Heterozygosity decrease in wild boar mating system - a case of outbreeding avoidance?

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Abstract

In sexually reproducing organisms, the specific combinations of parental alleles can have important consequences on offspring viability and fitness. Accordingly, genetic relationship between mates can be used as a criterion for mate choice. Here, we used microsatellite genetic markers to estimate the genetic relationship between mating pairs in the wild boar, *Sus scrofa*. Males, females and foetuses proceeding from Portugal, Spain and Hungary were genotyped using 14 microsatellite markers. The genetic relationship between mates was estimated using different measures of foetus heterozygosity. We found that the observed heterozygosity of foetuses was lower than that expected under random mating. This result occurred mainly when Sd^2 (relatedness of parental genomes) was used as the heterozygosity measure. After simulations, we concluded that the observed low heterozygosity was possibly due to outbreeding avoidance. Outbreeding avoidance based on genetically different genomes might play an important role in species evolution and its genetic conservation.

Introduction

In sexually reproducing organisms, offspring genotypes are the result of combinations of maternal and paternal genotypes. The specific combinations of parental alleles can have important consequences on offspring viability and fitness (Trivers, 1972; Penn, 2002). As both inbreeding and outbreeding can negatively impact fitness (Bateson, 1982; Charlesworth & Charlesworth, 1987), the most complementary mates should be those that do not produce inbred or excessively outbreed offspring (Trivers, 1972; Bateson, 1982). Populations might be expected to evolve mate choice preferences promoting optimal outbreeding, which would then maximize fitness by avoiding the hazards of both extreme inbreeding and extreme outbreeding (Penn & Potts, 1999).

Choice for genetically complementary mates occurs both in males and females (Andersson, 1994). However, in species where reproductive investment is female-biased, such as most of mammals, choice for complementary mates should occur mainly in females. This is because females pay a higher cost by producing offspring with low fitness (Trivers, 1972). In populations, female choice for complementary mates interacts with other criteria such male ornamentation or competitive ability (Andersson, 1994; Mays & Hill, 2004). Therefore, several selective pressures, with different outputs in offspring genotypes, act on a particular mating system to construct the genetic composition of the following generation. Choice for genetically complementary mates has direct consequences on offspring genotypes; a fact that can be easily assessed today in natural populations, thus leading to increase attention and empirical support (Mays & Hill, 2004).

Inbreeding, outbreeding and the genetic relationship between mates in the wild have been broadly estimated using molecular techniques. For instance heterozygosity-fitness correlation, a widespread goal in evolutionary biology studies, uses different estimates of heterozygosity at neutral markers (mostly microsatellite markers) to infer inbreeding and to derive correlations with fitness traits (Chapman *et al.*, 2009). Moreover, offspring heterozygosity measures have been used to estimate parental relatedness in the wild (Amos *et al.*, 2001). The inference of the inbreeding coefficient with heterozygosity estimates

has received criticisms because of their possible weak correlation (Balloux, Amos & Coulson, 2004). However, studies continue to show the utility of using heterozygosity at microsatellite markers in inferring inbreeding coefficient (Forstmeier *et al.*, 2012). Szulkin, Bierne & David (2010) show that multilocus heterozygosity can be used to estimate inbreeding and whole-genome heterozygosity, as long as the assessed loci present identity disequilibrium (ID). On the other hand, despite the widespread use of heterozygosity measures based on allelic difference (e.g. Coulson *et al.*, 1998; Coltman *et al.*, 1999; Da Silva *et al.*, 2009), there has been considerable heterogeneity in results and its ubiquitous application has been challenged (e.g. Tsitrone, Rousset & David, 2001; Coltman & Slate, 2003). Nevertheless, it has been shown useful in inferring outbreeding in individuals from genetically dissimilar populations (Coulson *et al.*, 1999; Da Silva *et al.*, 2009).

Wild boar (*Sus scrofa*) is a polytocous and polygynandrous mammal that has increased its distribution and population size throughout Europe (Apollonio, Andersen & Putman, 2010). Nevertheless, despite high population sizes, hybridization threats wild boar conservation. For hunting purposes, wild boar from different areas and individuals hybridized with domestic pigs in captivity, have been released to restock or increase local populations (Randi, Apollonio & Toso, 1989). Wild boar in these local populations can also hybridize with domestic individuals that escape from pig farms. Accordingly, studies found gene flow or genetic introgressions from domestic pigs to wild boar populations (Goedbloed *et al.*, 2013).

In wild boar populations, negative relationships between individual homozygosity and fitness have been described (Acevedo-Whitehouse *et al.*, 2005). Therefore, inbreeding avoidance might play an important role in mate choice. Moreover, due to the important role of hybridization in wild boar populations (Goedbloed *et al.*, 2013), outbreed mating might not have relevant consequences to individual fitness. Therefore, outbreeding avoidance might not be expected. On the other hand, processes such as sequential mating, multiple paternity, male-biased dispersal or male heterozygous advantage occur in wild boar populations and all contribute to increase genetic diversity and inbreeding tolerance (Kokko & Ots, 2006; Poteaux *et al.*, 2009; Pérez-González *et al.*, 2014; Podgórski, Scandura & Jędrzejewska, 2014). Consequently, inbreeding avoidance might lose its relative importance. Different mating processes acting on wild boar populations make genetic relationship between mates hard to predict. The knowledge of the genetic relationship between mates can be important to understand the relative role of different evolutionary processes shaping the wild boar mating system.

