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Runs of homozygosity provide a genome landscape picture of inbreeding and genetic history of European autochthonous and commercial pig breeds

G. Schiavo*, S. Bovo*, M. Muñoz†, A. Ribani*, E. Alves†, J. P. Araújo‡, R. Bozzi§, M. Čandek-Potokar¶, R. Charneca**, A. I. Fernandez†, M. Gallo††, F. García†, D. Karolyi‡‡, G. Kušec§§, J. M. Martins**, M.-J. Mercat¶¶, Y. Núñez†, R. Quintanilla***, Č. Radović†††, V. Razmaite‡‡‡, J. Riquet§§§, R. Savić¶¶¶, G. Usai****, V. J. Utzeri*, C. Zimmer††††, C. Ovilo† and L. Fontanesi*

*Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Viale Giuseppe Fanin 46, Bologna 40127, Italy. †Departamento Mejora Genética Animal, INIA, Crta. de la Coruña, km. 7,5, Madrid 28040, Spain. ‡Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Viana do Castelo, Escola Superior Agrária, Refóios do Lima, Ponte de Lima 4990-706, Portugal. §DAGRI - Animal Science Division, Università di Firenze, Via delle Cascine 5, Firenze 50144, Italy. ¶Kmetijski Inštitut Slovenije, Hacquetova 17, Ljubljana SI-1000, Slovenia. **Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Polo da Mitra, Apartado 94, Évora 7006-554, Portugal. ††Associazione Nazionale Allevatori Suini, Via Nizza 53, Rome 00198, Italy. ‡‡Department of Animal Science, Faculty of Agriculture, University of Zagreb, Svetošimunska c. 25, Zagreb 10000, Croatia. §§Faculty of Agrobiotechnical Sciences, University of Osijek, Vladimira Preloga 1, Osijek 31000, Croatia. ¶¶IFIP Institut du porc, La Motte au Vicomte, BP 35104, Le Rheu Cedex 35651, France. ***Programa de Genética y Mejora Animal, IRTA, Torre Marimon, Caldes de Montbui, Barcelona 08140, Spain. †††Department of Pig Breeding and Genetics, Institute for Animal Husbandry, Belgrade-Zemun 11080, Serbia. ‡‡‡Animal Science Institute, Lithuanian University of Health Sciences, Baisogala 82317, Lithuania. §§§GenPhySE, Université de Toulouse, INRA, Chemin de Borde-Rouge 24, Auzeville Tolosane, Castanet Tolosan 31326, France. ¶¶¶Faculty of Agriculture, University of Belgrade, Nemanjina 6, Belgrade-Zemun 11080, Serbia. ****Agris Sardegna, Loc. Bonassai, Sassari 07100, Italy. ††††Bäuerliche Erzeugergemeinschaft Schwäbisch Hall, Haller Str. 20, Wolpertshausen 74549, Germany.

Summary

ROHs are long stretches of DNA homozygous at each polymorphic position. The proportion of genome covered by ROHs and their length are indicators of the level and origin of inbreeding. Frequent common ROHs within the same population define ROH islands and indicate hotspots of selection. In this work, we investigated ROHs in a total of 1131 pigs from 20 European local pig breeds and in three cosmopolitan breeds, genotyped with the GGP Porcine HD Genomic Profiler. PLINK software was used to identify ROHs. Size classes and genomic inbreeding parameters were evaluated. ROH islands were defined by evaluating different thresholds of homozygous SNP frequency. A functional overview of breed-specific ROH islands was obtained via over-representation analyses of GO biological processes. Mora Romagnola and Turopolje breeds had the largest proportions of genome covered with ROH (~1003 and ~955 Mb respectively), whereas Nero Siciliano and Sarda breeds had the lowest proportions (~207 and 247 Mb respectively). The highest proportion of long ROH (>16 Mb) was in Apulo-Calabrese, Mora Romagnola and Casertana. The largest number of ROH islands was identified in the Italian Landrace ($n = 32$), Cinta Senese ($n = 26$) and Lithuanian White Old Type ($n = 22$) breeds. Several ROH islands were in regions encompassing genes known to affect morphological traits. Comparative ROH structure analysis among breeds indicated the similar genetic structure of local breeds across Europe. This study contributed to understanding of the genetic history of the investigated pig breeds and provided information to manage these pig genetic resources.

Keywords autozygosity, population genomics, selection signature, single nucleotide polymorphism, *Sus scrofa*

Address for correspondence

L. Fontanesi, Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Viale Giuseppe Fanin 46, Bologna 40127, Italy. E-mail: luca.fontanesi@unibo.it

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Introduction

Conservation programs of animal genetic resources, mainly constituted by numerous autochthonous breeds in all species, are usually challenged by their small effective

population size which, in turn, tends to increase inbreeding and reduce genetic variability (Charlesworth & Willis 2009). Inbreeding depression is considered the result of the increased level of autozygosity. Pedigree information is traditionally used to calculate the inbreeding coefficient (F_{PED}), defined as the probability that in a diploid individual, the maternal and paternal derived alleles at a randomly selected locus are identical by descent (Wright 1922). This definition is equivalent to considering F_{PED} as the proportion of autozygosity of an individual's genome. Then, the level of inbreeding of a population is expressed by averaging all F_{PED} individual values. The reliability of F_{PED} calculated in autochthonous breeds is in general lower than what it is possible to obtain for animals in commercial selection nuclei. This is mainly due to incomplete registration and incorrect recording of all mating events derived by the extensive production systems in which local breeds are usually raised (Gomez-Raya *et al*. 2008; Kios *et al*. 2012). In addition, it is clear that some assumptions used to calculate this pedigree-based coefficient are not correct and they are used as approximations in the methods of calculations: (i) all founder animals of the base population are expected to be unrelated, but this condition cannot be evaluated and it is usually not respected; (ii) recombinant events occurring during meiosis mix equally the individual's paternal and maternal haploid genome copies, but this condition mimics only average events and not what actually happens in each specific meiosis; and (iii) there are no selection biases on any parts of the genome, but this assumption is not respected considering that directional artificial selection and natural selection play important roles in shaping the genome of many domestic animal breeds (Leutenegger *et al*. 2003; Wang 2016; Knief *et al*. 2017).

Genome-wide analyses, usually based on SNP arrays, can be used to estimate the level of autozygosity of an animal genome by directly interrogating the genotype status at thousands of polymorphic sites (Kristensen *et al*. 2010). The proportion of the genome covered by ROHs of a certain minimal length has been considered one of the most precise estimations of the level of autozygosity, providing a measure of genomic inbreeding (F_{ROH}; Peripolli *et al*. 2017). ROHs are defined as continuous chromosome stretches in which all loci have a homozygous genotype (Gibson *et al*. 2006). Some ROH characteristics in a population (the average length of ROHs, the average proportion of the genome covered by ROHs and the patterns of ROH distribution across the chromosomes) are considered indicators of the origin and genetic history of a population (Ceballos *et al*. 2018). The high frequency of ROHs in some chromosome regions identifies selection signatures derived from a reduced haplotype variability around loci under natural or artificial selection (i.e. ROH island or ROH hotspots). By applying different strategies and methods, ROH islands have been used to detect signatures of selection in several livestock species (Purfield *et al*. 2017; Bertolini *et al*. 2018; Grilz-Seger *et al*. 2018; Mastrangelo *et al*. 2018; Peripolli *et al*. 2018), including the pig (Zhang *et al*. 2018; Gorssen *et al*. 2020; Schiavo *et al*. 2020a).

A lot of different pig breeds have been developed through the combined action of artificial directional selection and natural pressures that have contributed to shaping a large reservoir of genetic diversity within the *Sus scrofa* species (Porter 1993). A large fraction of these genetic resources is, however, constituted by autochthonous breeds of small population size, usually well adapted to their local agro-climatic and environmental conditions but less productive, compared with cosmopolitan breeds or lines. Conservation programs for these breeds, some of which are considered unexplored genetic resources, have different levels of managing actions that range from advanced herd book structures with specific breeding and selection plans to preliminary voluntary farmer-based herd books or primitive conservation programs (Čandek-Potokar & Nieto 2019). We recently analyzed major and candidate gene markers in 20 autochthonous European pig breeds from several different countries and obtained preliminary population structure results (Muñoz *et al*. 2018) that were refined using SNP array information (Muñoz *et al*. 2019) and whole genome resequencing data (Bovo *et al*. 2020a, b). Genome-wide data indicated that the average persistence and strength of LD between markers and SNP-based effective population size varied among breeds depending on the genetic structures and history of these breeds that had experienced different genetic events (e.g. admixture, bottlenecks and genetic drift). Selection signatures were also obtained using F_{ST} statistics by analyzing SNP chip genotyping and sequencing data (Muñoz *et al*. 2019; Bovo *et al*. 2020a). Genomic inbreeding analyses in these breeds could be used as additional information to refine their conservation programs, by controlling the level of autozygosity, and identify appropriate strategies to control inbreeding level and infer other population structures or features, such as breed-specific or subpopulation homozygosity hotspots.

