lower ACR_{SNP} than low-MAF SNPs (MAF <0.1) using the RAN method. Results suggest an LDP of at least 5 000 to 10 000 SNPs would suffice in maintaining less than 3% imputation errors for SA Drakensberger cattle and that SNP attributes such as MAF and LD should form the basis of SNP selection.

Key Words: cattle and related species, genotyping, imputation, genetic improvement

OP192 Accuracy of imputation from SNP array data to whole-genome sequence data in cattle. Y. Jiang*[1], Z. Zhang[2], L. S. Huang[2], Q. Zhang[3,1], and X. Ding[1], *[1]China Agricultural University, Beijing, China, [2]Jiangxi Agricultural University, Nanchang, China, [3]Shandong Agricultural University, Taian, China.*

The cost for sequencing large number of individuals in high depth is still high, despite the decrease per gigabyte data. But the same density markers comparable to sequencing can be obtained by imputation, which makes the most use of SNP array data and improves the power of genome association analysis, genomic selection, meta-analysis and fine-mapping. With the implementation of 1000 Bull Genomes Project, a large database for imputation was provided for cattle. However, the accuracy of imputation was not fully investigated, which varies greatly and measured differently across studies. Therefore, this study was further to investigate the factors affect imputation accuracy. In total, whole-genome sequencing data of 1585 Bos Taurus, 53 Bos Indicus and 27 Chinese yellow cattle were provided by the 1000 Bull Genomes Project (Run5.0). And the markers were masked to the Illumina BovineSNP150 BeadChip for target individuals, then imputed to the sequencing level by the new strategy, which we proposed based on Beagle4.1. Only bi-allelic SNPs in chromosome 1 were used for imputation, and all the sites with imputation and sequencing genotype were used to evaluate the accuracy of imputation, which was evaluated by the percentage of correctly imputed genotypes (PERCG) and the squared correlation between most probable REF dose and true REF dose (AR2). First, the 27 Chinese yellow cattle were used as target panel and the remaining individuals were used as reference panel, the individual level of PERCG varied from 75.94% to 95.92%, among which Mongolian achieved the highest imputation accuracy, followed by Yanbian, Hasake, Xizang cattle, which was 95.92%, 95.06%, 93.04 and 91.51% respectively. The Nanyang cattle achieve the lowest imputation accuracy, followed by Liping, Fujian, Dehong, values of 75.94%, 77.58%, 78.68% and 79.21% respectively. And other hybrid cattle, such as Dengchuan, Guanling, Luxi, Qinchuan ranges from 84% to 90%. Second, results showed that AR2 was sensitive to MAF. The variants in higher MAF were imputed more accurately and changed greatly from 0 to 0.65 when MAF increased from 0 to 0.15.

Key Words: imputation, sequencing, cattle

OP193 Genome-wide identification and characterization of InDels and SNPs in *Black Bengal goat* for breed identification. M. B. R. Mollah*[1], M. S. A. Bhuiyan[1], M. A. M. Y. Khandoker[1], M. A. Jalil[2], and G. K. Deb[2], *[1]Bangladesh Agricultural University, Mymensingh, Bangladesh, [2]Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh.*

The goat is a predominant livestock species in Bangladesh and it is land to the native Black Bengal goat (BBG), exotic breeds such as the Sirohi, Beetal and Jamnapari, and crossbreds between the BBG and exotics. The BBG is a dwarf-sized heritage goat, well known for its high fertility, excellent meat and skin quality. Mislabeling and selling of meat from exotic goats and crosses as BBG meat is evidently due to high demand and premium price of BBG meat in specialty market. Therefore, this study was carried out to find BBG breed specific SNPs and indels for identification of BBG breed. We sequenced the whole genome of a pedigreed BBG and Jamnapari goat by illumina Hiseq platform. A total of 1,500,245,820 raw reads consisting of 222.54 Gb nucleotides were obtained by sequencing a male BBG and Jamnapari goat. After quality filtering, the sequence reads from BBG were aligned to the San Clemente and the Yunnan black goat genome which resulted in 98.65% (properly paired, 94.81%) and 98.50% (properly paired, 97.10%) of the reads aligning, respectively. A total of 9,497,875 high quality SNPs (Q = 20) along with 1,023,359 indels, and 8,746,849 SNPs along with 842,706 indels were identified in BBG against the San Clemente and Yunnan black goat genomes respectively. Then we compared these indels and SNPs between BBG and Jamnapari to find unique indels and SNPs in BBG. Additionally, we utilized short read sequence data of 9 other goat breeds from NCBI short read archive (SRA) and aligned them within the identified SNP and indels regions of BBG by bwa-mem short read aligner, followed by visualizing in IGV browser. Indels and SNPs regions were manually curated to find unambiguous indels and SNPs unique in the BBG. By utilizing the whole genome data from BBG, Jamnapari and other goat breeds, we unambiguously identified 16,075 SNPs and 942 indels specific to BBG genome. These BBG-specific SNPs and indels could serve as marker tags for Black Bengal goat breed identification.

Key Words: goats and related species, genome sequencing, SNPs/Indels, sequence variation, breed identification

OP194 Whole-genome sequencing unveils helmeted guinea fowl (*Numida meleagris*) domestication in West Africa. M.-S. Peng[1,2,3,27], A. C. Adeola[1,2,27], Q.-K. Shen[1,2,3,27], S. Duan[4,27], Y.-W. Miao[5], Y. Du[4], M.-S. Wang[1,25,26], J. K. Lichoti[6], O. S. Charles[7], O. J. Sanke[8], P. M. Dawuda[9], A. O. Okeyoyin[10], J. Musina[11], P. Njoroge[11], B. Agwanda[11], M. G. Strillacci[12], E. Gorla[12], A. Bagnato[12], S. Kusza[13], H. A. Nanaei[14], R. Pedar[14], N. T. Abdulloevich[15], M. E. Afanasevna[15], K. B. Ibrohimovich[15], S.-F. Wu1, X. Chen[16,17], W.-K. Yang[16,17], N. O. Otecko[1,2,3], R. W. Murphy[18], L. M. Nneji[1,2], A. Esmailizadeh[1,14], Y. Dong[19,20,21], S. C. Ommeh*[11,22], and Y.-P. Zhang[1,2,3,23,24] *[1]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China, [2]Sino-Africa Joint Research Center, Chinese Academy of Sciences, Nairobi, Kenya, [3]Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, China, [4]Nowbio Biotechnology Company, Kunming, China, [5]Faculty of Animal Science and Technology, Yunnan Agricultural University, Kunming, China, [6]State Department of Livestock, Ministry of Agriculture Livestock Fisheries and Irrigation, Nairobi, Kenya,*

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The domestication and evolution of the helmeted guinea fowl (*Numida meleagris*) in Africa remains elusive. Herein, we report the de novo assembly of the domesticated helmeted guinea fowl genome and new whole-genome sequences for 135 helmeted guinea fowls including domesticated ones, wild ancestors and closely related species. Samples were collected from wild, feral and domestic helmeted guinea fowl populations from Nigeria, Kenya and other reference samples. DNA was extracted using QIAamp DNA Mini Kit (QIAGEN). The libraries were constructed and sequenced with the Illumina HiSeq 2500 system. This was followed by quality control, mapping and scaffolding and SNP calling. Total RNA was extracted from tissue samples using TRIzol Reagent (Invitrogen) and then purified the RNA using RNAeasy Mini Kit (QIAGEN). According to Illumina's standard pipeline, RNA-sequencing libraries were prepared from the total RNA of each of the 10 tissue samples, and then sequenced on the Illumina HiSeq 2500 platform. The raw data was filtered for quality control and assembled. Comparative genomic analyses with other avian species of economic interest revealed signals of selection on genes related to pathogen resistance and adaptation to harsh environments as those experienced in Africa. Inference on population history analysis indicates an origin in West Africa around 4,000–7,000 years ago. Scanning for selective sweeps detected a strong candidate gene *GRIK1* for neural change and behavior shift in domestication. In addition to presenting a missing piece to the jigsaw puzzle of domestication in poultry, our study provides valuable genetic resources for researchers and breeders to improve breeding and production of helmeted guinea fowls in Africa against a backdrop of climate change. These authors contributed equally to this work: MS Peng, AC Adeola, QK Shen and SC Duan.

Key Words: animal domestication, evolutionary genomics, genetic improvement, genome sequencing, poultry and related species

OP195 Genetic diversity of the *HSP70* gene in the native chicken (*Gallus gallus domesticus* L.) breeds of the Philippines. M. V. Valdez Jr*[1], R. V. F. Castillo[1], R. G. T. Romero[1], C. S. Daljog[1], R. C. Thomas Jr[1], and R. C. Santiago[2], *[1]Department of Biological Sciences, Institute of Arts and Sciences, Far Eastern University, Manila, Philippines, [2]National Swine and Poultry Research Center, Bureau of Animal Industry, Department of Agriculture, Quezon, Philippines.*

Climate change and population growth are major stressors on livestock production. Heat stress caused by climate change leads to high mortality and low productivity in the chicken livestock industry. Mitigation measures are needed to be implemented and improvements in the genetics of economically important livestock are considered as one of the most sound programs. Thus, this study elucidated the genetic diversity of the *HSP70*gene in 7 recognized native chicken breeds of the Philippines based on molecular analysis as a prior step in developing breed improvement. The partial exon of *HSP70* gene was amplified using primers designed from red-jungle fowl *HSP*70 gene sequence (J02579). The 5′UTR fragment was cloned in *puc19* vector before DNA sequencing. A total of 39 single nucleotide polymorphisms (SNPs) were identified. There were 14 observed haplotypes; 9 were breed-specific and 5 were shared between chicken breeds. The native chickens are characterized by low nucleotide diversity ($P = 0.003475$) and high haplotype diversity ($h = 0.796$). Haplotype distribution indicates unique haplotypes prevalent in breeds from the Southern Philippines. Analysis of molecular variance showed strong differentiation between breeds ($F_{st} = 0.22738, P > 0.05$). Lastly, the heat stress tolerant genotype A258A reported from native chickens found in other countries was detected in 4 out of 7 native chicken breeds of the Philippines. Thus, these native breeds of chicken from the Philippines might be a potential source population in developing heat stress tolerant chickens. Findings from this study will provide crucial baseline information regarding the molecular characteristics of the *HSP70*gene of the Philippine native chickens important in addressing the impacts of climate change on livestock production.

Key Words: heat tolerance, 5′ UTR of *HSP70* gene, SNP, haplotype diversity

OP196 Indigenous African sheep genomes reveal insights on fat-tail deposition and morphology. A. Ahbara*[1,2], H. Musa[3], E. Clark[4], C. Robert[4], M. Watson[4], A. Abeba[5], S. Latairish[6], O. Hanotte[7], and J. Mwacharo[8], *[1]School of Life Sciences, University of Nottingham, Nottingham, UK, [2]Department of Zoology, Faculty of Sciences, Misurata University, Misurata, Libya, [3]Faculty of Medical Laboratory Sciences, Khartoum, Sudan, [4]The Centre for Tropical Livestock Genetics and Health, The Roslin Institute, University of Edinburgh, Edinburgh, UK, [5]Debre Berhan Research Centre, Debre Berhan, Ethiopia, [6]Department of Animal Production, Faculty of Agriculture, Misurata, Libya, [7]LiveGene, International Livestock Research Institute, Addis Ababa, Ethiopia, [8]Small Ruminant Genomics, International Center for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia.*

African indigenous sheep represent 3 distinct tail phenotypes, fat-tail, fat-rump and thin-tail with clear geographic distribution across the continent. Current genetic data indicates that there is a strong genetic basis for these tail phenotypes. We generated and analyzed genome data from 60 long fat-tail, 32 short fat-tailed and 38 fat-rump sheep from Ethiopia and Libya (~30x coverage) and 20 thin-tailed sheep from Sudan (~10x coverage). Our findings, based on selection signature analysis, reveal 14 candidate regions under selection. The strongest region, spanning the *HOXB13* gene, suggests selection for vertebrate development and therefore tail length. Other candidate regions provide possible evidence for selection for stature, immune competency, thermal-response and fat-deposition. Our findings provide evidence implicating both human mediated and environmental selection pressures in molding genome variation associated with the tail phenotypes in sheep.

Key Words: genome, *Ovis aries*, Africa, fat-tail, thin-tail

OP197 Genomic selection: A pretentious paradigm needs implementation for river buffalo production in developing countries. M. Javed*, A. Nadeem, A. S. Hashmi, and W. Shehzad, *Institute of*

Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan.

Being an agrarian country, Pakistan and many other developing tropical countries are dependent on the livestock commodities for livelihood. According to a survey, nearly 62% of the rural population is directly or indirectly involved in livestock farming. River buffalo, being an efficient converter of poor roughage into valuable products like milk, is a unique animal which is well adapted to hot tropical climate. Genomic exploration of potentials of bovine resulted in many significant makers in cattle. Few of them are equally significant in buffaloes as well but many of them have not been found useful in buffalo. So there is dire need to explore the novel genomic loci which can be useful for substantial genomic improvement of buffalo. In the present research, the effect of genomic regions was studied for immunity, disease resistance, fertility and milk fat content by using candidate gene approach (CYP11b1, SCD, OLR1, INFg, Leptin, CYP19a1, OXT genes etc). Animal sampling was conducted in different phases. For dairy traits, animals were selected during the first month of second lactation and were categorized into 2 groups for low and high fat content (n = 250 in each group). For immunity against bovine tuberculosis (bTB), animals were selected after scanning the herds by tuberculin testing. For fertility (silent estrus behavior), animal selection was done after observing the signs of estrus for consecutive 3 cycles. After collecting blood samples, DNA was extracted by standard protocol. Then PCR amplification, by specific primer sets, and DNA sequencing was performed on pool samples of river buffaloes. Sequence alignment provided genomic variations in different study groups. Two SNPs were identified in CYP11b1, 2 in SCD and 1 was found in OLR1 gene. Restriction enzymes were selected for the genomic variants and PCR-RFLP was performed at larger population (n = 457). Association by One Way ANOVA revealed P17H in OLR1 gene and K158I in SCD gene was related to high fat content. Finally 3-D model of pertains was constructed and P17H was found in signal peptide of the OLR1 protein and K158I was found in transmembrane helix of SCD toward outer membrane of mitochondria and was found to enhance fat metabolism. Outcomes of the study are presenting the novel and informative genomic regions, which can be implemented in future buffalo breeding for selection of superior dairy animals.

Key Words: river buffalo, genomic selection, dairy, immunity, fertility

OP198 Advancing livestock genomics education and research in developing countries using strategies from the Virginia Tech PREP and IMSD training programs. E. Smith*, *Virginia Tech, Blacksburg, VA, USA.*

Between them, the PREP and IMSD programs have now yielded 64 PhDs who are pursuing careers in academia, research institutions, industry, and the government. Core strategies used to achieve this success include a cohort approach to graduate and undergraduate education, the use of Ombudspersons, peer and near-peer mentoring, active engagement with team members for each participant/trainee/ scholar. The demographics of our trainees, being from disadvantaged backgrounds, may make our approach a model for increasing genomics expects in developing countries.

Key Words: genomics education, developing countries

Microbiomes

OP199 A comprehensive strategy to assemble the rumen metagenome with long reads and detect high methane emitters. O. González-Recio*[1,2], M. Gutiérrez-Rivas[1], R. Atxaerandio[3], I. Goiri[3], J. Rey[3], J. Tamames[4], F. Puente-Sánchez[4], C. Gonzalez[1], J. A. Jiménez-Montero[5], and A. Garcia-Rodriguez[3], *[1]INIA, Madrid, Spain, [2]Departamento de Producciones Agrarias. Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas. Universidad Politécnica de Madrid, Madrid, Spain, [3]NEIKER-Tecnalia, Vitoria-Gasteiz, Spain, [4]Spanish Center for Biotechnology, CSIC, Madrid, Spain, [5]CONAFE, Valdemoro, Spain.*

To modulate the processes of fermentation in the rumen toward greater feed efficiency and lower methane emissions, it is crucial to understand the functions of these microbial genes and their relationship with the host animal. Third generation sequencing Oxford Nanopore Technology (ONT) provides long reads (several thousand bases), but with lower base calling accuracy than that from the considered gold standard (Illumina). The analysis of the microbiome presents 2 additional main limitations: the lack of a standardized analysis of the metagenome, and the peculiarity of dealing with compositional and zero inflated data in the statistical analyses. In this study we present an alternative to analyze the rumen metagenome obtained from ONT sequencing, and methods to analyze the compositional nature of the data. The METALGEN project has sequenced 204 samples of ruminal fluid with ONT from animals in 7 commercial farms, with individual methane emissions records during milking. A hybrid assembled metagenome was obtained from Nanopore and Illumina reads using Canu and Pilon with a total number of 24,818 contigs (N50 = 30,507bp). Subsequently, SqueezeMeta v0.4.3 was used to analyze the quality of the assembly, assign taxonomy, binning and gene detection. Sixty-one percent (15,125) of the contigs were assigned at the phylum level, and 28% (6851) at the genus level. The binning process assigned contigs in 71 genomes, 14 of which had a completeness >75%, with contamination < 10%, and were assigned to the genera of *Prevotella* and *Succiniclasticum*. A total of 420,555 genes were detected, but only 33% were assigned to genes with known KEGG function. To analyze the association between the metagenome and enteric methane, the compositional nature of the microbiota data was taken into account, performing a transformation of Aitchison on the abundances of the contigs detected in the samples. The centered log ratios of contig abundances were used to predict methane production using machine learning methods. The contigs with larger variable importance at classifying the high and low emitter animals were assigned to both known and unknown taxa, obtaining a classification accuracy between 60% and 95%. Our study confirms the lack of knowledge in the databases about the microbial genes that populate the rumen, but revealed that there is potential to use the information in an agnostic manner to classify phenotypes.

Key Words: rumen metagenome, Nanopore, methane, compositional data

OP200 Going full circle: Assembly of high-quality, single-contig microbial genomes from the rumen microbiome using long-read sequencing. A. Warr*[1], R. Stewart[1], M. Aufrett[2], A. Walker[3], R. Roehe[2], and M. Watson[1], *[1]The Roslin Institute, Edinburgh, UK, [2]SRUC, Edinburgh, UK, [3]The Rowett Institute, Aberdeen, UK.*

Ruminants such as cows and sheep are important livestock species. They convert low nutritional value plant matter into high-quality meat and dairy products. Within a specialized stomach called the rumen, microbes ferment the plant matter producing short-chain fatty acids from difficult to digest plant matter. The composition of the rumen microbial community can affect the animal's health, feed efficiency and level of methane production. Species in the rumen are typically difficult to culture and despite its importance, it remains an underexplored environment. DNA sequencing of the contents of the rumen offers the potential to identify microbial species without culture techniques. Here we sequence fluid from the rumen of a single cow using Nanopore sequencing. We show that despite these data coming from a highly complex microbial sample we can assemble high-quality, single-contig whole genomes and plasmids of known and novel species, including numerous circular contigs. Additionally, we compare and validate the

assemblies of these genomes with binned genomes generated from short read Illumina assemblies of 282 cow rumen samples. We show that the long read assembly outperforms the short read assembly in contiguity and in the incorporation of AMR genes and marker genes.

Key Words: metagenomics, cattle and related species, microbiomics

OP201 High-throughput metagenome sequencing for prediction of quantitative traits. M. Hess*[1], L. Zetouni[1], J. Budel[1], T. Van Stijn[1], H. Henry[1], R. Brauning[1], A. McCulloch[1], S. Hickey[2], A. Hess[1], M. Kirk[3], S. Kumar[3], N. Morton[4], H. Flay[5], S. Kittelmann[3], G. Henderson[3], S. Hendy[4], G. Wood[1], G. Attwood[3], J. McEwan[1], and S. Rowe[1], *[1]AgResearch Ltd., Mosgiel, New Zealand, [2]AgResearch Ltd., Ruakura, New Zealand, [3]AgResearch Ltd., Palmerston North, New Zealand, [4]Te Pūnaha Matatini, University of Auckland, Auckland, New Zealand, [5]DairyNZ, Hamilton, New Zealand.*

The rumen microbiome plays an important role in feed digestion and is associated with a range of economically and environmentally important traits e.g., methane production. Our objective was to develop a low-cost, high-throughput approach for metagenome sequencing using restriction enzyme reduced representation sequencing to obtain metagenome profiles for large-scale prediction of quantitative traits. DNA was extracted from freeze-dried and ground rumen samples then digested with the restriction enzyme *Pst*I. A library was generated from between 118 and 330 samples, depending on the data set; after selecting 193–318 bp fragments, the library was sequenced in one lane on a HiSeq2500. After demultiplexing and quality trimming, metagenome profiles were obtained using either a reference-based (RB) or reference-free (RF) approach. The RB approach involved BLASTing reads against the Hungate 1000 Collection of genome assemblies and assignment to genus. The RF approach identified common 65 bp tags, present in >25% of samples, within the data set and counted the frequency of each tag in each sample. Our approach has been tested on 3 data sets: D1) 236 sheep rumen samples from 118 high- or low-methane sheep; D2) 205 rumen samples from New Zealand lambs; D3) 186 rumen samples from New Zealand dairy cattle. The average number of reads per sample ranged from 760k to 2.7M, depending on the number of samples per lane. On average, 6–10% of reads were assigned at the genus level using the RB approach. On average 40–65% of the reads for each sample were captured in the metagenome profile using the RF approach. A comparison between D1 and 16S rRNA gene sequencing on the same samples showed more repeatable profiles with our approach than 16S (0.62 ± 0.06 vs 0.45 ± 0.08). Data sets were visualized using network analysis to identify relationships between samples or taxonomic groups. A microbial relationship matrix was added to models of methane emissions, showing the variance explained by the microbial component outweighs the host genetic component. Our approach will be used to sequence thousands of rumen samples over the next year and is easily adapted to other sample types.

Key Words: metagenomics, high-throughput sequencing (HTS), environment, network analysis, genomic prediction

OP202 Link-HD: A versatile integrative approach applied to across-kingdom microbial communities. M. L. Zingaretti*[1], G. Renand[2], D. Morgavi[3], and Y. Ramayo-Caldas[2,4], *[1]CRAG, Bellaterra, Barcelona, España, [2]INRA, Jouy-en-Josas, Paris, France, [3]INRA, Saint Genès-Champanelle, France, [4]IRTA, Caldes de Montbui, Barcelona, España.*

We present Link-HD, an approach to integrate heterogeneous data sets, as a generalization of STATIS-ACT ("Structuration des Tableaux A Trois Indices de la Statistique - Analyse Conjointe de Tableaux"), a family of methods to join and compare information from multiple subspaces. However, STATIS-ACT has some drawbacks since it only allows continuous data and it is unable to establish relationships between samples and features. To tackle these constraints, we incorporate multiple distance options and a linear regression based Biplot model to the traditional approach. We illustrate the usefulness of Link-HD to integrate ruminal metataxonomic communities (including bacteria, archaea and protozoal data) from 65 Holstein cows of which methane yield (CH4y, g CH4/kg feed intake) was individually measured. We assessed the relationships of predicted interactions with CH4y and we compared our results with predictions obtained with the integrative approach implemented in MOFA (Multiple Omics Factorial Analysis). Link-HD predicted a common sub-space composed by a mix of features from the 3 communities that allowed us to classify samples into 3 subgroups (C1, C2, C3). Sample cluster assignation was significantly associated with CH4y as cows that clustered within C1 had higher emissions (P = 0.006). The latent factor of MOFA analysis, which captures the co-variation between the 3 data sets, was consistent with our results and also classified samples into 3 clusters. Furthermore, MOFA confirmed the relevance of 52% of bacteria, 58% of archaea and 100% of protozoal features reported by Link-HD. According to both approaches, the ruminal microbiota of higher CH4-emitter cows was dominated by OTUs classified as Rumminoccaceae, Bacteroidales and Methanobrevibacter, and these cows showed a lower abundance of Succinivibrionaceae and Trichomitus. Consistent with literature reports, a co-exclusion between Succinivibrionaceae and Methanobrevibacter was observed. In summary, our results allow identifying interactions between microbial communities associated with CH4y emissions and suggest the usefulness of Link-HD to integrate heterogeneous data sets and explorer the impact of predicted interactions on socio-economical relevant traits.

Key Words: integrative genomics, microbiomics, computationalpipeline, cattle and related species

OP203 Metagenomic de novo assembly of *Corynebacterium bovis* in lactating Assaf sheep: A preliminary study. C. Esteban-Blanco*[1], F. Puente-Sánchez[2], B. Gutiérrez-Gil[1], H. Marina[1], J. Tamames[2], and J. J. Arranz[1], *[1]University of Leon, León, Castilla y León, Spain, [2]CNB-CSIC, Madrid, Madrid, Spain.*

The sheep milk microbiota is a complex community, which may have a major impact on host health and on the quality of the milk as a food product. High-throughput sequencing enables comprehensive microbial surveys with detection sensitivities higher than earlier molecular techniques as the 16S rRNA gene sequencing. In addition, the computational tools recently developed for metagenomic sequencing analysis attempt to classify the sequences present in a metagenomic data set into different species. In this context, the aim of this work was to characterize the milk microbiota retrieving taxa present in ovine milk samples using the binning approach. In total, 14 Assaf dairy ewes from a single flock (Zamora, Spain) and without clinical signs of mastitis were included in this study. The samples were classified as derived from healthy and subclinical mastitis dairy ewes based on somatic cell counts (SCC). The SqueezeMeta software pipeline was used in this study for retrieving individual genomes and for analyzing the structure and functionality of microbiomes. After removing host sequence reads (contamination), SqueezeMeta assembled 11.5 million bacterial raw reads into 31,902 contigs. After taxonomic assignment of contigs, the predominant phyla were *Actinobacteria*, *Firmicutes* and *Proteobacteria*. Some contigs were assigned to a "*Corynebacterium bovis*" bin. Interestingly, the contigs of 3 healthy samples (low SCC) make up almost the total of this bin. Our previous study based on 16S rRNA gene sequencing from 50 Assaf ewes reported that the *Corynebacterium* genus was also one of the most prevalent genera in the microbiota of the sheep mammary gland. In dairy cows, *Corynebacteria* are usually associated with low SCC milk, although specifically, *Corynebacterium bovis* is frequently isolated from milk samples of infected mammary glands. Here, we provide a recovered *Corynebacterium bovis* strain genome from milk sheep samples with low SCC values, which are associated with a healthy mammary gland status.

Key Words: sheep, metagenomics, high-throughput sequencing, animal health

OP204 Genomic surveillance of gut microbiome of Dohne merino sheep by MinIon sequencing. P. Soma*[1], R. Pierneef[2], B.

Kooverjee[1], and F. Muchadeyi[2], *[1]Agricultural Research Council, Animal Production, Irene, Pretoria, South Africa, [2]Agricultural Research Council, Biotechnology Platform, Onderstepoort, Pretoria, South Africa.*

Dohne Merino sheep of South Africa are bred and selected for high fertility, rapid lamb growth and wool production. The gastrointestinal tract (GIT) of sheep contains complex microbes that influence its health and development. Molecular approaches for microbial analysis of gut samples have become a widely accepted method to characterize microbial communities. The introduction of third generation sequencing platforms such as Oxford Nanopore Technologies (ONT) MinIon platform, has resulted in long sequencing reads that can be used to study human health, disease and antimicrobial resistance. Abomasum contents from 7 sheep naturally infected with *Haemonchus contortus* were collected and microbial DNA extracted using QIAamp DNA Microbiome Kit. Each DNA sample was barcoded and sequenced using the MinIon system. The bacterial composition of the abomasum was investigated at phylum, class, order, family, genus, and species levels using R packages. A total of 24 phyla were detected, where 16 were present in all samples. *Firmicutes* (78.35%) and *Bacteriodetes* (13.03%) were the most abundant phyla present in the GIT. Furthermore, *Cyanobacteria* (0.23%), *Patescibacteria* (0.19%), *Spirochaetes* (0.18%), *Epsilonbacteraeota* (0.14%), *Synergistetes* (0.13%), *Fibrobacteres* (0.10%) and *Elusimicrobia* (0.07%) were the least abundant phyla. A total of 39 genera (n = 7) with operational taxonomic units (OTU) abundance greater than 1% were identified across all samples. *Christensenellaceace R-7* group (9.93%), *Christensenellaceace R-7* group (rumen) (7.08%) and *Rikenellaceace RC9* gut group (5.18%) represents the top 3 core genera. A total of 3404 species were detected in all samples, however, they were dominated by uncultured bacterium of specific genera. Results indicate that several genera contribute to the differences in community composition between individuals. This is the first study to characterize the abomasum contents of South African Dohne merino sheep by use of ONT MinIon technology, expanding our knowledge of sheep microbiota. This information may allow the possible use of symbiotic bacteria as biocontrol of *H. contortus*.

Key Words: microbiomics, small ruminants, biodiversity, nematodes

OP205 Correlated responses to selection for intramuscular fat in the metagenomic profile on three gut sites in rabbits. M. Martínez-Álvaro, A. Zubiri-Gaitán*, A. Blasco, and P. Hernández, *Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Valencia, Spain.*

This study reveals the effect of divergent selection for intramuscular fat (IMF) on the rabbit's metagenomic profile. The novelty of this work lies on the study of the observed correlated response in the microbial genes abundances after a selection procedure, which implies a link between the genes of the individuals and the genes of their microbes. We considered 3 different gut sites, ileum, cecum and feces, to additionally discover whether selection has had a stronger effect in any of these sites. Samples were taken from 16 and 17 rabbits divergently selected for high (H) and low (L) IMF during 10 generations. The selection procedure showed a mean response of 0.5 standard deviations per generation. Counts of microbial genes in a sample are compositional variables, constrained by the sequencing depth of the instrument. This restriction creates dependencies between the microbial genes, since an increase in the abundance of one gene requires a decrease for some other genes. For this reason, we analyzed our data with specific compositional data statistic tools. We used *Selbal* algorithm to find the linear combination of the log-transformed microbial genes that better fits to the response variable, H or L line, in each gut site. We will reference this solution as the selected balance. Preliminary results showed that balances selected on each of the gut sites showed a similar high classification ability between H and L, showing R^2 ranging from 0.90 to 0.97 when being tested in a log regression model. Selected balances were composed by a low number of microbial genes in all sites, from 2 to 6. For instance, in cecum, glucose 1-dehydrogenase (K00034) with other 2 microbial genes from unknown pathways (K03439 and K03969) comprised the numerator of the selected balance, and malate-CoA ligase subunit β (K14067) comprised the denominator. H animals were associated with lower balance values; that is, larger relative abundances of K00034, K03439 and K03969 referred to K14067, in comparison to L animals. This preliminary analysis shows that selection for IMF led to a correlated response in the ileum, cecum and feces metagenomic profiles.

Key Words: metagenomics, selection, fat/lipid, compositional data, rabbits

OP206 Unraveling the effects of the gut microbiota composition and function on horse endurance physiology. S. Plancade[1], A. Clark[2], C. Philippe[3], J.-C. Helbling[4], M.-P. Moisan[4], D. Esquerre[5], L. Le Moyec[6], C. Robert[7,8], E. Barrey[7], and N. Mach*[7], *[1]MaIAGE, INRA, Université Paris-Saclay, Jouy en Josas, France, [2]Gastroenterology Department, Vall d'Hebron Institut de Reserca, Barcelona, Spain, [3]UMR 1319, INRA, AgroParisTech, Université Paris-Saclay, Jouy en Josas, France, [4]UMR 1286, INRA, Université Bordeaux, Nutrition et neurobiologie intégrée, Bordeaux, France, [5]UMR 444, INRA, Plateforme GET, Castanet-Tolosan, France, [6]Unité de Biologie Intégrative et Adaptation à l'Exercise, UBIAE, EA7362, Université d'Evry, Evry, France, [7]UMR 1313, INRA, AgroParisTech, Université Paris-Saclay, Jouy en Josas, France, [8]Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France.*

An integrated analysis of gut microbiota, blood biochemical and metabolome profiles in 52 endurance horses was performed. Clustering by gut microbiota composition revealed the existence of 2 communities that varied in taxa and functional composition. Community type 1 presented a low abundance of fibrolytic and cellulolytic bacteria as well as anaerobic fungal loads, but a higher abundance of pathobionts and the predicted lipopolysaccharide biosynthesis pathway. Moreover, its microbial diversity was higher than community type 2, meaning these individuals likely had a greater ability to respond to abiotic and biotic stressors in the gut. Presumably, the 2 communities were mainly driven by diet as host properties showed little effect.Community type 1 was associated with lower estimated daily forage and energy intakes, but higher proportion of acetate in feces and predicted pathways involved in lipid metabolism. Conversely,community type 2 was related to higher proportions of propionate and butyrate in feces together with predicted pathways related to carbohydrate metabolism, but also inflammation and fatigue, evoking an adaptation of gut microbiota to mitigate the effects of stress-induced gut dysfunction during endurance. However, the higher butyrate proportion in community type 2 was not associated with protective effects on telomere lengths. At resting time, community type 1 correlated with some blood metabolites, which may reflect compensatory mechanisms to render the gut mucosa less susceptible to endotoxin translocation during the race. Unexpectedly, the gut microbiota was neither associated with the blood biochemical markers nor metabolome during the endurance race, and did not provide a biomarker for risk of failure to finish the race or race ranking.The current data supports that the gut microbiota may exert a direct or indirect influence on physiological adaptations to endurance in horses.

Key Words: endurance, horse, microbiota, gut, systems biology

OP207 Microbiome and metabolome changes occurring in early *Salmonella* Typhimurium infection in pigs. H. Arguello*[1], S. Zaldívar-López[1], N. Bellido[1], F. Priego-Capote[2,3], Á. Jiménez-Marín[1], L. Morera[1], and J. J. Garrido[1], *[1]Animal Breeding and Genomics Group, Department of Genetics, University of Córdoba, Córdoba, Spain, [2]Department of Analytical Chemistry, Annex Marie Curie Building, Campus of Rabanales, University of Córdoba, Córdoba, Spain, [3]Institute of Biomedical Research Maimónides (IMIBIC), Reina Sofia University Hospital, University of Córdoba, Córdoba, Spain.*

Salmonella enterica infections, in particular those caused by the serovar *S.* Typhimurium, are mainly known by their food safety relevance. From a veterinary perspective, swine salmonellosis is character-

ized in the early infection by clinical signs such as diarrhea and fever associated with intestinal epithelial damage and the strong inflammatory response, particular intense at the ileum. *Salmonella* Typhimurium infection in pigs prompts changes in the gut microbiota. After a 2-d in vivo experimental infection with *S.* Typhimurium, metagenomic analysis of ileum mucosa and content (n = 12) was performed. Analysis of changes in 16S operational taxonomic units (OTUs) revealed a concomitant drop of abundance of representatives of the genus *Lactobacillus*, and other commensal beneficial bacteria such as *Bifidobacterium* and normal gut microbiota such as *Prevotella* in the ileum (content and mucosa). In addition, hierarchical clustering based on OTU abundances grouped separately ileum mucosa samples from infected and control samples. Anaerobic bacteria such as *Clostridium spp*., *Ruminococcus* or *Allestipes* were linked to integrity of the ileum mucosa (healthy pigs). Abundance of synergistic or opportunistic bacteria such as *Akkermansia muciniphila* or *Citrobacter* were increased after infection and correlated with damage of the epithelium of the ileum mucosa. These microbiome changes must be linked to changes in the gut environment. To accomplish this objective, we performed metabolomics (GC/MS-TOF and QTOF LC-MS/MS) in ileum content samples from *Salmonella*-infected and control pigs. More than 50 compounds linked to fatty acids, glucose or amino acid metabolism as well as other metabolic routes such as bile acids metabolism were detected in infected animals compared with controls. Interestingly, families such as *Desulfovibrionaceae, Pseudomonadaceae* and *Leptotrichiaceae* abundance was strongly correlated with variations in concentration of metabolites. At genus level, compounds such HODE linked to oxidative stress and inflammation was positively correlated with *Akkermansia* abundance, Dihydroxybutanoic acid was associated with the strict anaerobes Megasphaera or Dialister. These results demonstrate the link between microbiome and metabolome changes occurring within the intestine as consequence of *Salmonella* infection in pigs.

208 Gut microbiome, birth weight, and productive traits in pigs in Iberian × Duroc crossbred pigs. M. Vázquez-Gómez*[1], J. Estellé[2], C. Garcia-Contreras[3], R. Benitez[3], S. Astiz[4], J. L. Pesantez-Pacheco[5], C. López-Bote[1], A. Gonzalez-Bulnes[4], B. Isabel[1], and C. Ovilo[3], *[1]Faculty of Veterinary Medicine, UCM, Madrid, Spain, [2]GABI, INRA, AgroParis Tech, Université Paris-Saclay, Jouy-en-Josas, France, [3]Department of Animal Breeding, INIA, Madrid, Spain, [4]Department of Animal Reproduction, Madrid, Spain, [5]School of Veterinary Medicine and Zootechnics, UCuenca, Cuenca, Ecuador.*

Low birth-weight (BW) animals, which resulted from intrauterine growth restriction, have an altered intestinal microbiota at early stages that might produce long-lasting effects on metabolism. During the postnatal period, low birth-weight pigs show slow growth, metabolic dysfunctions and different meat composition with differences by sex. Thus, our goal was to study the effect of BW and sex on gut microbiome in adult pigs at slaughter and its possible relationship with different productive traits. In a commercial farm, the productive life of 52 Iberian × Duroc crossbreed pigs, assigned to low BW (LBW, 0.83 ± 0.07 g; 7 females and 6 males) and normal BW (NBW; 1.33 ± 0.23 g; 18 females and 21 males) groups at birth, were followed until slaughter. Males were surgical-castrated after birth and females were inmuno-castrated during the growing-fattening phase. Fecal microbiota composition at slaughter was analyzed by re-sequencing the V3-V4 region of the 16S rRNA gene in an Illumina Miseq. Bioinformatic analyses identified a total of 1,127 operational taxonomic units (OTUs), out of which 330 OTUs were new, distributed in 105 genera. LBW pigs showed a trend for higher observed species richness than NBW pigs ($P = 0.06$). However, no differences were found between BW groups in β-diversity or abundance analysis. On the other hand, the gut microbiota Bray-Curtis dissimilarity among samples at slaughter was significantly related to productive traits such as age at slaughter ($P < 0.005$), slaughter and carcass weights ($P < 0.01$), final average daily weight gain ($P < 0.01$), 7 mo-old back-fat depth, and Ω-6/ Ω −3 ratio in subcutaneous fat ($P < 0.05$). Regarding sex effects, there were differences between females and males using weighted-UniFrac distance ($P < 0.05$) and an OTU of *Clostridiales* had greater abundance in males than females ($P < 0.05$). Overall, no major effects of a low BW on gut microbiome were found. However, the detected associations between microbiome and growth and meat quality traits should be further explored.

Key Words: castration, Iberian pig, IUGR, metagenomics, slaughter

OP209 The gut microbiota composition at slaughter as a potential certification tool for the Iberian pig traditional farming system. J. M. García-Casco[1], M. Muñoz[1], G. Lemonnier[2], J. M. Babilliot[2], O. Bouchez[3], A. I. Fernández[1], F. R. Massacci[2], M. A. Fernández-Barroso[1], A. López-García[1], C. Caraballo[1], C. Óvilo[1], and J. Estellé*[2], *[1]INIA, Centro de I+D en Cerdo Ibérico, Dpto. Mejora Genética Animal, Zafra, Badajoz, Spain, [2]INRA, UMR1313 GABI, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France, [3]INRA, US 1426 GeT-PlaGe, Genotoul, Castanet-Tolosan, France.*

The traditional raising system for Iberian pigs includes an open-air fattening period performed in the *Dehesa* forest, where animals have a diet dominated by acorns. This system allows obtaining dry-cured products of unmatched quality like the *ibérico* ham with the *bellota* label, and contributes to the preservation of the legacy *Dehesa* landscape and its socioeconomic viability. However, the advances on nutrition science makes feasible to emulate *bellota* meat products in an industrial farming environment (the so-called *cebo* quality label) and there is a growing need for the identification of complementary certification approaches. The objective of this study was to evaluate the potential of gut microbiota composition analyses from fecal samples collected at slaughter as a new discrimination method of the *bellota* production system. To this end, the gut microbiota composition of 84 *bellota* and 131 *cebo* Iberian pigs sampled in 13 slaughter batches was determined by re-sequencing the bacterial 16S gene in an Illumina MiSeq device. NMDS and PERMANOVA analyses performed showed significant effects of diet and sampling batch on gut microbiota composition. The microbiota of *bellota* animals showed also a tendency toward a reduced microbial diversity. Differential abundance analyses confirmed these differences and showed that abundances of 2 thirds of the 1703 OTUs identified were significantly different between *bellota* and *cebo* pigs. Finally, sPLS-DA analyses allowed the identification of a subset of 15 OTUs that were able to predict the production system with more than 99% accuracy. Overall, our results suggest that the gut microbiota composition of Iberian pigs sampled at the slaughter could be used as a supplementary certification tool of traditional *montanera* Iberian pigs. Funded by European Union's Horizon 2020 (grant agreement 634476). The European Union Agency is not responsible for any use made of the information contained in this abstract.

Key Words: pig, microbiota, certification, Iberian ham, production system

OP210 Lightning Talk: Identification of microbial profiles that promote honeybee colony health. J. Gorrochategui*[1], A. Fernandez[1], M. Muñoz-Colmenero[1,2], M. Parejo[1,3], M. Kovacic[4], J. Filipi[5], R. Buechler[6], A. Estonba[1], and I. Zarraonaindia[1], [1]*Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Leioa, Spain*, [2]*Institute of Marine Research, Vigo, Spain*, [3]*Swiss Bee Research Center, Agroscope, Bern, Switzerland*, [4]*Josip Juraj Strossmayer University of Osijek, Osijek, Croatia,* [5]*University of Zadar, Zadar, Croatia,* [6]*Landesbetrieb Landwirtschaft Hessen, Kirchhain, Germany.*

The European honeybee has suffered a constant decline. Main contributors are both, the accumulation of pesticides used in apiculture and agriculture, and a greater pathogen incidence. By modulating the microbiome associated with bees and their hives, it is possible to reinforce bee's immune system, and consequently, improve their response to chemical treatments and their susceptibility to diseases. The ERLEMIKRO project aims to characterize the microbial taxonomic and functional diversity of the hive (bee's gut, pollen bread, brood, …) for the identification of profiles that harbour symbiotic properties beneficial for honeybee health, either because (1) they are involved in the defense

against pathogens such as *Varroa destructor*, or because (2) they provide detoxifying functions against the commonly used chemical treatments coming from beekeeping practices or agricultural management. Those microorganisms, and or their genes, could be optimal candidates for future probiotic development, that could be used as an alternative strategy to the actual indiscriminate use of chemical treatments against honey bee diseases. Next generation sequencing approaches, such as 16S amplicon rRNA and ITS sequencing, as well as metatranscriptomics, will be used (1) to characterize and compare the taxonomic and functional composition of bacteria and fungi present in the hive before and after applying an organic anti-Varroa treatment (Oxalacetic acid), (2) to monitor the microbial community of hives located in contrasting environments (agricultural vs. pristine), ones treated and others non-treated against Varroa, and finally, (3) to examine the microbiome of hives that show natural tolerance to Varroa.

Key Words: honeybee resilience, Varroa, chemical residues, microbiome, probiotics

OP211 Lightning Talk: Holofood: A holo'omic solution towards sustainable animal food production. S. Marcos*[1], I. Zarraonaindia[1], D. Sandvang[4], M. Limborg[2], J. Zentek[5], D. Jozefiak[6], E. Johansen[4], J. Tarradas[3], A. Estonba[1], T. Gilbert[2], and A. Alberdi[2], *[1]Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Leioa, Spain, [2]Department for Evolutionary Genomics at University of Copenhagen's (UCPH) Natural History Museum of Denmark (SNM), Copenhagen, Denmark, [3]Institute for Food and Agricultural Research and Technology (IRTA), Tarragona, Spain, [4]Chr Hansen A/S, Hoersholm, Denmark, [5]Department of Veterinary Medicine Free University of Berlin (FUB), Berlin, Germany, [6]Piast Pasze Sp. z o.o. (Piast Group llc.), Poland.*

With the planet's population approaching 9 billion, one of the key global challenges of this century is to secure that the growing food production is performed in a sustainable fashion and with a low-carbon signature. It is well known that gut microorganisms play a pivotal role in the homeostasis of animals, and a balanced gut microbiota is essential for an optimal food production. The gut microbiomes can be manipulated through the use of feed additives such as prebiotics and probiotics. However, the specific means of action of most additives on the microbiome and host organisms is not acknowledged. HOLOFOOD is a European H2020 Innovation Action comprised of 10 partners from 6 European countries that showcases a holistic approach to improve the efficiency of food production systems by deciphering the biochemical interactions between animals and their associated microorganisms. HoloFood will be running until 2022. Newly developed holo'omic framework will be implemented to understand the biochemical interactions between broiler chickens and their intestinal microorganisms through the analysis of whole animal genomes, deep intestinal transcriptomes, microbial metagenomes, microbial metatranscriptomes and intestinal metabolomes, all of them in relation to key performance indices. A total of 1300 chickens will be biochemically, physiologically and phenotypically characterized through the analysis of over 15000 samples. The knowledge generated will be used to optimize the feed additive administration strategies of already implemented products, by tailoring them to the genetic background and developmental stage of the animals as well as production environment. The ultimate goal is to improve the quantity, quality and safety of the produced food, as well as sustainability of food production and increased animal welfare. HoloFood will also serve to raise awareness about the importance of microbiomes in food production, and to establish bridges between companies and academia to foster science-based strategies.

Key Words: aviculture, hologenomics, microbiome, probiotics

Plenary Session IV

OP212 Analysis of the world's sheep reveals contribution of frequent genomic introgressions from congeneric wild species to local climatic adaptation in domestic breeds. M.-H. Li*, *Institute of Zoology, Chinese Academy of Sciences, Beijing, China.*

How animals, particularly livestock, evolve to adapt well to various climates or a changing climate over relatively short time periods has been a fundamental question of evolution and ecology which remained largely unsolved. Also, understanding the genetic mechanisms underlying climatic adaptation for local livestock breeds is important for enhancing sustainability of their production in an uncertain environmental future. By examining genome-wide SNP data in the world's wild and domestic sheep, we conducted a comprehensive analysis of interspecies introgression and climate-mediated selective sweeps. We identified strong selective signatures in genes involved in climate-mediated pressures for adaptive importance. We detected clear and consistent signals of genomic introgressions in adaptive genes from wild species into sympatric domestic breeds, which are a source for climatic and environmental adaptation. In particular, the gene PADI2 associated with ovine pneumonia shows significant signals of adaptive introgression, which could account for the pneumonia-resistance in some domestic sheep breeds. We conclude that genomic introgressions from congeneric wild species have contributed important adaptive variants in response of domestic sheep to various climates. Our results provide important insights into how local livestock breeds could have successfully adapted to various climatic and environmental conditions across the world after their migration out of their domestication center. The variants associated with climatic adaptation and different production traits are important resources for future breeding by combining adaptive and productive advantages.

Key Words: *Ovis* species, genome-wide SNPs, resequencing, genomic introgression, climatic adaptation

OP213 Application of genomics to resolve livestock production and adaptation issues in developing countries. F. C. Muchadeyi*, *Agricultural Research Council-Biotechnology Platform, Onderstepoort, South Africa.*

Genetic mechanisms underlying biological traits for environmental adaptation are unclear but unravelling them would be essential when designing methods to improve and sustain livestock breeds. In developing countries, indigenous animal genetic resources play a crucial role in sustaining the livelihoods of rural and resource-limited farmers. Local breeds thrive on adaptation to unfavorable environmental stressors such as extreme temperatures, worsening droughts and disease challenges that characterize most low input production systems. Livestock improvement programs strive to develop genotypes that are adapted to local conditions and are able to produce optimally and sustainably under constrained environments typical of most developing countries. Elucidating the intertwined relationship between production environments and genetics of animals, with the aim of establishing selection priorities and develop suitable improvement strategies, is critical. Previously, development of improvement programs has been stalled by the absence of performance data and pedigree records and exacerbated by such factors as uncontrolled livestock breeding practices on communal pastures. Advances in livestock genomics have facilitated the production of "big data" in genetics through the advent of genome-wide cover-

age which, together with environmental data, allows in-depth studies of the patterns of genetic diversity, identification of genes and clarification of processes underlying genetic adaptation in various indigenous livestock. We shed more light on the opportunities and challenges of implementing genomics programs in developing countries by presenting case studies designed to understand the genetics of adaption in indigenous livestock of Southern Africa.

Key Words: genetic adaptation, low-input systems, livestock improvement

POSTER PRESENTATIONS

Animal Epigenetics

P1 Identification of epigenetic related genes associated with carcass traits in Nguni cattle using Illumina BovineSNP50 BeadChip. K. T. E. Makua[1,2], A. A. Zwane[2], K. A. Nephawe[1], and B. Mtileni*[1], *[1]Department of Animal Sciences, Tshwane University of Technology, Pretoria, Gauteng, South Africa, [2]Animal Production Institute, Agricultural Research Council, Pretoria, Gauteng, South Africa.*

Nguni cattle play an important role in the South African beef industry. Carcass improvement in traits related to beef quality is the initial concern in beef production. Epigenetic changes such as DNA methylation and histone acetylation provide new molecular information that complements those obtained from genotyping data. The aim of this study was to identify SNPs in epigenetic related genes associated with carcass traits in Nguni cattle. Purebred Nguni cattle (n = 60) were sampled across South African provinces and genotyped using the Illumina BovineSNP50 BeadChip. Data from the Afrikaner (n = 41), Bonsmara (n = 46), and Holstein (n = 41) breeds were used as reference populations. Breed specific SNPs were identified using Reynolds F_{st} and extended Lewontin and Krakauer's (FLK) statistics. Five SNPs were found to be significantly ($P < 0.05$) associated with carcass and meat quality. Three SNPs were identified in epigenetic related genes (DNMT1, DNMT3a and DNMT3b) while 2 SNPs were identified in calpain 1 (CAPN1) gene. The SNP marker Hapmap43686-BTA-78412 found in DNMT1 gene was a silent mutation located in intron 2 of chromosome 7 and was found to be highly associated with meat color score (MCS). The SNP marker BTB-00455991 of DNMT3a located in intron 33 of chromosome 5 were found to be highly associated with carcass weight (CW) and marbling score (MS). Marker BTB-015467689 of DNMT3b located in intron 33 of chromosome 1 was found to be highly associated with carcass weight (CW). Markers UA-IFASA-8199 and BFGL-NGS-14073 of CAPN1 were found to be highly associated with carcass weight (CW) and dressing percentage (DP) respectively. These SNPs can be used to further investigate their functional effects on meat quality traits.

Key Words: epigenetic, SNPs, Nguni cattle, genome-wide, identification

P2 Unpredictable light schedule causes sex-specific epigenetic and transcriptional changes in the chicken's pineal gland. F. Pértille*[1,2], N. Mitheiss[2], P. Løtvedt[2], L. L. Coutinho[1], P. Jensen[2], and C. Guerrero-Bosagna[2], *[1]Luiz de Queiroz College of Agriculture from University of São Paulo (ESALQ/USP), Piracicaba, São Paulo, Brazil, [2]Linköping University, Linköping, Ostegötland, Sweden.*

The environment where production animals are reared is fundamental to determine their later health and wellbeing. Stress in production animals generated by common production practices is a frequent issue of concern. In animals undergoing distress, hormonal responses are produced. These include variations in testosterone, epinephrine, prolactin and cortisol. Animals constantly subjected to stress and systemic hormonal changes have their epigenome affected in a variety of cell types. The pineal gland is a region of vertebrate brains that regulates the circadian rhythm of individuals through the secretion of melatonin according to the patterns of light exposure they experience. These mechanisms require gene activation dependent upon light exposures, which strongly suggests the involvement of epigenetic mechanisms. To determine whether sustained stressful rearing conditions of chickens can produce long-term epigenomic effects in the pineal gland, we have investigated epigenetic and transcriptomic response in this organ after exposure to unpredictable light patterns. Hy-Line female (n = 18) and male (n = 16) chickens were randomly divided into 2 groups after day one after hatching. The control group was kept at standard 12:12 light-dark cycle for their entire life while the chronic light stress group was exposed to an unpredictable light exposure on a randomized schedule with intervals down to 3 h and up to 21 h. The pineal gland transcriptomic responses to the treatment was carried out by RNA-seq and confirmed by qRT-PCR. We found that the pineal gland of male chickens exhibits long-term expression changes after exposure to unpredictable illumination patterns. This effect was not observed in females. Moreover, we identified sex differences in the expression of autosomal genes in the pineal gland. Interestingly, when analyzing the methylome through MeDIP we found that normal sex differences in the pineal gland DNA methylation disappear in the stress group. Light exposure stress produces long-term sex-specific disruption both in methylomic and transcriptomic level in chickens, which could also be of relevance for human health.

Key Words: chicken, epigenomic, livestock, transcriptomic, stress

P3 Genetics of epigenetics in sheep. L. Drouilhet*[1], F. Plisson-Petit[1], D. Marcon[2], F. Bouvier[2], C. Moreno-Romieux[1], S. Fabre[1], and D. Hazard[1], *[1]Institut National de la Recherche Agronomique, UMR1388 GenPhySE, Castanet Tolosan, France, [2]Institut National de la Recherche Agronomique, UE0332 Domaine de la Sapinière, Osmoy, France.*

Recent studies highlighted that DNA or histone biochemical modifications, called epigenetic marks, influence adaptation and production traits. However, whether the epigenetic variations are under a genetic determinism or not remains unknown. To answer this question, we have considered DNA global methylation rate (DGMR) as a new phenotype in sheep. DGMR was obtained by LUminometric Methylation Analysis (LUMA) using a pyrosequencing approach and its variability was explored in blood cells and other various tissues, within breed and between breeds. Thus, 30 lambs from 2 sheep breeds (Romane and Martinik Black Belly; 16 males and 14 females) were bred simultaneously to share a common environment. At the age of 5 mo, they were slaughtered and 17 tissues per animal were collected. Concerning the non-reproductive tissues, the sex, breed and tissue effects and their interactions were tested. The tissue effect was highly significant ($P < 0.0001$), the sex effect was only significant for the pancreas and the kidney cortex (sex*tissue interaction P value = 0.03), whereas the breed effect was never significant. Concerning the reproductive tissues, the breed and tissue effects were significant ($P = 0.02$ and $P < 0.0001$, respectively), the DGMR in Martinik Black Belly being higher than in Romane, whatever the tissue. The correlations between the DGMR measured in blood cells and other tissues were low or not significant, indicating that blood DGMR would not be a predictor of the DGMR of tissue less accessible. The proof of concept of the existence of a genetic determinism of DGMR considered as a quantitative trait was obtained through LUMA measurement of 940 DNA blood samples from Romane lambs genotyped on the 50K SNP chip. Blood DGMR was variable between animals with an average of 70.7 ± 6.0%. Among the tested effects, the sex and the ram effects were significant. Blood DGMR was heritable (0.20 ± 0.05) and joint analyses combining linkage disequilibrium and linkage revealed 2 main genomic regions influencing this new phenotype. Such results indicate that blood DGMR could be used in genetic selection, but before, further analyses are in progress to investigate relationship between blood DGMR and productive or adaptive traits.

Key Words: sheep, genetics, epigenetics

P4 Epigenetic state of genes of immune responsiveness relevant to bovine mastitis. T. Zabek*[1], E. Semik-Gurgul[1], T. Szmatola[1], E. Kawecka[2], E. Kosciuk[2], and E. Bagnicka[2], *[1]National Research Institute*

of Animal Production, Krakow, Poland, [2]Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzebiec, Poland.

Pathogens are one of the environmental agents able to modify methylation of regulatory elements of genes. For instance, the occurrence of mastitis might lead to changes of methylation-pattern of genes which are involved in an immune response. The DNA methylation state was investigated for genes previously found to be upregulated in response to the inflammation of the cows' mammary gland. The source were tissue sections of mammary gland infected by coagulase-positive (24 samples) or coagulase-negative staphylococci (13 samples) and 11 control samples of bacteria-free tissue. Prepared DNA were bisulfite converted and PCR amplified using primers designed for converted DNA sequence of predicted regulatory regions of genes of Chemokine signaling pathway (CCL2, HCK), Cell adhesion molecules pathway (F11R), Antigen processing and presentation pathway (CD8A, PDIA3, LGMN, HSPA1A), and NOD-like receptor signaling pathway (IL18, NFKBIA). Amplified DNA were sequenced using PCR primers. The results of bisulfite SANGER sequencing showed hypomethylated state of predicted regulatory sequences of all loci with no differences between determined groups of infected and noninfected samples. The hypomethylated state of investigated DNA regions might be relevant for the continuous activity of immune-related genes being often increased in response to the inflammatory agents of mammary gland. Obtained data may emphasize the importance of gene hypomethylation as the epigenetic mechanism important for the preservation of immune responsiveness to chronic inflammation. The study were supported by the grant of the Polish Academy of Sciences No. 2015/17/B/NZ9/01561

Key Words: cattle, mastitis, DNA methylation, immune-related genes

P5 Abstract withdrawn

P6 Epigenetic and transcriptomic characterization of pure adipocyte fractions from obese pigs identifies candidate pathways controlling metabolism. M. J. Jacobsen[1], J. H. Havgaard[1], C. Anthon[1], C. M. Junker[1], S. Cirera[1], P. M. Sørensen[1], S. Pundhir[1], P. Karlskov-Mortensen*[1], C. S. Bruun[1], P. Lesnik[2], M. Guerin[2], J. Gorodkin[1], C. B. Jørgensen[1], M. Fredholm[1], R. Barrès[3], *[1]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark, [2]INSERM UMS 1166, Team A, Institute of Cardiometabolism and Nutrition (ICAN), Pierre and Marie Curie University, Pitié-Salpetrière Hospital, Paris, France, [3]Novo Nordisk Foundation Centre for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.*

Reprogramming of adipocyte function in human obesity is implicated in metabolic disorders like type 2 diabetes. In this study, we used the pig, an animal model sharing many physiological and pathophysiological similarities with humans, to perform in depth epigenomic and transcriptomic characterization of pure adipocyte fractions. Using a combined DNA methylation capture sequencing and Reduced Representation bisulfite sequencing (RRBS) strategy, we identified 2479 differentially methylated regions (DMRs) located at close proximity to-, or within genes. By sequencing of the transcriptome from the same adipocytes, we identified 276 differentially expressed transcripts with at least one or more DMRs. These transcripts were over-represented in gene pathways related to MAPK, insulin, and adipocytokine signaling. Using a candidate gene approach, we further characterized 13 genes potentially regulated by DNA methylation and identified putative transcription factor binding sites that could be affected by the differential methylation in obesity. The study show that the DNA methylation profile of adipocytes differs between lean and obese subjects. Our data suggest that methylation plays a role in the perturbation of the pathways involved in obesity and obesity related metabolic diseases. The association between methylation and expression of genes implicated in obesity and obesity related metabolic diseases supports a role of methylation in the development of obesity. Our cell-type specific analysis, using an animal organism with strong common physiological characteristics to humans, constitutes a valuable resource for future work investigating further the role of DNA methylation in the etiology of obesity and associated disorders.

Key Words: pigs and related species, epigenomics, RRBS sequencing, metabolism, biomedical model

P7 Investigation of genetic variation in global DNA methylation in bull semen and its relationship with semen quality and fertility parameters. Y. He[1], C. Maltecca[1], F. Tiezzi[1], A. Canovas[2], S. Bhattarai[3], and S. McKay*[3], *[1]Department of Animal Science, North Carolina State University, Raleigh, NC, USA, [2]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [3]Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT, USA.*

As an important mediator of gene expression, epigenetic modifications, such as DNA methylation, affect transcription and cause variation among phenotypes. DNA methylation has received much attention in studies of male fertility in human and livestock. In bulls, although epigenetic modifications have been found to significantly influence semen quality, the mechanisms and patterns underlying these modifications are not fully understood. Therefore, the objectives of the study were to determine the effects of global methylation on sperm quality parameters and to investigate genetic variability in semen DNA methylation among sire families (n = 27) and paternal lines (n = 4). Semen samples and semen quality parameters were obtained from bulls (n = 402) selected on the basis of pedigree and relevance of phenotypic information. From each sample, DNA extraction was performed and global methylation was measured. A linear mixed model was utilized to predict traits related to sperm morphology, motility, and viability, using global sperm methylation and sires as fixed effects. Additionally, variance components and heritability (h^2) were estimated for global meth-

ylation of overall sire families and paternal lines respectively. Mean methylation of the sampled bulls ranged from 0.383 to 1.994, and from 0.982 to 1.204 across sire families and paternal lines respectively. For the analyzed semen parameters, methylation was a significant effect for motility and normal spermatozoa ($P < 0.10$), while sire was a significant effect for viability ($P < 0.10$). Estimated variance for sire family and paternal line was 0.065 and 0.018 respectively. Heritability estimates for global methylation was 0.179 for sire family variance and 0.053 for paternal line variance. The current study has established the first association between overall methylation and semen quality parameters, contributing to the selection of a new criterion for fertility in dairy bulls.

Key Words: DNA methylation, semen quality, genetic variation

P8 DNA methylation dynamics across the bovine genome.
B. Cantrell[1], S. Bhattarai[1], E. Stassen[1], S. Liu[2,3], T. Buttolph[1], H. Lachance[1], J. O'Neil[1], J. F. Garcia[4], B. Murdoch[5], R. Schnabel[6], J. Taylor[6], R. Funston[7], R. Weaber[8], G. Liu[2], S. McKay*[1], *[1]University of Vermont, Burlington, VT, USA, [2]United States Department of Agriculture-ARS, Beltsville, MD, USA, [3]China Agricultural University, Beijing, China, [4]Universidade Estadual Paulista, Araçatuba, Brasil, [5]University of Idaho, Moscow, ID, USA, [6]University of Missouri, Columbia, MO, USA, [7]University of Nebraska, North Platte, NE, USA, [8]Kansas State University, Manhattan, KS, USA.*

The dynamics of DNA methylation is largely unknown in agricultural animals. Most studies have focused on differences between animals and use only a single method of analysis for DNA methylation. We present multiple approaches to characterize the dynamics of beef cattle methylomes in novel ways. The DNA methylome was characterized in multiple brain regions unstudied in most agricultural species. We quantified global DNA methylation, 5-mC, (n = 6) and hydroxymethylation, 5-hmC, (n = 2) in 9 functionally distinct brain tissues and blood using global 5-mC/5-hmC MethylFlash Kits (Epigentek, Farmingdale, NY) to determine the total amount of 5-mC and 5-hmC. To improve resolution, 5 of the 9 brain regions were sequenced in each of 8 cattle, and methylation was determined using Illumina HiSeq 150 bp paired end Whole Genome Bisulfite Sequencing (WGBS) and the BSseeker2 with bowtie2 program. Single nucleotide variations (SNV) in the WGBS data set were identified using CGmapTools to determine potential genetic relationships with DNA methylation. Furthermore, conservation of DNA methylation was examined in the bovine AMPK gene family using pyrosequencing of bisulfite converted DNA in liver samples of multiple cattle breeds (Nellore, Angus and Charolais) and bison. Global 5-mC levels were consistently higher than 5-hmC, but brain tissues showed elevated 5-hmC levels compared with blood. This indicates the importance of examining 5-hmC in cattle brain tissues. At a nucleotide level, we found DNA methylation in similar regions of the genome across samples and tissues, but the level of methylation at each site was variable by tissue and animal. Across tissues, average methylation ranged from 71 to 73% (CpG) and ~1% (non-CpG) of the genome. SNVs were identified at loci also variable in DNA methylation, indicating a potential relationship between genetics and epigenetics. SNVs were identified across the genome, including the *PRKAA1* gene of the AMPK gene family. We found evidence of conserved DNA methylation in 36 CpG sites in *PRKAA1* and *PRKAB1* genes across all breeds and species. These data show the importance of diverse methodology to interpret the complexities of DNA methylation in the cattle genome.

Key Words: DNA methylation, AMPK, SNV, WGBS

P9 Differential H3K27ac peaks within bursa tissue of two inbred chicken lines under NDV infection and heat stress.
G. Chanthavixay*[1], C. Kern[1], Y. Wing[1], P. Saelao[1], S. Lamont[2], R. Gallardo[3], N. Chubb[4], R. Gonzalo[4], and H. Zhou[1], *[1]Department of Animal Science, University of California, Davis, CA, USA, [2]Department of Animal Science, Iowa State University, Ames, IA, USA, [3]School of Veterinary Medicine, University of Califo, Davis, CA, USA, [4]Zoetis Inc, Kalamazoo, Mi, USA.*

Poultry within developing countries are greatly impacted by Newcastle Disease (ND), and additional abiotic stressors upon those birds are increasing due to global warming. B cells that develop within the avian-specific organ, bursa of Fabricius, aid in the clearance of pathogens and are crucial for future protection upon later infections of the same pathogen. Our project aims to study the genome-wide H3K27ac histone modifications within the bursa tissue to observe changes of activity in promoters and enhancers in response to treatment, and also to explore different H3K27ac profiles due to genetics. Therefore, we subjected 2 genetically distinct chicken lines, Leghorn and Fayoumi, at the age of 3 weeks to both infection with Newcastle Disease Virus and chronic heat stress, then performed ChIP-seq for H3K27ac on the bursa tissue collected at 6 d post infection (6DPI) for 2 individuals from each treatment-line group (4 groups). H3K27ac peaks were called using the MACS2 peak caller (q value < 0.01) for each sample, called peaks were confirmed between the 2 biological replicates for each group, and all sets of peaks were merged for one total set of 67,122 peaks. Read depth was quantified for each peak region in each sample and differential peaks were determined using DESeq2 (FDR < 0.05). There were many more differential peaks between treatment groups within Leghorn (n = 912) than within Fayoumi (n = 120). The majority of the peaks, 99% in Leghorn and 70% in Fayoumi, have decreased levels of H3K27ac in the non-treated group when compared with the treated group. Contrasting the lines, there were twice as many differential peaks between nontreated birds (n = 4,579) than treated birds (n = 2,113). The target genes of proximal H3K27ac peaks (within 2000 bp of transcription start site) were identified and used for GO term and KEGG pathway analysis. Additionally, all peaks from each differential analysis were used for transcription factor motif discovery using HOMER. Our data analysis suggests that H3K27ac changes due to treatment are affecting genes and pathways crucial to B cell differentiation and that the 2 lines have distinct bursa tissue response to treatment.

Key Words: poultry, epigenetics, Newcastle Disease, ChIP-seq, H3K27ac

P10 Abstract withdrawn

P10 Abstract withdrawn

P11 Inter-generational epigenetic inheritance patterns associated with growth traits in chickens. J. A. P. Marchesi*[1], C. G. Verruma[1], J. Peixoto[2], M. C. Ledur[2], and E. S. Ramos[1], *[1]Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil, [2]Embrapa Suínos e Aves, Concórdia, São Paulo, Brazil.*

Gene expression variations mediated by epigenetic mechanisms, as DNA methylation, has been frequently associated with phenotypic variations and may be transferred across generations in animals. This study aimed to investigate if methylation levels of the growth genes *Beta-Carotene Oxygenase 2* (*BCO2*), *Leptin Receptor Overlapping Transcript* (*LEPROT*), *Protein tyrosine phosphatase, receptor type S* (*PTPRS*) and *RUN and FYVE domain containing 3* (*RUFY3*) differ between chicken populations phenotypically divergent for growth traits originated from reciprocal crosses. Two experimental lines, broilers TT and layers CC, were used to create 2 F_1 populations CT (male CC × female TT) and TC (male TT × female CC). The crosses CT and TC differed significantly for body weight, abdominal fat percentage and breast yield at 41 d old. We used red blood cell samples for DNA extraction, and the promoter methylation levels of the growth genes were evaluated by the methylation-sensitive restriction endonuclease qPCR method. Statistical analysis was made using an ANOVA test ($P \leq 0.05$). In this study, no sex effect was found on promoter methylation of any gene. *BCO2* and *LEPROT* genes were hypomethylated showing no significant statistical differences between the populations. Significant difference was observed for *PTPRS* and *RYFY3* methylation between TT and CC lines. Furthermore, our results showed that for *PTPRS* (TC = 0.59 ± 0.09^{a}; CT = 0.67 ± 0.06^{b}; TT = 0.68 ± 0.08^{b}; CC = 0.59 ± 0.08^{a}) and *RUFY3* (TC = 0.71 ± 0.08^{ab}; CT = 0.81 ± 0.07^{bc}; TT = 0.85 ± 0.14^{c}; CC = 0.65 ± 0.15^{a}) genes, methylation of the F_1 populations was significantly different from their respective fathers and similar to their mothers. Evidence of the inter-generational effect in chickens has been shown in gene expression of offspring. In our study, we confirm that specific epigenetic patterns, in this case, DNA methylation, can be passed from mother to offspring. We suggest that in some regions, epigenetic changes caused by strong selection may be associated with a specific inter-generational epigenetic inheritance. This study provides a new insight of general mechanisms by which epigenetic variation forms phenotypic diversity of growth traits in chicken populations.

Key Words: poultry and related species, epigenomics, qPCR, trans-generational inheritance

P12 Tissue-specific characterisation of the ovine methylome. A. J. Caulton*[1,2], R. Brauning[2], B. M. Murdoch[3], and S. M. Clarke[2], *[1]University of Otago, Dunedin, New Zealand, [2]AgResearch, Invermay Agricultural Centre, Mosgiel, New Zealand, [3]University of Idaho, Moscow, ID, USA.*

Over the past decade, the application of genomic technologies to animal breeding has significantly advanced the profitability and sustainability of agricultural breeding programs by facilitating selection of genetically superior animals. However, genomic information alone explains only part of the phenotypic variance in quantitative traits. A portion of this so called 'missing variance' is embedded within the epigenome, a promising yet relatively unexplored resource to identify missing causality and heritability in complex traits and diseases. As one of the most common and stable epigenetic marks, DNA methylation plays a fundamental role in the regulation of growth and development in mammals. While the importance of DNA methylation is well established, tissue specific signatures of methylation have not been extensively characterized at the genomic level. We have employed a combination of whole genome bisulfite sequencing (WGBS) and 2 reduced representation methylation profiling assays to achieve single nucleotide resolution of DNA methylation across blood, muscle and skin tissue in sheep. This work sheds light on the level of conservation of DNA methylation between tissues and individuals and highlights the technical considerations when choosing an appropriate methylation profiling technology for research or commercial applications. In addition, as part of the Ovine Functional Annotation of Animal Genomes (FAANG) Project, we will be extending the WGBS work to characterize the methylome of over 60 tissue samples from a Rambouillet ewe whose DNA has previously been used for the de novo construction of the ovine reference genome (Oar_rambouillet_v1.0). The methylation data sets will be superimposed with gene expression profiles and chromatin architecture analyses to enhance functional annotation of the sheep genome and obtain a comprehensive picture of the ovine epigenetic landscape. Determining the functional potential of genetic variants will inform our understanding of the biological processes underlying phenotype and has application in animal breeding for use in weighted genomic selection predictions and the construction of genomic relatedness matrices using causative variants.

Key Words: methylation, sheep, functional annotation, animal breeding, epigenetics

P13 The genome-wide RNA-chromatin interactions revealed by GRID-seq in skeletal muscle of three pig breeds. L. Fu, J. Li*, Y. Liao, P. Zhou, X. Li, and S. Zhao, *Key Laboratory of Pig Genetics and Breeding, MOA China, Huazhong Agricultural University, Wuhan, Hubei Province, China.*

Chromosomes of eukaryotes are bound by a large number of coding and non-coding RNAs, providing an additional layer of epigenomic information. Therefore, the ability to identify these RNAs from actively transcribing genes associated with enhancers and promoters will advance understanding of the relationship between TF binding, chromatin status and the regulation of gene expression. Global RNA interactions with DNA by deep sequencing (GRID-seq) is a recently developed technique used to capture the entire repertoire of chromatin interacting RNAs and their respective binding sites. In this study, we use GRID-seq to capture the whole collection of chromatin-binding RNAs of 2-week skeletal muscle tissues in 3 pig breeds, Duroc, Large White and Enshi Black, focusing on the breed-specific landscape of the identity and binding-sites of essential functional RNAs during muscle growth. The number of detected RNAs varied in 3 breeds, with 189 novel RNAs highly enriched on chromatin in Enshi Black, while 300 and 287 novel RNAs were identified in Duroc and Large White, respectively. To further characterize these newly identified chromatin-interacting RNAs, we categorize their chromatin-interactions into local (±10 Kb from their encoding genes), cis (in the same chromosomes), and trans (across different chromosomes) and noted that the majority of RNAs exhibited local and cis-interacting preference, only a few exceptions of specific RNAs were highly engaged across the genome. However, compared with Large White and Duroc, we observed a much lower degree of global trans-interaction preference in Enshi Black, 37% compared with 42% of Duroc and 54% of Large White, suggesting potential regulatory differences of skeletal muscles between Asian and European breeds. Furthermore, single-nucleotide polymorphisms (SNPs) were also integrated to reveal potential key variants of RNA-interacting sites. Together, these findings demonstrate a novel RNA-chromatin interacting model in skeletal muscle tissue, where a large set of newly-identified RNAs could be further characterized with LncRNA identification and histone modification.

Key Words: pigs, epigenomics, GRID-seq, skeletal muscle, RNA-chromatin interactions

P14 Identification of orthologous tissue-specific enhancer-gene pairs across chicken, pig and cattle. M. M. Halstead*[1], C. Kern[1], Y. Wang[1], X. Xu[1], G. Chanthavixay[1], P. Saelao[1], S. M. Waters[1], J. F.

Medrano[1], A. L. Van Eenennaam[1], M. E. Delany[1], H. H. Cheng[2], C. K. Tuggle[3], C. W. Ernst[4], H. Zhou[1], P. J. Ross[1], *[1]University of California Davis, Davis, CA, USA, [2]USDA, ARS, ADOL, East Lansing, MI, USA, [3]Iowa State University, Ames, IA, USA, [4]Michigan State University, East Lansing, MI, USA.*

Distal regulatory elements, such as enhancers, contribute extensively to phenotypic differences between species. However, efforts to identify enhancers and link them to their target genes have proven difficult, as enhancers tend to evolve rapidly and their target genes can be hundreds of kilobases away. In mammals, Villar et al. (2015) observed that conservation of enhancer activity generally did not correlate with overall sequence conservation, although enhancers with conserved activity did show enrichment for shorter motifs, such as transcription factor binding sites (TFBS) – especially for transcription factors with tissue-specific functions. In liver, the subset of enhancers that demonstrated conserved activity across 20 mammals were enriched for liver-specific TFBS and flanked genes with liver-specific functions. It remains unclear why some enhancers are more conserved than others, given that most are not subject to strong evolutionary constraint. Here we identify orthologous tissue-specific enhancer-gene pairs across chicken, cattle, and pig in 5 tissues – adipose, brain, liver, lung, muscle and spleen. Active enhancers were predicted based on the presence of H3K27ac, H3K4me1, and chromatin accessibility. To narrow down the search space for potential target genes, topologically associated domains (TADs) were predicted from CTCF binding sites, determined from ChIP-seq, and tissue-specific enhancers were linked to orthologous genes with conserved tissue-specific expression within the same TADs. Nearly all orthologous genes with tissue-specific expression occurred in TADs containing tissue-specific enhancers, which were then characterized for TFBS enrichment and synteny. Broadly, tissue-specific genes and enhancers tended to co-occur in the same TADs. TADs containing more genes with conserved tissue-specific expression were enriched for enhancers with specific activity in the same tissue. These results indicate that conserved enhancers are not limited to mammals, and span more broadly across vertebrates. The functional conservation of this subset of enhancers over more than 300 million years of evolution suggests that they may be integral for modulating tissue-specific expression of orthologous genes.

Key Words: epigenetics, enhancer, conservation, livestock

Applied Genetics and Genomics in Other Species of Economic Importance

P15 Abstract withdrawn

P16 Abstract withdrawn

P17 Abstract withdrawn

P17 Abstract withdrawn

P18 Systematic analysis of non-coding RNAs Involved in the Angora rabbit (*Oryctolagus cuniculus*) hair follicle cycle by RNA sequencing. B. Zhao*[1], Y. Chen[1,2], S. Hu[1], N. Yang[1], M. Wang[2], M. Liu[1], J. Li[1], and X. Wu[1,2], *[1]College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China, [2]Joint International Research Laboratory of Agriculture & Agri-Product Safety, Yangzhou University, Yangzhou, Jiangsu, China.*

The hair follicle (HF) cycle is a complicated and dynamic process in mammals associated with various signaling pathways and gene expression patterns. Non-coding RNAs (ncRNAs) are RNA molecules which are not translated into proteins but are involved in the regulation of various cellular and biological processes. In this study, we explored the relationship between ncRNAs and the HF cycle by developing a synchronization model in Angora rabbits. Transcriptome analysis was performed to investigate ncRNAs and mRNAs associated with the various stages of the HF cycle. 111 long non-coding RNAs (lncRNAs), 247 circular RNAs (circRNAs), 97 microRNAs (miRNAs) and 1168 mRNAs were differentially expressed during the 3 HF growth stages. Quantitative real-time PCR was used to validate the ncRNA transcriptome analysis results. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses provided information on the possible roles of the ncRNAs and mRNAs during the hair follicle cycle. In addition, lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA ceRNA networks were constructed to reveal the underlying relationships between the ncRNAs and mRNAs. We found that LNC_002919 and novel_circ_0026326 could act as ceRNAs, participating in the regulation of the HF cycle as miR-320–3p sponges. Our research comprehensively identified candidate regulatory ncRNAs during the HF cycle by transcriptome analysis, highlighting the possible association between ncRNAs and the regulation of hair growth. This study will provide the basis for systematic further research and new insights on the regulation of the HF cycle.

Key Words: rabbit, non-coding RNA, sequencing, hair follicle cycle, ceRNA

P19 Analysis of candidate genes for the hygienic behaviour regulation in honey bees (*Apis mellifera*). P. Modesto*[1], C. Biolatti[1], M. G. Maniaci[1], D. Laurino[2], P. Mogliotti[1], F. Mutinelli[3], A. Manino[2], P. Barzanti[1], F. Ingravalle[1], M. Caramelli[1], M. Porporato[2], and P. L. Acutis[1], *[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy, [2]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Turin, Italy, [3]Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio nazionale di riferimento per le malattie delle api, Legnaro (PD), Italy.*

Hygienic behavior (HB) of honey bees is defined as the ability to detect and uncap cells with dead or diseased brood and remove it from the nest. HB is considered a major mechanism of resistance against pathogens. Many genes seem to be involved in the regulation of this complex behavior. Aims of the study were the molecular characterization of *Long Sarah- sra* and *Odorant binding protein- ASP1* genes, previously correlated with HB and the identification of polymorphisms associated with HB, to develop useful genomic tools for marker-assisted selection (MAS). The study lasted 3 years. The first year (2013), the freeze-killed brood assay, used to assess the HB, was performed twice in the 8 apiaries selected. For each apiary, the 3 colonies with the highest HB and the 3 with the lowest one were selected for the study. Twenty workers were sampled from each colony and stored at −80°C. The HB of the colonies that survived the winter were assessed for HB and sampled twice in 2014 and again in 2015. Fifty-four colonies were sampled in 2013, 43 in 2014 and 4 in 2015. HB decreased between 2013 and 2014 while increased between 2014 and 2015. This trend did not seem to be related to the variables studied. DNA and RNA were extracted from 244 honey bees and the coding sequence region of the *sra* and *ASP1* genes were amplified, sequenced and compared with the reference sequences available in GenBank. A descriptive statistical analysis was performed to evaluate how HB changed in correlation with time, apiaries, locations and environmental conditions. A linear mixed effect model was used to assess the associations between the SNPs observed in gene *ASP1* and the HB trend. Gene *sra* was amplified in 67 samples, 5 SNPs and 2 insertions/deletions were detected. Sixty complete sequences were available for *ASP1*, 8 SNPs were observed. Two were correlated with HB ($P < 0,05$): 110 C > T with low HB and 360 C > T with high HB. Although the role of these 2 SNPs needs to be confirmed with further studies, the high statistical significance found on a relatively small population suggests that the *ASP1* gene can be a candidate gene for a MAS of HB in queen bees and, consequently, resistance to pathogens.

Key Words: *Apis mellifera*, hygienic behavior, candidate gene, DNA sequencing, genetic resistance

P20 Genetic variability of Russian reindeer populations (*Rangifer tarandus* L., 1758) revealed by high-density SNP array. V. Kharzinova*[1], A. Dotsev[1], T. Deniskova[1], K. Layshev[2], T. Romanenko[3], I. Okhlopkov[4], K. Wimmers[5], H. Reyer[5], and G. Brem[1,6], *[1]L.K. Ernst Federal Science Center for Animal Husbandry, Dubrovitzy, Moscow, Russia, [2]North-West Center for Interdisciplinary Researches of Food Maintenance Problems, Federal Agency of Scientific Organizations, St. Petersburg, Russia, [3]Federal Center for Integrated Arctic Research (FCIARctic) Nenets Division, Agro-Experimental Station, Federal Agency of Scientific Organizations, Naryan-Mar, Nenets AO, Russia, [4]Institute for Biological Problems of Cryolithozone Siberian Branch of RAS, Yakutsk, Russia, [5]Institute of Genome Biology, Leibniz Institute for Farm Animal Biology [FBN], Dummerstorf, Germany, [6]Institute of Animal Breeding and Genetics, VMU, Vienna, Austria.*

Russia has always been a leading country for reindeer populations, including 4 officially recognized breeds and the largest wild reindeer population. The Nenets breed is the most numerous one with covering the territory from the Kola Peninsula in the west to Taimyr in the east. Wild reindeer are mostly concentrated in Taimyr and Yakutia regions. Here, we aimed to address the genetic architecture and differences between domestic and wild reindeer, using the Bovine HD BeadChip (Illumina Inc., USA). The domestic group was presented by 121 individuals of the Nenets breed of the Nenets (NEN, n = 75), Yamal-Nenets (YML, n = 13), Murmansk (MUR, n = 20), Archangelsk (ARH, n = 6) regions and Komi Republic (KOM, n = 7).The wild group included 70 reindeer of Taimyr (TAI, n = 33) and Yakut (YAK, n = 37) populations. PLINK 1.07, STRUCTURE 2.3.4, SplitsTree 4.14.5 software, R packages "diveRsity" and 'ggplot2' were used for statistical analyses. After filtering, 6511 SNPs in domestic and 8801 SNPs in wild reindeer

were remained. The level of genetic diversity was slightly higher for the wild group (*Ho* = 0.183, *He* = 0.178, A_R = 1.491) comparing to the domestic one (*Ho* = 0.178, *He* = 0.172, A_R = 1.465). Neighbor-Net tree, MDS and STRUCTURE analysis revealed the sharp distinctions and clear differentiation of domestic and wild animals. The unbiased observed heterozygosity in the Nenets breed ranged from 0.179 in ARH to 0.184 in MUR. Reindeer from Murmansk region were genetically distant from others, perhaps due to interbreeding with local reindeer and thus affecting genetic relationships with the Nenets populations inhabiting other regions. Wild reindeer were characterized by similar values of genetic diversity estimates and showed similar genetic background. Here, we obtained more detailed information on genetic variability of domestic Nenets breed, Taimyr and Yakut wild reindeer populations, which might be considered as the first crucial step to develop genetic improvement strategies and to design effective conservation programs for this valuable resource for entire population of the huge Russian North. The study was supported by Russian Science Foundation, Pr.no. 16–16–10068

Key Words: Reindeer, genetic variability, SNP

P21 Microsatellite markers based on next-generation DNA sequencing for parentage of Asiatic black bear population reintroduced in South Korea. S.-H. Han*, J.-Y. Moon, S.-J. Jeong, T.-W. Kim, J.-J. Kim, D.-H. Jeong, and D.-J. Song, *Species Restoration Technology Institute, Korea National Park Service.*

The present study was aimed to establish a parentage diagnostic system for the endangered Asiatic black bear reintroduced in South Korea. Microsatellite (MS) DNA markers were developed from whole genome sequences obtained by the next-generation DNA sequencing (NGS), and determined their genotypes of released and housed animals. After preliminary study, we selected a total of 15 MS markers for parentage test. MS markers consisted of 9 di-nucleotide (nt.), one tri-nt., 2 tetra-nt., 2 penta-nt., and one hexa-nt. tandem repeat unit markers. Mean number of alleles (*k*) was 6.267, and mean expected heterozygosity (H_e) was 0.724. The founder population reintroduced from Russia, China and North Korea had 5.867 of *k*, 0.722 of H_e, the second generation had 5.867 of *k* and 0.721 of H_e, and the third generation 4.467 of *k* and 0.694 of H_e. The third generation had lower levels of genetic diversity indexes than those of their parents, estimating that several superior males dominate the breeding activity of this population in the wild. Nineteen unidentified bears captured in the wild without visible tagging system, radio-telemetric or GPS device were successfully identified their parents by parentage test. In addition, an individual hit by a car on the highway in 2018 was also assessed by an identity test based on MS genotypes. This MS marker system allowed excellent results in both parentage and identity tests. The results of this study suggested that this molecular system may contribute to analyzing genetic diversity and planning for future conservation and ecosystem recovery of the Asiatic black bear restored in South Korea.

Key Words: Asiatic black bear, microsatellite, NGS, parentage

P22 Genetic association between residual feed intake and subcutaneous fat thickness in Nelore cattle. A. M. Maiorano*[1], R. A. S. Faria[1], M. E. Mercadante[2], L. G. Albuquerque[1], and J. A. V. Silva[3], *[1]Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil, [2]Instituto de Zootecnia, Sertaozinho, Sao Paulo, Brazil, [3]Universidade Estadual Paulista, Botucatu, Sao Paulo, Brazil.*

Residual feed intake (RFI) is a measure of feed efficiency that has an impact on the profitability of production systems. RFI is defined as the difference between the animal's actual feed intake and its expected feed requirements for maintenance and growth. The lower the RFI, the more efficient the animal is. Quantifying correlations for RFI and other economically important traits will contribute to the development of optimal selection indexes. The aim of this study was to estimate genetic parameters for RFI and subcutaneous fat thickness measured at the end of feedlot tests (BFT) in Nelore animals using a bivariate animal model. Phenotypic records for RFI and BFT were obtained from 946 sires between 2010 and 2017, with approximately 120 animals being evaluated each year in feedlot tests. A total of 956 animals were genotyped with a panel containing 777,000 SNPs, including the 946 sires with RFI records. Pedigree information of 4,682 animals was available for 6 generations. The variance components and heritabilities for the traits studied were estimated using the single-step genomic BLUP (ssGBLUP) method. The phenotypic and genetic correlations of RFI with BFT were of low magnitude (0.13 ± 0.03 and 0.05 ± 0.02, respectively). Heritability estimates were 0.26 ± 0.08 for RFI and 0.42 ± 0.08 for BFT. Selection for low RFI or more efficient animals can promote genetic gains in future generations without causing changes in subcutaneous fat thickness profiles. Therefore, the use of a selection index is recommended when genetic improvements in RFI and subcutaneous fat thickness are important for the breeding objective.

Key Words: cattle and related species, animal breeding, feed efficiency, heritability, genomic selection

P23 Genome-wide association study on fatty liver weight and body weight in geese using 2b-RAD. Y. Yang*[1,3], H. Wang[1], C. Wang[1], S. Gong[1], Y. Liu[1], G. Li[1], G. Cheng[2], and D. He[1], *[1]Institute of Animal Husbandry&Veterinary Science, Shanghai Academy of Agricultural Sciences, Shanghai, China, [2]Nanfeng Administration of Husbandry and Veterinary, Nanfeng, Jiangxi Province, [3]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden.*

Geese were reported widely to be resistant to extreme hepatosteatosis. Resistance performance are economically and medically important both in poultry industry and human medicine. Many genetic studies on this resistance have been carried out in last few years. The aim of this study was applying IIB-RAD approach (46,183 SNPs from enzyme BsaX1 and 26,420 SNPs from enzyme FalI) on Genome-Wide Association Studies (GWAS) in an outbred geese population, in which 343 geese were sampled and phenotyped both on FLW and BW. GWAS was carried out based on validated 62,023 SNPs after quality control. The most significant SNP(AA_01358_01: $P < 2.0e\text{-}5$) contributing to FLW were located in the gene with name of *LOC106466* on scaffold AA_01358. Liver weight of individuals with genotype CC(1,091.19 ± 201.44 g, n = 327) was significantly higher than those with CT(948.27 ± 118.03 g, n = 15) or CT(430 g, n = 1). However, detailed characterization of this gene remained unknow in geese but a gene with high sequence identity (>90%) was found in duck which was located in immunoglobulin (IGL) lambda light chain gene locus, inferring that *LOC1064636* was possibly related to immunity in overfed geese. We also attempted to find out genes that regulating body weight after overfeeding and genomic regions were located to scaffold AA_01358(9.07Mb ~11.91Mb) overlapping with LOC106466 and AA_01377(2.75Mb ~4.24Mb). For the most significant site in both regions was AA_01358_2($P < 7.0e\text{-}12$), body weight of individuals carrying TT(8,222.23 ± 724.88 g, n = 151) was extremely higher than CT(7,603.16 ± 671.91 g, n = 88) or CC(7,393.01 ± 630.33 g, n = 104). In conclusion, the most significant SNPs contributing to FLW were located in gene LOC106466 which is related to immunity possibly. Genomic regions regulating BW were located on scaffolds AA_01358, AA_01377 and the former one was overlapped with the gene LOC106466. The long range on these scaffolds found in this study suggests future searches for candidate genes and QTL refinements as well as potential use of the most significant SNPs in practical geese breeding programs.

Key Words: geese, fatty liver weight, body weight, 2b-RAD, GWAS

P24 Assessing inbreeding networks from partial correlations and information theory in rabbits. S. T. Rodríguez-Ramilo*[1], A. Reverter[2], J. P. Sánchez[3], M. Velasco-Galilea[3], O. González[3], and M.

Piles[3], *[1]INRA, Castanet-Tolosan, France, [2]CSIRO Agriculture & Food, Brisbane, QLD, Australia, [3]IRTA, Caldes de Montbui, Spain.*

Accurate estimates of inbreeding (F) are crucial for the management of populations in selection schemes. Traditionally, inbreeding has been estimated from pedigree-based information. Currently, genome-based estimates of inbreeding can be obtained from SNP chip data. The correlation between pedigree and different genome-based estimates of inbreeding is usually discussed in the literature. However, some of these correlations could be spurious. Using partial correlations and information theory (PCIT) it is possible to distinguish a significant association between 2 variables which is independent from associations with a third variable. The objective of this study is to implement PCIT to assess the relationship between different estimates of inbreeding and phenotypes using a selected population of rabbits. Pedigree data and genomic information from a 200K chip were available. After applying filtering criteria, the data set constituted 490 animals genotyped for 114,604 autosomal SNPs. Phenotypic records for average dairy gain (ADG) were also accessible for full (f) and restricted (r) feeding. Pedigree-based inbreeding estimates (ped) were decomposed in ancient (A), intermediate (I) and recent (R) inbreeding. Accordingly, genomic estimates of inbreeding based on runs of homozygosity (roh) were also decomposed in A, I and R. Other genomic estimates of inbreeding were also evaluated: VanRaden (van), proportion of homozygous SNPs (snp), proportion of homozygous SNPs for the minor allele frequency (PHoMA) and the compression efficiency (CE). The results indicate that FrohR is an essential node with 6 edges, one of which is negative with FrohA (−0.31). CE is a balanced node with 2 positive (Fsnp and FrohR) and 2 negative (FrohA and FpedI) edges. Fvan is the less related node with only one positive edge with PHoMA (0.70). When including phenotypic information, FpedI is correlated negatively with ADGf (−0.11) and positively with ADGr (0.10). In addition, ADGr has a negative edge with FrohA (−0.11). These results indicate that PCIT approach allows inferring meaningful associations between estimates of inbreeding.

Key Words: inbreeding, average genetic gain, rabbit, partial correlation, network

P25 Valuation of polymorphism 22 STR markers used for parentage control in pigeons in Poland. A. Radko*, A. Podbielska, and A. Szumiec, *National Research Institute of Animal Production, Krakowska, Balice, Poland.*

The aim of this study has been assessment the polymorphism of 22 microsatellite markers and their usefulness for parentage verification in pigeons. Two panels STR markers were tested on 445 randomly selected individuals. The first contained 17 *loci* and second contained 6 additional *loci*. The core panel involved the following STR: CliμD11, CliμT43, CliμD01, PIGN57, CliμT13, CliμD16, CliμD19, CliμT02, CliμD17, CliμD35, CliμT17, PIGN04, PIGN15, PIGN10, PIGN26, PIGN12 and CHD marker to identify the sex of birds. The additional panel contained: CliμD32, PG2, PG3, PG5, PG6, PG7. Genomic DNA was extracted from feathers and buccal swabs. Extracted DNA were amplified by PCR for the all microsatellites and CHD marker in 2 multiplex reaction. Primers was labeled with fluorescent dyes, respectively: 6-FAM, VIC, NED and PET. The alleles were amplified using the QIAGEN Multiplex PCR Kit, the amplified products were separated on ABI PRISM® 3130xl Genetic Analyzer and genotyped using GeneMapper Software (Applied Biosystems). Based on the frequency of identified alleles in all 22 microsatellite *loci* we calculated average values for expected heterozygosity (He) and observed (Ho) and were determined as Ho = 0.61 and He = 0.67, respectively. The average polymorphism information content (PIC) were 0.621. The lowest polymorphism in the present study was noted for the CliμD35 (PIC = 0.28 and Ho = 0.31), PG5 (PIC = 0.31 and Ho = 0.32) and CliμD19 (PIC = 0.39 and Ho = 0.40).The polymorphism of the rest markers were above 0.4. The highest polymorphism we observed for PIGN26 (PIC = 0.9 and Ho = 0.88). We estimated the cumulative probabilities of parentage exclusion, when one parent is known (CPE_1) and 2 parents is known (CPE_2), for core panel and for all 22 markers together. The cumulative probabilities of parentage exclusion CPE_1 and CPE_2 for 16 *loci* (core panel) were 0.9987 and 0.9999, respectively and when we used all 22 loci increased to 0.9998 and 0.999999, respectively.

Key Words: other species, genetic identification, parentage, microsatellite

P26 Abstract withdrawn

P27 Phenotypic biodiversity of Carniolan honeybee (*Apis mellifera carnica*) in Croatia. N. Raguz, B. Lukic*, M. Kovacic, P. Margeta, and Z. Puskadija, *Department for Animal Production and Biotechnology, Faculty of Agrobiotechnical Sciences, J.J. Strossmayer University of Osijek, Osijek, Croatia.*

Extensive research of Carniolan honeybee (*Apis mellifera carnica)* biodiversity in Croatia has not been conducted so far. In this study, a total of 161 bee samples were collected in Croatia and 21 samples in Slovenia (reference samples). One sample represents about 50 worker bees from one hive on single apiary. For morphological analysis, the left and the right fore wing of 20 workers from the sample were analyzed. In total, 3640 bees (7280 wings) were included in the analysis. Wings were removed from the bees, photographed and stored as a 2400 dpi photo. With the DrawWing software 19 points of venation intersections were marked on each wing and used in the analysis of geometric morphometry of shape and size of wings. Centroid size was calculated by use of markings on the wings in millimeters. The principal component analysis of the centroid size from 40 wings of the sample was performed. The origin of the samples was attributed to one of the 3 regions in Croatia: the Pannonian, the Mountainous and the Mediterranean region. Significant differences between the regions (F (2, 178) = 5.0727, P = 0.00720) were found in the PCA analysis. Specifically, significant difference was found between Pannonian honeybees in relation to Mountainous and Mediterranean, while differences between Mountainous and Mediterranean were not found. Since the results of morphological and genetic analysis with microsatellite markers often coincide, it is recommended to analyze the distribution of ecotypes using more precise genetic markers such as SNP-s, on the same population. For that reason, isolation of bee's DNA was performed. The soft tissue of the flight muscles was isolated and stored in insulating protocol tubes. All samples were successfully isolated with sufficient concentrations (≈50μg/ml). The analyzed population will be used for further genotyping and analysis of the ecotypes based on SNPs, with-

in the VaroGen project funded by the Paying Agency for Agriculture, Fisheries and Rural Development (PAAFRD) in Croatia.

Key Words: Carniolan honeybee, biodiversity, morphometry, SNP

P28 Identification of genomic signatures of divergent selection for birth weight environmental variance in a mice experiment. Preliminary analysis. I. Cervantes*[1], N. Formoso-Rafferty[1], JP Gutiérrez[1], and F. Goyache[2], *[1]Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain, [2]*Área de Genética y Reproducción Animal, SERIDA-Deva, Gijón, Spain.

A divergent selection experiment for birth weight environmental variability in mice has been successfully performed during 20 generations. Genetic control of the birth weight environmental variability is possible. Furthermore, this selection criterion had direct implications in other interesting traits in livestock. Selection for low variability (line L) was beneficial for production, welfare and robustness related traits, with higher heritability when compared with the high variability line (line H). This research aimed at the identification of selection signatures on the genome caused by that divergent selection. A total of 384 DNA samples belonging to 212 L-line and 172 H-line females were genotyped using the Affymetrix Mouse Diversity Genotyping Array. A preliminary analysis has been performed using 417,172 SNPs and the XP-EHH test as implemented in the software Selscan. As a complementary approach, the nSL statistics was computed per line, separately, as well. After normalization of the XP-EHH scores across chromosomes, a total of 25,159 SNPs had scores higher than |2|, being 11,692 SNP under selection in the H line and 13,467 in the L line. Regarding nSL test, 6,495 markers showed selection signatures in line L and 6,794 in line H. The number of SNPs which coincides in both methods was 2,357, most of them located on chromosome 17, 4 and 6. Some markers were in common with a previous GWAS analysis performed using these samples, 190 SNPs related with birth weight and its variation, 55 of these SNPs were located on mice chromosome 7 confirming those genomic areas as target for further identification of candidate genes involved in genetic control of environmental variability. After comparing the genomic locations of the identified candidate regions of selection with the available annotation of the reference mice genome, genes which putatively are been involved in this divergent selection could be identified.

Key Words: rodents, animal breeding, selection scan, selection, animal welfare

P29 Abstract withdrawn

P29 Abstract withdrawn

P30 Genomic regions associated with individual growth and cage feed efficiency in rabbits under two feeding regimes. J. P. Sánchez*[1], A. Legarra[2], M. Velasco-Galilea[1], M. Piles[1], O. Rafel[1], O. González[1], and M. Ballester[1], *[1]Animal Breeding and Genetics Program, IRTA, Caldes de Montbui, Spain, [2]UMR 1388 GenPhySE, INRA, Castanet-Tolosan, France.*

Our objective was to identify genomic regions associated with individual growth (ADGf), and cage feed intake (FIf), feed conversion ratio (FCRf) and residual feed intake (RFIf) in *ad libitum*-fed rabbits; as well as with individual growth (ADGr) in rabbits fed under restriction. Three genome-wide association methods were used to analyze the aforementioned traits on 438 rabbits genotyped for 114,604 SNPs. The methods were: i) regression on each SNP allele counting (Reg), ii) bivariate animal model between the performance trait and each SNP allele counting (Bi) and iii) random QTL model combining LD and linkage information (LDLA). LDLA returned the largest number of significant associations. Bi and Reg yielded significant associations only at chromosome (Chr) level. At this level, LDLA showed 22 chromosomal regions on Chr2, 3, 5, 6, 7, 9, 12, 14, 15, 18, 19 and 21 associated with ADGf. Reg yielded significant associations between ADGf and regions on Chr3, 5 and 21. Lastly, Bi revealed significant associations on Chr3, 5 and 16. All methods identified the region 102–113 Mb on Chr3 as significantly associated with ADGf. At Chr5, the 19–20 Mb region was associated with ADGf by Reg and Bi, while the 34–35 Mb region was associated with ADGf by Bi and LDLA. One region (0–2 Mb) on Chr13 and 2 regions (29–32 Mb and 38–39 Mb) on Chr9 were significantly associated with ADGr by Reg and Bi methods, respectively. Bi, the only method used to analyze traits recorded at cage level, revealed significant associations between FIf and region 3–4 Mb on Chr5, and between RFIf and regions Chr8:108–110 Mb and Chr21:7–8 Mb. No significant associations were identified for FCRv. A total of 36 chromosome regions were significantly associated with the traits under study, one of them (Chr21:7–8 Mb) having pleiotropic effect on ADGf and RFIf. After gene and functional annotation, 29 candidate genes on 22 out of these 36 significant regions were identified. It is remarkable that one of the identified candidate genes (*FTO*) was previously associated with growth in rabbits. In addition, candidate genes *FEZF2* and *PTPRG* for ADGr, are involved in the control of feeding behavior traits.

Key Words: genome-wide association, rabbits, growth and development, feed efficiency

P32 Disentangling the genetic background of environmental variance of litter size using whole-genome sequencing data in rabbits. C. Casto-Rebollo*[1], M. J. Argente[2], M. L. García[2], A. Blasco[1], and N. Ibáñez-Escriche[1], *[1]Institute for Animal Science and Technology, Universitat Politècnica de València, València, Spain, [2]Departamento de Tecnología Agroalimentaria, Universidad Miguel Hernández de Elche, Orihuela, Spain.*

Massive data generated for whole-genome sequencing (WGS) allow to identify large number of variants under selection. Thus, the genetic background of complex traits could be better characterized. Understanding the genetic background of the environmental variance of complex traits (VE) is increasing interest in animal breeding. VE is genetically determined and related to resilience. A divergent selection experiment for environmental variance of litter size in rabbits showed a clear response to selection and differences in mortality and health sta-

tus biomarkers (Cortisol, Leukocytes and acute-phase proteins). Thus, to identify variants underlying VE we sequenced the whole genome of 27 pooled DNA samples of each line. Alignment of sequence reads was performed using BWA algorithm and bam files were generated by SAMtools. Duplicates and Illumina markers were removed with Picard tools. The variant discovery and filtering were performed using GATK programs. The annotation of discovered variants was done with SnpEff. Result showed a total of 15,141,673 single nucleotide variants between both lines and the reference genome. 3,202,487 of them showed differences between the low and high VE line. Most of them were variants with low impact over the phenotype due their localization in intergenic and intron regions. However, 6,964 SNVs were identified as non-synonymous mutations with a higher impact over the amino acid coding of proteins. These polymorphisms could partly explain the phenotypic differences between the divergent lines. Potential loci of VE were identify in the chromosome 1, 3, 4, 8, 12, 13, 14 y 18 in the Oryctolagus cuniculus genome. This is the first study using WGS to disentangle the genetic background of VE.

Key Words: environmental variance, whole-genome sequences, single nucleotide variants, rabbits

P33 Identification and analysis of differential expressed microRNAs in subcutaneous of yak under nutrition pressure. M. Chu*[1,2], P. Yan[1,2], C. N. Liangn[1,2], X. Y. Wu[1,2], X. Z. Ding[1,2], X. Guo[1,2], L. Xiong[1,2], and J. Pei[1,2], *[1]Key Laboratory of Yak Breeding Engineering, Gansu Province, China, [2]Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Sciences, Lanzhou, China.*

Yak (*Bos gruniens*) are distributed in the Qinghai-Tibet Plateau and adjacent areas. Influenced by high altitudes and special climatic, the adipose tissue of yaks grow rapidly owing to plenty of pasture in warm season, while in cold season, the adipose tissue of yaks suffered severe weight loss under shortage of pasturage. In this study, high-throughput sequencing technology was used, 4 small RNA libraries of yak were successfully constructed, including subcutaneous fat from cold and warm season, and late to identify differential expressed microRNAs and analyze their functions so as to study the roles of microRNA in yak fat metabolism under nutrient stress. The results showed that 450 microRNA were identified, 70 microRNA of which expressed significantly different in tissues of 2 seasons. Among differential expressed microRNA, miR-335, miR-378, miR-378b, miR-200a, miR-200b, miR-200c and miR-210 played roles in yak fat metabolism. And then the function of miR-200a in regulating adipocyte differentiation in the yak was studied.Results showed that miR-200a increased the expression of adipocyte-specific genes involved in lipogenic transcription (PPARγ, ELVOL, and C/EBPα), fatty acid synthesis (ACC, ACS, SCD, and FAS), and fatty acid transport (DGAT, LPL, and FABP4). We also found that transfection of adipocytes with miR-200a resulted in suppression of the levels of noncanonical Wnt signaling transcription factors (Wnt5a, TAK1, and NLK). These results indicate that miRNA-200a plays an important role in promoting yak adipocyte differentiation that may operate via the suppression of noncanonical Wnt signaling. In conclusion, The results showed that microRNAs played important roles in regulating fat metabolism of yak under nutritional stress, and to provide a scientific basis and theoretical support for future study.