In this study, we used heterozygosity at microsatellite markers to estimate the genetic relationship between mates and to infer the existence of inbreeding or outbreeding avoidance in different wild boar populations. Here, we observed a heterozygosity decrease, but only when a particular heterozygosity measure was used. We employed different simulated scenarios to assess whether the obtained results can be supported by outbreeding avoidance or by alternative processes. As alternative processes, we assessed the advantage of homozygous males in mate competition and the mortality of highly heterozygous foetuses.

Materials and methods

Sample collection

The samples used in this work originated from Portugal, Spain and Hungary. We collected tissue samples from specimens legally culled by hunters. In Portugal, sampled individuals were obtained in two hunting events conducted in Alqueva and Vila Viçosa. In Spain, samples were collected in two hunting events conducted in Azagala and Santa Amalia. In Hungary, culled individuals were collected in one hunting event conducted in Kereki. All the samples were collected between November 2008 and February 2009.

For simulation analyses (see below) additional samples were collected. On one hand, around the Hungarian hunting place, we obtained samples from males harvested in Kisbajom, Lábod, Szulok, Cserénfa, Kereki Kapasi, Pusztaszemes, Karád and Tótokilap. Male domestic pigs were also sampled to assess the effect of hybridization on heterozygosity measures (see below). These domestic males belong to different Hungarian pig breeds such as Black Slavonian, Hungarian Large White or Mangalica.

Tissue samples were collected from 91 male wild boars, 79 pregnant females and 318 foetuses, as well as seven samples from male domestic pigs (Table 1). We recorded the foetuses belonging to the same litter and the mother of each litter. For additional assessments (see below), we weighted the foetuses from the hunting event with higher sample size (Azagala, $N = 135$ foetuses). See Supplementary Material and Pérez-González *et al.* (2014) for details on hunting locations and procedures, as well as sample processing.

Microsatellite genotyping and heterozygosity measures

All 495 samples used in this study were genotyped for a set of 14 microsatellite markers designed for parentage analyses in wild boar (Sw24, S0155, Sw936, Sw2410, S0005, Sw632, Sw857, S0226, Sw72, Sw240, S0068, S0101, Sw122 and Sw2008). See details in Costa *et al.* (2012) and Pérez-González *et al.* (2014).

Three heterozygosity measures were used: standardized heterozygosity (*SH*; Coltman *et al.*, 1999), heterozygosity by loci (*HetL*) and standardized d^2 (*Sd²*; Coulson *et al.*, 1998;

Table 1 Sample sizes across hunting events or populations. 'Other' refers to additional samples that were used to interpret the main results of the study (see Methods)

Hunting event/Population	Country	Males	Females	Foetuses
Alqueva	Portugal	10	11	45
Vila Viçosa	Portugal	28	11	44
Azagala	Spain	18	35	135
Santa Amalia	Spain	16	13	45
Kereki	Hungary	7	9	49
Other	Hungary	12		
Farm (pig)	Hungary	7		

Amos *et al.*, 2001). *HetL* equals 1-*HL*, being *HL* the homozygosity by loci index developed in Aparicio, Ortego & Cordero (2006). We used *HetL* instead of *HL* to simplify the interpretation of results. See Supplementary Material (Figure S1) for the relationship among the three heterozygosity measures in the studied wild boar populations.

The existence of ID was estimated using the g_2 parameter, which measures variance in inbreeding. For each hunting event we combined all loci to compute a single estimate of g_2 in REMS software (David *et al.*, 2007). We tested if g_2 differed from zero by resampling genotypes (10,000 iterations), with significant differences indicating ID (Szulkin *et al.*, 2010).

Genetic and statistical analyses

The genetic relationship between parents was assessed using the heterozygosity of the foetuses (see Amos *et al.*, 2001). We considered inbreeding or outbreeding avoidance in our data when the observed heterozygosity obtained in the sampled foetuses was significantly different from that expected under random mating. To simulate random mating, we randomly combined the genotypes of females and males from the same hunting event (see Supplementary Material; Figure S2). We randomly selected a haploid genotype of a female and a haploid genotype of a male from the same hunting event to create a diploid simulated offspring. This process was repeated 1,000 times for each female and the heterozygosity of its simulated offspring was quantified. The mean heterozygosity of the 1,000 simulated offspring was considered as the expected heterozygosity under random mating. Therefore, in our dataset each female had as many observed heterozygosity values as foetuses it gestated, but only one value for the expected heterozygosity under random mating. Observed heterozygosity and expected heterozygosity under random mating were compared using two linear mixed-effect (LME) models fitted by reduced maximum likelihood (REML). In the first LME, a general trend in our dataset was assessed using heterozygosity as the dependent variable, mating type (observed vs. random mating) as fixed factor and female within hunting event as nested random effects. In the second LME, we assessed whether the general trend was repeated in all hunting events. Therefore, heterozygosity was the dependent variable; mating type, hunting event and their interaction were included as fixed factors; female was included as random effect.

