

Parents' genetic dissimilarity and offspring sex in a polygynous mammal

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Abstract

Offspring quality may benefit from genetic dissimilarity between parents. However, genetic dissimilarity may trade-off with additive genetic benefits. We hypothesized that when sexual selection produces sex-specific selective scenarios, the relative benefits of additive genetic vs. dissimilarity may differ for sons and daughters. Here we study a sample of 666 red deer (*Cervus elaphus*) microsatellite genotypes, including males, females and their foetuses, from 20 wild populations in Spain (the main analyses are based on 241 different foetuses and 190 mother-foetus pairs). We found that parental lineages were more dissimilar in daughters than in sons. On average, every mother was less related to her mate than to the sample of fathers in the population when producing daughters not sons. Male foetuses conceived early in the rutting season were much more inbred than any other foetuses. These differences maintained through gestation length, ruling out intrauterine mortality as a cause for the results, and indicating that the potential mechanism producing the association between parents' dissimilarity and offspring sex should operate close to mating or conception time. Our findings highlight the relevance of considering the sex of offspring when studying genetic similarity between parents.

Keywords: *Cervus elaphus*, disassortative mating, inbreeding, red deer, sex differences, sexual selection

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Introduction

There is increasing support for the evolutionary role of genetic compatibility (complementarity; Piálek & Albrecht 2005) between sexual mates (reviewed in Mays & Hill 2004). Most compatible mates should be those that do not produce either inbred or excessively outbred offspring (Trivers 1972; Bateson 1982), which for spatially structured populations may mean more dissimilar mates than the average of available mates. Parental dissimilarity has been shown to positively correlate with fitness in a variety of organisms (Coulson *et al.* 1998; Slate *et al.* 2000; Amos *et al.* 2001; Hansson & Westerberg 2002; Penn 2002; Mays & Hill 2004). There is also empirical evidence that female choice is influenced by mate dissimilarity (Bateson 1982; Potts

et al. 1991; Wilson *et al.* 1997; Penn & Potts 1999; Tregenza & Wedell 2002; Mays & Hill 2004; Hoffman *et al.* 2007; Höner *et al.* 2007; but see Kotiaho *et al.* 2008a). In passerine birds, females may increase heterozygosity of their offspring by extrapair mating with mates more dissimilar than their social partners (Johnsen *et al.* 2000; Foerster *et al.* 2003; Stapleton *et al.* 2007). Dissimilar mating may also favour the maintenance of genetic diversity in populations subjected to strong inter-sexual selection (Amos & Balmford 2001; Kotiaho *et al.* 2001, 2008b).

However, selection for mate dissimilarity may be balanced by selection for additive genetic effects (reviews in Kokko *et al.* 2003; Charmantier & Sheldon 2006; Oh & Badyaev 2006; Kotiaho & Puurtinen 2007). In an experimental study with mice, Roberts & Gosling (2003) provided an example of flexible mating preferences by showing that females chose best males or more dissimilar ones depending on the relative variability of male quality and dissimilarity among the potential mates. Variation in mate choice for good genes vs. dissimilar

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genes should affect the genetic composition of offspring. Notably, if females chose mates for additive genetic effects they may mate randomly with respect to genetic dissimilarity, because the benefits accrued from these quality genes are not contingent of the genotype of the female (Mays & Hill 2004). This may lead to more inbred offspring when females chose heritable qualities than when they chose dissimilar mates.

Sexual selection (Darwin 1871; Anderson 1994) is a powerful evolutionary force that can produce contrasting trait-fitness relationships for males and females. Empirical evidence increasingly shows that alleles may confer different (even antagonistic) additive genetic benefits for males and females (Rice 1992, 1996; Forsman 1995; Holland & Rice 1999; Chippindale *et al.* 2001; Fedorka & Mousseau 2004; Robinson *et al.* 2006; Foerster *et al.* 2007). So, a male may contain different breeding values for fitness for his male progeny and for his female progeny. Then, even assuming that genetic dissimilarity may be beneficial for both sons and daughters, sex differences in the fitness effects of certain paternal alleles in species subjected to strong sexual selection predicts that the relative benefits of additive genetic effects vs. dissimilarity of mates may differ for sons and daughters. For parents, fitness return from mating is not realized until offspring are produced. Thus, fitness return from mating with a dissimilar mate may depend on the sex of the offspring produced thereafter. However, we are unaware of any study that has investigated how parental dissimilarity may differ in relation to the sex of offspring.