Key Words: yak, microRNAs, subcutaneous, nutrition pressure, animal breeding

Applied Sheep and Goat Genetics

P34 Variations in ovine leptin gene of Cholistani and Sipli sheep in Punjab, Pakistan. M. Safdar*[1] and Y. Junejo[2], *[1]Cholistan University of Veterinary & Animal Sciences, Bahawalpur, Punjab, Pakistan, [2]Virtual University of Pakistan-Multan, Multan, Punjab, Pakistan.*

The current study was carried to investigate the relationship of polymorphisms in exon 1, 2 and 3 of the Leptin gene with significant growth/economic traits in Cholistani and Sipli sheep breeds in Pakistan. Economic traits have impact on livestock which can improve genetically through marker assisted selection. The Leptin gene has a negative role in growth and skeletal muscle development. The genomic DNA was isolated and amplification products were purified and then sequenced. Mutations were found at exon 1 in Cholistani sheep as heterozygous G > T mutation while in a Sipli only synonymous T mutations were identified at position 3995. The genotype combination AA, AB and BB were defined, while allele frequencies A (0.2) and B (0.8) were calculated in Sipli and AB (0.5) in Cholistani. The obtained results showed that the Leptin gene polymorphism in Cholistani and Sipli sheep breeds were an important study to improve the sheep breeds in Pakistan. This is the first report of polymorphisms in the Leptin gene of the Pakistani sheep.

Key Words: polymorphism, leptin gene, Cholistani, Sipli, PCR

P36 Deciphering climatic conditions effect on the ovine sperm transcription by RNA-seq. I. Ureña*[1], C. González[1], M. Ramón[2], M. Gòdia[3], A. Clop[3,4], J. H. Calvo[5], M. J. Carabaño[1], and M. Serrano[1], *[1]Department of Animal Breeding and Genetics, INIA, Madrid, Spain, [2]IRIAF-CERSYRA, Valdepeñas, Ciudad Real, Spain, [3]Animal Genomics Group, Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Cerdanyola del Vallès, Barcelona, Spain, [4]Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain, [5]CITA-ARAID-IA2, Zaragoza, Spain.*

Climate factors can have strong effects on livestock reproductive efficiency, with obvious consequences in animal's fitness (Grazer & Martin 2012), which can result in large economic losses for farmers. In particular, sperm damage is influenced by the stage at which germ cells are exposed to heat stress (Hales et al., 2005). Sperm RNA profiles are proposed as a relevant source of markers for male fertility (Dadoune 2009, Hamatani 2012). To deepen these issues, this work investigates the effect of climatic conditions on the ram's sperm transcriptome using next generation sequencing. Ejaculates from 40 rams were collected in July under heat stress (Tmax = 37.4°C; 28 ejaculates) and in October under comfort conditions (Tmax = 21.4°C; 36 ejaculates). Differential gene expression analysis was measured using novel software for single-cell RNA-seq analysis to take into account the sperm RNA particular features of (ZINB-WaVE, Risso et al., 2018) in conjunction with Stringtie (Pertea et al., 2015) and DESEq2 (Love et al., 2014). Overall, 11,575 transcript genes were detected. Of these, 228 genes were differentially expressed (adjusted p-value <0.05) between both conditions: 220 were downregulated and 8 were upregulated under heat stress. Downregulated genes were significantly enriched for specific reproductive (14.3%) and metabolic (57.1%) biological processes, being 105 genes involved in the response to stimulus and reproduction. In relation to reproduction, we highlight *Adam2*, as it is involved in the binding of sperm to the zona pellucida, fusion of sperm to egg plasma membrane involved in single fertilization and positive regulation of gene expression. Among the downregulated genes related to response to stimulus, we found *Acod1, Chadl, Emc6, Fer, Il17rc, Ncoa3, Sprtn, Tgfb3* and *Tmed*. They are involved in immune and defense responses, protein folding and phosphorylation, androgen receptor, DNA repair, and embryonic development, among other functions. From these results, we

can conclude that thermal stress induces a pronounced transcriptional repression in sperm cells.

Key Words: sheep and related species, RNA-seq, gene expression

P37 Abstract withdrawn

P38 Abstract withdrawn

P38 Abstract withdrawn

P39 Genome-wide association study of sperm traits in Assaf rams. M. M. Serrano*[1], M. Ramón[2], J. H. Calvo[3], F. Freire[4], J. M. Vazquez[5], M. A. Jiménez[1], and J. J. Arranz[6], *[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA, Madrid, Spain, [2]Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal IRIAF-CERSYRA, Valepeñas, Ciudad Real, Spain, [3]Centro de Investigación y Tecnología Agroalimentaria de Aragón CITA-Fundación Agencia Aragonesa para la Investigación y el Desarrollo ARAID-IA2, Zaragoza, Spain, [4]ASSAF.E Asociación Nacional de Criadores de la Raza Ovina Assaf, Toro, Zamora, Spain, [5]OVIGEN Centro de selección y mejora genética del ganado Ovino y Caprino, Toro, Zamora, Spain, [6]Dpto. Producción Animal Universidad de León ULE, León, Spain.*

In artificial insemination (AI) centers, the volume (VOL), sperm concentration (CON) and the spermatozoa subjective motility (MOT) of rams' ejaculates are routinely evaluated before doses elaboration to ensure the fertilizing capacity of the spermatozoa and its suitability for AI. However, the relationship between these sperm parameters and ram's fertility is not clear. Moderate heritability estimates were found in sheep (David et al., 2007) for VOL, CON and number of spermatozoa (NSP) (0.12 to 0.33) and low for MOT (0.02 to 0.14). Current genomic tools could help to identify genomic regions associated with such low heritability traits, enabling to conduct a selection of the rams assisted by markers. In this work, a GWAS analysis of sperm parameters in Assaf rams was carried out using data of 27.886 ejaculates from 429 matured rams placed at OVIGEN AI center. A linear regression analysis including the ram age and herd of origin, the date of semen collection, the jump number during semen collection and the ram permanent environmental effects was conducted to obtain pseudo-phenotypes for the GWAS. The association analysis was performed with the GCTA software (Yang et al., 2011) using 342 rams genotyped with a custom 50K SNP Affymetrix microarray. The positional candidate genes were identified in the 250 Kb region on both sides of the significant SNPs. Genes and SNPs were mapped on the Oar v3.1 sheep genome. For VOL, CON and NSP, associations ($P < 0.01$) were found at the chromosome level. Putative causal genes were *TET2* (OAR6), *SLC4A4* (OAR6) and *DTNA* (OAR23) related to spermatogenesis, fertility and teratospermia, for VOL; *SERPINE3* (OAR10) and *SPEF2* (OAR16), which play role in fertility, spermatogenesis and flagellar assembly, for CON; and *GALNT14* (OAR3), *SNAP23* (OAR7), *CDYL2* (OAR14) and *SPR54* (OAR18) related to infertility, asthenozoospermia, sperm capacitation and gametogenesis, for NSP. For MOT, 5 genome regions at chromosomes 3, 7, 16, 19 and 20 showed significant associations ($P < 0.01$) at the genome level. Putative causal genes were *SEMA4F* (OAR3), *RBMS3* (OAR19) and *HSPA1L* (OAR20), which have been related to sperm fertility, teratospermia and binding of sperm to zona pellucida, respectively.

Key Words: sheep, genome-wide association, SNP, fertility, sperm traits

P40 Identification of variation in the growth differentiation growth factor 9 (GDF9) gene associated with litter size in New Zealand sheep (*Ovis aries*) breeds. H. A. Najafabadi*[1], J. Hickford[1], H. Zhou[1], and M. Khansefid[2], *[1]Lincoln University, Christchurch, Selwyn, New Zealand, [2]AgriBio Centre for AgriBioscience, Bundoora, VIC, Australia.*

Having the ability to control litter size is increasingly important for sheep farmers and breeders worldwide. Making genetic gain in improving reproductive performance using traditional breeding methods that are based on phenotypic assessment is very slow, so attention has turned to better understanding the genes that control reproductive performance. Of these genes, research has focused on the Growth Differentiation Growth Factor 9 (GDF9) gene (*GDF9*). In this study a Single-Strand Conformation Polymorphism (SSCP) approach was used to investigate variation in this gene in purebred Finnish Landrace sheep, Finnish Landrace × Texel-cross sheep and some composite sheep. Three GDF9 variants (named *A*, *B* and *C*) were observed, with the frequency of *A* (c.978A, c.994G and c.1111G) in the Finnish Landrace, Finnish Landrace × Texel-cross and composite sheep being 0.86, 0.78, 0.76 respectively. The frequency of *B* (defined by the presence of c.994A, c.978G) was 0.01, 0.03, and 0.23, and for *C* (c.1111A) was 0.13, 0.18, and 0.01, respectively. Sequence analyses of the 3 variants revealed 3 SNPs: c.978A > G, c.994G > A and c.1111G > A. Analysis of fertility data for Finnish Landrace × Texel cross breed revealed an association between litter size and the SNP c.1111G > A ($P = 0.036$), but this was not observed for the Finnish Landrace sheep ($P = 0.27$) and the composite sheep ($P = 0.17$). When all the sheep were analyzed together, the presence of c.1111A was associated with increased litter size ($P < 0.05$) compared with ewes that had c.1111G. Litter size did not differ between sheep with and without SNPc.994A in all 3 breeds investigated breeds. This study suggests that C.1111A is genetic marker for improving fecundity in New Zealand sheep breeds, and that it could be introgressed into other breeds, but analysis of more sheep may be required to confirm the associations observed here.

Key Words: NZ sheep, growth differentiation factor 9 gene, *Ovis aries*, litter size

P41 Differential selection patterns between sexes could be revealed by ROH analysis in dairy goats. S. Demyda-Peyrás[1,2], Y. Pirosanto[1,2], M. Ramón[3], T. Ziegler[1,2], M. Sanchez[4], M. E. Fernandez[1,2], M. E. Muñoz[4], M. Solé*[5], and A. Molina[6], *[1]Departamento de Producción Animal, Facultad de Veterinaria, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina, [2]IGEVET (CONICET, UNLP), La Plata, Buenos Aires, Argentina, [3]CERSYRA-IRIAF, Valdepeñas, España, [4]Departamento de Producción Animal, Universidad de Córdoba, Córdoba, Spain, [5]Department of Animal Breeding and Genetics Swedish University of Agricultural Sciences, Uppsala, Sweden, [6]7Departamento de Genética, Universidad de Córdoba, Córdoba, Spain.*

Runs of homozygosity (ROH) are becoming a valuable tool to analyze the evolution and genomic characteristics in animal populations. Here, we determined ROH patterns of 624 goats of the Spanish Florida breed to explore sex-related differences. Average ROH length, count and F_{ROH} (as a measure of the proportion of the genome covered by ROH) were obtained per individual, chromosome and sex group using DETECTRUNS package of R software, and differences were examined using general linear models (GLM) and LS preplanned comparison tests. Our results showed that males have longer ROH than females (2.559 vs 2.472 Mp respectively, $P < 0.05$), but with differences being only significant at CHI6 and CHI17 ($P < 0.01$). At the individual level, the number of ROH was highly variable and no significant differences were found between sex groups and chromosomes. Only for the CHI5, the number of ROH observed differed between males and females (1.54 and 1.85 respectively; $P < 0.01$). In a further analysis, the ROH counts were also determined in ROH >8Mb, normally associated with recent inbreeding events. It was noteworthy that only 36% of females showed at least 1 ROH >8Mb in comparison with 43% of the males, but with females showing an increased number of ROH in CHI4 (1.60 times higher, $P < 0.05$). The analysis of F_{ROH} showed statistical differences only in CHI23, where the mean value of males was 1.63 times higher than females. In the case of recent F_{ROH} (derived from ROH >8Mb), differences were observed in CHI8, where males showed a 3-times increased coverage. Finally, we also performed the analysis of the ROH incidence in an SNP basis which determined the existence of specific genomic regions were males showed increased ROH percentages of incidence than females and vice-versa. Those regions were more confined in females (covering part of 14 chromosomes) than in males (which included 17 chromosomes) in the general analysis. The analysis of ROH >8Mb showed the opposite, with ROH distributed over 8 chromosomes for females and only in 3 chromosomes for males, but with an important ROH concentration in CHI11 for the latter. The higher number of ROH >8Mb in males and the fact that they are confined to certain regions could be a consequence of recent selection (6 generations), with a greater selection intensity in the males, and in regions possible linked to productive traits. Our study suggests that ROH could be used to reveal genomic differences between sexes in goats.

Key Words: ROH, inbreeding, sex, goats

P42 Abstract withdrawn

P43 Genetic diversity of Nubian ibex in comparison to other ibex and domesticated goat species. L. Hassan*[1,2], D. Arends[3], S. Rahmatalla[3,4], M. Reissmann[3], H. Reyer[5], K. Wimmers[5], S. Abukashawa[2], and G. Brockmann[3], *[1]Wildlife Research Center, Animal Resource Research Corporation, Federal Ministry of Livestock, Fisheries and Rangelands, Khartoum, Sudan, [2]Faculty of Sciences, Department of Zoology, University of Khartoum, Khartoum, Sudan, [3]Albrecht Daniel Thaer-Institute for Agricultural and Horticultural Sciences, Humboldt-Universität, Berlin, Germany, [4]Faculty of Animal Production, Department of Dairy Production, University of Khartoum, Khartoum*

North, Sudan, [5]Institute for Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.

Capra nubiana is a wild ibex species that is in danger of extinction. This study aimed at assessing the genetic diversity and population structure of Nubian ibex (*Capra nubiana*, n = 8) in comparison to Alpine ibex (*Capra ibex*, n = 8), Bezoar ibex (*Capra aegagrus*, n = 4), and domesticated Taggar goats (*Capra aegagrus hircus*, n = 24). All animals were genotyped with the 50K goat SNP chip. Since commercial SNP chips are not designed for wild species, data analysis was done in 2 ways: (1) using all callable SNPs (33,698) and (2) with a reduced set of SNPs segregating within 3 out of 4 populations (662). Using these 2 sets of SNPs, the observed heterozygosity in Nubian ibex ranged from 0.02 to 0.44, in Alpine ibex from 0.01 to 0.38, and in Bezoar ibex from 0.13 to 0.38, when analyzing 33,698 or 662 SNPs, respectively. In domesticated Taggar goats, the values for the observed heterozygosity using all 33,698 callable SNPs and the reduced set of 662 SNPs were similar (0.40–0.41). Pairwise FST values for the differentiation between species ranged from 0.17 to 0.35 (Bezoar ibex vs. Taggar goats) to 0.47–0.91 (Bezoar vs. Alpine ibex), and was 0.33–0.90 between Bezoar and Nubian ibex, respectively, to the 2 sets of SNPs. The analysis of molecular variance among all animals revealed that 74–78% can be explained by differences between species, while the residual 22–26% results from differences among individuals, respectively. Cluster analysis of Nei's genetic distance allowed to detected 2 distinct clusters comprising Nubian and Alpine ibex on one hand and Taggar goats and Bezoar ibex on the other hand, and clear separation of all 4 breeds. Principal component (PC) analysis confirmed and further refined the clusters. SNPs thatContributed most to PC1 allowed us to identify genomic regions accounting for the distances between species. These regions contain known milk protein genes. The identification of milk protein genes as contributors to the differentiation between species provides insights into the domestication of wild Capra breeds.

Key Words: genetic diversity, Nubian ibex, Alpine ibex, Bezoar ibex, goat, conservation

P44 Entropion in Swiss White Alpine sheep is associated with the *CTNND1* region on chromosome 15. N. Hirter*[1], A. Letko[1], I. Häfliger[1], D. Greber[2], and C. Drögemüller[1], *[1]Institute of Genetics, University Berne, Berne, Switzerland, [2]Clinic for Ruminants, University Berne, Berne, Switzerland.*

Entropion is a known congenital disorder in different species including sheep. Lambs with entropion have an inward rolling of one or both lower eyelids since birth. The inward looking eyelashes and hairs lead to a constant irritation of the cornea and, in extreme cases, to blindness. In Switzerland, a recent survey showed that the most affected Swiss White Alpine sheep (SWA) breed showed a prevalence of entropion about 6%. Entropion in sheep is supposed to be heritable; however, so far no causative genetic variant causing this disease phenotype has been identified. In this study, 150 mostly SWA purebred and some SWA × Texel crossbreds were genotyped using the ovine high-density 600k SNP array. A genome-wide association study of 89 cases and 61 controls enabled us to identify an associated genome region on chromosome 15. The best-associated marker (p-value: 1.95 E-09) was located close to the *CTNND1* gene encoding catenin delta-1. In human, recent studies have identified pathogenic variants in *CTNND1* in blepharocheilodontic syndrome 2, a rare autosomal dominant disorder characterized by eyelid anomalies. Therefore, *CTNND1* represents an excellent functional candidate for entropion in sheep. We sequenced the genomes of 2 pairs of affected and normal full sibs of SWA, a single affected SWA, and case-control full sib pair of Texel sheep. The sequence reads were mapped to the latest sheep reference genome (Oar_rambouillet_v1.0) and single nucleotide variants were called. After filtering for disease-associated variants using control genomes of other unrelated sheep breeds, we found 2 intronic single nucleotide variants and one missense variant in the *CTNND1* gene. Genotyping of more than 300 sheep with known phenotypes showed no association of these 3 variants with entropion in SWA sheep. In conclusion, this study revealed a single locus associated with entropion in Swiss White Alpine sheep. Further sequence analysis is needed to identify the causative variant.

Key Words: sheep and related species, genome-wide association, SNP, genome sequencing

P45 Novel variants of growth differentiation factor 9 (*GDF9*) gene affect promoter activity and their relationship with litter size in Mongolia sheep (*Ovis aries*). B. Tong*[1,2], Y. Yang[1,2], J. Wang[1,2], and G. Li[1,2], *[1]State Key Laboratory of Reproductive Regulation & Breeding of Grassland Livestock, Inner Mongolia University, Hohhot City, Inner Mongolia Autonomous Region, China, [2]School of Life Sciences, Inner Mongolia University, Hohhot City, Inner Mongolia Autonomous Region, China.*

In sheep, litter size is an important economic trait in breeding and industry. Although several mutations increasing litter size or ovulation rate have been identified in various sheep breeds, the genetic variants have still to be revealed for some other sheep populations. The Mongolia sheep breed populations are the primitive sheep species and widely distributed from Mongolia plateau to Central Asia. Recently, the mutton of the Mongolia sheep is recognized as natural green food and become more and more famous in China. However, due to low prolificacy, the produce rate of Mongolia sheep was limited. The objectives of this study were to (1) perform association analysis between the 10 well-known variants including the *BMPR-IB* ($FecB^B$), *BMP-15* ($FecX^B$, $FecX^G$, $FecX^H$, $FecX^I$ and $FecX^L$) as well as *GDF9* ($FecG^H$, $FecG^E$, $FecG^F$ and G4) genes and litter size of the Mongolia sheep, (2) identify novel variants in the candidate gene *GDF9* and perform association analysis with litter size, (3) investigate the genetic diversity of variants in 6 Mongolia sheep subspecies, and (4) validate the effects of the variants in the promoter region of *GDF9* on their promoter activity. Association analysis showed that merely extra low allelic frequency of the $FecB^B$ mutation was distributed in this Mongolia sheep population and other 9 mutations were not detected. DNA sequence analysis identified 8 variants that exist in the promoter of *GDF9*. The mean litter sizes of the ewes with the *CT* genotype at the $g.41770932C > T$ SNP (included in haplotype I) were significantly higher than those of the *CC* genotype ($P < 0.0001$). All of these variants showed related low allelic frequencies and polymorphism information content in each Mongolia sheep subspecies. Moreover, *GDF9* promoter activity analysis showed that when the promoter was deleted to −177 bp, the promoter activity was significantly increased than to −460 from −1533 bp in H293T cell ($P < 0.01$). Furthermore, the *T* allele at the $g.41770932C > T$ SNP (included in haplotype I) could increase luciferase activity compared with that of the *C* allele ($P < 0.01$). Our results may be applied to effective marker-assisted selection to increase the litter size in the Mongolia sheep breed populations and other prolificacy sheep breed, also bring new insights into the key role played by *GDF9* in ovarian function.

Key Words: Mongolia sheep, little size, GDF9, variant, promoter activity

P46 Abstract withdrawn

P46 Abstract withdrawn

P47 Complete mitochondrial genome sequencing reveals new extinct group of snow sheep (*Ovis nivicola*). A. V. Dotsev*[1], E. Kunz[2], A. V. Protopopov[3], I. M. Okhlopkov[4], A. V. Shakhin[1], V. R. Kharzinova[1], M. S. Fornara[1], S. Krebs[5], J. Peters[6], D. G. Medvedev[7], T. P. Sipko[8], V. A. Bagirov[1], G. Brem[1,9], I. Medugorac[2], N. A. Zinovieva[1], *[1]L.K. Ernst Federal Science Center for Animal Husbandry, Podolsk, Moscow Region, Russia, [2]Department of Veterinary Sciences, Ludwig-Maximilians-University, Munich, Germany, [3]Mammoth Fauna Study Department, Academy of Sciences of the Republic of Sakha (Yakutia), Yakutsk, Russia, [4]Institute for Biological Problems of Cryolithozone, Yakutsk, Russia, [5]Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, Ludwig-Maximilians-University, Munich, Germany, [6]Department of Veterinary Sciences, Institute of Palaeoanatomy, Ludwig-Maximilians-University, Munich, Germany, [7]Department of Game Management and Bioecology, Irkutsk State University of Agriculture, Molodezny Settlement, Irkutsk region, Russia, [8]Severtsov Institute of Ecology and Evolution, Moscow, Russia, [9]Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria.*

The studies of fossils of snow sheep *(Ovis nivicola*) revealed different morphological characteristics from the animals of modern populations. To date, 3 extinct *O. nivicola* subspecies were described. Here for the first time we compared the complete mitochondrial genome of an ancient snow sheep with modern samples. The skull of the studied historical specimen was found on the bank of Amga river in Yakutia and it was carbon dated to be over 60,000 years old. The samples of modern snow sheep taken for this work cover all major habitats within its current distribution: Verkhoyansk Range, Chersky, Momsky, Stanovoy, Dzhungzhur and Suntar-Khayata ridges, Putorana Plateau and Kamchatka Peninsula Mountains. The sequencing was performed using NGS platform HiSeq 1500 (Illumina). The complete mitogenome sequences of the other *Ovis* species, *O. candadensis*, *O. dalli* and *O. ammon* were taken from GenBank as outgroups. The alignment of the sequences was done using the MUSCLE algorithm as implemented in MEGA 7.0.26. PartitionFinder 2 was used to select the best partitioning scheme and evolutionary models. Phylogenetic analysis based on the concatenated data sets of 13 PCGs and 2 rRNAs using the Bayesian inference method was performed in MrBayes 3.2.6. We found that the ancient specimen does not belong to any of the present-day snow sheep groups. In the phylogenetic tree it formed a sister branch to the currently living populations clade. Apparently, the studied ancient specimen inhabited the territory of Yakutia and went extinct during one of Pleistocene glaciation periods, while all the modern populations of snow sheep have the other ancestry. A similar evolution pattern was observed between ancient and present-day arctic ground squirrels (genus *Urocitellus*) which have the same habitat area as snow sheep. Thus, our work showed that snow sheep currently inhabiting the territory of Yakutia migrated to this area after extinction of the group to which belonged the studied ancient specimen. This work was supported by Russian Foundation for Basic Research (RFBR) grant 18–316–20009.

Key Words: snow sheep, *Ovis nivicola*, ancient DNA, mitochondrial DNA, complete mitogenome

P48 Effects of aluminium hydroxide adjuvant in ovine encephalon assessed by high-throughput RNA sequencing (RNA-seq). E. Varela-Martínez*[1], M. Bilbao-Arribas[1], N. Abendaño[1], J. Asín[2], M. Pérez[2], L. Luján[2], and B. M. Jugo[1], *[1]Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain, [2]Veterinary Faculty, University of Zaragoza, Zaragoza, Aragón, Spain.*

Aluminum (Al) is one of the most widespread compound used as adjuvant in both human and veterinary vaccines. Whereas they function as an excellent adjuvant, the underlying molecular mechanism and pathways have remained unclear. In fact, various pieces of evidence suggest that it has a propensity to accumulate in different compartments after a high level Al exposure and it has been linked with some neurodegenerative disorders such as Alzheimer (AD), amyotrophic lateral sclerosis (ALS) and Parkinson (PD). To characterize the mechanism of action of Al hydroxide as adjuvant, the molecular signature activated in the encephalon of sheep samples after a long-term exposure to different vaccines is analyzed. Animals were treated with either commercial vaccines (vaccine group), Al hydroxide only (adjuvant group) or PBS (control group), entailing a total of 19 inoculations through 475 d. Each animal received a total amount of 81.29mg of Al hydroxide (except control group). RNA was extracted from encephalon and total RNA and miRNAs were analyzed by RNA-seq technology with a differential expression analysis, a functional enrichment analysis and a co-expression analysis. In general, a dysregulation of different genes and miRNAs has been seen in the adjuvant group (with a total of 63 genes and 38 miRNAs differentially expressed [DE]), but nearly no changes in the vaccinated group (13 DE genes and 2 DE miRNAs). The DE mRNAs and miRNAs are clearly related to neuronal development, brain transport, brain injury and neurodegenerative diseases associated with Al like AD, ALS and PD. The top ranked enriched GO terms were associated with mitochondrial function. Taking all into account, it seems that in the adjuvant group there is a dysregulation of mitochondrial function, leading to oxidative stress. Moreover, miRNA pattern analysis links apoptosis pathways, mitochondrial dysfunction and ECM related pathways to the intensive vaccination with the adjuvant alone.

Key Words: sheep, RNA-seq, aluminum, brain, bioinformatics

P49 Abstract withdrawn

P49 Abstract withdrawn

P50 Inferring the population structure of six North African sheep breeds using a medium-density SNP chip. S. Ben Jemaa*[1], S. Kdidi[2], A. M. Gdura[3], A. S. Dayhum[4], and M. Boussaha[5], *[1]Institut National de la Recherche Agronomique de Tunisie, Ariana, Ariana, Tunisia, [2]Arid Lands Institute, Médenine, Médenine, Tunisia, [3]Ministry of Agriculture, Tripoli, Libya, [4]Faculty of Veterinary Medicine, Tripoli, Libya, [5]GABI, INRA, AgroParisTech, Université Paris Saclay, Jouy en Josas, Ile de France, France.*

This study aims at providing a detailed assessment of the population structure and the genetic origins of 6 North African sheep populations using the Illumina 50K ovine BeadChip and comparisons with 22 worldwide sheep and mouflon populations. To tackle this question, we genotyped 35 Barbarine (23 from Tunisia and 12 from Libya), 19 Black Thibar (BT), 15 Sicilio Sarde (SS), 17 West Thin Tail (WTT) and 6 D'man individuals on the OvineSNP50 Genotyping BeadChip. A fine-scale assessment of the genetic structure of the populations was provided using principal components Analysis (PCA), ancestry models implemented in ADMIXTURE software and Discriminant Analysis of Principal Components (DAPC). Patterns of splits and mixture of the populations of the study were carried out using TreeMix software. Furthermore, f3-statistics were used to provide further support for a past admixture between populations. Regardless of the analytical method used, patterns of multiple hybridization events were observed within all North African populations leading to a heterogeneous genetic architecture which varies according to the breed. The Barbarine population showed the lowest genetic heterogeneity with a major Southwestern Asian ancestry bringing a further support on the Asian origin of the North African fat-tail sheep. All other breeds presented substantial Merino introgression ranging from 15% for D'man to 31% for Black Thibar. We also identified several signals of ancestral introgression between North African and South European sheep. In addition, model-based clustering analysis identified 2 opposite gradients of ancestry, Southwestern Asian and Central European, occurring between North Africa and Central Europe. Our results bring further evidence for the weak global population structure of sheep resulting from high levels of gene flow between breeds occurring worldwide. At regional level, signals of recent admixture between North African populations were also detected resulting in change of the original genomic architecture of minority breeds.

Key Words: North African sheep, SNP, genetic structure, admixture

P51 Associations of single nucleotide polymorphisms in the ovine prolactin and prolactin receptor genes with milk traits in Assaf dairy sheep. M. R. Marques*[1,2], D. S. Ribeiro[3], S. Gomes[4], A. T. Belo[1], J. R. Ribeiro[1], A. P. Martins[4,5], and C. C. Belo[1], *[1]UEISPSA, INIAV Instituto Nacional de Investigação Agrária e Veterinária I.P, Vale de Santarém, Portugal, [2]CIISA, Centro de Investigação Interdisciplinar em Sanidade Animal, Lisboa, Portugal, [3]ESAC, Escola Superior Agrária de Coimbra, Coimbra, Portugal, [4]UTI, INIAV, Instituto Nacional de Investigação Agrária e Veterinária I.P, Oeiras, Portugal, [5]LEAF, Linking Landscape, Environment, Agriculture and Food, ISA, Lisboa, Portugal.*

Milk production is a process coordinated by a complex endocrine and nutritional signaling cascade in which genes from the somatotropic axis play a key role. Among them, the prolactin (*PRL*) gene is known to regulate mammary gland growth, lactogenesis, and galactopoiesis. The actions of PRL, and also the ones of growth hormone and placental lactogen, are mediated by PRL receptor (PRLR), which activate several intracellular signaling cascades. The aim of this work is to identify single nucleotide polymorphisms (SNPs) in *PRL* and in *PRLR* genes associated with milk traits in Assaf ewes. Nine SNPs were genotyped by SNapShot® analysis in 450 Assaf ewes. Data regarding 150 d adjusted milk yield (P150d), total milk yield (PTotal) and lactation length from 2007 to 2017, and milk quality traits (fat, protein, lactose, total solids and fat free total solids contents) evaluated in 184 ewes (one lactation), were analyzed by mixed model procedure to disclose associations between genotypes and milk traits. The 9 genotyped SNPs were found to be polymorphic (MAF ranging from 0.004 to 0.493). In the *PRL* gene, AA ewes at rs412263261 had higher P150d (372.8 ± 35.56 *vs* 229.1 ± 25.71 L/150d; $P < 0.01$), and PTotal (503.0 ± 58.10 *vs* 318.5 ± 42.02 L; $P < 0.05$) than CA ewes. TT ewes at rs406266481 had higher PTotal than GG ewes (412.5 ± 16.64 *vs* 346.8 ± 11.04 L; $P < 0.01$), but GG ewes presented shorter lactations (211.0 ± 3.50 *vs* 230.1 ± 5.23 d; $P < 0.01$). No associations were found between *PRL* genotypes and milk quality traits. In the *PRLR* gene, CT ewes at rs604784916 had higher PTotal (446.7 ± 31.17 *vs* 340.5 ± 32.22 L; $P < 0.05$), and longer lactations (241.1 ± 9.69 *vs* 208.4 ± 10.70 d; $P < 0.05$) than TT ewes. Regarding milk quality traits, CT ewes at rs600947105 produced milk with a significantly higher protein content than homozygous ewes ($P < 0.05$). The results suggest that SNPS rs412263261 and rs406266481 at the PRL gene, and rs604784916 at the PRLR gene may be useful as early selection markers for milk production traits in Assaf sheep. Funding: Project financed by European Fund for Regional Development (ERDF) [ALT20–03–0145-FEDER-000019]

Key Words: sheep, SNPs, milk traits, PRL, PRLR

P52 Effects of prolactin and prolactin receptor polymorphism upon milk composition and milk coagulation properties in Assaf ewes. M. R. Marques*[1,2], S. Gomes[3], D. S. Ribeiro[4], J. R. Ribeiro[1], A. T. Belo[1], A. P. Martins[3,5], and C. C. Belo[1], *[1]UEISPSA, INIAV, Instituto Nacional de Investigação Agrária e Veterinária I.P, Vale de Santarém, Portugal, [2]CIISA, Centro de Investigação Interdisciplinar em Sanidade Animal, Lisboa, Portugal, [3]UTI, INIAV, Instituto Nacional de Investigação Agrária e Veterinária I.P, Oeiras, Portugal, [4]ESAC, Escola Superior Agrária de Coimbra, Coimbra, Portugal, Coimbra, Portugal, [5]LEAF, Linking Landscape, Environment, Agriculture and Food, ISA, Lisboa, Portugal.*

Associations of 5 SNPs of the prolactin (*PRL*) and 4 SNPs of the PRL receptor (*PRLR*) genes with milk production, composition (fat, protein, lactose, total solids and fat free total solids content), pH, and coagulation properties assessed by Optigraph [clotting time (R), gel firmness after 20 min (A20), and after a 2R (AR) period and rate of firming (OK20)] were investigated in Assaf ewes. Milk production and composition were evaluated monthly until the sixth month of lactation in 184 ewes, and pH and coagulation properties were evaluated at the first and third month of lactation in 92 ewes. Data were analyzed using a mixed-model procedure with fixed effects of SNP and month of lactation, considering the linear and quadratic effect of ewe' lambing age covariate. In the *PRL* gene, SNP AMGL02030943.1:g.23731G > T genotypes affected milk production throughout lactation ($P < 0.01$). Regarding milk composition, rs406266481 genotypes influenced fat free total solids content ($P < 0.05$). Moreover, *PRL* gene SNPs tended to affect protein (rs406266481), and lactose (rs422713690) and fat free total solids (rs422713690, rs412263261) contents throughout lactation ($P < 0.10$). pH tended to be affected by rs406266481 ($P < 0.10$). Considering clotting time, R was affected by AMGL02030943.1:g.23731G > T ($P < 0.05$), rs412263261 ($P < 0.10$), and by rs408430940 genotypes throughout lactation ($P < 0.05$), and. *PRL* gene SNPs had no effect upon the oth-

er coagulation properties, except for SNP AMGL02030943.1:g.23731G > T which tended to affect A20 ($P < 0.10$). In the *PRLR* gene, SNP rs600947105 genotypes affected protein content throughout lactation ($P < 0.05$) and tended to affect total solids content ($P < 0.10$). It also influenced pH ($P < 0.05$), A20 ($P < 0.01$), and AR ($P < 0.01$). Results suggest that the aforementioned SNPs might be used in gene-assisted selection programs for the improvement of milk quality traits and coagulation properties in Assaf sheep. However, analysis should be extended to a larger number of animals to validate this results. Funding: Project financed by European Fund for Regional Development (ERDF) [ALT20–03–0145-FEDER-000019]

Key Words: sheep, SNPs, milk traits, PRL, PRLR

P53 Genome-wide association studies for somatic cell count in Assaf breed. Y. Öner*[1], M. Serrano[2], M. Ramón[3], M. P. Sarto[4], L. P. Iguacel[4], M. Joy[4], M. Blanco[4], O. Estrada[4], T. Juan[4], and J. H. Calvo[4], *[1]Bursa Uludag University, Bursa, Turkey, [2]INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain, [3]Centro Regional de Selección y Reproducción Animal (CERSYRA)-Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF-JCCM), Valdepeñas, Spain, [4]Centro De Investigación Y Tecnología Agroalimentaria De Aragón (CITA), Zaragoza, Zaragoza, Spain.*

Alteration in somatic cell count (SCC) is widely used as indicator of mastitis, being one of the most costly production-related infectious disease in dairy industry. The aim of this study therefore was, to identify SNPs and genes associated with somatic cell count in sheep by using the Illumina AgResearch Sheep HD (680K). The animals for association studies were selected from 3 flocks of the Spanish Assaf breed. These flocks belong to the Teruel Association of Dairy and Cheese Producers. Only multiparous ewes with 2 or more lactations and at least 3 test day records during one lactation were considered. In total, we used 6173 records from 1907 ewes with at least 3 test day records between 2 and 7 years old. SCC data were logarithmic transformed. The animal effects phenotype in the whole population (n = 1907) and the SCC phenotype were estimated by fitting a Repeatability Mixed Model that included the herd-test day, the number of lambs born and the days in milk as fixed effects and the ewe herself as a random effect. One hundred and 90 2 animals with extreme values for the animal effects for the SCC phenotype were selected for GWAS analysis (n = 96 for low SCC; and n = 96 for high SCC). The association analysis was performed with the GCTA software. Significance of associations was assessed using a false discovery rate (FDR) multi-test correction. Locations of SNPs and genes were identified based on the sheep genome Ovis aries v 3.1. Genes located within 250 kb on either side of the significant SNP were annotated. After quality control with PLINK 559,762 SNPs were used for association analyses. The MLMA analysis did not reveal any significant SNP at genome level. However, 4 SNP on OAR19 were significant at chromosome level ($P < 0.01$). The first SNP was located in *NUP210* gene (rs419096188), and the other 3 SNPs (rs415580501, rs410336647, and rs424642424) were close to *ARPP21* gene, and mir128–2, expressed in the mammary gland of lactating goats, localized in *ARPP21*. These genes have been involved in viral response, cholesterol homeostasis and stress response.

Key Words: Assaf sheep, GWAS, somatic cell count

P54 Identification of a new mutation responsible for epidermolysis bullosa in Mouton Vendéen sheep. L. Chantepie*, L. Drouilhet, C. Genêt, F. Plisson-Petit, J. Sarry, G. Tosser-Klopp, F. Woloszyn, and S. Fabre, *GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet Tolosan, France.*

Junctional Epidermolysis Bullosa (EB) is a severe congenital disease affecting the skin at the extremities of the limbs and the mucous membranes. In sheep, this recessive disease causes perinatal death of affected lambs. Multiple observations of EB cases were recently reported in the French Mouton Vendéen meat sheep breed. Skin biopsies of 6 affected lambs and when available, blood samples from the parents (n = 7) and unaffected full-sib lambs (n = 4) were collected for genomic DNA extraction. From a bibliography study and analysis of the OMIM database (Online Mendelian of Human Genes and Genetic Disorders), we have focused on 12 candidate genes mainly belonging to the collagen, laminin, integrin and keratin families. Based on the hypothesis of a recessive transmission of a deleterious variant, we have performed whole-genome sequencing of 2 unrelated EB-affected lambs (supposed homozygous carriers) and 1 unaffected full-sib (supposed heterozygous or non-carrier). Using the GATK workflow on a Galaxy platform, we have identified a novel SNP in the exon 23 of the *ITGB4* gene of the integrin family (OAR11_v4.0, g. 54799925 G > A (p.885 R > *)) whose variant allele causes a premature stop codon. By a specific RFLP assay, we have determined that all EB-affected lambs were homozygous for this variant allele, their parents were heterozygous and the full-sibs were either heterozygous or non-carrier, fitting well with the working hypothesis. Following this primary discovery, a larger set of Mouton Vendéen animals was genotyped. We estimated the population allele frequency at 6.8% by genotyping a cohort of renewal ewe lambs (n = 1227). We also found a 5.7% allele frequency among the breeding rams present in 2018 (n = 1007) in artificial insemination center, progeny-testing station, and for natural mating in farms. Moreover, the specific genotyping of producing ewes in the most EB-affected flocks revealed a variant allele frequency up to 13.3%, due to overuse of inbreeding strategy. In conclusion, the discovery of a new mutation in *ITGB4* causing EB in sheep will improve the selection scheme management of the Mouton Vendéen breed to limit the dissemination of this disease.

Key Words: sheep and related species, genetic disorder, genome sequencing, candidate gene

P55 Abstract withdrawn

P55 Abstract withdrawn

P56 Identification of polymorphism in *MC4R* gene and its association with dry matter and crude protein intake in post-weaned Bligon goats. L. Latifah*[1], A. Kustantinah[2], D. Maharani[1], and T. Hartatik[1], *[1]Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadja Mada, Yogyakarta, Indonesia, [2]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadja Mada, Yogyakarta, Indonesia.*

Melanocortin-4 receptor (MC4R), a prototypical G-protein coupled receptor (GPCR) that is highly expressed in the hypothalamus plays an important role in regulating feed intake and energy balance. The aim of this study was to identify the association between single nucleotide polymorphism (SNP) in MC4R gene with dry matter and crude protein intake in post-weaned Bligon goats. The dry matter and crude protein intake were estimated by in vivo method. Genomic DNA from Seventeen Bligon goats was extracted for genotyping analysis using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). One SNP (g.1079C/T) was identified by PCR-RFLP with *Kpn*1 restriction enzyme. The results revealed 2 genotypes (CC and CT), with frequencies of 0.71 and 0.29, respectively. Statistical analysis showed no significant association between MC4R genotype and dry matter and crude protein intake. In conclusion, SNP g.1079C/T in the MC4R gene is only useful for animal genotyping, but it cannot be used as a marker-assisted selection on dry matter and crude protein intake in Bligon goats.

Key Words: MC4R gene, dry matter intake, crude protein intake, Bligon goat

P57 Haplotype diversity and maternal origin of Swedish goat landraces. G. M. Tarekegn*[1,2], E. Jonas[1], and A. Johansson[1], *[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala Sweden, [2]Department of Animal Production and Technology, Bahir Dar University, Bahir Dar Ethiopia.*

Despite the fact that Swedish goats are considered among the highest milk yielders in the world, very little or no information is documented about their origin and level of diversity. We analyzed the control region (808 bp length) of mitochondrial DNA (mtDNA) to assess the maternal genetic variation and demographic dynamics of the indigenous goats in Sweden. In the study, 71 individuals from 3 populations (Svensk Lantrasget: n = 60; Jämtget: n = 6; Lappget = 5) were included. A total of 48 variable sites that generated 30 haplotypes were observed. The average haplotype and nucleotide diversities, respectively, were 0.931 ± 0.016 and 0.0134 for Svensk Lantrasget, 0.867 ± 0.129 and 0.00668 for Jämtget and 1.000 ± 0.126 and 0.01386 for Lappget. The analysis of molecular variance (AMOVA) revealed 17.92% of variation among the goat populations. This variation could possibly be attributed by accumulation of inbreeding within the populations as supported by neutrality test. The haplotype mismatch distribution analysis demonstrated presence of a onetime population expansion event in Swedish goats. To define the haplogroup of Swedish goats, we incorporated reference haplotypes from 6 globally defined haplogroups and the phylogenetic network revealed that all the haplotypes detected in Swedish goats clustered only to haplogroup A. This indicates that a narrow gene pool is available in the goat populations. However, genotyping the autosomal information may provide the full picture of the status of diversity.

Key Words: haplogroup, haplotypes, Swedish goat

P58 Unravelling ovine lncRNA expression in the presence of aluminium hydroxide adjuvants. M. Bilbao-Arribas*[1], E. Varela-Martínez[1], N. Abendaño[1], J. Asín[2], M. M. Pérez[2], R. Reina[3], D. de Andrés[3], L. Luján[2], and B. M. Jugo[1], *[1]Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Basque Country, Spain, [2]Veterinary Faculty, University of Zaragoza, Zaragoza, Aragón, Spain, [3]Institute of Agrobiotechnology, CSIC-UPNA, Pamplona, Navarra, Spain.*

Long non-coding RNAs (lncRNAs), defined as transcripts without coding capability and longer than 200 nucleotides, are increasingly being linked to physiological and pathological processes of many kind. Aluminum (Al) adjuvants have been added to vaccines for decades, yet their mechanism of action for activating immune responses is not fully understood. Recently, our group profiled the effect of aluminum adjuvants in vaccines by transcriptome-wide analyses in sheep. In a long-term experiment, sheep received commercial vaccines and adjuvant-only (Al-hydroxide) vaccines and their tissues' transcriptomes were sequenced by RNA-seq. Here, we study the expression profiles of lncRNAs and their relations with mRNAs and miRNAs in 2 tissues. Pairwise comparisons were made between the 3 groups in each tissue: control, adjuvant-inoculated and vaccinated animals. We classified 2284 and 3004 transcripts as lncRNAs in peripheral blood mononuclear cells (PBMCs) and brain tissue, respectively. Genomic conservation analyses revealed low sequence conservation at sequence level but several lncRNAs with syntenic conservation. In PBMCs, differentially expressed (DE) lncRNAs comprise 304 transcripts in the 3 comparisons, where the *adjuvant* vs. *vaccine* comparison showed the lowest number of DE genes. In the brain, 47 lncRNAs were DE and the *adjuvant* vs. *vaccine* and *adjuvant* vs. *control* comparisons had the most DE lncRNAs. In this tissue, the lncRNA TUNA, necessary for neural differentiation and pluripotency, was downregulated in the adjuvant group. Gene co-expression networks were constructed to determine with which genes lncRNAs showed similarities and shared functions, and interaction predictions were made in *cis* and *trans*. Globally, these results indicate that Al adjuvants have an effect on lncRNA expression, but in a different way depending on the tissue. The relevance of lncRNA dysregulation in the presence of Al remains to be further analyzed by functional studies, especially if it leads to or derives from Al-induced toxicity.

Key Words: sheep, lncRNA, RNA-seq, aluminium, animal health

P59 The use of an animal linear model to predict genotypes at a single locus of ungenotyped animals. M. N. Boareki*[1], D. Kennedy[2], L. R. Schaeffer[1], A. Suarez-Vega[1], F. S. Schenkel[1], and A. Cánovas[1], *[1]Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]2Ontario Ministry of Agriculture, Food and Rural Affairs, Elora, ON, Canada.*

Allelic variation at a single locus could be associated with the economically important monogenic traits. However, phenotypes may not be available for these traits. For example, sex-limited traits are expressed in one sex, and disease resistance traits are expressed in the presence of the infection. In such cases, the genotypic information at the locus of interest could be used to select for the favorable homozygous genotypes. However, in practice, the genomic information may be missing, because not all animals are genotyped. In this study, we propose the use of animal linear model to predict the favorable genotype of monogenic trait in ungenotyped animals. Pedigree and genotypes from 7,100 animals were simulated using QMSim software. The number of copies of favorable alleles observed on animals' genotypes (0, 1, or 2) were used as response variable in an animal linear model assuming complete heritability. The model was tested in 4 scenarios considering different sets of genotyped animals: 1) sires only; 2) 20% of animals; 3) 10% of animals; and 4) 5% of animals (randomly chosen out of 7,100). The genotypes of ungenotyped animals were predicted based on the additive relationship matrix. Correlations between the true genotypes and the predicted for ungenotyped animals were 0.51, 0.56, 0.48, and 0.39, when genotypes were available for sires, 20%, 10%, and 5% of the animals in the population, respectively. Selecting ungenotyped animals with predicted number of alleles (>1.5 alleles) increased the genotype frequency of favorable homozygous (>62.9%) and decreased

the frequency of unfavorable homozygous (<1.2%) among the selected group compared with the corresponding original genotypic frequencies of 22.7% and 28.1%, respectively. Thus, the predicted genotypes could be used for selection decisions to increase the frequency of favorable alleles for the desired monogenic trait. Furthermore, animals that are likely to have the favorable homozygous genotype could be identified and genotyped before breeding to confirm their genotype status. Therefore, an animal linear model could provide additional information that could be used in a breeding program to select for monogenic traits.

Key Words: monogenic trait, animal linear model, genetic improvement, selection

P60 Abstract withdrawn

P61 Association studies for the age at first lambing in Rasa Aragonesa ewes. L. P. Iguacel*[1], K. Lakhssassi[1], M. P. Sarto[1], B. Lahoz[1], J. Folch[1], M. A. Jiménez[2], M. Serrano[2], J. L. Alabart[1], and J. H. Calvo[3], *[1]CITA-IA2, Zaragoza, Spain, [2]Department of Animal Breeding and Genetics, INIA, Madrid, Spain, [3]CITA-ARAID-IA2, Zaragoza, Spain.*

In sheep, delays in the age at first lambing (AFL) give rise to unproductive periods and a decrease in productive life. Despite its proven influence on economic profitability, in the Rasa Aragonesa sheep breed the efforts in the last years to improve it have been scarce. The aim of this study was to identify new SNPs/genes associated with the AFL trait in sheep by using a SNP panel for parentage assigment that also include functional SNPs. For this purpose 191,114 fist lambing records (ewes mated without hormonal treatments) from 327 farms were analyzed using the GLM procedure (SAS), and were corrected for environmental effects. The model included $FecX^R$ genotype, farm, month and year of birth as fixed effects. Model residuals were used in the association studies. A total of 351 ewes from one farm were genotyped using KASP technology with a panel of 172 SNPs that included 153 SNPs for parentage assignment and 19 functional SNPs. The association analysis was performed with the GCTA software (Yang et al., 2011). Sixteen SNPs were found associated with AFL trait at $p_{nominal} < 0.05$. These SNPs were located in *BMP15*, *KISS1R*, *MTNR1A*, *SPTAN1*, *FA2H*, *TMEM154* and *MTUS1* genes. However, associations at the genome level ($p_{Bonferroni} < 0.05$) were only found for SNP rs421419167 (*BMP15*/$FecX^R$ allele). Associations at genome-wise suggestive significance ($p_{suggestive} \leq 1/n$) were found for SNPs rs421419167, rs398938610 and rs412567923 located in *BMP15* ($FecX^R$ allele), *KISS1R* and *SPTAN1* genes, respectively. The $FecX^R$ allele causes increased prolificacy in heterozygous and sterility in homozygous ewes. The SNP rs398938610 is a non-conservative mutation (p. C309F) located in *KISS1R* gene. This mutation was predicted as tolerated but with a low SIFT value (0.06) by VEP software. Kisspeptin (KISS1) and its receptor (KISS1R) form a system that regulate the release of GnRH that modulates the release of gonadotropins from the pituitary. KISS1/KISS1R system appear to be important for aspects of reproductive physiology, ranging from the initiation of puberty to the induction of ovulation. These results have to be validated in a bigger population before undertaking decisions about its management in the selection program of this breed.

Key Words: sheep, age at first lambing, SNP panel, KASP technology

P62 Genome-wide association study (GWAS) identifies the $FecX^{Gr}$ allele in BMP15 segregating in Rasa Aragonesa sheep breed. L. Chantepie[1], M. Serrano[2], M. P. Sarto[3], L. P. Iguacel[3], M. A. Jiménez[2], J. L. Alabart[3], J. Folch[3], B. Lahoz[3], S. Fabre[1], and J. H. Calvo*[4], *[1]Université de Toulouse, INRA, Toulouse, France, [2]INIA, Madrid, Spain, [3]CITA-IA2, Zaragoza, Spain, [4]CITA-ARAID-IA2, Zaragoza, Spain.*

Rasa Aragonesa is an autochthonous Mediterranean sheep breed, mainly reared in extensive or semiextensive farming systems and oriented to meat production. The Cooperative Oviaragon-Grupo Pastores carries out since 1994 a selection program for prolificacy in this breed, with 490,337 controlled ewes at present. A *FecX*-mutated allele called $FecX^R$ in *BMP15* gene was described in Rasa Aragonesa sheep breed. This polymorphism causes increased prolificacy in heterozygous and sterility in homozygous ewes. However, highly prolific ewes without the $FecX^R$ allele have been found in the population. A genome-wide association study (GWAS) was performed to identify other hyperprolific genetic variants in the Rasa Aragonesa sheep breed. The GWAS was performed with the GCTA software (Yang et al., 2011) using 158 ewes (73 high prolific *vs*. 85 low prolific ewes) with the Illumina AgResearch Sheep HD (680K) microarray. Ewes having at least 3 lambing records, and without the $FecX^R$ allele were selected from the whole Cooperative Oviaragon-Grupo Pastores database. These ewes came from 33 and 36 different farms for high prolific and low prolific ewes, respectively, and were selected as unrelated as possible based on their pedigree information. A significant association at the genome level ($p_{Bonferroni} < 0.05$) was found for SNP oar3_OAR27_50971170_dup located in *BMP15* gene (Oar3.1 X: g. 50971170C > T). This is the $FecX^{Gr}$ mutation (NM_001114767.1: c.950C > T) associated with increased prolificacy in other sheep breeds. Only 14 heterozygous animals were found (MAF = 0.04), all of them associated with high prolific ewes. These animals were sequenced for *BMP15,* finding only the $FecX^{Gr}$ allele. No homozygous animals were found for this allele. This allele was first described in Grivette (Demars et al., 2013), and recently in Mouton Vendeen (Chantepie et al., 2018), Romanov, Dorper and Ovella Galega (Vera et al., 2018) sheep breeds. These results could indicate the ancestry of this variant in sheep. The selection program should implement the genotyping of reproducers for the $FecX^{Gr}$ allele, to know its frequency and the size of its effects to be able to undertake decisions about its management.

Key Words: $FecX^{Gr}$, $FecX^R$, *BMP15*, Rasa Aragonesa, prolificacy

P63 Differential gene expression in pars tuberalis and hypothalamus tissue from Rasa Aragonesa sheep with different oestrous and anoestrous phases using RNA-Seq. K. Lakhssassi*[1], I. Ureña[2], B. Marín[3], M. P. Sarto[1], B. Lahoz[1], J. L. Alabart[1], J. Folch[1], J. H. Calvo[1,4], and M. Serrano[2], *[1]CITA-IA2, Zaragoza,Spain, [2]INIA, Madrid, Spain, [3]Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Zaragoza, Spain, [4]CITA-ARAID-IA2, Zaragoza,Spain.*

The mechanistic changes responsible for seasonal reproduction in ewes happen primarily at the level of the brain, being the Pars Tuberalis (PT) and Hypothalamus (HT) key tissues in sexual activity regulated by photoperiod. For understanding the molecular events behind follicular (F) and luteal (L) phases of oestrous cycle, and anoestrous phase (A), the PT and HT transcriptomes of 21 Rasa Aragonesa ewes (7 for each phase and tissue) were studied using RNaseq. Ewes were selected based on weekly repeated measures of progesterone to determine the ovarian phase, which was confirmed after animals sacrifice and ovary dissection. Sequencing was carried out through the Illumina Hi-Seq 2000 platform, generating paired-end reads of 76 bp. For differential expressed (DE) gene assessment, DESeq2 package was used (Love et al., 2014). We performed GO and KEGG pathway enrichment analyses using the DAVID online tool. In HT, 72 and 3 DE genes were found (p_adjusted <0.05) in the comparisons between F vs A and L vs A phases, respectively. Enrichment analysis of F vs A comparison showed significant enrichments for the oxytocin signaling, smooth muscle contraction, and focal adhesion KEEG pathways. Oxytocin signaling pathway regulates hypothalamic-pituitary-adrenal axis, modulating behavioral response toward stress and social behavior. Four DE genes downregulated in the follicular phase compared with the anoestrus in HT (*HTR2B*, *ITPR3*, *LTA4H* and *PTGIS*) have been related with oestrous behavior in dairy cows (Kommadath et al., 2011). In PT, the magnitude of gene expression differences between the different phases was greater than in HT. In PT, 6, 4 and 14 DE genes were found in the comparisons F vs A, L vs A and L vs F phases, respectively. *ITLN* was upregulated in the follicular ($Log_2FC = 17$) and luteal phases ($Log_2FC = 20$) compared with anoestrus. By contrast, *MRPL57* ($Log_2FC = -22$) and *IRX4* ($Log_2FC = -20$) were highly downregulated in the luteal compared with the follicular and anoestrous phases. The *DDC* gene, related to LH regulation, is upregulated in follicular phase ($Log_2FC = 1.92$; F vs A).

Key Words: sheep and related species, RNA-seq, gene expression

P64 Analysis of the allelic sequences in the DNA microsatellite loci used in parentage control in sheep: Preliminary studies. A. Szumiec, A. Radko, A. Piestrzynska-Kajtoch, A. Koseniuk*, A. Podbielska, and D. Rubis, *National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

Allelic ladder is important for accurate genotype determination and has been recommended by the International Society of Forensic Genetics. They are necessary to compare sizing measurements obtained from different instruments and conditions used by various laboratories. Alleles in the ladder should be confirmed by sequencing. The determination of a given allele should be not only the number of base pairs of the obtained PCR product, but above all the number of repeated motifs. The objective of the research was to create allelic ladders for sheep parentage control STR panel. To determine the allele's sizing, we analyzed polymorphism of 12 microsatellite markers recommended by ISAG for sheep parentage control. At first, we studied the CSRD247 marker. Only homozygous alleles were used for amplification. The PCR products were purified with ExoSAP-IT (USB Corporation) and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit. The sequencing reaction products were purified from residual dye terminators using BigDye® XTerminator Purification Kit. Electrophoresis was carried out on an 3500 Genetic Analyzer using POP-7 polymer. Data were analyzed using Variant Analysis Software® (Thermo Fisher Cloud Environment). Analysis of the CSRD247 sequences shown that the repeat motif consists of in AC nucleotides. Three alleles were sequenced (223, 227, 229) and we noticed that the AC-motif was repeated 20-, 22-, 23-times respectively. Next, other ovine STR markers recommended by ISAG will be analyzed. The study is in progress.

Key Words: sheep and related species, genetic identification, DNA sequencing, genetic marker, parentage

P65 Association of a nucleotide variant in Tenascin X with objective milk production traits in US dairy sheep. K. M. Hemmerling[1], T. W. Murphy[2], M. K. Herndon[1], A. T. Massa[1], M. U. Cinar[3,1], D. L. Thomas[4], S. N. White[5,6], and M. R. Mousel*[5,7], *[1]Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, [2]USDA, ARS, Meat Animal Research Center, Clay Center, NE, USA, [3]Animal Science Erciyes University, Kayseri, Turkey, [4]Animal Sciences, University of Wisconsin, Madison, WI, USA, [5]USDA, ARS, Animal Disease Research, Pullman, WA, USA, [6]Center for Reproductive Biology, Washington State University, Pullman, WA, USA, [7]School for Global Animal Health, Washington State University, Pullman, WA, USA.*

With the world population growing by 83 million people annually, it is vital to select livestock that efficiently produce high quality products to meet increasing worldwide nutritional needs. The US is the largest importer of sheep milk cheeses in the world, and has a small, but growing dairy sheep industry. Currently there is no genetic evaluation program in the US Therefore, identifying genomic regions that positively influence milk quantity and quality would be beneficial. In meat and wool breeds, a variation in the Tenascin X (TNXB) gene, E2004G, was associated with subjective milk score and mature bodyweight. To determine if there was an association between TNXB E2004G and objective 180 d adjusted milk production traits, 216 ewes originating from a US dairy sheep research flock were genotyped. Ewes were 2 to 6 years old and consisted of East Friesian (E), Lacaune (L), Awassi (A), and Katahdin (K) purebreds and crosses. The traits analyzed included individual milk, fat, and protein yield, and fat and protein percentage. A reduced mixed model was used with fixed effects of age, breed composition, and TNXB genotype and a random effects of sire nested within breed type and year of lactation. The effect of genotype on protein yield approached significance ($P < 0.06$) with AG ewes producing more protein than GG ewes. There was a numerical trend for each additional A to increase milk, protein, and fat yield but the low number of AA animals reduced the power to detect statistical differences. No genotype effects for the other traits were observed ($P > 0.10$). As expected, age impacted milk, protein, and fat yield ($P < 0.01$) with 5 year olds produced more than 4, 3, and 2 year olds. EL crossbreds had greater milk yield than ELA and ELK ewes ($P < 0.04$). Additional studies need to be conducted with more balanced genotype frequencies to validate these results. Gene expression in mammary tissue is being evaluated to determine whether differences in genotype affects expression. Understanding how TNXB E2004G impacts milk production may allow producers to use TNXB genotypes as a selection tool to improve dairy sheep production.

Key Words: dairy sheep, milk production, Tenascin X, breeds

P66 Framework for successful implementation of community-based breeding programs in small ruminants in Ethiopia. A. Haile*[1], T. Getachew[1], M. Rekik[2], A. Abebe[3], and B. Rischkowsky[1], *[1]International Center for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia, [2]ICARDA, Amman, Jordan, [3]Debre Berhan Agricultural Research Center, Debre Berhan, Ethiopia.*

Conventional breeding programs involving either nucleus schemes and/or importation of exotic germplasm for crossbreeding were not successful in most sub-Saharan Africa countries. Community-based breeding programs (CBBPs) are suggested as alternatives for genetic improvement of small ruminants. CBBP is different from other schemes in that it involves the various actors from the initial phase of design up until implementation of the programs. Livestock keepers' views are considered at each stage of the program design and implementation unlike the often-top down government run schemes. In Ethiopia, we piloted CBBPs since 2010 and the results show that CBBPs

are technically feasible to implement and result in measurable genetic gain and socio-economic impact. However, their successes depend on the following: 1) identification of the right beneficiary following a clear guideline on who should be a member; 2) clear framework for dissemination of improved genetics and up/ out scaling strategy to make impact at scale; 3) institutional arrangements including establishment of breeders' cooperatives to support functionality and sustainability of the programs; 4) capacity development of the different actors on general animal husbandry, breeding practices, estimation of breeding values and financial management; 5) mobile application for data collection and management that is easy to use and that works offline; 6) support for long periods with committed technical staff (either extension or research) mainly in data management, analysis and feedback of estimated breeding values; 7) complementary services including disease prevention and control, proper feeding, and market linkages for meat and breeding animals; 8) a system for certification of improved rams/ bucks by an authorized body to ensure quality control which will support and create demand for the breeding animals; and 9) evaluation of the program and assessment of impact of the scheme. The framework we developed which integrates these elements has resulted in successful and sustainable breeding programs in Ethiopia.

Key Words: small ruminants, genetic improvement, breeding schemes

P67 Use of whole-genome sequencing datasets to study the genetic variability of the *LALBA* gene across different sheep breeds. H. Marina, B. Gutierrez-Gil, C. Esteban-Blanco, R. Pelayo, and J.-J. Arranz*, *Departamento de Producción Animal, Facultad de Veterinaria, Universidad de Leon, Leon, Leon, Spain.*

Sheep milk is mainly used to produce a wide variety of high quality cheeses. The amount of milk produced is controlled primarily by the amount of lactose synthesized. Alfa-Lactalbumin forms the regulatory subunit of the lactose synthase and is also one of the in whey proteins. This protein is encoded by the *LALBA* gene located on sheep chromosome 3. In addition, a quantitative trait nucleotide (QTN) has been identified in the *LALBA* gene in relation to milk composition traits in Churra sheep (milk protein and fat percentages). The aim of this study was to assess the genetic diversity of the *LALBA* gene in relation to production specialization based on Whole Genome Sequences of 144 ovine genomes from 44 sheep breeds, and 3 wild sheep (*O. canadiensis, O. dalli, and O. orientalis*). A total of 87 sequences were obtained from the SRA archive whereas 57 were generated by our group. A variant calling analysis was performed with the GATK software and SAMtools software across the whole genome, to select those high quality variants detected by both pipeline. The nucleotide diversity detected in *LALBA* gene ($\pi = 0.00405$) was higher than that found in previous autosomal diversity studies in domestic sheep. In total, 40 variants were found within the *LALBA* gene, 4 of which were located in coding regions. Three of these variants were missense variants and were predicted to have a moderate impact over the protein (p.Val27Ala; p.Asp135Asn; p.Leu142Phe) whereas the other one was a synonymous variant of low functional impact. The rest of variants were located in UTR regions and in introns, 2 of them involving splice sites. Nine out of 40 variants were found only in the domestic sheep studied, of which 2 were located within exons. Conversely, 5 out of 40 variants were found only in *O. canadiensis* and *O. dally*, of which one was located in an exon and it was not previously described. The variant identified as a QTN variant (p.Val27Ala) for milk composition traits in previous studies was segregating in 32 breeds and did not show a clear pattern in relation to the production ability.

Key Words: sheep and related species, genome sequencing, bioinformatics tools, sequence variation

P68 A follow-up study on the genome-wide relationships among Merino and Merino-derived sheep breeds. S. Ceccobelli[1], E. Ciani[2], E. Lasagna*[1], F. S. Silva[3], G. Lühken[4], S. Kusza[5], M. Spehar[6], R. Niznikowski[7], M. Swiatek[7], V. A. Balteanu[8], G. Ciappesoni[9], T. Karsli[10], N. W. Kunene[11], F. Pilla[12], F. M. Sarti[1], *[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy, [2]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari "Aldo Moro," Bari, Italy, [3]Instituto Nacional de Investigação Agrária e Veterinária, I.P, Santarém, Portugal, [4]Institut für Tierzucht und Haustiergenetik, Justus-Liebig-Universität, Liebig, Germany, [5]Animal Genetics Laboratory, Institute of Animal Husbandry, Biotechnology and Nature Conservation, University of Debrecen, Debrecen, Hungary, [6]Croatian Agricultural Agency, Croatia, [7]Department of Animal Breeding and Production, Warsaw University of Life Sciences, Warsaw, Poland, [8]Institute of Life Sciences, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania, [9]Instituto Nacional de Investigación Agropecuaria, Las Brujas, Uruguay, [10]Department of Animal Science, Agriculture Faculty, Akdeniz University, Antalya, Turkey, [11]Department of Agriculture, University of Zululand, Richards Bay, South Africa, [12]Dipartimento Agricoltura Ambiente e Alimenti, Università degli Studi del Molise, Campobasso, Italy.*

Prompted the growing renewal interest in the exploitation of wool in various traditional as well as innovative application to the field, the genetic relationships among 18 Merino and Merino-derived sheep breeds from the Mediterranean, Central and Eastern Europe, South Africa, North and South America, Asia, and Australia were investigated. In this study a total of 44K SNP genotypes, that passed the quality control filters from the Illumina Ovine50 BeadChip, available for 408 animals were used. The multidimensional scaling plot of the IBS distances is highlighted known as well as the novel evidences of genetic proximity. Chinese Merino clustered with American Rambouillet. Polish Merino and German Merinofleischaf clustered close to each other, likely due to some influence of German Merino sheep stocks into the Polish one. Moreover, a strong influence of Australian Merino was detected in Merino breeds from South Africa and Uruguay. In addition, the Romanian Merino appeared close to the Australian group, which is consistent with the major genetic contribution of Australian Merino to the Romanian breed. The Croatian Paska was confirmed that have influenced by stocks from the other side of the Adriatic coast (Gentile di Puglia from Apulia). The Spanish Merino sampled in our study clustered in 2 separate groups, one corresponding to a current commercial population (animals sampled in the Estremadura region) and the other to the "historical" Spanish Merino population (animals preserved in the selection center, Andalusia). The Apulian sheep were those displaying the highest proximity with samples from the old-type Spanish Merino, this fact confirms the story of the Latin literature that described the importation of genetic material from Apulia in Spain. Interestingly, the Merino Branco in Portugal, was split in 2 separate clusters: the large group (19 animals) falls, as expected, close to the Spanish Merino (notably, the group from Extremadura), while the smaller (9 animals) branched close to the Italian Merino breeds. This phenomenon deserves further investigation to understand whether the observed genetic sub-structuring in the Iberian Merino population reflects recent or historical herd management practices.

Key Words: SNPs, *Ovis aries*, wool, genetic diversity

P69 Identifying genetic structure and admixture in sheep from terminal breeds in the United States. K. M. Davenport*[1], C. Hiemke[2], S. McKay[3], J. W. Thorne[4,1], R. M. Lewis[5], T. Taylor[6], and B. M. Murdoch[1], *[1]University of Idaho, Moscow, ID, USA, [2]Niman Ranch & Mapleton Mynd Shropshires, Stoughton, WI, USA, [3]University of Vermont, Burlington, VT, USA, [4]Texas A&M AgriLife Research and Extension Center, San Angelo, TX, USA, [5]University of Nebraska-Lincoln, Lincoln, NE, USA, [6]University of Wisconsin-Madison Arlington Research Center, Arlington, WI, USA.*

Selection for performance in diverse production settings has resulted in variation across sheep breeds worldwide. Although sheep are an important species to the United States (US), the current genetic relationship among many terminal sire breeds has not been well characterized. The objective of this study was to examine population structure

and admixture of US sheep from terminal breeds. Suffolk, Hampshire, Shropshire, Dorset, Oxford and Southdown (terminal) and Rambouillet (dual purpose) sheep (n = 256) sampled from different flocks were genotyped using the Applied Biosystems Axiom Ovine Genotyping Array (50K), and additional Shropshire (n = 26) using the Illumina Ovine SNP50 BeadChip. After merging data from the 2 genotyping platforms and excluding SNPs with a call rate of less than 0.90 and a minor allele frequency of less than 0.01, 45,864 SNPs were included in subsequent analyses. Relationships between breeds were investigated by calculating observed heterozygosity, inbreeding coefficients, eigenvalues, an identity by state (IBS) matrix and pairwise Wright's F_{ST} estimates. The mean observed heterozygosity for each breed ranged from 0.304 to 0.360 and is consistent with data reported in Australian sheep. Eigenvalue plots and a rectangular cladogram constructed from the IBS matrix revealed distinct clustering of Rambouillet away from terminal breeds; Suffolk from 2 different regions (Midwest and West) also clustered separately. These findings were supported by pairwise F_{ST} estimates for Rambouillet and terminal breeds, 0.16–0.23, and for Western Suffolk and Dorset and Southdown, 0.17–0.18, respectively. Admixture between breeds was examined using STRUCTURE, and based maximum likelihood estimates the best fit number of populations (clusters) was K = 6. The most admixture was observed for Hampshire, Suffolk, Shropshire, and Oxford breeds. When plotting eigenvalues, US terminal breeds clustered separately in comparison to sheep from other locations of the world. Understanding the genetic relationships between terminal sire breeds will inform us about the potential utility of production trait data derived from one breed for other closely related breeds.

Key Words: sheep, admixture, heterozygosity, breed diversity, population structure

P70 Abstract withdrawn

P71 Runs of homozygosity patterns of distribution and level of inbreeding in South African sheep breeds. E. F. Dzomba*[1] and F. C. Muchadeyi[2], *[1]University of KwaZulu-Natal, Pietermaritzburg, KwaZulu-Natal, South Africa, [2]Biotechnology Platform, Agriculture Research Council, Pretoria, Gauteng, South Africa.*

Production system and within-breed selection pressure impacts the genome architecture resulting in reduced genetic diversity and frequency of runs of homozygosity islands. In the current study, we determined autozygous segments based on runs of consecutive homozygous genotypes (ROH) for South African sheep breeds as well as various other sheep breeds obtained globally. We tested the hypothesis that production systems geared toward specific traits of importance such as mutton, wool, pelt or multiple traits (as with some dual purpose breeds) or absence of selection programs e.g., in non-descript breeds kept by smallholder farmers, could influence the occurrence and distribution of ROH. The Illumina OvineSNP50 BeadChip was used to genotype 400 sheep belonging to 13 breeds from South Africa. To gain insight into the South African breeds relative to global populations, 623 genotypes of sheep from worldwide populations were included in the analysis. After standard filtering, a total of 1017 animals and 43 556 SNPs were retained for analyses. Runs of homozygosity were computed at cut-offs of 0–6MB, 6–12MB, 12–24MB, 24–48MB and >48MB, using the R package detectRUNS. We identified 121 399 ROH in total with mean number of ROH per animal per breed ranging from 51.10 (Karakas) to 222.33 (Nguni). The average length of ROH across breeds was 5.88 Mb and ranged from 2.60 Mb (Afrino) to 6.90 Mb (Nguni). For all breeds, the majority of the detected ROH were in the smallest 0–6 Mb in length category (88.2%). The biggest were the least (n = 108) with most animals detecting no ROH in this category ROH >48 Mb. McArthur Merino had the highest value of inbreeding on the basis of ROH (F_{ROH} = 0.429), whereas Red Masai (F_{ROH} = 0.0624) and Australian Poll Merino (F_{ROH} = 0.0643) showed the lowest, and highest standard deviation values revealed high variability in autozygosity levels within the Nguni breed. Overall, the patterns of distribution of ROH revealed in this study showed peculiar patterns of inbreeding of sheep breeds that corresponded with levels of selection pressure typical of trait of economic importance as well as the production system typical of their rearing. There are similarities of South African breeds with other breeds reared across the world especially when comparing the Merino-type breeds. We also identified 41 genomic regions harboring most ROH across breeds as well as the associated candidate genes.

Key Words: sheep, production system, SNP genotypes, autozygosity, inbreeding

P72 Genetic structure of the Canary goat using genome-wide SNPs profiling. J. A. Bouzada*[1], V. Landi[2], A. M. Martínez[2], M. E. Camacho[3], J. Capote[4], N. Darmanin[4], M. M. Gómez[2], A. Torres[4], J. V. Delgado[5], and M. Fresno[4], *[1]Laboratorio Central de Veterinaria, Algete. Madrid. Spain, [2]Animal Breeding Consulting S.L, Córdoba, Spain, [3]Instituto de Investigación y Formación Agraria y Pesquera (IFAPA), Córdoba, Spain, [4]Instituto Canario de Investigaciones Agrarias (ICIA), Tenerife, Spain, [5]Dpto. de Genética, Universidad de Córdoba, Campus Universitario de Rabanales, Córdoba, Spain.*

This work aims to study the genetic structure and phylogenetic relationships of 3 goat breeds of the Canary Islands: the Palmera, Majorera and Tinerfeña breed. In the archipelago the caprine production represents an important economic sector both for the production of renowned dairy products, inserted in the tutelage circuit, and because this type of breeding, adapted to the ecosystem of the islands for centuries, promotes the fixation of the human population in rural areas in particular internal ones, far from marine tourism. In terms of numbers of animals, it is one of the largest goat populations in Europe. A total of 24 samples per breed were analyzed using the Illumina Goat 50K chip. The Murciano Granadina, Granadina, Murciana, Malagueña, Alpina and Saanen breeds were used as comparisons. After the application of various quality filters (MAF <0.01; Mind <0.1; Geno <0.1; Hwe <0.001) the final data set consisted of 49552 SNPs. The 3 Canary races appear to be very divergent from other continental and cosmopolitan races and a certain relationship can be observed between them (FST average 0.06)

and in particular between Majorera and Tinerfeña (FST 0.03). There is a considerable genetic distance with the rest of the breeds ranging from 0.161 with the Tinerfeña to 0.140 with the Majorera and to 0.214 with the Palmera. The genetic differentiation measured across AMOVA shows a variability of 11.14% ($P < 0.01$) between the groups (Canary vs Continetal) and 5.32% ($P < 0.01$) among the population within the group. The Baynesian analysis with FastStructure highlights the homogeneity of the 3 Canarian breed with an average membership coefficient of 0.96. The Palmera breed is still the most divergent evenly separating itself from low K values (2).

P73 Polled intersex syndrome (PIS) in goats—Nanopore sequencing revealed a complex structural variant and made it possible to devise a simple genetic test for identification of intersexual goats. R. Simon*[1], H. Tschanz-Lischer[2], I. Keller[2], I. Häfliger[3], A. Pienkowska-Schelling[3], C. Schelling[4], C. Drögemüller[3], and G. Lühken[1], *[1]Institute of Animal Breeding and Genetics, Justus Liebig Univerity, Giessen, Germany, [2]Interfaculty Bioninformatics Unit, University of Bern, Bern, Switzerland, [3]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [4]Clinic for Reproductive Medicine, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland.*

In goats the trait of polledness is, in contrast to other bovine species, connected with disorders in sexual development. The so-called polled intersex syndrome (PIS) is characterized by the fact that homozygous polled and genetically female (XX) individuals are infertile due to phenotypically diverse intersexuality, making the identification of such cases difficult. In 2001, a ~11.7 kb deletion was detected as PIS-associated genomic variant. After trials to establish genetic testing for PIS in goats with the existing molecular genetic information had failed, it was decided to take a closer look at the known variant using Oxford Nanopore sequencing, an emerging long read sequencing technology. We sequenced the whole genome of 2 goats, a PIS-affected polled and a horned goat as control. A genome coverage of 9x and 14x for the homozygous polled and the horned goat, respectively was reached. Mapping against the current goat reference genome (ARS1) was performed with Minimap2. Sequence data confirmed the presence of the published deletion on chromosome 1 in the homozygous polled goat but also indicated the presence of a more complex structural variant. Some of the around 4 kb long sequence reads map to the regions flanking the PIS-deletion however not continuously. Significant parts of the reads map to a region located about 20 Mb downstream on chromosome 1. In addition, within this particular region a copy number variant was observed in the polled goat. A closer look revealed that a duplicated copy of a segment (~500 kb size) is inversely inserted into the region of the known PIS-associated deletion. We succeeded to validate this structural variant by fluorescence in situ hybridization using BAC clones. Sanger sequencing confirmed the complex variant with PCR primers flanking the chromosomal breakpoints. Finally, a practicable proof of genetic horn status and sex of goats was established applying an easy to use and robust multiplex PCR. Meanwhile, more than 1000 goats of different known breeds, sex and horn status have successfully been tested. In conclusion, this study revealed a more complex structural variant causing PIS in goats based on whole genome long read sequencing. We report a simple genetic test that allows the determination of the polled genotype in goats for the first time.

Key Words: goats and related species, animal breeding, genome sequencing, fertility

P74 Selection signatures in goat breeds reveal the molecular basis for six different coat color phenotypes. J. Henkel*[1], R. Saif[1,2], V. Jagannathan[1], C. Drögemüller[1], C. Flury[3], and T. Leeb[1], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Institute of Biotechnology, Gulab Devi Educational Complex, Lahore, Pakistan, [3]School of Agricultural, Forest and Food Sciences HAFL, Bern University of Applied Sciences, Zollikofen, Switzerland.*

Domestication and human selection have formed diverse goat breeds with characteristic phenotypes. This process correlated with the fixation of causative genetic variants controlling breed-specific traits within regions of reduced genetic diversity, so called selection signatures or selective sweeps. We performed a comprehensive screen for selection signatures in 20 genetically diverse modern goat breeds and Bezoar goats, the wild ancestor of domesticated goats. We pooled DNA from 12 animals of each breed and sequenced the obtained breed pools to ~30x coverage. The sequence reads were mapped to the goat reference genome (ARS1) and single nucleotide variants were called. For each pool, heterozygosity scores within a sliding window of 150 kb were calculated and negative Z-transformed H_p scores ($-ZH_p$) were plotted. This approach revealed on average 111 selection signatures per breed with $-ZH_p > 4$. In 2 Pakistani goat breeds, the Pak-Angora and Barbari, we found a strong selection signature in a region harboring the *KIT* gene. *KIT* is a well known depigmentation gene and several previously discovered *KIT* variants result in diverse depigmentation phenotypes. The selection signature in the Pak-Angora goat breed revealed a copy number variant (CNV) downstream of *KIT*. The same locus in the Barbari goat breed harbored an alternative variant of the CNV. These CNVs could explain the white spotted phenotype of Barbari and the completely white phenotype of Pak-Angora. Another selection signature in Swiss goat breeds with white markings or specific symmetric color patterns was found at the *ASIP* locus encoding the agouti signaling protein. This selection signature revealed 4 different CNV alleles most likely affecting region-specific expression levels of *ASIP*. One of the CNV alleles corresponded to a previously published *ASIP* allele in Saanen goats, while the 3 other *ASIP* alleles have not been reported before. In conclusion, this study revealed new loci under selection in 20 different goat breeds. We report 6 structural variants at the *KIT* and *ASIP* loci that are likely to cause differences in caprine coat color phenotypes.

Key Words: goats and related species, evolutionary genomics, genome sequencing, selection scan, coat color

P75 Use of genetic and epigenetic tools to refine a genetic marker of host resilience to ovine lentivirus infection. A. T. Massa*[1], M. R. Mousel[2,3], B. M. Murdoch[4], J. B. Taylor[5], D. P. Knowles[1], and S. N. White[1,2], *[1]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA, [2]Animal Disease Research Unit, Agricultural Research Service, USDA, Pullman, WA, USA, [3]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, USA, [4]Department of Animal and Veterinary Science, University of Idaho, Moscow, ID, USA, [5]Range Sheep Production Efficiency Research, Agricultural Research Service, USDA, Dubois, ID, USA.*

Ovine lentivirus (OvLV), a retrovirus related to human immunodeficiency virus, causes maedi-visna in sheep. Economic losses are insidious since the incurable, chronic infection has long incubation and no effective treatment yet surveys demonstrate up to 80.7% of open range flocks in the US contain seropositive sheep. The best strategy to combat morbidity and mortality from OvLV is prevention. Previously we discovered an insertion/deletion associated with 50% decreased proviral concentration in the blood of multiple breeds. Proviral concentration correlates with severity of histological pneumonia, arthritis, and mastitis so this validated marker predicts resilience to disease. The marker is located near 4 zinc finger transcriptional repressor genes. None of the 26 mutations predicted to create amino acid substitutions or possible splice site mutations in these 4 genes were significantly associated with resilience in Rambouillet sheep. Our hypothesis is that the resilient phenotype is explained by a mutation within a regulatory element that influences gene expression of these zinc finger genes. Chromatin immunoprecipitation with high throughput sequencing (ChIP-seq) for histone 3 lysine 27 acetylation (H3K27ac) was completed for alveolar macrophages to identify active enhancers in sheep on a genome-wide level. An active enhancer region 1500 base pairs in length was identified approximately 3000 base pairs upstream of *ZNF389*. The current insertion/deletion marker lies within this active enhancer element. Three single nucleotide polymorphisms were also identified within this enhancer that are significantly associated with resilience ($P < 0.002$) in 147 Rambouillet. This led to our current model that ovine lentivirus re-

silience is explained by mutations in enhancer elements that alter gene expression of zinc finger transcription factors. Zinc finger transcriptional repressor genes have been shown to co-evolve with retroviruses in other vertebrate species. Future work includes validating these SNPs in multiple breeds which may lead to a commercially viable genetic test and confirming the enhancer with additional ChIP-seq biological replicates.

Key Words: ovine lentivirus, marker-assisted selection, ChIP-seq, sheep

Avian Genetics and Genomics

P76 Abstract withdrawn

P78 Abstract withdrawn

P78 Abstract withdrawn

P79 Fine-mapping of ancestral haplotype undergoing mosaic positive selection for body weight in the domestic chicken. Y. Wang*[1,2], X. Cao[2,3], and X. Hu[2,3], *[1]College of Animal Science and Technology, China Agricultural University, Beijing, China, [2]State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing, China, [3]College of Biological Sciences, China Agricultural University, Beijing, China.*

Artificial selection is a powerful force that not only contributes to an improvement in economically important production traits in agricultural species, but also in shaping their genetic architecture. In depth studies of loci contributing to selection responses can provide valuable basic knowledge about the genetic mechanisms facilitating selection responses to complex traits in general, and the specific agricultural trait in particular. A major effect quantitative trait locus (QTL) at the distal end of chicken GGA1 has been associated with growth traits in multiple studies, but there is still no consensus as to the underlying genetic mechanism. Here, this locus was dissected in detail using a 9-genera-

tion chicken advanced intercross population (AIL) including in total 1,119 birds (F_0, F_2 and F_9 generation) and the fine-mapped regions explored using 222 sequenced genomes of 10 high/low body weight (BW) chicken breeds. A series of progressive analyses including GWAS in F_2 and F_9, IBD mapping from F_0 to F_9 and haplotype sharing analysis in all re-sequencing individuals were employed to fine-mapping this QTL region. Finally, a shared 12 Kb haplotype in the high-weight breeds contained the *A* gene was identified and displayed the most significantly associations with BW. Individuals with the high-weight haplotype displayed higher expression of *A* in the duodenum and the polymorphism at the locus, which in total explained 10.88% of the variance in BW in the AIL. This haplotype associated with BW in the AIL demonstrated a mosaic pattern of haplotypes present in all evaluated domestic chicken breeds. It is an ancestral haplotype that has accumulated abundant haplotype diversity within the 12 Kb until the artificial selection occurred. The results illustrate the genomic consequence of long-term artificial positive selection in the domestic chicken, where a shared standing variant has likely been selected and the original haplotype has eroded due to long-term selection in isolated populations. It is a valuable example of the genomic complexity to be expected in studies of the genetic architecture of quantitative traits in segregating populations and provides new perspectives for the next generation of genomic selection breeding.

Key Words: chicken, body weight, fine-mapping, artificial selection, genetic architecture

P81 A genomic inference of the White Plymouth Rock genealogy. Y. Guo*[1], M. Lillie[1], Y. Zan[1], J. Beranger[3], A. Martin[3], C. Honaker[2], P. Siegel[2], and Ö. Carlborg[1], *[1]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden, [2]Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA, USA, [3]The Livestock Conservancy, Pittsboro, NC, USA.*

During the mid-19th century, several chicken breeds that had been introduced to America from Europe and Asia became the founders for those formed in the USA. Historical records about the genealogy of these populations are often unclear and inconsistent. Here, we used genomics in an attempt to describe the ancestry of the White Plymouth Rock (WPR) chicken. In total, 150 chickens from the WPR and 8 other stocks that historical records suggested they contributed to its formation were whole-genome re-sequenced. Here, the WPR was represented by the high (HWS) and low (LWS) body weight selected Virginia lines which were founded in 1957 as the progeny of crosses between 7 partially inbred lines of WPR. The admixture analysis of the autosomal and sex chromosomes showed that 2 stocks were major contributors (in essentially equal proportions) to the female lineage of the WPR, while one dominated the male lineage. These results were consistent and provided quantification with the historical records that they were the main (with others less) contributors to the WPR. The genomic analyses, however, revealed genome-wide contributions by 3 additional breeds and when viewed on an individual chromosomal basis, contributions varied considerably among stocks.

Key Words: White Plymouth Rock, genealogy, genomics

P82 Genome-wide detection of selection signatures in Ogye in comparison to the white leghorn chickens. Y.-S. Lee*, D. Shin, H.-K. Lee, and K.-D. Song, *Department of Animal Biotechnology, Chonbuk National University, Jeonju, Republic of Korea.*

The Ogye chicken, called silkies in western countries, has black bones, feathers, skin, eyes and even claws. Ogye is bigger than other chickens and has higher immunity, so even if it becomes infected, it is not killed immediately but sheds virus for a long time. In this regard, we investigated Ogye's evolutionary aspects using whole-genome sequencing and the reference population was white leghorn chickens (F and K subpopulation). White leghorn breed is commonly used as layer chickens in many countries of the world. We used the cross-population composite likelihood ratio (XP-CLR) and cross-population extended haplotype homozygosity (XP-EHH) to find Ogye's evolutionary characteristics. And we performed gene ontology (GO) and pathway analysis. In GO, the most enriched terms in XP-CLR (top 1% significant genes) were muscle cell differentiation, ribosome biogenesis and in XP-EHH, I-kappaB/NF-kappaB signaling and protein localization. In biological pathway analysis, the immune-related and viral activity were prominent. Our analysis could explain why Ogye has antiviral resistance.

Key Words: Ogye, selection signature, white leghorn, XP-CLR, XP-EHH

P83 Deciphering genome-wide selective signatures between Cornish and Korean native chicken (KNC) based on XP-EHH. G. Kim, Y.-S. Lee*, H.-K. Lee, D. Shin, and K.-D. Song, *Department of Animal Biotechnology, Chonbuk National University, Jeonju, Republic of Korea.*

Chicken has been domesticated before thousands of years and become one of the most important livestock in the world. The Cornish chicken is one of the neutralized breed in Korea, and the Korean native chickens (KNCs) have been raised traditionally and developed based on the conservation project to prevent extinction. The aim of our study was to identify the selection signatures of Cornish and KNCs using whole genome re-sequencing. After the first draft of the chicken genome was released, re-sequencing using NGS is efficient way to approach for a large scale, genome-wide SNP discovery and for the statistical analysis. The analyzing statistic was Cross-Population Extended Haplotype Homozygosity (XP-EHH). We found 553 KNC specific genes and 397 Cornish specific genes in XP-EHH analysis using the empirical P-value (P-value < 0.01). Using the gene ontology (GO) and gene pathway analysis, the significant genes, APOA4, APOA1 and APOA5 were revealed to be associated with lipid metabolism in KNC. In Cornish chickens, the significant genes, PRKCZ and WNT7A were associated with growth.

Key Words: XP-EHH, Korean Native chicken, Cornish chicken, selection signatures

P84 Additional molecular evidence that the Royal Palm is probably a turkey breed and not a strain. E. Smith*, J. Xu, J. Adikari, and K. Russell, *Virginia Tech, Blacksburg Virginia, US.*

In earlier investigations, we reported the uniqueness of Royal Palm from other commonly raise heritage turkeys. Here, we provide evidence from screening using primers specific for the Melanocortin 1 (MC1R) Receptor gene (located on turkey chromosome 13, accession number NC_015023). Five heritage strains, including Broad Breasted Bronze (48), Blue Slate (40), Midget White (40), Royal Palm (45), and Spanish Black (48) were included in the analyses of approximately 3300 base pairs of the *MC1R* gene. Seven SNPs were identified and validated. Among the haplotypes identified, only Royal Palm had unique haplotypes. When combined with previous investigations, we believe that the Royal Palm may be a breed and not a strain. It may therefore be the most viable resource for introgression with a goal of genetic improvement.

Key Words: turkey, MC1R, SNPs, haplotype

P85 Marek's disease virus infection induced mitochondria changes in two genetically divergent lines of chickens. Q. Chu*[1], Y. Ding[2], and J. Song[2], *[1]Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China, [2]Department of Animal and Avian Sciences, University of Maryland, College Park, MD, USA.*

Marek's disease (MD), caused by an avian α-herpesvirus, is characterized with lymphomas and immunosuppression. It is well reported that mitochondria are crucial cellular organelles in eukaryotes and participate in many cell processes including immune response. To evaluate the role of mitochondria in immunity in response to MDV infection, mitochondrial DNA (mtDNA) abundance and gene expression in immune organs were ascertained in 2 well-defined and highly inbred lines

of chickens, the MD-susceptible line 7_2 and the MD-resistant line 6_3. Young birds were randomly selected and divided into challenge and control groups on the fifth day after hatching, and birds from challenged groups were given a dosage of 500 plaque-forming units (PFU) of 648A passage 40 MDV intra-abdominally each. Bursa of Fabricius, thymus and spleen samples were collected at 5, 10 and 21 d post-infection (dpi) from 5 birds per line, per group. The relative content of mtDNA was determined using qPCR analysis of the mitochondrial gene *ND2* with the nuclear gene β-actin as a control. Meanwhile, the gene expression were studied using RNA sequencing in all 3 tissues at 21dpi. Our results showed that mitochondrial DNA contents decreased significantly at the transformation phase (21dpi) in spleen of the MD-susceptible line 7_2 birds in contrast to the MD-resistant line 6_3. However, the mtDNA-genes and the nucleus-genes relevant to mtDNA maintenance and transcription were significantly upregulated. The imbalance of mitochondrial contents and alteration of gene expression demonstrated the indispensability of mitochondria in virus-induced cell transformation and subsequent lymphoma formation, such as MD development in chicken. Furthermore, we found that *POLG2* may play a potential role leading to the imbalance. This is the first report on tumor-virus infection and mitochondria content changes following the infection in chicken, which provides important insights to advance the understanding on the bioprocesses in pathogenesis and tumorigenesis due to viral infection.

Key Words: chicken, Marek's disease virus, mitochondrial DNA copy number, RNA sequencing

P86 Genome-wide association studies for colour traits in Chinese Crested duck using whole-genome sequencing. X. Yuan*, Q. Guo, H. Bai, Q. Xu, G. Chang, and G. Chen, *Key Laboratory of Animal Genetics and Breeding and Molecular Design of Jiangsu Province, Yangzhou University, Yangzhou, Jiangsu, China.*

The color of feather, beak and shank are interesting quality color traits in birds. In general, the beak and plumage colors have a similar color pattern. The appearance of Chinese Crested duck (Fengtou Duck) usually have a black color at beak and shank with white color in plumage. However, the underlying mechanism of these colors in Chinese Crested duck is still unclear. In this study, we conducted a case-control genome-wide association studies (GWAS) using a Crested duck × Cherry Valley duck of 308 F_2 population to identify the genes associated with the colors at plumage, beak and shank. Significant signals for the white plumage were found on chromosome 11. The significant SNP on chromosome 11 was in the microphthalmia-associated transcription factor (MITF) gene region (p-value = 1.50e-20). The significant signals for beak (p-value = 1.79e-16) and shank (p-value = 8.84e-18) colors were discovered on chromosome 14. The candidate genes associated beak and shank colors are both related to Endothelin Receptor B2 (EDNRB2). MITF and EDNRB2 play an important role in the melanogenesis pathway. However, the mode of MITF and EDNRB2 regulated melanin deposition is different. We suggested that the variant of MITF leads the less deposition of melanin in plumage, while the mutant of EDNRB2 causes an excessive deposition of melanin in beak. In conclusion, MITF and EDNRB2 might be candidate genes for white plumage, black beak and shank, respectively. We speculated that the melanin deposition mechanism may be different between beak and plumage.

Key Words: Chinese Crested duck, GWAS, plumage color, beak color, shank color

P87 Unravelling kinship in a captive colony of greater flamingos (*Phoenicopterus roseus*) without a studbook. C. Biolatti*[1], C. Beltramo[1], A. Dogliero[2], V. Campia[1], S. Peletto[1], S. Colussi[1], P. Modesto[1], and P. L. Acutis[1], *[1]Regional Reference Centre for Exotic Animals, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy, [2]International Foundation for Wildlife Research - Rawdat Al Faras Houbara Breeding Center (Ministry of Municipality&Environment), Doha, State of Qatar.*

Genetic management of greater flamingos (*Phoenicopterus roseus*), highly social waterbirds and obligate colonial breeders, is challenging due to uncontrolled pairings. In this study we performed parentage analysis of a private collection of 45 greater flamingos to set the basis for translocation into a new destination. No data were available for any bird. DNA was extracted from blood samples and animals were genetically sexed. The following 10 microsatellites were chosen and combined in 2 multiplex PCRs: M1 (PrA3, PrA102, PrA110, PrD4, PrD5), M2 (PrC122, PrD3, PrD7, PrD102, PrD121). The STRs dye were labeled at the 5′ end of each forward primer. Sizing was carried out using ROX 500 size standard and GeneMapper v. Five software. STRs descriptive statistics was performed with *Demerelate* and CERVUS v.3.0.34 software. COLONY v.2.0.5.99 was run without candidate parent inputs to hypothesize full and half-sibship. CERVUS was then used to screen for mother/father-offspring relationships. Parentage analysis was run with COLONY, setting the animals of the dyads retrieved by CERVUS as candidate father/mother. All suggested pairs were checked manually to confirm parentage. The colony was composed of 19 males and 26 females. Population mean Hexp, Hobs, and PIC was 0.75, 0.69, and 0.70, respectively. The sibship reconstruction with COLONY retrieved 7 full and 2 half-sib dyads. The combined analysis with CERVUS and COLONY detected 20 parent-offspring relationships. While it is possible that these dyads are not actually parent- offspring, but closely related individuals, these data will be taken into account when managing the colony. This represents a white canvas to start taking decisions for designing a larger population management plan. In conclusion this study is an example of empirical verification of methodologies in a real-world situation.

Key Words: greater flamingos, genetic management, STRs, CERVUS software, COLONY software

P88 First preliminary overview of genetic diversity and conservation status of Ecuadorian creole chicken. P. Toalombo*[1,2], V. Landi[2], A. M. Martínez[2], M. M. Gómez[2], C. A. Camacho[1], M. E. Camacho[3], J. M. León[2], and J. V. Delgado[2], *[1]Escuela Superior de Chimborazo, Riobamba, Ecuador, [2]Universidad de Córdoba, Córdoba, Spain, [3]IFAPA Alameda del Obispo, Córdoba, Spain.*

In Ecuador, creole chicken is an important genetics resource being part of traditional diet and an important economic intake for country being exploited in backyard productions, whose meat and eggs have a high nutritional value and food, accessible to the entire population due to its low purchasing power. The current Creole chicken derives from the introduction of European animals during the Spanish colonization starting from the XV century and probably from pre-Columbian birds arrived with the first settlers of America coming from Asia via the Pacific Ocean. This genetic resource is at a high risk first due to the lack of productive information and breeding plans that expose it to the introduction of exotic genetic types and second because, lacking scientific information, it is not possible to quantify the degree of diversity or genetic erosion. For these reasons, the objective of this study was to evaluate the degree of genetic diversity of the Creole hens of all the regions of Ecuador and to give a first test of the internal structure of the population. We use samples a 244 birds (~40 for province) from the 6 provinces of Ecuador: Bolivar, Chimborazo; Cotopaxi, Guayas, Morona Santiago, Tungurahua. Samples were collected in FTA card and analyzed with the microsatellite marker panel from AVIANDiv project (30 loci) by standard PCR and capillary electrophoresis. Mean number of alleles was 5.67 ± 2.54 (range 5.37–6.20) and expected and observed heterozygosity were respectively 0.63 ± 0.03 and 0.55 ± 0.01. PCA analysis shows the formation of a cluster formed by Guayas, Bolivar and Cotopaxi population while Chimborazo, Tungurahua and Morona Santiago remain in independent position.

Key Words: poultry and related species, breed diversity, conservation, microsatellite

P89 Desmin and vimentin gene expression study in pectoralis major muscle of broilers affected by muscular abnormalities. M. Zappaterra*[1], F. Soglia[2], M. Mazzoni[3], M. Bordini[1], M. Di Nunzio[2], M. Petracci[2], and R. Davoli[1], *[1]Department of Agricultural and Food*

Sciences (DISTAL), Alma Mater Studiorum-University of Bologna, Bologna, Italy, [2]Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum-University of Bologna, Cesena, Italy, [3]Department of Veterinary Medical Sciences (DIMEVET), Alma Mater Studiorum-University of Bologna, Bologna, Italy.

Desmin (*DES*) and vimentin (*VIM*) genes code for proteins of primary importance for muscle cytoarchitecture and have been extensively investigated in human neuromuscular disorders. Since broiler pectoralis major (PM) muscles affected by white striping (WS), wooden breast (WB) and spaghetti meat (SM) abnormalities show phenotypic features similar to those of human dystrophies, the present study aims at assessing the expression of *DES* and *VIM* genes as well as the distribution of these proteins within PM of broilers affected by muscular abnormalities and in muscles having macroscopically normal appearance (NORM). Gene expression, protein quantitation and immunohistochemical assay were performed on PM muscles (5 samples/group) collected from the same flock of fast-growing broilers (males, 45-d-old, 3 kg live weight). Differences in gene and protein amounts between groups were assessed using one-way ANOVA. *VIM* mRNA levels gradually increased from NORM to SM groups, with significantly higher gene expressions in WB and SM samples compared with NORM group ($P = 0.009$ for WB *vs*. NORM and $P = 0.004$ for SM *vs*. NORM). Similarly, if compared with NORM, a 55% increase in VIM content was found in WB affected cases ($P < 0.001$). The *DES* gene expression showed an increase from NORM to WB ($P = 0.05$). With regard to the protein level, a significantly higher amount of DES (+53%; $P < 0.001$) was found by comparing WS and NORM, while the amount of DES assessed in WB did not differ from NORM. Immunohistochemical analysis showed an increased number of fibers immunoreactive to both VIM and DES in affected PM, while only few immunoreactive fibers were observed in NORM. Since high levels of DES and VIM are considered as markers of muscle regeneration, the results suggest that WS, WB and SM affected muscles are undergoing muscle regeneration. This study highlights for the first time a relationship between WS, WB and SM abnormalities and the expression and distribution of DES and VIM thus suggesting that the increase in their expression could be indicative of the occurrence of PM abnormalities in broilers.

Key Words: poultry and related species, gene expression, genetic disorder, meat production

P90 Discriminant and factor analysis of four strains of starter broiler chickens. F. E. Sola-Ojo and D.I Ibiwoye*, *University of Ilorin, Kwara State, Nigeria.*

This study was conducted to explore the relationship among body measurements in 4 strains of broiler chicken (Arbor Acre, Hubbard, Marshall, and Ross 308) using principal component analysis with the view of identifying those components that define body conformation in broilers, using stepwise discriminant analysis and cluster analyses to assess the magnitude of genetic diversity and interdependence of morphological traits. A total of 800 birds were used, 200 per strain. The parameters recorded throughout the 4-wk period were body weight, body length, keel length, shank length, body height, drumstick length, thigh length, wing length, shank circumference, comb length, body girth, neck length and beak length and were analysed using principal component analysis (PCA) procedure, stepwise discriminant analysis and cluster analysis at the starter phase. Four principal components were extracted in Arbor Acre which explained 56.678% of the total variation in the original variables. Also, 6 principal components were extracted in Hubbard, 3 principal components were extracted in Marshall, and 6 principal components were extracted in Ross strain accounting for 59.463% , 55.515% and 58.665% of the total variance respectively. Generally, PC1 had the largest share of the total variance and correlated highly with different traits in each strain. PC1 was termed the generalized form of broilers. These components could be used as selection criteria for improving body size of broilers. Stepwise discriminant analysis indicated that wing length and body girth had highest discriminating powers among the distinguishing variables. Discriminant analysis of morphometric traits in this study correctly classified 100% of the experimental birds. The hierarchical cluster analysis showed that the morphometric parameters of Arbor acre birds were similar to Hubbard and Marshall birds while the other genotypes were also similar to each other.

Key Words: strain, body parameters, loadings, variance maximizing orthogonality, cluster

P92 Analysis of the brain transcriptome in lines of laying hens divergently selected for feather pecking before and after light stimulation. J. Beier[1], C. Falker-Gieske[1], H. Iffland[2], S. Preuß[2], W. Bessei[2], J. Bennewitz[2], and J. Tetens*[1,3], *[1]Department of Animal Sciences, Georg-August-University, Göttingen, Germany, [2]Institute of Animal Science, University of Hohenheim, Stuttgart, Germany, [3]Center for Integrated Breeding Research, Georg-August-University, Göttingen, Germany.*

Feather pecking (FP) is a worldwide problem in the layer industry leading to serious impairments of animal welfare. The propensity to perform FP is a complex trait, which is influenced by numerous factors including a genetic component. Despite extensive research efforts, the motivation for this unwanted behavior is not completely understood. In the current study, we analyzed the brain transcriptomes of White Leghorn layer strains divergently selected for FP behavior with the aim to 1) unravel transcriptional differences between the strains possibly related to the general FP propensity and 2) characterize transcriptional changes associated with the onset of the actual behavior upon light stimulation. A total of 48 hens comprising 12 full-sib pairs from each strain (high vs. low FP) were phenotyped according to established protocols at 27 weeks of age and subsequently kept under low light conditions to prevent the occurrence of FP. One bird from each full-sib pair was then sacrificed and brains were immediately collected for RNA isolation. Thereafter, the remaining birds were kept under increased light intensity (= 50 lx) for several hours until they clearly showed FP and were then sacrificed as well. Brain transcriptomic profiling was done by RNaseq (Illumina HiSeq4000, 2x75bp PE) aiming for 30 mio. reads per sample. Differential gene expression analysis was done using DESeq2 and EdgeR. Comparing the 2 strains under base line conditions (low light intensity, no FP shown) revealed 626 significantly (FDR <0.05) differentially expressed genes (DEG), while 834 DEG were detected under high light intensity. Notably, a considerable difference was found with reference to DEGs upon light stimulation when comparing the 2 strains (266 DEGs in the high FP strain vs. 688 in the low FP strain). Considering both factors (strain and light intensity) in one model resulted in 536 genes differentially expressed between strains. A subsequent gene set enrichment analysis using GAGE revealed 3 significantly enriched (q <0.05) KEGG pathways: cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction, and cell adhesion molecules.