Additional assessments were performed to discuss the results. On one hand, we estimated the expected heterozygosity in offspring under random mating between females and two types of males: males from a different hunting event (Diff) and male domestic pigs (Pig; see Figure S2). In both cases we used the same procedure as that used to quantify the expected heterozygosity in offspring under random mating between females and males from the same hunting event (Same; see above). For the quantification of the expected heterozygosity under random mating between females and males from a different hunting event, we only used the males from the nearest hunting event. Therefore, for the females from Alqueva we used the males from Vila Viçosa (62 km apart); for the

females of Vila Viçosa we used the males from Alqueva; females from Azagala and males from Vila Viçosa (72 km apart); females from Santa Amalia and males from Azagala (92 km apart). For the females from Hungary, the males culled in different Hungarian hunting events were used (see above; mean distance = 36 km). We only used the nearest hunting event to simulated male dispersal in natural populations.

Diff was used to compare the genetic relationship between females and males from the same and different hunting event. This comparison was conducted by a LME with heterozygosity as the dependent variable, mating type (Same vs. Diff) as fixed factor and female within hunting event as nested random effects. Similarly, Pig was used to compare the genetic relationships between females and males from different hunting events and the genetic relationship between females and male domestic pigs. As above, a LME was conducted with heterozygosity as the dependent variable, mating type (Diff vs. Pig) as fixed factor and female within hunting event as nested random effects. Due to male domestic pigs were sampled in Hungary, we additionally compared Pig with Diff for only Hungarian samples (results in Supplementary Material).

On the other hand, we inferred the existence of any relationship between heterozygosity and the weight of foetuses to assess the possible effect of intrauterine mortality. Intrauterine mortality of highly heterozygous foetuses might affect the proportion of homozygous foetuses in late gestation. The gestation time (conception age in Hugget & Widdas, 1951) was estimated for each foetus using body weight. Therefore, we assumed the existence of relationship between heterozygosity and mortality in foetuses, in case of relationship between foetus heterozygosity and its body weight. We performed a Generalized Additive Mixed Model (GAMM) to assess the existence of relationship between heterozygosity and weight in foetus from Azagala (see above). Weight was the dependent variable, the smoothed term of heterozygosity was included as fixed factor, and female as random effect.

Statistical models were repeated for each of the three heterozygosity measures. Since we repeated three times each model we assumed as significant those differences with *P* values lower than 0.0167. Simulations and statistical analyses were conducted using R (R Core Team, 2012). We used the *nlme* package in R (Pinheiro *et al.*, 2012) to perform LME analyses, and *gamm4* package (Wood & Scheipl, 2015) for GAMM analyses.

Simulated scenarios

Outbreeding avoidance can explain the loss of heterozygosity in foetuses. However, similar results might be achieved under the effect of additional processes such as the advantage of homozygous males in mate competition and the mortality of highly heterozygous foetuses. To determine which processes can support a heterozygosity decrease, we created different simulated scenarios. In these scenarios we simulated outbreeding avoidance, advantage of homozygous males and mortality of heterozygous foetuses, and assessed whether the obtained results are compatible with the main results of this work. We

used the three heterozygosity measures within two contexts. On one hand, *SH*, *HetL* and *Sd²* were used as different measures over which selection might act. On the other hand, they were used as different consequences of the selection process on the heterozygosity decrease. In all three processes, we tried to simulate a similar strength of selection. For that, we ordered the individuals taking into account their heterozygosity and used the 75th percentile as threshold for selection (see Supplementary Material). In all simulations, females ($N = 11$) and males ($N = 10$) sampled in Alqueva (Portugal) were used as model.

We created three parameters: OUT_{diff} (Figure S3), ADV_{diff} (Figure S4) and MOR_{diff} (Figure S5). These parameters measure the effect of outbreeding avoidance, advantage of homozygous males and mortality of highly heterozygous foetuses (respectively) on the heterozygosity decrease in descendants. They were estimated as the standardized difference between the offspring expected heterozygosity under random mating and offspring heterozygosity under the selection processes. Therefore, the higher the values of the parameters, the larger the effects of the selection processes on heterozygosity decrease (zero means no effect). See details of simulation procedures in Supplementary Material.

Results

The molecular estimate of g_2 differed significantly from zero in the Azagala hunting event ($g_2 = 0.016$, [SD 0.013], $P = 0.017$). In the remaining hunting events g_2 did not differ from zero (Alqueva: $g_2 = -0.022$, [SD 0.015], $P = 0.939$; Vila Viçosa: $g_2 = 0.001$, [SD 0.013], $P = 0.452$; Santa Amalia: $g_2 = 0.016$, [SD 0.018], $P = 0.073$; Kereki: $g_2 = 0.007$, [SD 0.018], $P = 0.291$).