In this study, we analyzed the same 20 European autochthonous pig breeds from nine different countries (Croatia, France, Germany, Italy, Lithuania, Portugal, Serbia, Slovenia and Spain) and three other cosmopolitan-derived breeds to obtain genomic inbreeding information from whole genotyping datasets using ROHs and other genomic approaches, which were based on the variance of additive genetic values, on the correlation between uniting gametes and on SNP homozygosity. We also estimated the effective population size (N_e) of these breeds and compared this information with the ROH patterns. We then evaluated the distribution of ROH in the genome of these breeds and identified putative selection hotspot regions that might be originated by the different selection histories and structures of these pig genetic resources.

Materials and methods

Animals

Pigs included in this study were from 20 autochthonous breeds distributed in nine European countries (Alentejana and Bísara from Portugal; Iberian and Majorcan Black from Spain; Basque and Gascon from France; Apulo-Calabrese, Casertana, Cinta Senese, Mora Romagnola, Nero Siciliano and Sarda from Italy; Krškopolje from Slovenia; Black Slavonian and Turopolje from Croatia; Moravka and Swallow-bellied Mangalitsa from Serbia; Schwäbisch–Hällisches Schwein from Germany; Lithuanian Indigenous Wattle and Lithuanian White Old Type from Lithuania) and three commercial breeds (Italian Large White, Italian Landrace and Italian Duroc). Analyzed pigs were selected by avoiding highly related animals (no full- or half-sibs). All animals had standard breed characteristics and were registered to their respective herd books. Table S1 provides detailed descriptions of the investigated breeds and selected animals (Čandek-Potokar & Nieto 2019). Pictures of animals from the autochthonous breeds are reported in Muñoz *et al.* (2018, 2019) and Bovo *et al.* (2020a).

Genotyping and quality control

All pigs (39–55 for each breed; Table S2) were genotyped with the GeneSeek® GGP Porcine HD Genomic Profiler version 1 (Illumina Inc), which includes 68 516 SNPs evenly distributed with a median of 25 kb gap spacing. The average genotyping call rate was 0.94. SNPs were mapped on the Sscrofa11.1 genome version, following the procedure already described by Fontanesi *et al.* (2012, 2014). Only autosomal SNPs located in unique positions were considered. Genotyping data were then filtered using plink software version 1.9 (Chang *et al.* 2015). A call rate of 0.90 and HWE P of 0.001 were set as thresholds to keep SNPs. Although filtering for MAF is necessary as best practice in most SNP chip analyses, this approach excludes the SNPs that are homozygous for the whole breed; therefore it could cause an underestimation of the coverage in ROHs (Meyermans *et al.* 2020). For this reason, we analyzed ROHs without applying any MAF pruning. For comparison with other studies that applied a MAF threshold and to evaluate the impact of MAF on the calculated ROH parameters, we also used a MAF threshold of 0.01 (indicated as a method based on MAF > 0.01) and the results are included in the Supporting Information. All analyses in the text were derived without MAF pruning (indicated as a method based on MAF ≥ 0.00), if not stated otherwise. Animals were discarded if their call rate was less than 0.90. Table S2 reports the number of SNPs and animals considered for further analyses after filtering.

Multidimentional-plot analysis of pig breeds and effective population size

The first three dimensions for a MDS plot were obtained using plink software version 1.9 and plotted with the r package 'Scatterplot3d' (Ligges & Mächler 2003) to graphically visualize the genetic distances between the 23 pig breeds. Effective population size (N_e) at recent and remote generations was computed using SNP data with the software snep (Barbato *et al.* 2015). snep allows estimation of the historic effective population size by considering the linkage level (in terms of r^2) in bins of different widths and the recombination rate; the computation is based on the basic formula:

$$E(r^2) = (1 - 4N_e\mathbf{c})^{-1}$$

where the r^2 estimate $E(r^2)$ depends on the distance between SNPs in windows and $\mathbf{c}$ is the recombination rate, kept at 1×10^{-8} as default. snep software computes the N_e in past and recent generations by correcting the equation including the number of samples and the phasing information. Default parameters were used, except for the maximum distance in bp between SNPs to be analyzed, which was set to 10 Mb, and the binwidth for the calculation of LD that was set to 100 kb.

Identification of ROHs

ROHs were identified using plink software version 1.9 (Chang *et al.* 2015). No pruning was performed based on LD to avoid biases that could be derived by this practice (Marras *et al.* 2015; Meyermans *et al.* 2020), but a minimum length of 1 Mb was set to detect ROHs. This threshold may exclude short and common ROHs determined by markers in LD, as previously demonstrated (e.g. Ferenčaković *et al.* 2013; Marras *et al.* 2015). The following parameters, already used by Schiavo *et al.* (2020b), were considered to call ROHs: (i) the minimum number of consecutive homozygous SNPs included in the ROH was 15; (ii) the minimum length that constituted the ROH was 1 Mb; (iii) the number of heterozygous SNPs that were allowed in the ROH was 0; (iv) the minimum density of SNPs in a genome window was 1 SNP every 100 kb; and (v) the maximum gap between consecutive SNPs was 1000 kb. ROHs were placed into five size classes (Kirin *et al.* 2010; Ferenčaković *et al.* 2013; Schiavo *et al.* 2020b): 1–2, 2–4, 4–8, 8–16 and greater than 16 Mb, identified as ROH1–2 Mb, ROH2–4 Mb, ROH4–8 Mb, ROH8–16 Mb and ROH greater than 16 Mb respectively. The total number of ROHs (nROHs) was then obtained for each individual and for each length class. The average length of ROHs (L_{ROH}, in Mb) and the sum of all ROH segments by animals (S_{ROH}, in Mb) were calculated. These parameters were also calculated for each breed by averaging individual data.

Genomic inbreeding measures

The F_{ROH} was calculated for each pig as the proportion of the autosomal genome covered by ROHs. F_{ROH} was calculated using all of he detected ROHs with length greater than 1 Mb (F_{ROH1}) and also considering higher thresholds of length, namely greater than 4 Mb, greater than 8 Mb and greater than 16 Mb to obtain respectively, F_{ROH4}, F_{ROH8} and F_{ROH16} inbreeding coefficients. Averaged F_{ROH} values were calculated for each breed. In addition, chromosome (SSC) F_{ROH} (F_{ROHSSC}) values were also estimated for each breed: $F_{ROHSSC} = L_{ROHSSC}/L_{SSC}$ (Silió *et al*. 2013), in which L_{ROHSSC} is the total length of an individual's ROH in each SSC and L_{SSC} is the length of each chromosome covered by the involved SNPs.

Other genomic inbreeding coefficients were calculated: (i) the variance-standardized relationship minus 1 (F_{hat1}); (ii) the excess of homozygosity-based inbreeding estimate (F_{hat2}); (iii) the estimate based on correlation between uniting gametes (F_{hat3}); (iv) the values of the diagonal elements of the genomic relationship matrix, GRM (F_{GRM}; VanRaden *et al*. 2008); and (v) the difference between the observed and expected numbers of homozygous genotypes (F_{HOM}). The F_{hat1}, F_{hat2}, F_{hat3} and F_{GRM} GRM coefficients were calculated using PLINK1.9 with the ported functions of GCTA software version 1.92 (Yang *et al*. 2011). Among the latter methods, F_{GRM} and F_{hat1} are influenced by the frequency of alleles in the population and F_{hat3} takes into consideration the correlation between uniting gametes, which could come from the same ancestor in case of inbreeding. F_{hat2} and F_{HOM} are influenced by the excess of homozygosity, but do not consider the position of SNPs along the genome. F_{HOM} was computed with PLINK software version 1.9 (Chang *et al*. 2015). Pearson correlation coefficients (r) between all evaluated inbreeding coefficients were calculated.

Identification of ROH islands and annotation of genome regions

First, the proportion of SNPs residing within an ROH was calculated for a given breed by counting the number of times an SNP appeared in an ROH within the given breed divided by the total number of genotyped pigs of that breed. Then, to call ROH islands a threshold of frequency should be defined. A few methods have been proposed for this purpose, each with pros and cons (Purfield *et al*. 2017; Grilz-Seger *et al*. 2018; Gorssen *et al*. 2020). However, there is no general agreement on their use in different contexts and populations. In this study, we used three methods to identify ROH islands that differed on the threshold that was applied.