The red deer is a likely candidate to investigate how selection produces differences between the sexes (Clutton-Brock *et al.* 1982). In this species the mating system is highly polygynous (Clutton-Brock *et al.* 1988) and males strongly compete for females during the rutting season, which has favoured sexual size dimorphism and the development of antlers as weapons for male-male combats (Clutton-Brock *et al.* 1982, 1988). Red deer hinds typically produce one calf per birth (Clutton-Brock *et al.* 1982; Carranza 1996) so, for any mother in a breeding season, the genetic features of a particular sire are only transmitted to one offspring, either male or female. Heterozygosity positively relates to lifetime breeding success in red deer (Slate *et al.* 2000), which would predict that selection should favour parental dissimilarity. On the other hand, recent findings have shown that males with relatively high fitness fathered, on average, daughters with relatively low fitness (Foerster *et al.* 2007). This means that additive genetic effects associated with traits that confer competitive abilities to males (Kruuk *et al.* 2002) are not likely to benefit daughters. Under this sexual antagonistic scenario, selection may still favour parental dissimilarity for

offspring but also additive genetic effects mainly for sons, which might counteract selection for dissimilarity between parents mainly for sons, potentially leading to the production of more inbred sons than daughters.

Here we use data from culled male and female red deer and their unborn foetuses in 20 study areas in Southwestern Spain, to investigate if genetic dissimilarity between parents differs for sons and daughters.

Methods

Study area and data collection

The study was conducted between 2004 and 2006 (two hunting seasons) in the Spanish regions of Extremadura and Andalucía in the southwest of the Iberian Peninsula (see Supporting information). Each of the 20 areas included in the study was sampled only in one of the study years. Red deer stocks at these areas can be considered as distinct populations with regard to their current ranging and mating behaviour, either because of the geographical distance or due to the presence of artificial barriers between them. Study areas averaged ca.1000 hectares in size and were within private hunting estates. Every area typically included a part of a mountain range covered by Mediterranean scrubland (*Cistus* spp., *Erica* spp., *Genista hirsuta*, *Lavandula*, spp.) and forest (*Quercus* spp., *Arbutus unda*, *Olea europaea*, *Phyllirea* spp.) and a lower and flatter land, covered by open, oak (*Quercus* spp.) woodland called *dehesa*.

We obtained tissue samples from specimens culled by hunters. The hunting procedure was in all cases the Spanish montería or a similar type of hunt. In this type of hunt, packs of dogs are released within a shrub area to move the deer outwards to the sites where hunters are placed. Normally, every male deer of 2 years or more, or every female of any age, can be shot in commercial hunting focused on stags or in management hunt aimed at reducing density of hinds, respectively. Under these conditions there is little opportunity for hunting bias to particular individuals, and montería has been shown to be the least biased procedure to obtain data from hunted red deer (Martínez *et al.* 2005). This study never provoked hunters to shoot additional deer (see also Carranza *et al.* 2004).

From stags, pregnant hinds and foetuses we took a piece of tissue (muscle in most cases) and preserved it frozen at -20°C . Genomic DNA was purified by proteinase K digestion and salting out procedure (Miller *et al.* 1988). We were able to visually determine the sex for most of the foetuses, and used the amelogenin marker (Pfeiffer & Brenig 2005) for 8 incomplete foetuses and the very small ones. We also estimated adult females' age by counting cementum growth marks at

the interradicular pad under the first molar (Mitchell 1967; Carranza *et al.* 2004).