Observed heterozygosity, measured as *SH* and *HetL*, tended to be lower than expected heterozygosity under random mating (Same), but the difference did not reach significance (Table 2a, b, Fig. 1a,b). However, when $Sd²$ was used, observed heterozygosity was significantly lower than expected heterozygosity under random mating (Table 2c, Fig. 1c). When hunting

Table 2 LME coefficients for the comparison between observed heterozygosity in foetuses and expected heterozygosity under random mating (Same)

	Value	SE	d.f.	*t*-value	*P*-value
a) *SH*					
Intercept	0.978	0.016	317	59.796	<0.001
Mating type	0.040	0.021	317	1.893	0.059
b) *HetL*					
Intercept	0.624	0.031	317	19.929	<0.001
Mating type	0.027	0.013	317	2.093	0.037
c) *Sd²*					
Intercept	0.251	0.011	317	22.366	<0.001
Mating type	0.047	0.009	317	5.389	<0.001

Hunting event was included as random effect. Observed heterozygosity as reference. (a) *SH* as heterozygosity measure. (b) *HetL* as heterozygosity measure. (c) *Sd²* as heterozygosity measure.

event was used as fixed factor, the interaction between mating type and hunting event was not significant, so the pattern of lower observed heterozygosity than expected under random mating occurred in all hunting events (Table 3, Fig. 2).

Additional assessments

Same (expected heterozygosity under random mating between females and males from the same hunting event) was lower
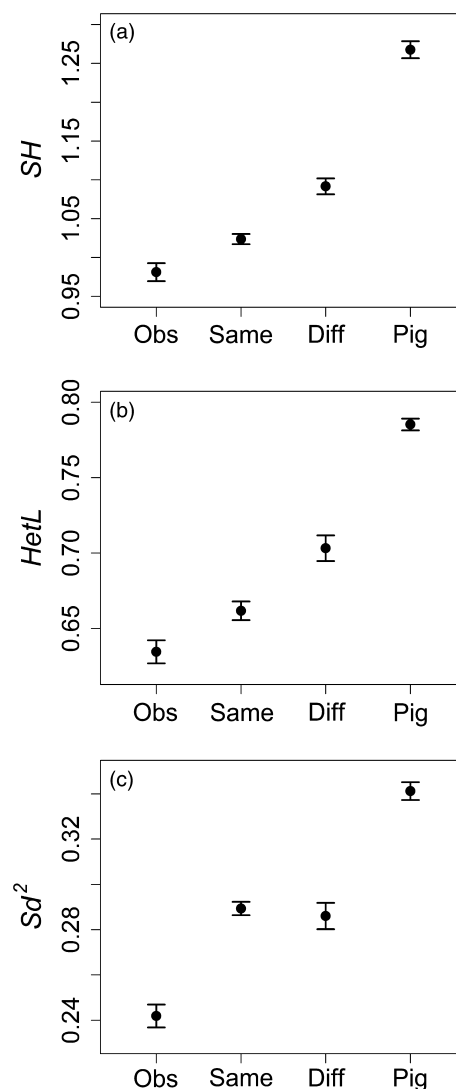
Figure 1 Mean heterozygosity and standard errors for each mating group. Obs: observed heterozygosity of foetuses; Same: expected heterozygosity in simulated offspring under random mating between females and males from the same hunting event; Diff: expected heterozygosity in simulated offspring under random mating between females and males from a different hunting event; Pig: expected heterozygosity in simulated offspring under random mating between females and male domestic pigs. (a) *SH* as heterozygosity measure. (b) *HetL* as heterozygosity measure. (c) *Sd²* as heterozygosity measure.

Table 3 LME results for the comparison between observed heterozygosity in foetuses and expected heterozygosity under random mating (Same)

	numDF	denDF	*F*-value	*P*-value
a) *SH*				
Intercept	1	313	6491.159	<0.001
Mating type	1	313	3.570	0.056
Hunting event	4	74	1.347	0.261
Mating type * Hunting event	4	313	0.285	0.888
b) *HetL*				
Intercept	1	313	7660.186	<0.001
Mating type	1	313	4.265	0.040
Hunting event	4	74	14.382	<0.001
Mating type * Hunting event	4	313	0.504	0.733
c) *Sd²*				
Intercept	1	313	2076.062	<0.001
Mating type	1	313	28.903	<0.001
Hunting event	4	74	4.504	0.002
Mating type * Hunting event	4	313	1.959	0.101

Hunting event was included as fixed factor. Observed heterozygosity as reference. *Interaction. (a) *SH* as heterozygosity measure. (c) *HetL* as heterozygosity measure. (c) *Sd²* as heterozygosity measure.
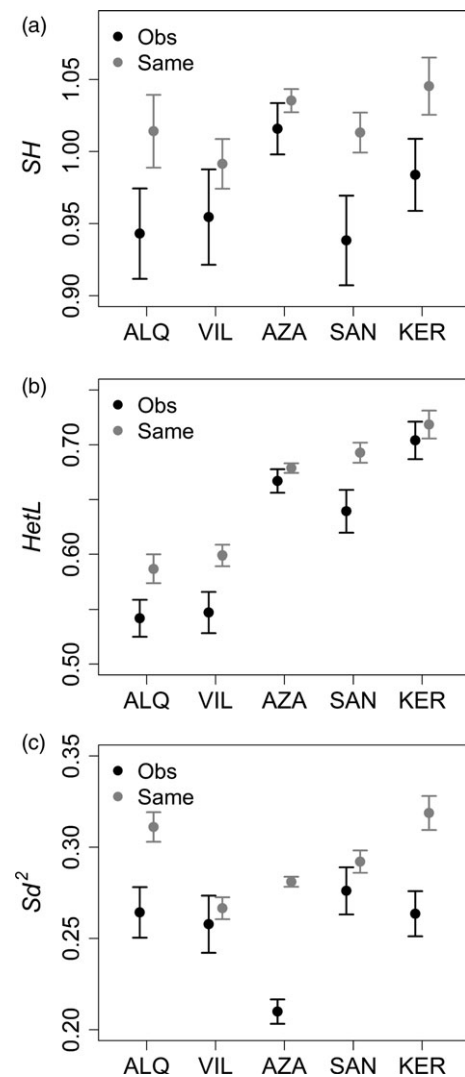
than Diff (expected heterozygosity under random mating between females and males from the different hunting events) when we used *SH* and *HetL*, but there was no difference when *Sd²* was used (Table 4, Fig. 1). Diff was lower than Pig (expected heterozygosity under random mating between females and male domestic pigs) for all heterozygosity measures, and this difference was the highest in all the assessed comparisons (Table 5, Fig. 1; see Table S1 for only Hungarian samples).