One method already reported in other studies (Grilz-Seger *et al*. 2018, 2019a,b) uses an empirical threshold defined as the percentage of animals (usually 50%) within a population that are positive for an ROH at each tested SNP (hereinafter called 50% of animals-based threshold). When the level of inbreeding is high, the identification of islands owing to the signature of selection based on a fixed percentage of animals having ROHs at each position of the genome might increase the number of false-positive ROH islands that indicate the presence of signature of selection. This method could increase the risk of type II errors when the level of inbreeding in the population is low. Another method, frequently applied for this aim (Szmatoła *et al*. 2016; Purfield *et al*. 2017; Bertolini *et al*. 2018; Mastrangelo *et al*. 2018; Zhang *et al*. 2018), defines a percentile threshold (99th percentile) based on the top 1% of SNPs observed in an ROH in each breed (hereinafter called the percentile-based threshold). Adjacent SNPs over this threshold are then merged into genomic regions corresponding to ROH hotspots. This method always identifies ROH islands as the threshold is defined on a percentile within the breed dataset and does not consider the structure of the population or its level of inbreeding.

Considering the problems that these two methods could have, we developed a third method where the identification of the threshold was chosen considering the average S_{ROH} level of the breed, which approximates the genomic inbreeding level of a population (hereinafter called S_{ROH}-based threshold). This method consisted of predicting the threshold after fitting a simple linear model in which the percentile threshold was a function of the average S_{ROH}. The basic model was:

$$y_i = \beta_0 + \beta_1 s_i + \varepsilon_i,$$

where y_i is the threshold value (minimum number of animals positive for an ROH) obtained using the percentile-based threshold for the i^{th} breed, s_i is the S_{ROH} value for the i^{th} breed, β_0 is the intercept term whereas β_1 is the corresponding regression coefficients and e_i is the error term. Based on this model, the values of S_{ROH} were used for the prediction of the new threshold value (minimum number of animals positive for a ROH).

ROH islands were then considered in the text and annotated based on the results derived by this latter method. The results obtained with the other two methods were used for a comparative analysis. ROH co-occurrence between different breeds was investigated by comparing the average homozygosity level in each breed at each island region. For this evaluation, each ROH island identified in at least one breed was considered.

Similarity among breeds was investigated by computing a first matrix $\mathbf{A}$ (n breeds × m ROH islands regions identified across all of the analyzed breeds) whose generic entry a is the average breed-specific frequency value of a given ROH island computed as follows: $a = \sum_i AF_i/n$, where AF_i is the allele frequency of the i^{th} SNP belonging to the ROH island and including n SNPs. This matrix was used to compute a similarity matrix $\mathbf{D}$ ($n \times n$), whose generic entry d is the Euclidean distance between pairs of breeds with values scaled in the range 0–1. A final dissimilarity matrix ($1 - \mathbf{D}$)

was obtained and used to produce a heatmap in R (package *corrplot*; Wei & Simko 2017) showing similarity among breeds.

Genes annotated in the Sscrofa11.1 pig genome version that mapped in the identified ROH islands were retrieved using the Ensembl Biomart tool (http://www.ensembl.org/biomart/martview/) and from the NCBI Sscrofa11.1 GFF file. Functional enrichment analysis was carried out with *Enrichr* (Chen *et al.* 2013) via Fisher's exact test. Analyses were carried on the Biological Process branch of the GO (Ashburner *et al.* 2000), by interrogating a total of 5103 functional terms that were covering 14 433 human genes. Breed-specific analyses were run using as the input set the list of genes included in the ROH islands. We considered as statistically over-represented terms those having: (i) at least two input genes from two or more different ROH islands; and (ii) an adjusted P lower than 0.10.

Results

Genomic relationships among breeds and effective population size

Genomic information on the analyzed breeds based on SNP data was graphically presented in a tri-dimensional MDS plot (Fig. S1). This plot showed that distinct groups of individuals were usually from the same breed. Several breeds were well separated from other groups. These distinct groups included breeds from several countries: Gascon and Basque from France; Italian Large White, Italian Duroc and Mora Romagnola from Italy; Iberian from Spain; and Turopolje from Croatia. Most of the other breeds formed a continuous large cluster showing a general geographical distribution gradient as already reported in PCAs that included the same autochthonous breeds (Muñoz *et al.* 2019).

Effective population size (N_e) estimated with the software SNEP for the 23 breeds is reported in Table S3. Five generations ago, the breeds with the lowest N_e values were Turopolje, Mora Romagnola, Apulo-Calabrese and Casertana (N_e = 15, 16, 22 and 22 respectively). The autochthonous breeds with the largest N_e were Iberian, Nero Siciliano, Alentejana, Majorcan Black, Sarda and Bísara (N_e = 69, 68, 61, 58, 57 and 55 respectively). The commercial breeds had a higher N_e than all other remaining autochthonous breeds. In Italian Duroc, Italian Landrace and Italian Large White the values of N_e five generation ago were 53, 59 and 61 respectively.

ROHs in the investigated breeds

Table 1 (MAF ≥ 0.00) and Table S4 (MAF > 0.01) show the average size and average number of ROHs (considering all ROHs >1 Mb) per pig (average L_{ROH} and average nROH respectively) and the average sum of ROH (S_{ROH}) values per animal in the 23 breeds. Minimum and maximum values for these three parameters are reported in Table S5. As expected, the parameters calculated without any MAF pruning were always higher than the parameters calculated using MAF greater than 0.01. The breeds that the highest mean nROH were Basque, Italian Duroc and Turopolje (n = 107, n = 104 and n = 80 respectively) and the breeds with the lowest mean nROHs were Nero Siciliano (n = 24) Sarda (n = 27) and Moravka (n = 30). The mean L_{ROH} in all autochthonous breeds was larger than that of all three commercial breeds. Three Italian local breeds (Mora Romagnola, Apulo-Calabrese and Casertana) had the largest values of L_{ROH} (14.38, 14.21 and 12.63 Mb respectively). Among the autochthonous breeds, the lowest L_{ROH} was observed in Alentejana (6.49 Mb), Iberian (6.50 Mb) and Majorcan Black (6.58 Mb). The maximum ROH length was observed in the largest chromosomes and reached 24.34 Mb in Mora Romagnola (SSC1), 23.36 Mb in Nero Siciliano (SSC1), 22.64 Mb in Moravka (SSC1) and 21.55 Mb in Apulo-Calabrese (SSC13). Mora Romagnola and Turopolje breeds had the largest mean S_{ROH} (totals of ~1003 and ~955 Mb respectively), whereas Nero Siciliano and Sarda breeds had the lowest mean values for this parameter (~207 and ~247 Mb respectively). The maximum S_{ROH} value was observed in one Mora Romagnola and one Black Slavonian pig that had about half of their genome covered by ROHs (Table S5).

Figure 1 shows the correlation plots between the S_{ROH} and the nROH values over the individual pigs in the 23 breeds. Basque and Gascon showed homogeneous plots, indicating that most pigs of these two breeds had similar within-individual ROH parameters (nROH, L_{ROH} and S_{ROH}). In contrast, heterogeneous distribution was observed in the Apulo-Calabrese, Bísara, Casertana and Turopolje breeds (Fig. 1).

Figure 2 reports the proportion of ROH of the five different length classes in each breed. Table S6 lists the corresponding values. The highest proportion of long ROH (>16 Mb) was in Apulo-Calabrese, Mora Romagnola and Casertana (about 25, 23 and 23% respectively). Apulo-Calabrese, Casertana, Mora Romagnola and Turopolje had the lowest proportion of short–medium ROH (ROH1–8). All three commercial breeds, Alentejana, Gascon, Iberian, Majorcan Black, Nero Siciliano, Lithuanian Indigenous Wattle, Lithuanian White Old Type and Schwäbisch–Hällisches had more than 50% of short ROHs (ROH1–2 and ROH2–4).

Genomic inbreeding parameters based on ROHs

Table 2 reports the mean and standard deviation of genomic inbreeding parameters calculated using ROHs from different size classes in the 23 breeds. Mora Romagnola, Turopolje, Apulo-Calabrese and Casertana were the autochthonous breeds with the highest F_{ROH} values,

Table 1 ROH parameters calculated in the 23 pig breeds obtained without any pruning for MAF, i.e. MAF ≥ 0.00. Parameters calculated using MAF > 0.01 are reported in Table S4.