All foetuses were weighed and their gestation length (days from conception) was estimated by using the equation proposed by Huggett & Widdas (1951) for mammals. Applying this formula to a particular species requires to know both the average birth weight and the gestation length up to full term of the species in order to estimate the gestation length of each foetus based on its weight. The average birth weight for the species in our study area was obtained from a sub-sample of 11 individuals captured and weighed within the day of their birth (males: 7760 g, $N = 6$; females: 7208 g, $N = 5$). These values were very similar to those obtained from the intercept of the regression between age (in hours from birth) and body weight, in a sample of 18 individuals captured within the five first days of life (males: 7763 g, $N = 10$; females: 7129 g, $N = 8$). We have no direct information on the completed gestation length in our study area, so it was taken from Rum population as 236 days for males and 234 for females (Clutton-Brock *et al.* 1982). Therefore, the equation was applied independently for both sexes. Date of conception was estimated by calculating the difference between the day when mothers were culled (montería date) and their gestation length (age in days). To make conception date comparable between years and the two study regions, we standardized conception date as the number of days since the earliest conception in our sample for that region and year.

Microsatellite genotyping

We typed individuals at 12 microsatellite loci: OarFCB193, OarCP26, OarFCB304, CelJP38, CelJP15, TGLA94, TGLA53, BM1818, CSSM22, CSSM66, ILSTS06, CSPS115 (Coulson *et al.* 1998; Marshall *et al.* 1998; Bonnet *et al.* 2002; Martínez *et al.* 2002; Kuehn *et al.* 2003). After polymerase chain reaction we used ABI3130 DNA sequencer and GeneMapper software (Applied Biosystems) to determine allele sizes. We combined the markers in five multiplex or simplex PCRs (1×6 , 1×3 and 3×1 ; number of PCRs \times number of markers). We made a thorough depuration of genotypes by retyping individuals, by eliminating doubtful cases and by eliminating from genetic analyses those individuals with less than 10 genotyped markers. After depuration our data set was reduced from $N = 910$ (292 males and 309 females (mothers) with their 309 foetuses) to $N = 666$ [191 males, 234 females (mothers) and 241 foetuses (among them, only 190 mother-foetus pairs maintained)]. For each foetus' genotype, we constructed paternal and maternal half genotypes of foetuses by comparing each foetus' genotype with its mother's

genotype (Jones *et al.* 2001). Ambiguity (i.e. foetus and mother shared the same heterozygous genotype; Fiumera & Asmussen 2001) existed in 13% of the cases. In these cases, we assigned the alleles to paternal lineage at random but with a probability proportional to the relative frequency of the ambiguous alleles in the males of the population which the foetus belonged to (see also Pérez-González *et al.* 2009).

We used an exact test implemented by Genepop 3.4 (Raymond & Rousset 1995) to assess the presence of linkage disequilibrium between loci. None of the possible pairs of loci presented linkage disequilibrium.

Genetic analyses

We used two genetic measurements in foetuses to estimate parents' genetic similarity. We firstly used standardized heterozygosity (SH) as the ratio of the heterozygosity of an individual to the mean heterozygosity of those loci at which the individual was typed. This standardization controls for possible differences in expected heterozygosities between loci (Coltman *et al.* 1999). Secondly, we used internal relatedness (IR), which gives more weight to homozygotes involving rare alleles (Amos *et al.* 2001). For IR determination we used the genotypes of our sample of adults from a given population to obtain allele frequencies, and used them to calculate the IR of foetuses in that population. For both genetic indices we obtained very similar results (see Amos *et al.* 2001) and reached at the same conclusions, so we only show the results for SH.

To obtain further information on the relationships between mothers and breeding males in the population we made the following calculations.

- 1 Mother-her mate: genetic relatedness between each mother and the paternally inherited half-genotype of her foetus, as an estimate of the relatedness between each mother and her mate.
- 2 Mother-fathers: genetic relatedness between each mother and the paternally inherited half-genotypes of all the foetuses in her population. This provided information on the relatedness between each mother and the whole set of breeding males (fathers) in her population.
- 3 Mother-fathers siring same sex foetuses: genetic relatedness between each mother and the paternally inherited half-genotypes of those foetuses of the same sex as her own foetus in her population. This provides information on the relatedness between each mother and those breeding males (fathers) producing the same-sex foetuses in her population. This calculation was included because we wanted to rule out the possibility that the differences in dissimilar-

ity between mates according to the sex of offspring could be an effect of features shared by the subset of breeding males siring foetuses of the same sex in the population.