There was no relationship between heterozygosity and body weight in foetuses from Azagala. The lack of relationship occurred in all three heterozygosity measures (smoothed term of *SH*, $F = 0.010$, $P = 0.922$; smoothed term of *HetL*, $F = 0.027$, $P = 0.871$; smoothed term of *Sd²*, $F = 1.504$, $P = 0.222$; see Figure S6).

Simulated scenarios

Outbreeding avoidance and mortality of highly heterozygous foetuses tended to decrease heterozygosity ($OUT_{diff} > 0 < MOR_{diff}$; Fig. 3). This effect was larger when we took into account *Sd²* as both the selection criterion and the measure used to assess the effect of the process on the heterozygosity decrease (*Sd²* in Fig. 3c). The results of these simulated scenarios match with the results we obtained in our data.

On the contrary, the advantage of homozygous males seemed to have a lower effect on heterozygosity decrease than the other processes. The difference was higher when we took into account *SH* and *Sd²* as the selection criteria and *Sd²* as the measure used to assess the effect of the process on the heterozygosity decrease (*Sd²* in Fig. 3a,c).

Figure 2 Observed heterozygosity of foetuses and expected heterozygosity in simulated offspring under random mating for each hunting event. ALQ: Alqueva (Portugal), VIL: Vila Viçosa (Portugal), AZA: Azagala (Spain), SAN: Santa Amalia (Spain) and KER: Kereki (Hungary). Obs: observed heterozygosity of foetuses; Same: expected heterozygosity in simulated offspring under random mating between females and males from the same hunting event. Figure shows means and standard errors. (a) *SH* as heterozygosity measure. (b) *HetL* as heterozygosity measure. (c) *Sd²* as heterozygosity measure.

Discussion

Observed heterozygosity of foetuses was lower than the expected heterozygosity under random mating, mainly when *Sd²* was used as the heterozygosity measure. This pattern was similar in populations from Portugal, Spain and Hungary. Simulations support that this result might be due to the existence of outbreeding avoidance in the wild boar mating system.

The genetic relationship between parents was inferred using foetus heterozygosity. The g_2 parameter was only significantly

Table 4 LME coefficients for the comparison between Same (expected heterozygosity under random mating between females and males from the same hunting event) and Diff (expected heterozygosity under random mating between females and males from the different hunting events)

	Value	SE	d.f.	t-value	P-value
a) *SH*					
Intercept	1.008	0.018	78	56.435	< 0.001
Mating type	0.068	0.010	78	7.050	< 0.001
b) *HetL*					
Intercept	0.649	0.030	78	21.509	< 0.001
Mating type	0.041	0.005	78	7.608	< 0.001
c) *Sd²*					
Intercept	0.283	0.012	78	23.637	< 0.001
Mating type	−0.003	0.006	78	−0.593	0.5546

Same as reference. (a) *SH* as heterozygosity measure. (b) *HetL* as heterozygosity measure. (c) *Sd²* as heterozygosity measure.

Table 5 LME coefficients for the comparison between Diff (expected heterozygosity under random mating between females and males from the different hunting events) and Pig (expected heterozygosity under random mating between females and male domestic pigs)

	Value	SE	d.f.	t-value	P-value
a) *SH*					
Intercept	1.083	0.021	78	50.304	<0.001
Mating type	0.176	0.014	78	12.539	<0.001
b) *HetL*					
Intercept	0.694	0.020	78	35.290	<0.001
Mating type	0.082	0.007	78	10.834	<0.001
c) *Sd²*					
Intercept	0.281	0.011	78	25.121	<0.001
Mating type	0.055	0.006	78	8.899	<0.001

Diff as reference. (a) *SH* as heterozygosity measure. (b) *HetL* as heterozygosity measure. (c) *Sd²* as heterozygosity measure.

different from zero in one hunting event (Azagala). Moreover, Sd^2 yielded the main result of the analyses. Thus, our results might be perceived as directly plunging into the controversy surrounding the use of microsatellite markers in the inference of whole-genome heterozygosity (Tsitrone *et al.*, 2001; Balloux *et al.*, 2004). However, our simulations indicate that the heterozygosity at the used markers might infer parents' genetic relationship. When individuals from different hunting events were randomly mated (Diff), the offspring heterozygosity (*SH* and *HetL*) was higher than that obtained after the mating of individuals from the same hunting event (Same; Fig. 1a,b). When Sd^2 was used as the heterozygosity measure, Same was not different from Diff, and Pig (heterozygosity of offspring obtained after the mating of wild boar and domestic pigs) showed the highest values (Fig. 1c). These results are expected to occur at the whole genome scale. Nonetheless, our findings should be confirmed in future studies with more powerful and genome-wide representative information.