Key Words: laying hen, feather pecking, transcriptome analysis, brain

P93 Discovery and detection of associated loci of plumage and shank color in chicken. G. Hua*, Y. Zhang, X. Dong, J. Chen, and X. Deng, *China Agricultural University, Beijing, China.*

In chicken there are several different loci controlling plumage and shank color. Some genes were reported, such as recessive white feather, dominant white feather, sex-linked barring feather and yellow shank. As for the shank skin colors, 3 pure patterns are white, yellow and black. The various shank skin colors are regulated by combination of specific genes that influence carotenoid and melanin pigmentations. In our study, some reported loci and new underlying loci for the plumage and shank color were identified through SNP-based genome-wide association study and selection signature in chicken. We constructed one F_2 chicken population based on white leghorn and DBW (dwarfism, black shank, recessive white feather). We collected the feather and shank color when one-day old and adult. 200 female chickens were genotyped by Affymetrix 600K HD chip. Filtering the SNPs using PLINK1.9, there were 510513 loci reserved. GEMMA software was used to perform the

mixed linear model. We downloaded the chicken resequencing data and used GATK4.0 to call snp. Fst, pi and Tajima'D were calculated by vcftools.For black feather, we proposed 3 genes as candidate genes, which are ZNF608, SNCAIP, SNX2. For white feather, TYR gene is responsible for recessive white feather in adult chicken and PMEL gene regulates the white feather in young chicken. For yellow feather, we proposed FKTN as candidate gene. For barring feather, CDKN2A gene was reconfirmed as the regulated gene. And we genotyped some breeds using one of the causative SNPs. For black shank color, the genes were located on ChrZ:66760305–81606091(Gga6). Three genes were suggested to verify. For yellow shank color, BCO2 gene has been mapped before, but the causative mutation need to be found.

Key Words: plumage, shank color, SNP, chicken, F_2 population

P94 Marker-assisted introgression of blue eggshell color into a white egg layer line. C. Dierks*[1], N. T. Ha[2], D. Cavero[3], H. Simianer[2], R. Preisinger[4], and S. Weigend[1,2], *[1]Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics, Neustadt, Germany, [2]University of Goettingen, Department of Animal Sciences, Goettingen, Germany, [3]H&N International, Cuxhaven, Germany, [4]EW GROUP GmbH, Visbek, Germany.*

As part of the EU project IMAGE (Innovative Management of Genetic Resources) under the umbrella of Horizon 2020 the aim of this study is the demonstration of the efficient transfer of a specific trait maintained in gene bank, here blue eggshell color, into a contemporary high performing white egg layer chicken line. Monogenic dominant inherited blue eggshell color is caused by a large retroviral insertion on chromosome 1 at 65.2 Mb upstream of *SLCO1B3* (Wang et al., 2013, Wragg et al., 2013). For the initial F_1, 6 Araucana cocks, homozygous for blue eggshell color, were mated with 10 White Leghorn (WL) hens. Based on 2 marker-assisted backcrosses (BC1 and 2) followed by an intercross (IC) a high performing blue layer WL-like line will be developed. Whole genome sequence data of the Araucana cocks and 580K SNP Axiom Genome-Wide Chicken Array data of the WL hens were used in search of breed/line specific markers. We identified 37 highly informative SNPs from 60.1 to 71.7 Mb on chromosome 1 surrounding the insertion. Thirteen of them were included in a customized 52K Genotyping Array which was used to genotype candidate birds. The additional 24 SNPs, the trait genotype and sex were determined by KASP technology. Criteria to select animals for the next generation were distinct breed/line specific haplotypes including the 37 markers surrounding the introgressed locus, the overall similarity to recipient white egg layer line as well as maximum diversity expected in future generations analyzed using the R package MoBPS. Introgression haplotypes were constructed with Merlin and we observed 30 paternal recombinants out of 137 male carriers. Out of them, 14 cocks with high proportion of recipient genome and highest degree of diversity were selected for the BC2 production. The proportion of the WL genome of the introgressed haplotype ranged from 56.9 to 76.5% in these 14 males. Out of 550 BC2 animals, 291 carriers were detected. From BC1 to BC2 the WL genome content increased in 37 animals by additional recombination up to 93.2% in the introgressed region.

Key Words: poultry and related species, genetic introgression, haplotype, breed/population identification

P95 Development of a SNP-based parentage verification panel for lovebirds. H. Van der Zwan[1], C. Visser[2], M. Schoonen*[1], and R. Van der Sluis[1], *[1]Focus Area for of Human Metabolomics, North-West University, Potchefstroom, South Africa, [2]Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, South Africa.*

The genus *Agapornis*, or lovebirds, are well known pet parrots across the globe.Despite breeder's dependency on pedigree records no genetic information exceptthe newly sequenced de novo genome of *Agapornis* roseicollis is available to confirmpedigrees for any of the 9 species. The whole genome of the parents of thereference genome individual were sequenced at a 30x coverage to identify SNPs bymapping the reads against the reference genome. By applying the Genome AnalysisToolkit (GATK) pipeline over 1.6 million SNPs were discovered shared by the Motherand Father. These SNPs were filtered to a panel of 480 SNPs based on qualityparameters recommended by GATK. This panel was analyzed in a population of 960lovebirds across 7 species using the QuantStudio 12K Flex platform to compile a parentage verification panel. The first panel consisted of 262 SNPs and werefurther reduced based on heterozygosity (H) and minor allele frequency (MAF) toinclude the lowest number of SNPs with the highest exclusion power for lovebirdparentage verification. Two additional panels consisting of 195 SNPs with MAF andH_O values ≥0.1 and a panel of 40 SNPs where these values were ≥0.3, wereconstructed. Parentage verification was performed on 43 families from differentspecies to assess the exclusion power of each panel. The 195-SNP panel with P_E99.9%, MAF and H_O values ≥0.1 was proposed as the *Agapornis* parentageverification panel.

Key Words: parrot breeding, pedigree confirmation, whole genome resequencing, Agapornis

P96 Transcriptome analysis reveals the effect of melanogenesis on shank color conversion in chickens. Z. Xin*, L. Shijun, C. Guoting, M. Changhuan, and R. Hongji, *Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei Province, China.*

Shank color of domestic chickens is controlled by the inhibitor of dermal melanin (ID) on Z chromosome. The id homozygous black shank cocks can cross with yellow shank hens to produce shank color auto-sexing chicks.The hens of the filial generation are having dark shanks, while the cocks are having light shanks. However, some hens were yellow shank when they are born, and then the shank would gradually turn dark. Until now, no report has explained the mechanism of shank color conversion. To figure out this issue, we collected shank skin tissues from the same part of the same hen at the age of one day, 10 d and 22 d. Experimental chickens included 14 hens, 4 of which were born black shank, 2 were born yellow, and 8 were converted ones. Then we extracted total RNA and performed RNA-seq. By comparing expressed genes of 1-d-old and 10-d-old, we found 1,044 differential expressed genes(DEGs), and between 1 d and 22 d old there were 724 DEGs. Gene ontology (GO) enrichment and KEGG pathway analysis showed that the DEGs were most enrichment in melanogenesis. But there are only 53 DEGs when comparing 10 d old with 22 d old. Meanwhile, we compared expressed genes of the 1-d-old black shank ones with converted ones. Nineteen DEGs were found. The significantly enriched GO terms among the DEGs included melanin biosynthetic process, developmental pigmentation, and transmembrane transport. These results indicated that melanin synthesis and transport functions of converted individuals are inhibited at birth, resulting in abnormal melanin pigmentation. We need further investigation to define the major genes that control shank color conversion. This study can lay a theoretical foundation for producing shank color auto-sexing chicks.

Key Words: chicken, melanin, shank color conversion, RNA-seq, auto-sexing

P97 Abstract withdrawn

P97 Abstract withdrawn

P98 Single-step methodology for genomic evaluation in turkeys (*Meleagris gallopavo*). E. A. Abdalla*[1], B. J. Wood[1,2], H. E. Begli[1], K. Pieters[3], P. van As[3], M. Bink[3], O. W. Willems[2], R. Vanderhout[1], F. S. Schenkel[1], and C. F. Baes[1], *[1]Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, [2]Hybrid Turkeys, Kitchener, ON, Canada, [3]Hendrix Genetics, Boxmeer, Netherlands.*

Genomic information can contribute significantly to the increase in accuracy of genetic predictions compared with using pedigree relationships alone; various studies show that the simultaneous incorporation of genomic and pedigree information may produce higher accuracy predictions. The objective of this study was to compare the prediction ability of pedigree-based best linear unbiased prediction (PBLUP) and single-step GBLUP (ssGBLUP) models. Feed conversion ratio, residual feed intake, body weight, breast meat yield, and walking ability data of a commercial pure line of turkeys was analyzed. For ssGBLUP, 3 different weighting factors were applied to combine the genomic and pedigree matrices. The performance of each model was assessed using 2 different approaches: 1) model-based theoretical accuracy and 2) correlation-based accuracy. Across the studied traits, ssGBLUP had higher heritability estimates and outperformed the PBLUP model, showing an increase in accuracy ranging from 15 to 41%. A weighting factor for the genomic matrix of 0.90 slightly increased the accuracy of prediction of ssGBLUP compared with other weights (0.95 and 0.85). In terms of prediction bias, only small deflation and inflation of estimates were observed across the models and traits. Therefore, incorporating genomic information into breeding programs will increase the prediction accuracy for young selection candidates in turkeys.

Key Words: single-step blending, genomic selection, pedigree BLUP, turkey

P99 Transcriptome sequencing reveals key potential long non-coding RNAs related to duration of fertility trait in the uterovaginal junction of egg-laying hens. A. Adetula*[1], L. Gu[1], C. Nwafor[2], X. Du[3], S. Zhao[1], and S. Li[1], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction, Ministry of Education, Key Laboratory of Poultry Genetics and Breeding of the Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei Province, China, [2]Faculty of Agriculture, Benson Idahosa University, Benin, Edo State, Nigeria, [3]College of Informatics, Huazhong Agricultural University, Wuhan, Hubei Province, China.*

Duration of fertility, (DF) is an important functional trait in poultry production and lncRNAs have emerged as important regulators of various process including fertility. In this study we applied a genome-guided strategy to reconstruct the uterovaginal junction (UVJ) transcriptome of 14 egg-laying birds with long- and short-DF (n = 7); and sought to uncover key lncRNAs related to duration of fertility traits by RNA-sequencing technology. Examination of RNA-seq data revealed a total of 9,977 lncRNAs including 2,576 novel lncRNAs. Differential expression (DE) analysis of lncRNA identified 223 lncRNAs differentially expressed between the long- and short-DF groups, with 81 upregulated and 142 downregulated. DE-lncRNA target genes prediction uncovered over 200 lncRNA target genes and functional enrichment tests predict a potential function of DE-lncRNAs. Gene ontology classification and pathway analysis revealed 8 DE-lncRNAs, with the majority of their target genes enriched in biological functions such as cellular response to cytokine, response to protein homodimerization, reproductive structure development, developmental process involved in reproduction, regulation of protein modification, osteoblast differentiation and ossification, in utero embryonic development, response to cytokine, carbohydrate binding, chromatin organization, response to growth factors, and immune pathways. Differential expression of lncRNAs and target genes were confirmed by qPCR. The discovery of these 2,576 novel lncRNAs in this study significantly expands the utility of the UJV transcriptome and our analysis identification of key lncRNAs and their target genes regulating DF will form the baseline for understanding the molecular functions of lncRNAs regulating DF and extend the knowledge of the molecular mechanisms underlying fertility.

Key Words: duration of fertility, long non-coding RNAs, egg-laying hens, uterovaginal junction, RNA-seq

P100 An open chromatin region on GGA1 has an important effect on regulating chicken growth. X. Cao*[1,2], Y. Wang[2,3], and X. Hu[1,2], *[1]College of Biological Sciences, China Agricultural University, Beijing, China, [2]State Key Laboratory of Agro-biotechnology, China Agricultural University, Beijing, China, [3]College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Body weight is one of the most important economic traits of chickens. Exploring the genetic mechanism of body weight has vital significance for chicken meat industry. In our previous study, a 1.2 Mb QTL and a 12 Kb haplotype in the QTL interval associated with body weight were detected on the chicken (Gallus gallus) chromosome (GGA) 1 using an advanced intercross population constructed by Huiyang Beard Chicken (a slow-growing domestic breed) and High Quality chicken Line A (a fast-growing broiler). In current study, we explored the 12 Kb haplotype block and its effect on regulating gene expression and chicken growth in duodenum at 7 weeks, as the digestion and absorption of food play an important role in gaining weight. The favorable allele for high body weight was defined as H haplotype utilizing 6 tag SNPs, in contrast to L haplotype for low body weight. Progeny test demonstrated that HH genotype chickens had a significantly higher body weight comparing to LL genotype chickens. In the 1.2 Mb QTL region, 3 genes presented different expression in duodenum between HH and LL individuals, which have been proved involving in gastrointestinal motility or energy metabolism. Through ATAC-Seq, we detected an open chromatin region containing 2 tag SNPs in the haplotype block and it suggested that 1) the open chromatin region might affect the expression of genes in the QTL region, and 2) the SNPs in the open chromatin region lead to the change of gene expression. Considering the effect of energy metabolism on growth, we measured protein levels of AMP-activated protein kinase (AMPK), the key regulator in energy regulation,through Western Blot. The increasing expression of both AMPK and phosphorylated AMPK in LL chickens comparing to HH chickens, suggested that the open chromatin region in the 12 Kb haplotype block might affect the body weight of chicken in a way of regulating energy metabolism.

Key Words: chicken, animal breeding, duodenum, genotyping, ATAC-Seq

P101 Abstract withdrawn

P101 Abstract withdrawn

Cattle Molecular Markers and Parentage Testing

P102 Abstract withdrawn

P103 Evaluation of genotyping concordance for commercial bovine SNP Chips using quality-assurance samples. J. Qiu*[1], X.-L. Wu[1], J. Xu[2], H. Li[1], J. He[3], Q. Xiao[1], R. Ferretti[1], B. Simpson[1], T. Mitchell[1], S. Kachman[2], and S. Bauck[1], *[1]Neogen GeneSeek, Lincoln, NE, USA, [2]Department of Statistics, University of Nebraska, Lincoln, NE, USA, [3]College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan, China.*

SNP arrays are widely used in genetic researches and agricultural genomics applications, and the quality of SNP genotyping is of paramount importance when interpreting the results. In this study, SNP genotyping concordance and discordance were evaluated for various commercial bovine SNP chips based on 2 types of quality assurance (QA) samples. Genotyping discordance rate (GDR), which was evaluated between chips, ranged from 0.06% to 0.037% based on QA Type I samples (which consisted of 292 GeneSeek internal control cattle DNA) and 0.05–0.15% based on QA Type II samples (which consisted of 96 USDA-MARC Beef Cattle Diversity Panel Version2.9 DNA). Average genotyping error rate (GER), which was estimated based on QA Type II data for single chips, varied between 0.02% and 0.08% per SNP and between 0.01% and 0.06% per sample. GDR tended to overestimate GER because it included genotyping errors resulted from 2 contrasting chips. Overall, genotyping concordance rate was high, which on averages was 99.63–99.95% between samples and 99.92–99.99% within samples. Nevertheless, mitochondrial DNA and Y chromosome had considerably elevated GDR (and GER) than the 29 autosomes and X chromosome. The majority of genotyping errors were from single allotyping errors, such as from AA to AB, or from BB to AB, which also included the allele "dropout" (i.e., from AB to AA or BB). In contrast, simultaneous allotyping errors on both alleles (e.g., mistaking AA for BB or vice versa) were relatively rare. A list of SNPs with > 1% GER was obtained. Interpretation of association effects of these SNPs, e.g., in whole genome association studies, needs to be taken caution by considering elevated uncertainty of their genotypes in statistical inferences.

Key Words: bovine, product quality, genetic improvement, bioinfomatics, SNP

P104 Birth size and birth weight in Brahman Cattle offspring and association with a PvuII polymorphism in the IGFBP-3 gene. T. Hartatik*[1], D. A. Priyadi[1], R. Y. Rahmawati[1], P. Panjono[2], S. Bintara[1], I. Ismaya[1], I. G. S. Budisatria[2], B. P. Widyobroto[2], A. Agus[3], and P. Leroy[4], *[1]Department of Animal Breeding and Reproduction, FAS, UGM, Indonesia, [2]Departemen of Animal Production, FAS, UGM, Indonesia, [3]Departemen of Animal Nutrition and Feed Science, FAS,UGM, Indonesia, [4]Faculty of Veterinary Medicine, University of Liege, Belgium.*

IGFBP gene is a potential marker of cattle growth. Polymorphisms at these loci have been associated with several production traits in bovine. The objective of this study was to identify Single Nucleotide Polymorphisms in Brahman crosses in the IGFBP-3 gene. This study focused on the association between the polymorphism with PvuII recognition site in IGFBP-3 gene, birth size (body length, height at withers and heart girth) and birth weight in Brahman Cattle offspring (BX, BXW and BXB). Genome DNA from 16 samples of Brahman Cross (BX), 8 sample of Brahman cross/Wagyu (BXW) and 10 Brahman Cross/Belgian Blue (BXB) were used in the analysis. Target sequence of IGFBP-3 gene in this study was located in the part of intron 2, exon 3 and part of intron 3. The gene targets were amplified using specific primers by Polymerase Chain Reaction (PCR) technique, resulting 563 bp amplification product. The result showed birth size and birth weight of BXW was smaller than in the 2 other breeds. The highest birth size ($P < 0.05$) corresponded to BXB with the size of body length, height at withers and heart girth of 67.70 ± 4.11 cm, 73.64 ± 4.89 cm and 75.93 ± 7.08 cm, respectively. Birth weight of BXW, BX, and BXB were 21.63 ± 1.99 kg, 32.40 ± 5.66 kg, and 28.93 ± 6.67 kg, respectively. This result were significantly different between breed ($P < 0.05$). The PvuII recognition sites were located in intron 2 at position 84 (A > A) and 172 (G > A). Based on the recognition by restriction enzymes *Pvu*II at position 172, 3 genotypes were observed as GG, GA and AA. The association genotype (except AA, 2 sample of BX only) and birth size and birth weight in Brahman cattle offspring were analyzed by 2x3 factorial model. However, according to genotype group (GG and AG) there were no significant different on birth size and birth weight between the 3 groups of Brahman cattle offspring. In conclusion, the differences of the performance of each breed may be affected by another candidate gene for growth traits.

Key Words: polymorphism, IGFBP gene, growth trait, Brahman cattle

P105 Average daily gain and single nucleotide polymorphism analysis of 211-bp growth hormone gene in crossbred cattle. T. Hartatik*[1], S. Bintara[1], I. Ismaya[1], P. Panjono[2], B. P. Widyobroto[2], A. Agus[3], I. G. S. Budisatria[2], and P. Leroy[4], *[1]Department of Animal Breeding and Reproduction, Faculty of Animal Science, UGM, Indonesia, [2]Departemen of Animal Production, Faculty of Animal Science, UGM, Indonesia, [3]Departemen of Animal Nutrition and Feed Science, Faculty of Animal Science, UGM, Indonesia, [4]Faculty of Veterinary Medicine, University of Liege, Belgium.*

The aim of this research was to investigate of average daily gain (ADG) and single nucleotide polymorphism (SNP) of partial growth hormone gene (211 bp) in crossbred cattle. Crossbreed cattle consist of Brahman cross, Belgian Blue cross, wagyu cross, Madura cattle, Madura Cross, and Friesian Holstein. Average daily gain was calculated from birth weight to 2 mo of age, and then the results was grouped into high and low category based on the average data. A partial fragment of growth hormone gene (211 bp) was amplified and digested using PCR-RFLP method with AluI restriction enzyme. The results show Belgin Blue cross and Friesian Holstein belongs to high birth weight and ADG. Frequency of genotype LL was higher for both category (high and low) birth weight and ADG. However, the frequency of genotype VV only occur in Belgian blue and Wagyu cross cattle. Frequency of LV and VV was increase in category high ADG. Conclusion, allele V ws probably one of the molecular genetics markers for growth trait in cattle.

Key Words: average daily gain, SNP, growth hormone, PCR-RFLP

P106 Associations between *MSTN* variants and milk fatty acid composition in New Zealand cross-bred Holstein-Friesian × Jersey cows. I. L. Haruna*, U. J. Ekegbu, Y. Li, H. Amirpour-Najafabadi, H. Zhou, and J. G. H. Hickford, *Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, Canterbury, New Zealand.*

The myostatin gene (*MSTN*), alternatively known as the growth and differentiation factor 8 gene (*GDF8*), encodes the myostatin protein (MSTN). *MSTN* has pleiotropic effects, and its expression has been associated with increased skeletal muscle mass and decreased adipogenesis as a result of the reduced secretion of leptin. The Polymerase Chain Reaction (PCR), coupled with Single Strand Conformation Polymorphism (SSCP) analysis, was used to reveal variation in bovine *MSTN* in 430 Holstein-Friesian × Jersey (HF × J)-cross dairy cows (Kiwicross™ cows). Subsequent sequence analysis of a 367bp fragment of the intron 1 region, revealed 5 variant sequences (*A*-*E*) with a total of 7 single-nucleotide substitutions, all of which have been previously reported. Using General Linear Mixed-effect Models (GLMMs), association analysis revealed that the presence of *A* in a genotype was associated with increased percentage of total fat in milk, whereas the presence of *C*, was associated with a decrease in percentage of total fat, and an increase in the amount of polyunsaturated fatty acids (C18:2 cis-9, trans-13 and C20:3 cis-8, 11, 14). These results were consistent in a genotype model, where the *AA* genotype was associated with an increase in C12.0 and a decrease in C18:2 cis-9, trans-13 fatty acid, whereas the *AC* genotype was associated with decreased C12.0 and increased C18:2 cis-9, trans-13 fatty acid. These associations in NZ cross-bred HF × J cows are all reported here for the first time, and they suggest that variation in bovine *MSTN* in NZ cross-bred Holstein-Friesian and Jersey cows could be exploited to increase the concentration of unsaturated fatty acids and decrease the concentration of saturated fatty acids content in milk.

Key Words: cattle, genotyping, SNP, genetic marker, milk production

P107 Developing a traceability system in Nguni cattle population using Illumina BovineSNP50 BeadChip. LS Modise[1], AA Zwane[2], B. Mtileni[1], and KA Nephawe*[1], *[1]Department of Animal Sciences, Tshwane University of Technology, Pretoria, Gauteng, South Africa, [2]Animal Production Institute, Agricultural Research Council, Pretoria, Gauteng, South Africa.*

The aim of the study was to identify single nucleotide polymorphisms (SNPs) for traceability in Nguni cattle. Purebred Nguni cattle (n = 50) were sampled across South African (SA) provinces and genotyped using the Illumina BovineSNP50 chip. Data from the Bonsmara (n = 30), Afrikaner (n = 48) and Holstein (n = 49) breeds were used as reference populations. The SNPs were filtered to remove those with the call rate of ≤ 98%, missing genotypes of ≤ 10% and a deviation from Hardy-Weinberg Equilibrium (HWE) ($P < 0.00001$). A total of 49 588 SNPs remained after filtering. The mean minor allele frequency (MAF = 0.184), Observed Heterozygosity (Ho = 0.248) and Expected heterozygosity (He = 0.248) for Nguni cattle population fell within the range of MAF = 0.157 to 0.214, Ho = 0.212 to 0.293 and He = 0.213 to 0.285 for Afrikaner and Holstein, respectively. The FIS ranged from −0.026 to 0.003 across the breeds. Breed informative markers were determined by SVS. The degree of differentiation between the breeds indicated that the highest genetic distance of 0.194 was observed between Nguni and Holstein breeds, while the closest genetic distance of 0.060 was observed between Nguni and Bonsmara breeds. There were 1 308 candidate breed specific SNPs identified with FST ≥ 0.60 across the breeds. Large number of SNPs with FST ≥ 0.3 were identified between Nguni and Holstein pairs (6403). Nguni and Bonsmara pairs had the smallest number of SNPs (719). Neighbor-joining showed a clear separation between African and European breeds. Breed assignment test revealed that all cattle breeds were successfully assigned to the correct breeds. The result showed that SNP markers can be successfully used as a part of an effective traceability method for identification of South African Nguni cattle.

Key Words: traceability, SNPs, breed-specific, polymorphism, Nguni cattle

P109 The allele and genotype distribution in SNP g. 408 C>G of the FABP4 gene in Kebumen Ongole grade cattle. A. Fathoni*, D. Maharani, S. Sumadi, and T. Hartatik, *Universitas Gadjah Mada, Yogyakarta, Indonesia.*

The FABP4 gene plays a role in the accumulation of fatty acids on the cell surface and affects animals growth. To evaluate the polymorphism of FABP4 gene in Kebumen Ongole grade Cattle, we genotyped a single nucleotide polymorphism (SNP) g. 408 C > G. One hundred blood samples were isolated and used for Polymerase Chain Reaction (PCR). The PCR product (590 bp) was digested with Hpy188I restriction enzyme to genotype using the PCR-RFLP method. The results revealed 2 alleles (C and G) and 2 genotypes (CC and CG). The frequency of the C allele (0.97) was higher than the G allele (0.03). The homozygous CC genotype frequency (0.94) was higher than the heterozygous CG genotype (0.06). The results of Chi-squared statistic showed that the population was in Hardy-Weinberg equilibrium. As a conclusion, the SNP g. 408 C > G of FABP4 gene may be suggested to determine the genetic diversity of Kebumen Ongole grade cattle.

Key Words: polymorphism, FABP4 gene, SNP, Kebumen Ongole grade cattle

P110 Strong signatures of selection in three Korean cattle breeds exposed to different selective pressures. K.-S. Kim* and Z. Edea, *Chungbuk National University, Cheongju, Chungbuk, South Korea.*

The Korean Peninsula harbors 3 phenotypically distinct cattle breeds. The brown Hanwoo cattle have experienced intensive selection for beef traits, whereas the Brindle Chikso and Heugu (Black cattle) have been less subjected to artificial selection for production traits. In this study, to identify signatures of selection associated with phenotypic and production traits, we performed a genome-wide scan on a total of 72 animals representing 3 Korean cattle breeds genotyped with the 777K chip. Principal component analysis showed a marked clustering of the 3 cattle breeds according to their phenotypic /coat color basic

discriminations. The highly differentiated and shared signatures of selection were detected on BTA18(14.28–14.82 Mb) in close proximity to the well-known pigmentation gene, *MC1R*. Our candidate genes such as *ADCY5, ADCY8,* and *MAPK1* have also been known to be involved in melanogenesis. These genes might have been contributed for the distinct coat color phenotypes of the target cattle breeds. Additionally, some of the candidate genes have previously been known to affect body size and growth (*DIAPH3, ATP8A1, UBR2, ZNF521, ADAMTSL1*), meat quality/, fatty acid metabolism and composition (*ADRB1, COL11A1, COL1A2, ASAH1, APBB2*), and feed efficiency (*ABCC8, PRKG1, ZNF521*). Gene Ontology(GO) analysis showed that that the candidate genes were associated with functional terms relevant to meat quality traits (lipid metabolism, fatty acid β-oxidation, adipose tissue development) and pigmentation.

Key Words: brindle cattle, candidate genes, Hanwoo cattle

P111 Abstract withdrawn

P112 Molecular genetic analysis of male infertility in Holstein Friesian cattle. S. Shan*[1], F. Xu[1], M. Bleyer[2], T. Melbaum[1], S. Becker[1], W. Wemheuer[1], M. Hirschfeld[1], M. Hölker[3], C. Wacker[1], E. Schütz[1], S. Zhao[4], and B. Brenig[1], *[1]University of Goettingen, Institute of Veterinary Medicine, Göttingen, Niedersachsen, Germany, [2]Pathology Unit, German Primate Center, Leibniz-Institute for Primate Research Göttingen, Göttingen, Niedersachsen, Germany, [3]University of Bonn, Institute for Animal Sciences, Königswinter, Nordrhein-Westfalen, Germany, [4]Key Lab of Animal Genetics, Breeding and Reproduction, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China.*

Before the approval of a bull for artificial insemination several semen characteristics, e.g., sperm motility, morphological abnormalities, concentration, velocity, are routinely assessed as predictors of fertility. However, the effective fertility of a bull can only be determined on the basis of the achieved conception rate. We have identified a Holstein Friesian sire who had been approved for artificial insemination based on his semen characteristics, but had no progeny after 412 first inseminations resulting in a non-return rate (NR-Abw) of −29. Using whole genome association analysis and next generation sequencing a nonsense mutation in the causative gene on bovine chromosome 13 (BTA13) was identified. The frequency of the mutation in the German Holstein Friesian population was determined to be 0.0018 using Fluorescence resonance energy transfer (FRET) and DNA chip genotyping of 222,645 cattle. The mutation was traced back to a potential founder (born in 1959). In vitro fertilization (IVF), lipidomic and protein analyses were performed to elucidate the so far unknown physiological and biochemical role of the causative gene. Western blotting and enzyme-linked immunosorbent assays of deep frozen semen samples using a polyclonal antibody failed to detect its protein signal, but it was detected in luminal side of seminiferous tubules in wild-type bull testis by immunohistochemistry. Therefore, a lipidome comparison of fresh semen of a heterozygous carrier and controls (n = 4) was performed showing significant differences in the concentration of Phosphatidylcholine (PC), Diacylglycerol (DAG), Ceramide (Cer), Sphingomyelin (SM) and Phosphatidylcholine (-ether) (PC O-) indicating that it plays a role in lipid biosynthesis similar to its other protein family members. IVF experiments revealed that deep frozen semen samples of homozygous carriers (n = 2) were able to fertilize cumulus-intact oocytes, implying that migration defects of mutated sperms through female reproductive tract lead to their infertility. The data presented here unravel the hitherto unknown biological function of the causative gene in lipid biosynthesis and fertilization.

Key Words: nonsense mutation, sperm lipidomics, bull infertility, sperm migration

P113 Abstract withdrawn

P114 Abstract withdrawn

P116 Abstract withdrawn

P117 Abstract withdrawn

P118 Identification of SNP in the *LMNA* gene in a heifer with suspected progeria. G. Smolucha, A. Koseniuk*, A. Kozubska-Sobocinska, A. Majewska, and B. Danielak-Czech, *The National Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

Lamins are structural proteins of the cell nucleus, which also affect the structure of chromatin, regulate gene expression, determine the distribution and stability of other proteins. In humans mutations of the *LMNA* gene cause of diseases (laminopathies) related to muscle tissue, fat, axons, as well as progeria or diseases manifested by premature aging. The classical premature aging disease in human - Hutchinson–Gilford Progeria Syndrome (HGPS) is most commonly caused by a *LMNA* dominant mutation (c.1824C > T; p.G608G) that activates a cryptic mRNA splice site leading to an internal in frame deletion of 50 amino acids that removes the *ZMPSTE24* cleavage site within prelamin A. The transgenic mouse with the human HGPS *LMNA* G608G mutation did not exhibit the external disease symptoms typical for humans, however revealed changes of the cardiovascular system including a progressive loss of vascular smooth muscle cells in the large arteries, seen usually in HGPS patients. Here, we present the case study of Polish Red heifer which was suspected of progeria on the basis of the phenotypic features like low weight and short stature at birth as well as insufficient growth rate up to the age of 2. The heifer was of normal karyotype 60,XX. The aim of our studies was molecular diagnosis of this case. Analysis of 233 bp fragment of the *LMNA* gene (exon 12) was performed using the PCR reaction, Sanger dideoxy sequencing and capillary electrophoresis. Sanger sequencing results not showed the HGPS-inducing mutation, however revealed 2 other mutations c.2098T > C and c.2115 A > G. The identified mutations occurred in heterozygous form in the examined heifer as well as in the mother and sister of normal exterior.

Therefore, we assume that the physiological state of the examined heifer does not result from identified mutations. However, further survey of this case will be undertaken.

Key Words: cattle and related species, functional genomics, diagnostics, polymorphism

P120 Abstract withdrawn

P121 Genome-wide CNV identification and CNVR association with carcass and meat quality traits. M. A. Andrade[1], A. C. Fernandes[2], A. S. Carmo[3], T. C. S. Chud[4], M. S. Carvalho[5], M. N. Bonin[6], E. C. Mattos[5], J. B. S. Ferraz[5], and F. M. Rezende*[1,2], *[1]Instituto de Biotecnologia - UFU, Patos de Minas, MG, Brazil, [2]Faculdade de Medicina Veterinária - UFU, Uberlândia, MG, Brazil, [3]Universidade Federal de Goiás, G, Brazil, [4]University of Guelph, Guelph, Guelph, ON, Canada, [5]Universidade de São Paulo, Pirassununga, SP, Brazil, [6]Universidade Federal do Mato Grosso do Sul, Campo Grande, MS, Brazil.*

The aim with this study was to detect copy number variations (CNV) and investigate the association of copy number variation regions (CNVR) with carcass and meat quality traits in Nelore beef cattle. Genotype data from Illumina BovineHD BeadChip were available for 407 bulls. The CNV detection was carried out using Hidden Markov model implemented by PennCNV software. The individual adjacent CNV calls were grouped into CNVR by CNVRuller software, when overlapping at least one base pair. The association analysis using phenotypes adjusted for non-genetic effects were performed applying a linear regression approach. Note that the framework of the classical animal model, using 658 non-castrated Nelore bulls with records for carcass and meat quality traits and a pedigree relationship matrix of 4.065 animals, was implemented to estimate non-genetic effects. In total, 8.161 CNV were detected in the autosomal chromosomes and aggregated in 626 CNVR, covering 2.24% of the bovine genome. These CNVR included 399 loss, 215 gain and 12 both (loss and gain within the same region) events, with mean length size of 107.4 kb, varying from 7.2 up to 2,910.2 kb. Of the 626 CNVR identified, 163 CNVR presented minor allelic frequency higher than 1% and were used for association analysis. CNVR associated (P-value < 0.05) with hot carcass weight, ribeye area, backfat thickness, tenderness after 7 d of aging, intramuscular fat content and intramuscular cholesterol content were identified across the entire bovine genome. Most of the CNVR associated with carcass and meat quality traits were located within olfactory receptors genes, which are known to be involved in different signaling pathways. In addition, CNVR in genes associated with muscle development and differentiation (*MBNL1, TRAK1*), cell migration, proliferation and differentiation (*TSPAN8, GADD45B*) and lipid and cholesterol metabolism (*MRPL28, TMEM8A*) were also identified. Overall, the CNV discovery and CNVR association analysis may improve our understanding about the phenotypic expression of the important economic traits. Our findings showed evidence of CNVR within genes directly implicated in carcass and meat quality traits in Nelore breed.

Key Words: *Bos indicus*, structural variants, beef cattle

Companion Animal Genetics and Genomics

P122 DNA polymorphisms in the *APC* gene in dogs with intestinal adetomatous polyposis. F. Rofes, G. Ramírez, and R. Pena*, *Departament de Ciència Animal, Universitat de Lleida, Lleida, Spain.*

Intestinal polyps are abnormal exophytic tissue proliferations that arise from the inner layer or intestinal mucosa and that grow toward the intestinal light, of benign character mainly. Unlike in humans, where the Family Adenomatous Polyposis (PAF) syndrome has a strong genetic component in dogs, family/breed predisposition is still unclear. Mutation in the *APC* gene (APC, WNT Signaling Pathway Regulator) has been described in the PAF syndrome and in early adenomas, and associated with the progression of these lesions to colorectal adenocarcinomas. Recently, this gene has also been characterized at the molecular level in dogs. This is a complex, 183 Kb-long gene that is expressed into at least 10 different transcripts in dogs, to generate 2556–2846 amino-acid-long proteins. In both species there are several mutation hot spots, which do not overlap fully. We investigated the sequence variability in the *APC* hotspots described in humans and dogs in samples from 8 unrelated dogs suffering from intestinal polyposis and 3 healthy control dogs. Breeds included Shar-Pei (n = 4), Cocker spaniel (n = 1), German shepherd dog (n = 1), American Staffordshire terrier (n = 1) and mixed breed dog (n = 1). Intestinal lesions consisted of variable-sized, exophytic growths derived from the small or large bowel mucosa, seldom eroded. They typically expanded edematous and fibrotic lamina propria with variable acute and chronic inflammation. They were also characterized by dilated distorted glands filled with mucin, or showed smooth muscle proliferation in the lamina propria. Dysplastic foci and carcinomatous change were also seen. DNA was isolated from paraffin-embedded colon and duodenum in blocks or mounted slides and used for PCR amplification of 3 PCR fragments covering the 15 Aa and 20 Aa repeat domain regions. We identified a total of 17 single nucleotide mutations (SNPs) in the last exon of the gene, 12 of which changed the amino-acid sequence of the protein. Among them, 7 of the mutations were considered not tolerant for the protein function, according to SIFT and PolyPhen-2 analysis. However, 4 of the animals showed no mutations

in the regions analyzed. Therefore, none of the mutations described can be considered a breed-specific polymorphism for this condition.

Key Words: dogs, genetic characterization, heritary disease

P123 Optimized fragment analysis kit to determine canine parentage with ISAG-recommended STR markers. D. Meza, R. Tebbs, S. Chadaram*, and R. Conrad, *Thermo Fisher Scientific, Austin, TX, USA.*

Microsatellites, also referred to as short tandem repeats (STRs), are the gold standard for parentage testing and identification including forensic analysis due to their Mendelian inheritance, and associated mutational diversity. Recently, the International Society of Animal Genetics (ISAG) added 3 new markers to the original 19-marker recommended panel for canine parentage determination, for a total of 22 loci. The Canine ISAG STR Parentage Kit (2014), from the AgriBusiness group at Applied Biosystems Thermo Fisher Scientific, is an optimized reagent kit for the analysis of the 22 STR loci recommended by ISAG in 2014. The Canine ISAG STR Parentage Kit (2014) includes primer mix, amplification master mix, and canine gDNA control. The kit allows for the simultaneous amplification of the 22 ISAG recommended STR loci in a single multiplex amplification reaction. Buccal swab and oral fluid samples were prepared using various DNA extraction kits following the protocols recommended by the manufacturer. Amplicons are sized using capillary electrophoresis on Applied Biosystems genetic analyzers and GeneMapper software is used to determine each animal's unique genetic profile. The performance of the Canine ISAG STR Parentage Kit (2014) has been demonstrated across more than 42 canine breeds, multiple different sample preparation methods, and across different genetic analyzer capillary electrophoresis instruments. The complete workflow takes less than one day from sample to results, and several samples can be analyzed together, depending on the genetic analyzer instrument capabilities.The Canine ISAG STR Kit (2014) enables customers to comply with ISAG recommendations with efficient out-of-the box optimized multiplex amplification reaction and fragment analysis while reducing risk of supporting complex homebrew panels. For Research Use Only. Not for use in diagnostic procedures.

Key Words: canine, genotype, parentage, microsatellites, multiplex

P124 A cell line model for mammary gland tumors: immunological characterization of CF33. P. Modesto*[1], B. Chirullo[2], P. Petrucci[2], C. Campanella[1], C. Pistoia[2], P. Pasquali[2], A. Ferrari[1], and E. Razzuoli[1], *[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy, [2]Istituto Superiore di Sanità, Rome, Italy.*

Mammary gland tumors (MGTs) are common neoplasms occurring in female dogs and they are malignant in about 50% of the cases. Many in vitro studies on MGTs are carried out on CF33 (canine mammary gland tumor) cell line; however, little is known about CF33 expression profile of genes involved in the innate immunity response, in DNA repairs, in cell cycle regulation. The aim of our study was to evaluate the expression of 31 immune-related and epithelial gene transcripts: the immune-related group consist of TNFa, IFNg, IFNb, IL1b, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL15, IL16, IL17, IL18, IL23, IL27, MYD88, NFKb/p65, TLR4, TLR5, MD2 and CD14, and the epithelial group was represented by CD44, CXCR4, RAD51, p53, PTEN, Erb2, TGFB, BCRA. CF33 cells were grown until confluence at 37°C with 5% in DMEM enriched with 10% (vol/vol) of fetal calf serum, a mixture of antibiotics and L-Glutamine 4 mM/L. Cells were tested at 37°, 39° and 42° passages; each experiment was repeated 10 times. Significant differences among data sets were found by ANOVA using PRISM software, setting the significance threshold at $P < 0.05$. Ribosomal protein S5 was used as reference gene. IL1b, IL-2, IL10, IL15, IL17, IL27, IFNg were not expressed in CF33. TGFb, IL4, IL6, IL12, IL-23, TNFa, NFKb/p65, TLR4, TLR5 and CD14 were expressed in an inconsistent manner among experiments. In the epithelial group ErbB2 was expressed in 6 out of 30 samples. The other genes under study were expressed in all samples. Gene expression of IFNb, IL-6 and BCRA2 was downregulated either between 37° and 39° or between 39° and 40° passage ($P < 0.05$). MD2, CD44 and IL-16 were downregulated between 37° and 39° passage, no differences were showed between 39° and 42°. The expression of CD44 and CXCR4 protein was assessed by immune-cytochemistry. Our results outline the basal expression in CF33 of important genes involved in innate immune response. Moreover, we demonstrated the presence of the stemness index CD44 in mammary tumor and of the CXCR4 receptor involved in cancer invasion. These results provide important data on CF33 basal gene expression profile useful for in vitro preliminary evaluation of new therapeutic approaches.

Key Words: canine mammary tumors, cell culture, gene expression, animal health

P125 Evaluation of alternative treatment for canine mammary tumors using CF33 cell line. P. Modesto*[1], B. Chirullo[2], P. Petrucci[2], C. Campanella[1], C. Pistoia[2], P. Pasquali[2], A. Ferrari[1], and E. Razzuoli[1], *[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy, [2]Istituto Superiore di Sanità, Rome, Italy.*

Canine mammary tumors (CMTs) are considered a good model for breast cancer studies due to the similarities of risk factors, biological expression and pathogenesis between human and canine neoplasia. On the other hand, the required reduction of animal experimentation led the attention of the scientific community on cell cultures. Recent studies used CF33 (Canine Mammary Gland Tumor), to evaluate in vitro pathogenesis and treatments for the CMT. Among alternative therapies, the use of attenuated strains of bacteria against different type of neoplasms is raising attention. However, the information available on CF33 cell line is currently incomplete, and its ability to interact with bacteria and to secrete cytokines is unknown. The aim of our study was to evaluate the response of this cell line to an infective stressor, such as *Salmonella Typhimurium* (ST). Bacteria were sub-cultured for 2 h a 37°C, re-suspended at 100 MOI/cell in DMEM and used to infect cells; untreated cells were employed as negative control. Bacterial penetration and innate immune response were evaluated as previously described (Razzuoli et al., 2018). Data were checked for significant differences by Test-t using PRISM software. The significance threshold was set at $P < 0.05$. Total RNA extraction and RT-qPCR reactions were carried out as previously described (Razzuoli et al., 2018). Ribosomal protein S5 was used as reference gene. Our results showed abilities of ST to invade CF33 and modulate gene expression and cytokines release. In particular, treatment with ST caused a pro-inflammatory effect characterized by significant increase of IL-8 and IL-6 gene expression and associated with IL-6 release (+ 80 pg/mL). Moreover, we observed a significant upregulation of CD14 gene expression and downregulation of MYD88, IL-18, NF-Kb/p65, TGFb, MD2 and TLR5. These data demonstrate the CF33 molecular response to ST cell penetration and suggest CF33 as suitable in vitro model to evaluate bacterial therapy effects for cancer treatment.

Key Words: canine mammary tumors, cell culture, immunogenomics, gene expression, animal health

P126 Investigation of canine APOA5 gene polymorphisms in some breeds. M. Takeda*[1], M. Hattori[1], H. Yasuda[2], and Y. Mizoguchi[1], *[1]Meiji University, Kawasaki, Kanagawa, Japan, [2]Yasuda Veterinary Clinic, Meguro,Tokyo, Japan.*

Hypertriglyceridemia is a common disease among canines that is characterized by high triglyceride (TG) levels. Primary hypertriglyceridemia is caused by environmental factors such as lack of exercise, whereas secondary hypertriglyceridemia is caused by genetic factors. Single nucleotide polymorphisms (SNPs) have been used to identify genes related to TG metabolism in humans. To explore the relationships between canine gene and hypertriglyceridemia, we focused on Apolipoprotein A5 (ApoA5) gene. ApoA5 is combined with lipoprotein, and functions to modulate its metabolism. ApoA5 SNPs have been shown to influence TG levels in humans. In the current study, we identified

SNPs and deletions in canine ApoA5 gene to explore the role in the high TG levels associated with hypertriglyceridemia. We collected blood samples at a veterinary clinic from 5 dog breeds (miniature schnauzer, miniature dachshund, toy poodle, Yorkshire terrier, and Pomeranian) and performed DNA extraction according to standard protocols. Blood sample TG concentrations were determined by the clinic using an automated spectrophotometric analyzer. Among 100 samples, we detected 9 SNPs and 3 deletions in ApoA5 gene, including the promoter region. One SNP found in exon 4 was a non-synonymous substitution. However, we detected no relationship between ApoA5 SNPs and blood TG levels. We will investigate SNPs in other genes related to TG metabolites in a future study.

Key Words: apolipoprotein A5, canine

P127 Analysis of ocular diseases in Shetland Sheepdog population and evaluation of candidate genes for oculocutaneous albinism. J. Bíla*, M. Bieliková, and A. Dudáš, *Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic.*

Inherited eye diseases in dogs represent a very important group of diseases, especially due to severe visual disturbances, leading in many cases to complete blindness. The molecular diagnosis of these defects offers important tool for reduction of the frequency of mutant allele in populations of affected breeds. Moreover, the canine model could be helpful for human medicine. We have focused on 3 genetic ocular diseases in Shetland Sheepdog breed: collie eye anomaly (CEA), progressive retinal atrophy (PRA) and oculocutaneous albinism (OCA). We used PCR-based and RFLP analyses for detection of causal mutations in population of 200 Shetland Sheepdogs. CEA is an inherited congenital visual impairment typical for herding dogs. Shetland Sheepdog belongs to the most affected breeds. The causative mutation is the deletion of 7.8 kb, which is located in the intronic part of the *NHEJ1* gene. Inheritance of this disease is autosomal recessive. The frequency of the mutant allele observed in the analyzed population was 37%. Progressive retinal atrophy (PRA) is autosomal recessive inherited disease that leads to degeneration of retinal photoreceptor cells. It is caused by the deletion of 4 base pair in *CNGA1* gene, which results in premature stop codon. The frequency of the mutant allele observed in the analyzed population was only 1%. OCA affects the coloration (pigmentation) of the skin, hair, and eyes. To date, 4 OCA causative mutations were identified in dogs. Three of them are located in *SLC45A2* gene: 4 081 base pairs deletion in exon 7 (Doberman pincher), c.1287delC and c.1773delC (Bullmastiff). The last mutation in the first intron of *OCA2* gene affects its splicing (German Spitz). We have evaluated the presence of all known mutations in affected Shetland Sheepdogs with OCA phenotype with negative result. To identify responsible defect further evaluation of candidate genes or genome wide analysis will be necessary.

Key Words: dog, ocular diseases, mutation

P128 Proteins and miRNAs in feline renal amyloid deposits. F. Genova*[1], S. Nonnis[1], E. Maffioli[1], F. Grassi Scalvini[1], N. Di Nanni[3], F. Cupaioli[3], E. Mosca[3], A. Mezzelani[3], G. Sironi[1], LA Lyons[2], G. Tedeschi[1], and M. Longeri[1], *[1]Department of Veterinary Medicine, University of Milan, Milan, Italy, [2]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO, USA, [3]Bioinformatics Group, Institute of Biomedical Technologies, National Research Council of Italy, Segrate, Italy.*

Amyloidosis is a group of diseases occurring in humans and animals, due to amyloid fibrils deposits in different organs. In humans, deposits were characterized using proteomics and recent studies have focused on miRNAs role in the pathogenesis. Among animals, the disease particularly affects Abyssinian and Siamese cats, with kidneys and liver as target organs, respectively. However, little is known about the mechanisms underlying the disease. This study aims to profile miRNAs and proteins in healthy and affected Abyssinians and evaluate their differential expression to clarify the pathogenesis mechanism. Formalin-fixed paraffin-embedded kidney slices were collected from 7 affected and 5 healthy cats and used for both proteomic and miRNAs analyses. Peptides were analyzed with an LTQ-Orbitrap Velos mass spectrometer (MS). MS spectra were searched against the cat sequence database vs9.0 by MaxQuant. Bioinformatic analysis was performed with DAVID and Panther software.MiRNAs were sequenced on the Illumina NextSeq500 platform. MiRDeep2 mapped reads on the cat genome vs9.0, identified putative miRNAs, quantified their expression and identified homologous human miRNAs. Proteins and miRNAs were filtered and those differentially expressed were identified with student *t*-test and moderate *t*-test respectively (p-value ≤0.05). Proteins belonging to affected or healthy cats were detected, as well as proteins commons for the 2 groups (n160), whereas 16 were upregulated and 18 were downregulated in the affected cats (p-value ≤0.05). Annotation suggested extracellular matrix and macromolecular complex subunit organization as the main associated pathways.MiRNA analysis detected 341 miRNAs, 22 differentially expressed between affected and healthy cats ($P < 0.05$). Six miRNAs out of 22 (4 with a p-value <0.009) are involved in Alzheimer Disease. Interestingly, miR-26a-5p (p-value 0.120) is involved in the human immunoglobulin light chain amyloidosis onset. This study has identified different miRNA and protein compositions in affected and healthy tissues. The pathways involving these molecules are under investigation, providing new insights for the pathogenesis understanding.

Key Words: cats and related species, functional genomics, bioinformatic tools, biomarker, animal health

P129 Genomic origin of Balkan livestock guardian dogs. M. Janeš[1], M. Zorc[2], M. Ferencakovic[1], I. Curik[1], P. Dovc[2], and V. Cubric-Curik*[1], *[1]University of Zagreb, Faculty of Agriculture, Department of Animal Science, Zagreb, Croatia, [2]University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Ljubljana, Slovenia.*

Livestock guarding dogs (LGDs) represent group of large sized dog breeds, often around 70 cm at the withers and more than 45 kg, that are formed to guard domesticated animals against wild predators. Karst Shepherd (KS), Sarplaninac (SP) and Tornjak (TOR) are FCI recognized breeds belonging to the LGD subgroup that are associated with the Balkan by geographic origin and considered as molosser-type mountain dogs. The aim of this study was to provide genomic characterization and position of those 3 breeds. Our analyses were based on high-throughput genomic information (Canine HD Bead chip with 173.662 SNPs) obtained from 14 KS, 14 SP, 24 TOR and 4 Croatian Grey Wolves as well as from publicly available genomic information (432 individuals) representing wolves, other well-known breeds and LGD breeds. Principal Component Analysis confirmed that KS, SP and TOR are closest to the other LGDs, although not so far from the wolves. On the NeighborNet derived from the Nei's distance, SP and TOR were classified together while KS was close but separated toward German Shepard breed. Our further analyses will be oriented toward genomic estimation of conservation status (inbreeding and effective population size) and potential admixture.

Key Words: dogs and related species, conservation genomics, SNP, breed diversity, breed/population identification

P130 Candidate gene search for canine hip dysplasia expression using genome-wide association study (GWAS) in Korean army dogs. J. M. Kang*[1], C. J. Gajaweera[1], S. B. Jang[1], Y. K. Kim[1], Y. J. Chung[1], S. H. Lee[1], D. H. Lee[1], Y. H. Ju[3], C. K. Lee[4], B. H. Choi[2], and S. H. Lee[1], *[1]Division of Animal and Dairy Science, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, South Korea, [2]Animal Genome and Bioinformatics Division, National Institute of Animal Science, RDA, Wanju, South Korea, [3]Department of*

Defense Military Observer Corps, South Korea, [4]Division of Biotechnology, Korea University, Seoul, South Korea.

Korean army dogs are made up of German Shepherd, Malinois and Labrador Retriever, and have an important role in preventing illegal distribution of dangerous goods and ensuring human safety. However, they are at a risk for canine hip dysplasia (CHD), musculoskeletal diseases that occur frequently in many dog breeds as well. If it is possible to predict this kind of disease, we can get positive effect on their performance. On the other hand, few genetic studies have been done for Korean army dogs so far. Therefore, present study was conducted to investigate the association between SNP data and CHD in Korean army dogs. We used total 69 Korean army dogs including Malinois(n = 12) and Shepherd(n = 57) and they were genotyped by Illumina CanineHD BeadChip 170K. Quality control was done by missing genotype 10% and call rate 95%. Genome-wide association study (GWAS) was used to identify the significant SNPs that associated with CHD for both legs. Norberg angle was used to determine the CHD score. Based on that 62 individuals were classified into a control group (normal) while 7 were classified as a case group (severe CHD). Candidate SNPs were identified on the chromosome 2 (n = 1), 4 (n = 1), 17 (n = 1), 25 (n = 6), 28 (n = 1), 31 (n = 2) for the left leg and the chromosome 19(n = 1), 25(n = 6) for the right leg. Candidate SNPs on chromosome 25 were common to left leg and right leg. The representative gene associated with these SNPs was NBEA gene and it is known to affect bone formation. Consistence with the previous studies our results also showed a polygenetic nature of the CHD.

Key Words: canine hip dysplasia (CHD), genome-wide association study (GWAS)

P131 An R-based methodology for remapping SNPs to nascent genome assemblies. W. Zhang*[1], J. McGrath[1], J. Brockman[2], D. Gunn-Moore[1], R. Mellanby[1], and J. Schoenebeck[1], *[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK, [2]Hill's Pet Nutrition, Pet Nutrition Center, Topeka, KS, USA.*

Probe-based genotyping assays are popular due to their cost-effectiveness. When new reference genomes are released, the position and strand information of SNP array variants requires updating so that the genotypes can be placed into the new context. Our R-based methodology enables users to remap array-based genotypes. We defined 2 classes of probes. "Golden" probes have precise positions in the new reference genome. Conversely, probes with imprecise mapping(s) are "ambiguous." Two pipelines were developed to detect and filter out ambiguous probes thus retaining golden probes. We tested our remapping strategy using a proprietary Illumina array whose 287,034 50 bp probes were designed based on Felis_catus_6.2. Using an R-based wrapper of BLAST, we remapped these probes to Felis_catus_9.0. This resulted in 265,403 (92.5%) golden probes. Among these, 231,818 (80.8%) probes matched perfectly to the updated reference, 246,449 (85.9%) probes had only one aligned subject, and 263,055 (91.6%) probes were relocated within the same chromosomes. Ambiguous probes totalled 11,209 (3.9%); they were classified as such due to the occurrence of tandem repeats or INDELs which made precise position assignment of the assayed SNP impossible. In this study, we designed a stringent protocol for remapping SNPs to newly released genome assemblies. An R-based package is forthcoming for distribution.

Key Words: probes, remapping SNPs, nascent genome assemblies

P132 cDNA cloning and variant analysis of the canine *CMAH* gene. Y. Uno*, S. Kawakami, K. Ochiai, and T. Omi, *Faculty of Veterinary Science, Nippon Veterinary and Life Science University, Musashino, Tokyo, Japan.*

Neu5Ac and Neu5Gc are generally the major sialic acids in mammals. Human influenza viruses and parvoviruses in dogs and cats bind to these acids. These acids are related to cat blood type antigens that cause hemolytic transfusion reactions. The enzyme cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) converts Neu5Ac to Neu5Gc. Humans are genetically defective in the gene encoding this enzyme, and *CMAH* mutations that modulate the AB blood group system are observed in cats. The presence of these sialic acids has also been reported in dogs, and Neu5Gc has been found on erythrocytes only in certain breeds of Asian origin. We performed cDNA cloning of the canine *CMAH* gene, sequenced the coding region, and detected DNA polymorphisms. cDNA derived from canine bone marrow was used for cDNA cloning of canine *CMAH*. To identify variants, the exons were amplified from the genomic DNA of 274 dogs representing 11 breeds. Sequencing was performed directly on RT-PCR or PCR products. The open reading frame (ORF) consisted of 1737 nucleotides and encoded 578 amino acids. The full-length ORF of canine *CMAH* has the same sequence length as that of feline *CMAH1a*. Canine *CMAH* exhibited 92% nucleotide sequence identity with its feline homolog. At the protein level, they are 93% identical. The cDNA sequence of canine *CMAH* in this study was assigned to the DDBJ with Accession No: LC 382414. Furthermore, the nucleotide sequences of 14 exons were determined, and we found 1 non-synonymous SNP and 3 synonymous SNPs. The frequency of the non-synonymous SNP varied among breeds. We are now investigating the relationship between parvovirus infection and Neu5Ac using SNPs as indicators. We expect that the genetic information obtained in this study will be an important tool in the future for analyzing relationships between sialic acids and infectious diseases.

Key Words: dogs and related species, genome sequencing, genotyping, cloning, dog viruses

P133 Genetic population study of STR loci (Finnzymes Canine Genotypes Panel 1.1) in Slovenian dog population. M. Cotman* and J. Zabavnik Piano, *University of Ljubljana, Veterinary Faculty, Institute of Preclinical Sciences, Ljubljana, Slovenia.*

Allele frequencies and population genetic parameters for 18 short tandem repeat (STR) loci and one sex determination locus were determined in the dog population from Slovenia to evaluate them for the application of the STRs for parentage testing and for forensic purposes. DNA samples from 85 unrelated dogs presented to our laboratory for parentage testing or individualization were used in the study. DNA from whole blood was isolated by Wizard Genomic Isolation kit (Promega) and from hair folicles using 5% Chelex and Proteinase K. STRs were amplified using Finnzymes Canine Genotypes Panel 1.1 Kit (Finnzymes Diagnostics, Espoo). Visualization of PCR products was made by capillary electrophoresis using the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Raw data were analyzed by Genemapper Software Version 3.7. Genepop version 4.0.7 was used to calculate Hardy-Weinberg Equilibrium, STR's allele frequencies and inter-population pairwise F(ST) values.. Test to evaluate a deviation from Hardy-Weinberg equilibrium showed no significant differences beetween observed and expected genotype frequencies ($P < 0.001$) at 4 loci (AHTk211, INU005, INU030 and REN162C04). All pairwise comparisons showed significant Fis values (Fis > 0).

Key Words: animal identification, animal forensics, allele frequencies, Hardy-Weinberg equilibrium, short tandem repeats

P134 Genetic background of aortic stenosis in Slovenian population of Boxer dogs. A. Perovic[1,2], A. D. Petric[1], I. D. Kusec[3], M. Zorc[4], and P. Dovc*[4], *[1]University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia, [2]Sonar d.o.o, Ljubljana, Slovenia, [3]University of*

Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia, [4]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia.

Aortic stenosis (AS) is a frequent congenital heart disease (CHD) in dogs, representing about 15% of all congenital heart defects. Among pure breed dogs, the incidence of AS is increased in herding, working, sporting, mastiff-like and retriever breeds. The fact, that the higher incidence of AS is associated with the increase of inbreeding coefficient in the population, supports assumption that AS has a genetic component. Genetic background of AS has been studied in several dog breeds with the aim to decipher its mode of inheritance and causal mutation for it. In some studies, the autosomal codominant mode of inheritance with moderate penetrance has been proposed. In Slovenian population of Boxers, the subvalvular type of stenosis was detected as the most frequent type of AS, which was frequently accompanied with pulmonic stenosis. Animals included in this study were divided into 2 groups based on severity of clinical sigs of aortic stenosis detected by ultra sound diagnosis, blood pressure and flow velocity. The blood pressure of the group with severe AS was higher than 80 mmHg and flow velocity was above 4.5 m/s. The control group without signs of AS had blood pressure lower than 20 mmHg and flow velocity less than 2.25 m/s. The genotyping of animals (n = 16) was performed using Illumina 170k CanineHD BeadChip. The quality control of genotypes and genome-wide association study (GWAS) analysis were performed using SVS Golden Helix V.8.8.3. The linear regression model, previously corrected for population structure, was used for GWAS and revealed candidate regions on chromosomes 8 and 11. On CFA8 *lincRNA* - ENSCAFG00000039469 was identified within the candidate region and on CFA11 2 candidate genes (*RPSAP37* and *GRAMD3*) were found within the candidate region. Both genes on CFA11 were already proposed to be involved in the angiogenesis.

Key Words: aortic stenosis, congenital heart defect, Boxer, GWAS, candidate genes

P135 An early onset retinopathy in Golden retriever dogs. S. Mäkeläinen*[1], K. Narfström[2], B. Ekesten[3], G. Andersson[1], and TF Bergström[1], *[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Section for Comparative Ophthalmology, College of Veterinary Medicine, University of Missouri-Columbia, Columbia, MO, USA, [3]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Progressive retinal atrophy (PRA) and other inherited retinopathies result in visual impairment and blindness in dogs and humans. In Golden retriever dogs, 3 different forms of PRA and the genetics behind them have been identified. We have recently observed a fourth form of retinopathy that affects the photoreceptors; more severely rods than cones. In comparison to classical PRA caused by variants in the *PRCD*, *SLC4A3* (GR PRA I) and *TTC8* genes (GR PRA II), this previously undiscribed retinopathy appears to have an earlier onset. Further, there is mainly a normal fundus appearance while clinical signs such as pupillary light reflexes and night vision are abnormal. Here, we used a whole-genome sequencing (WGS) approach of an affected dog, its unaffected sibling and their unaffected parents using Illumina paired-end sequencing with 150bp read length on a NextSeq500 platform. This resulted in an average coverage of 16.9X and identification of 4,764 exonic indels and 48,366 exonic single nucleotide variants (SNVs). Assuming an autosomal recessive mode of inheritance we used conditional filtering of the variants and identified 45 exonic indels and 842 SNVs. We then filtered these variants against 33 additional whole-genome sequenced dogs respresenting 8 different breeds and found 9 indels and 113 SNVs private to the affected individual. Eight of the indels resulted in frameshift insertions or deletions and one was a nonframeshift insertion. 46 SNVs were nonsynonymous and 5 of these were considered deletorious based on Polyphen-2 and Provean scores. These variants are currently being functionally evaluated bioinformatically and by Sanger sequencing of additional dogs. One of the nonsynonymous substitutions is a particularly strong candidate as a causative variant for this novel retinal disease.

Key Words: dog, monogenic disease, whole-genome sequencing, retinopathy, animal health

Comparative and Functional Genomics

P136 Discovery and characterization of lncRNA involved in lipogenesis and lipid composition in different types of adipose tissues of Wagyu cattle. J. Mi[1], Z. Zhao[1], X. Fang[1], A. Elke[2], S. Maak[2], and R. Yang*[1], *[1]Jilin University, Changchun, Jilin, China, [2]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Subcutaneous and visceral are 2 major types of adipose tissue and play different roles in development and metabolic processes of cattle, such as in insulin action,lipid composition and basal metabolic properties. It is known that visceral adipose tissues display a range of biochemical properties that distinguish them from adipose tissues of subcutaneous origin. However, we have little information on gene expression regulatory elements, either in relation to fat deposition or lipid composition. lncRNAs are an important class of pervasive genes involved in a variety of biological functions, and iIt can serve as key co-activators of proteins involved in transcriptional regulation. However, it remains unclear whether lncRNAs function during adipogenesis in different types of adipose tissues in cattle. In the present study, subcutaneous and visceral tissues were obtained from 6 adult wagyu cattle. The fatty acid composition of these adipose tissues was analyzed, and the lncRNA expression profile of 2 types of adipose tissues were characterized by RNA-seq. The 4,344 lncRNA transcripts were identified through 5 stringent steps and filtration by coding potential. 81 differentially expressed lncRNAs (34 upregulated, 47 downregulated) and 24 novel genes (11 upregulated, 13 downregulated) were identified to be differentially expressed in subcutaneous fat tissues relative to visceral fat tissues, respectively. The expression patterns of 15 lncRNAs and 20 mRNAs were further confirmed by qPCR, and Significant lncRNAs were selected to predict target genes through bioinformatics analysis. Function analysis of target genes showed that the most significant enriched biological processes of up/downregulated genes include fatty acid metabolism, fat digestion and absorption, PPAR signaling pathway, which are important mechanis of fat deposition. Several pivotal lncRNAs and targets were found (such as TCONS_15265585/KERA, TCONS_17061086/CPT1B) that could be used as candidate markers for adipose deposition prediction. This study revealed that the expression of lncRNAs differed between subcutaneous and visceral fat, suggesting that the molecular mechanism of adipogenesis is site dependent in beef cattle. These results provide important information and insights into the lipogenesis and lipid composition, and potential targets for the future improvement of meat quality of cattle.

Key Words: beef cattle, lncRNA, lipometabolism, molecular mechanism

P138 Optimising the number of SNP required to differentiate cows and somatic cell count in a bulk tank milk: The case of GenoCells. F. Perrin*[1], P. Lenormand[2], N. Taupin[1], M. Collet[1], M.-F. Quesnel[1], M. Foucher[1], and J.-B. Davière[2], *[1]AGRANIS Laboratory, Genomic department, Saint Berthevin, France, [2]SEENOVIA, Research and Development department, Saint Berthevin, France.*

Animal genotyping represents an important way to increase the genetic value related to animal health, functional and morphological traits and milk quality and productivity for farmers. Single nucleotide polymorphism (SNP) genotyping is used worldwide as a powerful tool

and the best technique in term of productivity and reliability using beadchip (10k, 50k, and 700k) technology.In a disruptive way, another technique passing through genotyping was developed: GenoCells. The GenoCells method allows to determine the somatic cell count (SCC) per milliliter of milk for each cow from a unique bulk tank milk sample.This technique requires to previous genotype each cow from the herd and then the bulk tank milk can be also genotyped.To differentiate each cow from a herd based on their individual genotype, the bulk tank milk was first genotyped with a 50k beadchip. However, this 50k beadchip leads to a long analysis time and is more expensive compared with the 10k chip for example. The aim of this work was to compare the tank milk analysis with 50k and 10k SNP genotype markers./ > Results demonstrate that the 10k beadchip is as efficient as the 50k one and give the same results. These results brings to light that a lower number of genetic marker is sufficient to identified multiple animals from a mix one.

Key Words: genomic, mastitis, SNP, milk quality, *Bos taurus*

P139 Data visualization toolkit for targeted genotyping-by-sequencing (GBS). P. Siddavatam, H. Suren, K. Gujjula, R. Willis*, and J. Schmidt, *Thermo Fisher Scientific, Austin, TX, USA.*

Traditionally, high-throughput genotyping has been carried out by array based technologies or simplex PCR techniques. AgriSeq GBS with Ion Torrent next generation sequencing (NGS) technology offers a faster, flexible, multiplexing, customizable, cost-effective alternative solution to study 50 to 5000 markers. However, the data formats and complexity of NGS can make the scientific interpretation challenging. For the wider adoption and usability, we need a better way of summarizing and presenting the data for easier interpretation. Unfortunately, there are no tools available to comprehensively visualize the genotyping outputs. We are developing a unified software tool to provide run summary metrics, genotype matrix table, genotypes in TOP/BOTTOM format, and additional features to view and compare the genotype calls. Preliminary toolkit consists of the following features: Genotype Summary - A summary report of the sequencing run with the high-level metrics of the sample call rates. GBSmatrix – Actual genotype alleles are displayed in a sample-by-marker matrix of all the samples from a single sequencing run. GenotypeTB (TOP/BOTTOM) - By default, AgriSeq™ reports genotype calls based on the positive strand alleles. To compare different genotyping technologies and calculate concordances, genotype calls are converted and displayed in TOP/BOTTOM format. The plugin enables researchers to visualize, interpret and troubleshoot the genotyping results better. In doing so, the tool helps them leveraging the informative power of NGS applied to targeted GBS. The data visualization toolkit will be distributed as an Ion Torrent Software Suite Plug-In.

Key Words: bioinformatics tools, multispecies, genotyping, DNA sequencing

P140 An intercross population study among domestic and wild sheep reveals genes associated with morphological and body conformation traits using a hybrid genome. X. Li[1,2] and M. H. Li*[1], *[1]CAS Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences (CAS), Beijing, China, [2]University of Chinese Academy of Sciences (UCAS), Beijing, China.*

Argali (*Ovis ammon*), one of the wild relatives of domestic sheep (*Ovis aries*), has a different number of chromosomes (2n = 56) from domestic sheep. However, they can crossbreed with domestic sheep (2n = 54) and generate fertile offspring. Here, we re-sequenced one argali, 36 Tibetan sheep (one domestic sheep breed on the Qinghai-Tibetan Plateau, China) and 367 individuals which are descendants of *Ovis ammon* × *Ovis aries* F_1 hybrid rams (2n = 55) and ewes of Tibetan sheep (2n = 54). Furthermore, we sequenced and de novo assembled one hybrid sheep with a diploid chromosome number of 2n = 55 through single-molecule sequencing and chromatin conformation capture to reveal the chromosomal recombination and structure variations during hybridization. Comparative genomic analyses of the new hybrid assembly genome with sheep reference genome Oar v.4.0 could help us further understand the genetic mechanism of hybrid fertility. Through further genome-wide associated studies (GWAS), we intend to identify the genomic regions and genes associated with several important morphological and body conformation traits (e.g., body size and body weight) showing heterosis.

Key Words: hybrid fertility, re-sequencing, *de novo*, GWAS

P141 Copy number variations in South African Holstein and Jersey cattle: Prevalence, characterisation and milk trait associations. M. D. Pierce*[1], L. H. Pickering[2], and K. Dzama[1], *[1]University of Stellenbosch, Stellenbosch, Western Cape, South Africa, [2]Unistel Medical Laboratories, Cape Town, Western Cape, South Africa.*

Copy number variation regions (CNVRs) comprise deletions, duplications and insertions larger than 1kb found within the genome. Increasing evidence demonstrates that these variations play pivotal roles in several adaptive, reproductive and production traits in cattle. CNVRs have been shown to influence dairy production traits. South African dairy cattle are primarily exotic breeds that demonstrate limited adaptability to the local climate which is circumvented through intensive management strategies. The prevalence of CNVRs within the South African (SA) dairy cattle populations has however not been ascertained. PennCNV software was utilized to identify CNVRs in the genome of SA Holstein and SA Jersey cattle. CNVRs identified in 3 or more animals across breeds and within breeds are reported. UCSC Genome Browser, the Panther databases and the Animal Genome QTL databases were used to asses CNVRs for gene content, gene ontologies, simple repeats and quantitative trait loci (QTL) overlap. Genome wide assocations of CNVRs with average milk yield, average percentage butter fat and average percentage protein were assessed. The incidence of CNVRs in areas of the genome holding QTLs and simple repeats was determined. In addition, genomic within breed associations of CNVRs with milk production traits are reported. This provides useful information regarding the prevalence of CNVRs in South African cattle and the associated dairy industry. Insight gained from this study will promote sustainability within the dairy industry.

Key Words: copy number variations, dairy cattle, genome-wide association

P142 Interaction effects on adipose tissue transcriptome in Iberian and Duroc pigs fed different energy sources. R. Benítez*[1], N. Trakooljul[2], Y. Núñez[1], B. Isabel[3], E. Murani[2], E. De Mercado[4], E. Gómez-Izquierdo[4], J. Garcia-Casco[1], C. López-Bote[3], K. Wimmers[2], and C. Óvilo[1], *[1]INIA, Madrid, Spain, [2]Leibniz-Institute for Farm Animal Biology (FBN) Institute for Genome Biology, Dummerstorf, Germany, [3]Departamento de Producción Animal, Facultad de Veterinaria,UCM, Madrid, Spain, [4]Centro de pruebas de porcino ITACYL, Hontalbilla, Segovia, Spain.*

We analyzed the effects of breed, diet energy source and their interaction on adipose tissue transcriptome in growing Iberian and Duroc pigs. The study comprised 29 Iberian and 19 Duroc males, which were kept under identical management conditions except the nutritional treatment (HO diet with 6% high oleic sunflower oil and CH diet with carbohydrates). All animals were slaughtered after 47 days of treatment, with 51.2 kg of average LW. Twelve animals from each breed (six fed each diet) were randomly selected for ham subcutaneous adipose tissue RNA-seq analysis. The bioinformatic analysis of RNA-seq data was performed using different pipelines. We detected 837-1456 differentially expressed genes (DEGs) conditional on breed, depending on the pipeline. Due to the strong effect of breed on transcriptome, the effect of the diet was separately evaluated in the two breeds. We identified 207 DEGs (FC 1.5-21.5) and 58 DEGs (FC 1.5-6.9) conditional on diet in Iberian and Duroc, respectively. Out of the DEGs conditional on diet in each breed only seven were common, one of them showing

an opposite response to the diet in both breeds (*SERPINE1*). Moreover, interaction breed*diet effects were tested with a complex design model in DESeq2 software. The functional analysis of DEGs showed the enrichment of functions related to inflammatory response, immune cell trafficking, CH metabolism and lipid metabolism being affected by diet in both Iberian and Duroc pigs. Nevertheless, diet had slightly different functional consequences in both breeds; for instance leukocyte movement, recruitment and migration were enriched in HO diet in Iberian and in CH diet in Duroc. The results, including the interaction effects, were technically and biologically validated by assessing the relative expression of 11 genes in all available samples (n=48) by qPCR. Interesting potential regulators for the expression differences were predicted, such as *TGFB1*, *SERPINE1*, *IFNL1* or *TNF*. The results indicate a more intense transcriptomic response to diet composition in Iberian than in Duroc animals and some interesting breed*diet interaction effects.

Key Words: Pigs and Related Species, RNA-Seq, Nutrigenomics, Metabolism and Fat/Lipid

P143 The European Variation Archive: Genetic variation archiving and accessioning for all species. C. Y. Gonzalez, J. M. Mut, S. Venkataraman, A. Silva, B. A. Koylass*, and T. Keane, *The European Bioinformatics Institute, Cambridgeshire, United Kingdom.*

The European Variation Archive (EVA, https://www.ebi.ac.uk/eva) is a primary open repository for archiving, accessioning, and distributing genome variation including single nucleotide variants, short insertions and deletions, and larger structural variants in any species. Since launching in 2014, the EVA peers with NCBI-based database dbSNP to form a worldwide network for exchanging and brokering of variation data. From 2017, issuing and maintaining variant accessions is divided by species: the EVA is responsible for non-human species and dbSNP for human. Other services include standard variant annotation, calculation of population statistics, and an intuitive browser to view and download queried variants in either variant call format (VCF) or comma-separated value (CSV) files. In addition, a comprehensive REST-API is available to query/export data that supports the htsget streaming protocol defined by the Global Alliance for Genomics and Health (GA4GH). The EVA also contributes to maintaining the VCF, implementing a validation suite (https://github.com/ebivariation) to ensure correctness of all the submissions made to the archive. The EVA has archived more than 770 million unique variants across 546 studies and 51 species. 280 million identifiers have also been imported from dbSNP, and 330 million new identifiers have been issued. The API is also species-agnostic and is extensively used by translational resources including Ensembl, Ensembl Genomes, Open Targets, WheatIS and the 1000 Sheep Genomes Project. A key function of the EVA as a long-term data archive is to provide standardised stable identifiers so that studies and discovered variants can be referenced in publications, cross-linked between databases, and integrated with successive reference genome builds. With these goals, the EVA will continue to act as a primary repository for variation data from any species.

Key Words: bioinformatics tools, computational pipeline, databases/repositories, multispecies

P144 Piglet body weight drives functional changes in hypothalamic and muscle transcriptome. R. Benitez[1], J. Segura[2], M. Vázquez-Gómez[2], J. Viguera[3], L. Calvo[4], JI Moríñigo[5], Y. Nuñez[1], C. López-Bote[2], and C. Ovilo*[1], *[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain, [2]Universidad Complutense de Madrid (UCM), Madrid, Spain, [3]Imasde Agroalimentaria, Madrid, Spain, [4]Incarlopsa, Cuenca, Spain, [5]Ibercom, Badajoz, Spain.*

Adaptation to extra uterine life is a challenge for the neonatal piglet. Body weight (BW) at birth and at early stages is a main factor affecting survival and growth performance. Moreover, low weight at early stages is associated with prenatal programming processes, which in turn are related to altered energy homeostasis, adipose tissue deposition and regulation of leptin production. In this work, we investigated functional genomics differences between piglets showing extreme values of BW at one week of age, in 2 tissues. Eight Iberian × Duroc crossbred females were selected from 20 contemporaneous litters, showing divergent values of BW (n = 4 in each group, high H and low L). Hypothalamus and Biceps femoris muscle samples were obtained for RNaseq analysis. BW difference was close to 2 SD (H: 3.2 vs L: 2.4 kg BW, $P = 0.001$), as well as for other body measures and viscera weights. Transcriptome results showed that BW significantly affected the expression of 85 and 44 genes (DEG), in muscle and hypothalamus, respectively. In muscle, DEGs were mainly involved in lipid and CH metabolism, Skeletal and Muscular System Development and Function and Connective Tissue Development and Function. Genes involved in lipid metabolism were upregulated in L group, such as *ADIPOQ*, *FASN* or *FABP4*. The activation of pathways related to immune system and pathogen response was also detected in L group. Interesting and complex regulatory networks were predicted to regulate the expression differences, with molecules such as *PPARG*, *AKT1* or *IRS1* having main roles. In the hypothalamus, DEGs were involved in growth-related, energy homeostasis and signaling functions. For instance, growth hormone signaling was predicted to be altered in L group. Many regulators, such as transcription factors, cytoquines and miRNAs, were also predicted to regulate transcriptome changes, which may be considered key molecules for piglet early growth. Results agree with a higher predisposition for lipid synthesis and deposition and metabolic alterations in low BW piglets and provide a comprehensive identification of candidate genes and regulators which may influence postnatal development.

Key Words: pigs, transcriptome, body weight, growth, energy homeostasis

P145 Conjugated linoleic acid promotes bovine intramuscular adipogenesis. N. Mizuta and Y. Mizoguchi*, *Meiji University, Kawasaki, Kanagawa, Japan.*

Beef marbling, which is related to intramuscular fat deposition, is an economically important trait in meat animal industries. Japanese black cattle have been characterized as having the most exceptional marbling traits worldwide. Conjugated linoleic acid (CLA) is an isomer of linoleic acid, a polyunsaturated fatty acid; it is produced by microbes in the bovine rumen, where it is thought to be a functional component. To evaluate the role of CLA in adipocyte differentiation, we used a clonal bovine intramuscular preadipocyte (BIP) cell line derived from Japanese black cattle (Aso et al., 1995) to compare the effects of different CLA concentrations after 6 d of culture on differentiation induction medium with treatments of 0, 5, 10, 25, and 50 μM CLA. We also investigated triglyceride accumulation, glucose uptake, and expression levels of the adipogenesis-related genes Fatty acid binding protein (FABP) 4, Glucose Transporter (GLUT) 1 and 4, Fatty Acid Synthase (FASN), and Stearoyl CoA Desaturase (SCD), according to standard protocols. Triglyceride accumulation, glucose uptake amounts, and expression levels of FABP 4, GLUT 4 and SCD increased with increasing CLA concentration, although no change in GLUT 1 and FASN gene expression was observed. Fatty acid synthesis was promoted by CLA stimulation through glucose uptake by GLUT 4 but not GLUT 1 to accumulate fat during bovine intramuscular adipogenesis. To understand the mechanism of bovine intramuscular adipogenesis through CLA in greater detail, metabolome analysis should be performed in a future study.

Key Words: adipogenesis, bovine, conjugated linoleic acid

P146 Abstract withdrawn

P146 Abstract withdrawn

P147 Abstract withdrawn

P147 Abstract withdrawn

P148 Agriseq targeted GBS is a customizable high-throughput genotyping technology that permits fast, easy, and inexpensive alteration of marker content. C. Carrasco, K. Gujjula, H. Suren, P. Siddavatam, and C. Adams*, *Thermo Fisher Scientific, Austin, TX USA.*

Attractive and valuable high-throughput genotyping solutions for parentage and breeding applications require the ability to simultaneously interrogate hundreds to thousands of genetic loci both easily and economically. One disadvantage of many high-throughput genotyping technologies is the lengthy lead times and considerable cost associated with changing the genomic marker content (targeted loci) in any particular assay. The Applied BiosystemsAgriSeq targeted genotyping-by-sequencing (GBS) solution for plant and animal genotyping does not suffer from this problem because the technology relies on a pool of PCR oligonucleotides that can be quickly, easily and inexpensively changed to accommodate always improving knowledge of genomic function. If and when the need arises to alter the content of a marker panel all that is required is the design and synthesis of additional PCR primers which are then simply spiked into existing assay pools. In addition, AgriSeq genotyping panels can be ordered in plate format in which primer pairs for marker-containing amplicons are individually aliquoted enabling the user to drop unneeded amplicons or re-formulate primer pools (panels) in any combination desired. Furthermore, individual panels targeting specific species can mixed together, creating a multi-species panel, while still enabling species-specific genotyping. For example, a mixture of 3 mid-density panels for multiple species not only allowed for accurate species-specific genotyping, but also enabled the accurate assignment of species to unknown gDNA samples being tested. Moreover, the flexibility of the workflow allows users to mix AgriSeq libraries from different species on a single chip for sequencing, which improves the overall economics. This unparalleled flexibility in a highly multiplexed genotyping platform provides users unlimited avenues for customizing their genotyping workflows.

Key Words: genotyping, next-generation sequencing, parentage, breeding

P149 Tannin supplementation in Mangalitsa pigs: Effects on muscle transcriptome. Y. Núñez*[1], C. Radovic[2], R. Savic[3], M. Candek-Potokar[4], R. Benítez[1], D. Radojkovic[3], M. Lukic[2], M. Gogic[2], L. Fontanesi[5], and C. Ovilo[1], *[1]INIA, Madrid, Spain, [2]Institute for Animal Husbandry-Pig, Belgrade-Zemun, Serbia, [3]University of Belgrade, Belgrade-Zemun, Serbia, [4]Kmetijski inštitut Slovenije, Ljubljana, Slovenia, [5]University of Bologna, Bologna, Italy.*

Tannins are plant polyphenols which may have detrimental effects on animal performance but have also demonstrated positive effects (antimicrobial, antioxidant, decrease of skatole production, PUFA increase). Thus, supplementation of pig diets with tannins has been proposed to improve meat quality and to reduce boar taint. The aim of the present work was to assess the potential effects of diet tannin supplementation on different growth, meat quality parameters, as well as on the muscle transcriptome. The study was carried out on 24 surgically castrated Mangalitsa male pigs: 12 corresponding to a control diet (C) and 12 supplemented with 2% chestnut wood extract containing hydrolysable tannins (T). All animals were slaughtered after 190 d of treatment (slaughter age 362 d). For the transcriptome study 5 animals from each group were randomly selected for muscle RNA-seq analysis. The phenotypic results showed that supplemented animals had lower average daily rate of gain ($P = 0.03$), higher loin intramuscular fat content ($P = 0.04$) and lower cholesterol content ($P = 0.03$). Transcriptome analysis showed 48 differentially expressed genes between experimental groups (DEGs, 8 overexpressed in T and 40 overexpressed

in C). The functional analysis of DEGs, carried out with IPA software, showed the enrichment, in T group, of functions related to cell death, apoptosis and decreased tissue development, while C group was enriched in cell survival, proliferation of muscle cells, glucose tolerance and growth-related functions. This may be associated with antinutritive/toxic effects of tannins at peripheral tissue level. Moreover, some key genes involved in lipid synthesis were upregulated in T, such as *SCD* or *ACLY*, in agreement with the higher muscle fat accumulation. Putative regulatory networks can be inferred from the gene expression data, which highlight relevant transcription factors which may be inhibited by tannins, such as *EGR1*. This work has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 634476.

Key Words: pig, transcriptome, tannin, nutrigenomics, meat quality

P150 Galectin encoding genes as indicators of selective susceptibility to endotoxin in ruminants. M. Worku*[1], E. Ekwemalor[1], E. Asiamah[2], S. Adjei-Fremah[1], and B. Osei[4], *[1]Department of Animal Sciences, North Carolina A&T State University, Greensboro, NC USA, [2]Department of Animal Sciences, University of Arkansas at Pine Bluff, Pine Bluff, AR USA, [3]Department of Biology, North Carolina A&T State University, Greensboro, NC, USA, [4]Functional and Chemical Genomics, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA.*

The objective of this study was to evaluate the effect of *Escherichia coli* lipopolysaccharide (LPS) on the expression of lectin, galactoside-binding, soluble (LGALS) genes. The expression of LGALS results in secretion of Galectins (Gal) that recognize and regulate the response to pathogens. In mice, Gal secretion is associated with selective resistance to LPS. Susceptibility to LPS is associated with impaired health and production outcomes in ruminants. Comparative evaluation of the impact of LPS on LGALS expression is needed. In this study, whole blood was collected from the jugular vein of BoerXSpanish goats(n = 3), St Croix sheep (n = 3) and Holstein Friesian cows (n = 5) into tubes containing an anticoagulant. Blood was incubated with 10 µg of *E. coli*-derived LPS (10 µg/mL) or with 200µL PBS for 30 min at 37°C, 5% CO_2, and 85% humidity. Total RNA was isolated using Trizol and converted to cDNA using the RETROscript kit (Qiagen). Real-time PCR was conducted on pooled cDNA samples from each species using specific primers for LGALS-1, −2, −3, −4, −7, −8, −9, −11, −12, and −14. Housekeeping genes GAPDH and β-actin were used to normalize the data. Fold change in transcript abundance was compared with control and calculated using the $2^{-\Delta\Delta Ct}$ method (Livak). Secretion of Gal-1, −3, and −9 was evaluated in cow and goat plasma using specific ELISA kits. Data were analyzed using PROC GLM in SAS 9.4 ($P < 0.05$). Differential transcription and fold change of LGALS was observed. LGALS-12 and −4 were upregulated in cow blood. All sheep LGALS were upregulated. In goats, 50% of LGALS tested increased while others were decreased. LGALS-2, −7 and −14 were upregulated in both sheep and goats. The highest fold change in response to LPS was observed with LGALS-14. Exposure to LPS had no effect on cow Gal but reduced the concertation of Gal-1, −9 and increased Gal-3 in goat plasma. Exposure of ruminant blood to LPS resulted in unique signatures of LGALS expression and Gal secretion. These results warrant further studies using more animals to determine if LGALS genes may serve as indicators of selective susceptibility to LPS in ruminant species to control disease

Key Words: immuno-genomics, diagnostics, candidate gene, cattle and related species, animal health

P151 Blood transcriptome analysis in a buck-ewe hybrid (geep) and its parents. C. Falker-Gieske[1], C. Knorr[1], and J. Tetens*[1,2], *[1]Department of Animal Sciences, Georg-August-University, Göttingen, Germany, [2]Center for Integrated Breeding Research, Georg-August-University, Göttingen, Germany.*

Cases of live hybrid offspring between sheep and goats have been described in literature, but, all cases reported so far involved the mating of goats with rams. Only one case of a buck-ewe hybrid (geep) has been reported in literature in 2016. Here, we present the analysis of the blood transcriptomes of that animal and its parents. Whole blood RNA of all 3 animals was sequenced on an Ion Torrent platform. Four widely used alignment methods were compared with map sequencing reads to the latest sheep and goat reference genome assemblies, respectively. Although TopHat was slightly superior in discriminating the origin of the reads, Star2pass exhibited almost twice the mapping efficiency with an acceptable species discrimination. Since 75% of the geep reads and 80% of the founder reads mapped to both reference genomes, species discrimination solely based on the alignments was not feasible. Hence, to discriminate between sheep and goat transcripts expressed the geep, an approach based on variant calling from RNaseq data was performed. Variants that were found alternatively homozygous in the founders were retained for further analyses. Since transcriptomic, not genomic, data were analyzed these variants are not exclusively heterozygous in the geep transcriptome (71.2% vs. sheep genome, 75.6% vs. goat genome). We identified 3,025 (vs. sheep genome) / 2,927 (vs. goat genome) genes in the geep's transcriptome with predominantly heterozygous variants and 2,520 (vs. sheep genome) / 2,182 (vs. goat genome) genes with predominantly homozygous variants. We performed a gene set enrichment analysis, which revealed that transcripts of genes involved in transcriptional regulation, cytoskeleton, nuclear and organelle metabolism predominantly stem from the goat and that those with a function in nucleotide metabolism, actin/myosin biology, and catabolic pathways are mainly expressed from the sheep. Variant effect prediction with snpEff revealed that the content of low impact variants is elevated where the geep is heterozygous (16.7%) compared with homozygous regions (6.3%). Taken together, our results provide unique insights into transcriptome regulation of an interspecies individual.

Key Words: interspecies hybrid, geep, blood transcriptome

P152 Functional genomics approach to characterize four adipose tissues in Finnish and Yakutian reindeer (*Rangifer tarandus*). M. Weldenegodguad*[1,2], K. Pokharel[1], I. Ammosov[3], M. Honkatukia[4], J. Peippo[1], T. Reilas[1], P. Soppela[5], N. Mazzullo[5], V. Fedorov[6], and J. Kantanen[1], *[1]Natural Resources Institute Finland (Luke), Jokioinen, Finland, [2]University of Eastern Finland, Kuopio, Finland, [3]Board of Agricultural Office of Eveno-Bytantaj Region, Batagay-Alyta, Russia, [4]The Nordic Genetic Resources Center (Nordgen), Ås, Norway, [5]Arctic Centre, University of Lapland, Rovaniemi, Finland, [6]Yakutian Research Institute of Agriculture (FGBNU Yakutskij NIISH), Yakutsk, Russia.*

Semi-domesticated reindeer (*Rangifer tarandus*) have adapted and survived in harsh northern and even arctic conditions where the temperature can fall below −50°C. Adipose tissues play vital role for cold adaptation by altering gene expressions in the tissues to supply energy and maintain thermogenic activity. To get insights into the seasonal (spring vs early winter) gene expression changes in different geographical areas, we conducted transcriptome profiling of 4 adipose tissues of different anatomical locations - perirenal, metacarpal, tailhead (no winter samples) and prescapular tissues from Yakutian and Finnish reindeer. We aimed at identifying genes and biological processes and pathways associated with seasonal changes and geographic origins. We used Illumina's HiSeq technology and generated a total of 238 Gb transcriptome data from 63 samples (19 metacarpal, 19 perirenal, 19 scapular and 6 tailhead). Alignment of the high quality reads against our draft reindeer genome assembly revealed on average 16,449 genes with mapping rate of 90% across all samples. Overall, tissue-specific gene expression pattern in metacarpal fat appeared to be more homogeneous and clustered separately (PCA analysis) compared with other tissues. The number of significantly differentially expressed genes due to seasonal changes in Finnish reindeer (metacarpal 445, perirenal 609,

prescapular 587) was higher than in Yakutian reindeer (metacarpal 63, perirenal 68, prescapular 280) which may indicate climatic and feeding practice differences between the regions. Moreover, when comparing the gene expression differences between the 2 regions, we observed the highest number of significantly differentially expressed genes in perirenal fat (n = 363) followed by prescapular (n = 236) and metacarpal (n = 163) fat. We observed several genes (*ACACA, AGPAT2, COX7A1, COX7B, COX7C, ELOVL6* and *ELOVL7*), biological processes and pathways (fatty acid metabolism, lipid metabolism and fatty acid elongation) associated with energy metabolism and cold adaptation. Taken together, this study provides new knowledge of reindeer genomics and the genomics of cold adaptation in general.

Key Words: reindeer, adipose tissue, transcriptome, fat, cold adaptation

P153 Differentially expressed tRNA fragments in bovine fetuses with assisted-reproduction induced congenital overgrowth syndrome. A. K. Goldkamp*[1], Y. Li[2], Q. Sun[1], L. Zhu[1], R. Rivera[2], and D. Hagen[1], *[1]Oklahoma State University, Stillwater, OK, USA, [2]University of Missouri-Columbia, Columbia, MO, USA.*

Assisted reproductive technologies (ART) are procedures used to conceive offspring. The use of ART has been associated with an increased risk of congenital overgrowth syndromes, including Large Offspring Syndrome (LOS) in cattle. The incidence of LOS in individuals conceived both naturally and via ART is increasingly more common. Both differential methylation patterns and a dysregulation of transcripts have been observed in bovine fetuses with LOS, suggesting causality of the phenotype. While there is no apparent relationship between DNA methylation and transcript dysregulation, some differentially methylated regions overlap tRNA clusters. Transfer RNAs can be processed to create tRNA-derived fragments (tRFs) from both precursor and mature tRNAs, giving rise to unique subtypes, characterized by their origin on tRNAs. These tRFs are typically between 16 and 27 nucleotides long. Another class, tRNA halves, are 28 to 36 nucleotides long. tRNA fragments have been shown to play a functional role in post-transcriptional gene regulation and epigenetic modifications. This led us to suspect tRNA-derived small non-coding RNAs as regulators for the syndrome. Here we identify differentially expressed tRNA-derived fragments associated with large offspring syndrome in cattle. Through analysis of expression profiles of predicted tRFs and their abundance in different tissues, these fragments could serve as biological markers or causative agents of gene regulation. We have sequenced small RNAs isolated from tongue, skeletal muscle, kidney, and liver of bovine fetuses from LOS and control individuals. We continue to analyze the differentially expressed tRFs to gain a better understanding of ART-induced LOS. Further, we will sequence mature tRNAs and assess relationships between tRNA expression and tRF abundance. We also plan to apply the methods and pipeline developed for this project on publicly available data sets to develop a tRF expression atlas.

Key Words: cattle and related species, non-coding RNA, development, computational pipeline, Functional Annotation of Animal Genomes (FAANG)

P154 Abstract withdrawn

P154 Abstract withdrawn

P155 Abstract withdrawn

P156 Prediction and quantitative expression of tRNA genes in bovine tissues. D. Hagen*[1], A. Goldkamp[1], T. Ji[2], and R. Rivera[2], *[1]Oklahoma State University, Stillwater, OK, USA, [2]University of Missouri, Columbia, MO, USA.*

Transfer RNAs (tRNAs) are universally expressed, non-coding RNAs, playing a central role in translation by converting information from mRNA codons to amino acids. Despite the critical role tRNAs play, they have been shown to vary in abundance in different human tissues. This heterogeneity suggests tRNA abundance contributes to the regulation of mRNA translation. The alteration of tRNA levels due to mutations in the tRNAs themselves or tRNA maturation has been reported to contribute to diseases in humans. Aside from their role in translation, tRNAs are now known to serve as a source of small functional RNAs. We have previously identified differentially methylated regions (DMRs) within skeletal muscle tissues of bovine fetuses associated with Large Offspring Syndrome (LOS). Some DMRs overlap current tRNA annotations in the UMD3.1 reference assembly, leading us to hypothesize that differential methylation of tRNA genes results in differential expression of mature tRNAs, leading to altered transla-

tional efficiency. The older UMD3.1 bovine genome assembly encodes more than 4,000 nuclear tRNA genes while the human genome encodes greater than 600, leading us to believe that tha number of tRNA is significantly overestimated in the older genome assembly. On the other hand, incomplete predictions in the newest bovine genome assembly (ARS-UCD1.2) hinder tRNA expression analysis. Moreover, tRNA-derived small RNA prediction and analysis is incomplete without reliable tRNA annotations. Using the new genome assembly, ARS-UCD1.2, in silico predictions, and ongoing high-throughput tRNA sequencing, we have improved the current tRNA gene set. Further, we report on ongoing efforts to identify differentially expressed tRNAs in 3 tissues; kidney, liver, and skeletal muscle, from fetal bovine tissues in LOS and control individuals. Methods we developed for this project will be valuable in the development of tRNA expression atlases for domesticated animal species.

Key Words: cattle and related species, bioinformatics, genome annotation, functional genomics, non-coding RNA

P157 Genetic control of temperament traits across species: Association of autism spectrum disorder genes with cattle temperament. R. Costilla*[1,2], K. Kemper[1], E. Byrne[1], L. Porto-Neto[4], R. Carvalheiro[5], D. Berry[6], D. Purfield[6], J. Doyle[6], S. Moore[2], N. Wray[1], and B. Hayes[2], *[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia, [2]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Australia, [3]Queensland Brain Institute, The University of Queensland, Brisbane, Australia, [4]Commonwealth Scientific and Industrial Research Organization (CSIRO), Agriculture and Food, Brisbane, Australia, [5]School of Agricultural and Veterinarian Sciences, Sao Paulo State University, Sao Paolo. Brasil, [6]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co, Cork, Ireland.*

Temperament (personality) traits are relevant to both human and cattle well-being. In humans, they correlate with psychological and psychiatric disorders. In cattle, they affect animal welfare and product quality and hence are of direct commercial importance. We hypothesized that genetic factors contributing to variation in temperament among individuals within a species will be shared across humans and cattle and indeed mammals. We conducted a series of whole genome sequence based genome-wide association studies on cattle flight time, a temperament phenotype defined as the time taken for an animal to cover a short fixed distance after being released from an enclosure, with a total sample of 9223 animals. Significantly associated regions were located on 4 bovine chromosomes and enriched in pathways related to synaptic transmission, regulation of the circadian rhythm, and neuron development functions. Given that human studies are better powered than those in cattle, we investigated the association of cattle temperament with polymorphisms in bovine orthologous genes reported to be associated with neuroticism, schizophrenia, autism spectrum disorders (ASD), and developmental delay disorders. We found enrichment of variants in, or close to, ASD susceptibility genes. These variants explained 7% of the total additive genetic variance in the biggest cattle cohort (randomized permutation test genome-wide P-value <0.02). ASD genes with the most significant associations were *GABRB3, CUL3,* and *INTS6*. We also confirmed these genes were highly expressed in bovine brain cerebellum and caudal lobe. These findings provide quantitative molecular evidence that genetic control of temperament traits might be shared across humans and cattle and highlight the potential for future analyses to leverage results between species.

Key Words: multispecies, genome-wide association, gene set enrichment analysis, complex trait, biomedical model

P158 Evaluation of RNA-sequencing pipelines for optimized power and accuracy of SNP and INDEL identification. S. Lam*[1], F. Miglior[1], J. Zeidan[1], I. Gómez-Redondo[1,2], A. Suárez-Vega[1], P. A. S. Fonseca[1], F. Schenkel[1], and A. Cánovas[1], *[1]Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Spanish National Institute for Agriculture and Food Research and Technology, Madrid, Spain.*

Evaluation of RNA-Sequencing (RNA-Seq) pipelines is critical to optimize power and accuracy of analysis for identifying genetic variants (SNPs and INDELs) in coding regions. Variant calling pipelines were compared with determine more optimized and accurate approaches for variant detection using RNA-Seq data. The new developed pipeline was used to identify unique variants associated with feed efficient beef steers. The RNA-Seq data (NCBI accession numbers: PRJEB15314 and PRJEB15314) used were from muscle and liver tissues from 12 Nellore beef steers (n = 6 low-RFI, n = 6 high-RFI), selected from 585 steers that had their residual feed intake (RFI) determined. Three multi-sample calling pipelines were compared including i) non-merged samples; ii) merged samples for low-RFI and for high-RFI for each tissue, and iii) merged samples for low- and high-RFI for both tissues. The data was aligned against UMD3.1 (release 94) assembly using STAR. Variants were called using BCFtools and variant annotation was performed using VeP and ToppGene. The approaches were compared by assessing filters (Phred score quality, location of variants, minimum read depth) and presence of variants unique to each approach using BCFtools. The use of approach i), i.e., non-merged calling, detected individual genotypes (GT) for each biological sample, revealing variability between individuals. The use of approaches ii) and iii) revealed greater read depth and power for GT calling and detection. In further analyses, approach iii), which had the greatest read depth and power, was used to detect variants within low- (SNPs: 13,145, INDELs: 371) and high-RFI (SNPs: 14,663, INDELs: 429) groups. Positional candidate genes located within variants (2,828 in low-RFI, 2,979 in high-RFI) were significantly ($P < 0.05$) associated with immune and metabolism pathways. RNA-Seq pipelines applied to detect genetic markers to be used for selecting for desirable traits in livestock should be chosen based on an experimental strategy that would maximize power of detection and accuracy of GT calling to increase confidence of variant detection.

Key Words: feed efficiency, RNA-Seq, SNP, transcriptome, cattle and related species

Comparative MHC Genetics: Populations and Polymorphism

P159 Bovine leukemia virus proviral load were associated with bovine MHC DRB3 and DQA1 alleles in Japanese Holstein population from 2011 to 2014. Y. Aida*[1,2], S.-N. Takeshima[1,3], A. Ohno[2], and L. Borjigin[1], *[1]Nakamura Laboratory, Baton Zone Program, RIKEN Cluster for Science, Technology and Innovation Hub, Wako, Saitama, Japan, [2]Viral Infectious Diseases Unit, RIKEN, Wako, Saitama, Japan, [3]Department of Food and Nutrition, Faculty of Human Life, Jumonji University, Niiza, Saitama, Japan.*

Bovine leukocyte antigens (BoLAs) are used extensively as markers of disease and immunological traits in cattle. BoLA-DRB3 gene is most polymorphic gene among class II genes, and it is well known that the polymorphism were associated with bovine leukemia virus (BLV) infection. The association of BoLA-DRB3*009:02 allele with low proviral load were predicted using low-resolution typing method, such as

PCR-RFLP, for Holstein cow. However, there is few association studies using high-resolution genotyping method, such as PCR-sequence based typing (SBT), for Holstein cow. Additionally, Yuan et al. suggested that the cow which showed the proviral load over 14,000 copies/10^5 cells secrete BLV provirus into nasal sample and it suggested that these cows were high-risk transmitter. Therefore, we here categorized the cow which shows the proviral load over 10,000 as the high-risk BLV spreader cow, and the cow which shows the proviral load under 10,000 as the low-risk BLV spreader cow. DNA samples were collected from 1290 Holstein cows belonging to BLV-positive commercial dairy farms located in the 23 prefectures of Japan, from 2011 to 2014. BoLA-DRB3 genotyping were performed by PCR-SBT. BLV proviral load were calculated by a quantitative real-time PCR method, BLV-CoCoMo-qPCR-2. We collected 1,290 blood samples from 1,290 heads of cow and there are 910 cows determined as BLV positive cow. The 910 cows were separated into 341 heads of "low-risk spreader" and 569 heads of "high-risk spreader." BoLA-DRB3 allele frequencies of these 2 groups were calculated and estimated p-values compared with each BoLA-DRB3 alleles and 2 spreader groups. From these 23 BoLA-DRB3 alleles, DRB3*002:01, *009:02, *012:01, *014:01 and *015:01 were determined as BLV provirus associated alleles. BoLA-DRB3*002:01, *009:02 and *014:01 were determined as resistant alleles (O.R. > 1), and BoLA-DRB3*012:01 and *015:01 were determined as susceptible alleles (O.R. < 1). In this study, we confirmed that BoLA-DRB3 was good marker for determine which cow spread the BLV and the result may be useful for eliminate BLV from farm without separate the cow into several cowshed.

Key Words: BoLA-DRB3, BoLA-DQA1, bovine leukemia virus, proviral load, Japanese Holstein

P160 Effectiveness of bovine leukemia virus (BLV) infection control strategies using cattle carrying resistant and susceptible bovine MHC DRB3 alleles. L. Borjigin*[1], L. Bai[2], T. Hirose[1], H. Sato[1], S. Watanuki[1], S. Yoneyama[3], M. Inokuma[4], K. Fujita[4], Y. Shinozaki[5], R. Yamanaka[6], A. Yasui[6], Y. Sohei[6], M. Baba[6], S.-N. Takeshima[1,7], Y. Aida[1], *[1]Nakamura Laboratory, Baton Zone Program, RIKEN Cluster for Science, Technology and Innovation Hub, Wako, Saitama, Japan, [2]Photonics Control Technology Team, RIKEN Center for Advanced Photonics, Wako, Saitama, Japan, [3]Kenou Livestock Hygiene Service Center, Utsunomiya, Tochigi, Japan, [4]Chuo Livestock Hygiene Service Center, Chiba, Japan, [5]Nanbu Livestock Hygiene Service Center, Chiba, Kamogawa, Japan, [6]Kumagaya Livestock Hygiene Service Center, Kumagaya, Saitama, Japan, [7]Department of Food and Nutrition, Jumonji University, Niiza, Saitama, Japan.*

Bovine leukemia virus (BLV) infects cattle worldwide and is causing serious economic damage. BLV mainly infects by horizontal transmission. The cattle with high proviral loads (PVL) are considered to be a major infectious factor in a population, while the cattle with low PVL are difficult to transmit BLV to other cattle. Previously, we identified "resistant (R) cattle" carrying bovine leukocyte antigen (BoLA)-DRB3*009:02 and *014:01:01 alleles which associated with a low PVL, and "susceptible (S) cattle" carrying BoLA-DRB3*015:01 and *012:01 alleles which associated with a high PVL in Holstein cattle. In this study, we investigated the distribution and actual PVL of these cattle at 5 farms in the Kanto region of Japan, and then we conducted the BLV control strategies using the R and S cattle. PVL of a total of about 350 cattle from 5 farms were estimated by an accurate PVL measuring system, CoCoMo-BLV-qPCR method, and BoLA-DRB3 alleles were identified by PCR-sequence-based typing method. Among 5 farms, the population of cattle carrying R and S alleles were 20.5% and 41.6%, respectively. Interestingly, PVL of the R cattle (mean 3,281 copies/10^5 cells) were significantly lower than that of the S cattle (mean 28,439 copies/10^5 cells). Next, we repeated the BLV control strategies twice within 2 years under the field condition. We first investigated whether R cattle are useful as a biological barrier to prevent new infection or not. Interestingly, no new BLV-infected cattle were detected in 2 farms for 3 or 5 mo after we placed the R cattle between the other infected and uninfected cattle. Second, we actively eliminated the S cattle with high PVL from the farms. As a result, the average of PVL were significantly reduced at all 5 farms and the BLV negative rate increased at the 4 farms. These results strongly suggest that concurrent approach of the effective utilization of the R cattle as a biological barrier to prevent new infection and the preferential elimination of the S cattle to decrease PVL is useful for BLV infection control and development of effective BLV eradication program.