Breed	Acronym	nROH (SD)[1]	L_{ROH} (SD)[2]	S_{ROH} (SD)[3]
Alentejana	AL	50.90 (10.67)	6.49 (2.48)	339.97 (167.31)
Apulo-Calabrese	AC	56.74 (11.67)	14.21 (3.60)	813.75 (266.55)
Basque	BA	106.62 (9.36)	7.21 (1.13)	764.56 (105.38)
Bísara	BI	43.88 (12.93)	7.59 (2.67)	352.18 (211.11)
Black Slavonian	BS	36.61 (14.72)	8.75 (3.29)	336.98 (230.97)
Casertana	CA	45.34 (11.20)	12.63 (4.04)	595.06 (268.90)
CintaSenese	CS	55.62 (15.47)	7.75 (2.28)	424.32 (144.99)
Gascon	GA	75.08 (8.52)	6.97 (1.06)	522.14 (89.18)
Iberian	IB	51.38 (11.97)	6.50 (2.25)	341.52 (148.95)
Krškopolje	KR	34.96 (7.36)	8.62 (2.72)	306.47 (138.31)
Lithuanian Indigenous Wattle	LIW	42.69 (7.07)	7.69 (1.74)	330.44 (98.97)
Lithuanian White Old Type	LWOT	56.27 (10.16)	6.59 (1.82)	373.55 (133.34)
Majorcan Black	MB	48.50 (10.47)	6.58 (1.95)	327.89 (147.08)
Mora Romagnola	MR	70.35 (7.37)	14.38 (2.48)	1003.13 (139.75)
Moravka	MO	30.14 (12.34)	8.48 (4.36)	289.36 (220.73)
Nero Siciliano	NS	24.15 (10.00)	7.30 (4.91)	207.33 (208.19)
Sarda	SA	27.46 (10.26)	7.77 (4.70)	246.77 (221.24)
Schwäbisch–Hällisches	SHS	49.14 (6.63)	7.28 (2.13)	360.16 (123.64)
Swallow-bellied Mangalitsa	SBMA	49.96 (8.11)	9.75 (2.04)	483.27 (115.50)
Turopolje	TU	79.76 (15.31)	11.91 (1.78)	955.04 (242.37)
Italian Duroc	IDU	104.00 (10.49)	6.33 (1.03)	655.35 (106.75)
Italian Landrace	ILA	65.56 (8.86)	5.27 (1.08)	347.80 (92.75)
Italian Large White	ILW	62.46 (12.90)	5.52 (1.00)	349.22 (107.11)

[1]nROH: the average total number of ROH and the standard deviation (SD) calculated for each breed.
[2]L_{ROH}: the average length of ROH (in Mb) considering all length classes and the standard deviation (SD) calculated for each breed.
[3]S_{ROH}: the average sum of all ROH segments (in Mb) by animals considering all length classes and the standard deviation (SD) calculated for each breed.

considering all ROH classes. For example, among these breeds the F_{ROH1} ranged from 0.409 (Mora Romagnola) to 0.243 (Casertana). Among the commercial breeds, Italian Duroc had the highest F_{ROH} values. The lowest F_{ROH1} levels were observed in Nero Siciliano (0.085), Sarda (0.101) and Moravka (0.118).

When considering only medium–long ROH to calculate other ROH-based inbreeding parameters (i.e. F_{ROH4}, F_{ROH8} and F_{ROH16}), the values decreased in all breeds, as expected. Among those with high F_{ROH1}, this drop was more evident in the breeds that had a high percentage of short ROHs than in breeds that had many long ROHs. For example, the Italian Duroc F_{ROH16} value was about 2.5 times lower than the F_{ROH1} value, whereas in Mora Romagnola, Turopolje, Apulo-Calabrese and Casertana the F_{ROH16} values decreased only 1.4–1.6 times compared with their respective F_{ROH1} values. The distribution of the F_{ROH} values in the analyzed breeds is shown in the boxplots of Fig. 3.

The genome-wide F_{ROH} information was also dissected by considering the average proportion of all ROHs covering the different autosomes (F_{ROHSSC}). Among all breeds, Mora Romagnola and Turopolje had the highest F_{ROHSSC} values for 10 (SSC1, SSC4, SSC8, SSC9, SSC10, SSC13, SSC14, SSC15, SSC16 and SSC17) and five (SSC2, SSC3, SSC5, SSC6 and SSC11) chromosomes respectively. Apulo-Calabrese had the highest F_{ROHSSC} values for SSC7 and SSC18 whereas Basque had the highest F_{ROHSSC} value for SSC12 (Fig. S2).

Mean F_{ROH1}, F_{ROH4}, F_{ROH8} and F_{ROH16} breed values were negatively correlated with the estimated breed N_e values five generation ago, defined as reported above ($r = -0.685$, -0.722, -0.737 and -0.716 respectively; $P < 0.0001$).

Other genomic inbreeding parameters and their correlations with F_{ROH}

Other parameters that have been proposed as estimators of the level of genomic inbreeding were calculated in the 23 breeds (Table S8). The average F_{hat1} value was positive in only two breeds (Mora Romagnola and Sarda) and ranged from −0.320 (Mora Romagnola) to 0.010 (Sarda), with large within-breed variability (the largest standard deviation was in Turopolje) and among-breed variability. These considerations could be also applied for the F_{GRM} parameter, which is equivalent to F_{hat1} (even if scaled in a different way). Negative F_{hat1} values correspond to lower relatedness, thus the results indicate that the individuals of the Mora Romagnola and Sarda breeds are more related to each other in comparison with individuals of the other breeds. The average F_{hat2} and F_{hat3} parameters had both of the extreme values for the same breeds (Lithuanian Indigenous Wattle with the lowest values and Apulo-Calabrese with the highest values) with similar within- and among-breed variability (Table S8). The average F_{HOM} values were negative in 11 out of 23 breeds and ranged from −0.070