In addition to outbreeding avoidance, other selective processes might explain the heterozygosity decrease. For instance
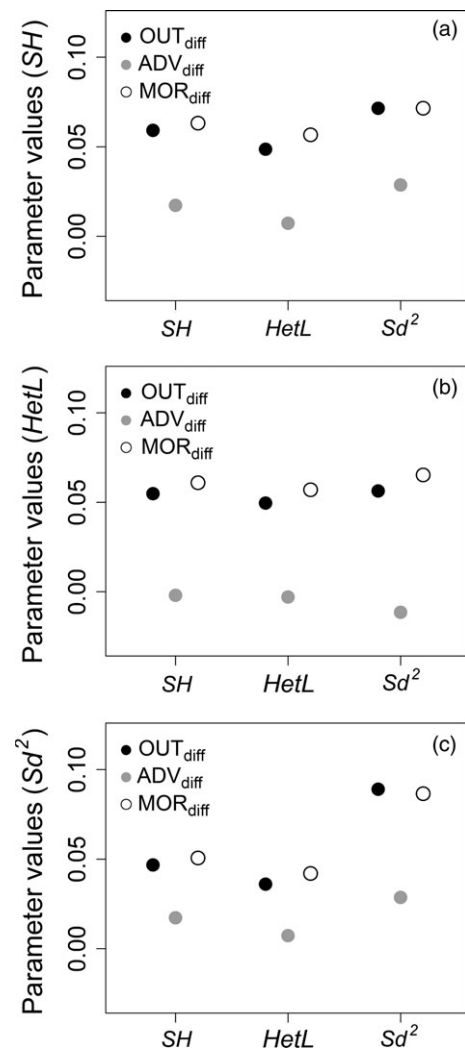
Figure 3 Results of simulated scenarios. Standardized difference in heterozygosity between that expected under random mating and that obtained under outbreeding avoidance (OUT_{diff}), advantage of homozygous males (ADV_{diff}) and mortality of highly heterozygous foetuses (MOR_{diff}, y axis). Heterozygosity was measured as *SH*, *HetL* and Sd^2 (x axis). a) Selection against heterozygosity taking into account *SH* index. (b) Selection against heterozygosity taking into account *HetL* index. (a) Selection against heterozygosity taking into account Sd^2 index.

in mate competition, outbred males might have lower fitness and heterozygosity of the following generation would tend to decrease (see Mitton *et al.*, 1993). However, we found that the effect of advantage of homozygous males on the heterozygosity decrease was relatively low. On the other hand, mortality of highly heterozygous foetuses might also explain the obtained heterozygosity decrease. Simulations support this possible effect, although we did not find any relationship between heterozygosity and gestation time (estimated by body weight) in foetuses from Azagala. The proportion of homozygous foetuses did not increase as gestation progressed. Results show

that low success of heterozygous males and mortality of highly heterozygous foetuses might not explain the heterozygosity decrease in the studied wild boar populations.

The simulated effects of outbreeding avoidance on heterozygosity decrease might be the best candidate to explain the results obtained in wild boar. Moreover, we do not have any evidence that this process might not occur. Despite we cannot rule out the effect of other processes, we propose outbreeding avoidance as the most likely process able to induce a heterozygosity decrease in wild boars. According to sexual conflict theory (Trivers, 1972), outbreeding avoidance should be mainly carried out by females. The avoidance of genetically dissimilar mates might occur as a precopulatory process dependent on olfactory assessments (Boulet, Charpentier & Drea, 2009) or a postcopulatory phenomenon based on sperm choice or gametic incompatibility (Eberhard, 1996).

Simulated scenarios show that individuals might be avoiding mating with genetically different potential mates, taking into account Sd^2 index, that is the difference in relatedness between parental genomes. The direct consequence of this selective process is that wild boars might be avoiding the mating with genetically different *taxa* such as domestic pigs. Our results show that the simulated random mating between wild boar females and male domestic pigs (Pig) produced the most heterozygous descendants. This mating produced simulated offspring whose heterozygosity was even higher than that obtained after the simulated mating between wild boar individuals from different hunting events (Diff). Therefore, despite the high frequency of contact among individuals and the importance of hybridization in the evolution of wild boar (Goedbloed *et al.*, 2013; Frantz *et al.*, 2015), there could be a process that tends to decrease the degree of hybridization between both *taxa*. Taking into account our results and the sexual conflict theory, we predict that wild boar males do not avoid mating with receptive female pigs. However, wild boar females might avoid mating with male pigs.