Key Words: bovine leukemia virus, proviral loads, BoLA-DRB3, resistant cattle, susceptible cattle

Domestic Animal Sequencing and Annotation

P161 SeqBreed: A python tool to evaluate genomic selection with sequence data. M. Perez-Enciso*[1,2], M. L. Zingaretti[2], and L. Ramirez-Ayala[2], *[1]ICREA, Barcelona, Spain, [2]CRAG, Bellaterra, Spain.*

Genomic selection (GS) is the procedure whereby molecular information is used to predict complex phenotypes. The discovery of massive numbers of genetic markers (SNPs) and high-throughput technology to genotype animals for thousands of SNPs in a cost-effective manner has made it GS to become widely used. Nevertheless, the advantages of GS depend on several factors that are difficult to assess analytically. In this paper, we have developed a versatile python forward simulation tool, SeqBreed, to evaluate GS strategies when using sequence or any molecular marker array. SeqBreed builds on previous fortran versions and is much easier to use, more versatile and incorporates many new functionalities, including GWAS, PCA graphs and several built-in evaluation methods: BLUP, GBLUP and single step among others. Other custom criteria can be easily programmed. SeqBreed accommodates sex chromosomes, mitochondria chromosomes and polyploidy. It can simulate any number of complex phenotypes, allowing a very flexible modeling of phenotypes suited to polyploids. As input, SeqBreed takes a vcf-format genotype data from the founder population, which can vary from single nucleotide polymorphisms (SNP) chips up to sequence, a list of causal variants for every trait and their heritabilities, and the pedigree. Generating interactively new individuals is also possible as is to run the program on its own. We illustrate the functionalities of SeqBreed with data from *B. taurus* and *B. indicus* crosses. Source code, examples and a complete manual will be freely available in GitHub https://github.com/miguelperezenciso/.

Key Words: genomic selection, sequence, forward simulation

P163 Rambouillet sheep transcriptome annotation resources. R. A. Harris[1,2], X. Qin[1,2], Y. Han[1], Q. Meng[1], T. P. Smith[3], M. P. Heaton[3], B. P. Dalrymple[4], F. Thibaud-Nissen[5], E. Clark[6], J. Kijas[7], N. E. Cockett[8], B. Murdoch[9], D. M. Muzny[1], K. C. Worley*[1,2], for the Ovine FAANG Project[1], *[1]Baylor College of Medicine, Human Genome Sequencing Center, Houston, TX, USA, [2]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX, USA, [3]USDA Agricultural Research Service, U.S. Meat Animal Research Center, Clay Center, NE, USA, [4]University of Western Australia, Institute of Agriculture, Perth, Western Australia, Australia, [5]National Center for Bio, Bethesda, MD, USA, [6]The Roslin Institute, The University of Edinburgh, Edinburgh, UK, [7]CSIRO, St. Lucia, Australia, [8]Utah State University, President's Office, Logan, UT, USA, [9]University of Idaho, Animal and Veterinary Science, Moscow, ID, USA.*

Functional genomic analysis requires high-quality reference genomes with comprehensive annotation. We report ongoing analyses of our FAANG (Functional Annotation of ANimal Genomes) quality samples for the Rambouillet breed sheep. Our high-quality reference

genome, with long, medium and short RNA sequence; CAGE data, ATAC-Seq and other assays are from multiple tissues from a single ewe, Benz2616. The high quality long-read de novo assembly with complete chromosome scaffolds (Oar_rambouillet_v1.0, GCA_002742125.1), is among the most contiguous, complete and correct reference genome. FAANG assays from over 100 Benz2616 tissues are underway. Assays include PacBio IsoSeq, Illumina RNaseq and miRNaseq, ATAC-Seq CAGE, and ChIP-Seq. We have made the pre-publication transcript data available (see BioProject PRJNA414087). Initial RNA sequence analysis of PacBio IsoSeq from 5 tissues sampled 76% of the previously annotated genes encoding proteins. The value of the IsoSeq data to identify alternative isoforms led us to perform additional IsoSeq sequencing of 3 more tissues with the latest PacBio Sequel technologies. MicroRNA data and Illumina RNaseq data are available for 30 and 29 tissues respectively. We are combining these data with CAGE data from the same samples to define high quality transcript isoforms. Transcript data available January 2019, including over 1,000,000 long-reads, were used as evidence for the preliminary RefSeq annotation of the genome. NCBI Sheep Annotation Release 103 includes 21,160 protein coding genes (with 42,390 CDSs), and 7,002 non-coding genes. Most of the 42,377 mRNAs (40,003, 94%) annotated have full evidence support. Non-coding RNA annotations include 154 miRNAs, 1,774 tRNAs, 3,943 lncRNAs, 694 snoRNAs, 1,148 snRNAs, 30 guide RNAs and 283 rRNAs. The new assembly combined with these transcript data greatly improves the annotation quality. Compared with Oar_v4.0, there are 14% fewer low-quality and 3% fewer partial genes. These high-quality genomic resources are setting the FAANG standard, providing for multi-omic analysis of agricultural ruminants. We will present the ongoing analysis of these data.

Key Words: genome, transcriptome, FAANG, sheep, IsoSeq

P164 Resolving the polled locus in Brahman (*Bos indicus)* cattle using Nanopore long read sequencing. H. Lamb*[1,2], B. Hayes[2], S. Moore[2], R. Lyons[3], and E. Ross[2], *[1]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia, [2]Centre for Animal Science, QAAFI, Brisbane, QLD, Australia, [3]School of Veterinary Science, The University of Queensland, Brisbane, QLD, Australia.*

Brahman cattle (*Bos indicus)* are adapted to thrive in tropical environments and form the foundation of beef production in many tropical regions. The vast majority of Brahman cattle are horned, with a low frequency of polled animals. Animals with the poll phenotype are increasingly desirable for welfare and economic reasons. Our aim was to characterize the polled mutation in the Brahman breed. The poll phenotype has been mapped to the poll locus on chromosome 1. Four alleles, each a copy number variant (CNV), have been reported across this locus in *B. indicus* and *Bos Taurus*. Oxford Nanopore Technology's minION was used to sequence 2 homozygous poll (PP) and 2 horned (pp) Brahman bulls to characterize the poll allele in this breed. An average sequencing depth of 2.5x was achieved across the samples. N50 scores between 9kb and 14kb were generated with the longest sequence being 131kb. Mapping the reads with minimap2 revealed a 198bp and 195bp insertion at the location of the Celtic allele in both poll animals. A BLAST of the insertion sequences aligned with 100% identity to the Celtic allele locus confirming the insertions are in fact duplications. Reads overlapping the location of the other 3 alleles were examined however no significant results were seen. The results prove the Celtic allele, which is of *B. Taurus* origin, is responsible for the poll phenotype in Brahmans, at least in the animals sequenced. Introgression of the allele likely occurred during breed formation which involved grading up from *B. taurus* cattle. These results also validate the minIONs ability to rapidly, accurately and cost effectively detect structural variants such as CNVs.

Key Words: cattle and related species, genome sequencing, DNA sequencing, copy number variation (CNV), animal welfare

P165 Abstract withdrawn

P166 A metadata ruleset solution for the Innovative Management of Animal Genetic Resources (IMAGE) unification of European Gene Bank Data. J. Fan*[1], P. Cozzi[2], A. Sokolov[1], O. Selmoni[3], E. Vajana[3], S. Joost[3], E. Groeneveld[2], G. Cochrane[1], P. Flicek[1], P. Harrison[1], and A. Stella[2], *[1]EMBL-European Bioinformatics Institute, Hinxton, UK, [2]2National Research Council, Milan, Italy, [3]*École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

The Innovative Management of Animal Genetic Resources (IMAGE) project focuses upon farm animal gene banks as important resources of genetic variation to manage and maintain farm animal breeds' adaptability and capability for use in the food industry (http://www.imageh2020.eu/). IMAGE aims to unify access to data from more than 60 gene banks with heterogeneous data stored across 20 countries, posing a major strategic challenge for the IMAGE consortium along with the integration of newly generated data. Our solution comprised 1) a well-defined metadata ruleset that enforces minimum metadata requirements from each gene bank and utilizes ontologies to improve data quality and comparability, 2) a custom Inject tool that applies the ruleset to import data from gene banks, unifies units, terms and languages, and submits the enhanced data to public EMBL-EBI BioSamples archive for long-term sustainability, 3) a bespoke data portal that integrates IMAGE data from all gene banks archived in BioSamples with generated 'omics data sets and cross-references to gene banks and breeding database resources. The IMAGE gene bank data can be accessed either via an interactive web interface or through programmatic APIs.

Key Words: multispecies, bioinformatics tools, gene bank

P167 Whole-genome sequencing analysis of six Eurasian native cattle breeds. C.-W. Lee[1], H.-S. Seong[2], W.-H. Chung[3], D.-H. Son[2], N.-H. Hwang[2], Y.-M. Kim[2,4], J.-B. Kim[2], B.-H. Choi[5], J. A. Lenstra[6], J. Kantanen[7], J.-W. Choi*[2], and D. Lim[5], *[1]Gangwon Province Livestock Technology Research Institute, Hoengseong, Republic of Korea, [2]College of Animal Life Science, Kangwon National University, Chuncheon, Republic of Korea, [3]Division of Food Functionality Research, Research Group of Healthcare, Wanju, Republic of Korea, [4]Division of Swine Science, National Institute of Animal Science, RDA, Cheonan, Republic of Korea, [5]Division of Animal Genomics and Bioinformatics, National Institute of Animal Science, RDA, Wanju, Republic of Korea, [6]Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands, [7]Department of Production Systems, Natural Resources Institute Finland (Luke), Helsinki, Finland.*

This study was conducted to report whole-genome sequencing results for 6 Eurasian cattle breeds including Busha (n = 10), Northern Finn (n = 10), Kalmykian (n = 10), Podolian (n = 10), Ukrainian Grey (n = 10), and Yakutian (n = 10). Initially, approximately 26.4 billion 150-bp paired-end reads, which yield an average of 65.9 Gbp for each individual were obtained from the Illumina HiSeq 2500 sequencing reactions. Using the bovine reference genome assembly (UMD 3.1), we detected a total of 31,793,943 single-nucleotide polymorphisms (SNPs) from all the cattle populations. Of the total SNPs, we discovered 16,830,363, 14,326,612, 19,014,937, 14,380,631, 16,648,845, and 16,289,411 SNPs for Busha, Northern Finn, Kalmykian, Podolian, and Ukrainian Grey, and Yakutian, respectively, while the number of overlapping SNPs between those 6 breed populations was 440,515 (1.4%). The average transition-to-transversion ratio for all the individuals was 2.2:1, which indicates most of discovered SNPs in this study have been called in a reasonable accuracy. The average ratio of homozygous versus Heterozygous SNPs was the highest in Kalmykian (1:2.5), and followed by Ukrainian Grey (1:2.0), Northern Finn (1:1.9), Podolian (1:1.8), Yakutian (1:1.7), and Busha (1:1.6). All the detected SNPs were deeply annotated to retrieve SNPs in gene regions, resulting in approximately 26.4% of the total SNPs in genic regions, and 73.6% in intergenic regions. Using 324,443 coding SNPs, we retrieved a total of 107,143 nonsynonymous SNPs in 15,380 genes. Furthermore, we identified a total of 24,465 deleterious nonsynonymous SNPs which were predicted to affect protein structure or function. In this study, we showed numerous genomic variants from the 6 Eurasian native cattle breeds. These results provide a valuable resource for further studies on identifying genetic characteristics of cattle especially for Eurasian native breeds.

Key Words: cattle, re-sequencing, SNP

P451 *De novo* assembly and analysis of a Banteng (*Bos javanicus*). W.-H. Chung[1], J.-W. Choi*[2], H.-S. Seong[2], D. Lim[3], D.-H. Son[2], Y.-M. Kim[2,4], J. A. Lenstra[5], and B.-H. Choi[3], *[1]Division of Food Functionality Research, Research Group of Healthcare, Wanju, Republic of Korea, [2]College of Animal Life Science, Kangwon National University, Chuncheon, Republic of Korea, [3]Division of Animal Genomics and Bioinformatics, National Institute of Animal Science, RDA, Wanju, Republic of Korea, [4]Division of Swine Science, National Institute of Animal Science, RDA, Cheonan, Republic of Korea, [5]Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.*

The Banteng (*Bos javanicus*) is a species of wild cattle mostly located at Indonesia in the Southeast Asia. The domesticated Banteng has been also called Bali cattle of Indonesia and it has been regarded as a different species from either typical European-origin cattle or zebus. In addition to its phylogenic interest, it is known to have promising characteristics such as its utilization of low-quality feeds as well as tolerance of several internal parasites. Despite the clear importance, the Banteng has not been well investigated particularly in the whole genome-wide level. In this study, we did sequence the genome of a Banteng using both technologies of a short read sequencing using Illumina HiSeq 2500 and of a long read sequencer, PacBio sequel platform. To assemble the Banteng genome, 70 X-fold of SMRT sequencing, 118 X-fold of paired-end sequencing, and 171 X-fold of mate-pair (5 Kb and 10 Kb) sequencing results were obtained. Basically, we used the FALCON assembler that was used to make an initial contigs that were constructed from the long sequence reads. After scaffolding, gap filling and error correction was performed using Meraculous. As a result, a total of 1,951 scaffolds were generated, which corresponds to approximately 2.55 Gbp with a mean scaffold size of 1.3 Mbp and N50 size of 3.4 Mbp. Those scaffolds cover approximately 93% of the bovine reference genome assembly (UMD 3.1.1) except for chromosome X. In addition, we predicted and annotated 21,002 coding genes and identified around 2 million repetitive elements which masked around 45.1% of the genome assembly. Banteng genome currently appear to have the least size among bovidae genome assemblies that are publically available; it might be caused by the condensation of the telomere and centromere regions. The number of coding genes is comparable of that of UMD 3.1.1. Although 93% of the autosomal region is covered by the UMD 3.1.1 assembly, only 60% of coding genes were placed as highly similar (>90%) genomic positions. The other genes were placed on the genomic divergent regions. The further improvement of genome assembly and comparative genomic study will reveal promising characteristics of the Banteng. We expect that this study should be a useful genomic resource particularly for genome-wide comparative genomic studies between bovine species.

Key Words: cattle, genome sequencing, genome assembly

Equine Genetics and Thoroughbred Parentage Testing Workshop

P168 Expression profiling of circulating miRNAs throughout the gestation period in pregnant mares. M. Kikuchi*[1], H. Kakoi[1], T. Tozaki[1], F. Sato[2], K. Hirota[1], and S. Nagata[1], *[1]Genetic Analysis Department, Laboratory of Racing Chemistry, Utsunomiya, Tochigi, Japan, [2]Hidaka Training and Research Center, Japan Racing Association, Urakawa, Hokkaido, Japan.*

Micro RNAs (miRNAs) are small non-coding RNAs critically involved in regulating gene expression. Circulating miRNAs present in bodily fluids are considered valuable biomarkers for various diseases and different physiologic states. Furthermore, some circulating miR-

NAs have been linked to pregnancy in some species. Although some pregnancy-associated miRNAs have been identified in horses, there is limited information on the expression of those miRNAs during the gestation period. Therefore, we investigated expression profiling of circulating pregnancy-associated miRNAs throughout the gestation period in normal mares. Plasma samples were collected from 8 pregnant Thoroughbred mares pre-pregnancy and at each month of gestation. The current samples were collected until 9 mo after mating. Total RNA was extracted from the plasma samples and subjected to cDNA synthesis. Referring to previous studies, 22 pregnancy-associated miRNAs were selected for subsequent analysis. Expression levels of the 22 miRNAs were measured by reverse transcription quantitative PCR (RT-qPCR) in accordance with the manufacturer's instructions. The expression levels of 11 miRNAs were found to be significantly changed compared with pre-pregnancy samples. One miRNA (miR-486–5p) was downregulated at both early and middle stage, and 3 miRNAs (miR-30c, miR-433 and miR-767–5p) were upregulated at early stage. Three miRNAs (miR-323–3p, miR-433 and miR-767–5p) were downregulated at middle stage and 7 miRNAs (miR-27a, miR-29c, miR-30c, miR-101, miR-140–3p, miR-323–5p and miR-874) were downregulated at both middle and late stage. The targets for those miRNAs are known to be involved in adherens junction, Wnt/β-catenin pathway and lipid metabolism; those pathways appear to be related to implantation and embryogenesis. This is the first study profiling the expression of pregnancy-associated miRNAs throughout the gestation period in horses. This study will present underlying data for researching biomarkers in pregnant mares. Further analysis is in progress for the remaining pregnancy stages and other miRNAs.

Key Words: horses and related species, microRNA, pregnancy, biopsy

P170 A medium-density SNP array for the horse genotyping. A. Fornal*, A. Piestrzynska-Kajtoch, and A. Radko, *National Research Institute of Animal Production, Balice, Poland.*

Horse parentage verification is currently based on the 17 microsatellite (STR) genotyping. However, SNP marker genotyping could be alternative and supplementary method. We tested medium-density SNP platform – QuantStudio 12K Flex with OpenArray block for the horse genotyping by using TaqMan MGB assays. In the presented study we used 67 SNPs selected from the Single Nucleotide Polymorphism database (dbSNP), the 53 JPN System and 101 Etalon SNPs. TaqMan primers and probes were designed for each SNP. SNPs covering all chromosomes in genome were designed including autosomes. Samples were selected from 4 breeds: Polish Coldblood, Malopolski Horse, Polish Konik and Hucul Horse. Genomic DNA was isolated from 72 samples of peripheral blood using Sherlock AX (A&A Biotechnology). All samples were analyzed, moreover 23 samples were made double for all assay as technical replicates. Samples were genotyped by Genotyping qPCR module (Thermo Fisher Scientific Cloud Environment). All the results were clustered in the Allelic Discrimination Plot, the amplification results were reviewed and assessed. We obtained about 96% of datapoints for all samples (without technical replicates), mostly because of OpenArray AccuFill (system for loading samples) errors. All assays were amplified. Most of the selected and detected SNPs were polymorphic, only tree assays seem to be monomorphic or doubtful. Nevertheless, average MAF was about 0,325. Despite small amount of tested samples results look promising. Our data and results were validated with horse comparison test samples. The study was financed by Ministry of Agriculture and Rural Development Multiannual Programme no. 03–17–27–90.

Key Words: horses and related species, SNP, genotyping, parentage

P171 STR polymorphism in donkey (*Equus asinus*) from Poland. A. Fornal*[1], B. Dlugosz[2], A. Piestrzynska-Kajtoch[1], and K. Kowalska[1], *[1]National Research Institute of Animal Production, Balice, Poland, [2]Agricultural University, Animal Science Institute, Krakow, Poland.*

Domestic donkey (*Equus asinus*) breeding in Poland is still developing. An institution dedicated to the registration and identification of donkeys is the Polish Horse Breeders Association, but, so far neither their race is recognized nor studbooks are kept. To support donkey breeding, we have undertaken the efforts to establish donkey parentage verification on the basis of horse STR panel. The aim of the study was to determine the utility of equine microsatellites panel for parentage verification in donkey based on Polish population. We tested 30 donkeys. DNA was isolated from peripheral blood or hair roots. Seventeen microsatellite markers in one multiplex reaction (Equine Genotypes Panel 1.1 Kit; Thermo Fisher Scientific) used in horse parentage verification were analyzed for donkey according to manufacturer recommendation. Amplification of STRs was conducted with SimpliAmp Thermal Cycler. Products of multiplex PCR amplification were analyzed on 3130xL and 3500xL Genetic Analyzers (Applied Biosystems). For STR profile determination GeneMapper Software v4.0 and v5.0 were used. STR profiles were obtained for all studied samples regardless of various DNA quality and amount. Some nonspecific artifacts were found in DNA profiles, but they did not disturb microsatellite's genotyping. One locus was excluded from the analysis due to lack of amplification (ASB2). Two loci were monomorphic (HMS1, ASB17). Moreover, LEX3 was excluded from the analysis due to X chromosome localization. Thirteen loci were polymorphic. For some alleles we had to adjust equine bin set (GeneMapper settings) and confirm it by performing single allele PCR. Two cases of parentage verification were conducted. Therefore, we conclude that horse STR markers could be an effective tool in donkey parentage verification in Poland. This study is presenting our preliminary analysis results and requires proceeding and verification in bigger population. The preliminary tests indicated the successful amplification of commercial equine microsatellites set in donkey, as reported in literature. A further research would include more parentage testing cases.

Key Words: horses and related species, animal breeding, breed diversity, microsatellite, genetic marker

P172 Innate effectors of neutrophil homing are increased in peripheral lung tissue during pasture-associated severe equine asthma exacerbation. K. A. Thomas, J. E. Bowser, C. A. Mochal, A. L. Eddy, A. Claude, S. Mukherjee, and C. E. Swiderski*, *Department of Clinical Sciences, College of Veterinary Medicine, Mississippi State University, Starkville, MS, USA.*

Severe equine asthma (SEA) is characterized by reversible airway obstruction, non-specific airway hyper-responsiveness and chronic neutrophilic airway inflammation. Two forms of SEA have been described. One is elicited by barn dust in association with indoor housing in continental climates. The second form is associated with grazing pastures during conditions of high heat and humidity. Neutrophilic airway inflammation that characterizes SEA also segregates with severe and corticosteroid refractory forms of human asthma. To date, the molecular mechanisms responsible for neutrophilic airway inflammation have not been elucidated. We hypothesize that the innate immune system provides integral signals for early and continued neutrophil recruitment. To test our hypothesis, we contrasted the transcriptome from serial thoracoscopic lung biopsies (RNASeq; 40M paired end reads/sample) derived from 6 horses with pasture-associated SEA during naturally occurring asthma exacerbation and remission. Reads were aligned to EquCab 3.0 and differential expression was determined using CLC Genomics Workbench (Qiagen). 162 genes were differentially expressed (FDR <0.05). Manual curation identified increased NLRP12, and TREM1 as innate immune effectors relevant to neutrophil recruitment during asthma exacerbation. Congruent with NFκB activation by NLRP12; CXCL1, CCL2, SELE, IL1β, IL1RN and IL6 were significantly increased in association with disease exacerbation. In mice, NLRP12 and TREM1 are required for neutrophil recruitment into the lung and both proteins have also been demonstrated to mediate IL-17A differentiation of CD4+ T

cells. Collectively, these findings indicate that NLRP12 and TREM1 are likely innate immune effectors responsible for initiating and sustaining neutrophilic inflammation in the airways of horses with EPA.

Key Words: severe equine asthma, NLRP12, TH17, horse, lung

Gene Function (jointly with FAANG)

P173 Proteomics recapitulates markers regulating pathways relevant to ovarian activity in pubertal *Bos indicus* heifers. M. S. Tahir*[1], L. T. Nguyen[1,2], B. L. Schulz[1], G. A. Boe-Hansen[1], M. G. Thomas[3], S. S. Moore[1,2], and M. R. S. Fortes[1,2], *[1]University of Queensland, Brisbane, Queensland, Australia, [2]Queensland Alliance for Agriculture and Food Innovation, Brisbane, Queensland, Australia, [3]Colorado State University, Fort Collins, CO, USA.*

Brahman heifers with superior fertility are desirable in beef production systems. Early-in-life reproductive traits in females appear as consequence of puberty. In gonadostat theory, ovarian function is essential to puberty development. Ovaries synthesize steroid hormones and paracrine factors, which in coordination with gonadotrophic hormones shift heifers from pre-pubertal state to post-pubertal. At puberty, heifers attain the ability of oocyte maturation, which were arrested at meiosis-I in their fetal life. Identification of differentially abundant ovarian proteins between pre- and post-pubertal heifers; and how their abundance levels govern the complex mechanism of puberty and subsequent reproductive traits were the objectives of this study. We used ovarian samples of 6 pre-pubertal and 6 post-pubertal Brahman heifers to conduct differential abundance analyses of protein profiles between the 2 physiological states. Extracted proteins were digested into peptides followed by identification with mass-spectrometry and quantification by SWATH-MS analysis. The DA proteins ($P < 10^{-5}$) were then analyzed for gene ontology and pathway enrichment using DAVID tools. We observed 566 DA proteins between pre- and post-pubertal heifers. Out of these, 89 were found directly involved in steroidogenesis (progesterone synthesis) and oocyte maturation. Our results identified important DA proteins controlling metabolic pathways, contributing acetyle-COA to subsequent steroid synthesis. Our results also identified DA proteins that suggestively control oocyte maturation/ arrest as they take part in progesterone, estrogen, retinoic acid, TGF-β and pleiotrophin signaling pathways. Genes of 24 DA proteins were also found to be associated with reproductive traits (data from the Animal QTL Database). Integration of these results with genomics analyses, like GWAS, may help to advance our knowledge on the genetics of early-in-life reproductive traits, and ultimately lead to better beef production in cow-calf operations.

Key Words: bovine, ovary, puberty, steroidogenesis, progesterone signalling

P174 Abstract withdrawn

P174 Abstract withdrawn

P175 Abstract withdrawn

P175 Abstract withdrawn

P176 The FAANG Data Coordination Centre: Infrastructure to enable functional annotation of livestock genomes. A. Sokolov*, J. Fan, G. Cochrane, D. Zerbino, P. Harrison, and P. Flicek, *EMBL-EBI, Hinxton, UK.*

The Functional Annotation of Animal Genomes (FAANG) Project aims to provide the scientific community with high quality functional annotation of livestock genomes. This is important to facilitate understanding of genotype to phenotype links in domesticated animals. The FAANG Data Coordination Centre (DCC) at EMBL-EBI is responsible for developing the core infrastructure to support this resource. A key focus is on providing high quality metadata to describe samples and experimental assays, and we support the community to achieve this with metadata validation tools (http://www.ebi.ac.uk/vg/faang/), an active helpdesk (dcc-faang@ebi.ac.uk), and file conversion software that facilitates submissions of data to the public archives and rapid pre-publication in concordance with FAANG's data release policy (http://www.faang.org/data-share-principle). To enable easy access to livestock functional annotation data from single access point the DCC has created the FAANG data portal (http://data.faang.org/home). From this portal users can get access to the wealth of livestock annotation data submitted by FAANG contributors and also to data from public archives imported under legacy standards. The portal provides direct links to download data from the archives, programmatic API access as well as rich filtering and searching capabilities that will help scientific community to identify appropriate data for their research. It is possible to search for organisms, specimens, data sets and experiment files in the FAANG portal as well as browse through available sampling and experimental protocols. All records can also be filtered based on whether they have been published, with information about published articles provided on details page for each particular item. The publication status of data records is automatically obtained by searching for record identifiers contained within the text of published literature. Through the services it offers, the FAANG DCC helps the research community to find and establish new genome to phenome links that are crucial for the future sustainability of agriculture.

Key Words: Functional Annotation of Animal Genomes (FAANG), bioinformatics, databases/repositories

P177 Identification of functional elements in dairy cattle. C. P. Prowse-Wilkins*[1,2], A. J. Chamberlain[1], and M. E. Goddard[1,2], *[1]Agriculture Victoria, Centre for AgriBiosciences, Bundoora, Victoria, Australia, [2]Faculty of Veterinary & Agricultural Science, The University of Melbourne, Parkville, Victoria, Australia.*

Regulatory regions are known to contain genetic variation which affects complex traits. Finding these genetic variants in bovine would improve the longevity and across-breed accuracy of genomic predictions in dairy cows. However regulatory regions are poorly annotated in the bovine genome. Using ChIP-seq and ChromHMM, this work describes the identification of putative regulatory regions in bovine mammary and liver. Chromatin immunoprecipitation was performed on mammary and liver tissue from 3 lactating Holstein dairy cows using the Magnify Chromatin Immunoprecipitation kit. Chromatin was assayed for the histone modifications H3K4Me3, H3K4Me1, H3K27Me3 and H3K27ac as these modifications are known to identify regulatory regions such as promoters and enhancers. DNA libraries were prepared using the NEBNext DNA Library Preparation kit. Sequence data were trimmed of adapters and poor quality bases and aligned to UMD3.1 with BWA mem. After filtering each ChIP and input sample had 100 million mapped reads. The quality of enrichment was checked with deepTools and SPP and peaks were called with MACS2. ChromHMM searches for patterns in the co-location of histone marks to segment the genome into reoccurring chromatin states. This tool was used for each cow and tissue and across all cows and tissues. Seven chromatin states were the most consistent and biologically informative, identifying poised and active enhancers and promoters. Each state corresponded appropriately to the location of known transcription start sites. This is the first study to assay the location of histone modifications in mammary tissue in dairy cows. This data can be used to identify regulatory regions important to lactation in dairy cattle and improve genomic predictions.

Key Words: FAANG, ChIP-seq, genomic prediction, genome annotation, epigenetics

P178 Functional analysis and association studies of bovine CDC10 gene with growth-related traits. B. Tong*[1], L. Wang[1], X. Kong[1], G. Cheng[2,3], L. Zan[2,3], T. Yamada[4], and G. Li[1], *[1]State Key Laboratory of Reproductive Regulation & Breeding of Grassland Livestock, Inner Mongolia University, Hohhot City, Inner Mongolia Autonomous Region, China, [2]College of Animal Science and Technology, Northwest A&F University, Yangling City, Shaanxi Province, China, [3]National Beef Cattle Improvement Center, Northwest A&F University, Yangling City, Shaanxi Province, China, [4]Department of Agrobiology, Faculty of Agriculture, Niigata University, Niigata City, Niigata ken, Japan.*

Growth-related traits of beef cattle are important economic traits, which are regulated by multiple genes. Among them, the *cell division cycle 10* (*CDC10*, also known as *septin7*) gene was located in the QTL loci of growth-related traits in some cattle breeds, and the expression level of *CDC10* in the skeletal muscle was positively correlated with the growth-related traits of Japanese Black beef cattle (JB) (Tong et al., 2015). These results implied the *CDC10* gene could be a candidate gene for growth-related traits in Chinese cattle breeds. In the part 1 of this study, we performed direct sequencing to find variant of *CDC10* in the Qinchuan (QC), Mongolia, Chinese Simmental and Luxi cattle breeds. The results showed that there are 23 SNPs in the promoter, exon, intron and 3′UTR regions of *CDC10*. Association analysis between the SNPs and the growth-related traits of 384 QC cows showed that the −842T > G and −323G > C SNPs were significantly associated with body weight by using MassARRAY (−323G > C SNP also associated with growth-related traits of JB (Tong et al., 2015)). In addition, the Dual-luciferase reporter assay results showed that the promoter activity of the *C* allele at −323 site was significantly stronger than the *G* allele. In the part 2 of this study, we employed the overexpression and interference of *CDC10* to validate the effects of *CDC10* on the proliferation and differentiation of C2C12 and bovine primary myoblast (BPM). EdU and WST1 assay results showed that the proliferation rate of C2C12 and BPM were significantly increased after overexpression of *CDC10*, and decreased after interference of *CDC10*. And then, we induced the differentiation of C2C12 and BPM, and stained MyHC after overexpression and interference of *CDC10*. The immunofluorescence results showed that the differentiation of C2C12 and BPM were promoted after interference of *CDC10*, whereas overexpression of *CDC10* showed the opposite results. Current data suggest that 1) the *CDC10* gene could be considered an important candidate gene for growth-related traits of beef cattle, 2) the −323G > C SNP could be a key mutation to affect the *CDC10* expression level, and be useful for effective marker assisted selection to increase beef productivity in beef cattle, 3) the −323G > C SNP could change the expression level of the *CDC10* gene, and then promote cell proliferation to improve muscle growth and finally increase growth rate of beef cattle.

Key Words: beef cattle, growth-related traits, CDC10, −323G>C SNP, myoblast proliferation

P179 Abstract withdrawn

P179 Abstract withdrawn

P180 Abstract withdrawn

P181 Abstract withdrawn

P182 Abstract withdrawn

P183 Genome-wide mapping of alternative polyadenylation sites in cattle. Z. Jiang*[1], J. J. Michal[1], X. Zhou[1], S. He[1], M. Stotts[1], Y. Zhang[1], X. Zhang[1], X. Leng[2], Y. Zhang[1], H. Wang[1], H. Jiang[2], M. Du[1], M. Maquivar[1], and L. K. Fox[1], *[1]Washington State University, Pullman, WA, USA, [2]Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.*

Alternative polyadenylation (APA) is one of the most important posttranscriptional events contributing to transcriptome diversity and functional dynamics. The objective of the present study was to detect APA sites harbored in the bovine genome. Using our whole transcriptome termini site sequencing method and bioinformatics analysis, we report 203,503 APA sites derived from 10 types of cells/tissues/organs from Wagyu or Angus cattle and endometrial cells from Holstein cows with or without endometritis. Among them, 137,359 sites were assigned to 20,801 currently annotated bovine genes. We observed that APA use frequencies, site-types and genomic neighborhoods were significantly different (P < 2.2e-16) among gene biotypes. There were 1.06, 1.35, 1.59, 2.82 and 7.62 APA sites per gene in average for tRNAs (transfer RNAs), miRNAs (microRNAs), pseudogenes, lncRNAs (long non-coding RNAs) and protein coding genes, respectively. Distal sites were used 50 – 70% of the time by tRNAs and miRNAs, while lncRNAs and protein coding genes used intronic APA sites 60% of the time. In comparison, pseudogenes used exonic APA sites most frequently. APA sites were located in A-rich stretch neighborhoods in 15%, 17%, 28%, 36% and 40% of tRNAs, miRNAs, lncRNAs, protein coding genes and pseudogenes, respectively. Pathways were enriched for cell cycle and division when satellite cells were cultured in growth medium for 48 h, but were enriched for muscle development and adaption pathways when cells were cultured in differentiation medium. Pathways with upregulated APAs in tissues collected from Angus cattle were related to muscle growth, development and function, but were associated with negative regulation of cell differentiation and xenobiotic metabolic process in Wagyu tissues. Endometrial epithelial cells from healthy Holstein cows had upregulated APAs enriched in pathways linked to cilium function, compared with cows with endometritis that had enriched pathways involved in immune and inflammation. Overall, profiling APA events reveals molecular mechanisms involved in growth, development and health/disease status. This work was supported by the NIFA USDA under Award Numbers 2016–67015–24470/2018–67051–27500.

Key Words: alternative polyadenylation, whole transcriptome termini site sequencing, gene biotypes, site-types, genomics neighborhood

P184 Gene expression of myogenic factors and its association with sex and growth period in cattle. J. Kyselova*[1], D. Rehak[1], D. Bures[1], L. Barton[1], and J. Simunek[2], [1]*Department of Genetics and Animal Breeding, Institute of Animal Science, Prague, Czech Republic,* [2]*Institute of Animal Physiology and Genetics CAS, Prague, Czech Republic.*

The aim of the study was to measure the mRNA expression of 4 myogenic factors (*MYF5*, *MYF6*, *MYOD1*, and *MYOG*) involved in transcriptional regulation in *Musculus longissimus lumborum* (MLL) of cattle throughout the life cycle and to estimate relationship among expression levels and sex or growth period. Biopsy samples (age of 11 and 15 mo) and slaughter samples (19 mo) of MLL were collected from 12 bulls and 12 heifers of Czech Fleckvieh. Relative levels of target and reference gene mRNA were determined using 2-step real time quantitative PCR with specific TaqMan Gene Bovine Expression Assays. The expression was calculated for each sample as a ratio of the target gene mean Cq (threshold cycle) to the 3 reference genes mean Cq using the Pfaffl formula based on gene assay efficiency and sample specific normalization factors. Gene expression was analyzed using mixed linear model (MIXED and GLIMMIXED procedures of SAS). The model was structured to determine the effects of sex, sampling date, and their interaction. Parameters were estimated by the REML method. Least squares means (LSM) were calculated, and multiple comparisons were made with P-values adjusted using Tukey's procedure. Expression data indicated a gender- and age-specific pattern. Earlier maturing heifers exhibited higher mRNA levels than later maturing bulls ($P < 0.05$), whereas these differences were less distinct at mature age. Expression of the factors was determined as following: 11mo > 15mo > 19mo of age at heifers. Generally lower but more balanced expression throughout life time was observed at bulls; with the highest values in the early age and with the lowest values observed in the middle age of 15 mo ($P < 0.05$). Surprisingly *MYF6* showed a different time expression scheme than other myogenic factors (MF). Sex and growth period interaction played a substantial role first of all in *MYOD1* expression ($P < 0.01$). Observed high positive correlation of expressions (Pearson) indicated cell coordination of the MF activity during formation of the polymerase II transcriptional complex. Acknowledgments: Ministry of Agriculture, Czech Republic MZE-RO0718

Key Words: biopsy, functional assay, qPCR

Genetics and Genomics of Aquaculture Species

P185 A transcriptomics overview of the biological changes in the liver after induced maturation of female European eel (*Anguilla anguilla*). F. Bertolini*[1], M. G. Pinto Jørgensen[1], C. Henkel[2], and J. Tomkiewicz[1], [1]*National Institute of Aquatic Resources, Technical University of Denmark, Lyngby, Denmark,* [2]*Department of Basic Sciences and Aquatic Medicine, Norwegian university of Life Science, Oslo, Norway.*

For the aquaculture industry, reproduction of European eel in captivity is of particular interest, given the critical status of the stock. While the process of European eel sexual maturation is unknown in nature, it can be induced by hormonal treatment in captivity. Understanding the major biological processes that occur during maturation is therefore of high relevance and high-throughput sequencing technologies can provide important tools to investigate those changes at a wide and deep scale. In this work, we explored changes in the liver during induced maturation of female broodstock. The liver of 16 female eels was collected before first treatment (n = 8) and after 9 weekly injections of carp pituitary extract (n = 8). Paired-end mRNA sequencing was performed for all the 16 samples. Trimmed sequenced reads were mapped against an improved version of the eel genome assembly, using hisat2, and mapped reads were filtered retaining only reads with mapping quality ≥30. Potential transcripts were then assembled with Stringtie. Read count was performed based on the coordinates of the potential transcripts with HTSeq and differential expression between the 2 groups was performed with DESeq2. Transcripts that in the comparison had Adjusted P-value <0.05 were considered as significant and compared with an *in silico* gene prediction of the eel genome, retaining the coordinates that overlap. The analyses showed that an average of 65% (±2.49) reads mapped with q ≥30 against the reference genome. A total of 9,313 genes were differentially expressed between the 2 states, with 4,682 genes upregulated and 4,631 genes downregulated. Vitellogenin transcripts were among the 10 most upregulated genes, while the hemopexin-like gene was one of the most downregulated. The expression level of this gene is also changing in the liver during maturation of Atlantic salmon. The gene enrichment analyses with the GOrilla online tool revealed pathways related to several metabolic functions (e.g., lipidic) and transport. These results are aligned with the massive phenotypical changes that occur in the liver during induced maturation, where European eel do not eat, but reallocates metabolic resources from particularly the muscle tissue to the growing gonads as well as meeting energy demands.

Key Words: RNA-seq, European eel, induced maturation, liver

P186 Expression analysis in Atlantic salmon (*Salmo salar*) liver tissue reveals miRNAs associated with parr-smolt transformation and seawater transfer. A. Shwe*[1], T.-K. Knutsdatter Østbye[2], and R. Andreassen[1], [1]*Department of Life Sciences and Health, Faculty of Health Sciences, Oslo Metropolitan University, Oslo, Norway,* [2]*Nofima AS, Ås, Norway.*

MicroRNAs (miRNAs) are non-coding small RNA molecules of ~22 nucleotides that regulate gene expression by repressing the translation of mRNAs. The aim of this pilot study was to analyze miRNA expression in Atlantic salmon liver to identify miRNAs that are associated with parr-smolt transformation (PST) and seawater transfer (SWT). Small RNA high throughput sequencing (HTS) and RT-qPCR were applied to analyze miRNA expression. The liver samples were collected before PST, after PST and 2 weeks post SWT. Small RNA sequencing of liver samples from each time points (n = 3) were carried out by use of Illumina Genome Analyzer IIx sequencing platform and DESeq2 was used to determine differentially expressed miRNAs. Then, 21 miRNAs with log fold change > 0.6 and Benjamini-Hochberg p-adjusted value < 0.1 revealed by the HTS analyzed material were further analyzed by RT-qPCR in a larger liver sample material (n = 9 per time points). The ΔΔCt method was applied to measure the relative difference in expression of miRNAs at time points compared. Eleven miRNAs showed satisfactory qPCR performance in terms of amplification specificity and effectivity, and could be further studied. Eight of these 11 miRNAs showed very good correlation between HTS and RT-qPCR results, while 3 miRNAs did not correlate well. The reason may be method related. Although 8 miRNAs correlated well between methods, only 5 of these showed significant differences ($P < 0.05$) when analyzed by RT-qPCR. The miRNAs confirmed as differentially expressed were let-7b-5p, miR-216a-5p, miR-454–3p, miR-221–3p and miR-3413–3p. They also revealed different patterns of change over the 3 time points. In other vertebrates, the Let-7 miRNAs have been reported as hypoxia responsive, miR-216 as stress responsive while miR-221 is involved in inflammatory response. However, further studies to identify their target

genes in Atlantic salmon are needed to understand their role in parr-smolt transformation and adaption to seawater.

Key Words: fish, microRNA, adaptation, qPCR, aquaculture

P187 Assessing isomiR-like HTS sequence artifacts and characterization of true isomiRs in Atlantic salmon. N. T. Woldemariam*[1], O. Agafonov[2], B. Høyheim[3], R. D. Houston[4], J. B. Taggart[5], and R. Andreassen[1], *[1]Department of Life Sciences and Health, Faculty of Health Sciences, OsloMet–Oslo Metropolitan University, Oslo, Norway, [2]Bioinformatics Core Facility, Department of Core Facilities, Institute of Cancer Research, Radium Hospital, Oslo University Hospital, Oslo, Norway, [3]Department of Basic Sciences and Aquatic Medicine, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway, [4]Division of Genetics and Genomics, The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom, [5]Institute of Aquaculture, University of Stirling, Stirling, Scotland, United Kingdom.*

IsomiRs are mature miRNAs different from their corresponding canonical mature miRNAs due to 5′ or 3′ post-transcriptional RNA editing. Such isomiRs could add variation to the miRNAome that are of biological importance. High-throughput sequencing (HTS) of small RNAs generate millions of reads. Some differing in length and sequence due to errors arising in the sequencing pipeline (RNA degradation, sequence errors introduced in cDNA synthesis or during sequencing). The erroneous sequence variants (ESVs) could be mistaken for isomiRs. It is therefore important to assess and remove isomiR-like ESVs when characterizing isomiRs. The aim of this study was to characterize Atlantic salmon isomiRs. Small RNA sequence reads from 48 fry libraries (Illumina) was trimmed to high quality reads applying a Phred score cut-off of 32. Errors arising before the sequencing was assessed by aligning the high-quality reads to 2 highly abundant and ubiquitously expressed Atlantic salmon RNAs. As the reads derived from anywhere out of these larger sized reference RNAs are not expected to be RNA edited, these RNAs could be used to measure proportion of ESVs. Aligned reads with single discrepancies compared with the references could be classified as ESVs or polymorphisms. This comparison showed that sequence errors found in ESVs showed a site-specific bias (toward the 3′ ends) and such ESVs could be misinterpreted as isomiRs. Using IsomiR-SEA and the ESV filtering 43 non-templated isomiRs and 5 allelic miRNA variants were discovered. RNA-hybrid was applied to measure the increase in putative target transcripts when isomiR variants were added to the canonical miRNAs. The biological significance (increase in target diversity) was very small. The allelic variation had large impact on target gene diversity if the variation was present in the seed sequence. Our results illustrate the importance of incorporating measurements of error beyond Phred score estimates to control errors likely to arise in the cDNA synthesis. Furthermore, the number of isomiRs discovered, and their impact on target gene diversity was small, indicating that isomiRs may have less biological impact than anticipated.

Key Words: fish, non-coding RNA, high-throughput sequencing (HTS), microRNA

P188 Transcriptome display sex difference of Chinese soft-shell turtle (*Trionyx sinensis*) in sex differentiation and mature period as revealed by RNA-Seq analysis. X. Wang*[1,2], X. Zhou[1,3], D. Zeng[1,2], P. Wang[1,2], Q. Qin[1,2], and Z.-N. Chen[1,2], *[1]College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan, China, [2]Collaborative Innovation Center for Efficient and Health Production of Fisheries in Hunan Province, Changde, Hunan, China, [3]Station of aquaculture in Xiangxi, Jishou, Hunan, China.*

The Chinese soft-shelled turtle (*Trionyx sinensis*) is one of the major commercially aquaculture species with high medicinal and nutritional values in China. The male Chinese soft-shelled turtle has good economic benefits because it grows faster 25 to 30 percent than female. To find out the information on sex differentiation of turtle, transcriptome of gonadal tissue were separately sequenced in sex differentiation and mature stages. The results showed that, in sex differentiation stage, there were 983 genes differentially expressed, and 769 genes were upregulated and 214 genes were downregulated in female compared with male, while in mature stage a total of 10,145 genes were found to be differentially expressed, including 4043 upregulated and 6,111 downregulated genes in female compared with male. A total of 1,981 and 16,736 of transcripts were found from 2 stages, which included 1,137 and 6,335 upregulated transcripts and 844 and 10,401downregulated transcripts. From these results, we found that the female turtle depended on gene and transcript more than the male in sex difference stage while in the mature stage was reversed. Combine with the results of tissue slice, we found that the female's sex differentiation was early than that of the male's.

Key Words: *Trionyx sinensis*, sex determination, gonad, RNA-seq

P191 Prediction of miRNA target genes in full-length Atlantic salmon (*Salmo salar*) mRNA transcripts from PacBio Iso-seq sequencing. S. Ramberg*[1], B. Høyheim[2], and R. Andreassen[1], *[1]Oslo Metropolitan University, Oslo, Norway, [2]Norwegian University of Life Sciences, Oslo, Norway.*

MicroRNAs (miRNAs) are an abundant class of endogenous small RNA molecules. They regulate expression post transcriptionally as part of the miRISC complex by partial complementary binding to the 3′ UTRs of target transcripts. Utilizing the characteristics of known miRNA-mRNA target sites several bioinformatics tools have been developed to predict which transcripts may be targeted by a given miRNA. The target genes control multiple biological processes like developmental timing, growth, stem cell division and apoptosis. Studies in humans and model organisms show that over 50% of all mRNAs may be regulated by miRNAs. miRNAs are well characterized in Atlantic salmon, and around 600 different mature miRNAs have been identified. However, the full-length sequence transcript resources for Atlantic salmon are limited. The aim of this study was to provide a comprehensive full-length transcript resource from head kidney and use this resource to predict putative miRNA target genes in Atlantic salmon. mRNAs from head kidney tissue sampled from healthy and Salmonid alphavirus challenged Atlantic salmon were sequenced using PacBio single molecule real-time (SMRT) circular consensus sequencing (CCS). This generated high-quality full-length transcripts with coding sequences (CDSs) and their untranslated regions (UTRs). From the healthy tissue, over 19000 unique isoforms from over 12000 different genes were characterized. In the challenged tissue, over 25000 unique isoforms from over 14000 genes were characterized. In both tissues, over 97% of the transcripts matched the Atlantic salmon genome to sequences predicted as genes. From these, CDSs and UTRs were extracted. All genes were annotated in the Gene Ontology (GO) framework. Their 3′UTRs were utilized to predict which genes could be regulated by any of the Atlantic salmon miRNAs and, subsequently, used to establish a miRNA target gene database. This database will provide a useful resource for functional studies of miRNAs.

Key Words: bioinformatics, microRNA, transcriptome, functional genomics, gene ontology

P192 Developing genomic information for *Holothuria polii* (Echinodermata: Holothuroidea), a novel potential aquaculture species. VJ Utzeri, A. Ribani, S. Bovo, V. Taurisano, and L. Fontanesi*, *Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy.*

Sea cucumbers (Holothuroidea) are ecologically important organisms for their bioturbation and alkalinization activities of the seabed. They are also important as luxury food and for the production of bioactive compounds and for these reasons they are extensively fished. A few initiatives are also evaluating sea cucumbers as novel species for cultivation in aquaculture systems. In this study we generated sequence information for *Holothuria polii*, a common sea cucumber species of the Mediterranean Sea, for which no detailed genomic studies have been

carried out so far. Whole genome sequence information was produced from an individual whose species identity was obtained by scanning electron microscope analysis of sclerites, which are anatomical features that can discriminate closely related species of the genus *Holothuria*. Illumina paired end sequencing produced a total of ~210 millions of reads for a about 21 Gbp. Generated data was analyzed against the genome sequence of other echinoderms to characterize several nuclear genes. In addition, the complete mitochondrial genome was assembled and used to evaluate the phylogenetic relationship of *H. polii* against the other few Holothuroidea species for which the whole mitochondrial DNA (mtDNA) was available. The 15,907 bp *H. polii* mtDNA sequence has the same gene order already reported for *H. scabra*, *H. forskali* and other species of the same family. *Cox1* gene sequences were informative for species identification across the genus and could be used for the authentication of commercialized *Holothuria* spp. These first genomic data we produced for *H. polii* may constitute starting information to obtain an assembled genome and analyze variability in the Mediterranean populations of this species.

Key Words: other species, genome sequencing, data mining, biodiversity, aquaculture

P193 Genome and transcriptome assembly of the Florida Pompano: Investigating genetic markers useful for aquaculture. L. E. King*[1], G. Ghosh[2], C. S. Perricone[1], E. Guisbert[2], P. S. Wills[1], R. Turingan[2], and N. J. Dickens[1], *[1]FAU Harbor Branch Oceanographic Institute, Fort Pierce, FL, USA, [2]Florida Institute of Technology, Melbourne, FL, USA.*

The objectives of this study are to sequence, assemble, and annotate the whole genome of the Florida Pompano using high-throughput sequencing technology. This will give us access to valuable genetic information that could ultimately be used to produce and maintain a healthy and diverse breeding stock for aquaculture. The Florida Pompano (*Trachinotus carolinus*) is a highly sought-after bony fish commonly found in warm waters of the US East Coast and Gulf of Mexico. Owing to its good taste and texture, Pompano commands high prices at market and is a prized catch for both commercial and sport fishermen. Studies have been conducted on the commercial viability of Pompano, and its aquaculture has advanced to the point where it is ready for commercialization. Here we describe an initial sequencing and assembly of the genome of Florida Pompano in combination with a draft transcriptome, which greatly enhances gene prediction and aids the identification of non-coding sequences. A pectoral fin clip from a captive-bred, juvenile fish was used for the genome assembly. Hybrid sequencing was applied with long reads on the Pacific Biosciences Sequel platform using their Single Molecule Real Time (SMRT) technology and Illumina high-throughput short read sequencing on the HiSeq platform. The length of the predicted genome was found to be 689 Mbp long. The contig N50 of the assembly was nearly 1.5 Mbp with the longest contig reaching 8.79 Mbp. We annotated 33,574 predicted protein-coding genes with our transcript-based assembly. Using OrthoMCL, 10,179 orthologs were discovered between our de novo and transcript-based assemblies and 4 different fish species. We can conclude from these results that we have built a representative draft of the Florida Pompano genome. Having access to the genome will provide a platform for future investigation of variant sites with the addition of other individuals. Our novel findings will facilitate selective breeding practices for the aquaculture of Florida Pompano by improving broodfish quality and production efficiency through the identification of genetic markers for growth, efficiency, and disease resistance.

Key Words: fish, aquaculture, genome assembly, genome annotation

P194 Analysis on the evolution of AMP repertoires between terrestrial and aquatic mammalian genomes. M. Kang*, B. Ahn, J. Yum, H. Cho, H. Jeon, N. Soundrarajan, and C. Park, *Konkuk University, Seoul, Korea.*

Antimicrobial peptides (AMP) are important innate-immune molecules to control pathogenic infection and good candidates to improve host resistance against pathogenic infection. We analyzed and/or compared the genome-level AMP repertoires of 6 terrestrial (human, cattle, pigs, goats, dogs, and African elephants) and 5 aquatic (minke whales, bottlenose dolphins, Yangtze river dolphins, walruses, and manatees) mammals. Our analysis resulted in the identification of 49 additional β defensin genes for the aquatic mammals comparing to the current gene annotation from public databases. In contrast, the results for non-β defensin AMPs were consistent to those of public databases. The average number of β defensin genes for terrestrial and aquatic mammalian species investigated in this study was 31.5 and 21.4, respectively, showing that the number was lower in aquatic than terrestrial animals. Phylogenetic analysis showed that a total of 296 BD genes from 11 species formed 27 clusters, suggesting the presence of large sequence variations in mammals. The synteny analysis of β defensin genes resulted in the identification of 8 conserved flanking genes which constitute 4 BD containing regions, PGK2-TFAP2D, BCL2L1-ZCCHC3, AGPAT5-GPM6A, and ADAM29-CTSB intervals. We observed the occurrence of chromosomal inversions, translocation and segmental deletions regarding to the intervals among different species, indicating that the expansion of β defensin genes occurred before separating into terrestrial and aquatic mammals. In contrast to the significant expansion of cathelicidins in the genomes of artiodactyls, the event did not occur in those of aquatic mammals although they are closely related to artiodactyls phylogenetically. In conclusion, our analysis suggests higher importance of AMPs for terrestrial than aquatic mammals. To understand the genetic diversity of the AMP subgenome could be important for improving the genetic potential of host resistance against pathogenic infection in livestock animals.

Key Words: aquatic mammals, evolutionary biology, phylogeny, antimicrobial peptides, animal health

P195 Mapping quantitative trait loci and identifying candidate genes affecting feed conversion ratio based onto three linkage maps in common carp (*Cyprinus carpio* L.). C. Lu*, X. Zhang, X. Zheng, D. Cao, X. Sun, *National Local Joint Engineering Laboratory for Freshwater Fish Breeding, Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Harbin, China.*

Feed efficiency is an economically important trait in aquaculture, which can be measured traditionally as feed conversion ratio (FCR). Because of the difficult measurement, genome-wide selection using quantitative trait loci (QTLs) affecting FCR may be an alternative for genetic improvement. In this study, QTLs for FCR based on three mapping panels (mirror carp and hybrid carp panels) were found in common carp (*Cyprinus carpio* L.). After that, candidate genes were identified by comparative genomics and verified by real-time fluorescent quantitative PCR. A total of thirty-eight QTLs were detected based onto three linkage maps. In the mirror carp panel (FAM-A, n=68), nine QTLs affecting FCR were detected in eight linkage groups (LGs) with two genome-wide QTLs (Lg1 and Lg21) explaining 32.3% and 35.6% of the phenotypic variation respectively. In the hybrid carp panel (FAM-B, n=92), nine QTLs affecting FCR were detected on eight LGs with four genome-wide QTLs (LG5, LG21, LG24, and LG33) explaining 29.3%~33.4% of the phenotypic variation. In another mirror carp panel (FAM-C, n=141), twenty QTLs were detected on four LGs with 7.1%~15.9% explained phenotypic variation based on 250 K SNP mapping array. A total twenty-seven candidate genes were identified using the whole-genomic browser on http://www. carpbase.org. Four genes (*gdf7*, *tgfbr2*, *Smad7*, *tgfb2*) are involved in the TGF-beta signaling pathway, while *tgfbr2* has been confirmed to be involved in the feed conversion process in chickens. The expression of *tgfbr2* gene in brain, muscle, liver, and intestine tissues was significantly different between large and small individuals fed in the same pool, while the expression of *gdf7* gene was significantly different in muscle tissues. Another four

genes (*mdh1ab*, *mecr*, *gapdh*, *pip5kl1*) are involved in glycolysis, fatty acid metabolism and other metabolic processes, which may be related to digestion and absorption of feed. The expression of genes in different tissues was significantly different between large and small individuals fed in the same pool. We believe that these genes are valuable candidate genes affecting feed efficiency, that might be used in MAS programs to improve performance in common carp.

Key Words: common carp, feed conversion ratio, quantitative trait loci, candidate genes, aquaculture

P196 Genomics of New Zealand trevally: Genomically enabling a new species for aquaculture. N. Valenza-Troubat*[1,2], P. Morrison-Whittle[1], D. Ashton[1], P. Ritchie[2], and M. Wellenreuther[1,3], *[1]The New Zealand Institute for Plant and Food Research, Nelson, New Zealand, [2]School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand, [3]Faculty of Science, University of Auckland, Auckland, New Zealand.*

Aquaculture is the fastest growing animal production sector in New Zealand but low species diversity is a barrier to long-term growth. New Zealand trevally (*Pseudocaranx georgianus*) is a promising candidate for aquaculture development, but growth rates need to be improved to ensure a successful breeding program. Modern genomics-informed breeding programmes can help accelerate these generational gains by focusing directly on the inherited components of traits and using parentage assignment to maximize family representation and control inbreeding.We present the first efforts to assemble a reference genome, reconstruct the pedigree of a captive-reared population to measure contribution levels of parents, measure inbreeding and genetic diversity, and identify key QTLs that influence growth-related traits using a combination of genotyping by sequencing (GBS) and whole-genome sequencing (WGS) data. This work represents the first genotype-phenotype map for *P. georgianus*, allowing us to gain fundamental insights into the genetic architecture of traits and the potential for selective breeding of this species.

Key Words: aquaculture, quantitative genetics, selective breeding

P197 Abstract withdrawn

P198 Detection of recent signatures of selection between three strains of Nile tilapia (*Oreochromis niloticus*) by whole-genome sequencing. M. I. Cádiz*[1], M. E. Lopez[2], D. Díaz-Domínguez[3], G. Cáceres[1], G. M. Yoshida[1], and J. M. Yáñez[1], *[1]Facultad de Ciencias Veterinarias y Pecuarias. Universidad de Chile, Santiago, Chile, [2]Department of Animal Breeding and Genetics. Swedish University of Agricultural Sciences, Uppsala, Sweden, [3]Departamento de Ciencias de la Computación. Universidad de Chile, Santiago, Chile.*

Nile tilapia (*Oreochromis niloticus*) is the second most cultivated group of fish in the world, mainly because of its favorable characteristics for production. Genetic improvement programs in this species began in 1988 with the aim of enhancing some traits of interest such as growth rate, disease resistance, cold and salinity tolerance. The implementation of these programs, together with the domestication process of Nile tilapia may have modified the genome through selective pressure, leaving signals that can be detected at the molecular level. In this work, genomic selection and domestication fingerprints were predicted using genome-wide SNP data and the Integrated Haplotype Score (|iHS|) method. Whole-genome sequencing of 326 individuals from 3 populations (A, B and C) of cultured tilapia of 2 countries (Brazil and Costa Rica) was carried out using Illumina HiSeq 2500 technology. After applying well established SNP-calling and quality-control pipelines, a total of ~2.8M high-quality SNPs were inferred and used as input for the |iHS| test. The analysis identified 33, 78 and 42 SNPs in populations A, B, and C respectively as potential selection signatures. Four SNPs were shared among all the populations, located in 3 different chromosomes (1, 3 and 22). We further inspected these shared regions, and we identified 113 genes that could be candidates for selection. These candidate genes represent genomic landmarks that could contain functions of biological and commercial interest.

Key Words: selection signatures, Nile tilapia, domestication, SNPs

P199 Genome assembly of a male Atlantic salmon provides insights into genome architecture related to sexual differentiation. S. McWillliam*[1], M. Menzies[1], B. Evans[2], K. Verbyla[3], P. Kube[4], M. Naval Sanchez[1], and J. Kijas[1], *[1]CSIRO Agriculture and Food, St Lucia, QLD, Australia*, *[2]Tassal Group Ltd., Hobart, TAS, Australia*, *[3]Data 61, Canberra, ACT, Australia*, *[4]CSIRO Agriculture and Food, Hobart, TAS, Australia*.

Sex determination in Atlantic salmon is likely to be controlled by the presence or absence of a master gene SdY. The gene exhibits Mendelian inheritance and is present on either chromosome 2, 3 or 6. Although presence of SdY it is strongly correlated with maleness, the association is not perfect. In attempting to characterize the male specific chromosomal region across 20 individuals with whole-genome sequencing and over 4000 individuals with genome wide SNP genotypes, we found evidence to show that the deletion containing SdY in female fish is of variable length. The current salmon reference genome (ICSASG_v2), being from a female fish, is of limited value in determining the structure of this region. Moreover the reference genome seems to contain a large deletion adjacent to the SdY insertion site. To overcome these challenges we used long-read single molecule real time (SMRT) sequencing with Pacific Bioscience technology to produce a genome assembly of a single male Atlantic salmon of North American origin. The resulting assembly contains 29000 contigs with an N50 of 334kb, and expands the genomic resources available for investigation in animals derived from North American stocks. We have used this assembly to evaluate the genome architecture present in both sexes, to more fully characterize the complex structural differences associated with sex differentiation.

Key Words: genome assembly, sex, differentiation

P201 RNA-seq analysis of two critical periods of induced ovarian development in European eel, *Anguilla anguilla*. M. G. Pinto Jørgensen*, F. Bertolini, and J. Tomkiewicz, *National Institute of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby, Denmark.*

The success of aquaculture lies in the domestication of aquatic species, allowing a closed cycle production and breeding programs.

Yet, culture of the endangered European eel remains capture-based, using wild-caught juveniles as basis for farming, which urges the need for hatchery technology. While, eels do not reproduce in captivity due to a dopaminergic inhibition at the brain-pituitary level, which prevents sexual maturation, hormonal treatments that stimulate ovarian development can overcome these maturational barriers. In this work, we investigated the molecular mechanism of genes involved in ovarian maturation through transcriptomic, morphometric, and histological profiles of ovarian tissue comparing females before sexual maturation and before spawning. To induce maturation, Carp Pituitary Extract (CPE) was administrated weekly to farmed broodstock females. Sampling included 6 female eels at wk 0 and 8 at wk 9. Analyses showed that fish at wk 0 were in the pre-vitellogenic phase (immature) with an ovary-body weight ratio (GSI) of 1.4 ± 0.3, while all fish at wk 9 were in the late vitellogenic phase (approaching spawning) with a GSI of 17.9 ± 6.7. For each female, total RNA was extracted from ovarian tissue and mRNA sequencing was performed. Sequenced reads were trimmed with Trimmomatic and aligned against the eel genome with Hisat2 using standard parameters. High quality mapped reads (q ≥30) were used to reconstruct potential genes using Stringtie and to perform differential expression analysis with Deseq2. The analyses detected 9,978 significantly differentially expressed genes (adjusted p-value <0.05), where 5,780 were upregulated and 4,198 were downregulated. The most differentially expressed genes were related to 2 major functions. First, genes such as Acyl-coa synthetases (*ACS*) and ATP synthetases (ATP) that are involved in biosynthesis of nucleosides, nucleotides and purines were downregulated. It has previously been discovered that these compounds inhibit meiotic resumption in fish. Second, aquaporin genes (AQP), which are connected to the hydration of fish eggs, were upregulated. Further analyses will clarify the role of each differentially expressed gene and their biological pathways.

Key Words: RNA-seq, fish, aquaculture, maturation, ovary

P202 Detection of genomic regions involved in sex determination in Nile tilapia (*Oreochromis niloticus* L.) using whole-genome sequencing. C. Giovanna*[1], L. M. Eugenia[2], C. M. Ignacia[1], Y. Grazyella[1], and Y. J. Manuel[1], *[1]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Universidad de Chile, [2]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Nile tilapia (*Oreochromis niloticus*) is one of the most cultivated and economically important species in world aquaculture. Commercial farming uses all-male populations, not only because males have a significantly higher growth rate and reach market sizes before females but also to avoid reproduction during growth which is a major problem that generates heterogeneous sizes of fish at the time of capture. For these reasons, the identification of genomic regions associated with sex determination is a research topic of great interest. Sex determination in tilapia is a complex trait, which has an important genetic basis. However, other genetic factors and environmental variables such as temperature may intervene in sex determination.The objective of this study was to identify genomic variants associated with sex determination in 3 commercial populations of Nile tilapia. The complete genome sequencing of 326 individuals was performed, and a total of 2.4 million high-quality bi-allelic single nucleotide polymorphisms (SNPs) were identified. A genome-wide association study (GWAS) was conducted to identify markers associated with the binary sexual trait (males = 0; females = 1). A mixed logistic regression model was fitted using the GenABEL package for R statistical environment. We detected a genome-wide significant signal comprising 36 SNPs, located on chromosome 23 spanning a genomic region of 536 kb. Ten significant SNPs hit a predicted gene which has been previously involved in the differentiation of male and female reproductive tissue at an early developmental stage. This gene has been strongly associated with sex determination in several vertebrate species, playing an essential role in the differentiation of male and female reproductive tissue in early stages of development. Also, 2 other genes involved in the regulation of sex hormones were recorded in this region.This study provides further evidence to better understand the genetic architecture and the mechanisms underlying sex determination in Nile tilapia.

Key Words: SNP, sex determination, quantitative trait loci, WGS, GWAS

P203 Optimising genotype imputation strategies for genomic selection in farmed Atlantic salmon. S. Tsairidou*[1], A. Hamilton[2], D. Robledo[1], J. Bron[3], and R. Houston[1], *[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK, [2]Hendrix Genetics Aquaculture BV/ Netherlands Villa 'de Körver', Boxmeer, The Netherlands, [3]Institute of Aquaculture, University of Stirling, Stirling, UK.*

Genomic selection is increasingly applied in aquaculture breeding to expedite genetic gain for key production and disease resistance traits. However, effective application in breeding programmes depends on large training data sets of phenotypes and genotypes. In livestock breeding, genomic information on large populations can be achieved in a cost-effective manner through genotype imputation. In typical aquaculture breeding programmes where large full-sibling families are available, the value of imputation is yet to be fully assessed. The aim of this study was to evaluate strategies for genotype imputation in Atlantic salmon breeding programmes, by assessing the trade-off between the cost of genotyping at a given density and the impact on genomic prediction accuracy. Optimal high density (HD) SNP panels were identified and imputation accuracies for a range of low density (LD) SNP panels were systematically assessed. Then, genomic prediction accuracies were compared. Analyses were performed via a software pipeline using: (a) PLINK and in-house built software in R and Shell for selection of HD and LD SNP panels and calculation of genomic relationship matrices; (b) FImpute for genotype imputation; and, (c) ASReml for estimating breeding values and cross-validated prediction accuracies. The study focused on (i) a Scottish Atlantic salmon breeding program population challenged with *L. salmonis*, and (ii) a Chilean population challenged with *C. rogercresseyi* (both Landcatch). Genomic prediction was assessed for sea lice resistance and growth traits, known to have polygenic genetic architecture. Relatively low density panels are sufficient to give near-maximal prediction accuracy in the absence of imputation, due to close relationships between training and validation populations. Although imputation to HD is likely to benefit the discovery of quantitative trait loci and fine-mapping of regions identified by genome-wide association analyses, genomic prediction appeared to reach near-maximum accuracy with less than 1,000 SNPs. However, the value of genotype imputation in genomic selection varies depending on the population and the trait.

Key Words: fish, animal breeding, bioinformatics, aquaculture

P204 Determination of genetic structure and selection signatures in Coho salmon (*Oncorhynchus kisutch*) populations by genome-wide SNP analyses. M. E. López*[1,2], A. Barría[2], E. Rondeau[3], B. Koop[3], and J. M. Yáñez[2], *[1]Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Universidad de Chile, Santiago, RM, Chile, [3]University of Victoria, Victoria, British Columbia, Canada.*

Coho salmon (*Oncorhynchus kisutch*) is one of the 3 most important species of farmed salmon in the world, and Chile is the main producer of this species. Phenotypes of farmed population have evolved due to the combined influence of domestication and selection through reproduction in captivity and human directional selective breeding. These events might have shaped the genetic diversity of these species throughout history, and their present genomes may contain traceable signatures of selection. Detecting genomic selection signatures is a main goal of modern population genetics as it enhances our knowledge of the molecular mechanisms shaping the genome as well as providing functional information on specific genomic regions that might have biological or productive interest. In this study we evaluate genetic diversity and structure and identify signatures of selection among the most important domestic strains of coho salmon in Chile using a medium densi-

ty SNP array (~130K). Population structure and signatures of selection were examined using principal component analysis (PCA), admixture analysis, pairwise distances (FST), integrated haplotype score (iHS) and runs of homozygosity (ROH). Based on the results of our study, we discuss the ability to detect genomic regions that may underlie important traits of practical interest for aquaculture and to better understand the effect of domestication in Coho salmon.

Key Words: fish, genotyping, animal domestication

P452 Breeding technology for aquaculture species in the post-genome era. X. Sun*, *National Local Joint Engineering Laboratory for Freshwater Fish Breeding, Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Harbin, China.*

It is very difficult that using changeable main QTL in the common carp breeding. Because a high explained variation locus in one family maybe lower down to very small explained variation locus sometimes. Even in same family, the main QTL has very big changeable between the different generation frequently. For common carp, the main QTLs of some quantitative traits such as body quality, body length etc. were changeable between families, but also some regular change of these main QTL were founded. Which are the balance between advantage of heterozygous loci and advantage of homologous loci for each quantitative trait. For example, in all the identified main QTL of body length, the heterozygous loci are 60~75%, and the homologous loci are 25~40% in many families. It is very interested that for other quantitative traits such as body quality, body wide etc. all have suited to this phenomenon. Based this phenomenon or balance hypothesis, and also based whole genome information, a breeding method and its operating software were made, and some common carp breeding works were tried using this method.

Key Words: main QTL, breeding, common carp, post-genomic

P453 First look at patterns of DNA methylation in Atlantic salmon and its involvement in sexual maturation. J. Kijas*[1], A. Reverter[1], B. Evans[2], H. King[3], and A. Mohamed[1], *[1]CSIRO Agriculture, St Lucia, Queensland, Australia, [2]Tassal Group Ltd., Hobart, Tasmania, Australia, [3]CSIRO Agriculture, Hobart, Tasmania, Australia.*

Atlantic salmon farming promotes rapid growth in conditions which mean animals may complete sexual development at weights below harvest size. This can lead to productivity losses and has prompted us to investigate the biological mechanisms that control the timing of sexual maturation. We performed a time course experiment, whereby animals were manipulated with photoperiod before tissues was collected through the time window when animals first enter the sexual development process. In addition to deep transcriptomic profiling, we performed whole genome bisulfite sequencing of 3 salmon tissues at both the beginning and end of the time course experiment. The results provide a global pattern of DNA methylation for each tissue, and we report both the sequence context and genomic distribution of hyper- and hypo-methylated regions at nucleotide resolution. Of the 3 tissues assayed, the methylome of the ovary underwent the most dramatic remodeling during the experiment. We identified differentially methylated regions for each tissue, before intersecting them with protein coding genes. We conclude epigenetic factors clearly play an important role the process of sexual maturation, and present an initial view of global patterns of methylation in this important production species.

Key Words: methylome, WGBS, salmon, sex, maturation

Genetics of Immune Response and Disease Resistance

P205 Genetic signature of strong recent positive selection at the *DRB-1* gene in goat. A. R. Asif*[1,2], A. Muhammad[1], S. Qadri[3], J. A. Bhatti[1], X. Du Du[2], and A. H. Saleem[1], *[1]University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan, [2]Huazhong Agricultural University,Wuhan, Wuhan, Hubei, China, [3]Livestock and Dairy Development Punjab, Jhang, Punjab, Pakistan.*

The identification of candidate genes that play a key role in phenotypic variation in livestock populations can provide new information about evolution and positive selection. Dopamine receptor binding 1 (DRB-1) gene is associated with the increased nematode resistance in small ruminants; however, the role of DRB-1 for the genetic control of different diseases in Chinese goat breeds is poorly described in scientific literature. Therefore, the current investigation was performed to better understand the molecular evolution and the positive selection of single nucleotide polymorphisms in DRB-1 gene. We used Fixation Index (FST) based method for the outlier loci determination and found that DRB-1 gene was present in outlier area with the provisional combined allocation of mean heterozygosity and Fst. Positively selected DRB-1 gene was significantly ($P < 0.05$) present in corresponding positive selection area. We also found that DRB-1 gene was related to positive selection sites by LRT using paired models (M1-M2, M7-M8). The nucleotide sequences of 8 mammalian species were used for the determination of the evolutionary selection of DRB-1 gene and found that formerly this gene was under no selection; however presently it is detected under positive selection. In the current study, we constructed a phylogenetic tree and found that goat DRB-1 has a close resemblance with sheep DRB1 and therefore they were placed in the same group. The sequences were compared in different species and found that the DRB-1 gene of goat shared 98% identity with sheep, 47% with rat and mouse, 46% with horse, 44% with camel and human and 41% with dog. Hence, our study provided novel information about the non-synonymous nucleotide variations in DRB-1 gene which may be helpful for the genetic control of diseases by enhancing the immune system in local Chinese goat breeds as well as in other analyzed vertebrate species.

Key Words: positive selection, DRB-1, goat, evolution

P206 Dynamic transcriptomic changes of goat abomasal mucosa during an experimental *Haemonchus contortus* infection in resistant and susceptible genotypes. H. M. Aboshady*[1,2], N. Mandonnet[3], A. M. Johansson[2], E. Jonas[2], and J. C. Bambou[3], *[1]AgroParisTech, Paris, France, [2]Swedish University of Agriculture Science, Uppsala, Sweden, [3]INRA-URZ, Petit-Bourg, Guadeloupe, France.*

One of the major constrains for sheep and goat production worldwide are gastrointestinal nematode (GIN) infections. Genetic selection for resistant animals is a promising strategy as there are no issues due to anthelmintic resistance and it aligns to demands for chemical-free food. Whole-transcriptome analysis via RNA-sequencing (RNA-seq) provides a key role to gain knowledge of mechanisms responsible for complex quantitative traits such as resistance to GIN infections. In this study, we use RNA-seq to follow the dynamic in transcriptome profiling of abomasal mucosa tissues from infected Creole goats and compared between resistant and susceptible (R and S) genotypes. A total of 8 cannulated kids, 4 susceptible and 4 resistant to GIN, were infected twice with 10,000 L3 *Haemonchus contortus*. During the second infection, abomasal mucosal biopsies were collected at 0, 8, 15 and 35 d post infection (dpi) from all kids for RNA-seq analysis, to identify differentially expressed genes (DEG) and characterize the mechanisms involved in the expression of genetic resistance in the mucosa. The multilevel partial least squares discriminant analysis for gene expression explained more than 70% of the variance between the S and R group. The R group showed early activation of biological processes related to the immune response. The top 20 canonical pathways of DEG for different comparison showed activation of immune response through many rel-

evant pathways for example: IL-8 signaling, Leukocyte extravasation signaling, Th1 pathway, and B cell receptor signaling. Results showed also a difference in time series activation in Th2 genes, indicating that immune response is earlier activated in R kids compared with S kids.

Key Words: GIN resistance, RNA-seq, immune response, goats

P207 Screening of potential markers in the JAK-STAT pathway related genes for mastitis resistance in dairy cattle at sub tropical conditions of Pakistan. T. Usman*[1], N. Ali[1,2], S. Niaz[1], Y. Wang[2], and Y. Yu[2], *[1]Abdul Wali Khan University Mardan, Mardan, Khyber Pakhtunkhwa, Pakistan, [2]China Agricultural University, Haidian, Beijing, China.*

Mastitis is the most common and costly inflammatory disease of the mammary gland of lactating dairy animals. Resistance to mastitis is a polygenic character that involves various pathways inside the cell contributed by numerous candidate genes. Many cytokines, growth hormone, and prolactin hormone use JAK-STAT signaling pathway inside the cell. This pathway plays a key role in many inflammatory responses as well as in the development and lactation of the mammary gland. The present study was designed to find out single nucleotide polymorphisms (SNPs) in the JAK-STAT pathway related genes (*JAK2, STAT5A*, *STAT5B* and *CD4*) and its association with mastitis resistance and production traits in different dairy cattle breeds maintained at various government dairy cattle farms at Khyber Pakhtunkhwa, Pakistan. Milk and blood samples (along with the primary data were collected) from 214 dairy cattle for somatic cell count (SCC), milk composition and DNA extraction, respectively. The genotype and phenotype associations were statistically analyzed using generalized linear model using GLM model in the SAS studio. Total 41 SNPs were detected in a pool DNA sample, of which 13 SNPs were selected and further validated in the whole population (n = 214) by Snapshot technique. The genotype and allele frequency of all the identified SNPs were in Hardy-Weinberg Equilibrium in the population ($P > 0.05$).The association analysis showed that total 13 out of 14 SNPs were significantly associated with either mastitis resistance or production traits ($P < 0.05$). Moreover, the analysis of the combination genotypes revealed that these genes in combination were also significantly associated with the mastitis indicator and production traits ($P < 0.05$). The findings of the present research showed that the identified SNPs in the JAK-STAT pathway related genes have a significant association with mastitis resistance and production traits. Thus, these SNPS can be used as powerful genetic markers for improving mastitis resistance and production traits in dairy cattle maintained at the subtropical conditions.

Key Words: bovine mastitis, marker-assisted selection, JAK/STAT pathway, production traits

P208 Host synaptogyrin-2 facilitates replication of PCV2b. L. Walker*[1], T. Engle[1], H. Vu[1], E. Tosky[1], D. Nonneman[2], T. Smith[2], T. Borza[3], T. Burkey[1], G. Plastow[4], S. Kachman[1], and D. Ciobanu[1], *[1]University of Nebraska-Lincoln, Lincoln, NE, USA, [2]USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA, [3]Dalhousie University, Truro, Nova Scotia, Canada, [4]University of Alberta, Edmonton, Alberta, Canada.*

Porcine Circovirus 2 (PCV2) is the smallest known virus capable of infecting mammals and the primary agent responsible for a set of symptoms and syndromes known as Porcine Circovirus Associated Diseases. However, infection with PCV2 does not guarantee the onset of clinical disease. Several factors, such as co-infection, are known to influence disease progression, but observed variation in severity between breeds and individuals suggested host genetics may play an important role as well. Genome-wide association analyses of ~1,000 pigs experimentally infected with PCV2b revealed 2 major QTL for PCV2b viral load, located on host SSC7 and SSC12. A combination of *ab initio* gene prediction, RNA sequencing, and genomic sequencing identified 66 novel polymorphisms across 5 positional candidate genes within the SSC12 QTL. Single marker association analysis of a subset of pigs with extreme high and low viral loads, identified a novel polymorphism accounting for >20% of the phenotypic variation. This polymorphism is a missense mutation (*p.Arg63Cys*) located within the second exon of the *SYNGR2* gene, which encodes a critical protein domain. In vitro siRNA mediated gene silencing of *SYNGR2* in PK15 cells resulted in a one-log reduction in PCV2b titer ($P < 0.05$) compared with scramble siRNA and non-transfected control cells. Additionally, gene editing using CRISPR-Cas9 ribonucleoprotein complexes generated a PK15 edited clone homozygous for a 106 bp deletion within the second exon of *SYNGR2* that exhibited a 2-log reduction in PCV2b titer following infection compared with wild-type PK15 cells ($P < 0.05$). Together, these findings indicate a direct role of *SYNGR2* in facilitating PCV2b infection. *SYNGR2 p.Arg63Cys* is the only missense mutation within this gene, and therefore a plausible QTN for PCV2b susceptibility. USDA is an equal opportunity provider and employer.

Key Words: PCV2, susceptibility, SYNGR2, host genetics, resistance

P209 Modulation of innate immune responses in jejunal epithelial cells by *Yersinia enterocolitica*. P. Modesto*[1], W. Vencia[1], E. Parisia[1], F. Lazzara[1], M. Amadori[2], T. Andreoli[1], C. Ercolini[1], A. Ferrari[1], and E. Razzuoli[1], *[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy, [2]Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna, Brescia, Italy.*

Yersinia enterocolitica (Ye) is able to infect humans and animals. Many studies described the molecular basis of the infection sustained by pathogenetic biotype 1B. Few data are available about 1A biotype, although it is often isolated during foodborne disease and in hunted wild boars. The aim of our study was to verify the ability of different Ye biotypes to modulate innate immunity in an epithelial cell line originating from the jejunum of pig (IPEC-J2). We tested 5 different Ye strains: 1B/O:8 (S1); 1A/O:9 (S2); 1A/O:5 (S3); 1A/O:8 (S4); 1A/O:5 (S5). IPEC-J2 were treated with 100 UFC/cell in DMEM/F12 medium for 3 h at 37°C to test immune modulation and at 4°C to evaluate adherence. Each strain was tested 5 times and the experiments repeated thrice. Data sets were checked for significant differences by ANOVA (the threshold was set at $P < 0.05$) using PRISM software. Based on our results, strains showed a different ability to adhere and modulate gene expression. All biotype 1A strains showed poorability to adhere to enterocytes compared with pathogenic biotype S1. Concerning immune modulation, S1 determined in IPEC-J2 a pro-inflammatory effect characterized by upregulation of IL-8 and TNF-α gene expression and downregulation of CD14, MD2, TLR1, TLR4 and TLR5. S2 caused increase of IL-8, TNF-α, bD1, bD2 expression and downregulation of NF-Kb1, bD4, MD2, and TLR4. S3 determined upregulation of IL-1β, IL-8, TNF-α, bD1, bD3 and downregulation of NF-Kb1, MYD88, MD2, TLR1 and TLR4. S4 caused an increase of IL-1β, IL-8, IL-18 and downregulation of bD3, CD14, MD2, TLR1, TLR4 and TLR5. S5 caused upregulation of IL-8, TNF-α, and decrease of bD1 and bD3. All the reported variations in up and downregulated genes were statistically significant ($P < 0.05$). Our data suggest a potential pathogenic role of Ye S3 and S4: they showed the ability to downregulate TLR1 expression, which appears to be essential in the host response to Ye. Moreover, IPEC-J2 treated with S4 showed a profile of up e downregulated genes similar to that of pathogenetic biotype S1. S2 and S5 had an expression profile similar to low pathogenic agents with TLR1 downregulation and upregulation of bDs.

Key Words: pigs and related species, cell culture, gene expression, adaptive immunity, animal health

P210 Hepatic transcriptome responses of chicken embryos to ochratoxin A. S. Y. Choi*[1], M. W. Hong[1], H. Lee[1], T. S. Park[2], and S. J. Lee[1], *[1]College of Animal Life Sciences, Kangwon National University, Chuncheon, Republic of Korea, [2]Institute of Green-Bio Science*

and Technology, Seoul National University, Pyeongchang, Republic of Korea.

Ochratoxin A (OTA) is a naturally occurring mycotoxins often found in cereals and animal feed as a contaminant. OTA has been shown to be nephrotoxic, hepatotoxic and genotoxic to human and several spices of animals. The aim of this study was to evaluate the toxic effects of in ovo exposure to OTA and to explore the molecular mechanism by RNA sequencing in the embryonic liver of chicken (gallus gallus). Embryos were treated with graded concentration of OTA (2.5 5, 10, 20 ug/egg per day) during d 9–11 of incubation. At 11 d, chicken embryos were terminated, measured growth performance of egg and collected liver tissue for histochemical and transcriptomic analysis. At the administration of 10 ug OTA/egg per day, embryo mortality began increased to 41.46% in control accompanied with injury of liver tissue. While liver weight and relative liver weight in 10 ug/egg OTA treatments was significantly reduced ($P < 0.001$), no statistical difference in fetus weight was observed. Based on these results, mRNA sequencing was conducted to determine the transcriptome expression using the liver tissue in 10 ug/egg OTA group and vehicle control (n = 3, respectively). The mycotoxin caused significant changes in the expressions of 3,320 genes between control and OTA treated group (absolute fold change > 2, p-value < 0.05, q-value < 0.01) and 140 GO terms were significantly enriched ($P < 0.05$). GO enrichment and KEGG pathway analysis showed that OTA induce the small molecule metabolic process, drug metabolism, oxidation-reduction and metabolism of xenobiotics by cytochrome P450 in chicken embryo. These results demonstrate the mode of action and toxicity of OTA in ovo system combined with transcriptomic analysis.

Key Words: poultry and related species, ochratoxin A, toxicogenomics, RNA-seq, gene expression

P211 Abstract withdrawn

125b, which is predicted to limit PRRSV viral levels, and Ssc-miR-145–5p shown to cause alternative macrophage priming.

Key Words: pigs, RNA-seq, non-coding RNA, immune system, animal health

P212 Abstract withdrawn

P213 Hypothalamic transcriptomic perturbations in mice developmentally exposed to perfluorooctanoic acid (PFOA). H. Kim*, M. W. Hong, H. Lee, and S. J. Lee, *Kangwon National University, Chuncheon, Republic of Korea.*

Perfluorooctanoic acid (PFOA), known as a ubiquitous environmental pollutant, target hormonal control of appetite and satiety, interrupt normal tissue development, and disturb with the endocrine system's homeostatic controls. In rodent, exposure to PFOA results in metabolic disorder including energy balance and appetite control that are regulated by the hypothalamus. Thus, we sought to identify the transcriptomic profile in the brain region of juvenile ICR mice offspring exposed to PFOA (0 or 1 mg / kg / day), during the perinatal period and lactation. Two weeks before breeding, mother mice were administered orally, 0 (control) or 1 mg PFOA / kg / day though drinking water, and continued until the end of the experiment. At 4 weeks, the brain of male offspring were collected, hypothalamic RNA isolated, and RNA-seq analysis performed. We identified 114 differentially expressed genes (DEGs) between control and 1 mg / kg / day PFOA treated mice hypothalamus, of which 75 were upregulated and 39 downregulated. *Nms* and *Plin4* were downregulated, whereas mt-*Atp8, POMC, Slc2a4* and *Agrp* were upregulated in the hypothalamus of PFOA exposed males. Comparison of transcripts differentially expressed in control and PFOA

groups revealed significant enrichment of KEGG pathway associated with adipocytokine signaling pathway associated with metabolic homeostasis and metabolic disorders. The results of this study show that perinatal exposure to PFOA can result in several transcriptomic alterations, including those associated with metabolic disorder, in the hypothalamus of mice. It remains to be determined whether these genes mediate PFOA-induced metabolic disorder disruptions.

Key Words: rodents, RNA-seq, gene expression, PFOA, endocrine system

P214 Polymorphisms associated with bovine paratuberculosis: Investigation of their role in DNA-protein interactions and transcriptional regulation. C. Beltramo, A. Dondo, K. Varello, M. Goria, A. Di Blasio, S. Nodari, S. Colussi, P. Modesto, P. L. Acutis*, and S. Peletto, *Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta.*

Genetic variants associated with disease resistance/susceptibility and located in gene regulatory regions may affect the binding sites for DNA-binding protein and gene expression patterns, thus influencing the host response following pathogen exposition. Previous studies led to identify 3 SNPs in putative regulatory regions of the *SLC11A1* and *CARD15* genes with association to paratuberculosis (paraTBC) in cattle. Aim of the study was to investigate the role of these mutations at the regulatory level by DNA-protein interaction analyses and transcriptome comparison between wild-type and mutated animals. Gene regions carrying the SNPs of interest were analyzed by bioinformatic tools to predict allele-dependent binding sites for transcription factors (TFBS). Putative TFBS were in vitro explored by Electrophoretic Mobility Shift Assays (EMSA). Three putative binding sites for GATA3, Sp1, and MYOD were identified in intron 10 of *CARD15* and in the promoter and intron 11 of *SLC11A1*. EMSA did not show specific gel shifts for any allele, indicating that these SNPs may eventually influence gene transcription without altering TFBS. Whole transcriptome expression analysis was performed on intestinal tissues of wild-type and mutated cattle by RNA-Seq to identify differentially expressed genes. Total RNA was sequenced on HiSeq Illumina system and data were compared by PCA and Cluster analysis. Differential regulation of 5 genes involved in innate immune system was detected. Specifically, *ULBP3* was downregulated, while *S100A8*, *S100A12*, *LOC510860*, and *IFI27* were upregulated. These significative modulations were linked to the SNP in intron 11 of *SLC11A1*, with the exception of *ULBP3,* related to the mutation in the *SLC11A1* promoter. In previous studies, *ULBP3*, *S100A8,* and *S100A12* resulted differentially expressed in cattle affected by paraTBC, suggesting a possible implication in the pathogen response. Further investigations are necessary to elucidate the functional role of these SNPs and to understand the gene network involved in the interactions between non-coding SNPs and other genome regions.

Key Words: paratuberculosis, SNP, EMSA, RNA-Seq

P215 Immune-related microRNA absorption in newborn calves. H. T. Do*[1,2], J. L. Williams[1], T. Chen[1], K. Petrovski[1], and C. D. K. Bottema[1], *[1]School of Animal & Veterinary Sciences, Davies Research Centre, University of Adelaide, Roseworthy, Australia, [2]Vietnam National University of Agriculture, Hanoi, Vietnam.*

In addition to immunoglobulin G (IgG), bovine colostrum contains many immune-related factors that are absorbed by the neonate, including microRNAs (miRNAs) which may stimulate immune development. If essential colostrum immune-related miRNAs can be identified, the information could be useful in breeding programs to improve calf immunity. Herein, the levels of colostrum immune-related miRNAs were compared between calves that received colostrum from different sources to determine if these miRNAs are absorbed by the calves. Thirty-eight bull calves were randomly divided into groups and fed equal amounts of colostrum from 2 sources (dam colostrum or colostrum pooled from 1 to 7 d postpartum cows). Dam colostrum was collected after birth (d 0), d 1 and d 2 postpartum and calf blood was collected at d 0 (before feeding), d 1 and d 7 postpartum. IgG concentration was measured by refractometry and ELISA. Five immune-related miRNAs were quantified by RT-qPCR with the addition of Cel-miR-39 as the internal standard. The differences between groups were analyzed using one-way ANOVA. Only miR-150 was moderately correlated with the IgG concentration in the dam colostrum at d 0. The concentration of miR-142–5p, miR-150 and miR-181a in the dam colostrum was highest at d 0 and decreased dramatically by d 1. The concentration of miR-155 increased over time though, while the level of miR-223 did not change. In the pooled colostrum, miR-223 was the only miRNA found at high levels and was the only miRNA at high levels in the calf blood at d 0. The concentration of all the miRNAs increased by d 1 in the calf blood, but returned to the d 0 levels by d 7. Interestingly, there was no significant difference between the calves fed the dam colostrum or pooled colostrum for any of the miRNAs at d 1 or 7 despite the absence of miRNA in the pooled colostrum. This suggests that the calves do not just absorb the miRNA from the colostrum but synthesize some or all of the miRNA themselves. Therefore, these miRNAs may not be good biomarkers of colostrum quality for breeding programs. However, the importance of the miRNAs for calf immune development and health is being further investigated.

Key Words: miRNA, bovine, colostrum

P216 Combined transcriptomic analysis of ileocecal valve and peripheral blood in Holstein dairy cattle at different stages of *Mycobacterium avium* ssp. *paratuberculosis* (*Map*) infection revealed CXCL8/IL8 as a common effector molecule. M. Alonso-Hearn*[1], M. Canive[1], C. Blanco-Vázquez[2], R. Torremocha[3], B. Soriano[4], A. Balseiro[2], J. Amado[5], R. Ramos[3], C. Llorens[4], and R. Casais[2], *[1]NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Derio, Bizkaia, Spain, [2]SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Deva, Asturias, Spain, [3]Science Park of Madrid, Genomic Unit, Madrid, Spain, [4]Biotechvana, Paterna, Valencia, Spain, [5]LSAPA, Animal Health Laboratory of the Principality of Asturias, Gijón, Asturias, Spain.*

Paratuberculosis (PTB) caused by infection with *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is a major endemic disease affecting global cattle production. Since the blood transcriptome is widely used as a source of biomarkers, we analyzed whether it recapitulates at least in part the transcriptome of the ileocecal valve (ICV), the primary site of MAP colonization. Total RNA was prepared from peripheral blood (PB) and ICV, and RNA-Seq was used to compare gene expression between animals with focal or diffuse histopathological lesions versus control animals. As expected, the number of differentially expressed (DE) genes was larger in ICV than in PB samples and in animals with diffuse versus focal lesions. Our results demonstrated both shared, and PB or ICV-specific regulation of gene expression in response to MAP infection. Among the identified DE transcripts in PB and ICV, there were 5 common transcripts irrespective of the type of lesion including the C-X-C motif chemokine ligand 8 (CXCL8), apolipoprotein L domain containing 1 (APOLD1), interferon α-inducible protein 27 (IFI27), KIAA1324-like and ArfGAP with RhoGAP domain ankyrin repeat (ARAP2). Two putative biomarkers were DE exclusively in PB and ICV of animals with focal lesions, the major histocompatibility complex class II (BOLA-DOβ) and ENSBTAG00000038080. Thirty 2 genes were DE only in animals with diffuse lesions; 17 appeared upregulated and 11 downregulated in both blood and ICV of cows with diffuse lesions. Five biological processes (BP) were enriched in ICV of cows with focal lesions; killing of other organisms (GO0031640), defense response (GO0006952, GO0050832), immune response (GO006955) and regulation of neutrophil chemotaxis (GO0090023). Two BP, GO0006952 and GO0006955, were also enriched in PB and ICV of cows with diffuse lesions. Some of the identified DE genes GO and metabolic pathways will be studied further to aid in better diagnostic tools, vaccines and/or immunotherapeutics.

Key Words: cattle and related species, immunology, RNA-Seq, infectious disease, animal health

P217 Abstract withdrawn

P218 Abstract withdrawn

P218 Abstract withdrawn

P219 CRISPR/Cas9-mediated precise genome editing of cellular host factor of avian influenza virus in chicken. Y. H. Park*, K. Chungu, S. B. Lee, Y. M. Kim, J. M. Kim, and J. Y. Han, *Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea.*

Avian influenza viruses (AIVs) have been seriously threatened to poultry due to their high pathogenicity and interspecies transmission competency, resulting in enormous economic loss worldwide over the past few decades. Since AIVs inevitably utilize the cellular machinery during the virus life cycle, much effort has focused on discovering and targeting host cellular factors to understand the viral life cycle and find anti-viral drug candidates as an alternative strategy to the vaccination. Despite the increased understanding of interaction dynamics among the AIVs and their cellular host factors, which host factors are involved in the species-specific differences against AIVs is still unclear. Here, we targeted the AIV-associated host factor using CRISPR/Cas9-mediated homology-directed recombination (HDR) to elucidate the functional role of the host factor on replication of AIV in chicken. First, we constructed the CRISPR/Cas9 vector and the donor plasmid vector for HDR, and we established the mutant clones and precise genome edited clones, which was verified by T7E1 assay and genomic DNA sequencing. The sequencing result showed that all established DF-1 clone had different bi-allelic mutation patterns, but HDR-mediated genome editing induced the precise deletion of the target sequence, showing the human host factor-like signature. Next, we revealed that the knockout of the host factor resulted in a significant reduction of replication in the AIV compared with wild-type DF-1 cells. Furthermore, we showed that the species-specific differences upon infection of AIV between the birds and mammals could be caused by differential expression of host factor family members through the enforced expression of human host factor family members into the chicken cells. Our study suggests that the functional assessment of species-specific cellular host factors can contribute to the understanding of replication of virus in the different hosts, and can be applied to discover an anti-viral drug target and to develop the AIV-resistant animal model in the near future.

Key Words: poultry and related species, genome editing, CRISPR/Cas9, infectious disease

P220 Allele specific and differential expression in the chicken splenic transcriptome in response to avian pathogenic *Escherichia coli*. M. Monson*[1], M. Kaiser[1], A. Wolc[1,2], and S. Lamont[1], *[1]Iowa State University, Ames, IA, USA, [2]Hy-Line International, Dallas Center, IA, USA.*

Avian pathogenic *Escherichia coli* (APEC) is the causative agent for colibacillosis, one of the most common and costly bacterial diseases for poultry production worldwide. Infections with APEC impair perfor-

mance and diminish welfare in chickens, reducing growth and egg production, while increasing mortality. The molecular pathways by which the host responds to APEC could provide targets to increase chicken resistance or improve vaccine efficacy. To identify genes and regulatory elements in these pathways, transcriptome changes from APEC can be characterized by differential expression (DE) analysis, while allele specific expression (ASE) provides markers for *cis*-acting regulatory elements impacted by APEC. For this study, F_1 progeny of reciprocal crosses between broiler (disease-susceptible) and Fayoumi (disease-resistant) lines were generated. At 14 d of age, birds were inoculated with either APEC O1:K1:H7 or sterile PBS by intra-airsac injection. Spleen tissue samples were harvested for bacteriology and RNA-sequencing (RNA-seq) after 1 or 2 d post infection (DPI). Transcriptome responses were measured by high-depth RNA-seq of 46 cDNA libraries (n = 5–6 libraries/cross/treatment/DPI) using the Illumina HiSeq 3000 to produce paired-end reads. Over 95% of these reads mapped onto the chicken genome (Galgal6a) and more than 16,000 genes were expressed at sufficient levels for DE and/or ASE analyses. At 1 DPI, 580 genes had significant DE in response to APEC, with greatest increases in pro-inflammatory genes (such as *IL22*, *IL17A*, and *PTX3*); only 157 genes were significant at 2 DPI, consistent with the decrease in bacterial load from 1 to 2 DPI. Allele specific counts were used to detect genes with significant ASE, shifts in allelic imbalance in response to APEC, and overlap with significant DE genes. Overall, DE and ASE in the splenic transcriptome provided insight on early immune response pathways and targets to investigate for improving chicken resistance to colibacillosis. Support: USDA-NIFA-AFRI #2015–67015–23093 as part of the joint NIFA-BBSRC Animal Health and Disease program and Hatch project #5424 and #5458.

Key Words: poultry, RNA-seq, allele-specific expression, infectious disease, animal health

P221 Porcine epidemic diarrhea virus induces APN gene expression to facilitate viral propagation. H. Wang*, C. Zhao, S. Wu, and W. Bao, *Yangzhou University, Yangzhou, Jiangsu Province, China.*

Porcine epidemic diarrhea virus (PEDV) causes acute watery diarrhea, dehydration and high mortality of suckling pigs. Expression of porcine APN gene which was previously indicated as the cellular receptor contributes to cell entry of PEDV. However, whether PEDV stimulates APN expression to facilitate viral propagation and the potential regulatory mechanisms remain unclear. To this end, we performed CRISPR/Ca9 mediated knockout of the APN gene in the intestinal epithelial cell IPEC-J2. Infection of $APN^{-/-}$ and wild type cells with PEDV at MOI = 0.1 showed that the viral titer in $APN^{-/-}$ cells was significantly lower than that in controls ($P < 0.05$), indicating the reduced PEDV infection by APN knockout. We detected APN expression at different time points (4, 6, 12, 24, and 48 h) after PEDV infection in wild type cells by quantitative PCR. Compared with the uninfected cells, significant upregulation of the APN gene at early time points (4, and 6 h) was observed in PEDV-infected cells ($P < 0.01$). Western blotting confirmed the APN protein upregulation in PEDV-infected cells. These indicated the potential of PEDV infection to stimulate the APN gene expression in host cells. CpG island DNA methylation in APN promoter was quantified by bisulfite sequencing PCR, and remarkably reduced DNA methylation was found in PEDV-infected cells ($P < 0.05$). Pearson correlation analysis revealed significantly inverse associations between APN expression and promoter methylation ($r = -0.72$, $P = 0.006$). Moreover, we quantified the enrichment of the activation mark H3k4me3 and the repressive mark H3k27me3 at APN promoter by ChIP-qPCR. Increased levels of H3k4me3 enrichment and decreased levels of H3k27me3 enrichment were observed in PEDV-infected cells, indicating that APN expression may be regulated by altering these epigenetic marks. These results indicated that PEDV could stimulate the APN gene expression by altering epigenetic modifications to facilitate viral replication. Our work provides insights into mechanisms of PEDV infection by inducing cellular receptor gene expression and may contribute to developing strategies for PEDV prevention.

Key Words: PEDV, APN gene, expression regulation, epigenetic modification

P222 Abstract withdrawn

P223 *PRNP* genotyping in sheep with scrapie disease. A. Piestrzynska-Kajtoch*[1], M. P. Polak[2], G. Smolucha[1], and J. F. Zmudzinski[2], [1]*National Research Institute of Animal Production, Balice, Poland,* [2]*National Veterinary Research Institute, Pulawy, Poland.*

Scrapie is one of the fatal, prion diseases and belongs to the group of transmissible spongiform encephalopathies (TSEs). Scrapie affects sheep and goats and active surveillance for this disease is obligatory in Poland. There are 2 scrapie types: classical and atypical (NOR98). They differ in etiology and genetics. Caused by prions (PrP^{Sc}), scrapie is connected with the *PRNP* gene. It has been shown that ovine *PRNP* gene polymorphisms influence scrapie susceptibility, especially in its classical type. Since 2009, we have sequenced the *PRNP* gene coding region in all sheep diagnosed with scrapie (Western blot) from Poland: 18 cases with classical scrapie and 67 cases of atypical scrapie. All classical scrapie individuals were imported to Polish slaughterhouses from abroad. We used BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and capillary electrophoresis (3130xl and 3500xl Genetic Analyzers) to perform the analysis. The data were analyzed in BioEdit Sequence Alignment Editor.We found 11 different genotypes in atypical scrapie group (AS) and 10 in classical scrapie group (CS). The most frequent genotypes in atypical and classical scrapie sheep were $A_{136}L_{141}R_{154}R_{171}$/ALHQ and ALRQ/ALRQ, respectively. Allele VLRQ, connected with the highest susceptibility to classical scrapie, was found in 5 classical scrapie sheep and in one atypical scrapie case (AFRQ/VLRQ). Allele ALRR, associated with classical scrapie resistance, had frequency of 42.54% (AS) and 22.22% (CS). Allele AFRQ, connected with atypical scrapie, was observed in 29.8% AS sheep and in 11.1% CS sheep, which significantly exceeds the allelic frequency observed in healthy sheep (2–6% according to different authors). We have also discovered other polymorphisms in the *PRNP* gene: M112T, H143R, N176K, R211Q, E224K. Different sheep origin (country and breed) may partially explain the *PRNP* gene variability. Our study confirmed the differences in atypical and classical scrapie genetic backgrounds. The study was financed by the Ministry of Agriculture and Rural De-

velopment Multiannual Programme no. 03–17–24–90 and Statutory Project no. 4–007.1.

Key Words: sheep and related species, DNA sequencing, polymorphism, animal health

P224 Identification of lncRNAs regulating variable stress-responding sheep naturally exposed to gastrointestinal nematode parasites. A. Suárez-Vega*[1], E. A. Borkowski[2], S. Dixon[1], N. A. Karrow[1], A. S. Peregrine[2], P. Menzies[3], B. A. Mallard[2], M. N. Boareki[1], F. S. Schenkel[1], J. J. Arranz[4], G. Tosser-Klopp[5], C. Klopp[6], and A. Cánovas[1], *[1]Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, [2]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, [3]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, [4]Departamento de Producción Animal, University of León, León, Castilla y León, Spain, [5]GenPhySE, Université de Toulouse, INRA, ENVT, Castanet Tolosan, Midi-Pyrénées, France, [6]Sigenae Platform, MIAT, INRA, Auzeville, Midi-Pyrénées, France.*

Better insight into the genetics of the ovine host response to gastrointestinal nematode parasites will enable improvement in integrated management strategies. Little is known about long noncoding RNA (lncRNA) function in the host response to nematodes infection. Samples from sheep naturally exposed to *Haemonchus contortus, Teladorsagia circumcincta* and *Trichostrongylus* gastrointestinal nematodes with High (n = 5) and Moderate (n = 5) cortisol responses, and non-exposed Controls (n = 4) were used to characterize lncRNA changes in liver using RNA-Seq. Samples were aligned to the sheep reference genome (Oar_v.3.1) using STAR software. Cufflinks and Stringtie software were used to construct 2 novel transcript models. FEELnc was used to identify lncRNAs in both approaches obtaining 3,688 and 4,854 lncRNAs with Cufflinks and Stringtie, respectively. The sensitivity and specificity for the lncRNA detection were 0.92. The coding potential threshold used to determine if a transcript was characterized as mRNA or lncRNA was 0.42. Among them, 3,307 lncRNAs were shared between the 2 transcript sets. When lncRNA models from the 2 assembly approaches and those shared among methodologies were compared with known sheep lncRNAs, those detected by both assemblers had the highest sensitivity (0.89) and precision (0.54) at the locus level. Common lncRNAs were used for further differential expression (DE) analyses between Control sheep and High and Moderate stress responders. The estimation of reads per lncRNA and DE analyses were performed using RSEM and DESeq2 software. A total of one, 16 and 23 DE lncRNAs were identified between High vs. Moderate, High vs. Control and Moderate vs. Control groups, respectively. Functional analyses performed using the list of genes located in close proximity (0.01–1 Mb window) to the lncRNAs suggested that regulation of steroid metabolism or oxidoreductase activity may influence the different responses to parasites in High and Moderate stress responder sheep. These results indicate that lncRNAs may be used as potential targets or indicators to control for gastrointestinal nematode parasites in sheep.

Key Words: sheep and related species, infectious disease, RNA-Seq, non-coding RNA, animal health

P225 PRRSV subverts reticulophagy by downregulating *Fam134b* expression. K. Guan*, X. Zhou, and B. Liu, *Huazhong Agricultural University, Wuhan, Hubei, China.*

Porcine reproductive and respiratory syndrome (PRRS) caused by PRRS virus (PRRSV) is one of most devastating diseases in swine industry. PRRSV replicates its RNA genome in endoplasmic reticulum (ER) and uses ER to facilitate its assembly and maturation. To maintain homeostasis, host cells initiate degradation of ER, known as reticulophagy or ER-phagy. Here, we reported that PRRSV subvert reticulophagy through downregulating ER-localized reticulophagy receptor FAM134B. RNAi-mediated depletion of FAM134B proteins or overexpression mutant FAM134B proteins can significantly disrupt ER-phagy and facilitate PRRSV replication in vitro. Furthermore, we showed that *Fam134b* gene expression at both transcriptional and post-transcriptional regulation is mediated by PRRSV and miRNA during PRRSV infection. Overall, our findings explore the important role for FAM134B-dependent reticulophagy in PRRSV replication, which might lead to a better understanding of the pathogenesis of PRRSV and further development of antiviral therapeutics.

Key Words: PRRSV, FAM134B, reticulophagy, endoplasmic reticulum

P226 Assessment of monthly tick count variation and mapping of genomic regions associated with cattle resistance to African ticks. N. O. Mapholi*[1], A. Maiwashe[2], O. Matika[3], V. Riggio[3], C. Banga[2], and K. Dzama[4], *[1]University of South Africa, Florida, Gauteng, South Africa, [2]Agricultural Research Council, Pretoria, Gauteng, South Africa, [3]The Roslin Institute and R(D)SVS, University of Edinburgh, Edinburgh, UK, [4]University of Stellenbosch, Stellenbosch, Western Cape South Africa.*

Ticks are among the most important livestock parasites and cause annual economic losses amounting to US$20–30 billion worldwide. Currently there is no sustainable tick control method. Acaricides and tick vaccines are expensive and difficult to manage. Genomic selection for host resistance presents an alternative tick control strategy that is less costly and more sustainable. The primary objective of this study was to assess variation in monthly tick count, as well as identify genomic regions associated with host resistance to ticks in South African Nguni cattle. Tick counts on the animal's perineum were recorded in the month of November for 2 years (2012 and 2014) from 586 Nguni cattle reared in 4 herds under natural grazing conditions. Tick count was transformed using log10(x + 1) and the resulting values were examined for normality. DNA of the animals was genotyped using the Illumina BovineSNP50 assay. After quality control (call rate >90%, minor allele frequency >0.02), 40,436 SNPs were retained for analysis. Heritability estimates were computed using mixed linear models, fitting univariate sire models. Fixed effects considered were location, sex, year and age as a covariate. An association analysis for tick resistance was carried out using a genome-wide association (GWA) analysis of the GenABEL package. The Bonferroni genome-wide ($P < 0.05$) corrected significance threshold was 1.24×10^{-6}, with 2.47×10^{-5} as the suggestive significance threshold ($P < 0.10$) (i.e., one false positive per genome scan) in the GWA analysis. A heritability estimate of 0.33 ± 0.17 was obtained for perineum tick count trait. Genome-wide significant regions were identified on chromosome 1, while other regions significant at the suggestive level were found on chromosomes 1, 11 and 15. The observed genetic variation of tick count in Nguni cattle forms the basis to explore genetic architecture of cattle resistance to ticks and may provide the potential for marker-assisted selection as an alternative tick control strategy.

Key Words: Nguni, tick species, heritability, GWAS

P227 Preliminary results: Heritability estimate for tick count and identification of genomic regions associated with tick tolerance in F_2 Nguni x Angus population. N. Mkize*[1,2], G. B. Hutang[1], B. Dube[1], K. Dzama[2], A. Maiwashe[1], and N. O. Mapholi[3], *[1]Agricultural Research Council-Animal Production Institute, Pretoria, Gauteng, South Africa, [2]Stellenbosch University, Stellenbosch, Western Cape, South Africa, [3]University of South Africa, Florida, Gauteng, South Africa.*

The presence of bovine ticks in cattle production is considered as one of main sources of diseases globally, attributing to serious economic losses in both developed and developing countries. The current used control strategies are not efficient and they are associated with substantial animal health and welfare consequences. Therefore there is a need for alternative tick control measures. A prospective approach to explore

genetic variation for bovine resistance to ticks is of importance, since the trait is genetically controlled. The aim of the study was to estimate heritability and identify SNPs markers associated with tick resistance in F_2 Nguni × Angus population artificially infested with *Amblyomma hebraeum* ticks. Heritability estimation was undertaken through bayesian inference using ASReml package in R program. Furthermore, llumina BovineSNP150 assay was used for genotyping and the genotyped data was subjected for quality control using Plink software (call rate > 90%, minor allele frequency > 0.01), after which 108452 SNPs were retained for further analysis. Genome-wide association study was undertaken in R program using GenABEL package. The estimated heritability for tick count was 0.23. Although, no SNPs were above the genome-wide significance threshold, 3 regions crossed the suggestive significance threshold and were identified on BTA 2 and 21 for tick count, and BTA 1 for skin thickness. Noting that this was a preliminary results, more data is required to increase the power in order validate regions identified in the current study.

Key Words: genotyping, genome-wide association, tick resistance

P228 Association of *TLR* gene variants with utility and health traits in Czech Simmental cattle population. K. Novák*[1], M. Bjelka[2], K. Samaké[3], and T. Valcíková[4], *[1]Institute of Animal Science, Prague-Uhríneves, Czech Republic, [2]Department of Genetics and Microbiology, Charles University, Prague, Czech Republic, [3]Breeding Company CHD Impuls, Bohdalec, Czech Republic, [4]Department of Genetics and Breeding, Czech University of Life Sciences, Prague, Czech Republic.*

The variants of the genes coding for the Toll-like receptors with anti-bacterial function were tested for the impact on milk utility traits and selected health and reproduction traits in Czech Red Pied (Czech Simmental) cattle population. The variants of *TLR1*, *TLR2*, *TLR4*, *TLR5* and *TLR6* present were identified by resequencing with 2 NGS technologies. Data from amplicon resequencing with PacBio technology and from the whole-genome resequencing with HiSeq X-Ten technology complemented each other, increasing the reliability of variant identification. Subsequently, a set of 150 bulls was genotyped with primer extension technique for 36 polymorphisms present. The effect of genotype classes was tested against 15 phenotypic traits with one-factor ANOVA. The traits, mostly expressed as breeding values, included 5 milk utility traits, 4 udder health traits and 6 reproduction or general health-related traits. The effect on the milk utility traits (fat and protein percentage and yield) was exerted mostly by the variants in *TLR2*, partially also in *TLR1*, *TLR6* and by 2 *TLR5* variants, but no effect was produced by the *TLR4* variation. On the other hand, the *TLR4* variants affected udder health traits, along with *TLR1*, *TLR6* and *TLR5* variants. Consequently, the effect of the *TLR2* variation on milk production is not mediated directly by infection resistance. Surprisingly, significant effects of variants in *TLR1*, *TLR6* and *TLR2* on calving ease, both paternal and maternal, were observed. Since the products TLR1 and TLR6 are known to form functional heterodimers with TLR2, the similar pattern of the effects is explainable. The observation might be related to the reported QTL #42040 for calving ease on chromosome 6, which is close to the location of *TLR1* and *TLR6* at 59.7 Mbp. Consistently, 3 most active variants of *TLR6* with respect of calving (rs68268250, rs43706434 and rs68268260) are missense and code for amino acid changes in the product. Although variants in *TLR4* did not produce a pronounced effect on the udder health traits, they affected calf vitality index, consistently with the role of infections compared with the adult animals.

Key Words: cattle, HTS, genotyping, innate immunity, animal health

P229 Time- and population-dependent genetic patterns underlie bovine milk somatic cell count. A. Miles* and H. Huson, *Cornell University, Ithaca, NY, USA.*

The objective of this study was to identify variation in genetic regulation of bovine milk somatic cell cont (SCC). A sample of 523 dairy cows from 2 farms in upstate New York were enrolled in a prospective cohort study involving 5 composite milk sample collections representing key physiological time points in lactation (3–5, 10–14, 50–60, 90–110, and 210–230 DIM). Of this cohort, 474 cows were genotyped for 777,962 SNPs on the Illumina Bovine HD bead chip and markers mapped using the bovine genome assembly UMD3.1.1. After quality control filtering, 458 cows and 581,669 SNPs had call rate = 0.09, minor allele frequency = 0.05, and allele # = 2. All phenotypic data transformation was performed in RStudio and all genetic analyses in Golden Helix SNP and Variation Suite software. Tracking the progression of SCS over time, we identified extreme populations of cows that remained "chronic" (SCS = 4) or "healthy" (SCS < 4) during the entire study period. Fixation indices were calculated and 2 SNPs identified that demonstrated moderate genetic differentiation between "healthy" and "chronic" populations (Fst = 0.4). Genome-wide association studies (GWAS) using an Efficient Mixed Model Linear Analysis (EMMAX) to account for underlying population structure revealed 4 SNPs associated with "healthy" or "chronic" disease status (FDR = 0.05) in a dominant inheritance model with farm and parity covariates. GWAS were performed for SCS at each of the 5 sampling time points using EMMAX recessive inheritance models with farm and parity covariates. These analyses identified 31 SNPs associated with SCS at 3–5 DIM; 113 SNPs associated with SCS at 10–14 DIM; and 2 SNPs associated with SCS at 210–230 DIM (FDR = 0.05). Three SNPs were common between GWAS at 3–5 DIM and 10–14 DIM. This study was designed specifically to capture the changes in SCC across lactation, given that there are non-pathological reasons for udder inflammation and that SCC is not exclusively an indicator of disease. Future investigation of candidate genes in the identified regions will help elucidate the genetic mechanisms regulating immune response in the bovine mammary gland and improve genomic selection for mastitis resilient cows.

Key Words: cattle and related species, disease resilience, genome-wide association, SNP, quantitative trait locus (QTL)

P230 Whole-genome re-sequence analysis reveals tick resistance and heat tolerance genes in Iraqi cattle breeds. A. Alshawi*[1,2], A. Essa[3], S. Al-Bayatti[3], A. Tijjani[1,4], S. Salman[3], and O. Hanotte[1,4], *[1]School of Life Sciences, Faculty of Medicine and Health Sciences, University of Nottingham, United Kingdom, [2]Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Baghdad, Iraqi Ministry of Higher Education and Scientific Research, Iraq, [3]Animal Genetics Resources Department, the Ministry of Iraqi agriculture, Baghdad, Iraq, [4]International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.*

In spite of the historic global importance of cattle in Iraq, a country at the crossroad of the species domestication centers, their genomes have not yet been characterized. Here, we present the results of full genome sequence autosomal scan of 20 native Iraqi cattle from 2 breeds (Jenoubi, n = 10; and Rustaqi, n = 10) for candidate signatures of positive selection associated with environmental adaptation, including acquired and adaptive immunity (e.g., tick resistance) and thermotolerance. Candidate selected regions and genes were uncovered following 2 within population (*Hp* and *Tajima's D*) and 2 among populations (*XP-EHH* and *Fst*) analysis. Gene's functions ontology analysis at significant regions were performed using PANTHER, KEGG and DAVID tools. In Jenoubi, we identified T-cell and B cell activation genes (*CD3D, CD3E, CD3G* and *PTPN6*), while *B2M* and *LIPH* genes were found in Rustaqi. These genes may play significant role into the resistance to tick infestation, a major external parasitic burden in Iraq. Heat tolerance genes were also identified in Jenoubi (e.g., *DNAJB1*) and Rustaqi (e.g., *DNAJC4*). Furthermore, we noticed *HOX* genes (e.g., *HOXC11* and *HOXC12*) that are related to thermoregulation activity in Jenoubi. Genes involved in the animal physiological responses to external stimulus and stress are detected in both breeds. Our results provide, the first full genome insights on the genome adaptation of Iraqi cattle

enlightening the importance to conserve and utilize these adaptations to control diseases and breeding improvement programs in the country.

Key Words: Iraqi cattle, genome sequencing, adaptive immunity, genomic selection

P231 Integrative functional genomics of the bovine host response to infection with *Mycobacterium bovis*. T. Hall*[1], M. Mullen[2], C. Correia[1], G. McHugo[1], K. Killick[1], J. Browne[1], N. Nalpas[4], S. Gordon[2,5], and D. MacHugh[1,2], *[1]Animal Genomics Laboratory, UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland, [2]Department of Life and Physical Sciences, Athlone Institute of Technology, Athlone, Ireland, [3]Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany, [4]UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland, [5]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland.*

Bovine TB (BTB), caused by infection with *Mycobacterium bovis*, is a major endemic disease affecting global cattle production. The key innate immune cell that first encounters the pathogen is the alveolar macrophage, which we have previously shown to be substantially reprogrammed during intracellular infection by *M. bovis*. In the current study we used differential expression with correlation- and interaction-based network approaches to analyze the macrophage transcriptional response to infection with *M. bovis* to identify core infection response pathways and gene modules. These outputs were then integrated with genome-wide association study (GWAS) data sets to enhance detection of genomic variants for susceptibility/resistance to *M. bovis* infection. The host gene expression data consisted of bovine RNA-seq data from alveolar macrophages infected with *M. bovis* at 24 and 48 h post-infection. These RNA-seq data were analyzed using 3 distinct analysis pipelines; novel response pathways and modules were further refined using cross-comparison and integration of the results. First, a differential expression analysis was carried out to determine the most significantly differentially expressed (DE) genes between conditions at each time point. Second, 2 networks were constructed at each time point using gene correlation patterns to determine changes in expression across conditions. Functional sub-modules within each correlation network were selected by statistical criteria for modularity. Third, a gene interaction base network of the mammalian host response to mycobacterial infection was generated using the GeneCards database (www.genecards.com) and InnateDB (www.innatedb.com). Differential gene expression data were superimposed on this base network to extract functional modules of interconnected DE genes. Bovine GWAS data were obtained from a published BTB susceptibility/resistance study. The results from the 3 parallel analyses were integrated with this data to determine which of the 3 approaches identified genes significantly enriched for SNPs associated with susceptibility/resistance to *M. bovis* infection. Our results indicate distinct and significant overlap in SNP discovery and demonstrate that network-based integration of relevant transcriptomics data can leverage substantial additional information from GWAS data sets.

Key Words: tuberculosis, macrophage, bovine, GWAS, transcriptome

P450 African Swine Fever Virus strains show different virulence associated with modulation of IFNs type I gene expression. E. Razzuoli[1], G. Franzoni[2], I. Ferretti[2], T. Carta[4], S. Zinellu[2], G. Galleri[4], M. Amadori[3], S. Dei Giudici[2], P. Modesto*[1], and A. Oggiano[2], *[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy, [2]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy, [3]Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna, Brescia, Italy, [4]Università degli Studi di Sassari, Sassari, Italy.*

African swine fever virus (ASFV) has a tropism for cells of the myeloid lineage, including macrophages. To develop a vaccine for ASFV we conducted an in vitro characterization of the interaction of unactivated porcine monocyte-derived macrophages (moMF) with 2 different ASFV strains: 22653/14 (high virulence strain; HV) and NH/P68 (low virulence strain; LV). Monocytes from 5 different swine were differentiated using 50 ng/mL of human monocyte Colony Stimuting Factor (hM-CSF) and infected with either HV or LV ASFV strains, along with a mock-infected control. Twenty-one hours post-infection (hpi) the expression of ASFV proteins (p72) and surface markers (MHC I) were assessed by flow cytometry. At different time points (3, 6, 9, 12, 21 hpi) the expression of genes coding for IFNB and 17 different IFNA subtypes was determined by RT qPCR. In each sample, the relative expression of the selected genes was calculated using the formula $2^{-\Delta\Delta Ct}$ where $\Delta\Delta Ct = \Delta Ct$ (mock) - ΔCt (target gene after infection). After calculation of 2^{-DDCt} and the Kolmogorov-Smirnov test, data were checked for statistically significant differences by the Friedman test, followed by a Dunn's test as implemented in PRISM software package. We observed that both isolates were able to infect macrophages, but only LV downregulated MHC I expression. Moreover, LV and HV strains induced a different set of IFN genes. HV infection caused upregulation of IFNA3 at 21 hpi, and IFNA9 at 9hpi and 21hpi. LV strain determined upregulation of IFNA5/6, -A8, -A10, -A12, -A13, -A15, -A16, -A17 and IFNB at 21hpi. All the reported variations resulted statistically significant ($P < 0.05$). In a recent study we demonstrated different anti-inflammatory effects and antiviral activity by IFNA subtypes. In particular, IFNA2, -A5, -A9, and -A10 showed high level of antiviral activity. In this study we observed downregulation of MCH I (which underlies the activation of NK cells) and an upregulation of IFNB and IFNA after LV infection. On the other hand, HV infection upregulated IFN9. These data suggest that virulent isolates are able to evade host immune response and promote their survival in infected pigs.

Key Words: pigs and related species, cell culture, adaptive immunity, animal health

Genome Edited Animals

P232 Genome modification in chicken for therapeutic protein production. Y. M. Kim*, Y. H. Park, J. M. Kim, J. S. Park, H. J. Lee, K. Y. Lee, and J. Y. Han, *Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea.*

Genome modification technology has been provided numerous opportunities for basic research and industry. Recent progress in genome modification technology such as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) system combined with primordial germ cell (PGC)-mediated germline transmission system allows more potential applications in genome modified poultry for diverse purposes. Especially, the practical use of these techniques was successfully adopted in producing genome edited chickens for application of industrial field including bioreactor system. Since the relatively short generation time, sufficient reproductive capacity and high egg production characteristics, chicken has been considered as the most efficient animal bioreactor for recombinant protein production. In addition, a half of the chicken egg white proteins was translated from the single egg white gene, *Ovalbumin*, the mass production of functional proteins in eggs has been highly expected. In this regards, we have applied the modified egg white promoter

for development of an efficient chicken bioreactor system. As results, the transgene was successfully integrated chicken genome, and the products derived from genome modified chicken showed consistent expression level and higher efficacy compared with commercial counterpart. The results suggest that chicken bioreactor developed by genome modification technologies could be an alternative system for producing human therapeutic proteins.

Key Words: poultry and related species, genetic engineering, transgenics, biomedical model

Horse Genetics and Genomics

P233 Detection of homozygous-by-descent segments revealed distinct age-related classes overlapping with known behavioural QTLs in Norwegian-Swedish Coldblooded Trotter. M. Solé*[1], B. D. Velie[2], C. F. Ihler[3], E. Strand[3], and G. Lindgren[1,4], *[1]Department of Animal Breeding & Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Faculty of Life and Environmental Science, University of Sydney, Sydney, Australia, [3]Department of Companion Animal Clinical Sciences, Norwegian School of Veterinary Science, Oslo, Norway, [4]Livestock Genetics, Department of Biosystems, KU Leuven, Leuven, Belgium.*

The process of horse domestication, followed by intensified selective breeding during the last hundreds years is associated with increased homozygosity at loci controlling valuable traits. Behavior and learning capacity likely contributed to the cognitive and social changes associated with domestication. Assuming the individual genome as a mosaic of homozygous-by-descent (HBD) and non-HBD segments, we applied a hidden Markov model (RZooROH package) to identify HBD segments in multiple age-related classes, tracing back different base populations. We hypothesize that a substantial proportion of the detected HBD segments in the Norwegian-Swedish Coldblooded trotter (NSCT) will overlap to known behavioral and cognitive QTLs of importance for racing success. To that end, genotypes from 658 NSCTs for 350,756 autosomal SNPs were considered after quality control (maf 0.01, geno 0.05, hwe 0.001). We defined a Mix10KR model (9 pre-defined HBD and 1 non-HBD class) to estimate the proportion of the genome belonging to different HBD classes (F_g). When considering the most remote base population, the F_g was equal to 0.17 on average, with a major contribution from old age-related classes (HBD segments of 0.29Mb on average). In total, we identified 138,505 HBD segments (from 182bp to 8Mb length). The loci on *E. caballus* (ECA) 7 known to have undergone positive selection during horse domestication (NTM/OPCML genes), partially overlapped with HBD segments of 12.2Mb on average in 32.7% of the individuals. Another QTL on ECA9 associated with horse tractability (VPS13B gene), partially overlapped with HBD segments of 6.4Mb on average in 24.3% of the individuals. Additional HBD segments partially overlapped genomic regions that harbour candidate genes associated with behavioral QTLs and physiological stress (e.g., PRRT2, XPO6, DRD4, SYD3, BDNF, ACTH, HSD17B3, SLC6A4, PRKCB and HTR1), albeit present in a lower proportion of the individuals (between 8 and 20%). These results encourage continued investigation on the genetic basis of cognitive ability in relation to physiological stress management and racing success.

Key Words: homozygosity, horses and related species, behaviour, nervous system

P234 Analysis of MSTN SNPs associated with speed in Polo Argentino breed. M. M. Martinez*[1], M. Costa[1], C. Ratti[1], and M. N. Corvalán[2], *[1]Laboratorio de Genética Aplicada, Sociedad Rural Argentina, CABA, Buenos Aires, Argentina, [2]Crestview Genetics, Luján, Buenos Aires, Argentina.*

Argentine Polo players and horses are worldwide famous due to their high performance. Despite its importance, the breed has not been genetically characterized. Unlike Thoroughbred, the best skills (docility, stamina, wisdom) of this breed are not measurable, making it difficult to carry out genome-performance association studies. Nowadays, Polo horses come mainly from thoroughbred stallions and mares. The purpose of this work was to analyze if gene variants related to racing performance in thoroughbreds are also important for Polo horses. Thus, 2 myostatin (MSTN) SNPs (g.65809482T > C and g.65868604G > T), associated with racing aptitude, were genotyped in 78 polo horses that were chosen based on its importance for the sport. Aguada herd (46 horses) was selected since it provides horses for different player skills. La Dolfina (25), with some of the most famous horses (like Dolfina Cuartetera, Aiken Cura), bred for just one player, Adolfo Cambiaso, ranked as the best player in Polo history. Finally, a small group of animals, representative of the old-type breed, was also tested. We found that the sprint MSTN genotypes were almost absent among horses analyzed. Genotypes were concentrated in mid-long distance phenotypes (95%), suggesting that stamina is more important than sprint for sport performance. Polo Argentino breed traced back to Criollo horses, used for work in argentine farms and available for the game. A test of the MSTN SNPs in criollos showed the stamina type expected for a breed characterized for its endurance. Those first Polo horses, showing high stamina, should have defined the standard of the sport, with horses playing a full chukker without breaks. Breeding later to English Thoroughbreds added sprint to the horses, making a faster version of the sport. Thus, horses are now changed more frequently during the game, and genotypes accordingly have moved to intermediate phenotype, a mix of sprint and stamina. The analysis of the old and new Polo herds, showed in this work, agree with this breed evolution. Polo Association is now planning to change the sport rules, moving closer to the old fashion style. This opens the opportunity to use the MSTN test to select horses suitable for these new conditions.

Key Words: horse, myostatin, athletic performance, animal breeding, polo breed

P235 Genetic diversity and relationships among native Japanese horse breeds and horses outside of Japan using genome-wide SNP data. T. Tozaki*[1,3,6], M. Kikuchi[1], H. Kakoi[1], K.-I. Hirota[1], S.-I. Nagata[1], D. Yamashita[2], T. Ohnuma[2], M. Takasu[3], I. Kobayashi[4], S. Hobo[5], D. Manglai[6], and J. Petersen[7], *[1]Genetic Analysis Department, Utsunomiya, Tochigi, Japan, [2]Japan Equine Affairs Association, Chuo-ku, Tokyo, Japan, [3]Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Gifu, Japan, [4]Sumiyoshi Livestock Science Station, Field Science Center, University of Miyazaki, Miyazaki, Miyazaki, Japan, [5]Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Kagoshima, japan, [6]College of Animal Science, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China, [7]Department of Animal Science, University of Nebraska–Lincoln, Lincoln, NE, USA.*

Eight horse breeds, Hokkaido (HK), Kiso (KS), Misaki (MS), Noma (NM), Taishu (TS), Tokara (TK), Miyako (MY), and Yonaguni (YN), are native to Japan and relatively free of artificial selection. Although Japanese native breeds are believed to have originated from ancient Mongolian horses, the phylogenetic relationships among these breeds are not well elucidated. In this study, we compared genetic diversity among 32 international horse breeds previously evaluated in the Equine Genetic Diversity Consortium (733 horses) and the 8 Japanese native breeds (HK: 42, KS: 23, MS: 21, NM: 24, TS: 25, TK: 24, MY: 35, YN: 24) using genome-wide SNP genotype data (20,287 SNPs pruned by LD from the EquineSNP70 BeadChip). The expected heterozygosity showed the 8 Japanese breeds (0.158–0.267) have low diversi-

ty compared with the other international breeds (all breeds: 0.277). The polymorphic proportion showed the 7 Japanese breeds (0.437–0.792) except for HK (0.864) have relatively low diversity compared with the other international breeds (all breeds: 0.833). Phylogenetic and cluster analyses demonstrated relationships among the breeds that largely reflect their geographic distribution in Japan. These data support historic records, suggesting a Mongolian origin of native Japanese horses, and refute the prior assumption that the northern and southern lineages were derived from separate founder populations. These data help to explain the history of the Japanese native horses, identify uniqueness within the breeds, and will also serve as a baseline from which to monitor future breed diversity and make conservation decisions for endangered populations.

Key Words: horse, equine, Hokkaido, SNP

P236 Genomic scan of conformation traits in Icelandic horses. M. K. Rosengren*[1], M. Solé[1], Å Wiklund[1], S. Eriksson[1], B. D. Velie[2], J. J. Negro[3], and G. Lindgren[1,4], *[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]School of Life & Environmental Sciences, University of Sydney, Sydney, Australia, [3]Department of Evolutionary Ecology, Doñana Biological Station, CSIC, Seville, Spain, [4]Livestock Genetics, Department of Biosystems, KU Leuven, Leuven, Belgium.*

The breeding goals for Icelandic horses comprise various conformation and riding ability traits. To aid the subjective conformation assessments in breeding field tests, 13 objective body measures (height, length and circumference) are recorded. Genetics has previously been shown to play a major role in conformation traits. Height at withers is the most studied trait where several candidate genes have been presented. However the genetics behind many of the other traits is still unknown. The aim of this study was to identify genomic regions associated with conformation traits in Icelandic horses by using objective measurements from breeding field tests. Eighty-three assessed Icelandic horses were included in the study. A genome-wide association study (GWAS) was performed using the 670K+ Axiom Equine Genotyping Array. Quality controls (QC) and GWAS analyses were performed using the package GenABEL in R. QC included call rate > 0.95, missing genotypes in individuals < 0.90, minor allele frequency < 0.05 and cut-off p-value 1e-10 in check for Hardy-Weinberg Equilibrium. A 5% Bonferroni was used as significance threshold. A suggestive genome-wide significance threshold was also applied at 1.0×10^{-5}. Sex was included as a covariate. HorseQTLdb was used to search for known quantitative trait loci (QTLs) for conformation and Variant Effect Predictor at Ensembl homepage to check the effects of significant SNPs. In total, 356037 autosomal SNPs and 82 horses passed the QC. A suggestive genomic region for body length was found on *Equus caballus* (ECA) 10 17.5–18.7 Mb (EquCab2.0) that overlapped with a known QTL for withers height. Five of the 7 significant SNPs were located within introns of gene *ZSWIM9*, *SLC8A2* and *CYTH2*. Six SNPs located on ECA1, ECA7 and ECA18 reached Bonferroni for the maximum circumference of the knee (carpus). None of these SNPs overlapped any known QTL for conformation and were not located within any gene. However, the 2 SNPs located on ECA7 were close to *FAT3* previously shown to be associated with bone density in humans and could therefore be a possible candidate gene. We are currently genotyping 94 additional Icelandic horses to confirm the results in a larger data set.

Key Words: horses and related species, genome-wide association, growth and development

P237 Jumping into selective sweeps for sport horse performance. M. Ablondi*[1], S. Eriksson[2], A. Sabbioni[1], Å. Viklund[2], and S. Mikko[2], *[1]Department of Veterinary Science, Università degli Studi di Parma, Parma, Italy, [2]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

In recent years, specialized breeding programs for sport horse performance have been developed to increase genetic gain and they have nowadays become common practice in elite studbooks. However, it is still not fully understood which regions of the genome such programs impact. This study aimed at identifying selective sweeps in the genome of 2 clusters of Swedish Warmblood horses (SWB) based on genetic relationships. These 2 clusters represent horses showing high breeding values either for dressage (DR) or show jumping (SJ) abilities. We analyzed high-density genotype data (670K) from 193 DR and 187 SJ horses belonging to these clusters. Haplotype selective sweeps were identified via XPEHH test by detecting those alleles that underwent directional selection in one population, while remaining polymorphic in the other one. Selection signatures were averaged over windows to minimize the number of false positives and to account for large standard variations. Only regions defined as a 500 kb-window showing an average XPEHH higher than 3.0 or lower than −3.0 ($P < 0.001$) were considered as putative signatures of selection. These regions were then compared with known QTL regions in the Horse QTL Database. In SJ horses, haplotype selective sweeps were detected on ECA7, ECA13, ECA21, ECA22, ECA26 and ECA31. On ECA22, the haplotype homozygosity exceeded the 500 kb window up until 2.5 Mb. In DR horses, ECA1, ECA4, ECA9, ECA25 and ECA28 showed potential selective sweeps, with 2 regions on ECA4 and 3 regions on ECA25. Three overlapping QTL regions in SJ horses associated with performance and health traits were found. In DR horses, haplotype selective sweep regions coincided with 2 QTLs related to sport performance abilities. The results from this study show that specialized breeding programs can lead to significant haplotype diversity within sub-populations belonging to the same breed.

Key Words: horses and related species, population genomics, selection scan, athletic performance, breed/population identification

P238 Intense artificial selection reflected by runs of homozygosity in Arabian horses. M. Stefaniuk-Szmukier*[1], T. Szmatola[2], A. Gurgul[2], K. Pawlina-Tyszko[2], E. Semik-Gurgul[2], I. Jasielczuk[2], K. Ropka-Molik[2], and M. Bugno-Poniewierska[1,2], *[1]University of Agriculture in Cracow, Kraków, Poland, [2]National Research Institute of Animal Production, Balice, Poland.*

Runs of homozygosity (ROH) are continuous homozygous segments within the DNA sequence. The length and frequency of ROH may reflect the directed artificial selection toward limited traits. The aim of this study was to characterize ROH patterns that may have occurred during selection for racing performance in Arabian horses.The study was performed on population of Arabian horses (A = 121) randomly selected from State Studs. Among them 20 horses represent remarkable horses of racing lines which were under strong artificial selection for racing purposes since more than hundred years (B = 20). DNA were genotyped using Neogen Equine Community BeadChip assay (Illumina). ROH were defined for each animal using cgaTOH software with a sliding window spanning 30 SNPs. After data filtering and ROH identification, the obtained results show the mean sums of the ROH lengths were 395.6 (SD = 56.9) for group A, and 400.4 for B (SD = 56.4). The analysis performed on the top 500 SNPs having higher occurrence within ROHs in both groups of animals show visible differences in the identified genes. The most interesting genes were those involved in calcium signaling, transition between fast and slow fiber, cardiac muscle maintenance. The presented results may indicate that intensive artificial selection is reflected by ROH patterns.The study was financed by the National Research and Development Center (Poland) under the Program: "Environment, Agriculture and Forestry" – BIOSTRATEG, with reference number BIOSTRATEG2/297267/14/NCBR/2016

Key Words: runs of homozygosity, intensive selection, Arabian horses

P239 Training-inducted modification of apoptosis related genes in Arabian horses during flat-racing schedule. K. Ropka-Molik[1], K. Piórkowska[1], AD Musial[2], and M. Stefaniuk-Szmukier*[3], *[1]National Research Institute of Animal Production, Balice, Poland, [2]Jagiello-*

nian University, Kraków, Poland, [3]University of Agriculture, Kraków, Poland.

Apoptosis is one of the adaptation mechanisms controlling and maintaining fitness and muscular strength via skeletal tissue remodeling. The programmed cell death is involved in muscle recovery due to the controlling of replacement of fatigued and damaged cell by new better suited to effort. The aim of research was to analyze the differences in expression profile of apoptosis-related genes in blood and muscle of Arabian horses during flat-racing training schedule.Samples of skeletal muscle (*m. gluteus medius*) and blood were collected (21 samples from each tissue) from 5 untrained horses and 8 horses during a one-year training period at 2 time points (after an intense gallop phase and at the end of the racing season). The previously obtained RNA-seq data was used to select the DEGs under flat-race training for expression levels evaluation by qPCR method. The differential expression of proapoptotic genes: *BAX, BCL2L11*, *NFATC1* and *PTNP1* occurred in both tissues during physiological effort. The differential expression between at least 2 training points was identified for genes - *NOTCH1*; *PIK3CA*, *RAS*, *p53* as well as *Hsp70*, *Hsp90* and *Hsp27*. The expression level of the antiapoptotic *Bcl-2* gene was the highest in blood and muscle tissues collected of horses at the end of racing season. The antiapoptotic gene *c-JUN* estimated in blood significantly increased from untrained vs end of racing season horses. The presented data indicated on strong relationship between apoptosis process and effort intensity. The described genes can regulate apoptosis via mitochondrial outer membrane permeabilization; caspase-related pathway; c-JUN and p53 pathways. The obtained results did not clearly pinpoint one apoptosis regulation pathway indicating the high complexity of the above process, which should be still investigated. Supported by the National Science Center (project no. 2014/15/D/NZ9/05256).

Key Words: apoptosis, Arabian horses, flat racing, qPCR

P242 Genetic variability of Lusitano horse breed reared in Italy. M. C. Cozzi*, P. Valiati, M. G. Strillacci, E. Gorla, F. Genova, and A. Bagnato, *Dipartimento di Medicina Veterinaria - Universitá degli Studi di Milano, Milano, Italy.*

The Lusitano Horse is one of the most ancient breeds. It originated from Portugal, but reared in many countries, primarily Brazil, France, and México. Since 1994, our Laboratory at UNIMI tested Lusitano horses breed in Italy for parentage control. This study aims to assess the genetic variability of 263 Lusitano horses reared in Italy and to evaluate their inbreeding level using microsatellite markers. In addition, the genetic relationships among Lusitano Horse, Spanish Pure Breed, local and cosmopolitan breeds were evaluated. Allelic frequencies, genetic equilibrium according to Hardy-Weinberg (P-val) and inbreeding coefficient (F_{IS}) were estimated using the GENEPOP software. The number of alleles (NA), the effective number of alleles (NE), the observed (Ho) and expected (He) heterozygosity were calculated with POPGENE v.1.32. Allelic richness (AR) per locus within the population was standardized for variations in sample size and was calculated using the FSTAT 2.9.3 software. The genetic relationships between Lusitano Horse and 692 horses from other 7 breeds, were analyzed using different approaches: *i*) Principal Component Analyses with R *ade4* and *adegenet* packages, *ii*) cluster assignment based on the Bayesian method obtained by STRUCTURE software and *iii*) individual animal-based Neighbor-joining dendrogram (NJD) using allele sharing distances with POPULATION software. One hundred 28 allelic variants were found on 17 microsatellites. The NA calculated on 16 microsatellites was 7.5, whereas the NE was 3.824. The mean value of AR was 7.5. The analyses of P-val showed 4 microsatellites not in genetic equilibrium. The Ho and the He mean values were 0.697 and 0.707, respectively, and the overall F_{IS} value for the population was 0.014, indicating very moderate inbreeding within the population. Well-defined population clusters were obtained through the PCA analysis, the cluster assignment and the individual based NJD.

Key Words: horse, microsatellites, genetic variability, genetic relationships

P243 *FAM174A*-11(G) allele is a poor indicator for equine metabolic syndrome. CE Rodríguez-Sanz*, N. Sevane, and S. Dunner, *Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, España.*

Equine metabolic syndrome (EMS) is a complex disorder characterized by insulin dysregulation and obesity associated with an increased risk of laminitis. A genetic basis for the EMS phenotype has been suggested, since some horse breeds, such as ponies, Andalusian and Arabian horses, are more commonly affected, and also due to the similarity with human metabolic syndrome. Recently, a polymorphism in the *FAM174A*3′ UTR gene region, consisting in 11 guanine nucleotides in length, has been proposed as a genetic marker for EMS in Arabian horses. Here, 14 horses were studied to define the presence of the *FAM174A* 11(G) allele in EMS affected individuals of breeds other than Arabian horse. All horses were exposed to the same environmental risk factors: high concentration of non-structural carbohydrates in the diet, limited exercise and housing conditions; but only 5 out of 14 developed EMS, and only 1 of them carries on copy of the *FAM174A* 11(G) allele, suggesting that it may have a low effect on the development of EMS. Our results confirm the existence of this allele in Andalusian horses, which is considered to be one of the most predisposed breeds to suffer from EMS; nevertheless, its correlation with elevated insulin values and increased frequency of laminitis is inconclusive, given its negligible presence in horses with EMS. Although this allele has been already validated in the Arabian horse, the questionable guidelines followed to identify EMS animals may have led to an inconsistent diagnosis and biased results. Taking these points into account, the *FAM174A* 11(G) allele seems a poor indicator of EMS incidence, probably due to a low effect of the *FAM174A* gene on the etiology of this disease and the complexity of the resulting phenotype. Therefore, further research defining the contribution of genetic factors in the development of the EMS is still needed to improve the management of horses of different breeds at risk of developing laminitis.

Key Words: equine metabolic syndrome, insulin resistance, laminitis, molecular marker, Andalusian horse

P244 Whole-genome population structure analyses of the Jeju horse using the equine high-density SNP array. H.-S. Seong*[1], S. A. Brooks[2], I.-C. Cho[3], B. C. Yang[3], Y. H. Choy[4], T.-J. Choi[4], J. H. Woo[3], S. M. Shin[3], M. C. Shin[3], J. H. Yoo[3], J.-Y. Choi[3], D.-H. Son[1], J.-W. Choi[1], and N.-Y. Kim[3], *[1]College of Animal Life Science, Kangwon National University, Chuncheon-si, Kangwon-do, Republic of Korea, [2]Department of Animal Science, University of Florida, Gainesville, FL, USA, [3]Subtropical Animal Research Institute, National Institute of Animal Science, RDA, Jeju, Jeju, Republic of Korea, [4]Animal Breeding and Genetics Division, National Institute of Animal Science, RDA, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

The Jeju horse is an indigenous Korean horse breed that is currently registered with the Food and Agriculture Organization of the United Nations. However, there are few studies available to compare in genome-wide the Jeju horse population with other horse breeds out of Korean peninsula. This study was conducted to investigate population structures of the diverse horse breeds using 3 horse populations inhabiting in South Korea (Jeju, Thoroughbred, and Jeju crossbred) and 6 horse breeds (Miniature, Fell pony, New Forest Pony, Mongolian, Tuva, and Thoroughbred). Equine 70K SNP Beadchip array was used to genotype the Korean horse populations and SNP data sets for the 6 horse breeds that were originally genotyped by Equine 50K SNP Beadchip were downloaded to be used in this study. We extracted 42,357 concordant markers between those 2 SNP data sets; subsequently the concordant marker set went through quality control, resulting in 242 horses

and 36,646 SNPs remained for further analyses. The genetic differentiation level was evaluated by F_{ST} calculation, exhibiting Thoroughbred sampled from United States had the lowest F_{ST} value (0.004) with other Thoroughbred from United Kingdom and Ireland. The F_{ST} value between Mongolian and Tuva horse also showed a low F_{ST} value (0.007), which might be due to their close geographic locations; whereas F_{ST} value was the highest (0.175) between Fell pony and Thoroughbred from United Kingdom. The Jeju horse had the lowest genetic differentiation level with Tuva (0.044) and Mongolian horse (0.044). The genetic relationship was further assessed by principal component analysis (PCA) and ADMIXTURE analysis. In the PCA result, Thoroughbreds were clearly separated from other breeds, and the result was further assured by the ADMIXTURE. Furthermore, both Jeju horse and Jeju crossbred showed somewhat dispersed clusters compared with each of other horse populations used in this study.

Key Words: horses, SNP, population genomics, admixture

P245 Genome-wide association study of muscular activity to identify genomic regions associated with Polysaccharide storage myopathy type 1 in Austrian Noriker horses. N. Khayatzadeh*[1], R. R. Zsoldos[1,2], T. F. Licka[3,4], G. Mészáros[1], B. Fuerst-Waltl[1], T. Druml[3], G. Brem[3], and J. Sölkner[1], *[1]University of Natural Resources and Life Sciences (BOKU), Vienna, Austria, [2]University of Queensland, Gatton, Queensland, Australia, [3]University of Veterinary Medicine Vienna, Vienna, Austria, [4]University of Edinburgh, Midlothian, Scotland, United Kingdom.*

Polysaccharide storage myopathy (PSSM) type 1 is an inherited glycogen disorder in horses with clinical signs of exercise intolerance, stiffness and muscle spasms. It is characterized by accumulation of abnormal polysaccharides and excessive glycogen in skeletal muscle fibers. The *GYS1* deleterious mutation on ECA10 (19.19–19.21 Mb), encoding *glycogen synthase 1* enzyme, is overexpressed in PSSM type 1 affected horses. The *GYS1* mutation is prevalent in Austrian Noriker horse, but strongly selected against since this has been possible, resulting in fewer heterozygous and very few homozygous carriers. A genome-wide association study (GWAS) was used to investigate possible associations between muscular activity and *GYS1* mutation. Sensors combining surface electromyography (sEMG) with accelerometry were used to measure muscle activity and motion cycles. Density of muscle activity within each motion cycle was counted as crossing the base line. Horses with valid signals were genotyped with the Affymetrix Axiom EquineHD Chip and after standard quality control, 339,334 single nucleotide polymorphisms (SNP) were used for 74 horses (53 G/G, 20 G/A, 1 A/A) for muscle activity in walk and 33 horses (28 G/G, 5 G/A) for muscle activity in trot, with 1,178 and 259 motion cycles, respectively. The baseline crosses within each motion cycle ranged from 9.93 to 162.21 for walk and 19.17 to 148.92 for trot. The GWAS results revealed significant regions on ECA2, 3, 6, 7, 11, 13, 14, 15, 16 and 18. We identified several novel genes responsible for glycolysis (*SLC25A18*, *ATP6V1E1*, *TNFAIP16*, *MIR141*, *MIR200c*, *PHB2* and *RBP5*), collagen and myocytes development (*CIITA*, *COL26A1* and *MYL10*). The most important find was the *NEB* on ECA18, related to glycogen synthase in skeletal muscle. No significant association was found for *GYS1* mutation and sEMG signals, which could be due to small number of measured horses, carrying *GYS1* mutation, especially in trot.

Key Words: GWAS, *GYS1*, muscular activity, Noriker, sEMG

P246 Population genetic structure evaluations of the Iranian horse breeds by SSR and SNP markers. M. Abdoli*[1], M. B. Zandi[1], and M. T. Harkinejad[1], *[1]University of Zanjan, Zanjan, Iran, [2]University of Zanjan, Zanjan, Iran, [3]University of Zanjan, Zanjan, Iran.*

The population genetic structure of Iranian horse breeds as primeval population in the globe has yet to be comprehensively studied, and the ancestral origins of Caspian, Iranian Arab, Kurdish, Turkmen and Dareshouri remain in query. In the present study by 11 ISAG microsatellite (STR) and 65k SNP markers the population structure and ancestral origins was studied. For this purpose, 565 STR result (113 for each breed) and 167 SNP marker results were applied. Based on STR markers the total number of observed alleles was 119 alleles, and the average number of alleles was also 10.818 alleles. Turkmen and Iranian Arab horse breed has highest (0.681) and lowest heterozygosity (0.624) respectively. Phylogenetic analyses showed that Caspian and Kurdish horse breeds have more genetic similarity and were placed into a single cluster and other breeds were placed in separate genetic cluster. We compared clustering and demographic differentiation of SNP with STR genotype and the results of both methods coincided. In general, the results of this study indicate that the genetic clustering of the populations is consistent with their geographic distances. These results confirm the assumption that the Kurdish and Caspian horses are close to Nisa horses, their ancestors.

Key Words: genetic structure, STR markers, SNP, Iranian horse breeds

P247 Abstract withdrawn

P248 Whole-genome sequencing reveals the genetic mechanisms underlying the high-altitude adaptation in Tibetan horses. X. Liu*, Y. Ma, and L. Jiang, *Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.*

Chinese horse breeds had experienced a very long history of breeding, with good adaptation to extreme environments. We tried to detect genome-wide selective signatures toward the high-altitude adaptation of Tibetan horse. Tibetan horse populations have evolved mechanisms that allow them to survive and perform as the major transporter for local farmers at an altitude of 4500 m on the Qinghai-Tibetan Plateau. We performed whole genome resequencing of about 138 horses from all around China, including Tibetan horses (65), lowland horses (61), Przewalski (5) and Thoroughbreds (7). A composite of multiple signals from fixation index (Fst), ZHp (Heterozygosity), pi-ratio (nucleotide diversity), and Tajima's D-test revealed the top candidate region for the altitude adaptation reside the gene of *EPAS1*, which has been identified in many species, including human, goat, sheep and yak. We found 2 significant SNPs in EPAS1 (HIF2a) gene. Interestingly, the overexpression of these 2 EPAS1 mutants in A549 cells showed significantly increased activities than the wild type protein. In large horse populations (n = 948) these 2 EPAS1 SNPs showed even more remarkable genetic differentiation between Tibetan breeds and lowland horses. By measuring the blood samples on spot, we found significant physiological difference between Tibetan (n = 88) and lowland (n = 85) horses, including lower HMG level and HCT but higher MCHC in Tibetan horse. We also found the metabolic difference between Tibetan and lowland

horses, including higher LDH and higher a-HBDH, suggesting a greater capacity for anaerobic lactate production in Tibetan horses. These results suggest that mutations in EPAS1 gene contribute to a hematology and metabolic basis in Tibetan horses, implying a quite similar adaptive mechanism of the Sherpa. Therefore, we tested the downstream target of EPAS1 gene, such as VEGFA, EDN, VHL, EGLN, and GLUT1 after the overexpression of 2 EPAS1 mutants separately in A549 cells. Both mutations lead to upregulation of all these 5 targets. Our study suggests that the 2 missense EPAS1 mutations represent key evolutionary changes underlying the adaptation to high-altitude hypoxia in Tibetan horses.

Key Words: Tibetan horse, hypoxia adaptation, *EPAS1*, genomic selection signatures, metabolic basis

P249 Identification of shared and species-specific k-mers in Equids and Caballines to characterize adaptive introgression events. K. de Silva*[1], E. Bailey[2], and T. S. Kalbfleisch[2], *[1]University of Louisville, Louisville, KY, USA, [2]University of Kentucky, Lexington, KY, USA.*

In a recent study, an allele was reported for the equine gene *CXCL16* that conferred susceptibility for persistent shedding of the Equine Arteritis Virus. It is comprised of non-synonymous variants at 4 positions in exon 1 of the gene producing 2 alternative proteins, one susceptible *CXCL16*S and the other resistant *CXCL16*R (1). The susceptibility allele contains a haplotype block that differs by between 15 to 18 SNPs from the resistant haplotype in the horse within the 1000bp window containing it. The susceptibility allele differed by far fewer SNPs (3 to 4) from corresponding locus in non-caballine equids. As such, this allele is likely the result of adaptive introgression from a non-caballine species. We are developing a method whose objective is the identification of the likely species of origin of this introgressed segment. Our method is a k-mer-based analysis which aims to provide new biological insight in our search for evidence of historical gene transfer events between species. The data consists of whole genome sequence from 5 female thoroughbreds including Twilight (the reference animal for the equine genome) and 8 non-caballines (Somali ass, Donkey, Kiang, Grevyi, Onager, Zebra, Quagga and Boehmi). We used Jellyfish v2.2.6 to count 21 bp canonical k-mers in Illumina WGS data with no bloom filter or high or low cut-off. We counted all k-mers, including those with low frequency to ensure a high proportion of unique k-mers between sample groups. K-mers from 4 caballines including Twilight were compared against the k-mers of 8 non-caballines to identify the caballine specific k-mers and those shared among all equids. Of a total 8,308,928,880 k-mers analyzed from caballine whole genome we identified 4,101,987,596 k-mers (49.37%) specific to caballines and 4,206,941,284 k-mers (50.63%) shared between both groups. When we compare k-mers in EquCab3 chr11, out of 250,865,144 k-mers 172,167,721 k-mers (68.63%) specific to caballines and 78,697,423 k-mers (31.37%) shared between both groups. We will identify k-mers specific to each species and those shared between horse and only some of the other non-caballines species and finally the corresponding genes which are ancestrally shared. The data will guide us to a more complete understanding of the CXCL16 introgression event and provide a foundation to study other events that have contributed to the evolution of the modern horse.

ISAG-FAO Genetics Diversity

P250 Population structure of Blanco Orejinegro (BON) cattle from germplasm bank using pedigree analysis. RJ Ocampo*, EJ Ramirez, and GJ Restrepo, *Corporación Colombiana de Investigación Agropecuaria AGROSAVIA, San Roque, Antioquia, Colombia.*

The Blanco Orejinegro breed is one of the 7 Colombian indigenous bovine breeds which are found in the country approximately 500 years ago and descend directly from the Spanish bovines brought to the American continent during the time of the conquest. Currently, almost all indigenous bovine breeds are at risk of disappearing due to the preferential use of foreign breeds and absorbent crosses to the Zebu. To conserve the indigenous breeds, the National Germplasm Banks System for Food and Agriculture (SBGNAA) was created, whose populations have been remained as closed nucleus over time, which can have an impact on the conservation of the genetic variability due to the increase of the homozygosis. The objective of this study was to characterize the genetic structure of the Blanco Orejinegro germplasm bank population maintained at the Nus research center of AGROSAVIA by pedigree analysis. A total of 4423 data collected between 1980 and 2018 were analyzed to estimate the generational interval, the pedigree completeness, the consanguinity and its evolution over time. The animals born between 2014 and 2018 were taken as reference population to estimate the effective population size and parameters derived from the probability of genetic origin. The average generational interval for the period studied was 4.58 years and the general consanguinity was 1.28%. For the reference population, the consanguinity was 2.8% and the effective population size was 122.6, while the effective number of founders and ancestors was 56 and 38, respectively. The results indicate that the population maintained in the germplasm bank maintains good genetic diversity and low levels of consanguinity. To maintain and increase the genetic variability, it is recommended to use a greater number of males in reproduction.

Key Words: cattle and related species, databases/repositories, effective population size, inbreeding, conservation

P251 Abstract withdrawn

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P252 Whole-genome assessment of goat breeds in Russia. T. Deniskova*[1], A. Dotsev[1], M. Fornara[1], M. Selionova[2], H. Reyer[3], K. Wimmers[3], G. Brem[1,4], and N. Zinovieva[1], *[1]L.K. Ernst Federal Science Center for Animal Husbandry, Podolsk, Moscow region, Russia, [2]All-Russian Research Institute of Sheep and Goat Breeding, Stavropol, Russia, [3]Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Mecklenburg-Vorpommern, Germany, [4]Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria.*

Being unpretentious to feed and easy to keep, goats are wide spread livestock among Russian smallholders and are used to produce milk (Karachaev, Dagestan Milk Aboriginal) and high-quality downhair (world famous Orenburg and Altai Mountain), and wool (Soviet Mohair). In addition, Saanen goats are bred at the large commercial milk farms as well. Whole-genome characterization of the Russian goat genetic resources will contribute into conservation of valuable genotypes of local breeds for future generations and will be used in selection of Saanen breed on the large goat-rearing milk farms. Using the Illumina Goat 50K SNP BeadChip, we genotyped 132 samples collected from Russian local goat breeds including Orenburg (OREF, n = 22), Altai Mountain (ALTM, n = 25), Dagestan Milk Aboriginal (DAGM, n = 17), Karachaev (KARA, n = 23) and Soviet Mohair (SOVM, n = 22) as well as from Saanen breed (SAAN, n = 23) which is very popular in Russia. QC and data processing were performed in PLINK v1.9. In addition, we used the R packages 'diveRsity', 'ggplot2' and software SplitsTree 4.14.5, Admixture v1.3 for data analysis. Within the studied breeds the lowest diversity was observed in KARA (He = 0.378; Ar = 1.970), while its highest level was found in SAAN (He = 0.412; Ar = 1.992) and DAGM (He = 0.428; Ar = 1.996). The MDS results showed a clear separation of SAAN from the other breeds by the First Component. KARA was isolated from the other groups by the Second Component. Besides, SAAN was connected with Russian local breeds via DAGM (Fst = 0.059) at the Neighbor Net graph. The different ancestry of SAAN was supported by the longest Fst values ranged from 0.107 with ALTM to 0.133 with KARA. The lowest Fst values were calculated between OREF and ALTM (Fst = 0.025). The Fst values between KARA and the remaining breeds were higher than 0.063. Summing up the key results of Structure analysis with K-values from 2 to 6, we found that 4 Russian local goat breeds as well as SAAN had strong population structure while DAGM was of admixed origin. Our study provides the first insight into current genetic status of Russian local goat breeds and creates the foundation for future breeding and conservation strategies. The reported study was funded by RFBR according to the research project No. 18–316–20006.

Key Words: goats, genotyping, population structure, biodiversity, breed diversity

P253 Phylogenetic analysis of Nepalese goats using mtDNA D-loop region and SRY gene sequences. Y. Nomura*[1], T. Kunieda[2], M. Shah[3], F. Kawaguchi[1], S. Sasazaki[1], and H. Mannen[1], *[1]Graduate School of Agricultural Science, Kobe University, Kobe, Japan, [2]Graduate School of Agricultural Science, Okayama University, Okayama, Japan, [3]Regional Agriculture Research Station in Kathmandu, Kathmandu, Nepal.*

Nepal is a landlocked country lying between India and Tibetan plateau and has diverse geographic altitudes range from less than 80 m above sea level (masl) in the south to 8848 masl on the northern border. In Nepal, goats are one of the most important domestic animals for meat and hair purposes. There are 4 indigenous breeds based on the different altitudinal regions of breeding; alpine (Chayngra), mountainous (Sinhal), hilly (Khari), and plain (Terai) regions. In this study, we investigated the phylogenetic relationships and genetic structures for 4 breeds of Nepalese indigenous goats using mtDNA D-loop region and *SRY* gene sequences. In addition, we estimated the genetic structures among Eurasian, African and Nepalese goats based on the frequencies of mtDNA and *SRY* haplotypes. We determined mtDNA HV1 (481bp) and *SRY* gene 3′ UTR (543bp) sequences for 136 Nepalese indigenous goats. We examined genetic relationships using neighbor-joining tree (mtDNA) and median-joining network (*SRY*), and then analyzed them in conjunction with previously published data from Eurasian and African populations, which are 4152 mtDNA and 1086 *SRY* sequences. In the present study, mtDNA sequences in Nepalese goats revealed 77 haplotypes, which were categorized into 4 haplogroups of A, B, D and G. The haplogroups A and B were observed in all breeds (n = 111 and 17, respectively), while haplogroup D was observed in Chyangra (n = 6) and Sinhal (n = 1), and haplogroup G (n = 1) was only in Chyangra. In male goats (n = 87), 3 *SRY* haplotypes (Y1A, Y2A and Y2B) were observed. The predominant haplotypes were Y1A (55%, n = 48) and Y2B (43%, n = 37), and minor haplotype of Y2A was observed only in Khari (2%, n = 2). The comparison among Nepalese, Eurasian and African populations by mtDNA and *SRY* data suggested that Chyangra has the genetic similarity with Tibetan goats, Sinhal and Khari with Bhutanese goats in the Himalayas country, and Terai with Indian goats. These results revealed the different genetic structures among Nepalese goat breeds, suggesting the different genetic backgrounds based on the geographic altitudes, genetic admixture and/or the propagation routes.

Key Words: diversity, goats, mitchondrial DNA, phylogeography, SRY gene

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P255 Genetic diversity and structure in 11 native Asian goat populations analyzed by high density SNP array. K. Iso*[1], S. Sasazaki[1], F. Kawaguchi[1], T. Yonezawa[2], J. Wu[3], K. Nomura[2], Y. Takahashi[2], E. Kobayashi[4], M. Shah[5], O. Faruque[6], J. Masangkay[7], M. Bakhtin[8], P. Kazymbet[8], T. Dorji[9], H. Mannen[1], *[1]Laboratory of Animal Breeding and Genetics, Agricultural Science, Kobe University, Japan, [2]Faculty of Agriculture, Tokyo University of Agriculture, Japan, [3]School of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan, [4]NARO Institute of Livestock and Grassland Science,*

Japan, [5]Regional Agriculture Research Station in Kathmandu, Nepal, [6]Faculty of Animal Husbandry, Bangladesh Agricultural University, Bangladesh, [7]University of the Philippines, Los Banos, Philippines, [8]Astana Medical University, Radiobiology Scientific Center, Kazakhstan, [9]Department of Livestock, Ministry of Agriculture, Bhutan.

In this study, we investigated the genetic diversity and population structure using the high density SNP array in Asian goats. We genotyped a total of 223 animals from 11 Asian native goat populations of Central Asia (Kazakhstan, Mongolia), South Asia (Bhutan, Nepal, Bangladesh), and Southeast Asia (Myanmar, Philippines, Laos, Vietnam, Cambodia mountain, Cambodia plain) by using 50k illumine BeadChip. The expected (*He*) and observed (*Ho*) heterozygosities were calculated in each population. The *He* ranged from 0.2656 to 0.4067, suggesting high genetic diversities in Kazakhstan (0.4067) and Mongolia (0.3914), and low in Cambodia mountain (0.2656). The correlation between the *He* and the geographical distance from goat domestication centers (Taurus, Turkey and West Zagros, Iran) was investigated to assess the trend of genetic diversity according to the distance. The correlation showed decreasing trend of genetic diversity ($r^2 = -0.76$). This is consistent with common consensus that West Asia is the major center of origin for modern Asian domestic goats. Neighbor-joining tree (NJ) and Neighbor-joining network (NW) were constructed using pairwise *Fst*. In both NJ and NW, 3 clusters were formed constructed based on the geographical locations (Southeast Asia, South Asia & Myanmar, Central Asia). In NW, reticulate connections were observed among Southeast Asian populations, and among South Asia and Myanmar populations. This result suggested the genetic admixtures among the populations. In addition, Cambodia mountain, Philippines and Vietnam were well diverged from other populations. Subsequently, we performed the ADMIXTURE analysis to estimate genetic structures. At K = 2, Cambodia mountain and Central Asia (Kazakhstan, Mongolia) were assigned to distinct clusters. At K = 6, Southeast Asian populations formed different clusters by each population, and South Asian populations and Myanmar were grouped into one cluster. These results indicated that the genetic structure of the 11 populations was assigned into 3 geographical areas of Central Asia, South Asia and Southeast Asia, and suggested genetic exchange between neighbor countries.

Key Words: diversity, goats, SNP, admixture

P256 Genetic diversity of *Bos taurus* and *Bos indicus* using developed indel markers. T. Yasui*[1], H. Yamanaka[1], F. Kawaguchi[1], S. Sasazaki[1], T. Yonezawa[2], J. Wu[3], and H. Mannen[1], *[1]Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Japan, [2]Faculty of Agriculture, Tokyo University of Agriculture, Atsugi, Japan, [3]School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, Japan.*

The indels (insertion/deletion) are useful genetic markers for identification of identical by descent (IBD). Since indels are considered to be low recurrence of mutations (back mutations), it could be suitable for estimating genetic structure and diversity. In the present study, we developed indel markers and investigated genetic diversity in 2 subspecies of cattle, *Bos taurus* and *Bos indicus*. At first, we carried out whole genome resequencing for 3 individuals of *Bos indicus* (native cattle in Bangladesh, Laos and Cambodia). Compared these with reference sequence of *Bos taurus*, we randomly selected 2 indels, which are more than 50bp and polymorphic between 2 subspecies, from each 29 autosomes. Using a total of 58 indel markers, 8 *Bos taurus* (breeds of Japanese Brown, Japanese Shorthorn, Japanese Black, Hanwoo, Holestein, Angus, Hereford, Limousine) and 8 *Bos indicus* (native cattle in Vietnam, Laos, Bangladesh, Cambodia, Myanmar) were genotyped. As results, 47 out of 58 indels were successfully amplified in 8 *Bos taurus* and 8 *Bos indicus* and were grouped into 5 types (Type-A to E) on the basis of polymorphic features: Type-A is monomorphic in all samples (5 indels), Type-B is polymorphic in both subspecies (15 indels), Type-C is polymorphic only in Bos indicus (17 indels), Type-D is polymorphic only in Bos taurus (6 indels), and Type-E genotypes completely differed between 2 subspecies (4 indels). The expected heterozygosity (He) is higher in Bos indicus (0.198) than in Bos taurus (0.136). Since Types-C, D and E polymorphisms indicated deviation of the genotypes in 2 subspecies, we further genotyped these 27 indels (Type-C, D, E) in 30 *Bos taurus* (Japanese Brown, Japanese Shorthorn Japanese Black, Hanwoo, Holestein, Angus and Hereford, Limousine) and 30 *Bos indicus* (native cattle in Vietnam, Laos, Bangladesh, Cambodia, Myanmar). Type-C (15 indels) is significantly more than Type-D (3 indels) ($P = 0.028$). These results suggested that *Bos indicus* has higher genetic diversity than *Bos taurus*.

Key Words: cattle, indel, diversity

P257 Changes in allelic frequencies when different genomic coancestry matrices are used for mantaining genetic diversity. E. Morales-Gonzalez*[1], J. Fernandez[1], R. Pong-Wong[2], and B. Villanueva[1], *[1]Departamento de Mejora Genetica Animal, INIA, Madrid, Spain, [2]The Roslin Institute and R(D)SVS, University of Edinburgh, Edinburgh, UK.*

One of the main objectives in a conservation program is to maintain genetic variability. However, it has been argued that the program should preserve genetic variation as closely as possible to that of the original population and thus it should aim at maintaining allelic frequencies. Nowadays it is accepted that the most efficient strategy to control the loss of genetic variability and the increase of inbreeding is the Optimal Contributions (OC) method. This method optimizes the contributions of candidates for minimizing coancestry and thus, under random mating, minimizes the expected inbreeding in the next generation. Thus, a fundamental element on the OC method is the coancestry matrix that contains the coancestry coefficients between all the candidates. Following the development of genomic tools, several measures of genomic coancestry have been developed and their relative efficiency for maintaining genetic variability when used in OC has been investigated. However, the choice of the genomic relationship matrix to be used will have an impact not only on the variability maintained, but also on the change in allelic frequencies. Thus, the objective of this study was to evaluate, through computer simulations, the genetic diversity maintained and the trajectory of allelic frequencies when different genomic coancestry matrices are used in OC. In particular, we compared 2 of the most commonly used genomic coancestry matrices: i) the matrix based on deviations of the observed number of alleles shared between 2 individuals from the expected numbers under Hardy-Weinberg equilibrium (θ_1); and ii) the matrix based on VanRaden's genomic relationship matrix (θ_2). The genetic variability was measured as the expected heterozygosity and the change in allelic frequencies was measured using the Kullback–Leibler divergence criterion. Results indicate that θ_1 not only maintain more genetic variability than θ_2 but also keep allelic frequencies closer to their original values.

Key Words: genetic diversity, allelic frequencies, genomic coancestry matrix, optimal contributions

P258 The genetic diversity studies for natural treasure chicken of South Korea. D. Seo*[1], D. H. Lee[1], S. S. Lee[1,2], S. H. Lee[1], and J. H. Lee[1], *[1]Division of Animal and Dairy Science, Chungnam National University, Daejeon, South Korea, [2]Yeonsan Ogye Foundation, Nonsan, South Korea.*

The Yeonsan Ogye is a chicken breed that has been passed down since the Gorye Dinasty. After Joseon Dynasty, it has been maintained and descended as a population by one family group and they were declared as one of the Korean natural treasure in 1980. The main morphological phenotype of this breed is their black color, including feathers, muscle tissue, skin and bone. It looks similar to Chinese and Japanese silky, but they have different characteristics. The Yeonsan Ogye breed has been managed as a closed population without pedigree and mating records. Thus, in recent years, awareness of the importance of inbreeding control has led to the planning of breeding programs. As a first step, we carried out genomic 600K high-density SNP genotyping of all males (136) and some of the females (54) to confirm genetic characteristics

and diversity of Yeonsan Ogye breeds. The analyses of the genomic information revealed that the genetic characteristics were distinct from other Korean chicken breeds and genetic components also clearly distinguished from other populations. In addition, it was confirmed that this population showed more than 0.2 of F_{st} value compared with other populations, and an r^2value of LD decay confirmed the very low converged value of less than 0.1 in other chicken populations. The results of heterozygosity decay showed low decline tendency from one generation to the next. As the result of genomic data analysis, Yeonsan Ogye population has been relatively well maintained in terms of their genetic diversity despite the lack of pedigree data recording. However, it seems necessary to establish a population conservation strategy that uses genetic information for efficient maintenance and management for the future population.

Key Words: Yeonsan Ogye, genetic diversity, linkage disequilibrium decay, heterozygosity decay

P261 Genomic analysis of sheep remains from the 4th to 5th century AD Roman villa at São Miguel de Odrinhas, Portugal.
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Seven thousand years ago, wild sheep were absent from the Iberian Peninsula. Zooarcheological evidence indicates they were probably introduced here by boat in the Early Neolithic. Also, genetic diversity found among extant Iberian native breeds supports the influx of new stock from overseas at some as yet unknown time. Diachronic ancient DNA studies are needed to investigate evolutionary trajectories in domestic animals, but genetic data from archeological specimens of sheep collected in Iberia are lacking. In 2004, sheep remains belonging to 7–8 individuals were recovered from a well in São Miguel de Odrinhas (SMO), a Roman villa located near Sintra, close to Lisbon, dated to the 4th/5th century AD. The Romans improved the quality of the wool of their sheep in southern Spain, this may have occurred at the same time in southern Portugal. Even today the commercial fine-wool sheep breed, the Merino, is reared in the southern part of the Iberian Peninsula whereas coarse-wool sheep prevail in central and northern regions. Nine of these bone/teeth remains were sub-sampled for ancient DNA analysis. Bone measurements indicate that SMO sheep are among the smallest recorded so far from Portugal. Whole-genome re-sequencing data (1.23–1.62% endogenous sheep DNA) were generated for 2 metacarpals and one astragalus. In addition, it was possible to assemble mitogenomes for these as well as for one metatarsal and one molar tooth. High nucleotide damage patterns and short fragment lengths, typical of ancient DNA, allowed us to authenticate these results. Phylogenetic analysis showed that all SMO sheep belong to mitochondrial haplogroup B, which is common worldwide in sheep, including Iberian Merino and coarse wool breeds. Genome-wide diversity will be used to investigate patterns of admixture and relationships among these Roman sheep and native breeds from Iberia and neighboring Mediterranean regions. To our knowledge, this is the first genomics study of ancient Iberian sheep. It aims to understand the development of Merino sheep during the Roman occupation of the Iberian Peninsula.

Key Words: Iberian native sheep, Roman sheep, whole-genome re-sequencing, mitogenome, ancient DNA

P263 Ancestry diversity versus genetic diversity in Creole cattle.
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Creole cattle suffered a long process of expansion and adaptation to different environments since their introduction in the Americas by the Portuguese and Spanish in the 15th Century. In the last years, important studies have been carried out to provide a deeper information about their genetic diversity and population structure. This knowledge has provided a rational basis for the conservation and possible use of American native cattle breeds as genetic resources with high levels of biodiversity. The objective was to study of the influence of the ancestry diversity of the breeds on their current genetic diversity. We used the information provided by a set of 19 microsatellites common to 109 cattle breeds distributed worldwide. The sampling strategy was designed in the context of the BioBovis Consortium and include a comprehensive representation of 40 Creole cattle breeds covering the whole American continent and cattle breeds as potential sources of genetic influence on Creole cattle from Portugal, Spain, British Islands, Continental Europe, Africa and American zebu. A model-based Bayesian clustering analysis was used to infer the number of populations (K) based on the e*stimated Ln Prob of Data* (LnPr(X/K)) and the level of admixture in the cattle breeds implemented in STRUCTURE v2.3. The inferred K value was 57. The ancestry diversity for each breed was calculated as $1-\Sigma(q_k)^2$, where q_k is the average fraction of the separate genetic clusters at the K value of 57 obtained in STRUCTURE analysis. We used as estimator of the present genetic diversity the unbiased expected heterozygozity estimates (He). Because the unbalance of the number of samples for each breed (N) we estimate the correlation of both parameters, $1-\Sigma(q_k)^2$ and He, with N. The results were no significant with p-values of 0.43 and 0.42 respectively. A linear analysis of expected heterozygosity as a dependent variable and breed ancestry diversity as explanatory variable was performed. Results showed that ancestry diversity account for 53% of the expected heterozygosity variability. The country of origin of the breed sampled had a significant effect ($P < 0.01$) both to the expected heterozygosity and breed ancestry diversity.

Key Words: cattle and related species, animal breeding, genotyping, biodiversity, conservation

P264 Abstract withdrawn

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P265 Genetic characterization of Spanish autochthonous chicken breeds using microsatellites. J. L. Vega-Pla*[1], A. M. Martinez[2,3], A. Pons[7], A. Arando[2], A. Canales[2], N. Garcia[4], M. M. Gomez-Carpio[2,3], C. Gonzalez-Felgueroso[5], V. Landi[2,3], J. M. Leon-Jurado[6], M. Macri[2,3], S. Nogales[2], G. Pizarro[2], S. Verges[8], M. E. Camacho[9], *[1]Laboratorio de Investigacion Aplicada, Servicio de Cría Caballar de las Fuerzas Armadas, Cordoba, Spain, [2]Departamento de Genetica, Universidad de Cordoba, Cordoba, Spain, [3]Animal Breeding Consulting S.L., Universidad de Cordoba, Cordoba, Spain, [4]CICYTEX Finca La Orden-Valdesequera, Guadajira, Badajoz, Spain, [5]Asociacion de Criadores de Pita Pinta Asturiana, Oviedo, Asturias, Spain, [6]Centro Agropecuario, Diputacion de Cordoba, Cordoba, Spain, [7]SEMILLA, Palma, Spain, [8]FEPIRA, Ibiza, Spain, [9]IFAPA Alameda del Obispo, Cordoba, Spain.*

Genetic diversity and population structure of 13 autochthonous Spanish chicken breeds were studied using 30 microsatellites as genetic markers. Two commercial purebred populations were used as reference populations for comparison. The analyses included DNA samples of 1296 animals. Observed and unbiased expected heterozygosities per breed ranged from 0.40 (Combatiente Español) to 0.57 (Utrerana Blanca) and from 0.44 (Combatiente Español) to 0.61 (Extremeña Azul) respectively, with slight differences among overall means for observed (0.49) and expected (0.54) heterozygosity. The values of genetic distances D_A and the values of F_{ST} between pairs of populations showed a greater genetic differentiation in the Leghorn breed. The Neighbor-Net method as implemented in SPLITSTREE software was used to compute a network based on Nei D_A genetic distances. Spain Southern breeds grouped together. Bayesian cluster analysis was carried out by STRUCTURE. When the existence of 2 populations is assumed (K = 2), the chicken of southern Spain differentiated from the rest of the populations; in K = 11, considered as the optimum K, all populations grouped independently except for the Blanca and Franciscana varieties of Utrerana breed, hence the Perdiz variety of Utrerana breed maintained its own cluster. In the Factorial Analysis of Correspondence, the first and second axis (accounting for 16.02% and 11.48% of the total inertia, respectively) differentiated the Pita Pinta breed from the rest of breeds. Moreover, Utrerana Perdiz population, Andaluza Azul and Mallorquina breeds could be differentiated when third axis (10.73%) is taken into account. Globally, there is a great diversity in the Spanish chicken populations although it is necessary to make an important effort to differentiate and catalog populations as breeds or varieties to ensure their conservation

Key Words: poultry and related species, animal breeding, PCR, breed diversity, breed identification

P266 Integrating in situ and ex-situ genomic data of domestic chicken breeds for conservation in China. M. Zhang* and K. Wu, *China Agricultural University, Beijing, China.*

The effective conservation and use of farm animal especially for chicken are vital for creating and maintaining sustainable increases in the productivity of healthy food for mankind. In situ and ex-situ conservation are 2 main protection strategies for Chinese domestic chicken breeds. However, few studies have compared the genomic diversity and population structure of conserved chicken between ex-situ and in situ populations in China. In the present study, a total of 361 individuals including 3 Chinese domestic chicken breeds collected from in situ and ex-situ populations were genotyped using genotyping-by-sequencing (GBS) to compare the genetic diversity and population structure. After sequence alignment, variant calling and quality control (QC), 1539911 high-quality SNPs were obtained for subsequent analysis. No matter in situ or ex-situ population, all 3 chicken breeds maintained relatively high genomic diversity in terms of heterozygosity (Ho, He), the proportion of polymorphic markers (P_N), allelic richness (A_R) and inbreeding coefficient based on runs of homozygosity (F_{ROH}). Population structure of the 3 native chicken breeds was analyzed using principal component analysis (PCA), neighbor-joining (NJ) tree, and STRUCTURE analysis, the results suggested that population stratification had occurred in different conserved populations. All F_{ST} values between in situ and ex-situ within a breed were higher than 0.05 (from 0.05 to 0.1), indicating that moderate genetic differentiation appeared. The Ne of the in situ population was higher than the ex-situ, for instance, the average Ne (for gga1–5) was 129.44 in YBEC (in situ), while only 50.34 was calculated in BEC15 (ex-situ). We concluded that being small and maintained in controlled environments, ex-situ conserved chicken populations would retain less genetic diversity than in situ. This study brought new insight into conservation genomics used in the actual conserved chicken population and provided the scientific basis for further optimizing the conservation programs in in situ and ex-situ population of Chinese domestic chicken breeds.

Key Words: Chinese domestic chicken, genomic diversity, conservation, in situ, ex-situ

P267 Towards a complete genomic characterization of African indigenous cattle. A. Tijjani*[1,3], K. Marshal[2,3], H. Kim[4,5], H. Jianlin[2,6], and O. Hanotte[7,8], *[1]International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [2]International livestock Research Institute (ILRI), Nairobi, Kenya, [3]Center for Tropical Livestock Genetics and Health (CTLGH), The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom, [4]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, [1]Republic of Korea, [5]C&K genomics, Seoul National University Research Park, Seoul, Republic of Korea, [6]ILRI-CAAS Joint Laboratory on Livestock and Forage Genetic Resources, Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, [7]LiveGene-CTLGH, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [8]Cells, Organism and Molecular Genetics, School of Life Sciences, University of Nottingham, Nottingham, United Kingdom.*

Genetic and genomic variations in African indigenous livestock populations remain huge resources, yet to be tapped. These resources are prerequisite for a complete characterization of local breeds toward genetic improvement contributing to food security and poverty reduction on the continent. The International Livestock Research Institute (ILRI) is leading an unprecedented research effort aiming at harnessing the genetic resources of indigenous cattle breeds across Africa. This is being achieved through an extensive collaboration of African national partners together with ILRI Livestock Genetics program, supported by the University of Nottingham (UK), the ILRI - CAAS Joint Laboratory on Livestock and Forage Genetic Resources (China), Seoul National University (South Korea), and the Centre for Tropical Livestock Genetics and Health (CTLGH) program based at the University of Edinburgh and SRUC (Roslin Institute, UK). So far, samples have been obtained from around 45 cattle breeds of zebu, taurine and admixed origins and comprising of over 1,500 individuals. High coverage (up to 30x) of whole genome re-sequencing of more than 25 breeds consisting of over 250 samples have now been completed. We aim to catalog the entire functional diversity, including SNPs and structural variations, of African cattle. Our current objectives include the identifications of the most informative SNPs for designing the African cattle reference SNP genotyping arrays to be applied to breeding improvement programs and the genomic regions underpinning adaptation and productivity, as well

as the capacity building among African collaborating scientists through training in bioinformatics and genomics. Future activities may include de novo genome sequencing and assembly of African cattle breeds and a transcriptome catalog of gene expression, paving the way to the pan-genome analyses of African cattle.

Key Words: African cattle, functional diversity, adaptation, genomic characterization, SNPs

Livestock Genomics for Developing Countries

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P269 Genome-wide association study of carcass quality using real-time ultrasound scans in South African Nguni cattle. J. de Vos*[1], E. van Marle-Köster[1], and DP Berry[2], *[1]Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, Gauteng, South Africa, [2]Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Co. Cork, Ireland.*

Real-Time Ultrasound (RTU) scans are a non-invasive, cost effective tool to measure the carcass quality on the live animal. In South Africa (SA), RTU scans are not routinely used in feedlots for evaluation of carcass quality in beef cattle. The aim of this study was to identify genomic regions associated with the RTU scans measured twice during a growth trial. The data set contained measurements of 200 Nguni steers finished in a growth trial; Nguni cattle are a Sanga type breed well adapted to the sub-tropical environment in SA. RTU scans of the backfat thickness (BF), rump fat thickness (RF) and eye muscle area (EMA) were measured at d 72 and 91 on test. Of the 200 steers used in the growth trial, 141 were genotyped using the 150k GGP HD SNP array; all animals had to have a call rate in excess of 90%; genotype quality control was imposed to discarded single nucleotide polymorphisms (SNPs) with a low MAF (i.e., < 0.02) or that deviated from Hardy-Weinberg equilibrium. After quality control, genotypes from 124,178 SNPs on 139 animals remained. PLINK as well as EMMAX software was used to perform the association study and a 5% confidence interval was applied. SNPs were associated at a threshold of $P < 10^{-5}$ for RTU measured at 72 d on trial for BF (BTA1, BTA16, BTA25), EMA (BTA2, BTA7, BTA8, BTA9, BTA13, BTA20, BTA25) and RF (BTA5, BTA9, BTA16). Similar SNP associations were detected for the RTU measurements at 92 d on trial with additional SNPs observed for BF on BTA2, BTA3, BTA5, BTA28 and X-chromosome and EMA on BTA12, BTA23, BTA29. Within the genomic regions associated with the traits, 10 genes were identified. The genes *NIPA1*and *SMYD3* were the most applicable to the traits studied and these are involved in binding function. Novel SNP associations were observed for BF: BTA3, BTA5, BTA25, BTA28 and X-chromosome; EMA: BTA25 and RF: BTA1, BTA16. This study is the first genome-wide association study in Sanga cattle for proxies for regarding carcass traits and provides insight into the genes involved in these traits.

Key Words: cattle, genome-wide association, SNP, quantitative trait loci (QTL), meat production

P270 Genome-wide characterization of selection signatures in Ugandan goat breeds. R. B. Onzima*[1,2], M. R. Upadhyay[1,4], H. P. Doekes[1], L. F. Brito[3,5], M. Bosse[1], E. Kanis[1], M. A. M. Groenen[1], and R. P. M. A. Crooijmans[1], *[1]Animal Breeding and Genomics, Wageningen University & Research, Wageningen, The Netherlands, [2]National Agricultural Research Organization (NARO), Entebbe, Uganda, [3]Department of Animal Biosciences, Centre for Genetic Improvement of Livestock (CGIL), University of Guelph, Guelph, ON, Canada, [4]Department of Veterinary Science,Ludwig Maximilian University of Munich, Munich, Germany, [5]Animal Sciences, College of Agriculture, University of Purdue, West Lafayette, IN, USA.*

Both natural and artificial selection are among the main drivers shaping genetic variation across the genome of livestock species. Selection typically leaves signatures in the genome, which are often characterized by high genetic differentiation across breeds and/or a strong reduction in genetic diversity in regions associated with traits under intense selection pressure. We evaluated selection signatures in 6 Ugandan goat breeds: Boer, and the indigenous breeds Karamojong, Kigezi, Mubende, Small East African and Sebei. After genotyping quality control, 45,294 autosomal single nucleotide polymorphisms (SNPs) remained for further analyses. A total of 394 and 6 breed-specific putative selection signatures were identified across all breeds, based on marker-specific fixation index (F_{ST}-values) and haplotype differentiation (hapFLK), respectively. These regions were enriched with genes involved in signaling pathways associated directly or indirectly with environmental adaptation, such as immune response (e.g., IL10RB and IL23A), growth and fatty acid composition (e.g., FGF9 and IGF1), and thermo-tolerance (e.g., MTOR and MAPK3). The study revealed little overlap between breeds in genomic regions under selection and generally did not display the typical classic selection signatures as expected due to the complex nature of the traits. In the Boer breed, candidate genes associated with production traits, such as body size and growth (e.g., GJB2 and GJA3) were also identified. This study provides insights into the effects of long-term selection in Boer and indigenous Ugandan goat breeds, which are relevant for implementation of breeding programs and conservation of genetic resources, as well as their sustainable use and management.

Key Words: *Capra hircus*, homozygosity, adaptation, genetic diversity, selective sweeps

P271 Abstract withdrawn

P271 Abstract withdrawn

P273 Abstract withdrawn

P274 Abstract withdrawn

P275 Genetic diversity and structure of the Mexican Lidia breed bovine based on SNP data. P. G. Eusebi*[1,2], O. Cortés[1], S. Dunner[1], and J. Cañon[1], *[1]Universidad Complutense de Madrid, Madrid, Spain, [2]VELOGEN.SL, Madrid, Spain.*

The bovine Lidia breed is special due to its low genetic exchangeability and its emblematic features in the culture of numerous countries, given its selection on aggressive behavior. The original Lidia population has its origin in the mid-XVIII century in Spain, using aggressive-selected bovines to participate in traditional festivities. In Mexico the Lidia breed was raised as an ancient heritage from the traditional Spanish festivities. The last decade of the XIX century was a determining phase in the specialization of the Mexican Lidia breed, when 2 families of breeders imported Lidia breed bovines from Spain with the aim of specializing their production. Alongside a third line arose, breeding local Mexican bovines selected for aggressiveness and discarding Lidia Spanish animals. This line to date is considered "impure" and sometimes rejected by several breeders. In this study both, genetic diversity and genetic structure of the 3 Mexican Lidia lines were analyzed. Samples of 306 individuals belonging to 32 Mexican herds and 4 lines (3 Mexican and one for recent Spanish imports) were taken for DNA extraction. Blood Samples were collected in Magic Buffer tubes and maintained at 15°C. DNA samples were genotyped on the Illumina BovineSNP50 chip. The softwares PLINK1.07, Admixture 1.3 and Arlequin 3.5 were used for statistical analysis. After quality control (GENO 0.1, MAF = 0.01 and discarding SNPs on sex chromosomes) 41,455 SNPs were taken for further analyses. Mean F_{ST} values ranged from 0.09 to 0.31, the lower genetic distances among herds are the consequence of a relatively frequent exchange of sires. Inbreeding values averaged computed as 1 – Ho/He ranging from −0.03 to 0.29. Population structure inferred by Multi Dimensional Scaling (MDS) and ADMIXTURE coincide in a clear separation between the 3 Mexican

lines, excepting a few Mexican herds that may have introduced recently Spanish animals. The herds arising from the "impure" Torrecilla line clearly cluster in a segregated branch which is coincident with their historical records. The results can be used to manage genetic diversity and further discover selected and shared genomic regions across Mexican lines.

Key Words: genetic variation, population differentiation, fighting bull, mexican cattle, genetic structure

P276 Linkage disequilibrium and haplotype block analysis of SNPs on the *CAST* gene in Boerka goat. D. Maharani*[1], A. Antonius[2], S. P. Ginting[2], S. Elieser[2], A. Tarigan[2], I. G. S. Budisatria[1], A. Batubara[2], D. N. H. Hariyono[1], and A. P. Z. N. L. Sari[1], *[1]Universitas Gadjah Mada, Yogyakarta, Indonesia, [2]Indonesian Goat Research Institute, Sei Putih, North Sumatera, Indonesia.*

The pattern of linkage disequilibrium (LD) in genome is powerful signal in the association gene study. Calpastatin (CAST) gene plays a role and encode in meat quality (tenderization). To define the SNPs, 20 one Boerka (Boer × Kacang) goats reared under control condition in Goat Research Institute, were used for blood sample collection. Five single nucleotide polymorphisms (SNPs) of the CAST gene located in chromosome 7 were found using direct sequencing. The SNPs are g.146C > A (SNP-1), g.224A > G (SNP-2), g.281G > A (SNP-3), g.373C > T (SNP-4) and g.431G > A (SNP-5). Those SNPs were analyzed for LD and haplotype block analysis using haploview software. Four types of haplotype block have been performed to indicate the D'prime, LD, r-square (r^2) and haplotype frequency. As a results, all block types indicated having high D'prime (= 1.0). Every block have different SNPs combination. Block A, B, C and D formed 10, 3, 2 and 1 SNPs combination. The highest LD (1.75) and r^2 (1.00) shown in block A with SNP1 and 2, SNP1 and 5, SNP2 and 5 combinations. The highest (97.6%) haplotype frequencies are defined in combination CA, CG and AG haplotypes located in SNP1 and 2, SNP1 and 5, SNP2 and 5, respectively. The lowest LD (0.01) and r^2 (0.0010) were found in SNP1 and 4, SNP2 and 4 and SNP4 and 5 combinations. In block B, 3 types of SNPs combination were made and indicated having average haplotype frequency (57.1%). For block C and D, 2 and one SNP combination observed with similar haplotype frequency (57.1%). In conclusion, these information of LD and characterization of haplotype block structure may be useful parameters for guiding the CAST gene association study with economic traits especially for meat quality in Boerka goat population in the future study.

Key Words: Boerka goat, CAST gene, haplotype, linkage disequilibrium, single-nucleotide polymorphism

P277 Abstract withdrawn

P277 Abstract withdrawn

P278 Abstract withdrawn

P279 Genome adaptation of indigenous Ethiopian cattle to high altitude and heat stress. E. Terefe*[1,2], G. Belay[1], K. Marshal[3,6], O. Hanotte[4,5], and A. Tijani[2,5], *[1]Addis Ababa University, College of Natural Science, Microbial Cellular Molecular Biology, Addis Ababa, Ethiopia, [2]International Livestock Research Institutes, Addis Ababa, Ethiopia, [3]International Livestock Research Institute, Nairobi, Kenya, [4]LiveGene – CTLGH, International Livestock Research Institute, Addis Ababa, Ethiopia, [5]School of Life Sciences, University of Nottingham, University Park, Nottingham, UK, [6]Center for Tropical Livestock Genetics and Health, The Roslin Institute, University of Edinburgh, Edinburgh, UK.*

Genetic background governs animal adaptation to environment. Adapted animals genetically withstand environmental challenges and reproduce. The genetic control of adaptive traits may be revealed following signature of selection analysis. Taking advantage of the diversity of Ethiopian agro-ecologies, we analyzed at autosomal genome level the diversity and genetic control of environmental adaptive traits of 4 indigenous Ethiopian cattle, representing populations living at high altitude (*HA*); Bale (3586*m*) and Semien (3732*m*), and low altitude (*LA*); Afar (729*m*) and Bagaria (680*m*). A total of 36,368,321 SNPs were identified following alignment against the new cattle genome of reference (ARS1-UCD). Genome-wide scan for candidate positive signature of selection were analyzed using within population pooled heterozygos-

ity (*Hp*) and between population differentiation (F_{ST}) tests. *Hp* analysis reveals 177, 130, 119 and 96 candidate genes under positive selection in Afar, Bagaria, Bale and Semien cattle, respectively, and 191 genes were identified in *Fst* test contrasting the 2 *LA* and *HA* populations. *RRAD, PSMA7 PSMD7, CXXC5* and *UBE2D2* are among the candidate genes with functions which might be related to *HA* adaptation. While *PFDN1, DNAJC18, DNAJB4*, *DCLK3,* and *TRANK1* may have function related to heat stress response. These results contribute to the ongoing effort to identify genetic and genomic variants related to adaptation and livestock improvement and conservation programs of indigenous African cattle.

Key Words: altitude adaption, genome re-sequencing, heat stress, selection signature, indigenous cattle

P280 Integrating Ecological Niche Modelling with genomics to dissect tropical adaptation in Ethiopian indigenous chicken. A. Vallejo*[1], A. Kabede[2,3], M. Lozano[4], D. Tadelle[2], N. Sparks[5], J. Smith[5], O. Hanotte[1,2], and A. Gheyas[5,6], *[1]Cells, Organism and Molecular Genetics, School of Life Sciences, University of Nottingham, Nottingham, United Kingdom, [2]LiveGene-CTLGH, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [3]Amhara Regional Agricultural Research Institute, Bahir Dar, Ethiopia, [4]Wageningen University & Research Animal Breeding and Genomics, Wageningen, The Netherlands, [5]The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom, [6]Centre for Tropical Livestock Genetics and health (CTLGH), The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom.*

Ecotypes of livestock species are genetically adapted to their local environments. Characterizing ecotypes of indigenous livestock species in terms of their genetic and environmental predictors may help preserve their livestock biodiversity and develop strategies for improved production. In this study, we apply an integrative approach of Ecological Niche Modeling (ENM) and genomics to identify ecotypes of indigenous chicken in the diverse agro-ecologies of the Ethiopian landscape and to dissect the genetic basis of their adaptation. Two hundred 53 chicken samples from 25 populations across Ethiopia were collected and genome sequenced. ENM was applied to characterize the ecological niches of these populations using 34 agro-climatic variables with the program MaxEnt. Based on the similarity of ecological niche distribution, the populations were grouped into 12 different ecotypes. To identify the genomic regions that may be driving local adaptations in these ecotypes, *Fst* based selection signature analysis was performed using genome-wide SNP data in sliding windows. *Fst* values > 0.3 (representing less than 1% of the windows) were considered as significant in pairwise ecotypes comparison. The analysis identified several candidate regions that overlapped with genes associated with various biological pathways. Some notable examples are *CPA6, DCLK1, SDK1* and *TCF12* (related to neuronal regulation and development), and *TEAD4* and *NRXN3* (related to skeletal muscle regulation and angiogenesis). These genes and the other regions identified may be related to local adaptations and can be used as candidates for further genotype-environment association studies in search for adaptations to tropical conditions. This study exemplifies the use of a landscape genomics approach for characterizing livestock species and for studying their adaptations to the local environment.

Key Words: poultry and related species, adaptation, landscape genomics, modelling, biodiversity

P281 Genetic diversity analysis of Creole cattle populations from Bolivia using a 50K SNP chip. G. Bottani Claros*[2,1], E. Jonas[1], and E. Strandberg[1], *[1]Sveriges lantbruksuniversitet, SLU, Uppsala, Sweden, [2]Universidad Mayor de San Simon, UMSS, Cochabamba, Bolivia.*

Creole cattle populations constitute an important reservoir of genes related to climate resilience and adaptation to harsh environments. Four of these populations were previously identified in Bolivia: Saavedreño, Yacumeño, Chusco and the Chaqueño Creole cattle. During recent years, an unplanned crossbreeding process is threatening the genetic diversity of these local populations in Bolivia and risking the conservation of valuable genes for the global animal genetic resources. With the purpose of generating information for conservation and breeding applications, 672 individuals were genotyped with a 50K SNP chip. The samples were collected from Pasorapa, El Chaco Area and from the Applied Ecology Center CEASIP. Sampling in El Chaco area covered the municipalities of Yacuiba, Villamontes, Puerto Margarita, Machareti and Boyuibe including samples from the Experimental Centers of El Salvador and Kilometer 20. Pairwise Fst were calculated to estimate population differentiation. The Fst values ranged from 0.017 to 0.087. Higher values of Fst were observed between the CEASIP herd and individuals from all other sampling areas with exception of Boyuibe. PCA analysis and Fst values showed that Pasorapa Creole cattle is grouped separately from the other populations. This study allowed us to describe a fifth population of Creole cattle in Bolivia.

Key Words: Creole cattle, 50K, SNP markers, diversity, population

P283 Abstract withdrawn

P284 Genome-wide patterns of selection in Malawi, South Africa, Zimbabwe indigenous village chickens and conserved chicken flocks. K. Hadebe*[1], E. F. Dzomba[2], and F. C. Muchadeyi[1], *[1]Agricultural Research Council, Biotechnology Platform, Onderstepoort, South Africa, [2]University of KwaZulu-Natal, Scottsville, South Africa.*

Indigenous chickens occur across heterogeneous landscapes, often under limited resource production environments. They demonstrate heritable adaptive variation across environmental gradients suggestive of local adaptation. The direction of adaptive differentiation is still underestimated and may have negative impact on the conservation programs. Illumina SNP60K BeadChip genotype data (47142 SNPs) from 260 indigenous chickens sampled from Malawi, South Africa, and Zim-

babwe were used to detect runs of homozygosity (ROH) and identify genomic regions that may be under selection. In addition, 4 conserved flocks (n = 70, Venda (VD), Naked Neck (NN), Potchefstroom Koekoek (PK) and Ovambo (OV)) were included. Among conservation flocks, VD had the highest degree of differentiation across all populations (F_{ST} range 0.21 to 0.12) suggesting genetic isolation. A total of 4214 ROH were identified across populations. Distribution of ROH according to their size showed that the majority of the detected ROH belonged to the 1–5 Mb length category and only 1 was >40 in the Zimbabwean population. We identified 4892 ROH islands across populations with chromosome 12 inhabiting the highest number. The univariate genotype-environment association analysis (latent factor mixed model) on uncorrelated 17 ($r < 0.90$) environmental variables, assuming population structure of K = 6 revealed 4022 SNPs were significantly ($P < 0.001$) associated with both temperature and precipitation. The mean of monthly (maximum temperature - minimum temperature; Bio2) was associated with the highest numbers of SNPs (n = 283). Functional analysis of the ROH islands and associated SNPs revealed genes affecting metabolic, cellular, immune system, developmental, climatic adaptation and reproduction processes. This study gives insight into genes under possible selection related to adaptation to prevailing environmental and production conditions.

Key Words: chickens, SNP, runs of homozygosity, adaptation

P286 Linkage disequilibrium and haplotype block partitioning in village, commercial and indigenous pigs of South Africa. N. Hlongwane[1,2], E. F. Dzomba[2], K. Hadebe*[1], P. Soma[3], and F. C. Muchadeyi[1], *[1]Agricultural Research Council, Biotechnology Platform, Onderstepoort, Gauteng, South Africa, [2]Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal, Scottsville, KwaZulu-Natal, South Africa, [3]Agricultural Research Council, Animal Production Institute, Irene, Gauteng, South Africa.*

Extent of linkage disequilibrium (LD) and haplotype block are prerequisites for a successful selection programs and GWAS because they provide insights into population genetic history. PorcineSNP60K BeadChip was used to examine patterns of LD, persistence phase and haplotype diversity in 191 pig samples from South African villages ecotypes (ALN, CAP, MOP, ORT) and commercial (DUR, LWT, SAL) and indigenous breeds (KOL, WIN). SNPs with a MAF < 0.02, call rates of < 85% and HWE < 0.0001 were excluded from downstream analyses. KOL had highest overall mean LD ($r^2 = 0.66 \pm 0.04$) while CAP had the least ($r^2 = 0.44 \pm 0.06$) attributed to either genomic selection, sample size or breed effects. DUR and KOL had the strongest LD decay average values of 0.88 to 0.69 at distance up to 100 Kb. LD decay started to decrease for all populations from > 1 Kb with village populations experiencing the sharpest decrease. The persistence phase between 36 pairs was the greatest among the commercial and indigenous populations. DUR and LWT presented the most ($r^2 = 0.79$) at distance > 1 Kb while a smaller value ($r^2 = 0.01$) was noted between ORT and SAL at distances > 4 000 Kb. The DUR population had the most haplotype blocks (3402), averaging 12 285 SNPs per block. The blocks comprised 13.15% of the genome with 355 the total sum of block length and 2 513 unique haplotypes. Overall, only 3 haplo-blocks were shared by the 9 populations and had 2 gene functions *DECR1* (Chr 4), *LENG9* (Chr 6), *TARS* and *ADAMST12* (Chr 16). The most unique haplotypes were containing 2 and 3 SNPs. Functional analyses of shaped haplo-blocks revealed genes important for carcass, growth rate and high altitude adaptation. There is evidence of levels of introgression in the South African pig populations. Linkage disequilibrium results demonstrated the effects of production system and effective population size while the haplotypes showed common ancestry between the populations.

Key Words: domestic pigs, GWAS, persistence phase, haplo-block structure, SNP

P287 Differential mitochondrial proteomics reveals plateau adaptability of polled yak. C. Liang*[1,2], D. Fu[1,2], X. Ma[1,2], C. Ji[1,2], X. Wu[1,2], Y. Gao[1,2], and P. Yan[1,2], *[1]Lanzhou Institute of Animal Science and Veterinary Pharmaceutics, CAAS, Lanzhou, China, [2]Key Laboratory for Yak Breeding Engineering, Lanzhou, China.*

Compared with the same cattle species in low-altitude areas, yak have higher survival pressure due to the unique conditions of low oxygen pressure, short grass period etc. So yak must have a more efficient energy-metabolism pathway. However, few reports and studies have deeply understood and studied these characteristics of yak from the proteome in mitochondria. Different proteomic analysis were performed to compare mitochondrial proteins from the brain tissue of polled yak and cattles using isobaric tags for relative and absolute quantitation (iTRAQ) technology combined with Western blot. A total of 57 proteins with different expression levels in mitochondria were screened. GO analysis showed that the molecular function of the differential protein group was concentrated on functions such as oxidoreductase and transaminase activity; biological processes mainly focused on metabolic processes and fatty acid metabolism; cell components were concentrated in cytoplasm and mitochondrial inner membrane. String protein interaction database analysis showed that there is a complex interaction between the enzyme family, the dehydrogenase family, the transaminase and the ATP synthetase. Reactome pathway analysis showed that the differential protein group was mainly involved in aerobic metabolic pathways such as lipid metabolism, citrate cycle, and amino acid anabolism. Western Blot results showed that the results of Western blotting of FKBP4 and ATPAF2 were consistent with the results of mass spectrometry. We concluded that some important proteins were associated with fatty acid β-oxidation, citric acid cycle, kElectronic transfer process etc during energy metabolism process. The results deepen our understanding of the molecular mechanisms underlying plateau adaptability.

Key Words: polled yak, proteomics, plateau adaptability, iTRAQ

P288 Autosomal genome evidence for introgression from other *Gallus* species into African and Middle East indigenous chicken. A. S. Al-Jumaili*[1,2], A. Gheyas[3,4], A. Kebede[5,6], J. Smith[3,4], and O. Hanotte[1,5], *[1]School of Life Sciences, University of Nottingham, University Park, Nottingham, United Kingdom, [2]University of Anbar, Anbar, Iraq, [3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian, UK, [4]Centre for Tropical Livestock Genetics and Health, The Roslin Institute, Edinburgh, UK, [5]LiveGene, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [6]Addis Ababa University (AAU), Addis Ababa, Ethiopia.*

Across species, gene flow is increasingly being recognized as playing an important role in the historical genetic background of a species. In particular, adaptive introgression may be related to environmental adaptation. Also, considering the geographic distribution of related wild species, it could help to trace the history of dispersion of a species. Here we investigate if introgression can be used to trace dispersion routes of indigenous chicken outside its putative centers of origins. Domestic chicken from 3 countries (Iraq n = 27, Ethiopia n = 283 and Saudi Arabia n = 5 NCBI accession no. SRP142580) beside the 4 *Gallus* species (Ceylon = 8, Green n = 12, Grey n = 3 and Red n = 6) were analyzed to assess their overall genetic background and frequency of introgressed haplotypes using the 4 taxons ABBA – BABA approach. All samples were analyzed using an Illumina HiSeqX sequencer and SNPs data were generated following the GATK best practices protocol restricted to bi-allelic sites. Haplotype maximum likelihood trees at 6 candidate introgressed regions were constructed allowing counting haplotype frequency in the studied population. The results have found no gene flow from Ceylon and Green junglefowl into the gene pool of Iraq, Ethiopia and Saudi village chicken at the opposite of Grey junglefowl introgression. Overall, the frequencies of the latter were low in Iraq and Saudi Arabia, South Ethiopian populations, compared with the North, Central-East and West Ethiopian populations. The results are adding

further support for an Indian subcontinent center of terrestrial dispersion for the Iraqi and Saudi village chicken. For Ethiopia, the situation is likely more complex and, with the country having likely witnessed both a terrestrial and maritime arrival of the domestic chicken, in agreement with the today geographic dispersion of Grey junglefowl.

Key Words: introgression, dispersion routes, indigenous chicken

Microbiomes

P289 Abstract withdrawn

P290 Effect of Bacillus probiotics on microbial gut diversity in weaner pigs. J. Rosenstrauch, E. van Marle-Köster*, and C. Jansen van Rensburg, *University of Pretoria, Pretoria, South Africa.*

Since the ban by the European Union on the use of in-feed antibiotics, alternative feed additives have been investigated to improve gut health and prevent post-weaning diarrhea. Probiotics holds potential to improve gut health and performance. In this study, a multi-strain *Bacillus* probiotic were applied to study the effect on the microbial gut diversity in weaner pigs. 40 weaner pigs (21 d of age) kept in individual pens were reared on a standard diet and subjected to 3 Bacillus probiotic treatments applied during cleaning and supplemented in drinking water for the duration of the trial. Fifteen fecal samples per treatment were collected at 35, 49 and 63 d of age directly from the anus after rectal stimulation. DNA was extracted using phenol-chloroform method, quantified and sent for 16S RNA sequencing at University of Michigan Medical School (USA). Data were analyzed using MOTHUR v.1.35.1. To determine the effect of treatment on the microbial population a sample independent Nonparametric Mann-Whitney U test was performed with a 95% significance level and Friedman's Two-way ANOVA by rank was performed with a 95% significance level. 1229551 sequence reads varied between 242 and 258 base pairs with an average length of 253 base pairs. 3275 OTUs were detected for all 3 sampling days, with only *Archaea* and *Bacteria* detected at a kingdom level. At a phylum level *Firmicutes* was the most abundant followed by *Bacteroidetes*, *Proteobacteria* and *Tenericutes*. Good's coverage exceeded 0.95 for all bacterial libraries over all 3 sample days with no significant differences between treatments ($P > 0.05$). Significant differences between *Erysipelotrichaceae* ($P < 0.05$) between 35 and 49 d and between 35 and 63 d were observed, *Prevotellaceae* between 35 and 49 d and *Ruminococcaceae* between 35 and 49 d for the probiotic treatment. *Firmicutes* were the most abundant bacteria at a phylum level with *Bacteroidetes* as the second most abundant phylum for both control and treatment at 35 d of age. The most abundant phylum at 63 d remains *Firmicutes*, followed by *Bacteroidetes* and *Proteobacteria*, with no significant differences between treatment and control. Overall the probiotic treatment seemed to have improved the quantity of beneficial microbes in the feces.

Key Words: Pigs, 16SRNA, microbiome, gastrointestinal tract

P291 Effect of selection for intramuscular fat content in the cecum microbiome content in rabbits. A. Zubiri-Gaitán*, M. Martínez-Alvaro, A. Blasco, and P. Hernández, *Universitat Politècnica de València, Valencia, Valencia, Spain.*

The objective of this study was to analyze the effect of selection for intramuscular fat content (IMF) in the microbiota composition of rabbits. Several evidences suggest that the gut microbiota is involved in the lipid deposition and that this microbiota is affected by the host genome. Cecum samples were taken from 16 and 17 rabbits from the high (H) and low (L) IMF line, respectively. The metagenome was obtained by means of Illumina NextSeq sequencing, and taxonomic classification of the reads was performed using Kaiju. 2653 operational taxonomic units at genus level were identified. All genera with zero counts in more than 25% of the individuals were discarded and 1435 genera were kept. The remaining zero counts were replaced by imputed values using a Bayesian-multiplicative treatment. The vector of counts of each individual was normalized to unit constant sum. The data was pre-processed using the centered log-ratio transformation to deal with its compositional nature. Microbial genera able to discriminate the lines were identified using projections to latent structure-discriminant analysis (PLS-DA). The selection criteria for the genera was the variable importance in projection (VIP), considering VIP > 0.8. The final PLS-DA model included 293 genera. After fitting the model, we obtained R^2: 0.998 and Q^2: 0.925. The marginal posterior distributions of the ratio H/L were estimated for the relative abundances of the 293 genera selected. The probability (P) of the ratio being greater or lower than 1 was obtained. 59 genera with $P > 0.95$ were kept. The most represented phyla were *Firmicutes* (40%) and *Proteobacteria* (34%). The 5 most important genera in the discrimination were *Firmicutes Peptococcus*, *Firmicutes Negativicoccus*, *Thermotogae Thermosipho*, *Proteobacteria Rubrivivax* and *Proteobacteria Pleomorphomonas*. All of them were overrepresented in the high line. These preliminary results showed that selection for IMF led to a modification in the gut microbiome composition.

Key Words: microbiome, divergent selection, intramuscular fat, rabbits

P292 Going full circle: Assembly of high-quality, single-contig microbial genomes from the rumen microbiome using long-read sequencing. A. Warr*[1], R. Stewart[1], M. Aufrett[2], A. Walker[3], R. Roehe[2], and M. Watson[1], *[1]The Roslin Institute, Edinburgh, UK, [2]SRUC, Edinburgh, UK, [3]The Rowett Institute, Aberdeen, UK.*

Ruminants such as cows and sheep are important livestock species. They convert low nutritional value plant matter into high-quality meat and dairy products. Within a specialized stomach called the rumen, microbes ferment the plant matter producing short-chain fatty acids from difficult to digest plant matter. The composition of the rumen microbial community can affect the animal's health, feed efficiency and level of methane production. Species in the rumen are typically difficult to culture and despite its importance, it remains an underexplored environment. DNA sequencing of the contents of the rumen offers the potential to identify microbial species without culture techniques. Here we sequence fluid from the rumen of a single cow using Nanopore sequencing. We show that despite these data coming from a highly complex microbial sample we can assemble high-quality, single-contig whole genomes and plasmids of known and novel species, including numerous circular contigs. Additionally, we compare and validate the assemblies of these genomes with binned genomes generated from short read Illumina assemblies of 282 cow rumen samples. We show that the long read assembly outperforms the short read assembly in contiguity and in the incorporation of AMR genes and marker genes.

Key Words: metagenomics, cattle and related species, microbiomics

P293 High-throughput metagenome sequencing for prediction of quantitative traits. M. Hess*[1], L. Zetouni[1], J. Budel[1], T. Van Stijn[1], H. Henry[1], R. Brauning[1], A. McCulloch[1], S. Hickey[2], A. Hess[1], M. Kirk[3], S. Kumar[3], N. Morton[4], H. Flay[5], S. Kittelmann[3], G. Henderson[3], *[1]AgResearch Ltd., Mosgiel, New Zealand, [2]AgResearch Ltd., Ruakura, New Zealand, [3]AgResearch Ltd., Palmerston North, New Zealand, [4]University of Auckland, Auckland, New Zealand, [5]DairyNZ, Hamilton, New Zealand.*

The rumen microbiome plays an important role in feed digestion and is associated with a range of economically and environmentally important traits e.g., methane production. Our objective was to develop a low-cost, high-throughput approach for metagenome sequencing using restriction enzyme reduced representation sequencing to obtain metagenome profiles for large-scale prediction of quantitative traits. DNA was extracted from freeze-dried and ground rumen samples then digested with the restriction enzyme *Pst*I. A library was generated from between 118 and 330 samples, depending on the data set; after selecting 193–318 bp fragments, the library was sequenced in one lane on a HiSeq2500. After demultiplexing and quality trimming, metagenome profiles were obtained using either a reference-based (RB) or reference-free (RF) approach. The RB approach involved BLASTing reads against the Hungate 1000 Collection of genome assemblies and assignment to genus. The RF approach identified common 65 bp tags, present in >25% of samples, within the data set and counted the frequency of each tag in each sample. Our approach has been tested on 3 data sets: D1) 236 sheep rumen samples from 118 high- or low-methane sheep; D2) 205 rumen samples from New Zealand lambs; D3) 186 rumen samples from New Zealand dairy cattle. The average number of reads per sample ranged from 760k to 2.7M, depending on the number of samples per lane. On average, 6–10% of reads were assigned at the genus level using the RB approach. On average 40–65% of the reads for each sample were captured in the metagenome profile using the RF approach. A comparison between D1 and 16S rRNA gene sequencing on the same samples showed more repeatable profiles with our approach than 16S (0.62 ± 0.06 vs 0.45 ± 0.08). Data sets were visualized using network analysis to identify relationships between samples or taxonomic groups. A microbial relationship matrix was added to models of methane emissions, showing the variance explained by the microbial component outweighs the host genetic component. Our approach will be used to sequence thousands of rumen samples over the next year and is easily adapted to other sample types.

Key Words: metagenomics, high-throughput sequencing (HTS), environment, network analysis, genomic prediction

Pig Genetics and Genomics

P294 Plant secondary metabolites affect pigs' gastro-intestinal health. H. H. Hofmann*, M. J. Proell-Cornelissen, M. Schulte-Rosier, K. Schellander, and C. Neuhoff, *Animal Breeding and Husbandry/ Animal Genetics group; University of Bonn, Bonn, North Rhine-Westphalia, Germany.*

Nutrigenomics connects genetics and feeding. In terms of health, the gastro-intestinal tract, especially the small intestine and the spleen play key roles. The type of ration fed and its defined nutritional components can influence gene expression in the intestine. Terpenoids, such as Carvacrol, have antimicrobial, antioxidative or digestive promoting effects as well as an antibacterial impact against *E. coli* and *E. faecalis* in the pig's intestine. We hypothesized whether a mixture of phytogenic ingredients can influence the transcriptional profile of immune related genes and can have an effect on the morphology of the small intestine's epithelium. To conduct our research, we produced 16 crossbreed piglets (GL × Pi). After weaning, 4 random selected piglets were sacrificed and the remaining pigs were fed in 2 different groups of 6 animals. The test group was fed a terpenoid mixture; all other parameters were identical to the control group. After a 14 d feeding period, the remaining pigs were euthanized. Jejunal, ileal and spleen tissues were collected and small intestine histological sections were produced and stained using H&E and PAS. Additionally, qPCR gene expression analysis of adaptive immune response genes and bacterial markers was performed. Statistical analysis was done via R using an ANOVA. PAS staining of jejunal and ileal epithelium revealed a lower production of mucus in the treated group compared with the control. The qPCR of both jejunal and ileal tissues revealed significantly lower expression levels of *TNF-α* ($P < 0.001$) as well as *IL-1β* ($P < 0.01$) in the treated group vs. the control group. In the ileal tissue, we found a significant reduction of $CD4^+$ mRNA level ($P < 0.01$) and *E. coli* ($P < 0.01$) within the treated group. In the spleen, gene expression of the Fas ligand was significantly increased ($P < 0.05$) in the treated group in comparison to the control. Taken together, these findings demonstrate that plant secondary metabolites are able to alter adaptive immune response gene expression in the pig's small intestine and spleen after weaning.

Key Words: pigs, nutrigenomics, qPCR, animal health

P295 Array genotyping and/or whole-genome sequencing facilitates detection of structural variants and chromosomal imbalance in pigs. A. Letko*[1], A. Grahofer[2], I. M. Häfliger[1], V. Jagannathan[1], A. Ducos[3], O. Richard[4], V. Peter[5], H. Nathues[2], and C. Drögemüller[1], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Clinic for Swine, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [3]GenPhyse, INRA, INPT, ENVT, Université de Toulouse, Castanet-Tolosan, France, [4]Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [5]Division*

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Palatoschisis or cleft palate is a known anomaly in pigs resulting in early death of affected individuals. However, little is known about its etiology. A detailed description of the phenotype was derived from necropsy and by CT revealing that 20 examined cases also exhibited hypodontia and renal cysts. Furthermore, a genetic origin was assumed due to dominant inheritance as all 20 recorded cases were confirmed offspring of a single boar. Single nucleotide polymorphism genotyping data were used to map the defect in the porcine genome and led to the detection of a chromosomal imbalance in the affected offspring. Whole-genome sequencing of an affected piglet and a normal full sib was used to identify a chromosomal translocation and to fine map the breakpoints in the genome. Finally, we proved that the boar, which sired the malformed piglets, carried a balanced translocation between Mb-sized segments of chromosome 8 and 14. This had not been previously observed during karyotyping. All affected offspring were shown being carriers of a partial trisomy of chromosome 14 including the *FGFR2* gene, which is associated with various dominantly inherited craniofacial dysostosis syndromes in man, and partial monosomy of chromosome 8 containing *MSX1* known to be associated with tooth agenesis and orofacial clefts in other species. The greatly improved genomic resources in pigs in combination with genome-wide genotyping and/or sequencing, facilitates early identification of the responsible boar and suggests possible causative mutations. By implementing systematic surveillance, it is possible to identify genetic defects at an early stage and avoid further distribution of congenital disorders.

Key Words: pigs and related species, genome sequencing, SNP, karyotype, chromosomal rearrangement

P296 Detecting deleterious variants in the pig. M. Johnsson*[1,2], R. Ros-Freixedes[1,3], A. Whalen[1], G. Gorjanc[1], D.-J. De Koning[2], C-Y. Chen[4], M. A. Cleveland[4], A. J. Mileham[5], S. Rounsley[5], W. Herring[4], and J. M. Hickey[1], *[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Midlothian, United Kingdom, [2]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden, [3]Departament de Ciència Animal, Universitat de Lleida, Lleida, Spain, [4]Genus plc, Hendersonville, TN, USA, [5]Genus plc, DeForest, WI, USA.*

Deleterious variants are an unavoidable fact of genetics and can cause substantial reduction in fitness. With the advent of affordable genome sequencing and effective pedigree-based imputation algorithms, it is becoming possible to observe deleterious variants directly in livestock breeding programs, and design new breeding strategies to improve fitness. In this work, we used a battery of bioinformatic algorithms to detect deleterious variants in sequence data from 8,500 commercial pigs and imputed them to 350,000 genotyped pigs from an elite breeding nucleus population. We used this data set to study the distribution of deleterious variants in the genome, between lines, and across generations of the breeding program. We also used the resource to score variant intolerance and evolutionary constraint genome-wide in the pig. Finally, we performed simulations to study the scope for future breeding strategies to remove deleterious variants and improve the fitness of livestock populations. These results form the basis for designing next-generation sequence based breeding strategies for pigs.

Key Words: deleterious variants, sequencing, genetic load

P297 Sustainability of extensive Iberian pig production through improved feed efficiency and genomic selection. W. M. Rauw*[1], L. A. García Cortés[1], J. M. García Casco[2], F. Gómez Carballar[3], E. de la Serna Fito[3], and L. Gomez-Raya[1], *[1]INIA, Dept de Mejora Genética Animal, Madrid, Spain, [2]INIA, Centro de I+D en Cerdo Ibérico, Zafra, Badajoz, Spain, [3]Sánchez Romero Carvajal Jabugo S.A, Jabugo, Huelva, Spain.*

Sustainability of extensive Iberian swine farming is limited to the amount of land suitable for pig production. A feed efficiency model for Iberian pigs feeding on acorns in extensive traditional production systems (Montanera) is investigated. Pigs with a higher feed efficiency could either reduce the amount of land needed for production or increase the number of animals that can be produced on the land size of a given farm. However, recording feed intake is extremely difficult in free ranging animals. The proposed model for selection of feed efficient animals in an extensive production system requires: 1) evaluation of feed intake and residual feed intake based on individually penned animals in a test population being provided a diet of acorns (intensive feed efficiency), 2) registration of body weight gain of relatives of animals in the test population that are free ranging and feeding on acorns in Montanera (extensive grazing efficiency), and 3) carrying out genomic evaluations of selection candidates that are also relatives of animals in the test population and in Montanera. The use of a genomic relationship matrix allows for the estimation of genomic breeding values in animals for which individual feed intake is not recorded. Computation of extensive grazing efficiency is based on the regression coefficients of the formula of residual feed intake estimation obtained in the test population. Additionally, feed intake is corrected for individual activity levels that can be monitored with a GPS tracking device. Preliminary results are presented on 30 Iberian pigs fed in 3 different periods: 1) intensively with concentrates, 2) and acorns, and 3) feeding on acorns in Montanera. The correlation coefficient between concentrate intensive feed efficiency and acorn intensive feed efficiency was −0.55, suggesting that feed efficiency on concentrate vs. that on acorns is not the same trait. This research is part of the Eranet SusAn "SusPig" project "Sustainability of pig production through improved feed efficiency" (www.suspig-era.net).

Key Words: pigs, animal nutrition, feed efficiency, animal breeding, genomic selection

P298 Comparative transcriptomic analysis of dorsal subcutaneous fat from Portuguese local pig breeds. A. Albuquerque*[1], C. Óvilo[2], Y. Núñez[2], R. Benítez[2], A. López-García[2], J. Ballesteros[2], F. García[2], M. Laranjo[1], R. Charneca[3], and J. M. Martins[4], *[1]ICAAM-Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Évora, Portugal, [2]Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain, [3]ICAAM, Departamento de Medicina Veterinária, Universidade de Évora, Pólo da Mitra, Évora, Portugal, [4]ICAAM, Departamento de Zootecnia, Universidade de Évora, Pólo da Mitra, Évora, Portugal.*

Portuguese local Alentejano (AL) and Bísaro (BI) pig breeds have a higher potential for subcutaneous and intramuscular fat (IMF) deposition than modern lean-type breeds. AL pig belongs to the Iberian branch, presenting many of the characteristics of this group such as a low growth rate, a precociously high adipogenic activity and a high level of unsaturated fatty acids (FA). BI pig is related to the Celtic group, sharing ancestors with breeds such as Large White and Landrace, and generally presents a poor but higher growth than AL, a reasonable amount of IMF and a good unsaturated/saturated FA ratio. Set within the framework of the TREASURE project, this work intended to explore the genetic diversity of AL and BI pig breeds to help better understanding the underlying physiological mechanisms associated with body fat accretion and lean muscle tissue deposition and therefore, meat quality of these breeds. Dorsal subcutaneous fat (DSF) samples were collected from adult AL and BI pigs with ~150kg body weight at slaughter. Total RNA was obtained and sequenced for transcriptome analysis. A total of 375 genes were found to be differentially expressed (DE) in DSF samples ($q < 0.05$). 335 DE genes were overexpressed in AL ($|FC| = 0.81$ to 6.47) and 40 in BI ($|FC| = 0.91$ to 7.11). Preliminary results also demonstrate that genes related to lipid metabolism such as *ACACA*, *FASN*, *SCD*, *LEP* and *ME1* are significantly overexpressed in AL when compared with BI which agrees with the higher lipid content of the AL breed. A functional enrichment analysis (metabolic pathways and GO enrichment) was performed of the DE genes, which showed that the main involved functions include lipid metabolism (synthesis of lipids and release of FA), inflammatory response, amino acid and car-

bohydrate metabolism. Potential regulators such as *MYD88*, *IL1*, *IL15*, *NR1D1* and *PPARGC1A* were also able to be predicted for these functions. This work was funded by European Union's H2020 RIA program (grant agreement no. 634476) and by Portuguese national funds through FCT/MCTES under project UID/AGR/00115/2019 and research grants SFRH/BD/132215/2017 and SFRH/BPD/108802/2015 to A. Albuquerque and M. Laranjo, respectively.

Key Words: Alentejano pig, Bísaro pig, RNA-seq, differentially expressed genes (DEGs), dorsal subcutaneous fat

P299 Runs of homozygosity provide a genome landscape picture of inbreeding and genetic history of European autochthonous and cosmopolitan pig breeds. G. Schiavo*[1], M. Muñoz[2], S. Bovo[1], J. García-Casco[2], A. Ribani[1], S. Tinarelli[1,3], I. Djurkin-Kušec[4], C. Radovic[5], R. Savic[6], M. Gallo[3], M. Candek-Potokar[7], A. Fernández[2], C. Óvilo[2], L. Fontanesi[1], TREASURE consortium[8], *[1]Department of Agriculture and Food Sciences, Università di Bologna, Bologna, Italy, [2]Departamento Mejora Genética Animal, INIA, Madrid, Spain, [3]Associazione Nazionale Allevatori Suini (ANAS), Roma, Italy, [4]Faculty of Agrobiotechnical Sciences Osijek, University of Osijek, Osijek, Croatia, [5]Institute for Animal Husbandry-Pig Research Department, Belgrade-Zemun, Serbia, [6]University of Belgrade, Faculty of agriculture, Belgrade-Zemun, Serbia, [7]Kmetijski inštitut Slovenije, Ljubljana, Slovenia, [8]TREASURE Consortium, Ljubljana, Slovenia.*

Runs of homozygosity (ROH) are long stretches of genomic DNA which are homozygous at each polymorphic position. The ROH length and the genome proportion covered by ROH are good indicators of the age, origin and level of autozygosity and thus inbreeding. In this work we investigated ROH in 20 European local pig breeds (Alentejana, Apulo-Calabrese, Basque, Bísara, Majorcan Black, Black Slavonian, Casertana, Cinta Senese, Gascon, Iberian, Krskopolje, Lithuanian indigenous wattle, Lithuanian White Old Type, Mora Romagnola, Moravka, Nero Siciliano, Sarda, Schwäbisch-Hällisches Schwein, Swallow-Bellied Mangalitsa and Turopolje) from 7 countries (Croatia, France, Germany, Italy, Lithuania, Portugal, Servia, Slovenia, Spain) and in 3 cosmopolitan breeds (Italian Large White, Italian Landrace and Italian Duroc). A total of 1129 pigs (45–50 per breed) were genotyped with the GGP Porcine HD Genomic Profiler v1 chip (68,528 SNPs). PLINK software was used to analyze this data set. ROH were placed into 5 size classes (1–2 Mbp, 2–4 Mbp, 4–8 Mbp, 8–16 Mbp and >16 Mbp). The genomic inbreeding coefficient, defined as the proportion of genome covered by all ROH and divided by the total length of autosomal genome (FROH), was computed for each animal. Alentejana pigs had the lowest averaged FROH (0.029 ± 0.018) whereas Apulo-Calabrese had the highest averaged FROH (0.103 ± 0.038). The highest FROH individual value was observed in a Black Slavonian pig (0.295). Apulo-Calabrese, Casertana and Sarda breeds had the highest number of ROH >16 Mbp, suggesting that their autozigosity was derived from recent ancestors whereas Italian Large White and Italian Landrace had a high frequency of short ROH, indicating a remote origin of common ancestors. Several ROH islands were found along the genome and their distribution among breeds indicated the presence of common or breed specific selection sweeps. This study contributed to understand the population genetic history of the investigated pig breeds and provided information that could be useful to manage these pig genetic resources. This study has been funded by European Union's H2020 RIA program (grant agreement No 634476).

Key Words: pigs and related species, population genomics, genotyping, inbreeding, conservation

P300 The transcriptomic impact of maternal dietary resistant starch on piglet's liver and colon when challenged with a high fat diet. M. Schroyen*[1], J. Leblois[2], J. Uerlings[1], B. Li[1], E. A. Sureda[1], J. Wavreille[3], J. Bindelle[1], and N. Everaert[1], *[1]Precision Livestock and Nutrition Laboratory, Teaching and Research Centre (TERRA), Gembloux AgroBioTech, University of Liège, Gembloux, Belgium, [2]Association Wallonne de l'Elevage (awé), Ciney, Belgium, [3]Production and Sectors Department, Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium.*

In the past several years, diet supplementation of resistant starch (RS), which acts as prebiotic, has extensively been studied in pigs, and this mostly in the critical period around weaning. RS is believed to exert beneficial effects on the gastrointestinal tract mainly due to higher levels of short chain fatty acids and an improved microbiota profile. In this study, possible maternal effects of RS on the transcriptome of the sow's progeny were assessed. During late gestation and lactation, 12 sows were fed a diet containing 33% of standard maize starch, considered as digestible (DS) while 12 others received a diet rich in pea starch, considered as resistant starch (RS). Thirthy-2 piglets were sampled, 16 of each maternal diet. Since RS is also described to have a positive effect on metabolic diseases, and to measure this effect likewise on a maternal level, half of these 16 piglets per maternal diet were assigned to a high fat diet, while the others were fed a control diet from weaning till the end of the trial at 10 weeks post-weaning. The transcriptome of liver and proximal colon scrapings of the piglets was examined. Since RNA-seq on liver and colon scrapings displayed no significant interaction effects, main effects of maternal and piglet diet were investigated. Only minor differences between the maternal diet groups were found. Twelve and 23 genes were differentially expressed in the liver and colon respectively, and no meaningful functional annotation could be performed. However, with regard to piglet diet, genes previously reported in relation to metabolic changes due to a high fat diet were found to be differentially expressed in the liver tissue. Network analyses also showed clusters of genes that were significantly correlated with piglet diet in the liver. These clusters were annotated for lipid metabolic processes and correlated well with backfat thickness, meat percentage, and total cholesterol measurements in fasted pig's plasma after 6 weeks on a high fat diet. In conclusion, there is only a minor impact of maternal dietary RS on the liver and colon transcriptome of the progeny. However, a high fat diet given to the progeny directly evokes metabolic changes in the liver, but they do not seem to be influenced by the maternal starch diet.

Key Words: pig, resistant starch, high fat diet, RNA-seq

P301 Differential expression analysis of genes correlated with reproduction in Gossypol-treated porcine granulosa cells. M. W. Hong*, S. Y. Choi, H. Kim, H. Lee, and S. J. Lee, *Kangwon National University, Chuncheon-si, Gangwon-do, South Korea.*

Gossypol (GP), a polyphenolic compound in the cottonseed is known to affect female reproduction. The aim of this research was to profile candidate genes affecting an estrous cycle and embryo developments by studying the difference expression genes (DEGs) in GP-treated porcine granulosa cells. The present study was investigated the RNA of cultured cells by treated 6.25 and 12.5 μM GP for 72 h, in vitro using RNA sequencing. A total of 21,074 genes were expressed in all samples. Between negative control (NC) and GP 6.25 μM, 91 genes were significantly upregulated and 288 genes were downregulated ($q < 0.05$). Comparing NC with GP 12.5 μM, 334 genes were significantly upregulated and 592 genes were downregulated ($q < 0.05$). Furthermore, expressed results of RNA and protein of several genes in GP-treated porcine granulosa cells using qPCR, tandem mass spectrometry (MS/MS) and Western blot showed equivalent expression with RNA-seq results. This study will provide as a fundamental data on the gene expressions associated with biological process of GP-treated porcine granulosa cells.

Key Words: gossypol, RNA-seq, gene expression, pigs and related species, granulosa cells

P302 Genomic mating as a sustainable breeding strategy in Chinese NingXiang pigs. J. He*[1,2], X. Wu[2,3], Q. Zeng[4], H. Li[2,3], H. Ma[1], J. Jiang[1,3], G. J. M. Rosa[3], D. Gianola[3], R. G. Tait Jr.[2], and S. Bauck[2], *[1]College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan, China, [2]Biostatistics and Bioinformatics, Neogen GeneSeek, Lincoln, NE, USA, [3]Department of Animal Sciences, University of Wisconsin, Madison, WI, USA, [4]Ningxiang Pig*

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Genomic selection (GS) has become an important, cost-effective tool for genetic improvement of animals and plants. However, collateral issues may involve an elevation of offspring inbreeding, with a subsequent more rapid depletion of genetic variability in the population. Optimum-contribution selection (OCS) represents an attempt to maximize genetic gain while constraining inbreeding within a targeted range. In the present study, genomic inbreeding was evaluated in 515 Ningxiang pigs, genotyped with either Illumina Porcine 60K SNP chip (68,528 SNPs) or GeneSeek Genomic Profiler (GGP) porcine 50K SNP chip (50,915 SNPs). The results showed that the average locus-wise inbreeding coefficient was 0.41 and 0.37 in the 2 sets of Ningxiang pigs; genomic inbreeding based on runs of homogeneity (ROH) was 0.24 and 0.25, respectively. The effectiveness of genomic OCS was evaluated with simulated phenotypes in a comparison with GS without inbreeding control. The genetic gain for average daily body weight gain per generation was between 19.74 and 22.24 g with genomic OCS, and it varied from 22.61 and 44.19 g under GS without inbreeding control. Nevertheless, the genetic gain obtained with GS had a steady decrease after the second generation of selection, and its difference with genomic OCS was close to nil at generation 10 and continued to go down thereafter, thus indicating the opportunity to continue exploitation of selection for growth. Further, the rate of inbreeding was maintained below 5% per generation with genomic OCS whereas, it increased to between 10.5% and 15.2% per generation with GS. Therefore, it is concluded that genomic OCS is appeared to be a sustainable strategy for genetic improvement of Chinese indigenous Ningxiang pigs.

Key Words: indigenous pigs, SNP, genomic selection, optimum-contribution selection

P304 Structural variants detection by large-scale sequencing data of pig genomes. H. Du* and J.-F. Liu, *China Agricultural University, Beijing, Beijing, China.*

Structural Variants (SVs), as an important genetic variation type, unlike the single nucleotide polymorphisms (SNPs) and Small Indels, can influence large segments of genomes and cause dosage-effect on genomes. To obtain highly convincing SV detection results of pig genomes, we employed a suite of commonly used methods which include 7 softwares based on 3 algorithms (Read-pair, Read-depth and Spilt-reads) depend on NGS data generated from 305 individuals of 53 porcine populations in our SV calling pipeline. The results of different softwares were merged based on our own standard, and genotyped the SV sites according to a unit criterion. We finally got the merged and genotyped SV release comprised of 31,967 deletions, 4,235 insertions, 799 tandem-duplications, 718 inversions, 31 translocations and 2,753 CNVs. The validation of detected SVs using Local Assembly method by TIGRA-SV and AGE showed that the average percentage is 94.08% for 53 different species. This means that the SV callset we archived followed by our own calling pipeline has the high accuracy. In our study, population diversity analysis based on SVs (especially deletions) indicated the similar results with the previous studies using SNPs as genetic markers. Intriguingly, Meishan pigs we found that unlike the phylogentic tree inferred from SNPs performed by previous studies that Meishan pig clustered with other Chinese domestic porcine populations located in Yangtze river region, the tree constructed with deletions indicated that Meishan pig separated from other Chinese domestic pig populations. This means that SVs may contain some unique genetic information which SNPs couldn't reveal. Besides, many insertion segments with about 300bp length were found enriched in the 45–95MB region of chromosome X. We suspect that repetitive sequences may enriched in this region of chromosome X and may tightly related to important traits of Meishan pigs. We hope our study will provided reliable genetic variation materials to deeply study population genetic structure, breed specificity and population evolution in the future.

Key Words: pigs and related species, population genomics, DNA sequencing, computational workflow, structural variants

P305 Genome-wide association and high-resolution QTL analyses identify a major locus on SSC12 influencing fatty acid composition in the *longissimus dorsi* muscle in pigs. J.-B. Lee[1], H.-B. Park*[2], Y. J. Kang[3], and I.-C. Cho[3], *[1]Korea Zoonosis Research Institute, Chonbuk National University, Iksan, Republic of Korea, [2]Department of Animal Resources Science, Kongju National University, Yesan, Republic of Korea, [3]National Institute of Animal Science, RDA, Jeju, Republic of Korea.*

Fatty acid (FA) composition is one of the most important parameters for the assessment of pork quality. To identify quantitative trait loci (QTLs) associated with FA composition in the *longissimus dorsi* muscle in pigs, we performed genome-wide association analyses using the PorcineSNP60K BeadChip in a resource population derived from a large intercross between Landrace and Korean native pigs (LK cross). A total of 39,474 SNPs from 976 F_2 progeny were analyzed for each FA composition trait after filtering for quality control. Data were analyzed by the Genome-wide Efficient Mixed Model Association (GEMMA) approach. A total of 269 significant SNPs ($P < 1.27 \times 10^{-6}$) on SSC8, SSC12, SSC14 and SSC16 were detected for FA composition traits in this study. Interestingly, several co-localizations of QTL on SSC12 for oleic acid (C18:1), linoleic acid (C18:2), arachidonic acid (C20:4), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) were observed. The QTL signal on SSC12 identified in the LK cross was replicated in an independent resource population between Duroc and Korean native pigs (DK cross). To refine the QTL region on SSC12, a haplotype-based linkage and linkage disequilibrium (LALD) analysis using the PHASEBOOK program was applied for both the LK and DK crosses. The LALD analysis refined the critical region to a less than 1-Mb region containing the cluster of myosin heavy chain isoforms (*MYH13*, *MYH8*, *MYH4*, *MYH1*, *MYH2*, and *MYH3*). The results obtained in this study provide valuable information to elucidate the porcine causal gene(s) for the FA composition traits in the further fine-mapping and functional studies.

Key Words: pig, genome-wide association, large-scale genomics, complex trait, meat production

P306 Piglet genotype, hypothalamic transcriptome, and their relationship with growth and viability traits. A. Heras-Molina*[1], C. García-Contreras[1,2], M. Vázquez-Gómez[3], R. Benítez[2], Y. Núñez[2], J. Ballesteros[2], J. L. Pesántez-Pacheco[1,4], V. Sanz-Fernández[1], S. Astiz[1], B. Isabel[3], A. González-Bulnes[1,3], and C. Óvilo[2], *[1]Department of Animal Reproduction, SGIT-INIA, Madrid, Spain, [2]Department of Animal Breeding, SGIT-INIA, Madrid, Spain, [3]Faculty of Veterinary Medicine, UCM, Madrid, Spain, [4]School of Veterinary Medicine and Zootechnics, UCuenca, Cuenca, Ecuador.*

In swine production, both commercial and traditional breeds (such as Iberian pig), are used, with commercial pigs being more efficient in reproductive and productive traits. However, recent studies have suggested a major growth potential in Iberian piglets during early developmental stages. The aim of this study was to determine the molecular events behind early development without potentially interfering effects of maternal environment. Hence, 16 sows were inseminated with heterospermic semen (from Iberian and Large White boars) to obtain 44 purebred (IBxIB) and 28 crossbred (IBxLW) littermates. Piglets were weighed and measured periodically until 7 m old. At 2 m old, the hypothalamus of 10 IBxIB and 10 IBxLW animals were obtained for RNA extraction and the transcriptome analysis was performed by RNA-seq technology. The growth patterns showed that crossbreds were heavier than pure piglets with a difference of one SD between 2 and 7 m of age ($P < 0.01$).Transcriptome analysis showed 245 differentially expressed genes (DE;156 upregulated in IBxIB and 89 in IBxLW), after

the following thresholds were applied: expression value equal or higher to 0.5 Fragments per Kilobase Million (FPKM) in one of the groups, Fold Change ≥1.25 and FDR <0.10. Some genes coding for hormones and related to organismal development such as *PRL, FSHB, GH1* and *TSH* were overexpressed in pure Iberian piglets, whereas genes related to muscle development and function (e.g., *TNNT1* and *SGCA*) were overexpressed in crossbreds. The functional analysis showed differences in the activation of physiological and biological functions, especially those linked to *organismal development*, *organ morphology* and *endocrine system development and function*, more expressed in Iberian piglets. There were also differences in the expression of some regulators associated with viability, such as *Jnk, RETLB* and *PRL*, also overexpressed in IBxIB. In conclusion, our results showed that genes, biological functions and upstream regulators related to viability, growth and endocrine system development were enriched in IBxIB animals, although the IBxLW individuals were heavier.

Key Words: pigs and related species, functional genomics, RNA-seq, development, husbandry

P307 MC1R gene polymorphism of Polish wild boar *(Sus scrofa scrofa)* and swine (*Sus scrofa f. domestica)*. A. Koseniuk*, G. Smolucha, D. Rubis, A. Szumiec, A. Radko, and K. Ropka-Molik, *The National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

According to the studies carried out elsewhere, the "wild" allele of MC1R (E^+) was identified and was proved to be unique to the wild boar population and absent from the main domestic pig breeds. To distinguish between wild boar and swine the analysis of the MC1R gene proved to be more effective than the most common method of species identification based on the polymorphism of selected mitochondrial DNA sequences. The aim of the study was to inquiry polymorphism of the MC1R gene to distinguish domestic pig (*Sus scrofa domestica*) from wild boar (*Sus scrofa scrofa*) in Poland. We extracted DNA from ear tissue samples of 22 wild boars from 2 Polish voivodeships (Opole and Silesia), and from blood samples of 67 pigs comprising 5 breeds: Polish Landrace (PL, n = 14), Polish Largewhite (PLW, n = 13), Zlotnicka White (ZW, n = 13), Pulawska (P, n = 10) and Duroc (D, n = 17). With the use of primers published elsewhere there were performed PCR and PCR-RFLP using *BspHI* enzyme. The MC1R gene fragment was sequenced (Sanger technique) with the use of PCR primers. Along the 672nt long MC1R sequence we found a total of 4 single nucleotide polymorphisms (SNPs) spanning 3 codons: c.370G > A (p.Asp124Asn), c.491C > T (p.Ala164Val), c.727G > A, c.729A > G (p.243Ala > Ala/Thr). We identified the "wild" G allele (c.370G > A) which was present in 20 of 22 samples of wild boar; in 2 remaining samples were identified heterozygotes AG. Interestingly the G allele was also found in 2 samples of Duroc (one heterozygote AG and one homozygote AA) and Zlotnicka biala (2 heterozygotes AG). The remaining samples were homozygotes AA. The *BspHI* digestion resulted in 2 bands of 472nt and 200nt length for homozygotes AA and 3 bands of 672nt, 472nt and 200nt for heterozygotes AG. The presence of the A allele in wild boars as well as the presence of wild allele (G) among commercial breeds (Zlotnicka white and Duroc) does not indicate the possibility of using polymorphism c.370G > A to distinguish between pigs and wild boars in Poland.

Key Words: pigs and related species, genetic identification, DNA sequencing, genetic marker, breed identification

P308 Integrative analysis of genomic data related with pig intramuscular fat. R. Gonzalez-Prendes*[1], Y. Ramayo-Caldas[2], R. Ros-Freixedes[1], E. Solé[1], J. Estany[1], and R. N. Pena[1], *[1]Department of Animal Science, University of Lleida–Agrotecnio Center, Lleida, Spain, [2]Animal Breeding and Genetics Program, IRTA, Caldes de Montbui, Barcelona, Spain.*

In this study, we performed an integrative analysis of genetic variants, transcriptomic data and lipid-related traits to identify transcription factors and candidate genes related to lipid metabolism. With this objective, a population of 256 Duroc pigs was genotyped with the 70k GGP Porcine BeadChip, and 22 traits of intramuscular fat (IMF) and its composition were measured in the gluteus medius muscle. From a subgroup of 40 pigs (20 fed with Vitamin A supplement and 20 without), the transcriptome of the semimembranosus muscle was sequenced in a HiSeq 2500XL (Illumina Inc.) at a depth of 60M reads. After an initial genome-wide association study (GWAs) analysis for the IMF-related traits, 290 genes and 555 SNPs from the candidate regions were included in the multi-omics factor analysis to discover the principal sources of variation between the genomic data sets and the phenotypes. With this approach, we identified 5 hidden factors that together explained 23% of variation in gene expression, 60% of SNPs and 68% of IMF-related traits. Latent Factor 1 (LF1) was relevant in all data sets (SNPs, RNaseq and phenotypes) whereas factors 2, 3, 4 and 5 were active in a single data type. Based on the loadings factors of LF1 in the RNaseq data, we selected the 40 most extreme genes (>2 SD). The main functions of many of those genes were related with lipid metabolism, i.e., SCD, COX15 and ABCC2, among others. Moreover, 30% of these genes co-localized with SNPs in the SSC14 (108–114 Mb) genome region indicating a gene expression correlated evolution (gene expression piggybacking effect). In addition, regulatory impact factor (RIF) was used to evaluate the regulative role of Vitamin A supplementation. According to the RIF values, the 60 (>2 SD) most extreme transcription factors (TF) were selected. The function of several of these TFs was related with lipid metabolism and their expression was affected by the levels of retinoic acid, i.e., RARB, LXRB, YY1, PPRC1, HOXA13 and HOXD8, among others. We are currently analyzing the gene expression networks related to these TF and to IMF-related traits. As a conclusion, the integration of phenotypic, genotypic and transcriptomic data allowed us to identify sources of variation between the expressed genes and the SNPs, as well as possible phenomena of gene expression piggybacking.

Key Words: fatty acids, pigs, integrative analysis

P309 Variant discovery rate and genotype concordance of sequencing at a moderate coverage. R. Ros-Freixedes*, R. González-Prendes, S. Gol, E. Solé, R. N. Pena, and J. Estany, *Departament de Ciència Animal, Universitat de Lleida–Agrotecnio Center, Lleida, Spain.*

Sequence data has the potential to empower identification of causal variants underlying quantitative traits or diseases. For that purpose, generally sequencing strategies that balance high discovery rate with high genotyping accuracy are required. One possible strategy is to sequence a set of individuals and then impute whole-genome data for the rest of the population. However, imputation with high accuracy for large populations may not always be feasible when sequencing resources are limited or when marker array genotypic data is unavailable. In those situations, it may be preferable to balance a smaller number of sequenced individuals with greater genotyping certainty, by increasing sequencing coverage to moderate levels of approximately 5x. This sequencing strategy could provide a useful platform for pre-screening of variants, especially if potential associations with traits of interest can be further validated by genotyping an extended set to compensate the smaller sample sizes. The objective of this study was to evaluate variant discovery rate and genotype and allele concordance between next-generation sequencing at ~5x, genotyping marker arrays, and genotyping by PCR-RFLP or PCR-HRM. Around 96% of the variants from a marker array were successfully discovered, with very high genotype (0.96) and allele (0.99) concordances. Genotype and allele concordances with markers genotyped by PCR were also high and indicated that sequencing was more accurate than PCR-RFLP. Sequencing design should be adapted to each application and sequencing at a moderate coverage (~5x) provided a suitable balance between variant discovery and genotyping accuracy for the discovery of variants potentially associated with traits of interest.

Key Words: animal breeding, pigs, sequencing, variant discovery

P310 The variance of gene expression in the porcine skeletal muscle changes in response to food intake. E. Mármol-Sánchez*[1], R. Quintanilla[2], TF Cardoso[3], J. Tibau[4], and M. Amills[1,5], *[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [2]Animal Breeding and Genetics Program, Institute for Research and Technology in Food and Agriculture (IRTA), Torre Marimon, Caldes de Montbui, Spain, [3]CAPES Foundation, Ministry of Education of Brazil, Brasilia D. F., Brazil, [4]IRTA-Monells, Monells, Spain, [5]Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.*

During the past few years, the analysis of RNA-seq data across different treatments has been mainly based on the contrast of expression means. Many studies have been conducted on diverse tissues and species, aiming to disentangle the complex responses at the gene level that different stimuli can trigger within the cell transcriptome. However, focusing on differences in gene expression means may have overlooked other valuable sources of biological information such as gene expression variance (GEV). We aimed to gain new insights into the relationship between nutrition and GEV by evaluating the dispersion of the expression of coding and non-coding genes in 48 Duroc pigs divided in 4 groups: pigs fed *ad libitum* and slaughtered under fasting conditions, pigs fed *ad libitum* and slaughtered 5 and 7 h after feeding, and pigs managed under restricted feeding during the first fattening phase and slaughtered under fasting conditions. *Gluteus medius* samples were collected and both RNA and small RNA fractions were subsequently sequenced. Novel long intergenic non-coding RNAs (lincRNAs) and natural antisense transcripts (NATs) were predicted by following the HISAT2-Stringtie pipeline and a combination of CPAT, CNCI, CPC2 and LncFinder softwares. Differences in GEV were assessed with the MDseq software. The Biological Coefficient of Variation (BCV) was calculated for each expressed protein-coding, long non-coding RNA and miRNA genes and differences across groups were evaluated. Highly expressed genes had more stable gene expression profiles than lowly expressed genes, which showed increased BCV values. Non-coding transcripts evidenced a reduced expression level compared with protein-coding genes and higher variance within analyzed groups. Genes having differential variance levels between conditions, *i.e.* differentially dispersed genes that, at the same time, did not show differences in mean expression, mostly belonged to DNA binding proteins and transcription factors (TFs), which mainly regulate the expression of many other transcripts by influencing the early steps of signaling pathways. These results might provide new hints to understand why the variance of gene expression changes across different experimental conditions.

Key Words: RNA-seq, nutrigenomics, gene expression

P311 Integration of phenomics, transcriptomics, epigenetics and glycomics to reveal the mechanism underlying the embryo-maternal interaction during implantation in pigs. F. Wang*, K. Han, J. Huang, D. Deng, W. Wang, and M. Yu, *Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China.*

The study was aimed to investigate the mechanism of embryo-maternal interaction in pigs which has not yet to be understood. First, by using digital slide scanning system, we performed systematical and large-scale examination on the morphological structure of porcine uterine lumen during implantation. Two phenotypic features involved in embryo implantation statuses were defined for the first time. Meanwhile, the 3-dimensional status of implanting embryo within uterus was imaged by taking advantage of a 3D reconstruction technology. Second, to obtain the in situ embryo-maternal interaction information at gestational d 12 and 15 (gd12 and gd15), we collected samples including conceptus and luminal epithelium from anti-mesometrial and mesometrial side adjacent to the conceptus, respectively, by using the laser capture microdissection technology. Then, we analyzed the RNA-seq and ATAC-seq data generated from these samples. The comparisons were as follows: (i) luminal epithelium at mesometrial vs anti-mesometrial side from gd12; (ii) luminal epithelium at mesometrial vs anti-mesometrial side from gd15; (iii) luminal epithelium of mesometrial side from gd12 vs those from gd15; (iv) luminal epithelium of anti-mesometrial side from gd12 vs those from gd15; (v) conceptuses from gd12 vs those from gd15. We found many differentially expressed genes and some regulatory pathways that may be associated with embryo-maternal interaction, for example, steroid hormone biosynthesis and metabolism pathway, retinoic acid signaling pathway, integrin-mediated signaling pathway. Third, the glycome of porcine endometrium was characterized for the first time and we found that sialylation of cell adhesion molecules has important roles in endometrium remodeling during implantation. Taken together, the integrative analyses of data described above revealed novel molecular pathways involved in implantation in pigs.

Key Words: pig, embryo implantation, phenomics, RNA-seq, ATAC-seq

P312 Combining metabolomics and genomics to elucidate physiological processes related to tail damage score in pigs. E. Dervishi*[1], L. van der Zande[2], T. da Silva Valente[1], I. Reimert[3], P. Mathur[2], M. S. Lopes[2,4], E. F. Knol[2], and G. S. Plastow[1], *[1]University of Alberta, Edmonton, Alberta, Canada, [2]Topigs Norsvin Research Center, Beuningen, The Netherlands, [3]Wageningen University & Research, Wageningen, The Netherlands, [4]Topigs Norsvin, Curitiba, Paraná, Brazil.*

The purpose of this study was to identify important metabolites related to tail damage (TDAM) score and to identify genomic regions associated with variation in the metabolites. We used 181 Tempo × Topigs-20 animals divided over 2 batches balanced for gender and selected for positive (n = 81) or negative (n = 100) indirect genetic effect (IGE) for growth. Half of the pigs were housed in a barren environment, and the other half in an enriched environment. The tail scores were recorded at weaning and thereafter once a week (score 1 no visible tail damage, score 2 hair removed from the tail, score 3 bite marks and score 4 clearly visible wound). Blood samples collected at 8, 9 and 22 weeks of age were used to determine metabolic profiles at The Metabolomics Innovation Centre (University of Alberta). A total of 53 compounds were quantified. Statistical analyses were performed in R version 3.5 using a generalized mixed model with repeated measurements. A single step genome-wide association study (ssGWAS) was performed to identify genomic regions associated with significant metabolites for tail biting score. Preliminary results show that serum levels of glycerol and isopropanol were significantly associated with tail damage score. Animals with TDAM 2 had greater glycerol concentration in blood when compared with animals with TDAM 3 and 4 ($P < 0.05$). In addition, animals with TDAM 1 had lower isopropanol concentration when compared with animals with TDAM 2 and 3 ($P < 0.05$). GWAS identified 2 candidate regions located on chromosome 6 associated with glycerol and isopropanol (at 45Mb, and at 149Mb respectively). The candidate genes identified in these regions were *ZFP14*, *DOCK7*, *ANGPTL3*, *USP1* and *KANK4*. Angiopoietin like 3 is a secreted protein encoded by *ANGPTL3* that is involved in the regulation of lipid and glucose metabolism. This protein is present at high levels in the liver where it can bind to adipocytes to activate lipolysis, releasing free fatty acids and glycerol. These results suggest that animals with tail damage (propensity to being bitten) might have impaired lipolysis processes.

Key Words: pig behavior, tail damage, metabolomics, genomics

P313 Investigation of gene expression profiles for correlation between female reproductive hormones and estrous cycle in the ovary, oviduct, and endometrium in swine. W. Park*[1], B.-H. Choi[1], J.-M. Kim[3], J.-E. Park[1], H. Ka[4], K.-T. Lee[2], and D. Lim[1], *[1]Animal Genomics and Bioinformatics Division, National Institute of Animal Science, RDA,Wanju, Republic of Korea, [2]Animal Genetics and Breeding Division, National Institute of Animal Science, RDA, Wanju, Republic of Korea, [3]Department of Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do, Republic of Korea,*

[4]Division od Biological Science and Technology, Yonsei University, Wonju, Republic of Korea.

Animal models are used in virtually all fields of biomedical research to help understand human pathologies and physiology. The pig is a representative animal model and an appropriate medical model for human pathologies and physiology in many areas of research because it having a similar and homology to human such as size, weight, organ, disease progression the availability of genomic, transcriptomic, proteomic tools and sexual maturity. So it is important to understand the morphological and functional similarities, and differences between the human and porcine female reproductive systems. The female pigs have an estrous cycle of about 21 d (range of 18 to 24 d). During the estrous cycle, female reproductive tracts have a variety of functions and play a major role in the secretion of the reproductive hormones. The purpose of this paper is to provide biological mechanism and function of genes that is significantly correlated gene between gene expression pattern and female reproductive hormones (LH, FSH, E2, P4 and Inhibin) value by each estrous cycle (0, 3, 6, 9, 12, 15 and 18 d) in female pig reproductive tracts (ovary, oviduct and endometrium). So we identified the trait associated genes (TAGs) in each female reproductive hormone in each reproductive tract. Therefore we found that these TAGs related with 2 major functional mechanisms such as hormone synthesis and neuron signaling

Key Words: estrous cycle, female reproductive tracts, female reproductive hormone, trait-associated genes, pig

P314 Whole-genome sequencing reveals selective signals for high-altitude adaptation in Tibetan pigs. Y. W. Zhang*, H. Zhang, and C. X. Wu, *College of Animal Science and Technology, China Agricultural University, Beijing, China.*

The Tibetan pig, a unique native pig breed to the Qinghai-Tibet Plateau, shows distinctive genetic adaptation to the high-altitude environment. High-altitude adaptation in animals is a complex trait that involves multi-genes and multi-channels regulatory mechanisms that mainly concern hypoxia adaptation, cold tolerance and radiation resistance. In this study, 179 samples that consisted of 135 domestic and 44 wild pigs were collected and whole-genome sequenced with Illumina HiSeq 2500 platform. Both the domestic and wild pigs were from 3 altitudes (high-, middle-, and low-altitude). We generated a total of 43 Mb of high-quality SNPs for population structure and signatures of selection analysis. The PCA, genome admixture and phylogenetic tree showed that the 71 Tibetan pigs appeared to be 3 populations corresponding to the distribution of rivers (the Yellow River, the Yangtze River and, the Yarlung Zangbo River). There was little overlap in selective signatures between Tibetan pigs and wild pigs. Many overlapped selective signatures were observed in comparing high- vs. low-altitude population and high- vs. middle-altitude in both domestic and wild pigs. Especially in domestic pigs, strong selection signals in Chr17 (Chr17:62,775,001–62,975,000) and Chr14 (Chr14:49,125,001–50,200,000) were tested. By utilizing ZFst, θπ and, mixed effect model for environmental adaptation (MEMEA) methods, we identify some positive selective genes (*SOX18*, *ZNF512B*, *PRPF6*, *PDE6A*, *SLC26A2*) and pathways (regulation of serine-type endopeptidase activity, calcium ion transmembrane transporter activity, response to UV-B, negative regulation of smooth and, muscle cell proliferation) related to high-altitude adaptation in Tibetan pig. The identified genes and pathways needed to perform further functional investigation. This research reveals that the independent evolution process of Tibetan pigs and wild pigs in high-altitude to adapt to the extreme plateau environment.

Key Words: Tibetan pig, high-altitude adaptation, whole-genome sequencing, altitude gradients

P315 Genetic relationship between Okinawa indigenous Agu pigs, wild boars in Japan and Asian pig breeds. A. Arakawa*[1], S. Touma[2], M. Taniguchi[1], T. Eguchi-Ogawa[3], S. Mikawa[1], and H. Uenishi[3], *[1]Institute of Livestock and Grassland Science, NARO, Tsukuba, Ibaraki, Japan, [2]Okinawa Prefectural Livestock and Grassland Research Center, Nakijin, Okinawa, Japan, [3]Institute of Agrobiological Sciences, NARO, Tsukuba, Ibaraki, Japan.*

There are many kinds of domesticated pig and wild boars occurring throughout eastern Asian regions. From Japanese archipelago studies, one domesticated breed, called Agu, and 2 sub-species of Asian wild boars have been recognized to inhabit since several centuries ago; the Japanese wild boar (*Sus scrofa leucomystax*) on the main islands of Japan and Ryukyu wild boar (*Sus scrofa riukiuanus*) on the Ryukyu. Our study was to investigate the genetic relationship between Agu, the wild boars in Japan and other *Sus scrofa* populations. A total of 131 Agu samples were collected from Okinawa Prefectural Livestock and Grassland Research Centre and Okinawa Prefectural Hokubu Agricultural High School. We also sampled 16 each of Japanese and Ryukyu wild boars for further genetic analysis. The samples of the Agu and the wild boars were genotyped using the PocineSNP60 BeadChip, and a series of quality control procedures for the acquired SNP data was carried out using PLINK v1.9. A part of SNP data of Asian pig breeds and wild boars published on a public database was used for this study. We performed discriminant analysis of principal component (DAPC), drew a phylogenetic tree using Nei's genetic distances by the R program. The admixture analysis using ADMIXTURE v1.3. The DAPC analysis and phylogenetic tree showed that the Japanese and Ryukyu wild boars were genetically related to the Asian wild boars, and the Agu was grouped into a clade which was far from European and Asian clades. In the results of the admixture analysis with K = 15, the Japanese and Ryukyu wild boars were found to have clusters of Asian wild boars, while we could not obtain fine evidence for inferred Asian origins of the Agu. Agu pigs have crossed with European breeds to improve meat productivity in the early twentieth century, which might bring about the complex genetic background in the Agu breed.

Key Words: Japanese native pig, Japanese wild boar, Ryukyu wild boar, SNP

P316 Fetal genotype effects on morphomics, fatty acids composition and transcriptomics in swine. C. Garcia-Contreras*[1,4], M. Vazquez-Gomez[2], O. Madsen[3], M. A. M. Groenen[3], S. Astiz[4], Y. Nuñez[1], R. Benitez[1], A. Heras-Molina[4], A. Fernandez[1], B. Isabel[2], A. I. Rey[2], A. Gonzalez-Bulnes[4], and C. Ovilo[1], *[1]Department of Animal Breeding. Instituto Nacional de Investigación y tecnología Agraria y Alimentaria (INIA), Madrid, Madrid, Spain, [2]Faculty of Veterinary Medicine, Universidad Complutense de Madrid (UCM), Madrid, Madrid, Spain, [3]Animal Breeding and Genomics, Wageningen University & Research, Wageningen, The Netherlands, [4]Department of Animal Reproduction. Instituto Nacional de Investigación y tecnología Agraria y Alimentaria (INIA), Madrid, Madrid, Spain.*

Iberian pigs are more prone to obesity and metabolic alterations than other lean swine breeds, due to leptin resistance and thrifty genotype, which makes the breed susceptible to changes in amount and composition of diet. This is especially manifested during prenatal development, where nutritional deficiencies in the diet of the mother may modify offspring's gene expression to cope with postnatal challenging conditions. This work aimed to assess the role of the genotype in the metabolic and transcriptomic changes of pig fetuses derived from undernourished pregnant sows. Pure Iberian sows were inseminated with heterospermic semen from Iberian and Large White boars to obtain IBxIB and IBxLW genotypes. The pregnancy was challenged by diminishing maternal intake to 50% of requirements during the last 2 thirds of pregnancy. Fifty-one fetuses were obtained at Day 77 of pregnancy and 32 of them (16 from each genotype) were selected at random. Assessment of body traits showed that IBxLW fetuses were heavier and more corpulent than their IBxIB littermates ($P < 0.05$). Analysis of liver fatty acids (FA) showed that IBxIB had higher level of saturated FA and monounsaturated FA and lower levels of polyunsaturated FA than IBxLW fetuses ($P < 0.0001$). Liver transcriptome analysis resulted in

249 genes differentially expressed (DE) between the 2 genotypes (q < 0.05 and FC >1.3). Functional annotation of the DE genes revealed a downregulation of biological functions related to growth and development (e.g.: *Cell survival, Cell movement, Quantity of connective tissue* or *Survival of organism*) in IBxIB fetuses. Potential transcription factors were assessed with IPA software, which allow the prediction of upstream regulators that may explain the molecular mechanisms responsible of transcriptome differences between genetic types. We highlighted 955 potential upstream regulators, some of them being involved in growth and development (e.g.: IGF1) and downregulated in IBxIB fetuses. Hence, our results indicate differential regulation of relevant functions and pathways involved in growth and development, which may explain the phenotypic differences among the fatty and lean swine genotypes.

Key Words: pigs and related species, functional genomics, RNA-seq, fat/lipid, product quality

P317 Selection and validation in the pig production chain of genetic markers associated with quality of thighs for dry cured ham. R. Davoli[1], M. Zappaterra[1], C. Schivazappa[2], N. Simoncini[2], R. Virgili[2], and P. Zambonelli*[1], *[1]Department of Agricultural and Food Sciences (DISTAL), University of Bologna, Bologna, Italy, [2]Stazione Sperimentale per l'Industria delle Conserve Alimentari (SSICA), Parma, Italy.*

The aim of this research was to validate a set of single nucleotide polymorphisms (SNPs) known to affect meat and green ham qualitative characteristics and to verify its utilization directly in pig production chain. Green ham weight and lean content, ultimate pH, weight losses and the amount of salt adsorbed after first and second salting were measured with a Ham-Inspector apparatus (lenz-instruments.com), on the thighs obtained from 230 commercial hybrid heavy pigs. *Semimembranosus* muscle samples were collected at ham processing plants from commercial hybrid pigs reared in 3 different farms and by crossing the 3 main breeds used for the production of Italian heavy pigs: Large White, Landrace and Duroc. The association study was carried using a custom panel of known 96 SNPs detected in 65 genes localized in 16 chromosomes. The analysis of this SNP panel revealed that 72 out of 96 SNPs were segregating in the utilized sample. The association study was performed with GenAbel package and a model where farms and slaughter day were considered. The results revealed some significant gene effects (P < 0.05) and in particular a SNP located in *Myopalladin* (*MYPN*) gene (P < 0.01) showed a significant association with green ham lean content. Ultimate pH resulted to be influenced by a polymorphism in *ATPase $Na^+/K+$ Transporting Subunit Alpha 2* (*ATP1A2*) gene (P = 0.016), and among the considered markers, a SNP on *Protein Phosphatase 3 Catalytic Subunit Alpha* (*PPP3CA*) gene showed significant effects on green ham weight (P = 0.015) and ham weight after both first and second salting (P = 0.011 and P = 0.012, respectively). Furthermore, it is worth noting that some SNPs were associated with more than one trait, in particular SNPs lying on chromosomes 8 and 2. The results show the feasibility of the use of a SNP set of genetic markers in the pig production chain and suggest that the genotyping with this panel could be e a useful tool to improve the quality of thighs for the production of protected designation of origin (PDO) dry-cured hams. Acknowledgments. This research was funded by AGER-ProSuIT project (rif 2017–2022) and Bologna University RFO funds.

Key Words: pigs and related species, genotyping, SNP, genetic marker, product quality

P318 Abstract withdrawn

P318 Abstract withdrawn

P319 Differences in longissimus transcriptome between Iberian pigs divergent for meat tenderness. M. A. Fernandez-Barroso*[1], J. M. García-Casco[1], L. Silio[2], C. Rodriguez[2], Y. Nuñez[2], F. Sanchez-Esquiliche[3], C. Caraballo[1], and M. Muñoz[1], *[1]Centro de I+D en Cerdo Ibérico,INIA, Zafra, Extremadura, Spain, [2]Departamento de Mejora Genética Animal, INIA, Madrid, Spain, [3]Sánchez-Romero Carvajal, Jabugo, Huelva, Spain.*

Traditional Iberian pig production is focused on the development of highly prized meat and meat products. Meat tenderness is a relevant trait directly related with meat quality. The analyses of the transcriptome of individuals with extreme values for meat tenderness would provide a better insight about the genes codifying proteins involved in pathways affecting this trait. The objective of the current study was to analyze through RNA-seq technique, differentially expressed genes (DEG) on longissimus dorsi (LD) muscle in 2 groups with extreme estimated breeding values (EBVs) for tenderness of a line of purebred Iberian pigs. LD samples from 950 castrated males were collected after slaughter. Tenderness was determined in cooked meat portions, measured as maximum shear force (kg/cm2) by Warner-Bratzler test (texturometer TA.XT Plus, Stable Microsystems). A total of 13 non sibling animals with extreme EBVs for shear force were selected for LD RNA-seq analysis, 7 of them with the higher values and 6 with the lower ones. Sequencing was performed using a llumina Hi-Seq 2000 equipment and analyzed using Tuxedo pipeline. Raw data was trimmed according standard criteria and mapped against Sscrofa11.1 assembly. Genes with a mean expression >0.5 FPKM, fold change between groups ≤ 0.5 and ≥ 1.5, and p-value ≤ 0.05 and q-value ≤ 0.10 were considered as differentially expressed. Functional analyses of DEG were carried out by examining GO enrichment with FatiGO. Differential expression analyses between divergent samples showed a total of 130 DEG and 156 new isoforms. There were an overrepresentation of DEG in 388 gene ontology biology process (GOBP) related with skeletal muscle tissue development (GO: 0007519), striated muscle contraction (GO: 0006941), regulation of calcium ion transport (GO: 0051924) and actin-myosin filament sliding (GO: 0033275). These processes are closely related with the routes involved in the transformation of muscle to meat. Some of the DEG were particularly relevant, for example, MSTN

gene codifies for myostatin protein, which is involved in the regulation of skeletal muscle cell proliferation and differentiation and DMD gene codifies for dystrophin protein, which is involved in the strengthening and development of muscle fibers.

Key Words: transcriptome, RNAseq, tenderness, DE genes

P320 Transcriptomic profiles can explain differences in meat quality between two Iberian pig varieties. A. Vilaplana[1,2], N. Ibanez-Escriche*[3], R. Pena[4], L. Muñoz[6], E. González[7], J. F. Tejeda[7], and J. L. Noguera[5], *[1]The Roslin Institute, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, UK, [2]Centre for Medical Informatics, User Institute, Edinburgh, UK, [3]Department for Animal Science and Technology, Universistat Politécnica de València, Valencia, Spain, [4]Departament de Ciència Animal, Universitat de Lleida-Agrotecnio Center, Lleida, Spain, [5]IRTA, Genètica i Millora Animal, Lleida, Spain, [6]NGA FOOD S.A, Almendralejo, Spain, [7]Tecnología de los alimentos, Universidad de Extremadura, Badajoz, Spain.*

Iberian pig produce a high quality meat characterized by enhanced deposition of intramuscular fat and high oleic fatty acid content. These 2 parameters present great variability among the varieties that make up the Iberian pig population. In a previous experiment, a complete diallelic cross generated using Retinto and Torbiscal varieties was used to describe differences in intramuscular fat (IMF) and fatty acids profile in *Longissimus thoracis*. We have now used whole transcriptome shotgun sequencing to study differences between lines at muscle transcriptomic level. Several genes differently expressed between varieties participated in metabolic pathways related to meat quality traits. Of relevance, *COX*, *ACA* and SDHB family genes involved in fatty acid biosynthesis, were overexpressed in the Retinto variety with greater content of IMF. Likewise, Torbiscal pigs, with less IMF than Retinto, overexpressed a hormone (*ADIPOQ*) and a transcription factor (*SREBF1*) related to the inhibition of the adipogenesis and lipid transport. Additionally, the phospholipase *PLA2G7*which is related to oleic acid transport, was also overexpressed in the Retinto line. Therefore, this study identified key genes and metabolic pathways that could explain the phenotypic differences of meat quality and adiposity between 2 Iberian pig varieties.

Key Words: Iberian pig, RNA-Seq, meat quality, differential expression, fatty acids

P321 Genetic relationship of linoleic to arachidonic acid pathway with intramuscular fat in pigs. S. Gol*[1], R. González-Prendes[1], L. Bosch[2], M. Tor[1], J. Reixach[3], R. N. Pena[1], and J. Estany[1], *[1]Department of Animal Science, University of Lleida-Agrotecnio Center, Lleida, Spain, [2]Department of Agriculture Engineering and Food Technology, University of Girona, Girona, Spain, [3]Selección Batallé, Riudarenes, Spain.*

Intramuscular fat (IMF) content is a relevant trait for high-quality meat products such as dry-cured ham, but increasing IMF has the undesirable correlated effect of decreasing lean growth. In pigs, the proportion of linoleic (C18:2) and arachidonic (C20:4) acids decline with fat deposition and they can be considered as indicators of fatness. Hence, the objective of this research was to assess the potential of these fatty acids as specific biomarkers of IMF. With this purpose, the heritability for C18:2 and C20:4 in IMF, as well as their genetic correlations with IMF and backfat thickness (BT) were estimated. Data on 162,494 purebred Duroc pigs, 91,448 of which had records of BT at 180 d and 1,371 had records of fatty acid composition in the muscle *gluteus medius*, were used for the analysis. Results showed that the C18:2 to C20:4 pathway is genetically determined. The absolute amount of C18:2 (mg/g of muscle) and the C20:4 to C18:2 ratio (C18:2/C20:4) in IMF displayed much stronger genetic correlations with IMF (0.88, for C18:2, and −0.59, for C20:4/C18:2) than with BT (0.07, for C18:2, and −0.10, for C20:4/C18:2). In particular, we found that selection for absolute C18:2 is expected to deliver similar genetic response outcome as selection for IMF at restrained BT. These findings support the search for molecular markers in genes encoding enzymes involved in C18:2 metabolism. One of them is the fatty acid desaturase-2 (*FADS2*), a rate-limiting enzyme in the conversion of C18:2 into C20:4. With this aim, we characterized the promoter region of *FADS2*, evaluating in particular the effect of the haplotype-tagging polymorphism rs321384923A > G. Pigs carrying the A allele showed enhanced *FADS2* expression, higher C20:4/C18:2 and lower absolute C18:2. Correlated effects on IMF and BT were in line with expected changes, with the A allele showing a negative trend toward decreasing IMF (allele substitution effect of -0.49 ± 0.23, $P < 0.05$) without altering BT and carcass weight. Our results would confirm that quantitative biological analysis can be a good approach to find new traits and candidate markers for efficient selection for IMF and lean weight.

Key Words: intramuscular fat, pig, selection

P322 Predicting variant deleteriousness in non-human species: Applying the CADD approach in pig. C. Gross*[1,2], M. Derks[3], H. J. Megens[3], M. Bosse[3], M. A. M. Groenen[3], M. Reinders[1], and D. de Ridder[2], *[1]Delft Bioinformatics Lab, Delft University of Technology, Delft, The Netherlands, [2]Bioinformatics Group, Wageningen University & Research, Wageningenm The Netherlands, [3]Animal Breeding and Genomics Centre, Wageningen University & Research, Wageningenm The Netherlands.*

Predicting deleteriousness of genomic variants has taken a step forward with the introduction of the Combined Annotation Dependent Depletion (CADD) for human genomes. CADD learns a model to differentiate variants that have experienced positive selection from those expected to have experienced negative selection over the evolutionary history of primates. This approach can in theory be applied to any species. The success of CADD led us to create a similar model for livestock species, for which far less genomic annotation data is available. Here we present p(ig)CADD, a model to score SNVs in pig genomes. We created 7,158,434,598 comparable scores to predict the potential deleteriousness of all possible SNV on all autosomes and X of the pig genome. To evaluate whether generated pCADD scores capture sites of biological meaning, we first tested how well pCADD scores discriminate different positions of a codon. We found that in 82% of all tested transcripts, the third codon position has a significantly lower pCADD score (i.e., lower predicted deleteriousness) than the 2 other positions, while the second position is highest scored on average. This is in agreement with the number of synonymous nucleotide substitutions for each position. Further, we tested whether regions coding for miRNA transcripts do get a higher pCADD score than their direct neighboring regions. The results suggest that both regions can be clearly distinguished. Finally, we scored the coding regions of genes that are specific for different tissues. The pCADD scores are relatively low for genes specific for salivary gland tissue, which can be explained by fast dietary adaptive evolution. Contrary, neuronal tissue is particularly highly scored. These results indicate that based on pCADD scores, regions with biological meaning can be identified and distinguished according to their rate of adaptation. In this way, observed SNVs of unknown effect can be prioritized for further analysis.

Key Words: bioinformatics, bioinformatics tools, genomic prediction, functional genomics, pigs and related species

P323 Genome-wide association studies for feed efficiency with imputed genotypes in pigs. E. Delpuech*, Y. Labrune, A. Aliakbari, H. Gilbert, and J. Riquet, *GenPhySE, Université de Toulouse, INRA, INPT, ENVT, Castanet-Tolosan, Toulouse, France.*

Genetic improvement of feed efficiency is a major challenge for the sustainability of monogastric animal productions. It also has positive consequences on the environmental impact of livestock, through the reduction of excretion. Two divergent lines are conducted at INRA considering Residual Feed Intake (RFI) as a criterion for selection. In each generation, first parity males were tested during growth to select the 6 boars with the lowest (LRFI line) or highest (HRFI line) RFI. A

second parity was produced to evaluate the correlated responses to selection on production and carcass traits on females and castrated males. In total, data comprised records from 1,632 sires and dams (parents from generation G0 to G9), and 2,426 response animals. Dams had no phenotypic records. Genotyping was completed on sires and dams with the Illumina PorcineSNP60 (MD) beadchip (n = 1,632). In addition, the 32 G0 animals most contributing to the pedigree (12 boars and 20 dams) were genotyped using the Illumina high-density (HD) porcine SNP chip. Using the FIMPUTE software (v-2.2), HD genotypes were imputed to all the parents of the design using linkage disequilibrium and pedigree information. Imputation accuracy was assessed based on the correlation between the true and the imputed genotypes, per animal (r = 0.982) and per SNP (r = 0.946). In a second step, genotypes for the 570,440 SNP of the HD SNP chip were imputed to the progeny with phenotypic records and no genotypes (second parity), using the average genotype of the 2 parents. Genome-wide association studies (GWAS) were then performed on those animals for 24 traits (RFI, related traits as average daily gain (ADG), backfat thickness (BFT), and FCR, together with traits related to carcass composition and meat quality). Results from GWAS revealed 198 associations with the traits, including 9 regions associated with RFI. GWAS performed separately in each line revealed significant associations in different genomic regions, indicating that different metabolic pathways were mobilized during the selection. Fine mapping of the detected regions will provide new insights into the genetic basis of feed efficiency in pigs.

Key Words: genetic improvement, genome-wide association, imputation, feed efficiency, pig

P324 Population structure and genome characterization of synthetic pig breed based on Korean native pig. D.-H. Son*[1], H.-S. Seong[1], J.-W. Choi[1], Y.-M. Kim[1,2], and E.-S. Cho[2], *[1]College of Animal Life Science, Kangwon National University, Chuncheon, Republic of Korea, [2]Division of Swine Science, National Institute of Animal Science, RDA, Cheonan, Republic of Korea.*

Woori-Heukdon (W) is a black pig breed introduced by crossbreeding Chookjin-Chamdon (C), Chookjin-Duroc (D) and crossbreds (D × C, DC × D). Chookjin-Chamdon is a Korean Native Pig breed that is known to have superior meat quality and strong adaption ability in Korean peninsula, although its growth rates and productivities are lower than typical European-origin commercial breeds such as Duroc, Landrace, and Yorkshire. To improve growth rates and productivities of C, W which is a hybrid from C, D, DC, and DCD had been developed in National Institute of Animal Science in South Korea. This newly synthetic pig breed had been influenced by C as its genetic composition is 37.5%. In this study, we compared whole genomes of the 7 pig populations including W (n = 253), D (n = 582), C (n = 91), DC (n = 1), DCD (n = 61), European Duroc (n = 10), and Berkshire (n = 10) to assess genomic characteristics of the W. Five pig populations sampled in South Korea (including C, D, W, DC, DCD) were genotyped by Illumina Infinium Porcine SNP60 v2 BeadChip, while we downloaded SNP data sets for European origin breeds (including Duroc, Berkshire) that were genotyped by SNP60 v1 BeadChip. We performed quality control (QC) procedures on the concordant SNPs in autosomes (49,087), resulting in 959 pigs and 24,260 markers remained for further analyses. In the population differentiation analysis (F_{ST}) and principal component analysis, W showed closer genetic relationship with the D than with the C. The average of the F_{ST} values were 0.20, 0.19, and 0.07 for the comparisons of 'D vs. C', 'W vs. C', and 'W vs. D', respectively. Also, C showed the highest genetic distance to Berkshire (0.37). Admixture and Treemix analyses also indicated that Chookjin-Chamdon was clearly separated from other populations. Furthermore, we found several candidate genes as potential selection signatures on the genome, which are known to be implicated with economically important traits in various pig breeds.

Key Words: pigs and related species, population genomics, population structure, crossbreeding

P325 QTL analysis of serum traits in an F_2 intercross between Landrace and Korean native pigs. I.-C. Cho*[1], M. U. Kang[2], M. S. Choi[2], N.-Y. Kim[1], M.-C. Shin[1], S.-M. Shin[1], J.-K. Son[1], J.-H. Woo[1], J.-H. Yoo[1], N.-G. Park[1], H.-B. Park[1], and B.-C. Yang[1], *[1]National Institute of Animal Science, Jeju, Jeju, South Korea, [2]Foundation of Agri. Tech. Commercialization and Transfer, Iksan, Jeollabuk-do, South Korea.*

The aim of this study was to identify quantitative trait loci (QTL) and detect causative gene locus in a genome-wide level affecting serum traits in a reciprocal intercross population between Landrace and Korean native pigs. Blood samples were collected following an overnight fast at 140 d after birth. Twenty-five phenotypes related to serum traits were measured in > 970 F2 progeny. All samples were subjected to genotyping analysis using 168 microsatellite markers located across the genome. The effects of reciprocal crosses were significant among 8 traits (ALT, K, GPT, Amy, ALP, CPK, Crea, and D-BIL) out of a total of 25 clinical-chemical phenotypes in the F2 animals. No significant effect of reciprocal crosses on other clinical chemical traits was found in either sex. Significant effects of reciprocal crosses were detected in both males and females for Amy, ALP, and Crea. An effect of reciprocal crosses on GPT was only found to be significant in females, while significant effects of reciprocal crosses on ALT, K, CPK, and D-BIL were detected only in males. In conclusion, the identified QTLs together with the positional candidate genes identified here could play an important role in elucidating the genetic structure of serum phenotype variation in humans and swine.

Key Words: serum traits, healthy index, QTL, reciprocal intercross

P327 Whole-genome resequencing reveals signatures of selection in European pig breeds and wild boars. S. Bovo*[1], G. Schiavo[1], A. Ribani[1], F. Di Palma[2], V. J. Utzeri[1], G. Moscatelli[1], C. Geraci[1], M. Gallo[3], M. Muñoz[4], A. I. Fernandez[4], G. Usai[5], J. Riquet[6], R. Charneca[7], I. Djurkin-Kušec[8], Č. Radović[9], R. Savić[10], J. P. Araujo[11], R. Quintanilla[12], V. Razmaite[13], M. J. Mercat[14], C. Zimmer[15], D. Karolyi[16], M. Candek-Potokar[17], F. García[4], Y. Núñez[4], C. Ovilo[4], L. Fontanesi[1] and TREASURE Consortium[18] *[1]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [2]Earlham Institute, Norwich, United Kingdom, [3]Associazione Nazionale Allevatori Suini (ANAS), Rome, Italy, [4]Departamento Mejora Genética Animal, INIA, Madrid, Spain, [5]AGRIS SARDEGNA, Loc. Bonassai, Sassari, Italy, [6]Génétique Physiologie et Système d'Elevage, INRA, Castanet-Tolosan, France, [7]Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade de Évora, Évora, Portugal, [8]University of Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia, [9]Institute for Animal Husbandry-Pig Research Department, Belgrade-Zemun, Serbia, [10]University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, Serbia, [11]Instituto Politecnico de Viana do Castelo, Viana do Castelo, Portugal, [12]Programa de Genética y Mejora Animal, IRTA, Barcelona, Spain, [13]Animal Science Institute, Lithuanian University of Health Sciences, Baisogala, Lithuania, [14]Institut du Porc, IFIP, Le Rheu, France, [15]Bäuerliche Erzeugergemeinschaft Schwäbisch Hall, Schwäbisch Hall, Germany, [16]Department of Animal Science, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia, [17]Kmetijski Inštitut Slovenije, Ljubljana, Slovenia, [18]TREASURE Consortium, Ljubljana, Slovenia*

Natural and artificial directional selection in cosmopolitan and autochthonous livestock and wild relative populations have shaped their genomes defining the level of variability and determining selective sweeps as final adaptation to different environmental conditions and production systems. In this study we analyzed the genetic variability and selection signatures in 19 European local pig breeds sampled from 7 countries (Croatia, France, Germany, Italy, Lithuania, Portugal, Serbia, Slovenia and Spain) (Alentejana, Apulo-Calabrese, Basque, Bísara, Majorcan Black, Black Slavonian, Casertana, Cinta Senese, Gascon, Krskopolje, Lithuanian indigenous wattle, Lithuanian White Old Type, Mora Romagnola, Moravka, Nero Siciliano, Sarda, Schwäbisch-Hällisches Schwein, Swallow-Bellied Mangalitsa and Turopolje), 3 cos-

mopolitan Italian breeds (Large White, Landrace and Duroc) and Italian wild boars. For each population, we prepared DNA pools using equimolar DNA from 30 to 35 animals. Whole-genome 150-bp paired-end sequencing was carried out on an Illumina HiSeq machine. More than 18.4 billion of reads were obtained and mapped on the Sscrofa11.1 genome version with BWA, obtaining a mean depth of sequencing of 42X, respectively. CRISP, coupled to an ad hoc bioinformatic pipeline, was used to detect more than 30 million of high-quality variants (18% not included in dbSNP yet). Variant annotation, carried out with VEP, highlighted that the 0.34% of the autosomal SNPs impacted genes at the protein level. Selection signature analyses (F_{st} and the Pooled Heterozygosity) highlighted more than 400 sweep regions distributed along the 18 porcine autosomes and scaffolds. Some of these genome regions harboured major genes affecting body shape/size (e.g., *NR6A1*, *PLAG1*, *LCORL* and *CASP10*), coat color (e.g., *KIT*, *MC1R*) and growth/fatness (e.g., *MC4R*), providing a first global variability analysis of European *Sus scrofa* populations. This study has been funded by European Union's H2020 RIA program (grant agreement No 634476).

Key Words: pigs and related species, genome sequencing, comparative genomics, breed diversity, conservation

P328 Identification and experimental validation of enhancers in 11 tissues in the genome of lean and fatty type pigs. Q. Xiaolong*[1], H. Mingyang[1], H. Ye[1], Z. Yuxia[1], L. Yu[1], L. Tingting[2], Y. Hongbo[2], Y. Feng[2], Z. Shuhong[1], and L. Xinyun[1], *[1]College of Animal Science & Technology College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China, [2]Department of Biochemistry and Molecular Biology, College of Medicine, The Pennsylvania State University, Hershey, PA, USA.*

With the continuous development of pig genome studies, functional annotation of genomic sequences, especially the identification of enhancers, has become as a bottle neck to further understand the complex function of the genome. To break through this problem, we carried out ATAC-seq and ChIP-seq for H3K27ac to comprehensively identify the open chromatin regions and enhancers of pig genomes in skeletal muscle, spleen, heart, kidney, liver, backfat, lung, thymus, small intestine (duodenum), cerebrum and cerebellum from Enshi Black, Meishan, Duroc and Large White pigs. In total, we generated 28 ATAC-seq and 183 ChIP-seq libraries. About 275,770 open chromatin regions and 146,399 putative enhancers were identified, among them, 15,758 putative enhancers were tissue specific. The GO analysis of above tissue specific enhancers were significantly associated with the function of tissue ($P < 0.01$).Enhancers identified by H3K27ac signals were validated by the reporter assay and ATAC-seq singals. The reporter assay was performed to validate the activity of above identified enhancers. We randomly selected 8 predicted enhancers for validation and found that 7 of them (88%) can upregulate the reporter gene expression. Moreover, ~50% of enhancers identified by H3K27ac were also enriched by ATAC-seq open chromatin signals, indicating the reliability of our data. Super enhancer, the ultra-long cis-regulator elements with transcriptional enhancement activity, were also identified in this study for an average of ~1,000 in each tissue. The expression of super enhancer associated gene were significantly higher than genes associated with regular typical enhancers ($P < 0.001$).

Key Words: ATAC-seq, ChIP-seq, H3K27ac, open chromatin regions, enhancer

P329 Analysis of porcine muscle transcriptome reveals regulators and pathways associated with feed efficiency. Y. Ramayo-Caldas*[1,3], E. Mármol-Sánchez[2], M. Ballester[1], R. González-Prendes[2], M. Amills[2,3], and R. Quintanilla[1], *[1]Institute for Research and Technology in Food and Agriculture, Caldes de Montbuit, Barcelona, Spain, [2]Department of Animal Genetics, Centre for Research in Agricultural Genomics, Universitat Autònoma de Barcelona, Barcelona, Spain, [3]Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Universitat Autònoma de Barcelona, Barcelona, Spain.*

In this study, we used muscle gene-expression data to identify candidate genes, biological pathways and predictors of feed efficiency (FE) in a Duroc pig population. Gene expression data of the *gluteus medius* muscle corresponding to 104 pigs was explored through a combination of differential expression (DE) and 2 multivariate methods, including sparse Partial Least Squares Discriminant (sPLS-DA) and regularized Canonical Correlation Analysis (rCCA). Two groups of animals (10 highly feed-efficient and 10 lowly feed-efficient) were used in the DE analysis to identify 991 differentially expressed genes. The same sub-set of samples were used in the sPLS-DA; the first component combined the expression pattern of 200 genes to clearly discriminate between the groups with extreme efficiency profiles. The rCCA procedure allowed us to explore gene expression and FE traits joint co-variation using the whole data set (104 individuals). The rCCA reported 350 genes that maximize the correlation between gene expression and FE related traits. We compared the list of candidates genes reported by each method. A total of 57 genes were commonly detected by the 3 approaches, and 221 genes were identified by at least 2 approaches. The functional annotation showed that these 221 genes belong to pathways and gene networks related to energy and carbohydrate metabolism, and to methionine, isoleucine and valine degradation. Moreover, it is worth mentioning that the set of genes detected with multiple approaches includes regulators reported in previous studies as associated with FE, including *ESRRG, ZNF473*, *NFATC3, RXRG, PPARGC1A* and *NFKBIZ*. In summary, our results evidence the predictive ability of muscle gene expression data for classifying pigs according to FE and provide also a list of candidate genes associated with FE in pigs.

Key Words: muscle transcriptome, multivariate, predictors, pig, feed efficiency

P330 Analysis of porcine miRNA-33b expression in liver and *longissimus dorsi* muscle and its role in fatty acid metabolism. L. Criado-Mesas*[1], M. Ballester[2], D. Crespo-Piazuelo[1,3], A. Castelló[1,3], and J. M. Folch[1,3], *[1]Plant and Animal Genomics, Centre de Recerca en Agrigenòmica (CRAG), Consorci CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, Spain, [2]Departament de Genètica i Millora Animal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Torre Marimon, Caldes de Montbui, Barcelona, Spain, [3]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain.*

miRNAs are small RNA molecules that are involved in post-transcriptional gene regulation and play important roles in diverse regulatory pathways of many cellular processes. In humans, the miRNA-33 family, which is composed of miRNA-33a and miRNA-33b, was reported to be co-transcribed with SREBP transcription factors and was involved in the regulation of lipid metabolism genes. miRNA-33b was the only member of the family studied in pigs and it was suggested that regulates fatty acid oxidation in adipose tissue. The aim of this work was to study the expression of porcine miRNA-33b in liver and *longissimus dorsi* muscle, to better understand its biological role in lipid metabolism. The expression of pig miRNA-33b was analyzed in 41 animals from an Iberian × Landrace cross in liver and muscle. RT-qPCR for miRNA-33b was performed with Taqman MicroRNA Assays, using miRNA-let7a and miRNA-26a as porcine reference miRNAs. TargetScan and miRGate algorithms were used to identify potential miRNA-33b targets. Liver and muscle mRNA expression of 44 lipid metabolism-related genes was analyzed in the same set of animals by Fluidigm Real-Time PCR. miRNA-33b expression levels were higher in muscle (mean = 5.01, SD = 0.64) than in liver (mean = 2.49, SD = 3.26) and a moderate correlation (r = 0.34, p-value = $3.5x10^{-02}$) was found between them, suggesting different regulatory mechanisms of miRNA-33b in both tissues. Overall, small correlations were found among the expression of miRNA-33b and lipid-related genes in liver (r = −0.26 to 0.31) and muscle (r = −0.35 to 0.37) tissues. In fact, no correlation was found between SREBPF1 and miRNA-33b. In liver, miR-

NA-33b showed a negative correlation with *CPT1A* gene (r = −0.26, p-value = $9x10^{-02}$), which was previously described in human as a target gene for mitochondrial β-oxidation. The obtained results suggest that miRNA-33b is involved in the regulation of the b-oxidation pathway in liver but not in muscle. Further analysis will be performed to study the regulatory role of miRNA-33b in adipose tissue and miRNA-33a in liver, adipose tissue and muscle to better understand the miRNA-33 family role in lipid metabolism.

Key Words: microRNA, pig, metabolism, lipid, qPCR

P331 From population genetics to single gene variants: Molecular background of breed specific effects at a QTL for thoracic vertebrae in pigs. M. van Son[1], M. Lopes[2], H. Martell[3], M. Derks[4], J. Kongsro[1], E. Grindflek[1], and B. Harlizius*[2], *[1]Norsvin SA, Hamar, Norway, [2]Topigs Norsvin Research Center, Beuningen, The Netherlands, [3]University of Kent, Canterbury, UK, [4]Wageningen University, Wageningen, The Netherlands.*

Large phenotype, genotype and sequence data sets from 2 different pig breeds (Duroc and Landrace) were analyzed extensively at the quantitative and the molecular level. Refining trait definition by counting vertebrae (NVE) and thoracic vertebrae (RIB) from CT scan data increased heritability from 0.28 for number of teats (NTE) up to 0.62 for NVE and 0.78 for RIB in Duroc. In Landrace, heritability for RIB was much lower (0.24) compared with NVE (0.59). Genome-wide association analysis identified a major QTL for NTE on chromosome 7 and explained a large proportion of the genetic variance (>80%) for NVE and RIB in both lines. But the lines differed considerably in the estimated size of the allelic effect for RIB which was 0.49 in Landrace but 0.83 in Duroc. At the molecular level, haplotypes in this QTL region derived from 660K SNP data identified a common haplotype of 7 SNPs in Duroc. Sequence analysis of 16 Duroc animals showed that 2 functional mutations reside on this haplotype that were recently shown to increase expression of Vertnin (VRTN) and number of thoracic vertebrae. In Landrace, the linkage disequilibrium extended over a region of more than 3 Mb also containing both VRTN mutations. In all sequenced Landrace animals, additional variants were found on the wildtype haplotypes surrounding the VRTN region including 2 missense mutations in the ABCD4 gene. Also, variants at other modifying loci can be expected across the genome causing the breed-specific effect because Landrace animals had one NVE and RIB more than Duroc animals in each genotype class of the wild type and mutant VRTN haplotypes. This might indicate that the developmental code of the homeobox-genes determining the identity of the vertebrae (thoracic or lumbar) is dissociated from the molecular segmentation clock in Landrace and that the capacity for RIB development is getting exhausted in the Landrace population. Together, we show specifically how the size of the effect at one locus and accuracy of rib counting are influenced by the genetic background changing genetic population parameters dramatically.

Key Words: number of vertebrae, Vertnin, QTL, pig, genetic parameters

P332 Transcription profile of *Semimembranosus* muscle in Italian Large White pigs with high and low intramuscular fat content and comparative analysis. M. Zappaterra*[1], S. Gioiosa[2], G. Chillemi[3], T. Castrignanò[2], P. Zambonelli[1], and R. Davoli[1], *[1]Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum-University of Bologna, Bologna, Italy, [2]CINECA Super-Computing Applications and Innovation Department (SCAI), Roma, Italy, [3]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), La Tuscia University of Viterbo, Viterbo, Italy.*

Intramuscular fat (IMF) content is a complex trait influencing the technological and sensorial features of meat products and determining meat quality. The purpose of the present work was to analyze through RNA-sequencing the *Semimembranosus* muscle (SM) transcriptome of Italian Large White pigs to study the gene networks associated with IMF deposition. Two groups of samples, each one composed of 6 unrelated pigs with extreme and divergent IMF (0.67 ± 0.09% in low IMF vs. 6.81 ± 1.17% in high IMF groups) were chosen from a population of 950 pigs and used for the present study. Paired-end RNA sequences were aligned to *Sus scrofa* genome assembly 11.1 and gene counts were analyzed using WGCNA and DeSeq2 packages. The functional annotation analysis was performed with DAVID v.6.8 on-line tool and Cytoscape v.3.5.1. Four WGCNA modules resulted significantly associated with IMF, and the most significant one was the grey60 module ($P = 0.003$). The genes comprised in the significant modules were then investigated through functional analysis. The most significant annotation cluster identified for grey60 module contained genes involved in primary cilia ($P = 0.004$), intraciliary transport ($P = 0.005$) and cell projection ($P = 0.02$). Interestingly, among the 58 differentially expressed genes (DEG) found with DeSeq2 (Fold Change >1.5 and adjusted $P < 0.05$) some were related to primary cilia organelles (such as *Lebercilin 5* gene), in addition to genes involved in the regulation of differentiating cells, in the control of RNA-processing, and in G-protein and ERK signaling pathways. Recent literature has reported the importance of primary cilia in the differentiation of fibro-adipogenic precursors to adipocytes, and dysfunctional primary cilia have been indicated as a possible key event in the pathogenesis of obesity. Our results seem to suggest that among the complex molecular processes affecting muscle fat depots, genes involved in primary cilia may have an important role. The research was funded by PRIN 2015 national project (no. 201549TZXB001).

Key Words: pigs and related species, functional genomics, RNA-seq, fat/lipid, product quality

P333 Abstract withdrawn

P334 Association of *SCD* and *LEPR* genes with litter size and weight in pigs. E. Solé*[1], R. N. Pena[1], M. Tor[1], J. Reixach[2], and J.

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In pigs, the AY487830:g.2228 T > C polymorphism in the promoter region of stearoyl-CoA desaturase (*SCD*) gene and the NM_001024587:g.1987C > T polymorphism in the leptin receptor (*LEPR*) gene have been associated with fatty acid composition and fat content, respectively. Although there is a link between fat metabolism and reproductive performance, their influence on reproduction traits has not been investigated. Previous experimental research has shown that *LEPR* is involved in embryo attachment and milk composition. The aim of this study was to investigate the effect of the *SCD* and *LEPR* polymorphisms on litter size and weight at weaning. Data on 1,547 litters from 621 purebred Duroc sows genotyped for both polymorphisms were used. In each farrowing, the total number of piglets born, number of piglets born alive, number of piglets born dead, number of weaned piglets and litter weight at weaning was recorded. No differences were found between *SCD and LEPR* genotypes for litter size, either at birth or at weaning, and between the sow's *SCD* genotype for litter weight. However, the sows carrying the C allele for *LEPR* had heavier litters at weaning as compared with TT sows. On average piglets from *LEPR-C* sows weighted at weaning around 150 g more than piglets from *LEPR-TT* sows. This finding is consistent with previous experiments indicating that the C allele exerts a negative-dominant effect on fatness and thus TT pigs are considerably fatter than *LEPR-C* pigs. In conclusion, our results indicate that *SCD and LEPR* genes do not affect litter size but that the T allele at the *LEPR* gene impairs litter performance due to increased body fatness.

Key Words: pig, *LEPR*, *SCD*, litter size, weight at weaning

P335 Low birth weight affects porcine intestinal gene expression and impairs intestinal development. M. Ayuso*[1], S. Van Cruchten[1], C. P. Walsh[2], R. Irwin[2], and C. Van Ginneken[1], [1]*Department of Veterinary Medicine, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Wilrijk, Belgium,* [2]*School of Biomedical Sciences, Faculty of Life & Health Sciences; Ulster University, Coleraine, United Kingdom.*

Proper gut development is needed for nutrient digestion and assimilation. The normal maturation of the gut may be altered when challenges occur during prenatal development, for example in intrauterine growth restricted pigs. However, the extent of the impact on gut maturation and its implications during the first weeks of life is not fully understood. This study aimed to characterize postnatal growth and intestinal development of 24 normal (NBW) and 24 low birth weight pigs (LBW). At 0h, 8h, 96h, 10, 28 and 56 d (d) of age, 4 NBW and 4 LBW were sacrificed and BW, gross and microscopic morphometry of the small intestine, gene expression (by qPCR) and methylation (in *OLFM4, ALPi, FZD5* and*FZD10* by pyrosequencing) were assessed. Mixed models were fitted to analyze the effects of BW and age. Pigs born with NBW had shorter relative intestinal length and longer and wider villi than LBW. The expression of genes coding for digestive enzymes (*ANPEP, SI*), intestinal development markers (*ALPi, OLFM4*), immune system (*TNFA2, IFNG*), intestinal barrier (*OCLN, CLDN4*) and cell proliferation and apoptosis (*PCNA, BAX*) was determined. The expression of *TNFA2* and *CLDN4* was higher in NBW than LBW pigs; *ALPi*expression tended to be higher in NBW. *OCLN* expression peaked in 10d old NBW but not LBW pigs, similar to the results obtained for *OLFM4*. *SI* expression was rather constant during the pre-weaning period and increased in 56d old NBW but not LBW pigs. *PCNA, ANPEP* and *IFNG* expression increased with age in NBW and LBW pigs in a similar manner. Additionally, changes in methylation were observed for the important regulatory genes *OLFM4* (decreased with age in both groups), *FZD10* and *FZD5* (methylation tended to increase and decrease, respectively in LBW piglets. Taken together, these results point toward a maturation deficit (as indicated by the lower expression of developmental markers) in LBW pigs, that could affect their digestion capacity, barrier function and innate immunity. Also, we demonstrate that some maturation processes are not complete at the generally accepted 3–4d of age and thus, longer studies should be used in the study of the development of the porcine intestine.

Key Words: piglet, low birth weight, small intestine, gene expression, methylation

P336 Transcriptome-wide analysis of glucocorticoid regulated genes in pigs in the context of a gain-of-function mutation in the glucocorticoid receptor. E. Murani*, N. Trakooljul, F. Hadlich, S. Ponsuksili, and K. Wimmers, *Leibniz Institute for Farm Animal Biology (FBN Dummerstorf), Institute for Genome Biology, Dummerstorf, Germany.*

Glucocorticoids (GC) play a major role in the maintenance of basal and stress-induced homeostasis by modulating many physiological processes including behavior, inflammatory response, and metabolism. Glucocorticoid signaling is mediated primarily via glucocorticoid receptor (GR), a ligand activated transcription factor. In spite of its importance little is known about the GC signaling pathway in farm animals. We have previously identified a gain-of-function mutation in the GR, Ala610Val, which causes profound compensatory reduction in GC (cortisol) production via downregulation of the HPA axis. To get insights into mechanisms of action of this mutation, and into GC signaling in general, we treated pigs with alternative GRAla610Val genotypes (in total n = 48 in the age of 7 weeks) either with saline or 2 different doses (10 or 60 µg/kg) of the selective GR agonist dexamethasone (DEX) for 3 h. Transcriptome responses were analyzed using RNA-seq in different tissues, along with physiological responses in plasma. Differential gene expression depending on treatment, genotype, and genotype × treatment interaction were analyzed using linear models implemented in LIMMA and DESeq2. Transcriptional and physiological responses were correlated using WGCNA. DEX induced vast transcriptome changes both in liver and brain, with about 30% and 8% of present genes, respectively, being regulated by the higher DEX dose at q <0.01. In both tissues genes responding to DEX were enriched particularly for immune-related functions. Comparison of differentially expressed genes between treatments revealed dose-dependently regulated genes, e.g., *FKBP5*, which can be used as sensitive biomarkers of GC action. We found a robust transcriptional signature of GRAla610Val comprising genes related to neuronal signaling in both tissues. Tissue specific changes associated with GRAla610Val included genes related to glucose metabolism in the liver. The transcriptional changes induced by GRAla610Val indicate its potential impact on stress resilience.

Key Words: glucocorticoid receptor, glucocorticoid sensitivity, stress response, transcriptome

P337 Genetic regulation of liver metabolites and transcripts linking to biochemical-clinical parameters. S. Ponsuksili, N. Trakooljul, F. Hadlich, E. Murani, and K. Wimmers*, *Leibniz-Institute for Farm Animal Biology (FBN), Genome Biology, Dummerstorf, Germany.*

Given the central metabolic role of the liver, hepatic metabolites and transcripts reflect the organismal physiological state. Biochemical-clinical plasma biomarkers, hepatic metabolites, transcripts and SNP genotypes of some 300 pigs were integrated by weighted correlation networks and genome-wide association analyses. Network-based approaches of transcriptomic and metabolomic data revealed conspicuous correlation between transcripts and metabolites of the pentose phosphate pathway (PPP). This finding was evidenced by the quantification of the PPP product NADPH and transcripts of key enzymes of this canonical pathway, HDAC4 and G6PD, as well as by RNAi knockdown experiments. Whereas amino acid metabolites correlated with

transcripts of immune or acute phase response signals, carbohydrate metabolites correlated highly with cholesterol biosynthesis transcripts. Genome-wide association analyses revealed 180 metabolic quantitative trait loci (mQTL) ($P < 10^{-4}$). Consideration of shared marker association with biomarkers, metabolites and transcripts revealed 144 SNPs associated with 44 metabolites and 69 transcripts that are correlated with each other, representing 176 mQTL and eQTL. For example, trans-4-hydroxy-L-proline, which is strongly linked to plasma creatinine, showed the strongest association with SNPs on chromosome 6 ($P = 6 \times 10^{-9}$), which had pleiotropic effects on PRODH2 expression, as shown by multivariate analysis. The identified associations link between variation at the genome, transcriptome, and metabolome level with clinically relevant phenotypes. This approach has the potential to detect novel biomarkers displaying individual variation and promoting predictive biology in medicine and animal breeding.

Key Words: mQTL, eQTL, metabolite, transcript, SNPs

P338 Profiling of miR-874 and its predicted target gene *citron kinase* during myogenesis *in vitro*. K. R. Daza*, L. M. Ford, D. Velez-Irizarry, N. E. Raney, and C. W. Ernst, *Michigan State University, East Lansing, MI, USA.*

MicroRNAs (miRNAs) are a class of noncoding RNAs known to post-transcriptionally regulate gene expression, ultimately affecting many biological processes and phenotypes. By combining miRNA and gene expression profiles with genotypic and phenotypic data from adult pig skeletal muscle samples, an integrated GBLUP-based GWAS analysis identified miRNAs potentially regulating important complex traits. MiR-874 was chosen as an intriguing candidate for further study, as it exhibited strong expression QTL (eQTL) and its predicted target genes co-localized with phenotypic QTL for meat quality traits. *Citron kinase* (*CIT*), a predicted target gene of miR-874, also exhibited eQTL and is known to effect cell division. Our objective was to use an in vitro myoblast model to confirm the expression of these genes and to define the effect of their putative regulatory relationship on myogenesis. C2C12 mouse myoblasts were cultured in growth media in a 5% CO_2 incubator at 37°C for 24, 48, or 72h, and the rate of proliferation was assessed by measuring DNA concentration. Cells were stimulated to differentiate by switching to serum-free medium, and cellular differentiation rate was assessed by measuring creatine kinase activity. Total RNA was isolated from proliferating cells at ~75% confluence, and from differentiating cells at 24, 48, or 72h after stimulation of differentiation. Transcript abundance of miR-874, its predicted targets *CIT*, *IGFBP5*, and *CDKN1A*, along with *RHOA* and *MYOG* were profiled using quantitative PCR. Significant increases in DNA concentration were found between all proliferative time points ($P < 0.002$). Significant increases in differentiation were identified between 24 and 48h ($P < 1x10^{-4}$) and 24 and 72h of differentiation ($P < 1x10^{-5}$). The expression of both miR-874 and *CIT* were confirmed. MiR-874 expression was consistent across time points. However, all 5 profiled genes exhibited significant effects of time on expression ($4x10^{-4} < P < 0.006$). Ongoing work will further assess the regulatory relationship between miR-874 and *CIT*, and its effect on myogenesis.

Key Words: pig, microRNA, muscle, growth and development, gene expression

P339 Genotype-environment interactions for quantitative traits of purebred pig population in Korea. D. Shin*, J.-D. Oh, K.-D. Song, and H.-K. Lee, *Chon-buk National University, Jeonju-si, Jeollabuk-do, Korea.*

Due to the lack of statistical power and confounding effects of population structure in pig population data, we want to perform genotype-environment interaction study for exploring how genotype and environmental factors interact to in their influence onto phenotype. We analyzed 5 pig quantitative traits in over 5,000 individuals on 51,945 autosomal single nucleotide polymorphisms (SNPs) collected from GGP(Grand-Grand-Parents) farms, and we estimated the statistically significant proportion of variance that could be explained by genotype-environment interactions in economic pig traits (P-value < 0.05), which is related to pig performance test. Our data suggested that the genotypes could have different effects on each traits in different environmental settings. In this study, we defined the genotype groups of individuals with similar genetic profiles based on the additive genetic relationships among individuals using SNPs. We observed the norms of reaction, and the differential phenotypic response of a genotype to a change in environmental exposure. This significant heritability estimate of genotype-environment interactions will lead to conceptual advances in our understanding of the mechanisms underlying genotype-environment interactions, and could be ultimately applied to selection of superior piglet in GGP farm.

Key Words: pig, genomics, genomic selection, environment, interaction

P340 Abstract withdrawn

P341 A genome-wide association study for the robustness of piglets at weaning. M. Revilla*[1,2], F. Blanc[1], R. Muñoz-Tamayo[2], G. Lemonnier[1], J.-J. Leplat[1], M.-J. Mercat[3], L. Ravon[4], Y. Billon[4], N. C. Friggens[2], J.-P. Bidanel[1], C. Rogel-Gaillard[1], N. Le-Floc'h[5], and J. Estellé[1], *[1]GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France, [2]MoSAR, INRA, AgroParisTech, Université Paris-Saclay, Paris, France, [3]IFIP-Institut du porc and Alliance R&D, Le Rheu, France, [4]GenESI, INRA, Surgères, France, [5]PEGASE, INRA, AgroCampus Ouest, Saint-Gilles, France.*

In industrial swine breeding conditions, piglet weaning is one of the most critical phases because it constitutes a complex stressful event characterized by diet, social, and environmental changes. In this crucial period, antibiotics are still widely used either in the management of the post-weaning diarrhea episodes or in preventive prophylactic strategies. In this context, finding sustainable alternatives to maintain piglet health

at the critical weaning period and limit antibiotic resistance becomes a high priority. The objective of this work is to perform a genome-wide association study (GWAS) for a set of relevant proxy phenotypes able to describe weaning robustness based on individual growth trajectories. To this end, over 400 French Large White pigs were monitored along the weaning period for health and production traits. All animals were weaned at 28 d of age and fed conventionally during the post-weaning period without antibiotic administration. The dynamics of body weight were evaluated with frequent measures from birth to 75 d of age for each animal. Also, individual diarrhea occurrence in each animal was evaluated with a fecal score (0–1–2) twice per week during the first 2 weeks after weaning, and blood hematological analyses were performed at weaning and one week later. A dynamic model based on the Gompertz-Makeham law already developed in our team was used to quantify the different degrees of growth perturbation and obtain synthetic weaning phenotypes able to inform on the amplitude and length of perturbation, and the rate of animal recovery. In parallel, animals were genotyped with the Affymetrix HD 650K chip. Genome-wide association studies revealed significant associations (FDR <0.05) for several traits. Interestingly, a genomic region on the chromosome 9 containing relevant genes for the gastrointestinal tract function was associated with parameter describing the overall weaning robustness. Overall, our results provide a step forward in the characterization of the genetic determinism of piglet robustness at weaning, and illustrate that breeding and selection strategies have the potential to limit the weaning complications in porcine production.

Key Words: pigs and related species, genome-wide association, SNP, biomarker, animal health

P342 Genetic parameters of protein of nitrogen efficiency in a Swiss Large White pig population: Preliminary results. C. Kasper*, I. Ruiz-Ascacibar, P. Stoll, and G. Bee, *Agroscope, Posieux, Switzerland.*

Pork production contributes to environmental pollution through the emission of nitrogen and phosphorus compounds. In addition, pig fattening in Europe usually requires imports of soybean, since the protein requirement of feed cannot be easily met by domestic plant-protein sources alone. It is therefore desirable to improve protein efficiency, i.e., to achieve a similar protein uptake in the carcass with a lower intake of dietary protein, through selective breeding. For a preliminary evaluation of the potential of breeding for higher nitrogen efficiency in this population, we used the results of previous experiments with protein-reduced feed to estimate genetic parameters of nutrient efficiency in Swiss Large White pigs. Protein efficiency could be determined by using automated feeders with individual pig recognition system allowing for detailed information of individual lifetime consumption. We determined protein contents of the feed and of the body fractions after slaughtering with wet-chemistry analyses. Heritability of protein efficiency in the whole body (including organs and cleaned gastro-intestinal tract) was estimated at 32% and in the carcass (including the head but entrails removed) at 16%. The common environment played a minor role in shaping nitrogen efficiency. Positive phenotypic correlations between the 2 traits suggest that selection for one trait will lead to an increase in the other trait. However, since protein-efficient pigs took longer to reach the target slaughtering weight, a slight delay in the growth of protein-efficient pigs might be expected. We have just begun a more thorough investigation of nutrient efficiency with studies yielding higher sample sizes to improve the quality of estimates and to conduct genetic correlation analyses with production traits. We further aim at obtaining information on the genes underlying nutrient efficiency in pigs as well as their functions.

Key Words: pigs, animal breeding, digestive system, animal nutrition, environment

P343 Application of STR markers and their evaluation for parentage verification of pigs in Poland. A. Radko, A. Koseniuk*, G. Smolucha, and A. Podbielska, *The National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.*

A routine parentage verification of pigs in Poland is conducted using 14 microsatellite sequences (STRs). The goal of the study was to estimate the genetic diversity based on the index of polymorphism (PIC), the observed and expected heterozygosity (H_o and H_E respectively) and the inbred index (F_{IS}). To evaluate the usefulness of the markers for parentage purposes in Polish pig population the parentage exclusion probability was calculated. The genomic DNA was extracted from peripheral blood and semen from 485 pigs comprising 3 breeds: Polish Largewhite (PLW, n = 326), Polish Landrace (PL, n = 93) and Pulawska (P, n = 66). The multiplex PCR was performed using 14 fluorescently dyed primers for microsatellite sequences recommended by ISAG: S0090, S0101, S0155, S0227, S0228, S0355, S0386, SW24, SW240, SW72, SW857, SW911, SW936 and SW951. Finally, the capillary electrophoresis was performed. The calculations were carried out with the use of statistical models implemented to the IMGBOVSTAT - IZOO PIB database – the property of the National Research Institute of Animal Production. We identified 85 alleles at 14 microsatellite *loci*. The PL and PLW breeds proved to be most polymorphic in the meaning of PIC (above 0.57), H_o (above 63%) and He (above 62%). In Pulawska breed 7 of total 14 analyzed STRs were of low polymorphism (PIC <0.47). The negative and low mean values of the F_{IS} calculated for all breeds indicate the lack of inbreeding. Finally, the parentage exclusion probability was calculated for all combined *loci* when either one or both parent's genotypes are known (PE_1 or PE_2 respectively). The estimated cumulative probability of exclusion in PL and PLW breeds was 0.98226 and 0.9994 (CPE1 and CPE2 respectively) and for Pulawska breed the values of 0.9043 for CPE1 and 0.9913 for CPE2 were calculated. The PE1 and PE2 values indicated that the panel of markers is sufficient for parentage analysis of abundant breeds (here: PL and PLW), whereas the parentage veryfication of breeds of limited number of individuals presumably needs to broaden of addition set of markers.

Key Words: pigs and related species, genetic identification, microsatellite, genetic marker, parentage

P344 Effects of fiber addition and dietary protein on phenotype and muscle transcriptome in Iberian pigs. A. López-García*[1], Y. Núñez[1], R. Benítez[1], L. Fontanesi[4], F. I. Hernández[2], I. Seiquer[3], P. Palma-Granados[5,3], M. Izquierdo[2], R. Nieto[3], and C. Óvilo[1], *[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain, [2]Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CICYTEX), Guadajira, Badajoz, Spain, [3]Estación Experimental del Zaidín (EEZ), CSIC, Granada, Spain, [4]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [5]Centro de I+D del Cerdo Ibérico, INIA, Zafra, Badajoz, Spain.*

Feed restriction during premontanera period in traditional Iberian pig production has been shown to negatively impact on animal welfare and contribute to final weight variability. Multiple approaches have been studied for reducing this restriction without compromising the established weight limit for montanera. Complementing growing diets with low-energy agricultural by-products might diminish feeding stress, and protein content adjustment could be helpful for reducing weight gain maintaining feed intake. However, physiological effects of these strategies remain mainly unknown. We analyzed the effects of fiber addition and variation in protein content in growing diets on muscle transcriptome, as well as on animal performance and meat quality. Two experiments were performed during the pre-montanera period. In the first one 10 castrated male pure Iberian pigs were fed a commercial control diet (C) or supplemented with rice fiber (HF, +12% fiber). In the second one, 2 isoenergetic diets with different CP/ME ratio were evaluated: 110 and 150 CP/kg DM diet (LCP and HCP). Carcass traits were measured at slaughter (100 kg LW), and *Longissimus dorsi* samples were taken for meat quality analysis and RNA-seq. Animals from HF group tended to accumulate more fat and had reduced muscle mass. Meat from HF tended to have a higher yellow (b) color value and MUFA. Twenty-two DE genes were found, some related with muscle

development and overexpressed in C group, in agreement with the trend to muscle mass reduction in HF animals. LCP treatment did not affect pig growth, although nitrogen retention was diminished and muscle tended to have higher red (a) and chroma values. RNaseq revealed 26 DE genes, some related with muscle development or animal size. At sight of our results, these dietary changes have moderate effects both at phenotypic and gene expression levels. Applying these strategies to improve animal welfare in premontanera period might be considered, although their truly potential to reduce feed restriction must be further explored. This work has received funding from the EU H2020 research and innovation program under grant agreement No 634476.

Key Words: Iberian pig, transcriptome, fiber, protein

P345 Genomic differentiation among varieties of Iberian pig. I. Alonso[1], N. Ibañez-Escriche[2], J. L. Noguera*[3], J. Casellas[4], and L. Varona[1,5], *[1]Universidad de Zaragoza, Zaragoza, Spain, [2]Universitat Politécnica de Valencia, Valencia, Spain, [3]IRTA, Lleida, Spain, [4]Universitat Autonoma de Barcelona, Bellaterra, Spain, [5]Instituto Agroalimentario de Aragón(IA2), Zaragoza, Spain.*

In this study, we used the Porcine v2 BeadChip to genotype 349 individuals from 3 varieties of Iberian pig (Entrepelado, EE; Retinto, RR; and Torbiscal, TT) and their crosses. After filtering the SNP markers, 47, 67, and 123 haplotype phases were identified that had EE, RR, and TT origins, respectively, which were used to calculate allelic frequencies of 31,180 SNP markers involved in the calculation of the average F_{ST} for sliding windows of 2Mb and centered at each SNP marker. The results confirmed the greater genetic closeness of the Entrepelado and Retinto varieties, and identified several genomic regions that had a degree of divergence greater than expected along the genome. The genes present in the genomic regions that had an average F_{ST} over the 95% percentile for each population pair were used to perform an Overrepresentation Enrichment Analysis (ORA) for the Gene Ontology (GO) terms for biological process based on the annotated genes with the *Homo sapiens* and *Sus scrofa* genomes. The analysis indicated that several groups of biological processes were overrepresented: a large group involving morphogenesis and development, and others associated with neurogenesis, cellular responses, or metabolic processes. These results were reinforced by the presence of some genes within the genomic regions that had the highest genomic differentiation; e.g., members of the HOXD family, and EVX2, SP9, NEUROD1, OLA1, ATF2, ACRT5, INSR, ADCYAP1, NWD1, MN1, FYN, CDK19, TRAF3IP2, and COL12A1.

Key Words: Iberian pig, genomic differentiation, SNP, founder haplotypes, candidate genes

P346 Estimation of genomic regions associated with assortative mating. L. Gomez-Raya*, L. A. Garcia-Cortes, and W. M. Rauw, *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria INIA, Madrid, Spain.*

It is well established that the mating of individuals with similar phenotypes (assortative mating) results in increased linkage disequilibrium, additive and phenotypic variance, and heritability. Phenotypes are the consequence of the expression of genes and, therefore, there must exist regions within the genome associated with assortative mating. There are, however, neither publications on estimation methods nor reports on the extent of genomic regions associated with assortative mating in any species of animals or plants. In this study: 1) a model for assortative mating is proposed in which an assortative parameter, α, increases (or decreases) the probability of alike gametes to join with respect to random association; b) a maximum likelihood method (MLE) for the joint estimation of 2-locus linkage disequilibrium (D) and α is developed; and 3) computer simulations using 10,000 replicates are used to test the MLE method with 435 individuals for varying α. A likelihood ratio test (LRT) is proposed in which the null model (α = 0) is compared with an alternative model (estimating both α and D) with 1 degree of freedom. Simulated allele frequencies were 0.5 at the 2 loci. The simulated D was 0.12. The simulated α was −0.5, 0, and 0.5 with a corresponding observed average (and standard deviation) of −0.501(0.066), 0.000 (0.114), and 0.501 (0.152) across replicates. The average of the estimates of D had the same value as the simulated D in all situations. The observed average of LRT across replicates was 1.20, 17.44, and 37.94 for α = −0.5, 0, and 0.5, respectively. Empirical statistical power for detecting α = 0.5 at a significance level of 0.01 was 0.85. Preliminary results on genomic regions associated with assortative mating (e.g., SSC11) in 435 Iberian pigs after being interrogated with the Illumina 60K array are presented.

Key Words: linkage disequilibrium, assortative mating, maximum likelihood estimation, swine

P347 Genetic determinism of immunity traits in pigs: An opportunity for selecting robustness. R. Quintanilla*[1], J. Reixach[2], O. González-Rodríguez[1], M. Pascual[1], Y. Ramayo-Caldas[1], M. Díaz[2], J. Tibau[1], and M. Ballester[1], *[1]Institute for Research and Technology in Food and Agriculture (IRTA), Torre Marimon, Caldes de Montbui, Spain, [2]Selección Batallé S.A, Riudarenes, Spain.*

Increasing animal robustness and disease resistance while improving production performance is crucial to the sustainability of pig production systems. The IMMUPIGEN project aims to study the genetic arquitecture of global immunocompetence in pigs as a first step to implement selection programs on pig health. In this study, we present preliminary results regarding the genetic determinism of immunity traits (ITs) measured in 432 health piglets, males and females aged 8 weeks, belonging to a Duroc commercial line. The set of measured ITs included circulating immunoglobulins (IgG, IgA and IgM) in plasma, secretory IgA in saliva, the acute phase proteins haptoglobin (Hp) and C-reactive protein in serum, γδ T-cell subpopulation, leukocyte phagocytosis, nitric oxide formation and several hemogram-derived cell counts. Additionally, some stress parameters were measured, and evolution during fattening was controlled in a subset (n = 125) of these animals, aiming to assess a putative relationship of immunity with animal welfare and production performance. Moderated to high heritability estimates were obtained for most ITs. Plasma concentrations of Ig showed the highest heretabilities (h^2 between 0.52 and 0.72); for the rest of ITs heritabilities ranged from 0.18 to 0.44. Only the IgA concentrations in saliva and the monocytes amount had heritability estimates bellow 0.10. The neutrophils/lymphocytes ratio showed positive but limited genetic correlations with some ITs such as plasma IgG and serum Hp concentrations. Regarding associations with production parameters, growth showed moderated genetic correlations with some ITs (e.g., negative correlation with Ig and positive with γδ T-cells), but residual feed intake was uncorrelated with most ITs. These preliminary results support the possibility of applying effective selection programs to improve both immunocompetence and production efficiency in pigs.

Key Words: immunity, pigs, robustness, selection

P348 Allele-specific expression in *longuissimus dorsi* muscle transcriptomes associated with phenotypic traits in pigs. D. Vélez-Irizarry*[1], K. R. Daza[1], R. O. Bates[1], N. E. Raney[1], J. P. Steibel[1,2], and C. W. Ernst[1], *[1]Department of Animal Science, Michigan State University, East Lansing, MI, USA, [2]Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, USA.*

Significant genetic gain in pork production has been achieved through the implementation of breeding programs focused on genetic improvement of economically important traits. Advancements in sequencing technology, improvements in the annotation of the pig genome, and development of quantitative genetic models have aided these efforts. Several quantitative trait loci (QTL) have been identified for growth, meat quality and carcass composition phenotypes, however, the biological mechanisms underlying most QTL remain unknown. Functional genomics can reveal insights on the genetic architecture of complex traits, and transcriptomic profiling of skeletal muscle during the initial steps leading to the conversion of muscle to meat can identify key

regulators of meat quality and carcass phenotypes. One such functional genomic approach is the study of allele-specific expression (ASE) which identifies cis-acting regulation of transcript abundance that can be associated with a measurable phenotypic difference. In this study, we tested for ASE in 69,502 *longissimus dorsi* coding SNP (cSNP), which were called directly from RNA-seq data of 168 pigs from an F2 Duroc × Pietrain pig resource population. A total of 18,234 cSNP with significant ASE were identified (FDR ≤ 0.01) using a Quasibinomial model. A meta-analysis merging cSNP p-values per gene identified 4,170 genes with significant allele-specific effects (FDR ≤ 0.01). A gene-wise conditional analysis fitting all ASE cSNP per gene for each phenotype identified 60 genes associated with growth, carcass composition, and meat quality phenotypes (FDR ≤ 0.05). Our study found ring and zinc finger transcription factors associated with 45-min pH, drip loss and 10th-rib backfat, and we confirmed allelic expression bias for these genes with pyrosequencing. Results support an important role for the activation of the PI3K-Akt-mTOR signaling pathway on meat quality traits. This study provides new information on the complex regulation of the pig longissimus muscle transcriptome and direct or indirect relationships with economically important phenotypic traits.

Key Words: ASE, RNA-seq, meat quality, pig

P349 Altered hippocampal epigenetic regulation underlying reduced cognitive development in response to early life environmental insults. K. M. Schachtschneider*[1,2], M. E. Welge[2,3], L. S. Auvil[2], S. Chaki[2], L. A. Rund[2], O. Madsen[4], M. R. P. Elmore[2], R. W. Johnson[2], M. A. M. Groenen[4], and L. B. Schook[1,2], *[1]University of Illinois at Chicago, Chicago, IL, USA, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [3]Mayo-Illinois Alliance for Technology-Based Healthcare, Urbana, IL, USA, [4]Wageningen University, Wageningen, The Netherlands.*

The hippocampus is involved in learning and memory and undergoes significant growth and maturation during the neonatal period. Environmental insults during this developmental period can affect epigenetic patterns and have lasting effects on brain structure and function. This study assessed hippocampal DNA methylation (reduced representation bisulfite sequencing) and gene transcription (RNA-seq) from 2 independent studies reporting reduced cognitive development stemming from early life environmental insults (iron deficiency and PRRSv infection) using porcine biomedical models. The Boruta machine learning approach was utilized to identify DNA methylation and gene expression features attributed to the reduced cognition phenotype. In total, 1,743 differentially expressed genes (DEGs) were identified between the reduced cognition and control groups, with samples clustering by group when comparing expression of the identified DEGs (ANOSIM R = 0.68, p-value = 0.001). GO term enrichment analysis resulted in the identification of 219 GO terms enriched for DEGs, including terms associated with immune responses, angiogenesis, cellular signaling, development, differentiation, and proliferation. In addition, 943 differentially methylated CpG regions (DMRs) were identified between the reduced cognition and control groups. While samples did not cluster by group when comparing methylation levels of the 30,696 tested regions (ANOSIM R = −0.07, p-value = 0.731), samples clustered by group when comparing expression levels of the 943 DMRs (ANOSIM R = 0.92, p-value = 0.001). These 943 DMRs overlapped with 891 gene regions. Overlapping genes were enriched for 171 GO terms, including terms associated with neurodevelopment, cellular signaling, development, differentiation, and proliferation. Finally, the identified DMRs overlapped with 67 DEGs, including 18 genes involved in neurodevelopment, function, and maintenance of the blood brain barrier. Together, these results support the role of altered hippocampal DNA methylation and gene expression in early life environmentally induced reductions in cognitive development across 2 independent studies.

Key Words: porcine biomedical model, DNA methylation, cognitive development, gene expression, environmental impact

P350 Estimating the shared genetic architecture between site-specific RNA editing and economically important traits in pigs. S. A. Funkhouser*, J. P. Steibel, D. Velez-Irizarry, and C. W. Ernst, *Michigan State University, East Lansing, MI, USA.*

The highly conserved post-transcriptional mechanism known as adenosine to inosine (A-to-I) RNA editing impacts gene function by converting adenosine to inosine molecules within specific regions of the transcriptome. The degree that specific sites are edited—the "editing level"—has been observed to vary within populations and can be considered a molecular quantitative trait hypothesized to influence higher-level phenotypes. How genetic variation influences editing level variation remains largely unknown, and the degree with which the genetic architecture of RNA editing overlaps with that of other phenotypes has yet to be investigated. Here we utilized the Michigan State University Pig Resource Population, consisting of 940 F2 animals, to investigate the shared genetic architecture between RNA editing and economically important pig traits. Using a subset of 144 animals with RNaseq data from longissimus dorsi muscle tissue, we quantified editing levels at previously detected A-to-I RNA edited sites and selected 3 within genes *OXCT1, CCNYL1*, and *BLOC1S6,* whose editing level variance was partially explained by the additive effects of all observed SNP markers (est. h_g^2 = 0.266–0.321; *p*-value = 0.004–0.03). We then utilized bivariate linear mixed models to estimate the marker-derived genetic correlation (ρ_g) between each selected A-to-I edited site and each of 67 carcass composition, meat quality, and growth traits. We observed 11 traits possessing nominally significant genetic correlations (*p*-value ≤ 0.05; 95% confidence interval (CI) did not contain zero) with at least one selected A-to-I edited site. The most significant genetic correlations estimated were between longissimus muscle moisture content and the A-to-I edited site within *OXCT1* (est. ρ_g = −0.770; 95% CI: −1, −0.287; *p*-value = 0.005), and between 45-min carcass temperature and the A-to-I edited site within *CCNYL1* (est. ρ_g = 0.734; 95% CI: 0.306, 1.0; *p*-value = 0.002). Both *OXCT1* and *CCNYL1* are known to be A-to-I edited in humans. Here we provide evidence that causal variants exhibit pleiotropy between RNA editing activity and complex traits.

Key Words: quantitative genetics, pigs and related species, RNA editing

P351 miRNA and mRNA differential expression in peripheral blood mononuclear cells of pigs exposed to topsoil in early life. M. de Souza[1], D. Koltes[1], H. Beiki[1], T. Tsai[2], M. Sales[2], C. Maxwell[2], J. Zhao[2], and J. Koltes*[1], *[1]Iowa State University, Ames, IA, USA, [2]University of Arkansas-Division of Agriculture, Fayetteville, AR, USA.*

Exposure of piglets to soil-borne microbes during lactation were related with modulation of gut microbiota, immune system, and increased growth performance. To understand the molecular mechanisms underlying these results, we analyzed the mRNA and miRNA expression in peripheral blood mononuclear cells (PBMC) of piglets daily exposed to topsoil (mRNA n = 6; miRNA n = 5) or not (mRNA n = 6; miRNA n = 8) from 4 d postpartum to the end of lactation (d 21). PBMC were collected from piglets at 11, 20, and 56 d of age and mRNA and miRNA extracted and sequenced (Illumina HiSeq4000). After sequence quality (FastQC) and low-quality read trimming (Trim Galore), reads were aligned to the Sscrofa11.1 genome using STAR for mRNA and miRDeep2 for miRNA. Differential expression (DE) analysis was performed using PROC Glimmix of SAS at a false discovery rate (FDR) of q <0.10. For both mRNA and miRNA models, topsoil treatment, piglet age, and the treatment by piglet age interaction were fit as fixed effects; RQI, parity of the sows, sex, and litter size were fit as covariate; and piglet as a random effect. For the mRNA model, lane was fit as a covariate as samples were run across multiple lanes. A total of 138 mRNA and 21 miRNAs were found DE for the treatment by age interaction. Eight clusters were enriched for GO terms by DAVID. Among these GO terms, process associated with carbohydrate and glycogen metabolism and energy reserve were enriched. These changes may alter regulation of cellular metabolic processes important in directing immune cell function and may contribute for growth performance. Genes, *PHKA1*, *PHKA2* and *GYS1,* were linked with glycogen stor-

age and metabolic disease in humans. The miRNA functional analysis, using DIANA-miRPath, showed similar GO term results as mRNA. Small RNAs and their target genes were enriched for biosynthesis of fatty acid, and lipid metabolism known to affect proliferation of T-cell. In total, 32 pathways were found enriched for miRNAs. Co-expression networks are in process and will contribute to finding the main miRNAs and target genes interactions that could be affecting the immune system and growth performance due to topsoil exposure.

Key Words: gene expression, immune system, PBMC, swine

P353 Abstract withdrawn

P354 Circular RNAs as biomarkers for porcine sperm motility.
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Male fertility in swine has been associated with sperm motility parameters. Sperm movement is regularly assessed in Artificial Insemination studs and provides an objective and reproducible measurement of semen quality. As sperm transcripts are highly fragmented and present at low abundances we seek to investigate circular RNAs (circRNAs), a novel class of non-coding RNAs with a closed loop structure, more stable than linear transcripts and potentially acting as miRNA sponges. We have performed total and small RNA-seq from 40 boar sperm samples. The objective of this study was to characterize the porcine circRNA repertoire, assess their potential role as miRNA sponges and the correlation between their abundance and sperm motility. We identified 1,393 potential circRNAs across all the 40 samples. The majority of the circRNAs were derived from exonic regions (63.6%), formed from less than 4 exons and had an average lenght below 500 bp. circRNA abundances ranged between 0.18 and 137 CPMs. Compared with other pig tissues, the sperm circRNA repertoire resembled most to testis, even thought 75% of the sperm circRNAs were sperm-specific. Gene ontology analysis revealed that sperm circRNAs involved genes related to epigenetic regulation, spermatogenesis and cilium assembly functions. We identified 69 circRNA-miRNA interactions. For example, miR-28–5p, a miRNA that is dysregulated in human infertile patients, presented 2 different target sites in a circRNA from *ZMYND10*, a gene required for motile ciliary function. We identified 148 circRNAs that correlated with different sperm motility parameters. We selected 10 circRNAs and validated the closed loop structure by RT-qPCR and Sanger Sequencing. The RNA abundance of 6 cirRNAs was validated by RT-qPCR and compared in 38 additional animals presenting extreme motility parameters. Three circRNAs presented significant differences in abundance between the groups. These included circRNA from *LIN7A* (P-value: 0.008), *LRBA* (P-value: 0.04) and *PAPOLA* (P-value: 0.026) genes, the functions of these genes are related to generating and maintaining cell membrane channels, vesicle trafficking and RNA and ATP binding, respectively.

Key Words: reproduction, RNA-seq, microRNA, pigs and related species, genomic selection

P355 An integrative GWAS and RNA-seq study to identify SNPs and transcripts related to sperm quality traits in pigs.
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For the last decades, boars have been selected for their genetic merit on carcass and meat quality traits. However, breeders and researchers are now paying attention to additional phenotypes including sperm quality. We carried a GWAS and a semen RNA-seq experiment in pigs with the aim to identify SNP markers for 25 sperm quality traits. The GWAS included 288 boars and genotypes from the Affymetrix Axiom Porcine 660K Genotyping Array. Sperm from 40 of these pigs were subjected to total and to small RNA-seq. The GWAS resulted in 345 SNPs significantly associated mostly to sperm head abnormalities and motility. The RNA-seq evidenced 4,120 protein coding genes and 95 miRNAs. 3,053 genes showed significant correlations with at least one semen quality trait. Independently for each phenotype, we then searched for eQTLs using only the GWAS SNP hits and the genes that correlated with the given semen trait. This yielded 119 eQTLs. Several of these hits involve genes with known function related to semen quality. For example, we found a hit on *ACTR2* and the percentage of head abnormalities. *ACTR2* has been involved in the morphogenic modeling of the sperm head. We also detected a hit between *NDUFS8* and sperm motility. *NDUFS8* is a known mitochondrial NADH subunit involved in the respiratory chain which is important for sperm capacitation and motility. Using the RNA-seq data, we also searched for the subset of 10 RNAs that explained the largest percentage of the variability for the semen quality phenotypes. To achieve this objective, we retrieved the genes that (i) were co-associated with traits at the SNP level by building an Associated Weight Matrix and finding correlations with PCIT, and (ii) also displayed co-abundant RNA levels (by PCIT). The resulting list of genes, together with the information of their correlated phenotypes, was used for network analysis. We identified a subset of 10 genes,

which included *EFHC1, ATP9A* and *THADA,* that explained between 16 to 67% of the different sperm quality parameters.

Key Words: reproduction, RNA-seq, genotyping, system genetics (eQTLs), microRNA

P356 Abstract withdrawn

P358 Effect of *MUC4* and *FUT1* genotypes on piglets infected with enterotoxigenic *Escherichia coli* F4 and F18. F. R. Massacci*[1,3], S. Tofani[2], M. Tentellini[2], S. Orsini[2], C. Forte[2], C. Lovito[2], D. Luise[3], C. Bevilacqua[1], M. Bertocchi[3], L. Marchi[2], C. Rogel-Gaillard[1], G. Pezzotti[3], J. Estellé[1], P. Trevisi[3], C. F. Magistrali[2], *[1]INRA, GABI, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France, [2]Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati," SC3RS, Perugia, Italy, [3]University of Bologna, DISTAL, Bologna, Italy.*

Enterotoxigenic *Escherichia coli* (ETEC) is one of the most important causes of post-weaning diarrhea (PWD) in pigs. The g.13:8227C > G SNP located on the *Mucine 4* (*MUC4*) gene and the g.6:54079560T > C SNP located on *Fucosyltransferase 1* (*FUT1*) gene have been associated with the susceptibility to ETEC F4 and ETEC F18 infection, respectively. The aim of this study was to validate the effects of the genotype of *MUC4* and *FUT1* in piglets naturally infected with ETEC F4 and F18. A total of 71 piglets produced in an Italian herd positive for both F4 and F18 was divided into 3 groups: 2 groups differing by antimicrobial administration routes – parenteral (A, 23 piglets) or oral (B, 24 piglets) and a control group without antibiotics (C, 24 piglets). All groups were balanced by weight, sex and litter. Animals arrived in the facility on weaning day (T0). For groups A and B, at T0 amoxicillin was administered during 5 d either parentally or orally. Animals were evaluated at the end of the amoxicillin administration (T1) and 7 d after the withdrawal of the antibiotic (T2). Fecal scores and body weight were recorded, and presence of ETEC F4 and F18 in fecal samples was assessed by PCR. Results revealed that 50/71 piglets were naturally infected by ETEC F4 and 21/71 by ETEC F18 at T0; 7 piglets were positive for both the pathogens and 7 resulted negative. Only F18 was detected at T1. Both strains ETEC F4 and F18 were resistant to amoxicillin. At T0, Fisher tests showed that *MUC4* genotype was significantly associated with the presence of ETEC F4 and the fecal scores ($P < 0.05$). Intriguingly, the *MUC4* resistant genotype was associated with ETEC F4 absence but also with a higher diarrhea score. At T1, *FUT1* was significantly associated with the presence of ETEC F18 ($P < 0.05$) but not with the diarrhea scores. Antibiotic administration showed a significant association with the presence/absence of F18 and the diarrhea score at T1 and T2 ($P < 0.05$). Overall, our results confirm that *MUC4* and *FUT1* genotypes are associated with the susceptibility to ETEC F4 and F18 infection and show the interest of considering these genetic markers in commercial farming industry.

Key Words: *MUC4*, *FUT1*, ETEC infection, piglets

P359 Genome-wide association study reveals candidate genes for growth relevant traits in pigs. Z. Tang*[1,2], J. Xu[1,2], L. Yin[1,2], D. Yin[1,2], M. Zhu[1,2], M. Yu[1,2], X. Li[1,2], S. Zhao[1,2], and X. Liu[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education & College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China, [2]Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei, China.*

Growth rate is a vital important economic trait and significantly affects pig production. Average daily gain (ADG) and Age (AGE) that adjusted to 100kg are directly related to the pig growth performance and commonly used in pig breeding. To reveal the candidate genes and advance the understanding of growth related traits, we performed genome-wide association study (GWAS) and genetic parameters estimation for ADG and AGE using the genomic and phonemic from a big population that includes 4,260 purebred pigs composed of 4 breeds (Duroc, Yorkshire, Landrace, and Pietrain). All analyses were performed by a multi-loci GWAS model, FarmCPU. The GWAS results of all 4 breeds indicate that 5 genome-wide significant SNPs were associated with ADG, and the nearby genomic regions explained 4.08% of the genetic variance and 1.90% of the phenotypic variance, respectively. For AGE, 6 genome-wide significant SNPs were detected, and the nearby genomic regions explained 8.09% of the genetic variance and 3.52% of phenotypic variance, respectively. In total, 9 candidate genes were identified to be associated with growth and metabolism. Among them, *TRIB3* was reported to associate with pig growth, *GRP*, *TTR*, *CNR1, GLP1R, BRD2, HCRTR2, SEC11C, and ssc-mir-122* were reported to associate with growth traits in human and mouse. The newly detected candidate genes will advance the understanding of growth related traits and the identification of the novel variants will suggest a potential use in pig genomic breeding programs.

Key Words: GWAS, pig, growth traits, ADG, AGE

P360 Circular analysis of breeding seasonality in Iberian sows under an intensive production system. M. M. de Hijas-Villalba*[1], L. Varona[2], N. Ibáñez-Escriche[3], J. P. Rosas[4], J. L. Noguera[5], and J. Casellas[1], *[1]Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Spain, [2]Departamento de Anatomía Embriología y Genética Animal, Universidad de Zaragoza, Zaragoza, Spain, [3]Departament de Ciència Animal, Universitat Politècnica de València, Valencia, Spain, [4]Programa de Mejora Genética "Castúa," INGA FOOD SA, Almendralejo, Spain, [5]Genètica i Millora Animal, Institut de Recerca i Tecnologia Agroalimentàries, Lleida, Spain.*

Breeding seasonality has economic relevance in the extensive livestock industry, despite its effect in intensive systems it is little known. Studies about the genetics of breeding distribution are scarce

due to the complexity of circular data analysis, though this must be softened by the recent development of a von Mises circular mixed model (i.e., breeding time distributes around a circular parametric space from January, 1st to December, 31st). In this study, parity time data from *Entrepelado* and *Retinto* Iberian pigs were analyzed. The *Entrepelado* variety had 3,200 parities between the years 2010 and 2017 from 739 sows, and the pedigree included 69 boars and 794 sows. *Retinto* data involved 4,744 parities from 922 sows between 2009 and 2017 (89 boars and 975 sows on pedigree). For each variety, the circular model included parity number (1 to 8 and >8), and litter size on previous parity (first parity, < 6 piglets, 6 to 10, and >10) as systematic effects; herd-year-season (81 levels for *Retinto* and 65 levels for *Entrepelado*) and sow as permanent environmental effects; and the infinitesimal additive genetic effect. Models were solved by Bayesian inference with 50,000 burning and 500,000 sampling iterations. Posterior mean (and 95% credibility intervals) for herd-year-season variance was 0.029 (0.013 to 0.067) in the *Retinto* variety, and 0.012 (0.004 to 0.075) in *Entrepelado*, whereas the variance for permanent sow effect reached 0.010 in both varieties (0.007 to 0.018 and 0.003 to 0.027, respectively). The additive genetic variance in the *Entrepelado* variety was 0.024 (0.009 to 0.039; h^2 = 0.02), and the deviance information criterion (DIC) favored the model without additive genetic background by 9.58 DIC units. Otherwise, *Retinto* individuals reported a slightly higher genetic variance (0.035, 0.028 to 0.050; h^2 = 0.03) and its model obtained a lower DIC (<1 unit). Therefore, the genetic effect on breeding seasonality of *Entrepelado* and *Retinto* Iberian sows under intensive production systems could be suggested as minimal, if any.

Key Words: pigs and related species, quantitative genetics, SNP, quantitative trait locus (QTL), genomic prediction

P361 Assessment of punctual Δ9-desaturase activity in porcine adipose tissue. L. Sarri*, G. De la Fuente, A. R. Seradj, J. Estany, R. N. Pena, J. Balcells, and M. Tor, *Animal Science Department, University of Lleida- Agrotecnio Center, Lleida, Spain.*

Δ9-desaturase activity is often measured through the monounsaturated/saturated fatty acids ratio of adipose tissue. This value is the result of the whole of anabolic and catabolic lipid processes that happened throughout the entire life of the animal and does not reflect the activity at a given time. Moreover, this value is masked by fatty acids deposited directly from the diet. To evaluate the punctual activity of the stearoyl-CoA desaturase enzyme (SCD) an experiment was carried out by adding deuterium-labeled stearic acid (C18:0D35) to the diet. Thus, 24 growing pigs weighing 25–30kg were used: 8 entire F2 (Pietrain sires × (Duroc × Landrace) dams) and 16 castrated purebred Duroc pigs, 8 CC/ 8 TT for the *SCD* genotype (g.2228T > C polymorphism in the promoter of the *SCD* responsible for enhanced biosynthesis of oleic acid by desaturating stearic acid). Each variety was subdivided in 2 groups that were fed for 7 d with 2 levels of crude protein [15% low protein (LP); 17% normal protein (NP)]. A 0.02% of C18:0D35 was incorporated in all diets. Blood samples were collected daily for 5 d before culling and subcutaneous adipose was sampled after culling. Desaturation ratio of stearic acid in adipose tissue (14.9 ± 0.49; 12.5 ± 0.49; 10.4 ± 0.45 for F2, OO and NN respectively) was different among genotypes and higher than those reported at 90kg of live weight. No diet interaction was detected. Nevertheless, punctual Δ9-desaturase activity measured as desaturation ratio of C18:0D35, reach much lower values and differences between genotypes were only significant in the LP diet (0.08 ± 0.014; 0.06 ± 0.012; 0.02 ± 0.012 for F2, OO and NN genotypes respectively). This study is part of the Feed-a-Gene project and received funding from the European Union's H2020 program under grant agreement n° 633531, as well as Spanish National funding by MINECO (AGL2017–89289-R).

Key Words: pig, nutrigenomics, mass spectrometry, fat/lipid, product quality

P362 Genome-wide identification of splicing QTL (sQTL) in the pig Longissimus dorsi muscle. Z. Zheng*[1,2], R. Zhang[1,2], Y. Liu[1,2], Y. Liu[1,2], and X. Xu[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education & College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China, [2]The Cooperative Innovation Center for Sustainable Pig Production, Wuhan, China.*

The alternative splicing (AS) is an important mechanism of eukaryotic posttranscriptional regulation of gene expression, which contributed large proportion of protein diversity and phenotypic variation. In the present study, alternative splicing events were quantitated with percent spliced in (PSI) based on RNA-sequencing of longissimus muscle, which was used as the molecular phenotype for splicing QTL (sQTL) mapping in the population of 189 Duroc × Luchuan crossed pigs. With mixed linear model, we identified 45,282 cis-sQTL (P < 0.014, fdr <0.05) and 38,816 trans-sQTL (P < 1.86e-05, fdr <0.05). Furthermore, we analyzed the correlation between splice events count and gene expression, and found the higher expression gene often has more isoforms. In addition, sQTL SNPs were enriched near to the alternative splicing site. Overlapping analysis of cis-sQTL and cis-eQTL associated genes and all available published candidate genes identified 27 genes related with meat quality traits, such as MEF2C (P = 2.26e-20 for leading SNP of cis-sQTL mapping, similarly hereafter) and PHKG1 (P = 5.18e-08). By association analysis, the polymorphism of one mutation site (rs699990442) in 5′UTR of MEF2C was significantly associated with meat color' lightness (L) (P = 7.76e-4). Finally, we developed a molecular phenotype database containing the data of sQTL and eQTL of human, pig, cow and mouse. In conclusion, the present study provided some new candidate genes and molecular markers for genetic analysis of porcine meat quality traits, and the molecular phenotype database will provide integration analysis services for scientific community of animal genetics.

Key Words: pig, sQTL, RNA-seq, meat quality

P363 Abstract withdrawn

P363 Abstract withdrawn

P364 Breed feature characteristics and genome variation based genome-wide association study on pigs. J. Xu*[1,2], Y. Fu[1,2], Y. Zhou[1,2], L. Yin[1,2], Z. Tang[1,2], D. Yin[1,2], M. Zhu[1,2], M. Yu[1,2], X. Li[1,2], X. Liu[1,2], and S. Zhao[1,2], *[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education & College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China, [2]Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei, China.*

There is a large amount of pig breeds distributed in the world, and their feature characteristics varied from one to another. Pig's breed feature characteristics are abundant resources and understand the underlying genetic mechanism can help the breeders to develop ideal pig breed. As a powerful tool for detecting candidate genes of phenotypes, Genome-Wide Association Study (GWAS) has been widely used for detecting candidate genes for the objective traits. In this study, we performed GWAS by standard mixed linear model using 3 kinds of genetic markers (SNP, InDel, and CNV) identified from public whole-genome sequencing data sets. A total of 469 pigs that composed of 58 breeds and approximately 19 million SNPs, 1.8 million InDels, and 18,016 CNVs are identified and analyzed in this study. Ten phenotypes, which were defined by the breed feature characteristics were used to identify the associated genome variations and candidate genes, including coat color, ear shape, gradient zone, body weight, body length, body height, sexual maturity, commercial lines, dressing percentage, and lean meat percentage. First, we detected many candidate genes that were reported previously, such as *KIT* for coat color and *PRKD1* for sexual maturity. Furthermore, incorporated the results of KEGG pathway and Gene Ontology enrichment analyzes, we find some new candidate genes, such as *ALKBH7* for body weight and *DCT* for gradient zone. In addition, we detected many InDels and CNVs that significantly associated with the traits, which are previously reported by the associated SNP markers only in pigs, such as *PRKCE* for body length and *ACSL4* for body weight.

Key Words: GWAS, pig, breed feature characteristics, SNP/InDel/CNV

P365 Mitochondrial and SNP data analysis confirm the complex origin of Bazna pigs from Romania. VA Bâlteanu*[1], T. Figueiredo-Cardoso[2], M. Amills[2], and A. Zsolnai[3], *[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania, [2]Center for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Campus Universitat Autònoma de Barcelona, Bellaterra, Spain, [3]NARIC-Research Institute for Animal Breeding, Nutrition and Meat Science (ÁTHK), Herceghalom,Hungary.*

Bazna is a native pig breed from Romania with a black coat and white belt. It has a very tasty and marbled meat, which is much appreciated by the local consumers. The story of the Romanian Bazna breed goes back to 1872 when it is documented the first crossing between a Berkshire boar and Mangalitza sows in Transylvanian region. Subsequently these populations were infused in different episodes with cosmopolitan pig breeds. Currently there are 78 Bazna sows and 14 boars registered in the herd book. Therefore the historical data suggest an admixed origin of Bazna pigs. To verify this hypothesis we sequenced the mitochondrial Cytochrome *b* gene in Bazna versus Red Mangalitza and Vietnamese pigs. Additionally, using the Illumina Porcine SNP60 BeadChip, we have genotyped a larger panel of breeds *i.e.* Bazna pigs versus Mangalitza group (Red, Blonde and Swallow-belly) and a cosmopolitan group (Hampshire, Landrace, Large White and Pietrain). The NJ tree generated using mitochondrial DNA data evidenced 2 district clusters *i.e.* European (Mangalitza) and Asian (Vietnamese). Some of the Bazna pigs clustered tightly within the Mangalitza group, suggesting the maternal contribution of Mangalitza sows to Bazna breed formation, while some of them formed distinct sub-clusters, suggesting additional, but still European, maternal lineages. On the other hand, several Bazna pigs clustered in the Asian group, an observation which is consistent with the presence of Asian alleles in the contributing British breeds. By using SNP data we built an MDS plot, in which Bazna and Hampshire pigs occupied an intermediate position between the other 2 groups, one composed of Mangalitza pigs and the other one including cosmopolitan breeds. The Structure analysis indicated a probable contribution of Mangalitza pigs to the formation of the Bazna breed and at the same time it was evident the contribution of the British breeds, which is historically documented. Due to its superior meat quality Bazna pigs can well be the next fatty pig success story, after Iberian and Mangalitza breeds, if conservation and reproduction plans are implemented.

Key Words: Bazna pigs, mitochondrial DNA, SNP, diversity, origin

Ruminant Genetics and Genomics

P366 Abstract withdrawn

P366 Abstract withdrawn

P367 Mapping the *SCURS* locus in the South African Bonsmara beef cattle breed. R. Grobler*, C. Visser, and E. van Marle-Köster,

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Scurs are loose protuberances not attached to the skull that exhibit large variation within and between breeds, specifically in terms of shape, size and length. The *SCURS* locus has been mapped to BTA19 using microsatellite-based linkage mapping. Subsequent studies could not confirm the location of the *SCURS* locus on BTA19 and genetic heterogeneity has been suggested. The composite South African Bonsmara breed has selected for polledness for the past decade. The challenge however is to also eliminate the scurs phenotype prevalent in this breed. In this study, a genome-wide association study (GWAS) was performed with the aim to identify genomic regions associated with the scurs phenotype in South African Bonsmara beef cattle. This is the first study on scurs in South African cattle breeds. A total of 161 heterozygous polled Bonsmara animals with clearly visible scurs or a smooth polled phenotype were genotyped with the GGP150K Bovine SNP chip; 97 Scurred animals and 64 heterozygous polled animals were selected as cases and controls, respectively. The genetic structure of the sample group was assessed by principal component analysis (PCA) using GCTA software. To map the *SCURS* locus, a trio family-based design was implemented and a case-control analyses was conducted between Pp Scurs and Pp Polled animals using the–assoc function in Plink. The GWAS analysis revealed a clear association peak above the 5% Bonferroni threshold on BTA3 and a suggestive association on BTA16, whereas single SNP associations observed on BTA4, BTA13 and BTA21 are likely to be spurious associations. A single SNP association which achieved genome-wide significance (-log10 5 $x10^{-8}$) was observed on BTA19, which corresponds to findings reported in a previous study. Some distinct peaks which failed to achieve the 5% Bonferroni significance were observed on BTA10 and BTA15. These results suggest a genetic heterogeneity of the *SCURS* locus and indicates that more than one gene might be responsible for the scurs phenotype in South African beef cattle.

Key Words: GWAS, heterozygous polled, polledness

P368 Bioinformatic investigation of the cattle *POLLED* variants. J. E. Aldersey*[1], W. Y. Low[1], N. Liu[2], R. Tearle[1], J. L. Williams[1], and C. D. K. Bottema[1], *[1]Davies Research Centre, School of Animal & Veterinary Sciences, University of Adelaide, Roseworthy Campus, Roseworthy, SA, Australia, [2]Bioinformatics Hub, School of Biological Sciences, University of Adelaide, North Terrace Campus, Adelaide, SA, Australia.*

The 4 known genetic variants for polledness found in Celtic, Friesian, Mongolian and Nellore cattle are all located in intergenic regions near the BTA1 centromere. Gene expression studies have shown that 2 long intergenic non-coding RNA (lincRNA), LincRNA#1 and LincRNA#2, are differentially expressed in the horned versus Celtic polled fetal horn bud. These lincRNAs are located near the *POLLED* variants and LincRNA#2 is duplicated in the Nellore variant. In addition, a topologically associated domain (TAD) has been previously predicted for bovine from human data that includes all the *POLLED* variants (chr1:1,226,028–2,201,452). TADs are regions of the genome that have high levels of interactions between loci within a domain, but few interactions with loci in different domains. TADs insulate regions of DNA to ensure appropriate enhancer-promoter interactions, and disruption of TAD boundaries may create interactions that would not normally occur. The bovine reference genome sequence (UMD3.1) was used herein to study genomic differences and possible effects on gene expression resulting from the Celtic, Friesian and Mongolian variants. The Nellore variant could not be investigated as the mutation has not been defined. The predicted TAD region was compared across horned (cow, yak, sheep, goat) and hornless species (pig, dog, human) to determine genomic differences, and Hi-C data from Angus and Brahman were used to analyze TADs to examine possible effects of the *POLLED* variants. An open reading frame (ORF) was predicted at site of the Mongolian variant, and the ORF length increased when the Mongolian duplication is present. None of the other variants interrupted the sequence of genes, predicted ORFs, lincRNAs or micro-RNAs. LincRNA#1 and LincRNA#2 are entirely conserved within bovids, but only partially conserved in hornless species. Overall, there is good conservation of synteny across species for the *POLLED* region (chr1:1,593,290–2,029,406). However, the TAD analysis of Angus (polled) and Brahman (horned) Hi-C data show dissimilar TAD boundaries in this region between subspecies. Further research will investigate whether TAD boundaries are altered for other *POLLED* variants and examine how the loci in the region may interact during horn bud formation.

Key Words: bovine, horn, polled

P369 Genetic parameter estimation and dynamics of fatty acid composition in Korean Holstein cattle. R. Umanthi[1], C. H. Park[2], C. G. Dang[3], and C. H. Do*[1], *[1]Chungnam National University, Daejeon, Korea, [2]Korea Animal Improvement Association, Seoul, Korea, [3]National Institute of Animal Science, RDA, Cheonan, Korea.*

Fatty acids (FA) in milk are considered to be important nutritional component of the human diets and noticeably affect human health. Making targeted modifications to the FA profile has the potential to improve the quality of milk and dairy products. The objective of this study was to identify the factors effecting elevated FA levels and evaluate the genetic parameters for milk FA content in the Korean Holstein population. Total 885,249 test-day records of milk composition were collected from 2012 to 2018 by the Korea Animal Improvement Association (KAIA). The test day milk records included milk yield, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), total unsaturated fatty acids (TUFA), fat and protein percentages of cows that were 1 to 305 d in milk at sampling. Genetic parameters, including genetic (co)variance components, were estimated by the restricted maximum likelihood procedure based on a repeatability model using the Wombat program. FA composition varies along with lactation and energy balance (EB). At the second week of lactation, during negative EB (−13.18 MJ nel/d) low level of SFA content (1.8 g/ dL of milk) was observed and it increased with lactation week (2.22 g/ dL of milk at 12 th week of lactation). TUFA level was 1.63 g/ dL of milk at the second week of lactation and it reduces to 1.16 g/ dL of milk at 12 th week of lactation. EB increased up to 63.40 MJ nel/d by the 12th week of lactation. Genetic and phenotypic correlations estimated among FA contents were positive. Heritability estimates for SFA, MUFA, PUFA, TUFA were 0.33, 0.42, 0.37, 0.41 respectively. According to the parity wise heritability analysis first parity cows had relatively low heritability of SFA (0.19). There is no significant difference of heritability along with parity in other FA groups. Heritabilities indicated that FA were under stronger genetic control and it indicate that selection of animals with improved FA profiles would be feasible due to genetic variation in FA composition.

Key Words: Holstein, fatty acid, heritability, parity, energy balance

P371 Effects of including sequence variants on imputation accuracy. M. Spengeler*[1], I. M. Häfliger[2], C. Drögemüller[2], H. Pausch[3], and F. R. Seefried[1], *[1]Qualitas AG, Zug, Switzerland, [2]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [3]Animal Genomics, Institute of Agricultural Sciences, Lindau, Zurich, Switzerland.*

Selection of SNPs for genomic selection is crucial since it affects both, accuracy of imputation and genomic prediction. It has been shown that the use of millions of sequence variants leads only to small gains in genomic prediction accuracy compared with dense SNP chips, if at all, while it increases the computational complexity drastically. One solution could be to include only a set of pre-selected SNPs. Since the number of sequenced animals is much lower than the number of genotyped animals, the accuracy of imputation could be reduced by introducing sequence variants. The aim of this study was to investigate the accuracy of imputation of SNPs obtained from sequencing data and their impact on accuracy of imputation within the region they are located. The study included the 2 existing sub-populations of the Brown Swiss breed: Brown Swiss and the Original Braunvieh since both share one

common traditional evaluation system and have shared pedigree links. The pre-selected SNPs have either been identified using genome-wide association studies (GWAS) in both sub-populations separately or they were SNPs, for which an impact on the phenotype has been described previously. The set of SNP obtained included 75 SNPs. These SNPs were extracted from sequencing data and added as an *in silico* chip to the imputing system. Sequencing information included data of 242 Animals in total. Final density during imputation system was 110K, which was selected based on MAF criteria from the GeneSeek UHD (150K) chip. Imputation accuracy was evaluated by masking 5% of the youngest 150K-genotyped animals from each sub-population downwards to the current LD-assay (47K). Subsequently effects of introducing sequence variants on region specific imputation accuracy were analyzed for both sub-populations. Imputation was performed using FImpute. The error rate of imputation for sequence SNPs was between 0% and 18%, while it was between 0% and 54% for all other SNPs. Overall imputation accuracy was very similar with or without the inclusion of sequencing SNPs.

Key Words: cattle, animal breeding

P372 Expressions of β-defensin family genes in cisternal lining epithelial cells of dairy cattle mammary gland infected with staphylococci. E. Bagnicka[1], E. Kosciuczuk[1], J. Jarczak[2], P. Lisowski[1], M. Rzewuska[3], M. Zalewska[1], E. Kawecka[1], T. Zabek*[4], S. Marczak[1], S. Petrykowski[1], D. Sloniewska[1], and L. Zwierchowski[1], *[1]Institute of Genetics and Animal Breeding PAS, Jastrzebiec, Poland, [2]Biobank Lab, Department of Molecular Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland, [3]Department of Pre-Clinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Warsaw, Poland, [4]National Research Institute of Animal Production, Balice, Poland.*

We studied the β-defensin gene expressions in cows' udder cisternal-lining-epithelial cells (CLEC), depending on the milk microbiological status. The study was conducted on 40 dairy cows of Polish HF breed with naturally occurring chronic mastitis, being between 1st and 4th parities. The "first milk" samples were microbiologically examined before their slaughter. One or 2 quarter CLEC samples were taken from each cow. Six sample groups were distinguished: infected with coagulase-positive staphylococci in 1st or 2nd parities and 3rd or 4th parities (CPS1/2; CPS3/4; n = 14 in each group), coagulase-negative staphylococci (CNS1/2; CNS3/4; n = 9 in each), and bacteria-free samples (H1/2, H3/4; n = 8 in each). Tracheal antimicrobial peptide (*TAP*), β-defensin 1, 4, 5, and 10 (*DEFB1*, *DEFB4*, *DEFB5*, *DEFB10*), and lingual antimicrobial peptide (*LAP*) gene expressions were analyzed using qPCR method with hypoxanthine phosphoribosyltransferase1 (*HPRT1*) and TATA box-binding protein (*TBP*) as a reference genes. The mRNA levels were transformed into a natural logarithmic scale. The variance analyze was done. The mRNA of all studied genes, except *TAP,* were identified in all samples. However, the higher level of *DEFB1* mRNA was observed only for animals infected with CPS being in their 1st or 2nd parity vs. CNS1/2 and H1/2, while *DEFB4* and *DEFB10* mRNA only for older animals (CPS3/4 vs. CNS3/4 and H3/4). CPS groups had extremely high expression of *DEFB5* gene in contrary to CNS, or H, regardless of the parity. The *LAP* mRNA was found at barely detectable level in all samples. However, its higher level was found in CPS1/2 and CPS3/4 vs. H, from analogous parities. Moreover, its higher level was also found in CPS1/2 and CPS3/4 vs. CoNS1/2 and CNS3/4 (in analogous parities). It seems that DEFB4, DEFB5 play an important role in the defense of CLEC, especially against the coagulase-positive staphylococci. The constitutive expression of 5 β-defensins was proved. The TAP does not take part in protection of CLEC against staphylococci, since its expression was not detected in this tissue. The research was funded by the Polish NSC (grant No. 2015/17/B/NZ9/01561).

Key Words: dairy cattle, mammary gland, cisternal lining epithelial cells, qPCR, defensin

P373 Differences in miRNAs expression between blood leukocytes and milk somatic cells of SRLV-seropositive and seronegative goats. E. Bagnicka[1], D. Reczynska*[1], M. Czopowicz[2], J. Kaba[2], J. Jarczak[3], D. Sloniewska[1], K. Horbanczuk[1], and L. Zwierzchowski[1], *[1]Institute of Genetics and Animal Breeding PAS, Jatrzebiec, Poland, [2]Warsaw University of Life Sciences, Laboratory of Veterinary Epidemiology and Economics, Faculty of Veterinary Medicine, Warsaw, Poland, [3]Biobank Lab, Department of Molecular Biophysics, Faculty of Biology and Environmental Protection, University of Lódz, Lodz, Poland.*

In our previous studies, the differences between expression of some immune system genes at the mRNA and protein level were found which was explained by epigenetic regulations e.g., miRNAs activity, influenced by the small ruminant lentivirus (SRLV) infection. The aim of the present study was to analyze expressions of selected miRNA in blood leukocytes (BL) and milk somatic cells (MSC) of SRLV-seropositive and SRLV-seronegative goats. The study was conducted on 24 dairy goats divided into 2 groups: SRLV-seronegative (n = 12) and SRLV-seropositive (naturally infected; n = 12). The milk and blood samples for RNA isolation were collected 5 times during lactation: just after kidding, on the 30th, 60th, 140th, and 200th day of lactation. The expression of chi-mir-24–5p, chi-mir-93–5p, chi-mir-30e-5p, chi-mir-214–3p, chi-mir-29b-3p, chi-mir-141, and chi-mir-221–5p miRNAs was measured by RT-qPCR performed with U6 snRNA as a reference. miRs expressions were logarithmically transformed (natural logarithm) and compared between groups using the ANOVA. DIANA-tools-TarBase v7.0 was used in silico functional analysis. Constitutive expressions of 4 miRs: miR-214–3p, miR221–5p, miR-24–5p, miR-29b-5p were found in BL and MSC from both seropositive and seronegative goats. Expression of miR-141 was found only in MSC, while miR-93–5p only in BL of goats from both groups. Moreover, miR-30e-5p was found only in BL of seropositive goats. The levels of miR-214–2p and miR-221–5p were lower in BL than in MSC, but only in SRLV-seropositive goats, with no differences in seronegative animals. Both miRs regulate the expression of many genes related to the immune system and the response to viral infections. Thus, they could regulate the production of cytokines, T cell differentiation, transport of viral RNA to the host cell cytoplasm, and show antiviral activity, e.g., *via.* interferon. The expression of miR-141, miR-93–5p, and miR-30e-5p only in one biological material may indicate their tissue-specific expression. The higher expression of miR-214–3p and miR-221–5p in MSC vs. BL of seropositive goats may mean that immune response in the udder is local and independent from the general response of the immune system. Financed by the NSC Poland, grant No. 2016/21/N/NZ9/01508

Key Words: goat, SRLV, blood leukocyte, milk somatic cells, miRNA

P374 Genetic and genomic analyses for predicted methane-related traits in Japanese Black cattle. Y. Uemoto*[1], M. Takeda[1,2], A. Ogino[3], T. Nozaki[3], K. Kurogi[3], T. Yasumori[4], S. Ogawa[1], M. Satoh[1], and F. Terada[1], *[1]Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan, [2]National Livestock Breeding Center, Nishigo, Fukushima, Japan, [3]Maebashi Institute of Animal Science, Livestock Improvement Association of Japan Inc, Maebashi, Gunma, Japan, [4]Cattle Breeding Department, Livestock Improvement Association of Japan Inc, Tokyo, Japan.*

Methane emission in cattle has become an important research area, because of linking to global warming. Recent studies on cattle have revealed the existence of a heritable variation in methane production (CH4). However, measuring CH4 directly from cattle on a long fattening period is difficult, and predicting CH4 can be performed without undertaking costly experiments. The objectives of this study were to estimate heritability and to perform genome-wide association study (GWAS) for predicted methane-related traits in Japanese Black cattle. Dry matter intake (DMI), body weight, daily gain, total digestible nutrients, and roughage ratio were measured every 2 mo from 9 to 21 mo of age. The methane-related traits, which are CH4, CH4 per DMI (CH4/DMI), and methane conversion factor (MCF), were predicted by using

these records. The methane-related traits were predicted from 4,578 Japanese Black steers which were progenies from 362 sires. Genotypes of the 362 sires were obtained using an Illumina BovineSNP50 array, and they were then imputed to an Illumina BovineHD array. A total of 546,866 SNPs after quality control were used. For 3 methane-related traits, single-trait animal model was used to estimate heritabilities in 7 different fattening periods and the total fattening period. In addition, the SNP effects within a 100 kbp-window were estimated using a single-step GWAS for these traits in the total fattening period. The estimated heritabilities for CH4, CH4/DMI, and MCF were different in each fattening period, ranging from 0.42 to 0.52, 0.27 to 0.36, and 0.27 to 0.37, respectively, and the estimated heritabilities for the total fattening period (0.61, 0.58, and 0.59, respectively) were greater than those for these short fattening periods. The SNP effects with more than 5% of the genetic variance were found on BTA3 in all 3 traits and on BTA8 in CH4/DMI and MCF. These results indicated that the methane-related traits are heritable and higher estimated heritabilities were obtained in a long fattening period. In addition, some SNP windows had higher effects on the methane-related traits.

Key Words: GWAS, heritability, Japanese Black cattle, methane emission, SNP

P376 Simulation study on the power of QTL detection and genomic prediction accuracy using small effective population sizes. M. Takeda*[1,2], Y. Uemoto[2], and M. Satoh[2], *[1]National Livestock Breeding Center, Nishigo, Fukushima, Japan, [2]Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan.*

Japanese Black cattle have a small effective population size, and knowledge of the optimal reference population size and its composition for performing genome-wide association studies (GWAS) and genomic predictions (GP) is required. This study's objective was to evaluate the power of QTL detection and GP accuracy on different population sizes to determine if the reference population in the simulation study accounted for the small population size. Data were simulated using the QMSim software. A historical population was simulated to create a mutation-drift equilibrium, with the effective population size set at 20. Of the simulated 12 generations of the recent population, the first 4 were used only for pedigree information. A total of 300 sires from the 5th to 10th generations and their offspring in groups of 1500, 4500, or 9000 from each of the 6th to 11th generations were randomly selected. All individuals had true breeding values (TBVs), phenotypes, and 50K SNP genotypes. Six scenarios (S1–S6) with different reference population set sizes were considered; S1–S3 had 9000, 4500, and 1500 offspring with phenotype and genotype data, respectively, and S4–S6 had 9000, 4500, and 1500 offspring with phenotype data but only 300 sires with genotype data, respectively. QTLs were positioned on the SNP chip with the S1 as the true result (S0). The power of QTL detection in S1–S6 using weighted single-step GWAS was calculated using S0 as a 100% marker. Genomic estimated breeding values (GEBVs) of 100 sires from the 11th to 12th generations in S1–S6 were calculated using single-step genomic best linear unbiased predictions (ssGBLUP); then the GP accuracy as the correlation coefficient between TBV and GEBV was obtained. All processes were replicated 10 times. The power of QTL detection was dependent on the number of QTLs and phenotyped individuals. For more than an 80% power of detection with QTLs, 4500 individuals with phenotypes were required. GP accuracy depended on the heritability of the trait and the number of genotyped individuals. For a GP accuracy greater than 0.7, 4500 individuals with genotypes were required.

Key Words: cattle and related species, animal breeding, computational workflow, quantitative trait locus (QTL), genomic prediction

P377 A genome-wide association analysis for dairy traits in Murciano-Granadina goats. D. Guan*[1], V. Landi[2], M. G. Luigi[1], J. V. Delgado[2], A. Castelló[1,3], B. Cabrera[1,3], E. Mármol-Sánche[1], J. F. Alvarez[4], A. Martínez[2], X. Such[3], J. Jordana[3], and M. Amills[1,3], *[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Spain, [2]Departamento de Genética, Universidad de Córdoba, Córdoba, Spain, [3]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Spain, [4]Asociación Nacional de Criadores de Caprino de Raza Murciano-Granadina (CAPRIGRAN), Granada, Spain.*

With a census of more than 100,000 individuals, Murciano-Granadina is the most important dairy goat breed in Spain. The average milk production is 498 Kg (normalized lactation of 210 d), with 5.1% and 3.6% of fat and protein percentages, respectively. However, we still do not know the main genetic determinants of milk yield and composition traits in Murciano-Granadina goats. The study presented here aimed to map loci with potential effects on 4 milk traits, *i.e.* Fat Percentage (FP), Protein Percentage (PP), Dry Matter (DM), and Milk Yield (MY). A total of 1,023 goats with available phenotypic records and Goat SNP50 BeadChip genotypes were used to perform genome- and chromosome-wide association analyses by fitting a univariate linear mixed model implemented in the GEMMA software. These analyses revealed a significant genome-wide association for PP on chromosome 6 (78–93 Mb), while several additional regions were significantly associated with other dairy traits at the chromosome-wide level. Interestingly, the QTL on chromosome 6 encompassed the casein cluster (α_{s1}-CN, α_{s2}-CN, β-CN, κ-CN), which has a strong impact on dairy traits as well as on cheese yield and texture. A next step would be to identify the casein variants that have causal effects on milk composition in Murciano-Granadina goats.

Key Words: GWAS, dairy traits, QTL, casein, goat

P378 ASIP: Transcripts and expression in llamas with different coat color. M. Anello*[1], M. S. Daverio[1,2], S. R. Romero[3], L. B. Vidal Rioja[1], C. Renieri[4], and F. Di Rocco[1], *[1]Instituto Multidisciplinario de Biología Celular (IMBICE), CONICET-UNLP-CIC, La Plata, Buenos Aires, Argentina, [2]Cátedra de Biología, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina, [3]Instituto de Investigaciones y Desarrollo Tecnológico para la Agricultura Familiar (IPAF). INTA, Maimara, Jujuy, Argentina, [4]School of Pharmacy, University of Camerino, Camerino, Marche, Italia.*

The Agoutí gene (ASIP) is one of the most important genes for coat color determination in mammals. It has a complex structure with different promoters and alternative non-coding first exons that are transcribed into mRNAs with different 5′UTR. Studies in domestic animals indicate these transcripts can control pigment distribution. Otherwise, white coat is usually produced by mutations in KIT, MITF and TYR genes. However, this phenotype has also been associated with overexpression of ASIP in some species. In llamas (*Lama glama*), the molecular mechanism responsible for white is still unknown. We have recently studied the coding regions of KIT, MITF, TYR and ASIP, but no mutations were found to be associated with white coat in this species. Here, we characterized ASIP transcripts and studied their expression levels in the skin of llamas to further understand the role of ASIP in coat color determination. Skin biopsies were taken from white, pheomelanic (reddish brown) and eumelanic (black) llamas and total RNA was extracted. Then PCR-RACE was used to amplify the 5′UTRs of ASIP and these products were cloned and sequenced. Seven ASIP transcripts that differed in the 5′UTR were found. Most of them exhibited a structure similar to some ASIP transcripts already described in other mammalian species and we identified the homologous exons 1A, 1A', 2A, 3A, and 1C. Additionally, one transcript (T6) presented a 5′UTR of 120 bp that showed similarity with NCOA6 gene. Then, we studied the global expression of ASIP by qPCR in the 3 phenotypic groups. White and brown groups showed an overexpression of ASIP in comparison to black ($P < 0.0001$) but no significant difference between white and brown was observed. Besides, we analyzed how T6 contributes to the global expression and found it was highly expressed in white and brown but not detected in black. Therefore, T6 is contributing to the

expression of ASIP in white and brown while other transcripts account for ASIP overall expression in black llamas. These results suggest ASIP transcripts have a role in coat color determination and we will carry out a more thorough study to evaluate the expression of each transcript and assess their contribution to color.

Key Words: New Word Camelids, biochemical genetics, qPCR, pigmentation

P379 Mapping copy number variation in Murciano-Granadina goats. D. Guan*[1], A. Castelló[1,3], V. Landi[2], M. G. Luigi[1], J. V. Delgado[2], J. F. Alvarez[4], A. Martínez[2], X. Such[3], J. Jordana[3], and M. Amills[1,3], *[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Spain, [2]Departamento de Genética, Universidad de Córdoba, Córdoba, Spain, [3]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Spain, [4]Asociación Nacional de Criadores de Caprino de Raza Murciano-Granadina (CAPRIGRAN), Granada, Spain.*

Copy number variation (CNV) plays an important role in phenotypic diversity and evolutionary adaptation in livestock. In this study, we have searched for caprine CNV regions (CNVRs) by using Goat SNP50 BeadChip data from 1,036 Murciano-Granadina goats. Two softwares, PennCNV and QuantiSNP, have been employed to call CNVs in this resource population. The CNVRs were defined as genomic regions containing CNV that overlap at least 30% by 2 callers. The CNV scan resulted in the identification of 486 CNVRs that were consistently detected with PennCNV and QuantiSNP. Interestingly, one of the CNVRs mapped to the casein cluster, so it would be worth to investigate its association with milk composition. Indeed, one of the null alleles of the *CSN1S1* gene involves a partial deletion of this locus and causes a marked decrease in milk protein content. Moreover, another CNVR mapped to the *ASIP* gene, which has been reported to be associated with white color in sheep and goats. To further validate the presence of the *ASIP* CNVR, a qPCR assay was developed to estimate its copy number in several goat populations. We have found evidence of the presence of structural variation in the *ASIP* locus in goat populations, such as Murciano-Granadina, which have a solid non-white color.

Key Words: copy number variation, *ASIP*, goat, Murciano-Granadina

P380 Interplay among miRNA, gene expression, and mineral metabolism in Nelore cattle. W. J. S. Diniz*[1,2], P. Banerjee[2], G. Mazzoni[2], L. L. Coutinho[4], A. S. M. Cesar[4], J. Afonso[3], C. F. Gromboni[5], A. R. A. Nogueira[3], H. N. Kadarmideen[2], and L. C. A. Regitano[3], *[1]Federal University of São Carlos, São Carlos, São Paulo, Brazil, [2]Technical University of Denmark, Kgs. Lyngby, Denmark, [3]Embrapa Pecuária Sudeste, Empresa Brasileira de Pesquisa Agropecuária, São Carlos, São Paulo, Brazil, [4]University of São Paulo, Piracicaba, São Paulo, Brazil, [5]Bahia Federal Institute of Education, Science and Technology, Ilhéus, Bahia, Brazil.*

Growing evidence suggested that miRNAs not only modulate gene expression but are also involved with mineral homeostasis. However, our understanding of the interplay among miRNA-gene expression-mineral metabolism in cattle is still minimal. In this study, we analyzed mRNA and miRNA expression as measured by RNA-Seq in *Longissimus thoracis* muscle of 50 Nelore steers and established miRNA and mRNA networks based on Weighted Gene Co-expression Network method. By applying a linear model, we identified, respectively, 9 and 15 miRNA and mRNA modules, which were mainly associated with Ca and Fe concentration, followed by Na, Mg, Cu, S, Zn, K, P, and Mn ($P < 0.05$). Further, we carried out a multi-level miRNA-mRNA integration based on the module eigengene correlation. We identified 41 miRNAs and 1,185 target genes inversely correlated by intersecting the associated modules ($P < 0.05$) with predicted interactions from TargetScan. The majority of genes was targeted by the bta-mir-29 family (miR-29a, 29b, 29c, 29d-3p, 29e), which can act redundantly and cooperatively to modulate them. Among the gene targets, we found transcription factors mineral-miRNA-modulated such as *HIF1A*, *HIF3A*, and *TFEB*, as well as genes involved with Fe homeostasis (*TFRC*, *IREB2*). Based on the pathway over-representation analysis, those genes are mainly acting in the AMPK, insulin, mTOR, and thyroid hormone signaling pathways. These pathways are interrelated and central to cell metabolism, being modulated by nutrients, growth factors, and miRNAs-mineral affected. We reported for the first time a complex interplay among miRNAs, mRNAs, and minerals likely acting in a feedback loop to maintain the cellular homeostasis in Nelore cattle muscle. Unrevealing the role of minerals in miRNA, gene expression, and metabolism regulation opens up several possibilities to potentially develop predictive biomarkers for mineral concentration in meat, and to improve animal nutrition.

Key Words: cattle and related species, functional genomics, integrative genomics, network analysis, miRNA

P381 Inclusion of herdmate data improves genomic prediction for milk production and feed efficiency traits within North American dairy herds. N. Schultz* and K. Weigel, *University of Wisconsin, Madison, WI, USA.*

Genomic data are widely available in the dairy industry and provide a cost-effective means of predicting genetic merit to inform selection decisions and increase genetic gains. As more dairy farms adapt genomic selection practices, dairy producers will soon have genomic data available on all of the animals within their herds. This is a very rich, but currently underutilized, source of information. Herdmates provide an excellent indication of how a selection candidate's genetics will perform within a given herd, noting that herdmates often include close relatives that share a similar environment. The study objective was to evaluate the utility of incorporating herdmate data into genomic predictions in a data set comprised of 3303 Holsteins from one herd in Canada and 6 herds throughout the United States. Within-herd prediction accuracy was assessed for milk production and feed efficiency traits determined from genomic best linear unbiased prediction under 4 different scenarios. Scenario one did not include herdmates in the training population. Scenarios 2 through 4 included herdmates in the training population while scenarios 3 and 4 also included modeling of herd-specific marker effects. Leave-one-out cross validation was used to maximize the number of herdmates in the reference population in scenarios 2 through 4, while maintaining constant reference population size with scenario one. Results from the present study reveal the importance of incorporating herdmate data into genomic evaluations. Scenarios 2, 3, and 4 improved mean within-herd prediction accuracy across the 6 milk production and feed efficiency traits by 0.06 ± 0.01, 0.07 ± 0.01, and 0.08 ± 0.01, respectively, in comparison to scenario one which did not include herdmates in the training data. Herds with higher within-herd heritability and low genomic correlation with the remaining herds benefitted most from the inclusion of herdmate data.

Key Words: genomic prediction, genotype by environment interaction, dairy herd

P382 Genome-wide tests and sequencing point to candidate gene variants for body temperature maintenance under the cold stress in Siberian cattle population. A. V. Igoshin*[1], A. A. Yurchenko[1], N. M. Belonogova[1], D. V. Petrovsky[1], R. B. Aitnazarov[1], V. A. Soloshenko[2], N. S. Yudin[1,3], and D. M. Larkin[1,4], *[1]The Federal Research Center Institute of Cytology and Genetics, The Siberian Branch of the Russian Academy of Sciences (ICG SB RAS), Novosibirsk, Russia, [2]Siberian Research Institute of Animal Husbandry, Krasnoobsk, Russia, [3]Novosibirsk State University, Novosibirsk, Russia, [4]Royal Veterinary College, University of London, London, UK.*

Genetic mechanisms of adaption to cold are important to study to improve livestock performance in countries with cold climates. We used Siberian populations of related Kazakh Whiteheaded and Hereford breeds to perform genome-wide association studies (GWAS) and scans

for signatures of selection to identify genome intervals related to body temperature maintenance under the acute cold stress. A single-point (EMMAX software) and haplotype-based (HTR test) GWAS were carried out on 183 animals bred in Siberia and genotyped on a 150K SNP array. The area under the curve of the in-ear temperature measured over the period of 5 coldest winter days (–20 to –32°C) was used as a proxy phenotype for the acute cold stress resistance. A significant signal reported by both methods was obtained for SNPs near *GRIA4* and *MSANTD4* genes. The same region was among the intervals reported for the same 183 animals in our signatures of selection scan utilizing the DCMS approach which combines the H1, H12, Tajima's D, and nucleotide diversity statistics in a single framework. We then tried RNASeq of blood samples for 12 individuals with the largest and the smallest (6 animals per group) values for our proxy phenotype. Unfortunately, both candidate genes were found not expressed in blood. Therefore, we performed whole-genome resequencing of the same 12 individuals to ~15X coverage and identified 3 missense and 2 splicing form changing SNPs in *GRIA4*. Two of these genetic variants (one missense and one splicing form changing) had different frequencies in the 2 animal groups suggesting that they could contribute to cold resistance phenotype. *GRIA4* encodes for one of the subunits of the AMPA receptor. It was previously shown that expression of the receptor in hypothalamus contributes to thermoregulation in rats. Therefore, this work provides a novel candidate gene to be involved in cold adaptation in the Siberian cattle and with candidate SNPs which should be tested on a larger cohort of animals and tissues before be recommended for marker-assisted selection purposes.

Key Words: body temperature, GWAS, cattle, selection, cold adaptation

P383 RNA-seq analysis of the effect of melatonin on the expression of genes related to hair follicle development in Inner Mongolia cashmere goats. Y. Zhao*, Y. Wang, Q. Mu, Z. Wu, and Z. Liu, *College of Animal Science, Inner Mongolia Agricultural University Hohhot, Inner Mongolia.*

This study aimed to explore the detection of genes related to hair follicle development in Inner Mongolia cashmere goat after the implantation of melatonin. The RNA expression was analyzed mainly by transcriptome sequencing technology on tissues of cashmere goat taken in February, September and December. Results showed that 532 differentially expressed genes were screened in February, 641 differentially expressed genes in October and 305 differentially expressed genes in December. At the same time, the effect of melatonin on the expression level of relevant genes in the development of hair follicle of Inner Mongolia cashmere goat was detected by fluorescence quantitative PCR. The results showed that *Wnt10b, esf-catenin, FGF21, SFRP1* and *TCHHL1* genes were all expressed in different levels in the skin tissue of cashmere goat implanted with melatonin.

Key Words: Inner Mongolia cashmere goat, melatonin, villi development, gene expression

P384 Validation of candidate SNPs for fat percentage in bovine rib-eye area using genotyping integrated fluidic circuit. R. Yamamoto*[1], S. Toyomoto[1], N. Kohama[2], T. Akiyama[2], E. Yoshida[3], E. Kobayashi[4], K. Oyama[5], H. Mannen[1], and S. Sasazaki[1], *[1]Graduate School of Agricultural Science, Kobe University, Kobe, Hyogo, Japan, [2]Northern Center of Agricultural Technology, General Technological Center of Hyogo Prefecture for Agriculture, Forest and Fishery, Asago, Hyogo, Japan, [3]Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries, Kasai, Hyogo, Japan, [4]Division of Animal Breeding and Reproduction Research, Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan, [5]Food Resources Education & Research Center, Kobe University, Kasai, Hyogo, Japan.*

Fat percentage in rib-eye area (FPR), which is measured by image analysis of carcass cross-section, would be an attractive alternate for evaluation of beef marbling. In our previous study, we performed GWAS using illumine bovine SNP50 BeadChip to identify the genomic region associated with FPR, and detected 2 significant SNPs (No.1, 2 SNP) in BTA7. Considering the location of 2 SNPs and previously reported QTLs for beef marbling, we assumed the range of 10–30Mbp in BTA7 as the candidate region. We also conducted whole-genome resequencing to detect all polymorphisms which include responsible polymorphism in the candidate region. As a result, we detected 127,090 polymorphisms within the region. The objective of the present study is to verify the effects of these candidate SNPs. We selected 96 candidate SNPs from all SNPs detected by resequencing according to the information such as linkage disequilibrium (LD) with No.1 SNP, function of the gene, and annotation of the SNP. We genotyped these 96 SNPs in Japanese Black population (n = 435) using Integrated Fluidic Circuit (IFC) to investigate their effects on FPR using ANOVA (ANOVA). As a result, we obtained the genotypes of 69 SNPs in all 435 animals. P-value from ANOVA of the 69 SNPs ranged from 0.99 to $3.56e^{-5}$, and 27 SNPs showed lower p-value than No.1 SNP ($P = 1.90e^{-3}$). We also calculated LD coefficients between each SNP and No.1 SNP. The r^2-values of the 27 SNPs with No.1 SNP ranged from 0.314 to 0.722. These 27 SNPs were located on 13 genes. Out of them, ALDH7A1 and SLC27A6 have been reported to be related to fat metabolism and marbling. In this study, we identified 2 candidate genes for beef marbling by validating SNPs within candidate region detected by GWAS. The result would be useful information to identify responsible gene and polymorphism for beef marbling, and would contribute to selective breeding of Japanese Black cattle.

Key Words: beef marbling, GWAS, Japanese Black cattle, integrated fluidic circuit

P385 Effect of stage of lactation and polymorphisms in *CSN1S1* 5′ flanking region on milk production traits in White Fulani cattle breed in Benin. I. Houaga*[1,2], A. W. T. Muigai[3], F. M. Ng'ang'a[4], F. Stomeo[4], I. A. K. Youssao[2], V. C. Yapi-Gnaoré[1], and E. M. Ibeagha-Awemu[5], *[1]Centre International de Recherche-Développement sur l'Elevage en zone subhumide, Bobo-Dioulasso, Burkina Faso, [2]Department of Botany, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya, [3]Biosciences Eastern and Central Africa-International Livestock Research Institute (BecA-ILRI) Hub, Nairobi, Kenya, [4]Laboratory of Animal Biotechnology and Meat Technology, Department of Animal Health and Production, Polytechnic School of Abomey-Calavi University, Cotonou, Benin Republic, [5]Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada.*

White Fulani is the main indigenous zebu cattle breed in Benin. However, there is limited data on the variability of their milk production traits. The aim of this study was to investigate the effect of the stages of lactation and the single nucleotide polymorphisms (SNPs) in 5′flanking region of *CSN1S1 gene* on milk production traits in White Fulani. Thus, 99 White Fulani cows were genotyped at *CSN1S1* gene using Sanger sequencing. The milk production traits in relation to stages of lactation were recorded (Test-day milk yield, fat%, Protein %, milk urea nitrogen, lactose %, test-day fat and protein yields).The cows at late lactation stage presented milk with higher fat (6.79 vs 4.35 vs 5.11%, $P < 0.001$) and protein (4.26 vs 3.44 vs 3.60%, $P < 0.001$) contents than cows at early and mid-lactation stage respectively. On the contrary, the White Fulani cows at late lactation stage showed lower content of milk urea nitrogen (6.88 vs 8.46 vs 8.60 mg/dl, $P < 0.05$) and lactose content (4.33 vs 4.63 vs 4.49%, $P < 0.05$). Three different SNPs (g.10331 A > G, g.10359 T > C and g.10430 G > A) were discovered in *CSN1S1* 5′ flanking region. The TT genotype of *CSN1S1* g.10359 T > C was associated with higher ($P < 0.01$) test-day milk yield and higher ($P < 0.05$) test-day fat and protein yields. The GG genotype of *CSN1S1* g.10430 G > A was associated with higher ($P < 0.01$) test-day milk yield. The identified SNPs in *CSN1S1* 5′ flanking region could serve as potential genetic markers in the improvement of milk yield traits in indigenous White Fulani cattle breed in Benin. However, further studies with a

larger population of the White Fulani breed are needed to better understand the genetic variability of their milk production traits.

Key Words: White Fulani, *CSN1S1*, SNPs, milk production traits, Benin

P386 Accuracy of genomic estimated breeding value in Hanwoo (Korean Native Cattle) cows. D. J. Lee*[1], S. H. Lee[2], S. E. Park[1], and D. H. Yoon[1], *[1]Department of Animal Science and Biotechnology, Kyungpook National University, Sangju, Korea, [2]Division of Animal and Dairy Science, Chungnam National University, Daejeon, Korea.*

This study was conducted to compare the accuracy of estimated breeding value (EBV), genomic estimated breeding value (GEBV), and single-step genomic estimated breeding value (ssGEBV) for carcass traits of 608 Hanwoo cows. The carcass traits considered in this study were carcass weight (CW), eye muscle area (EMA), backfat thickness (BFT), marbling score (MS). The EBV analysis was performed using the best linear unbiased prediction (BLUP) method by constructed a numerator relationship matrix (NRM) using the pedigree information of 608 cows, with pedigree and phenotype data of the candidate bulls and their steers in the National Breeding Program. The GEBV analysis was performed genomic best linear unbiased prediction (GBLUP) method by constructing a genomic relationship matrix (GRM) using SNP 50K information of 608 cows, and phenotype and genomic data of 3,821 steers as the reference population. The ssGEBV analysis was performed single-step genomic BLUP (ssGBLUP) based on the relationship matrix H, which is constructed from the numerator relationship matrix (A) augmented by the genomic relationship matrix (G). As the results, the EBV accuracy of 54,213 heads in NRM were between 0.334 ~0.362, the GEBV accuracy of 4,429 individuals in GRM were between 0.470 ~0.508, and the ssGEBV accuracy of mixed pedigree with genomic relationship matrix (H) were between 0.460 ~0.493. The differences in accuracies of 3 estimated breeding values for CW, EMA, BFT and MS traits for 608 cows showed that the accuracies of GEBV were the highest accuracy for all traits except EMA. As a result, the ssGBLUP method with genomic information on Hanwoo cows gives higher accuracy than BLUP. However, when the analyzed data has all the genotypes, GBLUP method might represent more accurate estimation, and it could be used as a basic data for genomic selection.

Key Words: Hanwoo cow, SNP, estimated breeding value, genomic selection

P387 Abstract withdrawn

P387 Abstract withdrawn

P388 Metabolome fingerprinting and potential biomarkers of hypocalcemia in dairy cows. E. Dervishi*[1], M. Zhu[1], M. Colazo[2], L. Li[1], and G. S. Plastow[1], *[1]University of Alberta, Edmonton, Alberta, Canada, [2]Alberta Agriculture and Forestry, Edmonton, Alberta, Canada.*

The purpose of this study was to screen serum samples to identify biomarkers predictive of hypocalcemia in dairy cows. Samples were collected prospectively 7.7 ± 4.5 d before calving from 1,096 cows in 11 dairy herds in Alberta, Canada. Samples from 30 cows subsequently diagnosed with hypocalcemia were chosen for untargeted metabolomics analysis. Samples from 56 control cows from the same herd (accounting for parity, body condition score and time of calving) with no diagnosis of disease were used to search for differences in metabolic signatures. A 4-channel isotope labeling LC-MS technique was applied to study the amine/phenol, ketone/aldehyde, carboxylic acid and hydroxyl submetabolomes. For each labeling one aliquot of the sample was incubated with 12C-isotope labeling reagent. A pooled serum sample was prepared, and for each labeling one aliquot of pool was incubated with 13C-isotope reagent. The 12C-labeled individual sample and the 13C-labeled pooled sample were mixed in equal amount and analyzed by LC-FTICR-MS. A receiver operating characteristic (ROC) curve of each significant metabolite was generated to determine the sensitivity and specificity for differentiating hypocalcemia from control. Preliminary results showed that 5,464 peak pairs were commonly detected from the 4 submetabolomes. The volcano plot of hypocalcemia versus control showed 1,904 significantly changed peak pairs from the 4-channel labeling. The amine/phenol submetabolome revealed that in cows with hypocalcemia, 98 peak pairs were downregulated and 122 peak pairs were upregulated (FC >1.5, q <0.05 ($P < 0.016$)). In the ketone/aldehyde submetabolome, 144 peak pairs were downregulated and 153 were upregulated (FC >1.5, q <0.05 ($P < 0.027$)). In addition, the carboxylic acid submetabolome revealed 753 downregulated peak pairs and 506 upregulated peak pairs (FC >1.5, q <0.05 ($P < 0.025$)). Finally, the hydroxyl submetabolome revealed 108 downregulated peak pairs and 99 upregulated peak pairs (FC >1.5, q <0.05 ($P < 0.026$)). Many of the significantly changed metabolites had 98–100% sensitivity and specificity. These metabolites could be potential biomarkers predictive of hypocalcemia in lactating dairy cows.

Key Words: metabolomics, biomarkers, dairy cows, hypocalcemia

P389 Genetic relationship of maternal haplotypes of the endangered long-tailed goral (*Naemorhedus caudatus*) populations in South Korea. S.-H. Han*, J.-Y. Moon, Y.-H. Lee, S.-J. Jeong, G.-C. Kim, D.-W. Lee, J.-I. Son, and C.-U. Cho, *Species Restoration Technology Institute, Korea National Park Service, Gurye, South Korea.*

The population size of long-tailed goral, designated as an endangered species grade I, has been declined by illegal hunting and habitat destruction in 20th Century, then protection and recovery programs have been carrying out in national parks in South Korea since 2012. At present the animal number is increased in most managing sites, and several populations have been also recently found in new habitats such as high mountainous areas and demilitarized zone between North and

South Korea. However, there was little information about genetic background and evolutionary relationship among the populations of long-tailed goral. In the present study, we analyzed the genetic structure of maternal haplotypes of the endangered long-tailed goral (*N. caudatus*) in South Korea based on the nucleotide and haplotype diversity to reveal the genetic relationship between the local populations. Nucleotide sequences of mitochondrial DNA (mtDNA) *cytochrome oxydase I* (*COI*) gene were obtained from fecal samples collected from natural habitats, and *COI* haplotypes were determined and compared with each other. A total of 4 *COI* haplotypes were identified. Among those, H01 was common in all populations tested, and H03 was also found predominantly in most populations showing geophylogenetic correlation in the Baekdudaegan Mountain Range (BMR) of the geographical alpine backbone of Korean Peninsula, estimating that the populations might have a common genetic and evolutionary history related among the populations via maternal lineages within the BMR area. However, among those haplotypes, H02 was specific in a western population and H04 was only found in 2 eastern populations. These western and eastern locations are separated by the BMR, estimating that local differentiation might be estimated in western and eastern populations. In addition, several populations showed lower haplotype diversity than 0.5, estimating undergoing genetic bottleneck. To makeup genetically stable condition, more active protection and conservation plans such as translocation using meta-population and captive breeding are needed for increasing population viability and genetic diversity in the future.

Key Words: *COI*, haplotype, long-tailed goral, *Naemorhedus caudatus*, population structure

P390 Whole-genome sequence analysis reveals insights into the genetic diversity and structure of the Cuban Charolais, a taurine breed adapted to tropical climate. L. C. Ramírez-Ayala*[1], S. E. Ramos-Onsins[1], J. Leno-Colorado[1], Y. Rodríguez-Valera[2], D. Rocha[3], Y. Ramayo-Caldas[4,5], and M. Pérez-Enciso[1,6], *[1]Plant and Animal Genomics, Centre de Recerca en Agrigenòmica (CRAG), Consorci CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, Spain, [2]Facultad de Ciencias Agropecuarias, Universidad de Granma, Granma, Cuba, [3]GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France, [4]Animal Breeding and Genetics Program, Institute for Research and Technology in Food and Agriculture (IRTA), Torre Marimon, Caldes de Montbui, Barcelona, Spain, [5]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain, [6]Institut Català de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.*

Early in the nineteenth century, Cuban farmers imported directly from France Charolais cattle. These animals have become adapted to tropical conditions and have been maintained as a "pure" breed without introgression from others local breeds or Indicine animals. These animals are adapted to rough environmental conditions that include long periods of drought and food shortage, all accompanied by extreme temperatures that commercial taurine cattle can hardly endure. In this study, we used whole-genome sequence data to estimate the genetic diversity, structure and putative ancestral origin of the 'Charolais de Cuba' (CHCU) breed as well as to identify regions with selective sweeps that may have had an important role in the adaptation to tropical conditions. A total of 12 CHCU samples and 49 samples from 5 breeds Brangus (BRG, n = 5), Brahman (BRM, n = 10), Canadian Charolais (CHCA, n = 15), French Charolais (CHFR, n = 15) and Texas Longhorn (TXL, n = 4) were analyzed. After quality control, 25,520,388 autosomal SNPs were selected for further analysis. To estimate signals of adaptation to tropical climate, we estimated nucleotide variability and differentiation values (F_{ST}) between the different populations. This was accompanied by the analysis of the Extended Haplotype Homozygosity (EHH), which is a reliable approach to detect regions of the genome under recent selective pressure. CHCU average nucleotide variability (0.0017) was similar to the rest of taurine breeds but lower than those in BRM or BRG (0.0031 and 0.0026, respectively). As expected, CHCU showed the lowest differentiation with CHFR (F_{ST} = 0.058) and CHCA (F_{ST} = 0.045). CHCU showed a larger degree of differentiation with the BRM (F_{ST} = 0.332) and similar values with BRG and TXL breeds (F_{ST} = 0.0870 and 0.0805, respectively).

Key Words: genetic diversity, tropical condition, adaptation, cattle

P391 Abstract withdrawn

P392 Abstract withdrawn

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P393 Genome insights into past and recent dynamics of goat pastoralism in northeast and eastern Africa. N. Khayatzadeh[1], G. M. Tarekegn[2], T. Dessie[3], A. Djikeng[4], A. Haile[5], B. Rischkowsky[5], and J. M. Mwacharo*[5], *[1]University of Natural Resources and Life Sciences, Vienna, Austria, [2]Department of Animal Production and Technology, Bahir Dar University, Bahir Dar, Ethiopia, [3]International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, [4]BecA-ILRI Hub, Nairobi, Kenya, [5]Small Ruminant Genomics Group, International Centre for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia.*

The genetic history of goat pastoralism and the genome dynamics of the species in the African continent remains largely unknown. Through the analysis of 50K SNP data in 25 indigenous goat breeds spanning the North-east and eastern Africa region, we reveal the genetic signatures of origin and dispersal of the species. Our analysis supports the initial entry of 2 independent caprine genetic pools into the continent via 2 geographic points. The earliest genetic pool arrived through North-East Africa while signatures of dispersal of the second genetic pool are detected in the Horn of Africa. While the first genetic pool only shows a southward dispersal, the second gene pool shows an expansion northwards and southwest wards across the region. The 2 genepools occur almost at equal proportions in Sudan suggesting it to be an ancient meeting point of pastoral societies from the North and the East Africa region. Signatures of recent interactions and dispersals are also revealed, through higher K values in admixture analysis, and uniquely between goats in Sudan and Egypt while within Ethiopia and Uganda the interactions occur between populations and individuals within the country. Unexpectedly, interactions between 2 populations (Keffa in Ethiopia; Kigezi in Uganda) and the others are minimal or absent as they retain a unique autosomal genome composition. The genome analysis also reveals an imprint of a population expansion event that commenced at different time periods (Uganda and Ethiopia = 620 generations ago; Sudan and Egypt = 860 generations ago) that is followed by a contraction at 180 (Uganda and Ethiopia) and 230 (Sudan and Ethiopia) generations ago, respectively. The decline in population size persists to present time. This suggest most likely a catastrophic (diseases epidemic, droughts etc) event may have occurred in the region between 360 and 460 years ago (assuming a generation interval of 2 years) that resulted in heavy mortalities in the species. This result correlates well with the reported occurrence, around 240–750 years ago, of 3 exceptionally favorable climatic periods with relatively shorter dry spells in the wider eastern Africa region which may have influenced the population dynamics of livestock. Our results suggest that past and recent anthropological and natural phenomena have influenced the genomes of extant African indigenous goats and therefore their evolutionary dynamics.

Key Words: population genomics, SNP, population structure

P394 Identification of genomic regions associated with morphological traits in Murciano-Granadina goats. M. G. Luigi-Sierra*[1], V. Landi[2], D. Guan[1], J. V. Delgado[2], A. Castelló[1,3], B. Cabrera[1,3], E. Mármol-Sánchez[1], J. Fernández-Alvarez[4], A. Martínez[2], X. Such[3], J. Jordana[3], and M. Amills[1,3], *[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [2]Departamento de Genética, Universidad de Córdoba, Córdoba, Córdoba, Spain, [3]Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [4]Asociación Nacional de Criadores de Caprino de Raza Murciano-Granadina, Granada, Granada, Spain.*

Morphological traits are of great importance to dairy goat production given their strong incidence on milk yield and longevity. However, their genomic architecture has not yet been extensively characterized. Murciano-Granadina is one of the most important goat dairy breeds in Spain, and its breeding program includes the measurement of 17 morphological traits related to udder, feet and body conformation. Our aim was to identify genomic regions associated with these 17 different morphological traits by performing a genome-wide association analysis (GWAS). A total of 722 Murciano-Granadina female goats with linear evaluation records of each morphological trait were genotyped with the Goat SNP50 BeadChip. Raw phenotypes were corrected for fixed factors (farm, age, number of births and milking stage at the scoring date). After quality control of the data, genome-wide association analyses and chromosome-wide association analyses were performed by using the genome-wide efficient mixed-model association (GEMMA) software. We found no significant associations between the typed SNPs and the studied traits at the genome-wide level. However, the chromosome-wide analysis made possible to identify 3 SNPs associated with diameter and positioning of the nipples in chromosomes 26 and 28. Interestingly, one significant SNP on chromosome 26 was located downstream the *FGFBP3* gene, which modulates FGFR signaling, one of the main pathways determining the proper development of mammary stem cells. With regard to body conformation, SNPs on chromosome 17 were found to be associated with the chest width. These SNPs mapped to the genes *ZNF268* and *ZNF827*, which encode zinc finger proteins playing important roles in cell growth, proliferation, development, apoptosis, and intracellular signal transduction. Our results suggest a strong additive polygenic background for these morphological traits since we were unable to identify any region on the genome with major effects on their phenotypic variance.

Key Words: genome-wide association, physical trait, goats and related species, milk production

P395 Using RNA-seq data in the genomic prediction model to increase the accuracy of Hanwoo (Korean cattle) intramuscular fat-related traits. S. B. Jang*[1], Y. J. Chung[1], J. M. Kang[1], D. Seo[1], D. J. Kim[2], J. H. Lee[1], and S. H. Lee[1], *[1]Chungnam National University, Daejeon, South Korea, [2]TNT research, Anyang-si, South Korea.*

Intramuscular fat (IMF) is a key trait to determine meat quality in Hanwoo industry. It also influences the tenderness, and flavor of Hanwoo meat. Therefore, IMF and Marbling Score (MS) are the main breeding objective traits in Hanwoo breeding program. Genomic prediction has a large potential on genetic improvement of traits such as IMF and MS that are difficult to measure and it will be more accurate in prediction of genomic breeding value if functional genes are involved in the prediction model. To investigate the improvement of breeding value accuracy when functional genes are involved, we conducted 2-step analyses: 1) identification of functionally related differentially expressed genes (DEGs) from RNA-seq analysis. 2) identification of SNPs in and near the DEGs region on the whole Hanwoo reference genome. Two data sets were used in RNA-seq analysis. First data has 24 Hanwoo muscle samples with high-low MS and second data has 40 Hanwoo muscle samples with IMF contents. From both RNA-seq results, we had 374 DEGs to do genomic prediction. The genomic prediction was performed to see the prediction accuracy of SNPs from functional DEGs (320k) compared with 50k, imputed 777k, and im-

puted Whole Genome Sequence (WGS) data. For genomic prediction, we used 1,160 Hanwoo cattle which has been genotyped using Illumina Bovine SNP50 BeadChip (50k) with the phenotype of MS and IMF content of *longissimus dorsi muscle* and *semimembranosus muscle*. We imputed 50k to 777k and WGS levels. Finally, we then estimated genomic breeding value with 50k, imputed 777k, imputed WGS, and DEGs using GBLUP and Bayes R model. In the GBLUP result, DEGs panel showed relatively lower accuracies (0.26 ~0.32) than other panels (0.33 ~0.42) for all the traits. The most interesting results were that Bayes R model showed higher prediction accuracies (0.41 ~0.52) than GBLUP model (0.26 ~0.42) and for the MS, DEGs panel showed higher accuracy (0.51) than other 2 panels (0.41, 0.45 respectively). This results showed that DEGs panel in Bayes R model indicated high prediction accuracy of MS so that RNA-seq can be a useful way to improve genomic prediction.

Key Words: cattle and related species, animal breeding, SNP, fat/lipid, genomic prediction

P396 Effect of the *PLAG1* gene polymorphism on carcass weight and oleic acid percentage in Japanese Black cattle populations. F. Kawaguchi*[1], H. Kigoshi[1], R. Yasuzumi[1], K. Oyama[2], H. Mannen[1], and S. Sasazaki[1], *[1]Laboratory of Animal Breeding and Genetics, Kobe, Hyogo, Japan, [2]Food Resources Education & Research Center, Kasai, Hyogo, Japan.*

The pleomorphic adenoma gene 1 (*PLAG1*) on BTA 14 has been reported to be a causative gene for certain economically important traits in cattle growth such as carcass weight (CW). On the other hand, QTL for fatty acid composition have been reported in the region near the *PLAG1* gene on BTA14. Furthermore, several studies have reported an association between the *PLAG1* gene polymorphism and fatty acid composition. The objective of this study is to investigate the association between *PLAG1* and fatty acid composition in various Japanese Black cattle populations and to evaluate the effectiveness of the *PLAG1* polymorphism as a DNA marker for improving oleic acid percentage (C18:1). We genotyped the *PLAG1* polymorphism (rs109231213) for 4 Japanese Black cattle populations (JB1: n = 900, JB2: n = 560, JB3: n = 456, JB4: n = 450) and then analyzed the effects on CW and C18:1 by statistical ANOVA (ANOVA) and Tukey-Kramer's honestly significant difference (HSD) test. As a result, the polymorphism showed highly significant effect on CW in 4 populations ($P < 0.001$), in agreement with previous reports. In addition, it was also significantly associated with C18:1 in JB1, JB2 and JB3 ($P < 0.05$). However, considering that the effect of *PLAG1* differed among populations, it was suggested that the *PLAG1* polymorphism would not have a direct effect on C18:1 and would be in linkage disequilibrium (LD) with a causative mutation. Turkey-Kramer's HSD test revealed that the qq genotype had a higher percentage of C18:1 than the QQ genotype in JB1 and JB2 (1.62 and 2.23, respectively), while the qq genotype showed lower CW in all 4 populations. These results suggest that the *PLAG1* gene polymorphism would be useful as a DNA marker for C18:1 in Japanese Black cattle populations and this information could contribute to the identification of a causative mutation on BTA14 for fatty acid composition.

Key Words: pleomorphic adenoma gene 1, carcass weight, oleic acid percentage, Japanese Black cattle

P397 Expression profiling identifies candidate drivers of sexual dimorphism in bovine placenta and somatic tissues. R. Liu*[1], R. Tearle[1], T. Chen[1], D. Thomsen[1,2], T. P. L. Smith[3], J. L. Williams[1], and S. Hiendleder[1,2], *[1]Davies Research Centre, School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, South Australia, Australia, [2]Robinson Research Institute, The University of Adelaide, Adelaide, South Australia, Australia, [3]USMARC, USDA-ARS-US Meat Animal Research Center, Clay Center, NE, USA.*

Sexual dimorphism significantly affects the phenotype of many mammalian species and impacts the production traits of farm animals. Sex-specific phenotypes frequently develop *in utero*, involve the placenta, and manifest in differences in birth weight that lead into further differentiated postnatal phenotypes. At midgestation, the bovine conceptus enters accelerated growth and is therefore particularly suited to dissect the molecular basis of sexual dimorphism. We used expression profiling to identify candidate genes that drive sexual dimorphism in placenta and somatic tissues (brain, liver, lung, and skeletal muscle) of Day 153 concepti with purebred and reciprocal cross *Bos taurus taurus* (Angus) and *Bos taurus indicus* (Brahman) genetics. We generated >70M RNaseq reads for each tissue from each of 12 male and 12 female concepti and mapped them to UOA_Angus_1 and UOA_Brahman_1 genome assemblies, which contain improved sex-chromosomes compared with previous bovine genome assemblies. Irrespective of their genetics, all fetal tissues showed a clear separation of males and females based on expression profile. A total of 57 genes (FDR < 0.05) were differentially expressed (DE) between males and females, of which 41 are located on the sex chromosomes. The majority of the identified autosomal DE genes were tissue-specific. Sixteen genes were shared across all 5 tissues, 12 of which are XY homologs. We compared the expression levels for the combined X and Y homologs in males with the X homolog expression in females. Only 4 of the 6 XY homologous pairs indicated complete dosage compensation. There were pronounced tissue-specific differences in expression levels for the remaining 2 pairs, with a strong male bias. Lung was distinct with a consistent female bias for 5 XY homologous pairs. In conclusion, we have demonstrated that a remarkably small number of DE genes, whose functions include cell cycle regulation and protein synthesis, discriminate between male and female placenta and fetal somatic tissues.

Key Words: cattle and related species, functional genomics, gene expression, growth and development

P398 Genomic diversity in a local Swiss cattle breed. M. Bhati*, D. Crysnanto, and H. Pausch, *Animal Genomics, Institute of Agricultural Sciences, ETH, Zurich, Switzerland.*

Original Braunvieh (OB) is a local dual purpose cattle breed which is well adapted to harsh conditions in Swiss alpine areas with scarce food. However, the genomic architecture of the OB cattle breed is not well known so far. The analysis of whole-genome sequencing (WGS) data may yield insights into the genomic constitution which might be useful for the sustainable breeding and genetic monitoring of the breed. Therefore, the objectives of this study are to characterize genomic variation and identify genomic regions that show evidence of past or ongoing selection in the Swiss OB population. We analyzed WGS data of 49 key ancestors of the current Swiss OB cattle population. We discovered 17.3 biallelic variants (SNPs and Indels) using *Graphtyper*. These variants were annotated based on the gene annotation of UMD3.1 assembly using the Ensembl Variant Effect Predictor. We detected 10,738 missense deleterious variants including 2,763 high impact variants that had lower minor allele frequency than neutral variants. We assigned ancestral alleles for 9.05 million SNPs. These variants were used to identify runs of homozygosity (ROH module of BCFtools) and signatures of selection (Sweepfinder software and the rehh R package). The percentage of the genome in ROH ranged from 24% to 29% per animal, where average contributions of short (<100 Kb), medium (100Kb-1Mb) and long ROH (>1Mb) size classes were 6.8%, 13.8% and 5.4%, respectively. Short ROH resulting from ancient inbreeding were more frequent than long ROH resulting from recent inbreeding. We were able to detect ROH that were between 203 bp and 23 Mb of length, thus providing detailed insights into past and ongoing demographic processes of the OB cattle breed. Using 2 complementary methods (CLR and iHS) to detect signatures of selection, we identified 98 and 145 regions, respectively, in the top 0.5% of all windows analyzed. Candidate genes located within the most strongly associated selection signatures are related to stature, fetal size, fertility, and feed efficiency in cattle. Our study provides a comprehensive overview of genomic variation segregating in OB cattle, which will be useful for a sustainable breeding and genetic monitoring of the breed.

Key Words: cattle and related species, genome sequencing, selection scan, homozygosity

P399 Multi-variate mixed-models for the normalization of RNA-Seq data: Application to onset of puberty in beef cattle. L. Tusell*[1], I. David[1], A. Canovas[2], M. G. Thomas[3], and A. Reverter[4], *[1]GenPhySE, Université de Toulouse, INRA, INPT, ENSAT, Castanet-Tolosan, France, [2]Department of Animal Biosciences, Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, [3]Department of Animal Sciences, Colorado State University, Fort Collins, CO, USA, [4]CSIRO Agriculture & Food, St. Lucia, Brisbane, Australia.*

Methods based on univariate mixed-models are used to normalize RNA-Seq data as an initial step to detect differentially expressed (DE) genes based on the gene by experimental condition interaction term. Character state models are classically used in quantitative genetics to assess genotype by environment interactions in discrete environments. This approach, considers phenotype measurements in different environments as different traits (or character states). Thus, the interaction variance can be estimated as a function of the genetic variances and covariances of the genetic effects in the environments. In this study, we propose a multi-variate mixed model approach (i.e character state model approach) to normalize RNA-Seq data to detect DE genes as well as potential interaction variance between the genes and (i) pre and post- puberty periods and (ii) several tissues (i.e., muscle, fat, liver, uterus, ovary, pituitary gland and hypothalamus) in composite beef cattle. A total of 1,087,752 base-2 log-transformation Reads Per Kilobase of transcript per Million mapped reads (RPKM) were analyzed in the pre and post- puberty physiological states as 2 different traits in a bivariate model. The model includes the systematic effect of library (61 levels) and the random effects of gene (17,832 genes) and gene × tissue (142,656 levels). The bivariate model allowed detecting DE genes in a similar way than the univariate did (98% of DE genes in common). The interaction variance between the genes and the puberty physiological states was small (0.02) because the estimated correlation of the genes was close to unity (0.98) and the gene variances in the 2 physiological states were of similar magnitude (6.78 and 6.76 in pre and post-puberty environments, respectively). Further research is warranted to assess the optimality of a multi-variate mixed model to evaluate the interaction variance across 8 different tissues. In this second model, the log-transformed RPKM reads measured in the tissues will be considered to be different traits, while the differential expression of interest will still be between pre and post-puberty physiological states.

Key Words: RNA-Seq data, pre and post- puberty physiological states, character state model, multivariate model, beef cattle

P400 Genomic characterization of a set of endangered Spanish bovine breeds: Morenas Galegas. M. A. García-Atance[1], J. Cañon[1], P. G. Eusebi[1], S. Dunner[1], C. J. Rivero[2], R. Justo[3], and O. Cortes*[1], *[1]Veterinary Faculty, University Complutense of Madrid, Madrid, Spain, [2]Centro de Recursos Zooxenéticos de Galicia, Ourense, Spain, [3]Federación de Razas Autóctonas de Galicia-BOAGA, Ourense, Spain.*

The region of Galicia, in the Northwest of the Iberian Peninsula hosts a set of local cattle breeds called Morenas Galegas that encompassing 5 bovine breeds (Cachena, Caldela, Limia, Vianesa and Friairesa) classified as endangered. Since 1990 the local government increased their efforts to preserve this important genetic resource, well adapted to the specific Galician orography and climatology, and promoted the development of ex situ and in situ conservation programs. However these breeds have not been included in any scientific molecular study to elucidate the genetic structure and relationships between them and other Spanish or European cattle breeds. In this current study, we aimed at investigating for the first time the amount of genetic diversity, population structure and level of inbreeding among Morenas Galegas and their genetic relationships with a comprehensive set of Iberian Peninsula and European bovine breeds using the Illumina Bovine SNP50 array. Preliminary results evidenced little heterozygosity variation among Morenas Galegas breeds ranging from 0.3 (Frieresa) to 0.34 (Caldela). The principal component analysis resulted in the separation of Cachena, Caldela and Frieresa, while Limia and Vianesa were grouped in a single cluster. Population structure analyzed by admixture reveal at k = 3 a clear separation of Cachena and Caldela, while Limia, Vianesa and Frieresa evidenced a certain degree of admixture among them. At k = 5 Limia formed a single cluster and Vianesa and Frieresa at k = 6. Detailed analyses will achieve the genetic relationships among Morenas galegas and Spanish and European bovine breeds. In addition Runs of Homozygosity (ROH) patterns and their distribution through the genome among Morenas Galegas breeds were analyzed. The results of this study will be useful for the development of breeding strategies as well as conservation programs for the Morena Galegas bovine breeds.

Key Words: Morenas Galegas, bovine, SNP, genetic variability

P401 Epigenetic changes of photoperiod responsiveness were identified in DNA methylation maps and transcriptome profiles using ovariectomized and estradiol treatment (OVX+E_2) sheep. X. He*[1], R. Di[1], X. Wang[1], W. Hu[1], X. Zhang[2], J. Zhang[2], Q. Liu[1], and M. Chu[1], *[1]Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Tianjin Institute of Animal Sciences, Tianjin, China.*

The change of photoperiod is the key factor affecting the reproductive seasonality in sheep. Emerging evidences have indicated that epigenetic modification participated in expression regulation of hypothalamic peptides which controlled reproductive activities, but the mechanism is still unclear. In the present study, whole genome bisulfite sequencing (WGBS) and RNA-seq were used to explore the differences of DNA methylation and transcriptome profiles in the hypothalamus with the photoperiodic change. Sunite ewes, one of typical seasonal estrus breeds in China, were selected to establish ovariectomized and estradiol-implanted (OVX+E_2) model, then housed in light-controlled rooms as 3 groups (short photoperiod, SP; long photoperiod, LP; short photoperiod transfers to long photoperiod, SP-LP). Secretion patterns of serum reproductive hormones showed that the concentrations of FSH, LH and PRL were closely related to the photoperiod. Dynamic DNA methylation and transcriptome profiles of hypothalamus were measured at different time points under artificial SP or LP, respectively. Here, comparison with the reference genome, around 1500 differentially methylated regions (DMRs), 1664 differentially expressed mRNA (DEM) and 59 differentially expressed lncRNA (DEL) were identified respectively after the photoperiod switching. Functional annotation of the DMRs, DEM and DEL showed that circadian entrainment, oxytocin signaling and GnRH signaling pathway were significantly enriched from SP to LP. The interaction analysis of lncRNA and mRNA found that XR_173415.3 has a negative regulatory effect on Thyroid Hormones (*TH*) and Insulin-like Growth Factor 2(*IGF2*). Combination analysis of methylation and mRNA showed that the expression of *IGFR* and *THR* were downregulated by DNA methylation after the photoperiod switching from SP to LP. These results suggested that lncRNA and methylation, as epigenetic regulatory factors, play a vital role in modulating genes associated with seasonal estrus. Those findings provided a new idea for us to understand the effects of photoperiod on seasonal estrus of sheep.

Key Words: OVX+E_2 sheep, photoperiod, hypothalamus, DNA methylation, transcriptome

P402 Identification of new *DGAT1* and *Casein* sequence variants in goats using capture sequencing. S. A. Rahmatalla*[1,2], D. Arends[1], M. Reissmann[1], L. M. A. Hassan[1,3], S. Krebs[4], and G. A. Brockmann[1], *[1]Albrecht Daniel Thaer-Institut für Agrar- und Gartenbauwissenschaften, Humboldt-Universität zu Berlin, Berlin, Germany, [2]Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Khartoum North, Sudan, [3]Wildlife Research Center, Animal Resource Research Corporation, Federal Ministry of Livestock, Fisheries and Rangelands, Khartoum, Sudan, [4]Laboratory*

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The diacylglycerol O-acyltransferase (*DGAT1*) gene was proposed to be a positional and functional candidate for fat content in goat milk. The 4 casein proteins (αS1-casein, αS2-casein, β-casein, and k-casein) in goat milk are encoded by the genes *CSN1S1*, *CSN1S2, CSN2*, *and CSN3*, respectively, which are located in a cluster on chromosome 6. Our study aimed at finding novel SNPs using capture sequencing in different goat breeds: Sudanese goats (Nubian, Desert, Taggar, and Nilotic), Saanen, Nubian ibex, Alpine ibex, and Bezoar ibex. In *DGAT1*, one novel non-synonymous variant in Alpine ibex and additional 4 synonymous SNPs were detected in Alpine ibex. In *CSN1S1*, one novel non-synonymous variant was identified in Alpine ibex and an additional 5 novel synonymous variants were found, one segregating only in Alpine and Nubian ibex, 3 SNPs in Saanen, Desert, Taggar, Nilotic, Nubian and Bezoar ibex, and one in Nubian and Nilotic goats. In *CSN1S2*, 4 novel non-synonymous SNPs were identified: one in Taggar and Saanen goats, whereas the other 3 were found only in Nubian ibex. In *CSN2*, 3 novels non-synonymous and one synonymous variant were detected in both Nubian and Alpine ibex. In the *CSN3* gene, 2 novel non-synonymous and one synonymous SNPs segregated in Alpine ibex. In conclusion, 22 non-synonymous and 18 synonymous SNPs were identified within *DGAT1* and the *casein* genes in the 7 goat breeds investigated. Among all SNPs, 11 non-synonymous and 11 synonymous were novel. The high variability in coding regions affects the protein quality and could eventually also influence the yield of milk proteins and milk fat. To identify phenotypic effects of these novel SNPs, further research is required. Furthermore, we would like to emphasize that most of the novel SNPs were found only in the critically endangered Nubian ibex; highlighting the importance of preservation and studying rare and endangered breeds.

Key Words: goats and related species, genome sequencing, DNA sequencing, breed diversity, conservation

P403 Annotation of selection signatures in the bovine breed Asturiana de Valles. C. Paris[1], S. Boitard[1], B. Servin[1], N. Sevane[2], and S. Dunner*[2], *[1]GenPhySE, Université de Toulouse, INRA, INPT, INP-ENVT, Castanet Tolosan, France, [2]Facultad de Veterinaria. Universidad Complutense de Madrid, Madrid, Spain.*

Past events of positive selection leave characteristic signatures in the genetic diversity of a population, which can be detected by genome-wide scans based on present time molecular data. However, determining the adaptive trait or the onset and intensity of selection at a given locus is often difficult from such data. By providing direct access to the temporal evolution of allele frequencies, the analysis of genomic data extracted from gene banks might significantly improve our understanding of selection history in livestock species. The aim of this study is to evaluate whether the analysis of genomic samples collected at different times in the recent past allows (i) detecting recent selection events and (ii) annotating selection signatures found by classical approaches based on present time data only. To answer these questions, we considered the case study of the Spanish bovine breed Asturiana de los Valles (RAV), for which genotyping data was available for 137 animals with birth dates between 1980 and 2010. Fifteen additional RAV animals born in 2008–2013 were sequenced at ~8X coverage. These data were used to detect historical selection signatures in RAV using a classical statistic (nSL) based on a single sampling time. A new statistical approach allowing detection of selection from genomic time series was applied to the combined data set including 9 distinct generations. The time series approach combined with a statistical method allowing to detect clusters of small p-values pointed out several candidate regions with a clear shift in allele frequencies over the few last generations. The time series and nSL approaches detected 13 candidate regions under selection in RAV including genes related to carcass and meat traits (such as *MSTN*, *RBPMS2* or *OAZ2*), immunity (*GIMAP7, GIMAP4, GIMAP8)*, olfactory receptors (*OR2D2, OR2D3, OR10A4, 0R6A2)* and milk traits (*ARFIP1*). Thus, the combined time series and nSL approach are complementary and should be extended to other populations where temporal data can be extracted from gene banks. These results outline that gene banks represent a great resource for the understanding of breed history and the detection of relevant functional genes and variants.

Key Words: livestock, selection signatures, time series, candidate genes

P404 Abstract withdrawn

P405 Genome-wide association analyses of blood metabolites in crossbred beef cattle. J. Li*[1], E. Akanno[1], T. Valente[1,2], M. Abo-Ismail[1,3], B. Karisa[4], Z. Wang[1], and G. Plastow[1], *[1]Livestock Gentec, Dept. of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, Canada, [2]Ethology and Animal Ecology Research Group, Dept. of Animal Science, Sao Paulo State University, Jaboticabal, Brazil, [3]Department of Animal and Poultry Production, Damanhour University, Damanhour, Egypt, [4]Alberta Agriculture and Forestry, Edmonton, Canada.*

Metabolites are intermediate products of metabolic reactions in organisms that lie between the genomic level and the external phenotypes. They can explain phenotypic variation and reveal biological mechanisms underlying the associations between genomic and external phenotypes. Thus, the aim of this study was to identify genomic regions and candidate genes associated with 33 distinct metabolites in crossbred beef cattle. Blood metabolites were quantified using nuclear magnetic resonance for 501 Canadian crossbred beef cattle that were genotyped using Illumina BovineSNP50 Beadchip. After quality control, 45,266 SNPs were used for further analyses. Genomic breed com-

position predicted using Admixture software was included as covariate in a mixed model to account for population stratification and breed effects. The mixed model also included contemporary groups (defined as year of test, location and sex) as fixed effect and random animal effects. A single-step genomic BLUP approach was used to estimate the SNP solutions that calculated the variance for windows of 10 SNPs, and the regions that accounted for more than 1% of the additive genetic variance were used to map candidate genes. We identified 74 genomic regions that are associated with 16 metabolites whose heritability ranges from 0.01 to 0.43. The region that explained the largest additive genetic variance (4.99%) was associated with betaine on chromosome 21. Four regions were associated with 2 metabolites, such as the SNP window (7:13336301–13632174) containing 7 genes associated with both L-histidine and choline. Additionally, we identified several important candidate genes within the associated regions including choline kinase α (*CHKA*), ARP6 actin-related protein 6 homolog (*ACTR6*), acetyl-CoA carboxylase α (*ACACA*) and glutaminase (*GLS*). These genes encode biological functions directly related to the metabolites (choline, acetone, acetic acid and L-glutamine respectively). The results provide evidence for genetic components underlying the individual variation of 16 bovine blood metabolites explaining 2.49% to 13.44% of the additive genetic variance of these metabolites.

Key Words: beef cattle, single-step genomic BLUP, metabolic quantitative trait loci

P406 Relationship among the gene expression of SCD1, SCD5, SREBP1 and the fatty acid profile of Holstein-Friesian steers finished under different pasture systems. D. Gamarra[1], N. Aldai[2], A. Carvajal[3], M. M. de Pancorbo[1], M. Taniguchi*[4], and R. Morales[3], *[1]Biomics Research Group, Lascaray Research Center, UPV/EHU, Vitoria-Gasteiz, Álava, Spain, [2]2Lactiker Research Group, Lascaray Research Center, UPV/EHU, Vitoria-Gasteiz, Álava, Spain, [3]3Instituto de Investigaciones Agropecuarias, INIA Remehue, Osorno, Chile, [4]Animal Genome Unit, Institute of Livestock and Grassland Science (NARO), Tsukuba, Ibaraki, Japan.*

In southern Chile, beef is normally produced under grazing systems which is characterized by a low amount (2–3%) of fat and a nutritionally healthy fatty acid (FA) profile. Fat unsaturation can be regulated endogenously by stearoyl CoA desaturases (*SCD*1 and novel *SCD*5) which are controlled by transcription factor sterol regulatory element binding proteins (SREBP). Feeding systems could affect the expression of lipogenic genes conferring variations in FA profile. Therefore, our objective was to study gene expression of *SREBP1* and *SCD* isoforms, and to determine the correlations among these lipogenic genes and the backfat FA profile obtained from steers reared under different pastures. Holstein-Friesian steers (n = 42) were finished under different pasture treatments: T1) perennial ryegrass (One50AR1 + white clover ≥10%Bounty; n = 14); T2) perennial ryegrass high in sugar (AbermagicAR1; n = 13); T3) improved natural pasture (CTL; n = 15). Total RNA was extracted from backfat (RNeasy Lipid Kit) and mRNA of each gene was measured in duplex using TaqMan qPCR based on *18S rRNA* gene as an internal control. Backfat total FAs were analyzed by double GC/FID (100m SP2560 & SLB-IL111 columns) and desaturation indexes (DI) were calculated. ANOVA and Tukey were used to analyze gene expression data. Pearson correlations among DI and mRNA expressions were calculated for each gene (SPSS). Gene expression of *SCD*1, *SCD*5 and *SREBP*1 were higher in steers grazed in T2 compared with CTL pastures, whereas *SCD*1 expression was higher in steers grazed in T1 compared with CTL pastures. Linear regression between *SCD*5 and *SREBP*1 of steers grazed in T1 and CTL pastures was significant ($P < 0.05$), suggesting that pasture type could affect the regulation of *SCD*5 expression by *SREBP*1. In terms of correlations, *SCD*5 gene obtained the highest number of significant correlations with DIs (9*c*-16:1, 9*c*-17:1, 9*c*,11*t*-18:2 and total) compared with other genes. However, correlations were, in general, negative under T2 pasture for all genes studied while positive under T1 pasture. Overall, it is observed that pasture type affects differently to backfat desaturation.

Key Words: backfat, lipogenic enzymes, unsaturated fatty acids, ryegrass pasture, desaturation

P407 Influence of the genotype array density on copy number variants identification with PennCNV. A. M. Butty*[1], T. C. S. Chud[1], F. Miglior[1], F. S. Schenkel[1], P. Stothard[2], I. M. Häfliger[3], C. Drögemüller[3], and C. F. Baes[1,3], *[1]University of Guelph, Guelph, ON, Canada, [2]University of Alberta, Edmonton, AB, Canada, [3]University of Bern, Bern, BE, Switzerland.*

Structural variants, especially copy number variants (CNV), along with well-known SNP, have been strongly linked to multiple traits of interest, such as reproductive traits in cattle breeding. CNV identification methods were developed around the compromise existing between high true positive and low false positive discovery rates. Multiple algorithms were developed that relied on whole-genome sequences or genotype array data. CNV studies relying on genotype array information were often carried out with the software program PennCNV (Wang et al., 2007) that can be used with different SNP panels. The influence of the SNP density on the CNV false positive discovery rate for variants positioned on the latest bovine reference genome (ARS-UCD1.2) have not been quantified thus far. In this study, we compared the CNV identified with PennCNV on 70 Holstein animals genotyped with the Illumina BovineHD Beadchip and reduced SNP panels mimicking the GeneSeek® Genome Profiler Bovine 150K and the Illumina BovineSNP50 Beadchip. We described the change in number, type, and length of the identified CNV, and proposed a quantification of the change in the proportion of false positive variants detected. All values presented are within the 95% confidence interval produced through bootstrapping. We observed a reduction of 41% and 78% in the number of identified CNV, by decreasing the number of markers from 777K to 150K and 50K, respectively. CNV were identified with the reduced panels, that had no equivalent variants when applying PennCNV on the full SNP panel. The lack of intermediate genotyping information for these genomic segments in the reduced panel led to their improper identification while the information of the additional markers in the same genomic region in the full data set showed no CNV pattern. Thus, those CNV suggested false positive discovery rates of at least 22% and 12% for the 150K and the 50K variant panels, respectively. To conclude we showed that the number of SNP has an important impact on the false discovery rates of CNV.

Key Words: dairy cattle, SNP, variation

P408 Association of SNPs related to Johne's disease with Holstein bulls EBVs for milk ELISA test scores. S. Mallikarjunappa[1,2], F. S. Schenkel[1], L. F. Brito[1,3], N. Bissonnette[4], K. G. Meade[2], F. Miglior[1], J. Chesnais[5], M. Lohuis[5], and N. A. Karrow*[1], *[1]Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, [2]Teagasc Animal and Bioscience Research Department, Grange, Co. Meath, Ireland, [3]Department of Animal Sciences, Purdue University, West Lafayette, IN, USA, [4]Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, [5]The Semex Alliance, Guelph, ON, Canada.*

Johne's disease (JD) is a chronic intestinal inflammatory disease caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection in cattle. Since there are currently no vaccine and treatment options available to control JD, genetic selection may offer an alternative to enhance JD resistance. Numerous single nucleotide polymorphisms (SNPs) have been found to be associated with MAP infection status based on published genome-wide association and candidate gene studies. The objective of this study was to validate these JD SNPs by testing their association with Canadian Holstein bulls' estimated breeding values (EBVs) for milk ELISA test scores, an indirect indicator of MAP infection status in cattle. A total of 500 bulls were classified into high and low groups (n = 250 in each group) based on their EBVs for milk ELISA test scores. Both groups were then genotyped using a customized SNP

panel comprising 155 of the most prominent JD SNPs reported in the literature, including studies using phenotypes other than milk ELISA to define the case-control populations. General Quasi Likelihood Scoring (GQLS) genome-wide association analysis based on logistic regression was used to carry out the validation using SNP1101 software. Three SNPs, rs41810662, rs41617133 and rs110225854, located on *Bos taurus* autosome (BTA) 16, 23 and 26, respectively, were confirmed as significantly associated with Canadian Holstein bulls' EBVs for MAP infection status (FDR < 0.01). These findings highlight the need to further investigate the genomic regions harboring these SNPs. Since JD is a polygenic disease, where the accurate phenotyping of infected, resistant and disease-free animals remains difficult, the use of JD associated SNPs in a selective breeding program to increase resistance to MAP infection should be preceded by validation studies, preferably involving different dairy cattle populations.

Key Words: cattle and related species, genome-wide association, genotyping, genetic marker, genetic improvement

P409 A transcriptional landscape of long noncoding RNAs in tissues from cattle differing in metabolic efficiency. R. Weikard*[1], W. Nolte[1], H. M. Hammon[1], R. M. Brunner[1], E. Albrecht[1], A. Reverter[2], and C. Kuehn[1,3], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Commonwealth Scientific and Industrial Research Organisation (CSIRO) Agriculture and Food, Brisbane, Queensland, Australia, [3]University of Rostock, Faculty of Agricultural and Environmental Sciences, Rostock, Germany.*

Numerous studies have shown that long non-coding RNAs (lncRNAs) have an important function in the regulation of gene expression and epigenetic processes. Variation in their functional contribution is known to be associated with the pathogenesis of many diseases and specific differentiation and growth processes. Based on the functional relevance of lncRNAs in other species, we postulate that lncRNAs may also play a regulatory role for complex metabolic processes in cattle. In our study we analyzed the transcriptome of key tissues associated with nutrient conversion (liver, skeletal muscle, jejunum and rumen) of 24 cows and 24 bulls from a dairy × meat type cross population using whole transcriptome sequencing (RNA-seq) with a focus on lncRNAs. The selected animal groups differed in metabolic efficiency (residual feed intake in bulls, milk yield in cows) and showed a clear clustering based on plasma metabolic profiles. The transcripts were assembled using an annotation-guided approach enabling discovery of yet unannotated transcripts. The number of expressed genes across tissues ranged from 13,952 to 17,742 including 930 genes expressed tissue-specifically. In addition to known protein coding genes, a total of 3,784 lncRNAs were detected across gender and tissues using the bioinformatic lncRNA prediction tool FEELnc. The expression of 244 lncRNAs was tissue-specific. Across gender, animals differing in their metabolic efficiency displayed a total of 292 differentially expressed (DE) lncRNAs (liver: 206, jejunum: 51 and skeletal muscle: 53). Gender-specific differential expression analysis revealed the highest number of DE lncRNAs in the liver (170 in bulls and 405 in cows) compared with skeletal muscle (13 in bulls and 99 in cows), rumen (107 in bulls and one in cows) and jejunum (2 each in bulls and cows). This indicates that lncRNAs may play a prominent regulatory role in the liver. For further functional annotation of the DE lncRNAs, integrated co-expression network analysis with correlated genes in combination with knowledge based pathway analysis and GWAS were performed.

Key Words: transcriptome, RNA-seq, non-coding RNA, feed efficiency, cattle and related species

P410 Genomic selection through single-step genomic BLUP improves the accuracy of evaluation in Korean Hanwoo cattle. M. N. Park*[1], M. Alam[1], S. Kim[1], B. Park[1], S. H. Lee[2], and S. S. Lee[3], *[1]National Institute of Animal Science, Rural Development Administration, Cheonan, Republic of Korea, [2]Chungnam National University, Daejeon, Republic of Korea, [3]Hanwoo Genetic Improvement Center, NongHyup Agribusiness Group Inc, Seosan, Republic of Korea.*

Accuracy of genomic evaluation can limit successes of genomic selection (GS) in domesticated livestock species. Therefore, we investigated both pedigree BLUP (PBLUP) and genomic BLUP (GBLUP) methods and compared the accuracy of estimates among different Hanwoo bulls. A total of 9,952 Hanwoo (young, candidate and progeny-tested) bulls born between 1997 and 2018 under performance, and progeny test programs were studied. We studied body weight at 12 mo (BW12) and carcass traits (CWT, kg), backfat thickness (BFT), eye muscle area (EMA), and marbling score (MS) at 24 mo of age after slaughter. About 7,387 bulls were genotyped using Illumina 50K BeadChip Arrays. Multiple-trait animal models were used for estimation of breeding value solutions BLUPF90 software programs. Accuracy of traditional and genomic EBVs, i.e., (G)EBV, were calculated in both population ($r_{(G)EBV}$) and individual (r_{ACC}) level. Generally, r_{ACC} accuracies were larger than average individual r_{ACC} measures among animal groups. (G)EBV accuracy varied among various groups of animals. For all animals in the population, accuracy for all traits were somewhat similar and the lowest (: 0.41–0.45,: 0.40–0.44). Almost similar accuracy (r_{GEBV}: 0.43–0.52, r_{EBV}: 0.37–0.49) were also observed with candidate bulls. However, accuracy of evaluation was relatively higher than 70% in proven bulls with both GBLUP and PBLUP. Animals with both genotypes and phenotypes were relatively better estimated than those with phenotypes only. The young bulls, candidate bull, and proven bulls were 21–35.7%, 3.3- 9.3% and 2.8–6.1% higher than r_{EBV}. Baseline accuracy also greatly improved by GBLUP methods in the population. Average relatedness between animals also influenced accuracy measures. In overall, GBLUP yielded higher accuracy of evaluation than PBLUP. Our result indicated that GBLUP would be beneficial for all types of bulls and it would be good start point to ensure more effective sire evaluation.

Key Words: single-step, genomic BLUP, evaluation accuracy, genomic selection, Hanwoo cattle

P411 Long non-coding RNAs modulate metabolic efficiency in cattle and are linked to arginine biosynthesis. W. Nolte*[1], R. Weikard[1], R. M. Brunner[1], E. Albrecht[1], H. Hammon[1], A. Reverter[2], and C. Kühn[1,3], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Mecklenburg-Vorpommern, Germany, [2]Commonwealth Scientific and Industrial Research Organisation (CSIRO), Brisbane, Queensland, Australia, [3]University of Rostock, Rostock, Mecklenburg-Vorpommern, Germany.*

Long non-coding RNAs (lncRNA) modulate chromatin accessibility and gene expression and are suspected to modify resource efficiency and nutrient transformation in mammals. We aimed to identify and characterize the role of lncRNAs for metabolic processes in cattle. The transcriptome, genome and metabolome were analyzed for animals from both sexes of a F2-population (Holstein × Charolais). Based on residual feed intake (RFI) in bulls, milk yield (MY) in cows and intramuscular fat content in both genders, the animals were split into 2 groups: high and low metabolic efficiency. Tissue samples from liver, skeletal muscle, jejunum and rumen were collected and subjected to a ribosomal depletion protocol for transcriptome analysis. A tissue specific differential expression (DE) analysis yielded most numerous differences in liver (1,286 DE genes, see poster Weikard et al.). Based on these genes, a pathway analysis showed significant enrichment for metabolic processes and arginine biosynthesis. With a prioritised set of genes (DE, tissue specific, QTL (RFI, MY) containing, lncRNA), we used the regulatory impact factor and partial correlation and information theory analysis to build a network for the high and for the low metabolic efficiency group with 1,923 and 2,410 nodes each. MSTRG.14864 is one of the 2 novel liver specific lncRNAs that are present in both networks and have a minimum connectivity of 10. This lncRNA is linked to different biological pathways in the 2 metabolic types. In highly efficient animals, a pathway analysis from genes correlated with this element showed enrichment for arginine biosynthesis, while connections from the low efficiency group network were enriched for collagen fibril organiza-

tion, extra cellular matrix structural constituent and cellular response to amino acid stimulus. We postulate that MSTRG.14864 modulates biosynthesis of arginine, which is known to increase muscle gain and reduce body fat. This assumption was supported by significant group differences in plasma metabolites related to this pathway.

Key Words: cattle and related species, Functional Annotation of Animal Genomes (FAANG), RNA-seq, metabolism, genetic improvement

P412 A case of pulmonary hypoplasia and anasarca syndrome in Holstein cattle due to trisomy of chromosome 19. I. M. Häfliger*[1], J. S. Agerholm[2], and C. Drögemüller[1], *[1]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark.*

Pulmonary hypoplasia and anasarca (PHA) syndrome is a known rare recessive disorder reported in several breeds of cattle and sheep. For Cika, Maine Anjou and Shorthorn cattle, DNA tests for PHA are available but the causative variants remain unpublished (OMIA 001562–9913). The herein presented single case was a female purebred Holstein calf sired by a progeny-tested AI sire. No further PHA-affected offspring of the sire and dam are known. WGS using Illumina HiSeq3000 was performed using DNA from sperm of the sire, blood of the dam and skeletal muscle of the affected offspring. The sequence reads were mapped to the latest reference genome (ARS1-UCD1.2) and SNVs were called. After filtering for disease-associated variants using 404 control genomes of various breeds, we found a single protein-changing variant in *SERPINB6* in accordance with the assumed recessive mode of inheritance. As the variant allele occurs quite frequently in heterozygous state in unrelated controls, we also looked for de novo variants private to the sequenced case. Thereby, we identified a single missense mutation in *RAB9A* that was present only in the genome of the PHA-affected calf. As for this gene only a role in pigmentation is known we have no further evidence that this variant might cause PHA. Interestingly, in the offspring we could also show that read depth was in average 0.5 times higher for bovine chromosome (BTA) 19 compared with the rest of the genome. This indicates an aneuploidy of BTA19 in the PHA affected calf while evidence for trisomy of BTA19 was absent in both parents. The source of the sequenced DNA samples was key for determining the parental origin of the extra chromosome. Due to the fact that the genomic data of the sire, which is representing his germline, does not show any sign of trisomy, it can be assumed that the aneuploidy was caused by a wrong disjunction in the gametogenesis in the dam. In conclusion, we showed that the presented PHA case is most likely due to a trisomy of BTA19 due to non-disjunction in the germline in the dam. This indicates that possible monogenic recessive disorders are genetically more heterogeneous than expected.

Key Words: cattle and related species, genome sequencing, reproduction, genetic disorder

P413 Hong Kong feral cattle: A distinct genetic pool? M. Barbato[1], M. Reichel[2], M. Passamonti[1], W. L. Low[3], L. Colli[1], R. Tearle*[3], J. Williams[3], and P. Ajmone-Marsan[1], *[1]Department of Animal Science, Food and Nutrition - DIANA, Università Cattolica del Sacro Cuore, Piacenza, Italy, [2]Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong, [3]Davies Research Centre, School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, Australia.*

In Hong Kong a feral cattle population of ~1,200 individuals of uncertain origin and diversity is present. This population shows heterogeneous morphology, both in body type (Taurine and Indicine features) and pigmentation. Local government veterinary officers hypothesize that this population is either a rare group of indigenous cattle related to Indonesian cattle, or a heterogeneous group of South China cattle derived from animals released into the wild by farmers. To shed light on the origin and diversity of Hong Kong feral (HKF) cattle we genotyped 21 animals with the Illumina BovineSNP50 v2 BeadChip. Of the ~50,000 SNPs successfully genotyped, a subset of 3,943 SNPs polymorphic in 2 wild *Bos* species, Gaur (*Bos gaurus*) and Banteng (*Bos javanicus*), was extracted for further analysis, to reduce ascertainment bias. We compared the genotypes of HKF to those of Gaur, Banteng and 379 animals from 18 cattle breeds: 10 European, 2 African Taurine, 2 African Sanga and 4 Asian Indicine. Preliminary results show HKF to be genetically homogeneous, distinct from Taurine, Indicine and Sanga cattle, and admixed with wild Asian cattle species. Further studies are underway to explore the origin of this unique population.

Key Words: cattle, feral, admixture

P414 Imputation of copy number variants from flanking single nucleotide polymorphism haplotypes in cattle. P. Rafter*[1,2], I. C. Gormley[2], A. C. Parnell[3], J. F. Kearney[4], and D. P. Berry[1], *[1]Teagasc, Fermoy, Cork, Ireland, [2]University College Dublin, Belfield, Dublin, Ireland, [3]Maynooth University, Maynooth, Kildare, Ireland, [4]Irish Cattle Breeding Federation, Bandon, Cork, Ireland.*

Copy number variants (CNVs) are a type of genomic variation formed by the deletion or duplication of a stretch of DNA. Most cattle genotypes are from medium density (MD) single nucleotide polymorphism (SNP) panels; the aim of this study was to quantify the ability of MD genotypes to identify CNVs in cattle. CNVs were called from high density (HD) (713,162 SNPs) and generated MD (45,677 SNPs) genotypes on the same 991 Holstein Friesian, 1,015 Charolais, and 1,394 Limousin bulls using available CNV calling algorithms. CNVs called from the HD and MD genotypes of the same animals were compared; for 84.8% of CNVs called from the HD genotypes, the same genomic region on the MD genotype had less than 3 SNPs. The software used to call the CNVs, PennCNV, requires a minimum of 3 SNPs per CNV, and therefore the MD panel could never have identified those CNVs. Given that the MD genotype panel could not identify most of the CNVs called from the HD genotypes, attempts were made to impute CNVs using flanking SNP haplotype structure of the HD genotypes. Imputation was carried out within breed using either FImpute or Beagle imputation software suites, with between 10 to 500 SNPs flanking each side of the midpoint of each CNV. A CNV was considered to be correctly imputed when the called copy number of the CNV matched the imputed copy number; the accuracy of imputation was calculated for each CNV as the proportion of animals in which the CNV was correctly imputed. The average accuracy of imputation for deletion CNVs across all breeds was 0.28, with a standard deviation of 0.29. Across all breeds the called copy number of duplication CNVs did not match the imputed copy number; therefore, the accuracy of imputation for duplication CNVs was zero. The absence of a CNV (i.e., the normal state) was imputed with an average accuracy of 0.98 and a standard deviation of 0.02. In conclusion, the MD genotype panel failed to identify the vast majority of CNVs detected with HD genotypes. Furthermore, CNVs in our cattle breeds could not be accurately predicted, at least from flanking SNP haplotypes from the BovineHD panel, using imputation algorithms routinely used in cattle.

Key Words: copy number variation, cattle and related species

P415 Choosing animals for a reference population that maximize both the captured variability and the probability of correct imputation. C. Diaz*[1], C. Meneses[1], M. J. Carabaño[1], M. A. Toro[2], and K. J. Abraham[3], *[1]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Depto. Mejora Genética Animal, Madrid, Spain, [2]Universidad Politécnica, UPM, Depto. de Produción Agraria, Madrid, Spain, [3]University of São Paulo, Ribeirão Preto SP, Brazil.*

We propose an algorithm for choosing animals to start a reference population by selecting mutually unrelated animals such that each animal has a substantial degree of relatedness to various other animals. In that way we capture the maximum haplotype diversity and we also

maximize the probability of correct imputation for those animals in the target population. Initially, the user defines 2 thresholds, a lower threshold, which is defined such that animals whose degree of relationship falls below the lower threshold are considered unrelated. The upper threshold is selected such that animals whose degree of relationship is above the upper threshold are sufficiently closely related to permit accurate imputation of genotypes. Thus, for each animal it is possible to define the number of close relatives as well as the degree of relatedness to animals that are not close relatives. In a population with a small effective population size, we expect that imputation may be facilitated by the existence of many relatives with a low-medium degree of relationship. This is the justification for using both criteria in the algorithm. The algorithm is stochastic. It starts by searching for an animal for which a combination of the number of close relatives and non-close relatives is large. Then the algorithm searches for a second animal for which the same combination is large but which is unrelated to the first animal, and so on. The algorithm terminates once a user-defined number of animals is chosen or when it is no longer possible to include additional animals distantly related to all preceding animals. The code provides multiple sets of animals and the best set can be selected according to some user dependent requirements. This algorithm has been applied to select animals to be genotyped as reference population in the Avileña-Negra Ibérica breed, a local Spanish beef cattle population. We have the coancestry matrix of 726 males and 27888 females, genetically alive and with EBVs for several characters, and from them we chose 500 individuals that fulfill the above criteria and that will constitute the starting reference population.

Key Words: imputation, reference population, genetic variability, bioinformatic tool, local breed

P416 Identification of expressional quantitative trait loci (eQTL) influencing gene expression after *Mycobacterium avium* ssp. *paratuberculosis* infection in Holstein Friesian cattle using a genome- and transcriptome-wide approach. M. Canive*[1], N. Fernandez-Jimenez[2], J. R. Bilbao[2], R. Casais[3], and M. Alonso-Hearn[1], *[1]NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Derio, Bizkaia, Spain, [2]University of the Basque Country, UPV/EHU, Leioa, Bizkaia, Spain, [3]SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Centro de Biotecnología, Deva, Asturias, Spain.*

Paratuberculosis (PTB), caused by infection with *Mycobacterium avium* subsp *paratuberculosis* (MAP), is a major endemic disease affecting global cattle production. Genome-wide association studies have led to the identification of PTB risk-associated genetic variants in the bovine genome. However, the disease causal variant is still unknown. Causal variants associated with complex diseases such as PTB are likely to be located in noncoding regulatory regions of genes and produce effects on gene expression. In the current study, (cis-) expression quantitative trait loci (eQTL) analysis in PTB relevant tissues such as peripheral blood (PB) and ileocecal valve (ICV) was used to identify single nucleotide polymorphisms significantly associated with changes in gene expression levels (eSNPs). For the analysis, PB samples collected from 15 cows from a Spanish herd with high prevalence of PTB were genotyped using the LD EUROG10K BeadChip (Illumina). PB and ICV samples were collected at the culling time and used for high-throughput transcriptome sequencing (RNA-Seq). Our data set including 52887 SNPs and 27570 transcripts per animal was analyzed using Matrix eQTL separately for PB and ICV. Predicted eSNPs had P-values lower than $1x10^{-6}$, false discovery rate ? 0.05 and minor allele frequency >5%. eQTL analysis predicted that 13 genes in PB and 88 genes in ICV were controlled by 13 and 151 eSNPs, respectively. Two of the identified eSNPs influence the expression of the major histocompatibility complex, class II, DQ β (BOLA-DQβ) gene in PB of PTB-infected cows. In ICV, some of the identified eSNPs influence the expression of the chromosome 24C18orf32 apoptosis inhibitor, AP-1 transcription factor (FOSB), acyl-CoA synthetase medium chain family member 1, and the peptidoglycan recognition protein 1 after MAP infection. PTB-associated eQTLs could be exploited via marker-assisted selection to breed for PTB resistance in cattle.

Key Words: cattle and related species, system genetics (eQTLs), SNP, genetic marker, genomic selection

P417 Abstract withdrawn

P419 Genome-wide association study for hair coat length in Brahman-Angus crossbred heifers. K. M. Sarlo Davila*[1], F. Rezende[1], S. Dikmen[2,1], and R. G. Mateescu[1], *[1]University of Florida, Gainesville, FL, USA, [2]Bursa Uludag University, Bursa, Turkey.*

Thermal stress in hot and humid conditions limits beef cattle production. Over 65% of the world's cattle (beef and dairy) reside in tropical or subtropical climates known for their hot and humid conditions. In the US alone, thermal stress results in a loss of almost $370 million each year due to reduced animal performance. A shorter, slicker hair coat is a key thermoregulative adaptation that allows cattle to lose heat through conductive, convective, and evaporative cooling at the hair-skin interface more efficiently. The objective of this study was to identify genetic variants associated with hair length of the undercoat and topcoat of crossbred cattle. Hair samples were collected from the shoulder, 4 inches down from the spine and halfway along the horizontal axis from 1,058 heifers in 2016 and 2017. Five long and 5 short hairs from each individual were measured for length using ImageJ software. The length of the topcoat and undercoat were evaluated for each individual by av-

eraging 5 long and 5 short hairs, respectively. DNA was extracted from blood samples and genotyped with the Bovine GGP F250 array. After quality control, 182,664 SNP were available for association analyses for both additive and non-additive effects performed in a 2-step mixed-model-based approach using the R package MixABEL. The function VIFGC implemented in the R package GenABEL was used to correct for a possible inflation of the test statistics. To correct for multiple tests, the false discovery rate was constrained to 0.2 and 46 and 65 significant SNP were identified for additive effects for undercoat and topcoat length, respectively. Only 2 of these SNP were significant for both phenotypes, one of which is located on BTA 4 close to the *PIP (*prolactin induced protein) gene. Functional mutations in the prolactin signaling pathway have been documented to impact both thermoregulation and hair morphology. For the undercoat length, 4 SNP were identified as having a recessive effect within a 100 kb region on BTA20. This region includes *IRX1*, a homeobox gene which regulates the differentiation of ectodermal appendages, including hair. These identified variants may improve the thermotolerance of cattle in hot, humid environments by contributing to shorter, slicker hair coats.

Key Words: thermoregulation, hair length

P420 A genomic landscape of mitochondrial DNA insertions (NUMTs) into the cattle genome. G. Schiavo, S. Bovo, A. Ribani, H. Kazemi, and L. Fontanesi*, *Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy.*

Mitochondrial DNA (mtDNA) insertions have been detected in the nuclear genome of many eukaryotes, including mammals. These mtDNA inserted fragments are called nuclear DNA sequences of mitochondrial origin or NUMTs. They are pseudogenes that derives from mtDNA regions (including both coding and non-coding regions) which were integrated into the nuclear genome through horizontal transfer mechanisms. *NUMTs* are considered sequence fossils of the nuclear genomes and have contributed to shape their genomic architecture and evolution. Some of these regions have high homology with the original mtDNA genome, as they derive from recent insertion events in terms of evolutionary time. A few studies have reported the presence of *NUMTs* in the genome of several livestock species. In this study we obtained a detailed genome map of *NUMT* regions in the *Bos taurus* genome and compared their distribution between the latest assembled versions, UMD3.1 and ARS-UCD1.2. The 2 genome versions were aligned with the reference linearized mtDNA sequence using LAST and BLASTN software. A total of 424 and 443 *NUMTs* (which covered the whole mtDNA sequence) were identified in the UMD3.1 and ARS-UCD1.2 versions, respectively. A few NUMTs were validated by amplifying and then sequencing the corresponding regions in animals of cosmopolitan and autochthonous European breeds. *NUMTs* had from 70% to 100% identity with the corresponding mtDNA genome region. A few *NUMT* positions were different in 2 genome versions. Some of these mtDNA integrations were complex and several rearrangements were observed. These results provide a first comparative genomic landscape of mtDNA insertions between the 2 cattle genome versions.

Key Words: cattle and related species, evolutionary genomics, data mining, biodiversity, heritage

P421 Inbreeding across generations after the crossbreeding in a composite breed in cattle. T. P. Paim*[1,2], E. H. A. Hay[3], C. Wilson[4], M. Thomas[5], L. A. Kuehn[6], S. R. Paiva[7], C. McManus[2], and H. Blackburn[4], *[1]Instituto Federal de Educação, Ciência e Tecnologia Goiano, Iporá, GO, Brazil, [2]Universidade de Brasília, Brasília, DF, Brazil, [3]Fort Keogh Livestock and Range Research Laboratory, Agricultural Research Service, USDA, Miles City, MT, USA, [4]National Center for Genetic Resources Preservation, Agricultural Research Service, USDA, Fort Collins, CO, USA, [5]Department of Animal Sciences, Colorado State University, Fort Collins, CO, USA, [6]US Meat Animal Research Center, Agricultural Research Service, USDA, Clay Center, Nebraska, USA, [7]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.*

Taurine-indicine hybrids have been used to explore heterosis, complementarity, and productivity. Brangus is an example of a composite breed formed by Angus (62.5%) and Brahman (37.5%). We sampled foundational and subsequent generations (1 to 10) of Brangus to evaluate inbreeding and homozygosity dynamics. Genotypic data (777,962 SNP, BovineHD Beadchip) from 68 Brahman, 95 Angus and 59 Brangus born from 1970 to 2010 were evaluated. The number of equivalent generations of each Brangus was calculated using the optiSel package in R 3.4.2. Runs of homozygosity (ROH) analyses were conducted in SNP & Variation Suite® v8.7 (run length > 1,000 kb and > 70 SNP, up to 2 heterozygotes and 5 missing genotypes, gap < 50 kb and SNP density > 1 SNP per 50 kb). Genomic inbreeding coefficient based on ROH (F_{ROH}) was calculated by the sum of the length of ROH regions divided by the whole genome length. Brangus cattle had close to 6% inbreeding and the first generation had close to 2% of inbreeding. The F_{ROH} increased ≈1% per generation in Brangus (F_{ROH} = 0.0196+0.0097*generation, *Radj2* = 0.19, *p*-value = 0.0004). This corresponds to an effective population size (Ne) of 51.55 (Ne = 1⁄2?F). FAO Guidelines for in vivo conservation of animal genetic resources suggest, the inbreeding rate per generation should not exceed 1 percent (equal to Ne = 50). Inbreeding estimates based on pedigree and F_{ROH} were similar at the whole genome and individual chromosome (1, 4, 7, 10, 13, 15, 26 and 29) levels. Regressing F_{ROH} rate on generation yielded a positive and significant trend for chromosomes 4, 10, 13, 15, 23, 26 and 29. But the remaining chromosomes did not show a similar trend. The lack of uniformity in inbreeding among chromosomes suggests increased homozygosity among specific regions of a chromosome may be the result of selection for traits of interest. Brangus breeders have emphasized selection for traits more closely associated with Angus, inspection of some of the significant chromosomal regions indicates increased Angus prevalence (>62.5%). These results suggest as new hybrids are formed various forces of natural or artificial selection influence homozygosity and accumulated inbreeding levels.

Key Words: population genomics, homozygosity, heterosis, hybridization, population structure

P422 Identifying genomics regions affecting meat tenderness in *Longissimus Dorsi* muscle in crossbred beef cattle. S. Nayeri*[1], G. Plastow[2], G. Vander Voort[3], F. Schenkel[1], M. M. Magalhães[1,4], L. Gãlvao de Albuquerque[4], M. McMorris[3], R. Ventura[5], M. Miller[2,6], and A. Cánovas[1], *[1]Centre for Genetic Improvement of Livestock, Department of Animal Bioscience, University of Guelph, Guelph, ON, Canada, [2]Department of Agricultural, Food and Nutritional Science, University of ALberta, Edmonton, AB, Canada, [3]AgSights, Elora, ON, Canada, [4]Department of Animal Science, School of Agricultural and Veterinarian Sciences, São Paulo State University (Unesp), Jaboticabal, São Paulo, Brazil, [5]Department of Animal Nutrition and Production, FMVZ-USP, Pirassununga, São Paulo, Brazil, [6]Neogen Corporation, Enterprise Square, Edmonton, AB, Canada.*

Meat quality and consistency in beef cattle are important factors for consumer satisfaction. Meat tenderness has a moderate to high heritability (ranging from 0.12 to 0.53) and can be improved through genetic selection within and across breeds. There is a positive correlation between Warner-Bratzler shear force values and sensory assessment of beef tenderness. Genome-wide association studies (GWAS) for shear force and sensory traits have revealed several well-supported associations. However, there has been few genomic studies of meat tenderness in multi-breed beef cattle. This study is aimed at identifying genomic regions affecting tenderness of *Longissimus dorsi* muscle at 7, 14 and 21-d post-mortem (LM7, LM14, and LM21, respectively) in beef cattle. A total number of 1,740 purebred and crossbred bulls were genotyped, using Illumina BovineSNP50 Beadchip. These animals were mainly crossbreds of 6 major breeds (Angus, Charolais, Simmental, Piedmontese, Limousin, and Gelbvieh). Three generations of ancestors were traced back, totaling 2,284 individuals in the pedigree used. Qual-

ity control was performed and 37,239 polymorphic markers were used for a GWAS in a single-step genomic BLUP analysis using Blupf90 software. Preliminary results show a significant region on chromosome 29 (44 Mb) with a very strong signal (log10(pvalue) = 7.9) for LM7, LM14. This region and the associated gene (*Calpain 1)* were reported to be previously associated with tenderness, evaluated by Warner-Bratzler shear force. The GWAS result also pointed to an additional region on chromosome 17 for LM7 explaining a high proportion of genetic variance for this trait, which was not reported in previous studies. These results will be combined with a GWAS using a large population of approximately 2000 animals and transcriptomic analysis from muscle (n = 100) and fat (n = 100) tissues of animals divergent for shear force in purebred (Angus and Nellore) and crossbred beef cattle. The final findings of this research may increase the efficiency of genetic selection for tenderness and other meat quality-related traits in beef cattle.

Key Words: GWAS, meat tenderness, shear force, blupf90

P423 Abstract withdrawn

P424 Parent- and offspring-specific transmission ratio distortion on the cattle sex chromosome. S. Id-Lahoucine*[1,2], J. Casellas[2], A. Suárez-Vega[1,3], P. Fonseca[1], F. Schenkel[1], S. Miller[1,4], M. Sargolzaei[1,5], M. Lohuis[3], J. Medrano[6], and A. Cánovas[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Universitat Autònoma de Barcelona, Barcelona, Spain, [3]The Semex Alliance, Guelph, ON, Canada, [4]Angus Genetics Inc, St. Joseph, MO, USA, [5]Select Sires, Inc, Plain City, OH, USA, [6]University of California-Davis, Davis, CA, USA.*

Significant deviation from Mendelian inheritance expectations (transmission ratio distortion, TRD) has been linked to a broad range of biological causes (e.g., germline selection, gametic competition, meiotic drive, imprinting, diseases, inbreeding, embryonic lethality, postnatal viability). Despite the recent interest in targeting the whole genome for finding regions with TRD or, in particular, for the absence of homozygous haplotypes in livestock species, TRD on sex chromosomes has not yet received attention. In this study, TRD regions were scanned using an allelic model including parent- (sire and dam) and offspring-specific (male and female) TRD parameters. The data analyzed comprised 436,651 genotyped Holstein cattle with 3,832 heterosomal SNPs (X-chromosome) and 400 pseudoautosomal SNPs spanning the length of the sex chromosomes. Results on the pseudoautosomal region showed 2 main TRD patterns. First, an opposite sire-TRD pattern between male- and female-offspring was identified displaying decisive evidence (Bayes factor $\geq$ 100) on 149 SNPs. This pattern displayed strong sire-TRD at the beginning of the pseudoautosomal region and gradually reduced along this region until being null at the extreme of the chromosomes. This finding revealed unique SNPs linked to a specific-sex (Y- or X-) chromosome and with a gradual decline of this linkage suggesting a consistent level of recombination across the short pseudoautosomal part of cattle genome. Second, specific sex-offspring sire-TRD were observed on 15 SNPs, being distorted only via male (8 SNPs) or females (7 SNPs). On the other hand, only 13 SNPs were identified with dam-TRD on the heterosomal part of X-chromosome. This latter result suggests a low prevalence of TRD across the heterosomal part of the X-chromosome in comparison to pseudoautosomal regions and autosomal chromosomes. The discovered genomic regions will be further investigated, as they could potentially be linked to specific biological mechanisms affecting fertility with interesting implications in the cattle industry.

Key Words: transmission ratio distortion, sex-offspring, sex chromosomes, recombination, cattle

P425 A comparative profile of the mRNA and microRNA transcriptome in immature and mature bovine testes. X. Fang[1], Z. Zhao*[2,1], L. Qin[3], Y. Zhao[3], A. Elke[4], S. Maak[4], and R. Yang[1], *[1]Jilin University, Changchun, Jilin, China, [2]Guangdong Ocean University, Zhanjiang, Guangdong, China, [3]Jilin Academy of Agricultural Sciences, Changchun, Jilin, China, [4]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Fertility is one of the most important economical traits in livestock production. Bull requires the continuous production of high quality spermatozoa in abundance. Spermatogenesis is a complex process, and sperm vitality and quality may affect by multiple factors including genetics, feed-related factors, fiber and energy intake, dietary fats, as well as seasonal and regional effects. To screening potential regulators of fecundity, the bovine testes of immature and mature in Chinese Red Steppes were performed by a genome-wide analysis of mRNAs and miRNAs. Compared with born testicular tissue, 6051 upregulated genes and 7104 downregulated genes in adult bovine testicular tissue were identified as differentially expressed genes (DEGs) ($\log2^{FC} > 1$ or < -1, FDR <0.05). The DEGs were significantly enriched in 808 GO terms ($P < 0.05$), some of which are involved in sperm biological activity, including male gonad development, male genitalia development, spermatogenesis, sperm motility, spermatid development, sperm chromatin condensation, *etc*. Moreover, DEGs were also significantly enriched in 105 KEGG pathways ($P < 0.05$), such as focal adhesion, insulin secretion, cGMP-PKG signaling pathway and calcium signaling pathway, *etc*. To explore the miRNA-regulated gene expression network integrated analysis was used between DEGs and differentially expressed miRNAs (DERs). Correlation prediction and analysis found that 896 differentially expressed target genes, such as USP42, AXL, GLI1, PRM2 and SERPINA5, were negatively correlated with the expression levels of 31 differentially expressed bovine miRNAs. Our results identified novel

candidate DEGs and DERs correlated with male reproduction, which will be valuable for future genetic and epigenetic studies of sperm development and maturity, as well as provided valuable insights into the molecular mechanisms of the male production in cattle.

Key Words: cattle, microRNA, RNA-seq, fertility

P426 The effect of acyl-CoA synthetase long chain family member 5 on triglyceride synthesis in bovine preadipocytes. X. Yu[1], Y. Liu[1], H. Xiao[1], X. Fang[1], M. Wang[1], Z. Zhao[2,1], and R. Yang*[1], *[1]Jilin University, Changchun, Jilin, China, [2]Guangdong Ocean University, Zhanjiang, Guangdong, China.*

The gustatory quality of beef mainly contingents on the deposition of fat and the most important is intramuscular fat deposition. Studies proved that intramuscular fat deposition mainly refers to triglyceride metabolism. It has been known that acyl-CoA synthetase long chain family member 5 (ACSL5) was a member of acyl-CoA synthetase long chain families, and it played a key role in fatty acid metabolism. In contrast to earlier findings, however, no evidence of the function of ACSL5 in the triglyceride metabolism was detected. In this study, we proved an association between the ACSL5 gene and triglycerides synthesis at the cellular level in cattle. Primary cells were isolated from bovine perirenal adipose tissue. The overexpression plasmid (pBI-ACSL5) and the interference plasmid(pGPU6-ACSL5) were constructed and transfected into bovine preadipocytes by electroporation. The expression levels of ACSL5 were detected by real-time quantitative PCR and Western blot. The triglyceride content was detected by a triglyceride kit. The results indicated that the expression levels of ACSL5 mRNA and protein in the pBI-ACSL5 transfected group were increased significantly compared with those in the control group. Furthermore, the pGPU6-ACSL5 transfected group were decreased significantly compared with those in the control group. A cell triglyceride test showed that overexpression of the ACSL5 could increase the content of cellular triglycerides, while in the silencing group could decrease. These results all accorded with our earlier observations, which showed that the ACSL5 expression could impact triglycerides metabolism. This research could provide both theoretical and technical support in the study of the function of candidate genes for fat metabolism in beef cattle.

Key Words: ACSL5 gene, bovine preadipocytes, triglycerides, fat metabolism pathway

P427 Alternative measures of robustness for body weight in Nellore cattle. D. C. B. Scalez*[1,2], A. Reverter[2], L. H. S. Iung[1], L. R. Porto-Neto[2], L. G. Albuquerque[1,3], and R. Carvalheiro[1,3], *[1]Department of Animal Science, School of Agricultural and Veterinarian Sciences, Sao Paulo State University (UNESP), Jaboticabal, SP, Brazil, [2]CSIRO Agriculture & Food, Brisbane, QLD 4067, Australia, [3]National Council for Scientific and Technological Development, Braslia, DF, Brazil.*

Each animal is exposed to a diversity of environmental challenges during its lifetime. For commercial and animal welfare perspectives, it is desirable to develop the capacity to cope with these challenges and to recover quickly when changes occur. The capacity of the animal to be minimally affected by disturbances or to rapidly return to the state pertained before exposure to a disturbance is defined as resilience. Similarly, the combination of high production potential with resilience to external stressors is called robustness. The aim of this study was to evaluate alternative measures of robustness, as measured by coefficient of variation (CV), skewness (Sk) and kurtosis (Ku), for yearling weight (YW) in Nellore cattle based on within-family data. Data for YW from 132,924 cattle born between 1986 and 2016 and sired by 368 Nellore bulls were extracted from the Alliance Nellore database. The number of progeny per sire averaged 361 and ranged from 20 to 12,531. Sires were genotyped with the Illumina® BovineHD chip. Cattle were raised on pasture in herds from Brazil and Paraguay, and YW was measured at an average of 533 d of age, with a minimum of 338 d and a maximum of 627 d. The alternative measures of robustness were computed based on within-sire progeny groups (PGs) comprised of at least 10 progeny (maximum of 30) from the same sex, farm and year of birth. Similarly the 3-way combination of sex, farm and year was used to create contemporary groups (CGs) to be fitted as fixed effect in the model for the estimation of genetic parameters. A multiple trait single-step GBLUP model was adopted, considering the genotypes of the sires and the pedigree information relating a given PG with its sire. Preliminary results indicate a very high estimate of heritability (h^2) for YW (i.e., h^2 ~90%) likely due to using PG averages rather than individual observations. On the other hand, preliminary estimates of h^2 for the alternative measures of robustness are in the low range (i.e., h^2 ~5%). Further research is warranted to assess the impact of group sizes in forming PGs as well as competing parameterization such as re-defining CGs or treating CG as random effects.

Key Words: animal breeding, beef cattle, genomic selection, heritability

P428 An update from the International Sheep Genomics Consortium. S. Clarke*[1], S. McWilliam[2], H. Daetwyler[3], A. McCulloch[1], and R. Brauning[1], *[1]AgResearch, New Zealand, [2]CSIRO, Australia, [3]Agriculture Victoria, Australia.*

The long-term goals of the International Sheep Genomics Consortium (ISGC) to develop underpinning resources for the sheep research community has resulted in continued improvement of the sheep genome assembly and development of low, medium and high-density Illumina SNP chips. The ISGC members have continued to make available whole genome sequence data to the community that has been captured via the Sheep Genomes Database, an initiative of the ISGC that extends the consortiums recent achievements. SheepGenomesDB is an electronic warehouse containing sequence variants called from the expanding collection of sheep genomes. Through the application of a single harmonised pipeline for read QC, mapping, variant detection and annotation, SheepGenomesDB makes available variant collections derived in a standardised manner. Run 2 has seen ~1000 animals analyzed with variant collections positioned on the OAR V3.1. The consortium is now in the process of Run 3 that will include an additional ~300 animals utilizing Rambouillet v1 genome assembly with the aim of providing users with tools to obtain variants defined by chromosomal location, SNP annotation results or via animals and breeds of interest. An update of the ISGC's activities will be presented.

Key Words: sheep, SNP, genome database genome assembly

P429 Survey of allele-specific expression in bovine muscle. G. Guillocheau and D. Rocha*, *INRA, GABI, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France.*

Allelic imbalance is a common phenomenon in mammals that plays an important role in gene regulation. An Allele Specific Expression (ASE) approach can be used to detect variants with a *cis*-regulatory effect on gene expression. In cattle, this type of study has only been done once in Holstein. In our study we performed a genome-wide analysis of ASE in 19 Limousine muscle samples. We identified 5,658 ASE SNPs (Single Nucleotide Polymorphisms showing allele specific expression) in 13% of genes with detectable expression in the *Longissimus thoraci* muscle. Interestingly we found allelic imbalance in *AOX1*, *PALLD* and *CAST* genes. We also found 2,107 ASE SNPs located within genomic regions associated with meat or carcass traits. To identify causative *cis*-regulatory variants explaining ASE we searched for SNPs altering binding sites of transcription factors or microRNAs. We identified one SNP in the 3′UTR region of *PRNP* that could be a causal regulatory variant modifying binding sites of several miRNAs. We showed that ASE is frequent within our muscle samples. Our data could be used to elucidate the molecular mechanisms underlying gene expression imbalance.

Key Words: allele-specific expression, bioinformatics, genome regulation, muscle, single nucleotide polymorphism

P430 Large-scale mitogenome sequencing as a strategy to identify mitochondrial genetic disorders in domestic animals: Cattle mitogenome sequencing as a proof of the concept. D. Novosel[1], V. Cubric-Curik[1], V. Brajkovic[1], M. Simcic[2], P. Dovc[2], and I. Curik*[1], *[1]University of Zagreb, Faculty of Agriculture, Department of Animal Science, Zagreb, Croatia, [2]University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domžale, Slovenia.*

Mitochondrial DNA (mtDNA) is crucial for the aerobic respiration while among many functions it contribute to cellular activities such as are apoptosis, iron storage, calcium homeostasis, hormone signaling/synthesis and thermogenesis. Considerable efforts in human medicine resulted in identification of several disorders caused by dysfunctional mitochondrial mutations. In contrast to humans, no single mitochondrial disorder has been reported in cattle so far. Here we presented a case study where we performed large scale mitogenome sequencing in cattle (>800 individuals, > 120 breeds), together with screening for the human known detrimental mitogenome mutations. Among several mildly detrimental mutations located in the NADH dehydrogenase gene complex, we have identified 2 mutations, one in Cika (T10663C in ND4L gene) and the other in Croatian Buša (C4171A in ND1 gene) cattle, each known to cause Leber's Hereditary Optic Neuropathy (LHON) in humans. After anamnesis and clinical inspection of some individuals within "detrimental mitolineage" of Cika cattle, one cow has clear symptoms of exophthalmia, a well-known consequence of the same mutation in humans. Here, we further discuss algorithmic (Magellan software) and molecular strategies that can increase cost-efficiency in identifying harmful mitogenome mutations in domestic animal populations. Such efforts will enable establishment of animal models that will contribute to the better understanding of mitogenome disorders as well as to the breeding of healthier animals.

Key Words: cattle and related species, functional genomics, high-throughput sequencing, genetic disorder, animal health

P431 Inbreeding in Angus cattle via pedigree and runs of homozygosity. D. Lu*[1], M. Sargolzaei[2,3], D. Moser[1], and S. Miller[1], *[1]Angus Genetics Inc., American Angus Association, St. Joseph, MO, USA, [2]Select Sires Inc, Plain City, OH, USA, [3]Dept. of Pathobiology, University of Guelph, Guelph, ON, Canada.*

Understanding and monitoring inbreeding is important. Inbreeding is traditionally estimated using pedigree relationships, which can face limited pedigree depth as well as potential errors. Alternatively, inbreeding can be estimated using runs of homozygosity (ROH) determined from moderate density Single Nucleotide Polymorphism (SNP) genotypes. The ROH is expressed as proportion of the genome covered by segments of consecutive homozygous SNP. The exercise reported herein was performed on 567,164 Angus cattle born between 1969 and 2018 genotyped on one of 5 commercial SNP chips, 2 of which were low density (<50K) and imputed to a common 50K. Common SNP (44,818) reported on 29 autosomes from the 5 panels were used in this analysis. Pedigree-based inbreeding (F_p) was calculated in a pedigree of 1,061,099 individuals, with maximum pedigree depth of 10 generations. The ROH was determined with 3 minimum window sizes (15, 30, and 45 SNP) using snp1101 software, and ROH-based inbreeding (F_{ROH}) was reported as proportion of total number of SNP in ROH segments to total number of SNP used in the analysis. Average F_p was compared with average F_{ROH} for the same individuals born between 2000 and 2018. Average F_p increased from 2000 (0.023 ± 0.021) to 2018 with the highest at 0.044 ± 0.028 in 2011. Average F_{ROH} calculated from the 3 window sizes were all much higher than F_p, and revealed a clearer increasing trend. For minimum window sizes 15, 30, and 45 SNP, the lowest and highest average F_{ROH} were 0.168 ± 0.023 and 0.193 ± 0.028, 0.125 ± 0.024 and 0.149 ± 0.029, 0.105 ± 0.024 and 0.129 ± 0.029, respectively. Our analyses of effective population size and linkage disequilibrium of this data set revealed that F_{ROH} estimated using window size of 45 SNP was comparable to the F_p. The F_{ROH} determined with larger SNP is indicative of more recent inbreeding, where smaller windows represent more ancestral inbreeding. The relatively high levels of recent inbreeding as determined through ROH might be closer to the true inbreeding than pedigree-based estimates, thus should be taken into account in breeding programs in efforts to improve cattle performance while reducing or preventing inbreeding depression.

Key Words: cattle, animal breeding, ROH, inbreeding, genetic improvement

P432 Digital PCR methods to detect and quantify ruminants in complex samples: a comparison with real-time qPCR. A. Sanz*, C. Cons, P. Zaragoza, and C. Rodellar, *Laboratorio de Genética Bioquímica-LAGENBIO (Universidad de Zaragoza), Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA), Instituto de Investigación Sanitaria de Aragón-IIS, Zaragoza, Spain.*

The detection of ruminant species in animal-derived materials is an issue of concern with disease transmission, most notably prions associated with transmissible spongiform encephalopathy from ruminant tissues. Quantitative real-time PCR (qPCR) have been widely implemented for ruminant species detection and quantification in different types of samples. Recently, digital PCR (dPCR) methods have been reported in this field, since provides absolute quantification, eliminates the need for a standard curve as in qPCR, improves accuracy for quantifying target DNA at low concentrations and have reported to be less sensitive to inhibitors compared with qPCR. In this work, we compare the multicopy qPCR-based protocols for detecting ruminant DNA species with digital PCR developed methods in complex biological samples. Our ruminant digital assay is based on the standard European procedure, and the identification of the specific ruminant species such bovine, caprine or ovine have been performed using other non-standardized assays. To ensure calibration of the method we used Certified Reference Materials (CRM) for ruminant DNA, or samples prepared from single species materials. Quantification by qPCR and dPCR was performed for the 4 targets using a 6-point 10-fold calibration curve and CRMs. Both methods showed a high degree of linearity and the concentrations of DNA correlated with the dilution factor used between the samples, although an underestimation of copy number was detected in the analyzed CRMs. A good linear dynamic range was observed by qPCR over 5 orders of magnitude, while by dPCR the estimated limit was lower. We also observed that the dPCR assay exhibited superior tolerance to inhibitors in inhibition-prone samples than qPCR. Data provided by this work are important to select a suitable analytical tool according to matrix complexity and experimental requirements.

Key Words: species detection, ruminant DNA, qPCR, digital PCR

P433 Epistatic interactions of scurs and polled in beef cattle. C. Ketel and M. Asai-Coakwell*, *Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.*

In beef cattle, scurs (loose horns) are an undesirable trait that can affect the financial outcome of the producer. Its complex inheritance has hindered eradication of the phenotype and identification of the mutation causing it. Scurs are inherited in a sex-influenced manner, with females requiring 2 scur alleles (ScSc), and males requiring one or 2 alleles (ScSc or Scsc) to display the phenotype. Additionally, scur presence is influenced by the polled mutation. Identification of a mutation resulting in polled cattle in European breeds, excluding Holstein-Friesians, known as the Celtic polled (P_C) was previously reported. We examined through PCR amplification, 3 embryo transfer families segregating for scurs, with a total of 11 scurred, 7 horned and 18 polled offspring. We found that all male offspring with scurs genotyped as heterozygous polled (Pp). An additional 58 male or female scurred animals were genotyped for P_C and all carried a horned allele (Pp). Thus, all 69 scurred animals in this study were heterozygous for the P_C allele. This provides evidence that scurs appear only when cattle are heterozygous polled. Furthermore, it has postulated that in both males and females, homozygosity for the polled mutation will mask the appearance of scurs. We examined 3 smooth polled male offspring from 3 scurred females (Pp, ScSc). These male offspring are obligate scur carriers, and all males

were homozygous polled (PP), further supporting this hypothesis. By defining the inheritance pattern of scurs and its interactions with the polled locus, identification of the gene and mutation responsible for the scurred phenotype may be possible.

Key Words: cattle, scurs, polled, epistasis, sex-influenced

P434 Genomic analysis in Bolivian highland Creole cattle revealed signatures selection that could be related to the Andean high-altitude adaptation. A. Rogberg-Muñoz[1,2], P. Álvarez Cecco[1], A. H. Falomir-Lockhart[1], A. Pereira Rico[3], A. Loza Vega[3], O. N. Arce Cabrera[4], M. E. Fernández[1], and G. Giovambattista*[1], *[1]Instituto de Genética Veterinaria (IGEVET, CONICET), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Provincia de Buenos Aires, Argentina, [2]Facultad de Agronomía, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina, [3]Facultad de Ciencias Veterinarias, Universidad Autónoma Gabriel René Moreno, Santa Cruz de la Sierra, Santa Cruz, Bolivia, [4]Facultad de Ciencias Agrarias y Veterinarias, Universidad Técnica de Oruro, Oruro, Provincia de Cercado, Bolivia.*

Latin-American Creole cattle are direct descendants of the bovines introduced into America by the Spanish and Portuguese conquerors in the 16th century. Currently, Creole cattle are spread all over American countries and became adapted to different environments, evolving under low levels of breeding management, exhibiting a high degree of resistance to tropical disease and a high level of fertility. The spread of biospheres includes for example tropical rainforest, subtropical dry forest and highland steeps. In Bolivia, some Creole populations had adapted to the Andean highland plains wich are around 4000 m altitude. Those populations had been under those conditions for centuries, adapting to such environments, which could have left signatures of selection within the actual genomes. The aim of this research was to study a population of Bolivian Creole cattle from Andean highland for selective footprints. For this purpose, 67 animals were genotyped using the 50K ArBos1 microarray and compared with lowland populations. After genotyping, quality control of raw data was performed, considering call rate (?97%) by sample and by SNP. *Shape-IT* software was used to determine the haplotypes in all the individuals and *rehh* package, included in R software, to compute the *i*HS index within population. The results showed 101 significant SNPs ($P \leq 0.01$) among the 29 autosomes. Chromosomes BTA2 and BTA14 showed the highest number of significant SNPs, while the highest associated peak was detected in BTA17 ($P \leq 1.10–8$). Those genomic regions could be related to the adaptation of Creole populations to the over 4,000 m Andean biosphere. Further analysis needs to be done to increase the understanding of cattle adaptation to high-altitude environments.

Key Words: altitude, adaptation, creole, cattle, SNP

P435 Gene-specific co-expression distribution anticipates gene functionality: Proof of concept with beef cattle RNA-sequence data. A. Reverter*[1], M. Naval-Sanchez[1], L. T. Nguyen[2], M. R. S. Fortes[3], and L. R. Porto-Neto[1], *[1]CSIRO Agriculture & Food, Brisbane, QLD, Australia, [2]QAAFI, Brisbane, QLD, Australia, [3]University of Queensland, Brisbane, QLD, Australia.*

In the quest to go beyond differentially expressed (DE) genes, gene co-expression studies are becoming increasingly popular. A gene co-expression study requires the computation of the co-expression correlation coefficient between a given gene and all the other genes under scrutiny, potentially numbered into the thousands. For each gene pair the correlation is computed across the samples using the normalize expression as the input data. We hypothesize that a random gene, irrelevant to the biological questions being examined, will present a null distribution with the majority of co-expression correlations around zero and only a few extremes, likely false positives, on either boundary, ± 1. On the other hand, a gene of relevance will reveal a distribution skewed toward the extremes reflecting the (higher than average number of) genes to which it significantly interacts. We construct a template of 8 possible distribution shapes, from extremely boring (flat) to undoubtedly puzzling (bimodal). We test our hypothesis with a real data set comprising 16,978 genes, 5 tissues and 60 samples concerning the onset of puberty in Brahman cattle. Using a naïve distance-based classifier, we assign each gene to one of the 8 distributions. We found an enrichment of DE genes among negatively skewed distributions (i.e., with an overabundance of positive co-expressions), and of transcription factors (TF) among bi-modal distributions. We reveal a set of DE TF genes with a distribution of correlation co-expression either highly skewed or extremely bimodal. Among these we highlight ELF5, ESRRG, NPM2, PITX1, and WNT4 for their reported role in beef cattle growth and fertility. While these preliminary results are encouraging, further research is warranted to fully assess the importance of the shape of distribution of correlation co-expression as a novel metric to prioritise genes of functional importance.

Key Words: computational pipeline, RNA-Seq, puberty

P436 Identifying putative genomic signatures of selection between the Brahman and Afrikaner cattle of South Africa. S. Mdyogolo*[1,2], M. Walugembe[3], J. Chinchilla-Vargas[3], M. F. Rothschild[3], and M. L. Makgahlela[1,2], *[1]Agricultural Research Council-Animal Production, Pretoria, South Africa, [2]University of the Free State, Bloemfontein, South Africa, [3]Iowa State University, Ames, IA, USA.*

Genomic variation due to natural or artificial selection in cattle breeds exists as selective sweeps and selective pressure that can be associated with functional and production traits. The Brahman and Afrikaner breeds display adaptive characteristics that include heat tolerance and tick resistance. The objective of this study was to identify putative signatures of selection between the Brahman and Afrikaner breeds. A total of 264 and 373 South African Brahman and Afrikaner cattle were genotyped on the Illumina 7K chip and Geneseek Genomic Profiler 150K, respectively. Markers with minor allele frequency <0.02, call rate <0.95 and individuals with call rate <0.90 were excluded. The Brahman 7K markers were imputed to 150K using influential South African individuals as reference animals. After quality control, a total of 83,306 autosomal markers common for the breeds were retained, as well as 239 and 330 Brahman and Afrikaner cattle, respectively. Runs of homozygosity per breed and pairwise F_{st} between breeds were determined to identify chromosomes with possible signatures of selection. Population stratification displayed ancestral admixture, however, the 2 breeds clustered separately. Runs of homozygosity exhibited by 30% of the animals per breed showed possible regions of selection on chromosomes 5, 6, 10, 14, 20 and 24 for Brahman and chromosomes 5, 7, 8, 11, 12, 14 and 15 for Afrikaner. One region of selection on chromosome 14 in Brahman (22.1 – 26.2Mb) overlapped with a similar region in Afrikaner (24.8 – 25.5Mb). This region consists of *TOX, NSMAF, TRNAC-GCA* and *LOC107133116* genes which are associated with feed efficiency and growth traits. Chromosomes 6 and 21 between the Brahman and Afrikaner had the highest average F_{st} (0.66) differences. Chromosomes 6 and 21 with regions 77 – 78Mb and 68.5 – 69.5Mb, respectively, consist of the *ADGRL3*, *LOC100140587, CEP170B* and *AKT1* genes which are associated with immune response and milk production traits. The results suggest that further investigation of these selective sweeps from these 2 breeds will be valuable.

Key Words: genetic variation, selection signatures, adaptation

P437 Multi-trait metanalysis to identify markers with pleiotropic effect in economically important traits in beef cattle. A. Cánovas*[1], P. A. S. Fonseca[1], M. Muniz[1,2], L. Albuquerque[2], M. R. S. Fortes[3], F. S. Schenkel[1], L. R. Porto-Neto[4], M. G. Thomas[5], and A. Reverter[4], *[1]University of Guelph, Department of Animal Biosciences, Centre for Genetic Improvement of Livestock, Guelph, ON, Canada, [2]São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil, [3]The University of Queensland, School of Chemistry and Molecular Biosciences,*

Brisbane, Queensland, Australia, [4]CSIRO Agriculture and Food, Queensland Bioscience Precinct, Brisbane, Queensland, Australia, [5]Colorado State University, Department of Animal Science, Fort Collins, CO, USA.

Pleiotropy drives genetic correlations between complex traits. The combination of SNP effects from genome-wide association studies (GWAS) may lead to the identification of key-regulators of pleiotropic effects. A total of 35,041 SNP effects from GWAS were combined for 16 traits related to meat quality, fatness, body composition and feed efficiency. Preliminary analyses were performed using 800 animals composed by different proportions of 6 beef breeds (Angus, Charolais, Simmental, Piedmontese, Limousin, Gelbvieh). A multi-trait statistic was calculated for each SNP following: $\chi^2 = t'_i V^{-1} t_i$; where t_i was the vector of signed t-values of SNP_i for the traits and t'_i was its transposed, V^{-1} was an inverse of the correlation matrix among the traits, and P-values obtained from a χ^2 distribution with 16 degrees of freedom. In total, 74 SNPs distributed across all the autosomes were significantly associated (P-value $<10^{-3}$) with pleiotropic effects. BTA6 (12) and BTA14 (6) were the chromosomes with the highest number of significant pleiotropic SNPs. Furthermore, 31 SNPs showed significant pleiotropic effect with 6 or more traits (absolute t-value >3). Interestingly, the most important SNP located in BTA6 (P-value $<10^{-5}$), close to a transcription factor, showed the highest number of related traits (13) with pleiotropic effect. In addition, clusters of QTLs were estimated using the correlation between the t-values for each pair of significant markers on BTA6 and BTA14. Two clusters of SNPs were identified on BTA6 (6 SNPs/each) and BTA14 (3 SNPs/each). Clear differences were observed between traits for the percentage of related SNPs within clusters on BTA14, e.g., lean carcass composition (cluster1 = 100%; cluster2 = 33%). Further research is warranted to validate these results and will be done using a larger population of ~15,000 animals including representation of several Taurine (*Bos taurus*) and Indicine (*Bos indicus*) breeds and over 30 traits related to meat quality, fatness, body composition, feed efficiency, reproduction and health. The results could help to avoid the unfavorable indirect genetic selection of genetically correlated traits in beef cattle.

Key Words: cattle and related species, genetic improvement, comparative genomics, functional genomics, complex trait

P438 Mammary RNA-seq data can better understand the genetic architecture of milk production traits in dairy cattle. W. Cai*[1,2], L. Fang[2], J. Cole[3], P. VanRaden[3], S. Zhang[2], and J. Song[1], *[1]University of Maryland, College Park, MD, USA, [2]China Agricultural University, Beijing, China, [3]USDA, Beltsville, MD, USA.*

Even with genome-wide association studies (GWAS) observed thousands of genomic variants associated with milk production traits in dairy cattle, the differentially expressed genes (DEGs) and long non-coding RNA (lncRNAs) in mammary gland across lactations on which those genomic variants act remain largely unknown. Here, we used multi-variance component approaches to test whether variants in DEGs regions and lncRNAs across lactations will explain more variance in milk production traits than others. To investigate the landscape and dynamic changes of genes and long non-coding RNA across lactations in dairy cattle, we reanalyzed 115 mammary RNA-seq samples, including 49 lactating and 66 nonlactating samples, which were collected from available data sets. Accounting for study effect, we identified 8,553 genes and 5,140 lncRNAs differentially expressed between nonlactating period and lactating period. Our population data set consisted of 3.2 million SNPs in 19,575 dairy cattle with records for 5 milk production trait phenotypes from USDA. We found that genomic variants in DEG regions captured the dynamic proportions of the variance ranged from 26.0% to 72.4% for different traits. Genomic variants in DE lncRNAs regions could explain 7.3% ~32.4% of the variance depends on different traits. Upregulated lncRNAs seemed more important than downregulated lncRNAs based on their SNPs explanation proportions. Then, we divided the DEGs into 8 groups based on their fold change values. We found that sequence variants in small fold change groups (−2~2) captured the greatest across all traits. The upregulated DEGs could explain a higher proportion of the variance than downregulated DEGs. We also found genomic variants in fold change >8 group regions, captured 16% of the variance for protein percentage trait. For per genomic variant, variants in fold change >8 group regions explain the highest proportion of variance for protein percentage, fat percentage, and milk traits. The proportion of variance captured by DEG regions with a fold change < −4 was very small. Overall, the results underscore that the use of mammary biological priors such as DEGs and lncRNAs enhances our insight into the genetic architecture underlying phenotypic diversity. Variants found in differentially expressed lncRNAs regions, explained considerable variation in milk production traits, should be further explored in detail.

Key Words: cattle, RNA-seq, DEGs, long non-coding RNA, SNPs explanations

P439 Investigation of genomic variation of coat color genes in Italian goat breeds. S. Frattini*[1], M. Cortellari[1], A. Talenti[2], A. Negro[1], M. Caprioglio[1], and P. Crepaldi[1], *[1]Department of Veterinary Medicine, University of Milan, Milan, Italy, [2]The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, United Kingdom.*

Coat color, a distinctive trait described in the breed standards, allows the identification of many native and cosmopolitan breeds.The aim of this work is to evaluate the presence of signals of selection in genes involved in pigmentation processes of *Capra hircus*.Starting from genotyping data (GoatSNP50 BeadChip) of 423 goats belonging to 25 Italian breeds/populations provided by the Italian Goat Consortium. For every breed, an Integrated Haplotype Score (iHS) analysis was performed. Animals were then classified in 5 groups depending on their coat colors patterns (solid eumelanic, solid pheomelanic, pied eumelanic, pied pheomelanic and white). A reduced data set consisting of 467 SNPs included in regions surrounding 40 candidate genes (+0.25 Mb) was generated. Using this data set, a canonical discriminant and allelic frequencies analyses on the 5 groups previously defined were performed.The iHS, calculated with the Selscan software, allowed the identification of 44 relevant signal (>0.6) in 17 out of 25 breeds. These signals of selection are about 4% of all the genomic regions investigated, and fall in 26 genes. The canonical analysis highlighted that genes involved in the dilution of the eumelanins (e.g., *OCA2* and *MYO5A*) and in the formation of the white patches (e.g., *DOCK7* and *PAX3*) have a major role in differentiating these groups of breeds. The analysis of the allele frequencies of the 467 SNP was focused on extreme frequencies (<0.2 or >0.8) and allowed the identification of 13 genes with at least 67% of extreme SNPs in 8 different breeds. Another noteworthy result is the high level of extreme SNPs observed for the *EDNRB* gene (white patches) only in the Maltese population and in the Vallesana breed which are characterized by a wide white extension in their coat.In conclusion, the results show that, despite the lack of selection signals within breed likely due to a reduced standardization of coat color in goat, canonical discriminant analysis highlight the relevance of regions around genes involved in withe patches and eumelanin dilution. However, the pigmentation control in the species is a complex system that deserves to be better studied from a phenotypical/genomic point of view.

Key Words: goat and related species, genetic identification, genotyping, coat color, breed standardization

P440 A complex structural variant at the *KIT* locus in Alpine cattle with a unique white spotting pattern. L. Küttel[1], A. Letko[1], I. Häfliger[1], S. Joller[1], G. Hirsbrunner[2], H. Signer-Hasler[3], G. Mészáros[4], J. Sölkner[4], C. Flury[3], and C. Drögemüller*[1], *[1]University of Bern, Vetsuisse Faculty, Institute of Genetics, Bern, Switzerland, [2]University of Bern, Vetsuisse Faculty, Clinic for Ruminants, Bern, Switzerland, [3]Bern University of Applied Sciences, School of Agricultural, Forest and Food Sciences HAFL, Zollikofen, Switzerland, [4]University of*

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The beauty of Austrian Pinzgauer cattle is based on the polarization of pigmented head and sides, and depigmented back, tail and lower side. This line-backed spotting pattern, sometimes referred to as finching is supposed to be inherited incompletely dominant in several more or less unrelated breeds including the Tux-Zillertaler, another local breed of Austria. A GWAS using 777k SNV genotypes of 27 white-spotted and 16 solid-colored Tux-Zillertaler cattle enabled us to identify an associated genome region on chromosome 6. Subsequent haplotype analysis localized a 122 kb-sized critical interval upstream *KIT*, a well-known depigmentation gene. All white-spotted Tux-Zillertaler shared a haplotype present in at least one copy, whereas all but one of 141 Pinzgauer cattle with available 777k SNV data were homozygous for the corresponding haplotype. The whole genome of a single Pinzgauer was sequenced, which revealed no evidence for the presence of associated private SNVs in the critical region after filtering against 400 control genomes. In contrast, a genome-wide scan for private CNVs revealed a 9.4 kb deletion and a 1.5 kb inversion within the mapped interval on BTA6, in addition to a 310 kb-sized duplication on BTA4. We confirmed and refined this complex structural variant mainly characterized by the fusion of the duplicated BTA4 segment into the deleted BTA6 region by visual inspection of paired-end reads and Sanger sequencing of PCR products flanking the breakpoint regions, which all mapped to interspersed non-homologous repeat elements. A diagnostic PCR was developed for straightforward genotyping of carriers for this structural variant that allowed ensuring that the most likely causative variant was present in all white-spotted Pinzgauer and Tux-Zillertaler cattle. Interestingly, we detected the variant allele also in all so far tested Slovenian Cika, British Gloucester and Spanish Berrenda en Negro cattle showing a similar spotting pattern. In addition, the variant occurs in some white-spotted animals of the Swiss Valais breeds Evolène and Eringer, which nicely confirms the reported historical relationship of these breeds with Austrian Tux-Zillertaler.

Key Words: cattle and related species, genome-wide association, genome sequencing, copy number variation (CNV), coat color

P441 Exploring the genetic resistance to *Haemonchus contortus* infection in Dohne Merino sheep using RNA-Seq. T. M. Ramantswana[1,2], D. P. Malatji[2], P. Soma[3], and F. C. Muchadeyi*[1], *[1]Agricultural Research Council, Biotechnology Platform, Onderstepoort, Pretoria, South Africa, [2]University of South Africa, Florida, Gauteng, South Africa, [3]Agricultural Research Council, Animal Production Institute, Irene, Pretoria, South Africa.*

Small ruminant farming plays an important role in South African agriculture and holds promise in mitigating challenges associated with unsustainable farming practices that contribute to global food insecurity. In developing countries, small ruminants make an important contribution to human livelihoods by providing food (i.e., animal fiber, meat and dairy products), income and other important non-market services. The South African Merino is the sheep breed with the highest wool production per head which are bred with the specific aim of maximizing wool and mutton produce, such as the Dohne Merino. The main aim of the study is to use RNA-Seq and differential gene expression profiling of the abomasum and small intestines of Dohne Merino sheep that are either resistant or susceptible to *H. contortus*to investigate genes and associated pathways that play a role in conferring either resistance or susceptibility to this pathogen in sheep. A total of 6 Merino sheep were slaughtered and fecal samples along with abomasum, jejunum, ileum and duodenumtissue samples were collected from each animal.Total RNA was extracted from the tissue samples of 3 resistant and 3 susceptible Dohne Merino sheep and preserved in RNAlater (Ambion) solution,Illumina HiSeq2500 was used to generate the average of 66,912,43 reads. The overall alignment rate of the quality-controlled reads to the reference genome (*ovis aries*4.0) was 90.88% using HISAT2 followed by transcript assembly and quantification using StringTie. Empirical Analysis of Digital Gene Expression Data in R will be used for differential gene expression analysis. For understanding high-level functions of genes, biological processes, cellular components, and molecular processes will be annotated based on the gene ontology (GO) categories using enzyme code and enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. The study will provide insight on the genes and associated pathways that play a role in conferring either resistance or susceptibility to this pathogen in sheep.

Key Words: sheep and related species, RNA-seq, gene expression

P442 Pre-selection of SNP markers to improve carcass quality prediction in Korean cattle (Hanwoo). S. de las Heras Saldana*[1], N. Moghaddar[1], D. Lim[2], S. H. Lee[3], and J. van der Werf[1], *[1]School of Environmental and Rural Science, University of New England, Armidale NSW, Australia, [2]Animal Genomics & Bioinformatics Division, National Institute of Animal Science, RDA, Jeonbuk, South Korea, [3]Division of Animal and Dairy Science, Chungnam National University, Deajeon, South Korea.*

In beef cattle, the improvement of carcass quality traits is important to meet market expectations, particularly in Korean Hanwoo cattle where high levels of intramuscular fat result in high prices. Genomic selection can be applied to select for carcass traits based on phenotypes measured outside the breeding nucleus. Improving the accuracy of genomic prediction is important and the integration of genomic and transcriptomic data could show promise in this field. The aim of this study was to assess the value of combining information from Genome-Wide Association Studies (GWAS), gene expression significantly associated with the trait (GSA) and expression QTL (eQTL) results in the pre-selection of SNPs used in the genomic prediction for carcass traits. We imputed low-density genotypes to medium density and finally to sequence level in genotypes on 13,717 Hanwoo steers. Four carcass traits corrected for fixed effects were used for the analysis (marbling score-MS, back fat thickness-BFT, and eye muscle area-EMA and carcass weight-CWT). From these, 3000 animals were used as a discovery data set to perform a GWAS. Using k-means clustering the rest of the animals were split based on their genetic relationship to perform a 10-fold cross-validation with around 1,072 animals in the validation data sets while around 9,645 animals were included in the training data set. From a time series experiment, we obtained RNA sequence data on 45 Hanwoo steers at 8, 12, 18, 24 and 30 mo of age to obtain eQTL and GSA for each carcass trait. Markers selected from GWAS, eQTL and GSA regions were evaluated for improving genomic prediction accuracy. This accuracy was evaluated for each trait using the genomic best unbiased prediction (GBLUP) fitting one genomic relationship matrix (GRM) from the standard 50k chip array or fitting 2 GRMs (one for 50k and one for selected SNPs). There was a 0.02, 0.03, 0.05 and 0.06 increase in accuracy for EMA, MS, BFT and CWT respectively, when selected SNPs were added to the standard 50k SNP array. The accuracies reached were 0.48, 0.51, 0.61 and 0.53 for MS, EMA, CWT and BFT. The integration of transcriptomics and genomics to improve prediction accuracy is therefore promising.

Key Words: genome-wide association, complex traits, gene expression, genomic prediction

P443 Effects of copy number variants on birth and weaning weights in a Nellore-Angus population. Y. Xing, A. Dabney, and C. Gill*, *Texas A&M University, College Station, TX, USA.*

Copy number variants (CNV) are insertions or deletions of 1 kb or larger with a variable number of copies that can affect phenotypic expression. We have previously identified CNV in the Nellore and Angus founders of a mapping population. In this study, we developed an approach to use SNP in high linkage disequilibrium (LD) with CNV to determine the association of CNV with growth traits by genome-wide association studies (GWAS), and developed approaches to incorporate CNV into genomic selection. Phenotypic data (ID, sex, year and sea-

son of birth, weaning age, birth weight, and weaning weight) for 995 animals from a Nellore-Angus crossbred beef cattle population were used in this study. Relative copy numbers were calculated for each CNV region (CNVR), and biallelic CNV were coded like SNP. Linkage disequilibrium between CNVR and SNP from additive, dominance and recessive SNP models were then calculated, and SNP having $r^2 \geq 0.8$ were selected for GWAS in GEMMA and for predictive modeling. Four predictive models including Bayesian sparse linear mixed model (BSLMM), multivariate linear regression model, regression tree, and random forest were fitted. The proportion of variance explained by each significant SNP tagging a CNVR was up to 2.2% for birth weight and weaning weight. The most accurate predictive models for these traits were random forest and BSLMM. The 3 SNP models yielded similar results, but the additive SNP model had slightly better performance than dominance and recessive models.

Key Words: copy number variants, SNP, genome-wide association study, genomic selection, predictive modeling

P444 Impact of a 1-Mb region on heifer pregnancy and rebreeding in *Bos indicus-Bos taurus* crossbred cattle. B. N. Engle, G. Wang, A. D. Herring, and C. A. Gill*, *Texas A&M University, College Station, TX, USA.*

Heifer fertility is of critical concern to beef cattle producers, as reproductive performance is a key driver of herd profitability. However, *Bos indicus* and *Bos indicus*-influenced females are later maturing and older at the onset of puberty than *Bos taurus* breeds, reducing heifer productivity in herds with these breeds. Therefore, the aim of this analysis was to identify differential patterns in the underlying genetic architecture influencing heifer pregnancy and rebreeding in *Bos indicus*-influenced cattle. A herd of *Bos indicus-Bos taurus* (Nellore and Angus) cows (n = 303) were assessed for this analysis. A GWAS was conducted using both 50k and HD genotypes via the univariate procedures of GEMMA, and a 1 Mb region on BTA 5 containing the lead SNP was identified from GWAS summary statistics. Using a sliding window, this region was found to explain the largest proportion of the model variance (PVE = 0.11), and haplotype analysis of this region revealed a significant relationship between haplotype breed-of-origin and heifer fertility ($P < 0.05$). At this location, individuals with 2 Nellore haplotypes significantly underperformed in heifer pregnancy and rebreeding rates in comparison to individuals with a Nellore and an Angus haplotype, and those with 2 Angus haplotypes. Within this region, the number of de novo SNP per kilobase identified from WGS is grossly inflated and differs between Nellore and Angus, suggesting structural variation between the breeds. Additionally, this window immediately proceeds a downstream region characterized by high levels of repeated elements and gaps in the assembly. There appears to be a mechanistic difference between *Bos indicus* and *Bos taurus* cattle at this region, driving the differential phenotypic expression of heifer pregnancy and rebreeding.

Key Words: *Bos indicus*, heifer, fertility, beef cattle

P445 Genome-wide association study shows sex-specific differences in the development of scurs in *Bos indicus* influenced beef cattle. G. Wang and C. Gill*, *Department of Animal Science, Texas A&M University, College Station, TX, USA.*

Scurs are corneous growths that range in size from buttons to large horn-like structures and develop in the same location as horns in cattle. In *Bos taurus* breeds, scurs typically are not firmly attached to the skull, but in *Bos indicus* influenced cattle, scurs can be more firmly attached to the skull and misclassified as horns. Development of scurs in *Bos taurus* cattle is due to a sex-influenced epistatic interaction between the *polled* (*P*) and *scurs* (*Sc*) loci. Scurs are only expressed in obligate heterozygotes for *polled* in *Bos taurus* breeds. Although the inheritance pattern for scurs is still debated, one model is that the *Sc* allele is dominant in males and recessive in females in *Bos taurus* cattle. The objective of this study was to characterize the inheritance pattern of scurs using a *Bos indicus* influenced (Nellore-Angus) mapping population and to detect genome-wide associations for scurs by modeling males and females separately. Nellore-Angus F_2 steers were harvested at 18 mo of age and any corneous growths were removed from the skull, bisected and photographed. Females from F_2, F_3, and F_4 generations were retained for breeding purposes and horned, scurred, or polled phenotypes scored on the live animal. Only animals that were genotyped as heterozygotes at the *polled* locus were included in this study and presence or absence of scurs was scored as a binary trait. Genome-wide associations were detected separately for males and females using the univariate procedures of GEMMA that fitted the genomic relationship matrix to account for genetic covariance among animals. The Benjamini and Hochberg false discovery rate was constrained to 0.05 to correct for multiple tests. Significant associations were found on BTA 2, 8, and 27 for steers, and on BTA 5 and 12 in females. Our results are suggestive of different contributors to the development of scurs in males and females.

Key Words: cattle, scur, GWAS

P446 Genome-wide association studies of digital cushion thickness in Holstein cattle. C. R. Stambuk[1], E. A. Staiger[1], B. J. Heins[2], and H. J. Huson*[1], *[1]Department of Animal Science, ornell University, Ithaca, NY, USA, [2]Department of Animal Science, University of Minnesota, St. Paul, MN, USA.*

The bovine digital cushion is a compression pad comprised of connective and adipose tissue between the distal phalanx and sole. Its thickness is a strong predictor of claw horn disruption lesions and lameness. Our objective was to identify QTL and candidate genes associated with digital cushion thickness (DCT) to improve biological understanding of its regulation and potential use for genetic selection to reduce lameness. To this end, 432 Holstein cows from 5 farms underwent digital sonographic examination of the digital cushion at the typical sole ulcer site for the right front and hind foot at <137 d prepartum and 86–127 DIM corresponding to periods where the digital cushion is thickest and thinnest. Genome-wide association studies using 579,743 SNPs compared results specific to hoof, digit, time point, and average measures. Cow and SNP data were retained for analysis given a call rate of >0.9, MAF >0.05, and HWE >0.0001. A genomic relationship matrix was used to correct for population structure using EMMAX. Six SNPs passing a Bonferroni corrected p-value of <0.05 highlighted QTL on BTA2, 8, and 9 using recessive mixed models with covariates of batch plate and parity group. Five of the SNPs were located in the *RUNX3, ZDHHC21, and AFDN* genes. The average heritability estimated from the GWAS was 0.42. Of the 35 candidate genes identified in the associated regions, 7 were highlighted as the most biologically relevant for potentially effecting DCT. These genes influence T cell differentiation, keratinocyte differentiation, epidermal differentiation, adherens junction maintenance, cholesterol homeostasis, magnesium ion transport, and bone morphogenetic protein (BMP) binding. In all, GWAS identified novel QTL and candidate genes potentially regulating DCT in dairy cows. These results provide a foundation for future functional studies and potential markers for genomic selection to reduce lameness.

Key Words: cattle, genome-wide association, animal health, animal welfare, genomic selection

P447 Epigenetic factors to face environmental variations in small ruminants. L. Denoyelle*[1,3], P. de Villemereuil[1], F. Boyer[1], M. Khelifi[1], C. Gaffe[1], F. Alberto[1], B. Benjelloun[2,1], and F. Pompanon[1], *[1]Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, Grenoble, France, [2]Institut National de la Recherche Agronomique Maroc (INRA-Maroc), Centre Régional de Beni Mellal, Beni Mellal, Morocco, [3]GenPhySE, INRA, INPT, ENVT, Université de Toulouse, Castanet-Tolosan, France.*

Species can optimize their fit to environmental conditions. Individuals can move to places with better conditions, or populations can adapt by natural selection of the best suited traits. Adaptation is a long-term evolutionary mechanism requiring several dozens to hundreds

generations to increase the frequency of the adapted alleles. Besides this genetic-based response short-term mechanisms based on phenotypic plasticity may mediate the response to environmental stresses. Phenotypic plasticity, which is the ability of a same genotype to express different phenotypes in different environments, may rely on the presence of epigenetic marks (such as DNA methylation) in the genomes, that regulate gene expression. This presentation focuses on the environment-related variation of DNA methylation patterns along the genome in goat (*Capra hircus*) and sheep (*Ovis aries*), living in field conditions in Morocco. For each species, we studied 2 groups of animals from environments with contrasted ambient temperatures (desert vs. Mediterranean climates). For this purpose, individuals methylomes were generated by sequencing of DNA methylated fragments, previously retrieved by immunoprecipitation. Then, we identified 5 and 2 differentially methylated genomic regions between the 2 groups for goat and sheep, respectively. We didn't find any homologous regions that are differentially methylated between the 2 species. Finally, we identified 4 genes for goats and 2 genes for sheeps that could be differentially expressed in relation to the variation of ambient temperatures.

Key Words: DNA methylation, *Ovis aries*, *Capra hircus*, local acclimatation

P448 Introgression with domestic goats has expanded the genetic variability of the Spanish ibex. T. Figueiredo-Cardoso[1], R. Tonda[2], M. G. Luigi-Sierra[1], A. Castelló[1,3], B. Cabrera[1,3], A. Noce[1], S. Beltrán[2], R. García-González[4], A. Fernández-Arias[5], J. Folch[6], A. Sánchez[1,3], A. Clop[1], and M. Amills*[1,3], *[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [2]Centre Nacional d'Anàlisi Genòmica-Centre for Genomic Regulation (CRG), Barcelona, Barcelona, Spain, [3]Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain, [4]Instituto Pirenaico de Ecología (IPE-CSIC), Spain, [5]Servicio de Investigación Agroalimentaria, Spain, [6]Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Zaragoza, Spain.*

The Spanish ibex (*Capra pyrenaica*) is a wild goat species distributed in the Iberian Peninsula. Based on phenotypic criteria, 4 subspecies have been defined: *C. p. hispanica* (CPH, south and east of the Iberian Peninsula), *C. p. victoriae* (CPV, center and northwest of the Iberian Peninsula), *C. p. lusitanica* (CPL, Galicia and north of Portugal) and *C. p. pyrenaica* (CPP, Pyrenees mountains). Hunting, epidemics and habitat loss caused the extinction of CPL (disappeared in the 19th century) and CPP (extinct in the year 2000) as well as severe population bottlenecks decreasing the diversity of CPV and CPH. By using a high throughput genotyping approach, we have demonstrated that interspecific hybridization with domestic goats has been an important source of novel variability for Spanish ibexes living in Tortosa-Beceite. Individual sequencing of one of the last CPP representatives (× 16.6 coverage) and Pool-sequencing (× 39 coverage) of 30 CPH and 23 CPV individuals revealed an extensive sharing of SNPs (96%) between the CPP individual and the extant CPV and CPH subspecies, thus suggesting that the extinction of CPP did not cause a major loss of diversity in *Capra pyrenaica*. Sequencing experiments also revealed that the genome of one of the last CPP representatives contains stop-gained mutations, with heterozygous genotypes, in the WASF2, RBM17 and SERPINB10 genes. The inactivation of WASF2 and RBM17 causes embryonic lethality, while SERPINB10 belongs to a family of serin proteases with key roles in immunity and other biological processes. Our results suggest that the dramatic reduction of the CPP population during the19th-20th centuries led to the progressive accumulation of mutations with harmful effects (genomic meltdown) that probably contributed to its extinction by limiting fitness and reproductive success.

Key Words: conservation, hybridization, goats and related species

P449 A *de novo* mutation causes polledness and a modified shape of the skull in Fleckvieh cattle. L. Gehrke[1], M. Upadhyay*[2], K. Heidrich[2], E. Kunz[2], D. Seichter[3], A. Graf[2], S. Krebs[2], A. Capitan[4], G. Thaller[1], and I. Medugorac[2], *[1]Christian-Albrechts-University Kiel, Kiel, Schleswig-Holstein, Germany, [2]Ludwig Maximillians University Munich, Munich, Bavaria, Germany, [3]Tierzuchtforschung e.V. München, Grub, Bavaria, Germany, [4]GABI, INRA, AgroParisTech, Paris, France.*

Genetic heterogeneity refers to the phenomenon where mutations in different loci (locus heterogeneity) or within the same locus (allelic heterogeneity) lead to a similar phenotype. In cattle, allelic heterogeneity is observed for the polled condition. In fact, at least 3 different alleles at the *polled* locus have been identified in cattle. In this study, we describe a case of a polled Fleckvieh bull born to horned parents that also implies locus heterogeneity of polledness. Genotyping of the case bull, its sire, grandsires and its polled and horned offspring was carried out using the bovine50K SNP array to determine the genetic basis of the de novo polledness condition. Additionally, Illumina paired-end and Oxford Nanopore sequencing technologies were employed to identify the exact candidate mutation for the polledness. Later, sanger sequencing technology was also used to validate the candidate mutation. The approach identified an 11-bp de novo deletion as the candidate mutation for the polled condition that first arose in a Fleckvieh bull and later passed onto its offspring. The 11-bp deletion event encompassed the second exon of the *ZEB2* gene and led to a translational frameshift. The frameshift caused a premature termination of translation, leading to a truncated protein. Compared with the wild type, the truncated *ZEB2* protein is predicted to be shortened by about 91%. Mutations in the *ZEB2* gene cause multiple congenital anomalies in humans as well as in cattle. However, apart from displaying polledness, a modified shape of the skull and presumably a short stature, the individuals carrying the 11-bp deletion in *ZEB2* gene did not display any other clinical symptoms. Because the *ZEB2* gene encodes a Smad Interacting Protein 1 (SIP1) that plays a vital role in epithelial-mesenchymal transition, it can be hypothesized that the truncated *ZEB2* protein might have lacked essential domains associated with the differentiation of horn buds. To conclude, the results of this study point toward a complex genetic pathway involved in bovine polledness that requires further investigation.

Key Words: cattle, polledness, ZEB2 gene, de novo deletion, frameshift

P450 Follows P231

P451 Follows P167

P454 A draft genome of Drung cattle (*Bos frontalis*) and its adaption to life at high altitude. Y. Chen*[1], X. Gao[1], J. Li[1], T. Zhang[1], W. Yang[2], W. G. Zhang[1], and B. Su[3], [1]*Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]The Co., Ltd. of 1 Gene Technology, Hangzhou, China, [3]Drung Cattle Nature Reserve, Gongshan,Yunnan China*.

Drung cattle (*Bos frontalis*), locally called "Panda cattle," is a unique semi-wild bovine specie that mainly inhabits the rain-forests of "Grand Canyon of the East" located in the Gaoligong Mountains and Drung River Basin of Yunnan Province, China. Here, we reported the ~2.74 Gb draft genome sequence of an adult male Drung cattle using Illumina, PacBio and 10X Genomics sequencing platforms. Compared with the previous gayal genome assembly, the scaffold N50 raised from 2.74 Mb to 4.08 Mb and the contig N50 boosted from 14.41 kb to 157.67 kb, respectively corresponding to ~2.6-fold and 10.9-fold improvement. Speciation time estimation showed that Drung cattle diverged ~1.20 Mya earlier than the clade of domestic cattle, indicating that *Bos frontalis* is clearly distinct from Bos taurus. This result strongly supports the contention that gayal is not the modern domestic cattle in lineage. To characterize the mechanisms underlying a chromosome fusion event leading to the formation of gayal chromosome 1, we analyzed bovine satellite I sequences and found concentrated tandem repeat regions located at the terminal end of 2 chromosomes in *Bos taurus*, corresponding to 29 satellite repeats at BTA2 and 57 repeats at BTA28, respectively. In our Drung cattle assembly, we identified one scaffold sequence (Fragscaffold60) having the presence of cattle satellite I with 28 tandem repeats, in which 17 genes were detected to be aligned to BTA2

and BTA28 with average identity 97.75%. Phylogenetic analysis of all 114 satellite sequences showed that satellite I repeats in Fragscaffold60 were clustering into an independent branch and formed a distinct satellite subfamily, indicating that the satellite sequences of the centromeres appear to be species-specific in genus *Bos*. Furthermore, 915 unique genes, 1102 orthologous genes under positive selection, and 228 expanded gene families were obtained that were involved in cardiac and vascular smooth muscle contraction or regulation of actin cytoskeleton pathways for adaption to life at high-altitude alpine ecosystem. In conclusion, our study provides valuable insights into the mechanisms of chromosome fusion and adaptation in the Drung cattle.

Key Words: Drung cattle, genome assembly, comparative genomics, adaptation, chromosomal fusion

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