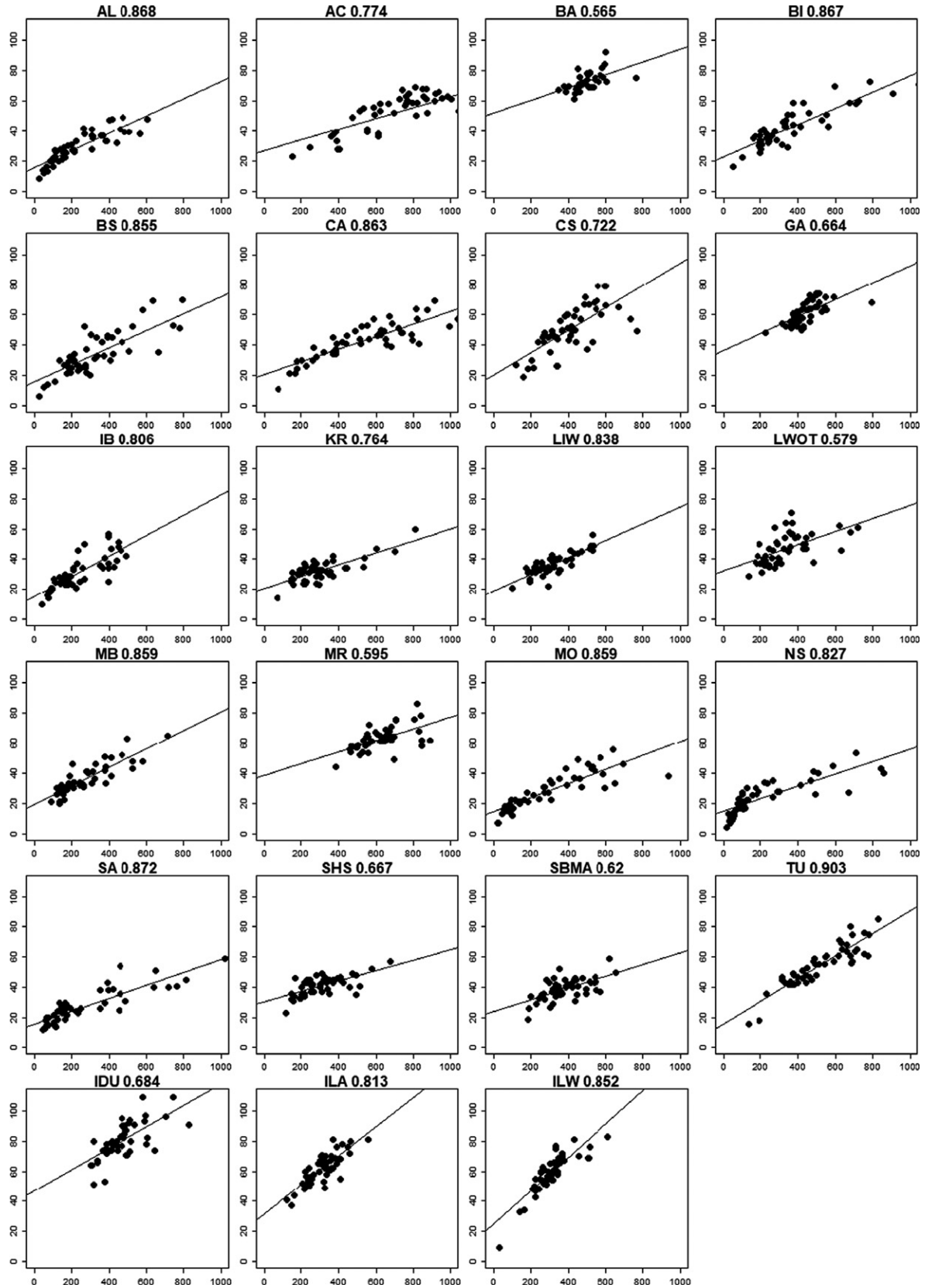

Figure 1 Correlation plots between nROH (*y*-axis) and S_{ROH} (*x*-axis) for the 23 pig breeds including all animals. Acronyms of the breeds and are defined in Table 1 and Table S1. Pearson correlation coefficient is reported beside the acronym of each breed.

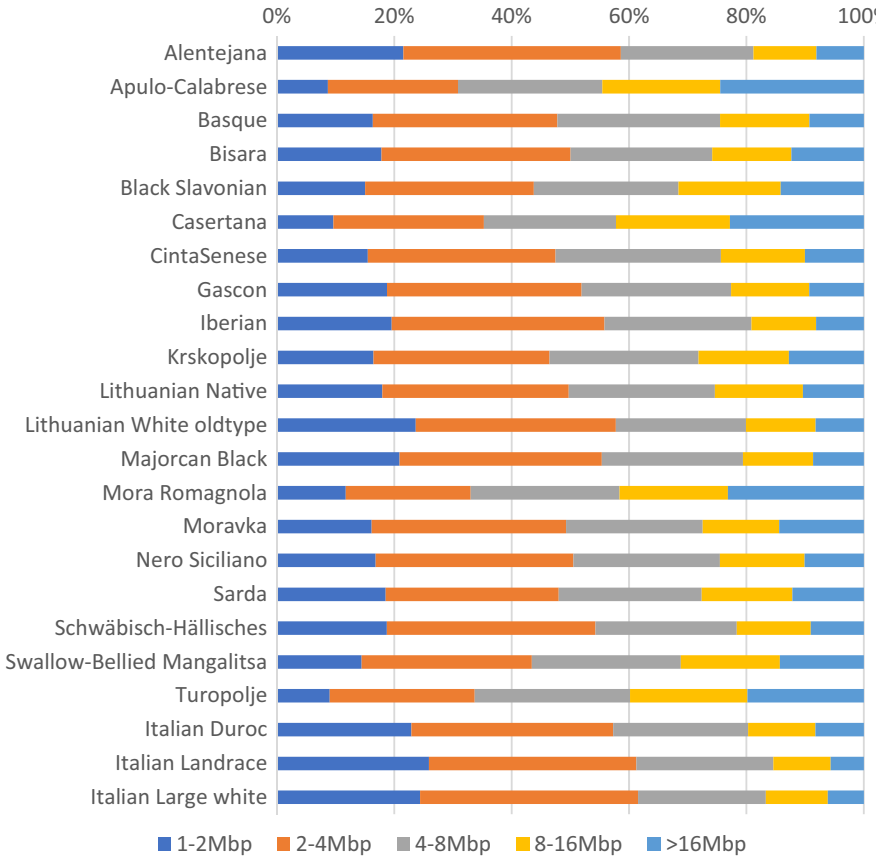

Figure 2 Proportion of ROHs of different class sizes in the 23 pig breeds. ROH classes were defined according to their size: 1–2, 2–4, 4–8, 8–16 and >16 Mb, identified as ROH1–2, ROH2–4, ROH4–8, ROH8–16 and ROH > 16 respectively.

in Lithuanian Indigenous Wattle to 0.124 in Apulo-Calabrese. Turopolje had the largest standard deviation for this parameter (0.24). Distribution plots of the F_{hat1}, F_{hat2}, F_{hat3} and F_{HOM}, parameters in the analyzed breeds are reported in Figs S3 & S4.

Correlations between all F_{ROH} parameters and all other genomic inbreeding measures for each breed are reported in Table S9. The F_{HOM} always had high and consistent correlations with the ROH-based measures over all breeds. For example, correlations with F_{ROH1} and F_{ROH4} ranged from 0.819 and 0.814 for the Nero Siciliano breed to 0.987 and 0.982 for the Bísara breed. Correlations between F_{hat2}, F_{ROH1} and F_{ROH4} had some lower values even if they were again high and consistent across breeds (they ranged from 0.447 or 0.450 in Swallow-bellied Mangalitsa to 0.909 and 0.906 in Casertana). The F_{hat1} and F_{hat3} showed inconsistent correlations compared with those of the other measures, also including negative values (Table S9). All of these other genomic inbreeding measures had low negative correlations with N_e (from −0.11 to −0.18).

ROH islands

Table 3 summarizes the number of ROH islands and the fraction of the genome covered by ROH islands identified using the S_{ROH}-based threshold in the 23 pig breeds. Figure 4 includes the Manhattan plots of a few breeds with extreme numbers of ROH islands. Figure 5 reports the pairwise similarities between breeds when overlapping ROH islands across breeds were considered. Some common features across breeds were evident.

The largest number of ROH islands was identified in the Italian Landrace (n = 34), Cinta Senese (n = 26) and Lithuanian White Old Type (n = 22) breeds. The largest covered fraction of the genome was observed in the Italian Duroc (92.85 Mb), Turopolje (80.82 Mb, with the largest averaged size of ROH islands) and Italian Landrace (75.03 Mb). No ROH islands were observed in Apulo-Calabrese and Sarda breeds.

Table S10 compares the results obtained using the S_{ROH}-based threshold method with the results obtained using the other two methods considered in this study (the 50% of animals-based threshold and the percentile-based threshold methods, see Materials and methods). The Manhattan plots for all breeds and including the thresholds derived by the three methods are reported in Fig. S5. Breeds with the highest level of genomic inbreeding estimated using F_{ROH} measures, like Mora Romagnola, Turopolje and Basque (Table 2), showed the highest number of ROH islands and the largest fraction of genome covered by ROH islands with the 50% of animals-based threshold method (n = 91 with 756 Mb in Mora Romagnola, n = 129 with 747 Mb in Turopolje and n = 93 in Basque with 312.9 Mb). Using the percentile-based threshold method, the number of ROH

Table 2 Mean F_{ROH} values calculated in the 23 pig breeds using all ROH >1 (F_{ROH1}), >4 (F_{ROH4}), >8 (F_{ROH8}) and >16 (F_{ROH16}) Mb. Standard deviation is in parentheses.

Breed	F_{ROH1}	F_{ROH4}	F_{ROH8}	F_{ROH16}
Alentejana	0.139 (0.072)	0.110 (0.071)	0.084 (0.062)	0.059 (0.061)
Apulo-Calabrese	0.332 (0.111)	0.314 (0.110)	0.281 (0.102)	0.229 (0.101)
Basque	0.312 (0.042)	0.261 (0.052)	0.194 (0.053)	0.120 (0.042)
Bísara	0.144 (0.093)	0.122 (0.082)	0.098 (0.081)	0.071 (0.062)
Black Slavonian	0.138 (0.091)	0.121 (0.091)	0.101 (0.092)	0.072 (0.071)
Casertana	0.243 (0.112)	0.226 (0.110)	0.202 (0.110)	0.162 (0.100)
Cinta Senese	0.173 (0.064)	0.147 (0.063)	0.111 (0.052)	0.075 (0.050)
Gascon	0.213 (0.042)	0.175 (0.042)	0.132 (0.041)	0.087 (0.031)
Iberian	0.139 (0.063)	0.111 (0.061)	0.082 (0.060)	0.056 (0.050)
Krškopolje	0.125 (0.061)	0.109 (0.060)	0.089 (0.063)	0.065 (0.052)
Lithuanian Indigenous Wattle	0.135 (0.042)	0.114 (0.040)	0.089 (0.044)	0.060 (0.032)
Lithuanian White Old Type	0.152 (0.052)	0.122 (0.050)	0.093 (0.051)	0.063 (0.050)
Majorcan Black	0.134 (0.061)	0.108 (0.060)	0.081 (0.051)	0.055 (0.052)
Mora Romagnola	0.409 (0.062)	0.386 (0.062)	0.345 (0.060)	0.286 (0.061)
Moravka	0.118 (0.092)	0.103 (0.091)	0.087 (0.080)	0.068 (0.071)
Nero Siciliano	0.085 (0.084)	0.073 (0.082)	0.059 (0.081)	0.043 (0.072)
Sarda	0.101 (0.092)	0.088 (0.094)	0.073 (0.092)	0.053 (0.070)
Schwäbisch–Hällisches	0.147 (0.051)	0.120 (0.052)	0.093 (0.052)	0.065 (0.051)
Swallow-bellied Mangalitsa	0.197 (0.052)	0.175 (0.050)	0.146 (0.050)	0.107 (0.042)
Turopolje	0.390 (0.101)	0.362 (0.101)	0.311 (0.093)	0.238 (0.081)
Italian Duroc	0.267 (0.043)	0.211 (0.041)	0.157 (0.041)	0.104 (0.042)
Italian Landrace	0.142 (0.042)	0.104 (0.040)	0.069 (0.031)	0.041 (0.031)
Italian Large White	0.143 (0.041)	0.106 (0.042)	0.075 (0.040)	0.046 (0.030)

islands and the total length of the genome fractions covered by these regions were similar in all breeds and ranged from $n = 7$ (Mora Romagnola) to $n = 20$ (Italian Landrace) and from 19.83 Mb (Casertana) to 44.51 Mb (Turopolje). These methods could capture different information from the analyzed populations. It seems, however, that these two latter methods are, to some extent, biased by the genetic structure of the analyzed populations and by the methodologies that are applied.

The complete list of ROH islands identified in the investigated breeds, using the S_{ROH} based-threshold method, including the genes annotated in these regions, is reported in Table S11. Several breeds had ROH islands encompassing genes that are well known to affect exterior traits, which might contribute to differentiate these pig breeds. For example, Gascon and Turopolje had an ROH island on SSC6 that includes the *melanocortin 1 receptor* (*MC1R*) gene and Krškopolje and Turopolje had another ROH island on SSC8 which includes the *v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog* (*KIT*) gene. These two genes are well known to affect coat colour and colour patterns (Fontanesi & Russo 2013). Two genes that are known to affect vertebral number (*nuclear receptor subfamily 6 group A member 1*, *NR6A1* on SSC1; and *vertnin*, *VRTN* on SSC7; Mikawa *et al.* 2007, 2011) were in two ROH islands observed in Italian Landrace and in Schwäbisch–Hällisches breeds respectively. Moravka and Schwäbisch–Hällisches breeds had an ROH island on SSC5 including the *methionine sulfoxide reductase B3* (*MSRB3*) gene whose variants have been associated with ear size in pigs (Chen *et al.* 2018; Bovo *et al.* 2020a). Cinta Senese and Italian Duroc had an ROH island including other genes that have been shown to affect body size (*caspase 10*, *CASP10*; and *non-SMC condensin I complex subunit G*, *NCAPG*; Rubin *et al.* 2012).

A functional overview of breed-specific ROH islands identified using the S_{ROH}-based threshold method was obtained via over-representation analyses of GO biological processes (Table S12). A few terms characterizing ROH islands were detected in two breeds (Krškopolje and Swallow-bellied Mangalitsa) only. Terms were general and included pattern recognition receptor signaling pathway, toll-like receptor signaling pathway, zymogen activation, cellular response to radiation and negative regulation of cell differentiation.

Discussion

The demographic history of a population can be inferred using information from the average distribution, coverage, size and patterns of ROH that can be identified in the individuals belonging to the population using high-density SNP data (Ceballos *et al.* 2018). In this study, we detected ROHs in the genome of pigs from 20 autochthonous and three commercial breeds and compared the obtained ROH genome landscapes patterns. These breeds represent populations derived from several countries and originating in different production systems that largely contributed to shape their genetic structures.

This study could allow reconstruction, to some extent, of the genetic events and history that contributed to defining

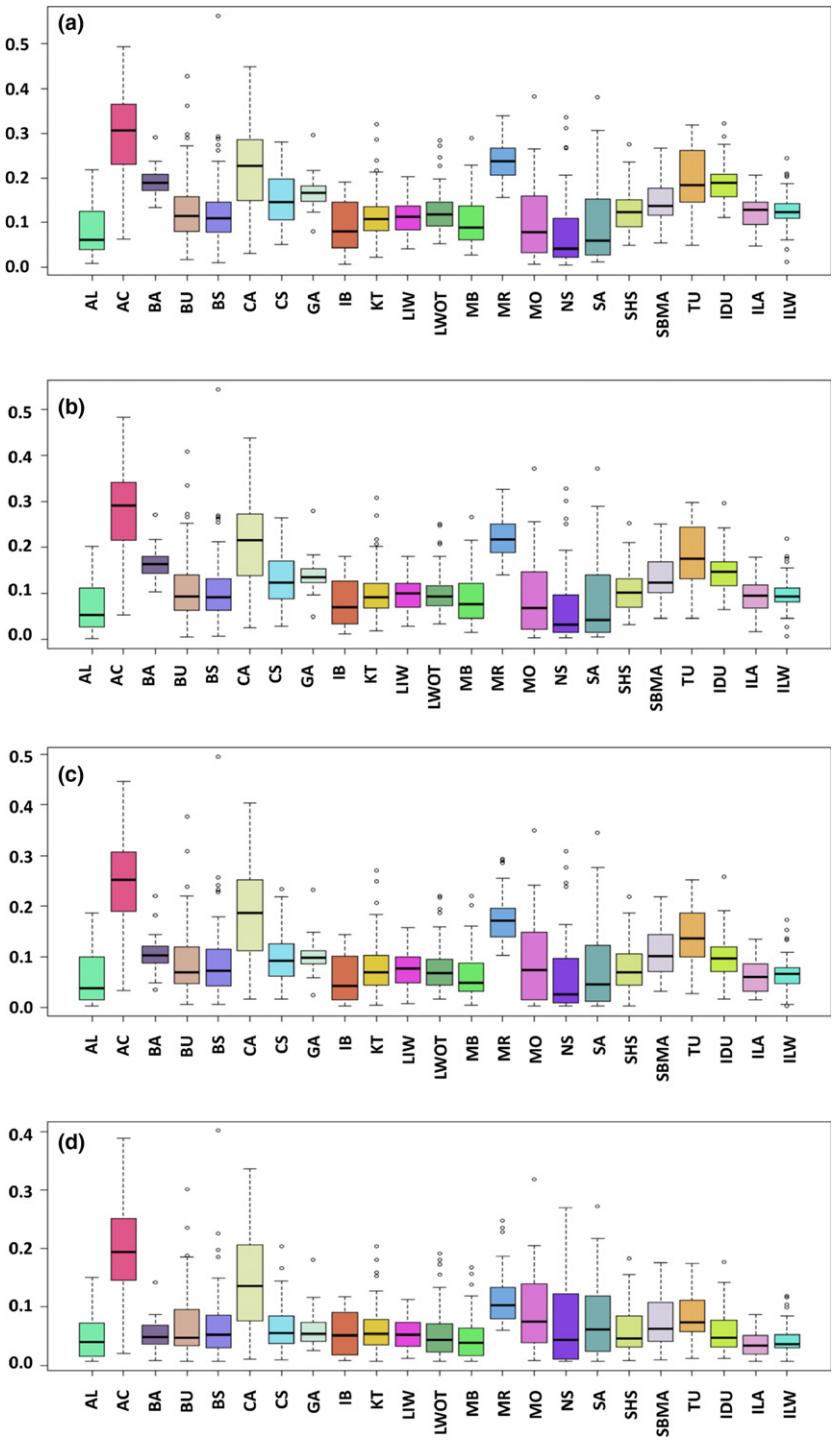

Figure 3 Boxplots of the F_{ROH} distribution in the 23 breeds: (a) F_{ROH1}; (b) F_{ROH4}; (c) F_{ROH8}; (d) F_{ROH16}. Acronyms of the breeds are explained in Table 1 and Table S1.

the current genetic pools of the investigated breeds. ROH-based fingerprints are left in the analyzed breeds and can be used to divide the 23 breeds into a few macro-groups that could have independently experienced similar genetic trajectories, as explained below.

The ROH complement of recently inbred populations is defined by a large number of ROHs with large size and a large fraction of the genome covered by ROHs (high S_{ROH}), owing to recent pedigree inbreeding loops, accompanied by a small N_e. The large S_{ROH} standard deviation indicates a low uniformity of the animals, which means that there might be different substructures or heterogeneities in the population or that an original bottleneck or founder effect could have increased the range of ROH size. Recent inbreeding features accompanied by a constituting bottleneck series of events can be clearly evidenced in a few

Table 3 The number of ROH islands and information on the genome covered by ROH islands identified in the 23 pig breeds with the method that used the S_{ROH} based-threshold.

Breed	Frequency[1]	Number of ROH islands	Genome covered (Mb)[2]	Average length (Mb)[3]
Alentejana	19/48 (40%)	12	35.88	2.99 (2.25)
Apulo-Calabrese	38/53 (72%)	0	–	–
Basque	36/39 (92%)	7	16.58	2.37 (1.84)
Bísara	20/48 (42%)	7	13.32	1.90 (1.36)
Black Slavonian	19/49 (39%)	3	2.64	0.88 (0.44)
Casertana	29/53 (55%)	7	10.23	1.46 (1.52)
Cinta Senese	23/53 (43%)	26	69.37	2.67 (2.42)
Gascon	27/48 (56%)	12	27.99	2.33 (2.00)
Iberian	19/48 (40%)	15	36.74	2.45 (1.49)
Krškopolje	18/52 (35%)	15	34.89	2.33 (2.14)
Lithuanian Indigenous Wattle	19/48 (40%)	15	41.81	2.79 (2.00)
Lithuanian White Old Type	21/48 (44%)	22	44.84	2.04 (2.19)
Majorcan Black	19/48 (40%)	12	27.23	2.27 (1.87)
Mora Romagnola	46/48 (96%)	4	12.34	3.09 (3.41)
Moravka	17/49 (35%)	9	19.11	2.12 (2.65)
Nero Siciliano	14/48 (29%)	4	7.41	1.85 (1.83)
Sarda	16/48 (33%)	0	–	–
Schwäbisch–Hällisches	20/49 (41%)	17	36.40	2.14 (1.76)
Swallow-bellied Mangalitsa	25/50 (50%)	8	23.41	2.93 (1.89)
Turopolje	44/50 (88%)	17	80.82	4.75 (3.50)
Italian Duroc	32/48 (67%)	19	92.85	4.89 (6.48)
Italian Landrace	20/48 (42%)	32	75.03	2.34 (2.48)
Italian Large White	20/48 (42%)	12	46.51	3.88 (2.57)

The three blocs indicate the two different thresholds that can be used to define an island. For each block, there is information about the number of animals that is used as threshold to define an island, the number of islands identified, the total length of genome that is covered by islands and the average length of islands.

[1]Frequency of the SNPs in an ROH, which identifies the threshold to declare an ROH island. The frequency has been calculated by dividing the number of animals needed to reach the defined level by the number of animals retained after genotyping (see Table S2).

[2]Sum of the length of the chromosome regions in the genome covered by ROH islands in Mb.

[3]Average length of the ROH islands (standard deviation) in Mb.

Italian local breeds, i.e. Apulo-Calabrese, Casertana, Mora Romagnola and Turopolje. The high level of inbreeding could have masked regions that harbor selection signatures as most of these breeds showed a low number of ROH islands (from 0 to 7, considering the S_{ROH}-based method; Table 3), apart from Turopolje, which seems to maintain a quite high level of ROH-specific regions ($n = 17$; Table 3). These breeds need to be carefully managed to reduce or control the high level of inbreeding. Programs in this direction are currently under way in the Italian breeds (ANAS 2020).

Other breeds have a quite high S_{ROH} levels but with short ROHs, indicating the occurrence of a past bottleneck and then a quite good isolation of the genetic pool. This is a case that can be observed in the two French breeds, Basque and Gascon, and in the Italian breed Cinta Senese. Differences in the three breeds are evident in the number of ROH islands that might indicate a low–medium level of specific signatures of selection in the French breeds (7 in the Basque that also had the largest nROH among the three – and 12 in the Gascon) and a high level of characterizing signatures in the Cinta Senese (26 ROH islands) probably due to different levels of selection pressures and adaptation of the three considered populations. A similar genetic history seems evident in the Italian Duroc breed (which, however, had a larger N_e; Table S3), reflecting deeper parental relatedness and consistent with an original strong bottleneck that occurred at the beginning of the 1990' when the heavy pig selection programme was defined and differentiated the Italian Duroc breed from other Duroc lines (Bosi & Russo 2004).

Breeds that experienced recent admixtures had, in general, a low nROH and as a proportion, had a higher frequency of short–medium ROHs than long ROHs, with high N_e. This group included the two breeds that had nROH less than 30, S_{ROH} less than 300.00 Mb and N_e greater than 55, i.e. Nero Siciliano and Sarda, for which the ROH-derived landscape was in agreement with the large variability observed in candidate gene markers and SNP chip data (Muñoz *et al.* 2018, 2019). Other breeds (i.e. Alentejana, Black Slavonian, Krskopolje, Lithuanian Indigenous Wattle and Moravka) had similar ROH patterns to those described for these two Italian breeds even if not so extreme (nROH < 40, S_{ROH} < 350.00 Mb). They are a heterogeneous group of populations that might have experienced some moderate introgression over the period

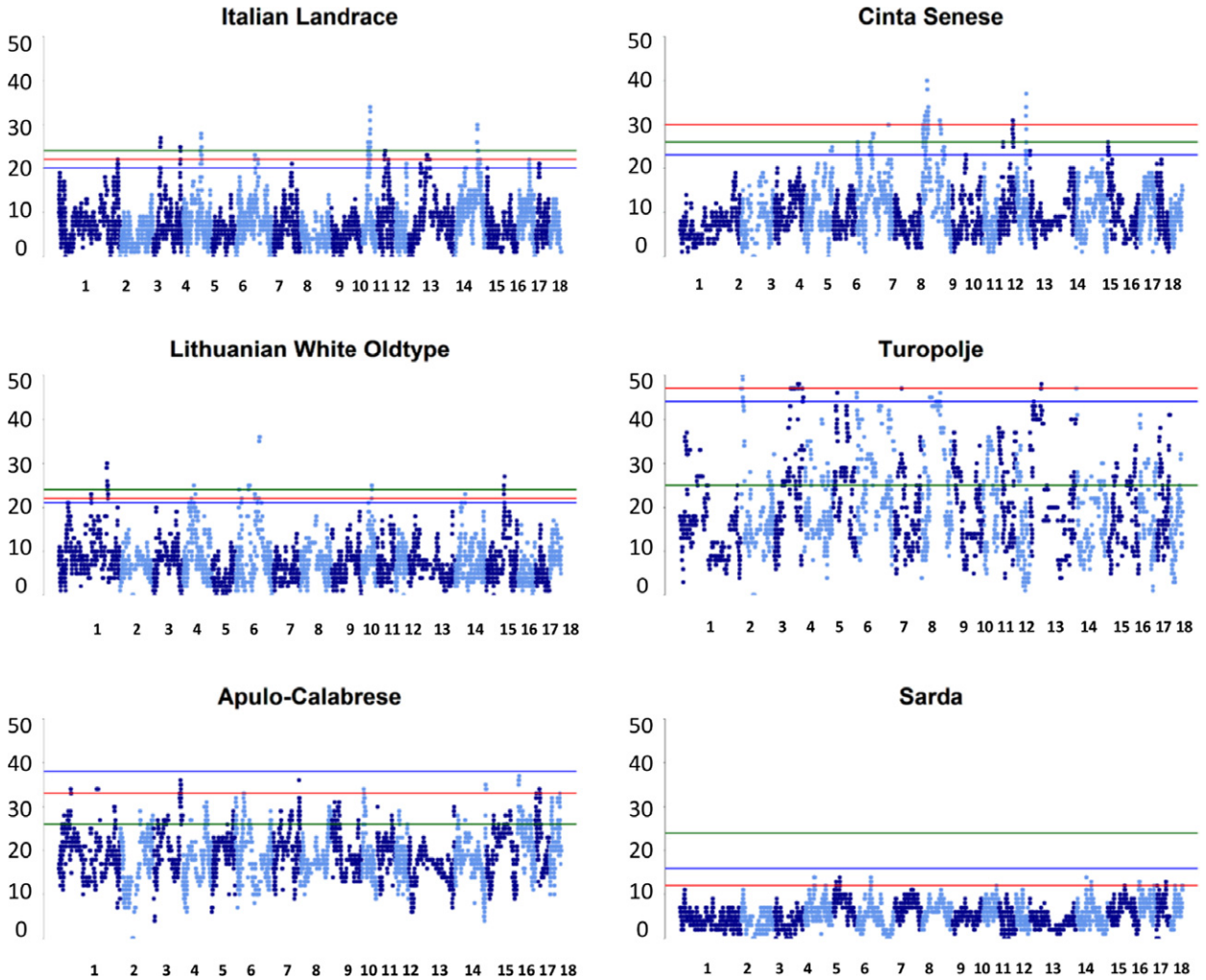

Figure 4 Manhattan plots showing ROH islands in a few analyzed pig breeds with extreme patterns. The blue line indicates the S_{ROH}-based threshold, the red line indicates the frequency corresponding to the top 1% most frequent SNP in the population and the green line indicates the 50% of individuals within the population. The *y*-axes indicate the number of animals carrying that SNP in an ROH.

of their constitution or these events might have occurred in the past and at present they maintain a moderate level of variability. The low–medium number of ROH islands (from 3 for Moravka to 15 for Krskopolje) indicates a low–medium level of differentiation in terms of specific ROH features. Another group of intermediate breeds (with some features partially overlapping with those of the previous group) with medium nROH and, in general, with a medium level of inbreeding (nROH > 40 and S_{ROH} > 300) includes Bísara, Lithuanian White Old Type, Majorcan Black, Schwäbisch–-Hällisches and Swallow-bellied Mangalitsa.

Three other breeds, i.e. Iberian, Italian Landrace and Italian Large White, had characteristic ROH-derived features of commercial breeds or large populations, as expected from their large population size (consistent with the large N_e). The two Italian breeds had some indicators of more specific differentiations and signatures of selection with a higher number of nROH, lower N_e and larger fraction of the genome included in ROH islands than the Iberian breed. This fact could also be due to the high level of genetic diversity observed within the Iberian breed, sometimes higher than in some other European pig breeds (Fabuel *et al*. 2004). This is consistent with the structure of these three populations, with the two Italian breeds being derived by a small selection of nuclei specifically addressing a selection program for heavy pigs. The presence of common features among breeds raised in different countries suggests that a few ROH islands might capture some adaptive features that are shared across populations and production systems.

The general picture depicted by the ROH profiles was able to summarize the main elements that characterize the population structure of the analyzed breeds. For a few of them, the potential burden derived by the ROH should be evaluated with attention. An increased homozygosity for (partially) recessive detrimental mutations maintained at

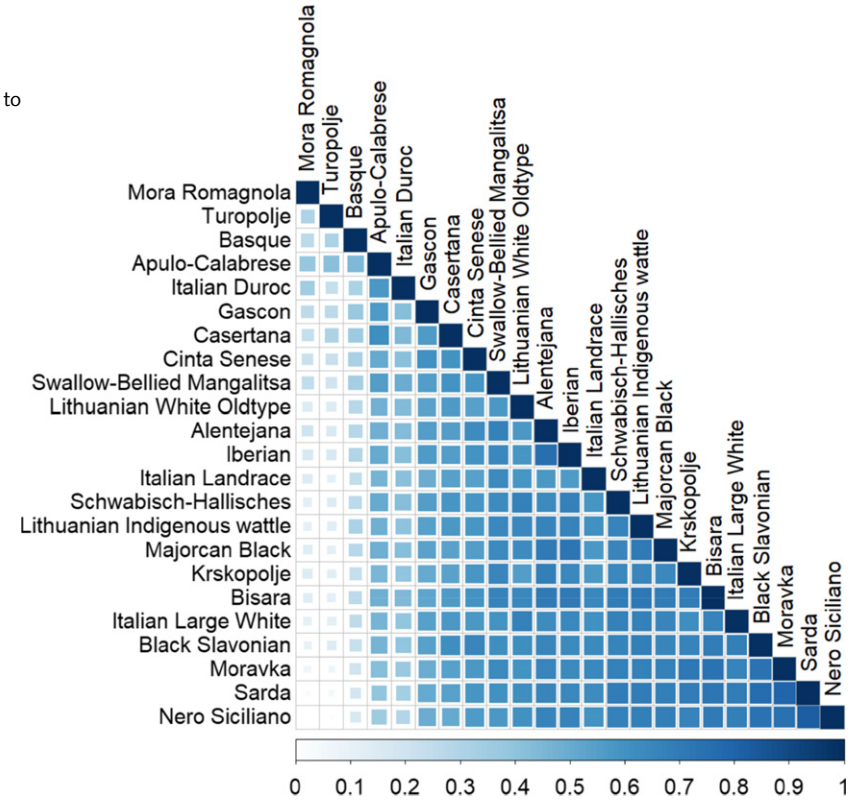

Figure 5 Similarity plot between patterns of homozygosity between pairs of breeds. Color intensity and size of the squares are proportional to the similarity values.

low frequency in populations by mutation–selection balance has been suggested to be one of the main causes of inbreeding depression (Charlesworth & Willis 2009).

Genomic inbreeding measures can help to manage all of these pig populations. In this study, we also calculated several other genomic inbreeding parameters (F_{HOM}, F_{hat1}, F_{hat2}, F_{hat3} and F_{GRM}) that have already been proposed to capture the inbreeding level from genomic information (VanRaden *et al.* 2011; Yang *et al.* 2011) with the main aim being to evaluate their relationships with F_{ROH}. The correlation between F_{ROH} and the other genomic inbreeding parameters in the analyzed breeds was in general low expect for F_{HOM}. Long ROH can be due to a general high homozygosity level in the population. F_{ROH}-based measures seem to be more appropriate than all other calculated parameters and are highly correlated with N_e, indicating that they better reflect the population structure and then the effective inbreeding level of the animals, as we already reported comparing these measures with pedigree-based inbreeding estimations (Schiavo *et al.* 2020b). For all 20 autochthonous breeds, the results confirmed the general low N_e for most breeds as already reported by Muñoz *et al.* (2019), who applied a similar estimation method.

The method used to identify ROH islands considers the level of inbreeding of the breeds to reduce the biases derived by the large fraction of the genome covered by ROH in highly inbred populations and to increase the probability of capturing the signatures of selection able to explain morphological or adaptative features that characterize the uniqueness of these genetic resources. Some of the ROH islands contained genes responsible for domestication signatures related to exterior traits and morphological adaptation (i.e. coat colour genes, *MC1R* and *KIT*, Fontanesi & Russo 2013; vertebral number, *NR6A1* and *VRTN*, Mikawa *et al.* 2007, 2011; parts of the body and body size, *CASP10*, *MSRB3* and *NCAPG*, Rubin *et al.* 2012; Chen *et al.* 2018), indicating that fixation or increased frequency for some haplotypes containing breed-specific alleles or features differentiating the domestic pool from wild boars could be captured by ROHs.

ROHs can complement other methods that have been applied to extract signatures of selection in these pig breeds (Muñoz *et al.* 2018, 2019; Bovo *et al.* 2020a,b) and can provide additional information that is useful to design conservation plans and mating strategies to maintain the diversity of these pig genetic resources.

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Conflict of interest

The authors declare they do not have any competing interests.

Data availability

Genotyping data of the autochthonous breeds can be shared after the signature of an agreement on their use with the TREASURE Consortium. Genotyping data of the commercial breeds can be shared after the signature of an agreement on their use with the University of Bologna. Please address all requests to luca.fontanesi@unibo.it.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. MDS plot of the 23 pig breeds.

Figure S2. Genomic inbreeding based on F_{ROH} across chromosomes (F_{ROHSS}).

Figure S3. Boxplot of the inbreeding coefficients estimated with all of the different methods.

Figure S4. Boxplot of the inbreeding coefficients estimated with all of the different methods.

Figure S5. Manhattan plots showing ROH island patterns in all investigated pig breeds.

Table S1. Analyzed breeds, their country and region of origin and other information useful to describe the breeds.

Table S2. Number of animals and analyzed SNPs before and after the filtering steps.

Table S3. Effective population size (N_e) calculated for each breed.

Table S4. ROH parameters using MAF greater than or equal to 0.01.

Table S5. Minimum and maximum values for the number and size of ROHs (nROH and L_{ROH} respectively) and for the sum of all ROH segments by animals.

Table S6. Proportion of the five different ROH classes for each breed.

Table S7. Mean F_{ROH} values calculated using different ROH lengths and MAF > 0.01.

Table S8. Average values for several genomic inbreeding measures.

Table S9. Correlation between all genomic inbreeding parameters in all breeds.

Table S10. The number of ROH islands and information on the genome covered.

Table S12. Results of the gene enrichment analysis on all ROH islands.

Table S13. Results of the gene enrichment analysis on ROH islands that overlapped previous work regions identifying the selection signature.

Table S11. ROH islands and annotations.