Outbreeding avoidance can be explained by several selective pressures. For instance it can be expected when excessive outbreeding has negative fitness consequences (Bateson, 1982) and an optimal outbreeding is the best strategy to maximize offspring fitness (Penn & Potts, 1999). Further, theory predicts that inbred mating can be adaptive because it increases the inclusive fitness of reproducers (Bengtsson, 1978). In addition, females might prevent the contact (and copulation) with males bearing dissimilar genomes to reduce the risk of disease transmission (Møller, Dufva & Allander, 1993). We did not find clear evidences supporting some of these selective pressures, but future studies on the genetic consequences of wild boar mating should explore the effect of outbreeding depression and disease transmission risks.

Wild boar populations present a male-biased dispersal pattern in which females are normally philopatric and males show high dispersal rates and large ranges (Poteaux *et al.*, 2009; Podgórski *et al.*, 2014). If females avoid outbreeding, male dispersal would be non-adaptive. However, our results show that male dispersal might be adaptive under conditions of outbreeding avoidance. As pointed out above, individuals might avoid mating with genetically different potential mates taking into account the difference in relatedness between parental genomes. We found that the Sd^2 of foetuses was not different when we simulated random mating between individual of the same (Same) and different (Diff) wild boar populations. Therefore, outbreeding avoidance based on the avoidance of genetically different parental genomes might not affect the reproductive success of dispersing males among different wild boar populations. Future studies should take spatial information into account (e.g. home ranges, size of study areas or population densities) to improve the interpretation regarding social behaviour and landscape genetics of the European wild boar.

We did not find a significant heterozygosity decrease using other parameters such as *SH* and *HetL*. However, there was a clear trend and we might expect a reduction in these parameters values in successive generations. The outbreeding avoidance based on different parental genomes might produce problems of genetic diversity conservation and, hence might reduce the fitness of individuals (e. g. Acevedo-Whitehouse *et al.*, 2005). This trend might be counteracted with other processes that favour the genetic diversity maintenance such as seasonal partner switch, litter production, multiple paternity, sex-biased dispersal and mate competition (Karl, 2008; Delgado-Acevedo *et al.*, 2011; Pérez-González *et al.*, 2014).

Wild boar presents a polygynandrous mating system in which several evolutionary phenomena interact to shape the genetic composition of the next generation. Our data suggest the existence of an additional process: the outbreeding avoidance based on genetically different genomes. This process might play an important role in the current genetic context where introgression of domestic pigs is an important threat for wild boar conservation.

Acknowledgements

References

Acevedo-Whitehouse, K., Vicente, J., Gortazar, C., Hoefle, U., Fernández-de-Mera, I.G. & Amos, W. (2005). Genetic resistance to bovine tuberculosis in the Iberian wild boar. *Mol. Ecol.* **14**, 3209.

Amos, W., Wilmer, J.W., Fullard, K., Burg, T.M., Croxall, J.P., Bloch, D. & Coulson, T. (2001). The influence of parental relatedness on reproductive success. *Proc. R. Soc. B* **268**, 2021.

Andersson, M.B. (1994). *Sexual selection*. Princeton: Princeton University Press.

Aparicio, J.M., Ortego, J. & Cordero, P.J. (2006). What should we weigh to estimate heterozygosity, alleles or loci? *Mol. Ecol.* **15**, 4659.

Apollonio, M., Andersen, R. & Putman, R. (2010). *European ungulates and their management in the 21st century*. Cambridge: Cambridge University Press.

Balloux, F., Amos, W. & Coulson, T. (2004). Does heterozygosity estimate inbreeding in real populations? *Mol. Ecol.* **13**, 3021.

Bateson, P. (1982). Preferences for cousins in Japanese quail. *Nature* **295**, 236.

Bengtsson, B.O. (1978). Avoiding inbreeding—at what cost? *J. Theor. Biol.* **73**, 439.

Boulet, M., Charpentier, M.J. & Drea, C.M. (2009). Decoding an olfactory mechanism of kin recognition and inbreeding avoidance in a primate. *BMC Evol. Biol.* **9**, 281.

Chapman, J.R., Nakagawa, S., Coltman, D.W., Slate, J. & Sheldon, B.C. (2009). A quantitative review of heterozygosity-fitness correlations in animal populations. *Mol. Ecol.* **18**, 2746.

Charlesworth, D. & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**, 237.

Coltman, D.W. & Slate, J. (2003). Microsatellite measures of inbreeding: a meta-analysis. *Evolution* **57**, 971.

Coltman, D.W., Pilkington, J.G., Smith, J.A. & Pemberton, J.M. (1999). Parasite-mediated selection against inbred Soay sheep in a free-living island population. *Evolution* **53**, 1259.

Costa, V., Pérez-González, J., Santos, P., Fernández-Llario, P., Carranza, J., Zsolnai, A., Anton, I., Buzgó, J., Varga, G., Monteiro, N. & Beja-Pereira, A. (2012). Microsatellite markers for identification and parentage analysis in the European wild boar (*Sus scrofa*). *BMC Res. Notes* **5**, 479.

Coulson, T., Pemberton, J., Albon, S., Beaumont, M., Marshall, T., Slate, J. & Guinness, F. (1998). Microsatellites reveal heterosis in red deer. *Proc. R. Soc. B* **265**, 489.

Coulson, T., Albon, S., Slate, J. & Pemberton, J. (1999). Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. *Evolution* **53**, 1951.