To measure genetic relatedness we used Ritland's index (Ritland 1996). This index is appropriate for our analyses because it is recommended when very polymorphic markers, such as microsatellite loci, are used (Vekemans & Hardy 2004), and because it allows working with haploid information (Hardy & Vekemans 2002). SPAGeDI software (Hardy & Vekemans 2002) was used to compute Ritland's relatedness index.

Statistical analyses

To investigate whether SH differed between male and female foetuses, we used a Linear Mixed Model fitted by restricted maximum likelihood (REML: SPSS software vs. 13, SPSS Inc.), with sex as fixed factor and populations as random factor. For this analysis we had 241 different foetuses.

A similar model (Mixed Model, REML procedure), but with repeated effects, was used to see whether the genetic relatedness between each mother and her mate differed from her genetic relatedness with the breeding males (fathers) in her population, for the two groups of male genotypes we had considered (see above), and according to the sex of her foetus. Thus, we ran two models (see below Table 1), one for fathers in general and another for those fathers siring same sex foetuses as the mother's foetus. In both cases the procedure was identical. The genetic relatedness between each mother and her mate and between her and fathers in her population were considered as repeated measures per mother, and the selected subject variables in the model were population and mother (mothers nested within populations). In a mixed model, subject variables are

used to identify repeated observations, thus defining the independent cases for the analysis (in our case $N = 190$ different mothers with foetus, both genotyped). The chosen covariance structure type was 'Unstructured', as the repeated measures per mother were significantly correlated and presented non-constant variance. Sex of foetuses and group of males (fathers or mates) were included as fixed factors, and their estimated effects were calculated by using Type III sums of squares method. Finally, to assess the effect of the fixed factors adjusting for population variation, population was included as random factor with Variance Component as the covariance structure for the random effects. This model showed the best-fitting values of the information criteria (-2 Restricted Log Likelihood and AIC: Akaike's Information Criterion).

To see if sex differences in SH of foetuses might potentially result after differential intrauterine mortality during gestation, we conducted another model similar to that used to investigate sex differences in SH but by adding gestation length and conception date of foetuses as independent variables (covariates) to the former model. Both variables were estimated on the basis of foetus weight but relative to the date when mother and foetus were collected, so that they were only slightly related (conception date = $37.73 - 0.095$ gestation length; $R^2 = 0.035$; $F_{1,231} = 8.421$, $P = 0.004$). Also, they have different biological meaning that justify their inclusion as different covariates. We checked anyway that similar significant results were obtained when both covariates were analyzed separately. Finally, mother's age and its quadratic term were also added to the model as further covariates to account for the fact that mature mothers, rather than young or very old ones, tended to conceive early in the season (conception date = $47.30 - 6.44$ age + 0.48 age²; $R^2 = 0.058$; $F_{2,220} = 6.833$, $P = 0.001$). We initially ran a full factorial analysis and afterwards the

Table 1 Effect of foetus sex factor on the genetic relatedness between mothers and fathers according to different groupings of sires: in (a) sire grouping tests the difference between mother's mate and fathers in general with respect to their genetic relatedness to the mother; in (b) sire grouping compares it between mother's mate and only those fathers siring foetuses of the same sex than the mother's foetus. Table shows the estimate differences in intercepts and slopes with mother's mate and female foetuses as references

| Parameter | (a) Sire grouping: mates vs. fathers in general | | | | (b) Sire grouping: mates vs. fathers siring foetuses of the same sex than the mother's foetus | | | |
|---------------------------------|---|------|----------|----------|---|------|----------|----------|
| | Estimate (SE) | d.f. | <i>t</i> | <i>P</i> | Estimate (SE) | d.f. | <i>t</i> | <i>P</i> |
| Intercept | -0.055 (0.008) | 164 | -6.654 | <0.001 | -0.055 (0.008) | 124 | -6.453 | <0.001 |
| Sire grouping: mate vs. fathers | 0.022 (0.007) | 188 | 3.142 | 0.002 | 0.023 (0.006) | 188 | 3.660 | <0.001 |
| Foetus sex: males vs. females | 0.027 (0.011) | 189 | 2.545 | 0.012 | 0.027 (0.011) | 190 | 2.539 | 0.012 |
| Sire grouping × foetus sex | -0.025 (0.010) | 188 | -2.515 | 0.013 | -0.030 (0.009) | 188 | -3.313 | 0.001 |

Random factor (population). (a) variance ± SE = 0.0002 ± 0.00009 , Wald $Z = 2.360$, $P = 0.018$.

(b) variance ± SE = 0.0003 ± 0.0001 , Wald $Z = 2.143$, $P = 0.032$.

non-significant interactions were removed by using a backward stepwise procedure. Conception date was also used as a categorical variable for illustrative purposes according to percentiles 33 and 66.

Results

Foetuses' standardized heterozygosity (SH) was higher in female foetuses (mean \pm SE = 1.057 ± 0.016 , $N = 124$) than in male foetuses (mean \pm SE = 1.009 ± 0.016 , $N = 117$; REML with study populations as random factor, sex factor: $F_{1,239} = 4.275$, $P = 0.040$; random factor: variance \pm SE = 0.001 ± 0.001 , Wald $Z = 0.744$, $P = 0.457$).

Genetic relatedness between mothers and paternally inherited half genotype of their foetuses showed that mothers of female foetuses had mated with sires who were more dissimilar to them than fathers on average in the population, and that this was not the case for mothers of male foetuses (Table 1a; Fig. 1; Bonferroni corrected pairwise comparisons: mean difference \pm SE = -0.027 ± 0.011 , $t_{189} = -2.454$, $P = 0.012$). Moreover, such sex differences in relatedness maintained even if we separate fathers according to the sex of offspring they sired: mothers that produced daughters had mated with males whose genotypes were still more dissimilar than the average genotypes of the

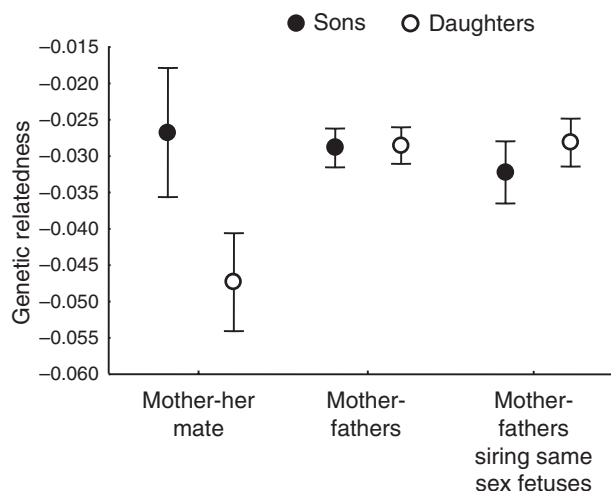


Fig. 1 Genetic relatedness between each mother and three groups of male genotypes, for male and female foetuses. 'Mother-her mate' means the relatedness between each mother and the paternally inherited half-genotype of her foetus; 'Mother-fathers' means the genetic relatedness between each mother and the paternally inherited half-genotypes of foetuses in her population; and 'Mother-fathers siring same sex foetuses' refers to the genetic relatedness between each mother and those fathers that sired foetuses of the same sex than her own foetus. Figure shows mean \pm SE of observed data. Analyses' results for these data are shown in Table 1.

subsample of daughters' fathers, demonstrating that the effect on the genotypes of daughters was not a consequence of the genetic features of a set of sires that produced daughters (Table 1b; Fig. 1).

Sex differences in SH of foetuses might potentially result after differential intrauterine mortality during gestation, from conception to the date when we collected each foetus. Additionally, conception date and mother's age might affect SH of foetuses. We conducted a mixed model (REML procedure) to investigate the effect of foetus sex, gestation length, conception date and mother's age (included as polynomial effect), with study populations as random factor (see Methods). The foetus sex factor remained significant whereas the gestation length of foetuses or its interactions with conception date or sex had no significant effects on SH. However, we found a significant effect of the interaction between conception date and sex on SH of foetuses (Table 2). For male foetuses, SH increased significantly as conception date progressed (estimated slope \pm SE = 0.0026 ± 0.0009 , $t_{211} = 2.695$, $P = 0.008$, from model in Table 2; see Fig. 2a). However, for female foetuses, SH did not change with conception date (estimated slope \pm SE = -0.0002 ± 0.001 , $t_{211} = -0.894$, $P = 0.373$, from model in Table 2; see Fig. 2a). The estimated difference in slope was significant (difference \pm SE = 0.0033 ± 0.001 , $t_{211} = 2.745$, $P = 0.007$). Fig. 2b, with conception date separated in three categories by percentiles 33 and 66, illustrates how SH increased for male foetuses and how the sex difference in SH was mainly caused by early-conceived foetuses (Fig. 2b).

Discussion

Our results for red deer show that sons were produced with lower standardized heterozygosity than daughters.

Table 2 Results of full factorial ANOVA (mixed model, REML procedure, with populations as random factor) to study the effect of sex, conception date, gestation length and mother's age on the standardized heterozygosity (SH) of foetuses. Table shows main effects and significant interactions that maintained after a backwards stepwise procedure. The random factor (populations) did not contribute to explain the variability in SH and its covariance parameter was redundant

| Effect | d.f. | F | P |
|---------------------------|-------|---------|--------|
| Intercept | 1,211 | 300.398 | <0.001 |
| Sex | 1,211 | 11.742 | 0.001 |
| Conception date | 1,211 | 2.562 | 0.111 |
| Gestation length | 1,211 | 0.483 | 0.488 |
| Mother' age | 1,211 | 1.264 | 0.262 |
| Mother's age ² | 1,211 | 1.346 | 0.247 |
| Sex x conception date | 1,211 | 7.535 | 0.007 |

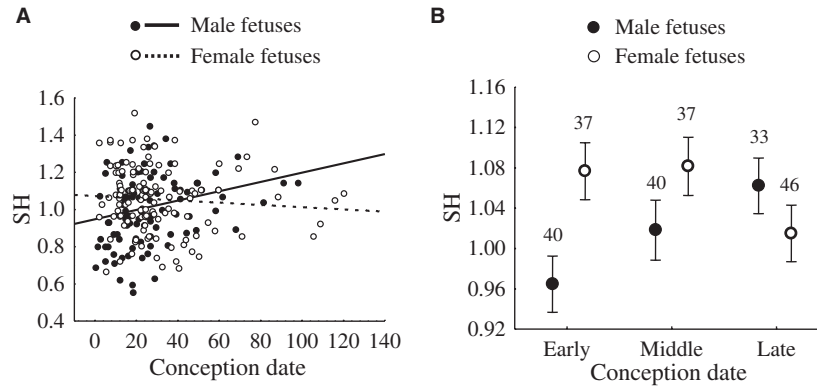


Fig. 2 Standardized heterozygosity (SH) of male and female foetuses as a function of the date when they were conceived (conception date). (A) Conception date as a continuous variable. Figure shows observed individual data. (B) Conception date categorized in three levels according to percentiles 33 and 66. Figure shows mean \pm SE of observed data. Numbers over whiskers represent sample sizes for male and female foetuses. Four male and four female foetuses could not be weighed, so sample sizes here are 113 male and 120 female foetuses.

This difference resulted because parents of daughters were more dissimilar than expected from random mating, while parents of sons did not differ from average similarity between breeding males and females in the population. This result was not caused by potential influences on the sex of offspring due to some genetic characteristic shared by the set of males siring offspring of one or another sex (see e.g. Gomendio *et al.* 2006), since the same relationships maintained within sets of males siring sons or daughters.

Although microsatellite markers are broadly used to estimate genetic relationship between parents (inbreeding coefficient), some studies have shown a weak correlation between the measures based on microsatellite markers and actual inbreeding coefficient (Balloux *et al.* 2004; Slate *et al.* 2004). Other studies, however, show a stronger relationship under certain circumstances such as strong population subdivision, small population sizes or extreme mating systems (polygyny or selfing) (Hedrick *et al.* 2001; Balloux *et al.* 2004; Da Silva *et al.* 2009; Ruiz-López *et al.* 2009). Our red deer populations are not small but they may be subdivided (Martínez *et al.* 2002) and present high levels of polygyny (Carranza *et al.* 1990, 1996; Carranza & Valencia 1999; Pérez-González *et al.* 2009). Furthermore, our results on differences between sons and daughters occur at the same loci and within the same population, which may reduce the potential influence of variations between markers and populations in the accuracy of using microsatellite markers to infer inbreeding.

Differences between sons and daughters might have resulted after sex-differential intrauterine mortality related to inbreeding. We can rule out this possibility as a main cause for our results, since the period of gestation when we collected the foetus had no significant effect on its heterozygosity. Rather, we found that sex

differences in standardized heterozygosity already occurred at early gestation, which suggests that the process causing these differences must have operated close to conception time. Therefore, these findings point to a relationship between the genetic relatedness of each breeding pair and the sex of offspring, and suggest that some process promoting parents' dissimilarity may be acting in association with the production of daughters but not so strongly for sons. Indeed this poses intriguing, unsolved questions on the potential behavioural and physiological mechanisms involved. In this sense our results provide an interesting insight at least on the temporal pattern, by showing that sex differences in inbreeding are higher at early rut and tended to decrease as the rut progressed. This change was due to SH of male foetuses, which was very low for those conceived at the beginning of the rut and increased onwards in the rutting period.

Thus, a question rises on what relevant differences occur between early and late rut? In Iberian red deer, most dominant males establish territories or hold harems at the beginning of the rutting period (Carranza *et al.* 1990, 1996; Carranza 1995, 2000; Carranza & Valencia 1999). These master stags become exhausted after several days of intense defense of harems or territories against rival males, so that it is uncommon that any of them may display intense rutting behaviour after 20 days or so of activity (Clutton-Brock *et al.* 1982; Carranza *et al.* 1990). As a consequence, the proportion of these most competitive males in the mating areas usually decreases in late rut while the presence of less competitive males increases (Clutton-Brock *et al.* 1982; L. Castillo, unpublished), which in south Iberia is associated with the dilution of territorial defense (Carranza *et al.* 1990). Therefore, if sex differences in foetuses' standardized heterozygosity were the result of

a trade-off between male qualities and dissimilarity, we may hypothesize that these differences should occur especially at early rut, when most dominant males are settled in the arena, rather than in late rut, when the least competitive males predominate.

Could female behaviour or physiology produce these results? Females might influence the association between mate dissimilarity and offspring sex at different stages. One of them is at mating. Behavioural preferences for dissimilar males have been shown for females in other species of mammals (Hoffman *et al.* 2007; Höner *et al.* 2007). Although rutting stags try to retain females within harems or territories (Carranza 1995), hinds commonly move between harems (Clutton-Brock *et al.* 1982) and collect in some preferred areas and harems (Carranza & Valencia 1999). These movements might be influenced by male characteristics such as physical appearance or olfactory cues, although so far only roaring rates have been shown to influence female mating behaviour (Charlton *et al.* 2007). But the most striking question is whether mate choice may relate to the sex of offspring produced thereafter. Some mother's features such as dominance or condition, may act as indicators that relate to biases in the sex of offspring. It was shown for red deer that high-ranking females, or females in above average body condition, produced a greater proportion of male offspring than low ranking or poor condition females, and these differences could hardly be explained by foetal mortality (on the basis of the low number of non-pregnant hinds) but by mechanisms acting before implantation (Clutton-Brock *et al.* 1986). A working hypothesis may be that high-ranking females might choose and compete for the attention of 'successful' males more than low-ranking females, who may mate with other males, for instance at less competitive areas, among which they should mate with those who are more dissimilar to them. Mating in red deer normally includes several mounting attempts before the female accepts copulation (Clutton-Brock *et al.* 1982), so there is opportunity for females to very closely evaluate cues related to male's genetic features before mating, although probably not so for competitive ability in intrasexual competition. Male dominance in the context of rutting competition might provide valid cues for female choice if she is going to produce a son rather than a daughter. For daughters, the results by Foerster *et al.* (2007) suggest that they might obtain potential additive genetic benefits from sires with low lifetime reproductive success. However, the evolution of mate choice for males with low reproductive success is difficult to conceive. Furthermore, intrasexual competition highlights the identity of most successful males while those with the lowest lifetime reproductive success may be much more difficult to

identify. Therefore, a more feasible strategy for females producing daughters might be to avoid dominant males and select mates on the basis of genetic dissimilarity, although this hypothesis deserves further research work.

Female mating behaviour could also act in combination with potential male influences on offspring sex. For instance, if females accept copulations only if the male is dominant enough or otherwise if he is at least notably dissimilar to her. Then, male features would play a role if dominants tend to sire more sons than subordinates [e.g. due to higher sperm quality (Gomendio *et al.* 2006) or testosterone levels (James 1986)]. However, empirical testing for any potential association between different criteria for female mating preferences and male features is pending.

Alternatively, females might associate sire dissimilarity and offspring sex only on the basis of postcopulatory mechanisms. Red deer females normally produce only one offspring per birth and tend to mate only once per ovulatory cycle (Clutton-Brock *et al.* 1982), so there is no opportunity for selective litter reduction or postcopulatory discrimination among several potential sires. But even so, they could assess sire's features after mating and perform postcopulatory adjustment of the sex of offspring (Rosenfeld & Roberts 2004) as recently found for birds (Pryke & Griffith 2009). After fertilization, mothers could still promote abortion and re-mating. These failures, however, would delay conception to the next ovulatory cycle some 19 days later, which means that the seasonal conditions during late gestation and lactation are likely to be suboptimal with fitness costs for offspring (Clutton-Brock *et al.* 1982, 1987). These costs suggest that pre-fertilization mate/sperm discrimination should be favoured. Nevertheless, our results only demonstrate that the association between mates' dissimilarity and offspring sex is already present at early gestation, and we cannot discard that several processes, either pre- or post-copulatory might act in concert.

Female mate choice for genetic benefits is argued to face a trade-off between preferences for non-additive compatible genes and directional choice for additive good genes (Colegrave *et al.* 2002; Mays & Hill 2004). Here we suggest that this trade-off may differ according to offspring sex. Indeed we expect this relationship not to be exclusive for red deer. Sexually antagonistic selection (Rice 1992; Chippindale *et al.* 2001; Fedorka & Mousseau 2004; Robinson *et al.* 2006; Foerster *et al.* 2007) predicts that additive genetic benefits from mates should be gender-specific. The operation of different mating processes depending on offspring sex may add a new complexity to the genotype-fitness relationships and may represent a new conceptual tool in the lek paradox debate (Hoffman *et al.* 2007; Reid 2007; Kotiaho

et al. 2008b; Neff & Pitcher 2008). We believe that the inclusion of offspring sex in the study of mating arrangements opens up an interesting avenue for research, with potential implications in our understanding of the operation of sexual selection, with consequences at the level of individual behaviour, as well as in individual and population genetics.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Sample sizes of foetuses (F Males: male foetuses; F Females: female foetuses) and mother-foetus pairs for each population (P Males: mother-male foetuses pairs; P Females: mother-female foetuses pairs)

Fig. S1 Location of the 20 studied populations in the study areas in Extremadura region (a) and in Andalucía region (b) in Spain.

Fig. S2 Frequency distribution of SH of foetuses. Left: male foetuses. Right: female foetuses.

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