Da Silva, A., Gaillard, J.M., Yoccoz, N.G., Hewison, A.J.M., Galan, M., Coulson, T., Allainé, D., Vial, L., Delorme, D., Van Laere, G., Klein, F. & Luikart, G. (2009). Heterozygosity-fitness correlations revealed by neutral and candidate gene markers in roe deer from a long-term study. *Evolution* **63**, 403.

David, P., Pujol, B., Viard, F., Castella, V. & Goudet, J. (2007). Reliable selfing rate estimates from imperfect population genetic data. *Mol. Ecol.* **16**, 2474.

Delgado-Acevedo, J., Zamorano, A., DeYung, R.W., Campbell, T.A., Hewitt, D.G. & Long, D.B. (2011). Promiscuous mating in feral pigs (*Sus scrofa*). *Wildl. Res.* **37**, 539.

Eberhard, W.G. (1996). *Female control: sexual selection by cryptic female choice*. Princeton, NJ, USA: Princeton University Press.

Forstmeier, W., Schielzeth, H., Mueller, J.C., Ellegren, H. & Kempenaers, B. (2012). Heterozygosity–fitness correlations in zebra finches: microsatellite markers can be better than their reputation. *Mol. Ecol.* **21**, 3237.

Frantz, L.A.F., Schraiber, J.G., Madsen, O., Megens, H.J., Cagan, A., Bosse, M., Paudel, Y., Crooijmans, R.P.M.A., Larson, G. & Groenen, M.A.M. (2015). Evidence of long-term gene flow and selection during domestication from analyses of Eurasian wild and domestic pig genomes. *Nat. Genet.* **47**, 1141.

Goedbloed, D.J., van Hooft, P., Megens, H.J., Langenbeck, K., Lutz, W., Crooijmans, R.P.M.A., van Wieren, S.E., Ydenberg, R.C. & Prins, H.H.T. (2013). Reintroductions and genetic introgression from domestic pigs have shaped the genetic population structure of northwest European wild boar. *BMC Genet.* **14**, 43.

Hugget, A.S. & Widdas, W. (1951). The relationship between mammalian foetal weight and conception age. *J. Physiol.* **114**, 306.

Karl, S.A. (2008). The effect of multiple paternity on the genetically effective size of a population. *Mol. Ecol.* **17**, 3973.

Kokko, H. & Ots, I. (2006). When not to avoid inbreeding? *Evolution* **60**, 467.

Mays, H.L. & Hill, G.E. (2004). Choosing mates: good genes versus genes that are a good fit. *Trends Ecol. Evol.* **19**, 554.

Mitton, J.B., Schuster, W.S.F., Cothran, E.G. & De Fries, J.C. (1993). Correlation between the individual heterozygosity of parents and their offspring. *Heredity* **71**, 59.

Møller, A.P., Dufva, R. & Allander, K. (1993). Parasites and the evolution of host social behaviour. *Adv. Study Behav.* **22**, 65.

Penn, D.J. (2002). The scent of genetic compatibility: Sexual selection and the major histocompatibility complex. *Ethology* **108**, 1.

Penn, D.J. & Potts, W.K. (1999). The evolution of mating preferences and major histocompatibility complex genes. *Am. Nat.* **153**, 145.

Pérez-González, J., Costa, V., Santos, P., Slate, J., Carranza, J., Fernández-Llario, P., Zsolnai, A., Monteiro, N.M., Anton, I., Buzgó, J., Varga, G. & Beja-Pereira, A. (2014). Males and females contribute unequally to offspring genetic diversity in the polygynandrous mating system of wild boar. *PLoS ONE* **9**, e115394.

Pinheiro, J., Bates, D., DebRoy, S. and Sarkar, D. & the R Development Core Team (2012). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-104.

Podgórski, T., Scandura, M. & Jędrzejewska, B. (2014). Next of kin next door – philopatry and socio-genetic population structure in wild boar. *J. Zool.* **294**, 190.

Poteaux, C., Baubet, E., Kaminski, G., Brandt, S., Dobson, F.S. & Baudoin, C. (2009). Socio-genetic structure and mating system of a wild boar population. *J. Zool.* **278**, 116.

R Core Team (2012). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Randi, E., Apollonio, M. & Toso, S. (1989). The systematics of some Italian populations of wild boar (*Sus scrofa* L.): a craniometric and electrophoretic analysis. *Z. Saeuget.* **54**, 40.

Szulkin, M., Bierne, N. & David, P. (2010). Heterozygosity-fitness correlations: a time for reapprasial. *Evolution* **64**, 1202.

Trivers, R.L. (1972). Parental investment and sexual selection. In *Sexual Selection and the Descendant of man*: 136–179. Campbel, B. (Ed). Chicago: Aldine.

Tsitrone, A., Rousset, F. & David, P. (2001). Heterosis, marker mutational processes and population inbreeding history. *Genetics* **159**, 1845.

Wood, S. and Scheipl, F. (2015). *Generalized additive mixed models using mgcv and lme4*. R package version 0.2-3. Available online: http://cran.r-project.org/web/packages/gamm4/index.html

Supporting Information

Additional Supporting Information may be found in the online version of this article: