Molecular Ecology (2009) **18**, 1591–1600 doi: 10.1111/j.1365-294X.2009.04150.x

Polygyny can increase rather than decrease genetic diversity contributed by males relative to females: evidence from red deer

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Abstract

Polygyny is expected to erode genetic variability by reducing the diversity of genetic contribution of males to the next generation, although empirical evidence shows that genetic variability in polygynous populations is not lost as rapidly as expected. We used microsatellite markers to study the genetic variability transmitted by mothers and fathers to offspring during a reproductive season in wild populations of a polygynous mammal, the red deer. Contrary to expectations, we found that males contributed more genetic diversity than females. Also, we compared study populations with different degrees of polygyny to find that polygyny was not related to a decrease in genetic diversity contributed by males. On the contrary, when population genetic diversity was relatively low, polygyny associated with higher genetic diversity of paternal lineage. Our results show that sexual selection, by favouring heterozygote individuals, may compensate the potential reduction of effective population size caused by polygyny, thus contributing to explain why genetic diversity is not depleted in polygynous systems.

Keywords: *Cervus elaphus*, heterozygous advantage, mating system, population genetics, sexual selection

Received 29 July 2008; revision received 21 January 2009; accepted 23 January 2009

Introduction

Theory predicts that the lower the number of males that take part in reproduction with respect to females, the smaller the effective population size with respect to census size (Wright 1938; Chesser 1991; Nunney 1991, 1993). Populations with small effective size tend to experience reductions of their genetic diversity through generations (Wright 1931, 1938; Nei *et al*. 1975; Young *et al*. 1996). Therefore, polygyny is expected to reduce genetic diversity due to its negative effects on effective population size (Briton *et al*. 1994). In agreement with this assumption, broadly used estimations of effective population size to predict genetic diversity loss are based on the variance in reproductive success among individuals in the population (Nunney 1991, 1993; Nunney & Elam 1994). However, these estimations assume that breeding individuals represent a random sample of the genetic diversity present in the adult population, an assumption that is likely violated if selection favours the reproduction of the most genetically diverse individuals.

Empirical evidence shows that genetic variability in natural populations is not lost as rapidly as expected (see, e.g. Wenink *et al*. 1998; Hmwe *et al*. 2006; Keaeuffer *et al*. 2007). Selective processes such as the interaction with predators and parasites may confer disadvantages to homozygous individuals (Keller & Waller 2002) and contribute to the maintenance of genetic diversity (Lewontin *et al*. 1978). In polygynous species, selection may be much more intense in males than in females and there is evidence that sexual selection may favour heterozygous males (Anderson 1994; Rose *et al*. 1998; Slate *et al*. 2000). Also, male–male competition has been shown to enhance selection against inbred individuals (Meagher *et al*. 2000). If sexual selection favours genetically diverse males, then the potential loss of genetic variability when only few males take part in reproduction might be compensated by the elevated genetic variability of the breeding males.

Surprisingly, and despite the largely accepted assumption that polygyny should reduce genetic diversity transmitted by males (Wright 1938; Chesser 1991; Briton *et al*. 1994), we

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are unaware of any study that has investigated the relative roles of males and females in the genetic variability transmitted to the next generation under a polygynous breeding system in natural conditions. The objective of this work was to analyse genetic diversity contributed by males and females within a breeding season in a polygynous species, the Iberian red deer (*Cervus elaphus hispanicus*), for which we can also compare study areas with different levels of polygyny to study the effect of polygyny over genetic diversity contributed by males.

Methods

Study area and data collection

The study was conducted between 2004 and 2006 in 20 areas located in Mediterranean ecosystems in Southwestern Spain (throughout Extremadura and Andalucía regions). Each area was studied only in one of the study years. Study areas averaged *c*. 500 ha in size and were located within private hunting estates. Every area typically included a part of a mountain range covered by Mediterranean scrub (*Cistus* spp., *Erica* spp., *Genista hirsuta*, *Lavandula,* spp.) and forest (*Quercus* spp., *Arbutus unedo, Olea europaea, Phyllirea* spp.) and a lower and flatter land, covered by open oak woodland or dehesa (*Quercus* spp.). Areas covered by Mediterranean scrub and forest constitute feeding and refuge areas. Dehesas constitute feeding areas and rutting arenas.

During the rutting period, we carried out car journeys along rut arenas and counted individuals to determine the sex ratio in every estates. All counts were performed by the same observer at the peak of the rut. We have already shown that most red deer clump at this time in mating areas (Carranza & Valencia 1999), so we considered that our counts were useful to estimate the relative numbers of males and females interacting during the rut. We obtained tissue samples from specimens legally culled by hunters in these areas the next hunting period. Sampled individuals in each area belonged to a Mediterranean forest zone associated with a particular rutting arena that we previously characterized on the basis of observations during the rutting season. Therefore, our data were collected from groups of animals that could interact during the previous rutting period within a mating behavioural unit. 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 less-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 the 20 study areas, we obtained samples of 292 stags, 309 pregnant hinds and their 309 foetuses. We determined the proportion of pregnant females by inspection of the reproductive tracts. In our populations, overall proportion of pregnant females was 0.78 (338 pregnant out of 433 inspected females). We used in the analyses only those pregnant females whose foetuses were sufficiently developed to obtain tissue samples. Samples of individuals consisted of pieces of muscle that were preserved frozen at –20 °C. Genomic DNA was purified by proteinase K digestion and salting out procedure (Millar *et al*. 1988).

Microsatellite genotyping

We typed individuals at 12 microsatellite loci: OarFCB193, OarCP26, OarFCB304, CelJP38, CelJP15, TGLA94, TGLA53, BM1818, CSSM22, CSSM16, 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 (PCR), we used ABI 3130 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). For each of five PCRs we created a microsatellite panel within GeneMapper software. For each panel we included all our typed samples as reference samples. We used Auto Bin function of GeneMapper to convert allele sizes into allele classes. To assess the accuracy of genotyping, 60 individuals were retyped returning identical genotypes. These retyped individuals included males, females and foetuses, and belonged to our sample stock (most of them included in this work). Retyped individuals were selected from those individuals without missing genotypes. For the samples used in this study, coincidence of at least one allele between a mother and her foetus was 99.6%. This percentage could be due to manual errors, mutations or some null alleles. Overall, our rate of missing data was 7.2%.

Estimating genetic diversity

We constructed paternal and maternal half genotypes of foetuses by comparing each foetus' genotype with its mother's genotype (Jones *et al*. 2001). There were 13% cases of ambiguity (i.e. foetus and mother shared the same heterozygous genotype) (Fuimera & Asmussen 2001). 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 belongs to. Two ploidy levels

were created: diploid individuals (stags and hinds) and haploid lineages (paternally inherited genetic component in foetuses or paternal lineage, and maternally inherited genetic component in foetuses or maternal lineage). Thus, we obtained four genotype sets (two diploids and two haploids). To calculate genetic diversity of different genotype sets with both ploidy levels, we used three different indices. One of them, the effective allele number computed as the sum of the squared frequencies of alleles (Kimura & Crow 1964), yielded almost identical results than Shannon's diversity index, so we finally omitted it. Thus, we present the results for the two following indices:

1 Shannon's diversity index (Lewontin 1972; see also Lowe *et al*. 2004) as:

$$H = -\sum p_i \ln(p_i)$$

where p_i is the proportion of the i allele in the genotype set. This index takes into account the number of different alleles and the frequency of each allele.

2 Simpson's diversity index (Simpson 1949; see also Lowe *et al*. 2004) as:

$$Dg = 1 - \{\sum[n_i(n_i - 1)]/N(N - 1)\}$$

where n_i is the absolute frequency (count) of allele i within a sample of N genotypes (diploid genomes contribute with two genotypes per locus). This index varies from 0 to 1 and mainly captures for a given locus how evenly frequencies distribute throughout different alleles. This index includes some correction by the maximum number of genotypes (N) and hence, it is little sensitive to sample size or ploidy (Lowe *et al*. 2004).

Both indices provide complementary information. Shannon index is a classical measurement of diversity in many fields, it being in the present use highly sensitive to the number of genotypes (hence to sample size and ploidy), whereas Simpson index is less sensitive to sample size or ploidy and mostly captures the evenness of the distribution of allele frequencies (Lowe *et al*. 2004). Simpson index is very similar to gene diversity or expected heterozygosity (Nei 1973), although the term heterozygosity may not be appropriate when applying it to haploid genotypes. Here we use the specific names of each index (Shannon and Simpson) and the more general term genetic diversity when referring to the results in general from either index. We computed both indices for the four sets of genotypes (adult stags, adult hinds, paternal lineage and maternal lineage of foetuses). Indices were calculated individually for samples of each study area (20) and each microsatellite marker (12), hence resulting in 20×12 data points for each of the four sets of genotypes. We included in calculations a set of genotypes for a microsatellite locus only if this set contained at least five genotypes, to avoid computing diversity indices with very low sample size, so the figure of 20×12 was slightly lower in some cases.

Genetically connected areas

To establish genetically connected areas in our data set, we used Structure 2.0 (Pritchard *et al*. 2000). This program uses Bayesian methodology to determine the level of genetic substructure in the data set independently of sampling areas (see Supporting information for detailed methods and results on genetically connected areas).

Polygyny degree

To estimate the polygyny degree in our study populations, we first obtained the observed number of fathers in each sample of foetuses from each population by using the Parentage software (Emery *et al*. 2001). This program uses a combination of Markov chain Monte Carlo techniques to select the most probable set of fathers from the distribution of parents and sibling relationships that could have produced the observed sample. Second, we used computer simulations to obtain the expected number of fathers for the same sample of foetuses according to the structure of the population (adult sex ratio), assuming random mating and equiprobability of paternity for all adult males. Then we computed the polygyny level index for each population by dividing the expected number of fathers by the genetically observed one. This index increases as the number of sires decreases with respect to the number of expected fathers under no competitive conditions, and hence it captures the level of competition among males in the population (detailed method and results of parentage analysis and polygyny degree in Supporting information).

Statistical analyses

To investigate whether paternally and maternally inherited genetic diversity of foetuses differed, we used repeated measures linear mixed models fitted by restricted maximum likelihood (REML: SPSS software version 13, SPSS Inc.). The dependent variables were the foetuses' diversity indices (both Shannon and Simpson indices) calculated individually for loci within parental lineage within estates ($2 \times 12 \times 20$ data points in total). Microsatellite genetic markers and estates were used to define the independent subjects ($N = 12 \times 20$) for the repeated measures effect (maternal and paternal lineage for each marker and estate), since the two lineage diversity indices for the same locus within estate may be correlated. Male or female adult genetic diversity was included as covariate for each respective lineage and microsatellite genetic markers and estates were also

introduced in the models as random factors. We chose the sum of squares type III and the 'unstructured' covariance structure for repeated effects. These models showed the best-fitting values of the information criteria [–2 restricted log likelihood and AIC (Akaike's information criterion)]. The final models included the fixed factor (parental lineage), the covariate and the interaction between them. Similar repeated measures mixed models were used to compare adults' genetic diversity and that of the parental lineage of foetuses (males vs. paternal lineage and females vs. maternal lineage).

To investigate the effect of polygyny on offspring genetic diversity, we conducted other mixed models by using only the paternal lineage's genetic diversity as dependent variable and males' genetic diversity as covariate. The predicted variable (polygyny values calculated for each estate) was categorized into two levels: low polygyny (values ranging from 0.97 to 1.20) and high polygyny (values ranging from 1.30 to 1.82) and added to the model as a fixed effect. Microsatellite genetic markers and estates were introduced in the models as random factors.

Simulations

We made computer simulations to see whether homozygous disadvantage could increase genetic diversity under a polygynous mating system. We used our samples of N_i adult males from the 20 populations and computed genetic diversity (both indices). From these initial populations, we took a sample of males m_i = number of fetuses/2 in order to generate a number of genotypes similar to that in the sample of paternal lineages for each population. Sampling of m_i males was performed with replacement, so any individual male could be represented more than once in the sample, as in a polygynous system. We performed three simulations with the following criteria: (i) polygyny without selection (random choice of m_i males to breed); (ii) polygyny with low homozygous disadvantage (random choice of m_i males after removing the $(N_i - m_i)/2$ more homozygous individuals in each population); and (iii) polygyny with strong homozygous disadvantage (random choice of m_i males after removing the $N_i - m_i$ more homozygous individuals in each population). Each simulation type was repeated 10 times.

Results

Genetic diversity transmitted by males and females within populations

For both indices, genetic diversity did not differ between adult males and females within populations (Fig. 1a, b). When comparing adults and parental lineage of foetuses by using the Shannon index, the results showed that the genetic diversity of adults was higher than that obtained for parental lineage of foetuses (mean ± SE: adult males = 1.386 ± 0.028, paternal lineage of foetuses = 1.309 ± 0.027; adult females = 1.394 ± 0.026, maternal lineage of foetuses = 1.220 ± 0.027; Table 1a, Fig. 1a). However, for Simpson index, paternally inherited genetic diversity was not lower but even significantly higher than the diversity of adult males (mean ± SE: adult males = 0.710 ± 0.010, paternal lineage = 0.732 ± 0.010; adult females = 0.710 ± 0.010, maternal lineage = 0.695 ± 0.011; Table 1b, Fig. 1b). When comparing both parental lineages of foetuses, our results showed that, contrary to expectations for a polygynous species, foetuses' genetic diversity inherited from paternal lineage was notably higher than genetic diversity inherited from maternal lineage, regardless the index of genetic diversity that we used (Table 2a and b, Fig. 1a and b). Furthermore, genetic diversity contributed by males with respect to females' contribution was higher in situations of

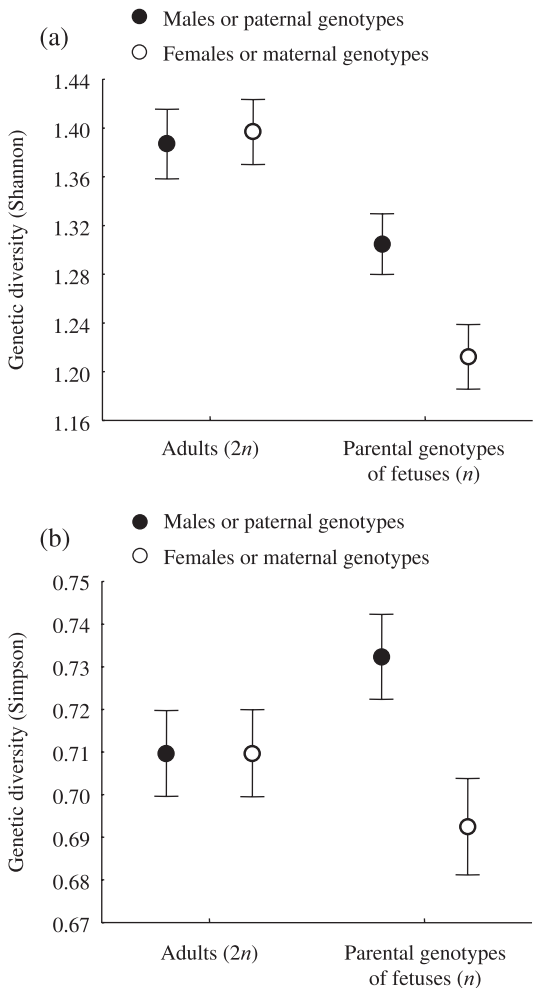

Fig. 1 Genetic diversity in adults and parental lineages of foetuses. Figure shows the observed values (mean ± SE) for Shannon (a) and Simpson (b) diversity indices.

Table 1 Comparison between adult genetic diversity and that of the parental lineage of foetuses (up, males vs. paternal lineage; down, females vs. maternal lineage). Results from repeated measures linear mixed model analyses (REML procedure) with genetic diversity as dependent variable

	(a) Shannon index				(b) Simpson index			
Parameter	Estimate (SE)	d.f.	*t*	*P*	Estimate (SE)	d.f.	*t*	*P*
Intercept	1.307 (0.083)	15	15.700	< 0.001	0.730 (0.030)	14	24.314	< 0.001
Males vs. paternal lineage	0.093 (0.020)	209	4.630	< 0.001	–0.017 (0.008)	207	–2.217	0.028
Intercept	1.215 (0.086)	16	14 068	< 0.001	0.691 (0.032)	14	21.746	< 0.001
Females vs. maternal lineage	0.192 (0.014)	219	13.195	< 0.001	0.022 (0.006)	219	3678	< 0.001

Random factors. a) Shannon index: males vs. paternal lineage: variance ± SE = 0.021 ± 0.008, Wald Z = 2.517, $P = 0.012$ for estates; variance ± SE = 0.067 ± 0.029, Wald Z = 2.255, $P = 0.024$ for microsatellite markers; females vs. maternal lineage: variance ± SE = 0.025 ± 0.010, Wald Z = 2.253, $P = 0.012$, for estates; variance ± SE = 0.070 ± 0.0313, Wald Z = 2.240, $P = 0.025$ for microsatellite markers. b) Simpson index: males vs. paternal lineage: variance ± SE = 0.001 ± 0.001, Wald Z = 1.980, $P = 0.048$ for estates; variance ± SE = 0.009 ± 0.004, Wald Z = 2.228, $P = 0.026$ for microsatellite markers; females vs. maternal lineage: variance ± SE = 0.002 ± 0.001, Wald Z = 1.927, $P = 0.054$ for estates; variance ± SE = 0.010 ± 0.004, Wald Z = 2.204, $P = 0.028$ for microsatellite markers.

Table 2 Effect of parental lineage (maternal vs. paternal lineage) on offspring genetic diversity: Shannon and Simpson indices. Results from repeated measures linear mixed model analyses (REML procedure). The table shows the estimate differences in intercepts and slopes with maternal lineage as reference

	(a) Shannon index				(b) Simpson index			
Parameter	Estimate (SE)	d.f.	*t*	*P*	Estimate (SE)	d.f.	*t*	*P*
Intercept	0.074 (0.055)	36	1.330	0.192	0.010 (0.033)	88	0.301	0.764
Paternal lineage	0.370 (0.078)	247	4.747	< 0.001	0.209 (0.049)	261	4.289	< 0.001
Adult diversity	0.810 (0.036)	37	22.534	< 0.001	0.954 (0.044)	98	21.523	< 0.001
Paternal lineage × adult diversity	– 0.196 (0.053)	249	–3.654	< 0.001	–0.239 (0.067)	262	–3.570	< 0.001

Random factors. a) Shannon index: variance ± SE = 0.007 ± 0.003, Wald Z = 2.233, $P = 0.026$ for estates; variance ± SE = 0.0003 ± 0.0009, Wald Z = 0.291.043, $P = 0.771$ for microsatellite markers. b) Simpson index: variance ± SE = 0.0003 ± 0.0002, Wald Z = 1.109, $P = 0.267$ for estates; variance ± SE = 0.000 ± 0.0002, Wald Z = 0.547, $P = 0.585$ for microsatellite markers.

low genetic diversity (see estimates for slope differences in Table 2a and b).

Our analyses assumed that sampled adult males for each study area represent the genetic composition of males interacting in the arena during the previous rutting period. We know that stags move during the rut to mating arenas that may be apart from the ranges that they use during the rest of the year (Clutton-Brock *et al*. 1982). If some of the males who mated in a particular area belonged to adjacent areas, genetic diversity of sampled males in a particular area may not include such wandering males and hence it may underestimate the genetic diversity of males actually interacting during the rut (Wahlund effect: Wahlund 1928). To avoid this problem, we repeated the analyses at a higher spatial scale, grouping areas at the level for which we could safely assume no movement of individuals between them, either because the areas were fenced estates that prevent deer movement between them or because there were geographical distances or discontinuities that produce fragmentation of populations which was assessed on the basis of gene flow (see Supporting Information). For this new grouping of areas ($N = 12$ instead of 20), the results were almost identical: all mean diversity values were higher as a consequence of the larger size of the areas (Shannon index: males mean ± SE = 1.481 ± 0.035, females mean ± SE = 1.491 ± 0.033, paternal lineage of foetuses mean ± SE = 1.402 ± 0.033, maternal lineage of foetuses mean ± SE = 1.337 ± 0.032; Simpson index: males mean ± SE = 0.732 ± 0.012, females mean ± SE = 0.734 ± 0.011, paternal lineage of foetuses mean ± SE = 0.749 ± 0.013, maternal lineage of foetuses mean ± SE = 0.718 ± 0.013), but the relationship between them maintained largely unaffected (Table 3).

Simulation results

To see whether our previous results could be achieved by a mechanism of selective disadvantage of homozygous males in a polygynous system, we performed computer

Table 3 Effect of parental lineage (maternal vs. paternal lineage) on offspring genetic diversity after grouping those genetically connected areas. Results from repeated measures linear mixed model analyses (REML procedure). The table shows the estimate differences in intercepts and slopes with maternal lineage as reference

	a) Shannon index				b) Simpson index			
Parameter	Estimate (SE)	d.f.	t	*P*	Estimate (SE)	d.f.	t	*P*
Intercept	0.099 (0.065)	41	1.505	0.140	0.009 (0.001)	59	0.032	0.975
Paternal lineage	0.347 (0.095)	139	3.643	< 0.001	0.202(0.058)	142	3.507	0.001
Adult diversity	0.830 (0.040)	40	20.713	< 0.001	0.976 (0.052)	64	18.885	< 0.001
Paternal lineage × adult diversity	–0.180 (0.062)	140	–0.897	0.004	–0.229 (0.077)	142	–2.961	0.004

Random factors. a) Shannon index: variance ± SE = 0.006 ± 0.003, Wald Z = 1.733, $P = 0.083$ for areas; variance ± SE = 0.0003 ± 0.001, Wald Z = 0.319, $P = 0.750$ for microsatellite markers. b) Simpson index: variance ± SE = 0.0002 ± 0.0002, Wald Z = 0.738, $P = 0.460$ for areas; variance ± SE = 0.000 ± 0.0002, Wald Z = 0.379, $P = 0.704$ for microsatellite markers.

Table 4 Effect of polygyny on paternally contributed genetic diversity. Results from a linear mixed model (REML procedure). The table shows the estimate differences in intercepts and slopes with high polygyny as reference

	a) Shannon index				b) Simpson index			
Parameter	Estimate (SE)	d.f.	*t*	*P*	Estimate (SE)	d.f.	*t*	*P*
Intercept	0.651 (0.095)	90	6.844	< 0.001	0.271 (0.055)	119	4.891	< 0.001
Low polygyny	–0.256 (0.128)	166	–2.005	0.047	–0.083 (0.078)	195	–1.072	0.285
Male diversity	0.455 (0.062)	153	7.298	< 0.001	0.641 (0.076)	140	8.460	< 0.001
Low polygyny × male diversity	0.199 (0.084)	193	2.379	0.018	0.116 (0.106)	195	1.099	0.274

Random factors. a) Shannon index: variance ± SE = 0.006 ± 0.004, Wald Z = 1.545, $P = 0.122$ for estates; variance ± SE = 0.009 ± 0.006, Wald Z = 1.559, $P = 0.119$ for microsatellite markers. b) Simpson index: variance ± SE = 0.001 ± 0.0001, Wald Z = 1.254, $P = 0.210$ for estates; variance ± SE = 0.0001 ± 0.0004, Wald Z = 0.335, $P = 0.737$ for microsatellite markers.

simulations. Simulation results show that polygyny without selection decreases genetic diversity due to a reduction of genotype sample size. However, this decrease compensated as polygyny included a stronger selective disadvantage for homozygotes (Fig. 2a, b). For Simpson index, selection against homozygotes increased genetic diversity even above that of all males in the population due to the increase of the relative frequency of rare alleles compared to most common alleles (Fig. 2b).

Effects of polygyny

Polygyny level was not related to a decrease in genetic diversity transmitted by males, as it might be expected. For Shannon's index, in situations of low genetic diversity, males transmitted more genetic diversity under high polygyny conditions than under low polygyny conditions (Fig. 3, Table 4a). Conversely, in situations of high genetic diversity this result reverted (Fig. 3, Table 4a). For Simpson's index, the pattern was the same although the effect was not significant (Table 4b).

Discussion

We have shown for the first time that males in a polygynous species transmit more genetic diversity than females, especially in situations of low population genetic diversity. This result maintained when our study populations were grouped in larger, genetically connected areas, indicating that the higher genetic diversity in paternal lineage compared with maternal lineage was not caused by rutting males immigrating from other areas. Also, paternally transmitted genetic diversity was not only higher than maternally transmitted one, but it was also higher than the genetic diversity in the population of adults when we used Simpson index to estimate genetic diversity, indicating that allele frequencies in paternal lineages followed a more even distribution than in the population of adults. Simulation results show that selection against homozygous males can explain these results. We also found that, in situations of low levels of genetic diversity, polygyny was not related to a decrease of paternally inherited genetic diversity of foetuses; rather, it was positively related to higher paternally

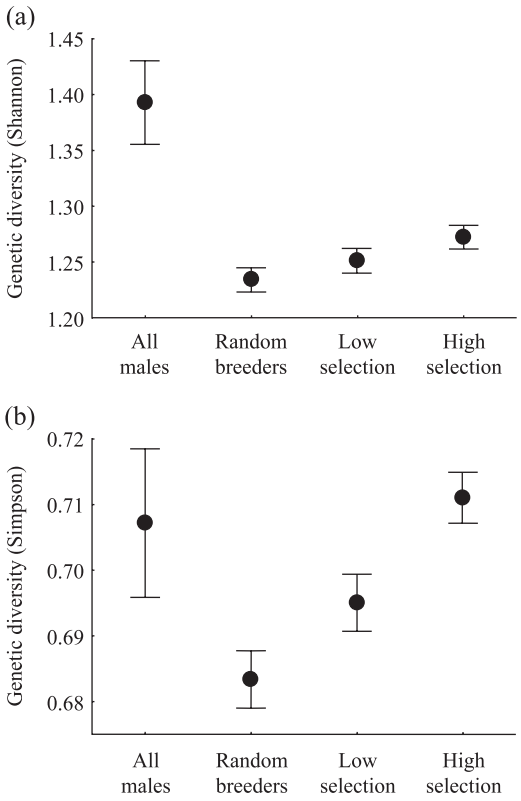

Fig. 2 Simulation results. Figure shows genetic diversity (mean ± SE) of the sample of males for the study populations (all males), compared with simulation results when a subsample of breeding males was chosen at random from the males of every population to simulate polygyny either without selection (random breeders), with low homozygous disadvantage (low selection), or with high homozygous disadvantage (strong selection). See details in Methods. Mean value for all males varied slightly from that shown in Fig. 1 because when computing diversity to compare it with parental lineages of fetuses we avoided some markers with very small sample size (less than 5 genotypes, see Methods), and it was not necessary here in the simulations. Each simulation type was repeated 10 times. (a) *Shannon index*: ANOVA for differences between simulated situations: $F_{3,585} = 7.458$; $P < 0.0001$. (b) *Simpson index*: ANOVA for differences between simulated situations: $F_{3,585} = 7.579$; $P < 0.0001$.

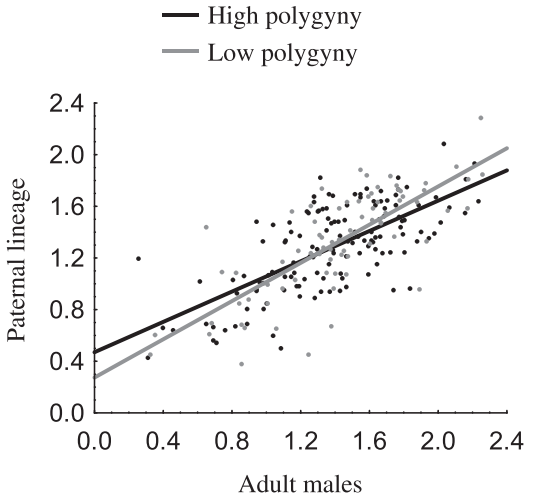

Fig. 3 Relationship between males' genetic diversity and the diversity of paternal lineage of fetuses (measured by Shannon index for all estates and microsatellite markers) under two polygyny conditions: high polygyny (black line) and low polygyny (grey line).

inherited genetic diversity (Shannon index) of foetuses. This effect reverted as population genetic diversity increased (see Fig. 3).

For a polygynous mammal like the red deer, the actual level of polygyny may depend on the operational sex ratio, on the degree of spatial aggregation of females and on the age structure of male subpopulation that confer different mating abilities to some males relative to others (Clutton-Brock *et al*. 1982, 1988; Shuster & Wade 2003). Polygyny may have opposite consequences on the genetic diversity contributed by males: on one hand (i), the decrease of the number of breeding males may reduce the genetic diversity of paternal lineage (Wright 1938; Chesser 1991; Nunney 1991, 1993), but on the other hand (ii), an increase of male competition may prompt the heterozygous advantage process (Meagher *et al*. 2000) and hence it may tend to increase the genetic diversity of paternal lineage (e.g. Lewontin *et al*. 1978). Our results suggest that in situations of low genetic diversity, the effect of heterozygote advantage to increase genetic diversity might outweigh the effect of the lower number of breeding males to decrease it.

Many studies have shown a link between overall heterozygosity at molecular markers and fitness (Mitton & Grant 1984; Allendorf & Leary 1986; Ledig 1986; Houle 1989; Mitton 1993; Coulson *et al*. 1998; Hansson & Westerberg 2002). There are several hypotheses that connect heterozygosity and fitness (Hansson & Westerberg 2002; Keller & Waller 2002). For instance, the overdominance effect may confer advantages to heterozygous individuals and the dominance relationship between alleles can decrease the risk of expressing recessive deleterious alleles (Charlesworth & Charlesworth 1987). Heterozygote advantage can be due to an increase of developmental homeostasis obtained by enzyme heterozygosity (Mitton & Grant 1984; Mitton 1993). Also it can be due to the advantage against infections favoured by major histocompatibility complex (MHC) heterozygosity (Penn *et al*. 2002). However, how exactly heterozygosity acts on fitness remains often unclear. For example, the correlation heterozygosity–fitness is normally interpreted as the result of the connection between inbreeding and heterozygosity, but it has been observed that individual heterozygosity and inbreeding coefficient can be weakly correlated (Balloux *et al*. 2004; Slate *et al*. 2004).

In red deer, homozygous males experience disadvantages at different stages of their life histories, notably as differences in mortality (Coulson *et al*. 1998) or breeding success (Slate *et al*. 2000). Mortality of homozygotes might have occurred for males during their lives before we took our sample of adult males and may also act at the level of intrauterine mortality before we took our sample of foetuses. For adult males, our results of high paternally inherited diversity are relative to the sample of adult males, so they are not affected by previous mortality in the cohorts of adult males. For intrauterine mortality to be relevant as an explanation for our results, we should expect differences in the proportion of pregnant females among populations that relate to population genetic diversity, but this was clearly not the case ($r = –0.026$, $N = 19$, $P = 0.915$). On the other hand, intrauterine mortality related to population diversity could hardly explain our result of a relationship between polygyny and paternally inherited genetic diversity when population genetic diversity is controlled in the analysis. Therefore, the advantage of heterozygous males relevant for our results should operate mainly at competition for mates, in agreement with recent results for mice (Meagher *et al*. 2000).

Selection has been proposed to produce lower than expected effective population size by increasing heritable-based fitness-variability (Robertson 1961; Santiago & Caballero 1995). Our results suggest an opposite effect of the intensity of selection to produce higher than expected genetically effective population size, for which a likely mechanism is the advantage of heterozygous males in the competition over mates. Some studies support the advantage of heterozygous males (Keller *et al*. 1994; Kempenaers *et al*. 1996) including red deer (Slate *et al*. 2000) and that the fitness reduction experienced by homozygous males is magnified under conditions of male–male competition (Meagher *et al*. 2000).

The actual effect of sexual selection to increase effective population size with respect to theoretical estimations may be even higher than our data indicate. Storz *et al*. (2001) suggested that small effective population size in one reproductive season caused by few males contributing to reproduction might be compensated by higher turnover of males compared to females since they have shorter reproductive lifetime, which is the case for red deer, where males have much shorter reproductive lifespan than females (Clutton-Brock *et al*. 1982; Carranza *et al*. 2004).

At the interspecific level, polygyny has been observed to relate to higher levels of genetic variability in birds (Møller *et al*. 2008). This association might be caused by increased germline mutation rate in polygynous males (Møller & Cuervo 2004; Petrie & Roberts 2007) or because male breeding success may depend on genetic diversity (Mays & Hill 2004). Germline mutation rate has been suggested to increase in conditions of strong sperm competition (Møller & Cuervo 2004; Petrie & Roberts 2007). The occurrence of sperm competition has not been investigated in depth in red deer. However, behavioural observations indicate that males strongly compete to monopolize groups of females before copulation and that females normally copulate only once per ovulatory cycle (Clutton-Brock *et al*. 1982), which suggests that the role of sperm competition may not be strong in this species. But anyway, although germline mutation rate might be considered as a possible cause for interspecific differences in genetic variability, it cannot explain the differences in genetic variability observed here between paternal and maternal lineages, since they occur within one generation. By contrast, recent studies increasingly show that the role of sexual selection favouring genetic diversity rather than heritable quality may be much higher than previously assumed (Mays & Hill 2004), which may contribute to explain why genetic variability is not depleted under conditions of strong sexual selection (Hoffman *et al*. 2007; Keaeuffer *et al*. 2007; Petrie & Roberts 2007; Kotiaho *et al*. 2008).

Much emphasis has been put on the demographic estimation of effective population size (see Engen *et al*. 2005 and references herein) but recent works indicate that demographic estimations may not reflect actual genetic size of the breeding population (Wenink *et al*. 1998; Hmwe *et al*. 2006; Keaeuffer *et al*. 2007), so that assumptions on the relationship between polygyny, effective population size and genetic variability should be revised. Our results for red deer indicate that variance in male mating success may contribute to effective population size if mating success depends on genetic diversity. The generalization of these results to other species deserve further research to see whether sexual selection may compensate the effects of genetic drift on allele frequencies, and hence polygynous species may be less vulnerable to the loss of genetic variability than previously thought (Briton *et al*. 1994; Frankham 2005).

Acknowledgements

We thank Armando Caballero, David Coltman and five anonymous reviewers for comments to the manuscript. We also thank Ian Wilson for assistance in the use of PARENTAGE software. Autonomic goverments of Extremadura (Junta de Extremadura) and Andalucía (Junta de Andalucía) and owners of hunting estates provided permissions and facilities for field work. C. Galán, J. L. Fernández-Garcia, J. Torres, L. Castillo, S. Alarcos, C. Sánchez-Prieto, M. J. Palacios, A. Flores, J. Valencia, S. Martín, M. Barbosa and Y. Moreno, helped in field and laboratory work. Financial support came from Spanish Ministry of Education and Science, projects REN2001-1524, CGL2004-05993 and CGL2007-63594.

References

Allendorf FW, Leary RF (1986) Heterozygosity and fitness in natural populations of animals. In: *Conservation Biology: The Science of Scarcity and Diversity* (ed. Soulé ME), pp. 57–76. Sinauer & Associates, Sunderland, Massachusetts.

Anderson M (1994) *Sexual Selection*. Princeton University Press, Princeton, New Jersey.

Balloux F, Amos W, Coulson T (2004) Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology*, **13**, 3021–3031.

Bonnet A, Thevenon S, Maudet F, Maillard JC (2002) Efficiency of semi-automated fluorescent multiplex PCRs with 11 microsatellite markers for genetic studies of deer populations. *Animal Genetics*, **33**, 343–350.

Briton J, Nurthen RK, Briscoe DA, Frankham R (1994) Modelling problems in conservation genetics using *Drosophila*: consequences of harem. *Biological Conservation*, **69**, 267–275.

Carranza J, Valencia J (1999) Red deer females collect on male clumps at mating areas. *Behavioral Ecology*, **10**, 525–532.

Carranza J, Alarcos S, Sánchez-Prieto CB, Valencia J, Mateos C (2004) Disposable-soma senescence mediated by sexual selection in an ungulate. *Nature*, **432**, 215–218.

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, **18**, 237–268.

Chesser RK (1991) Influence of gene flow and breeding tactics on gene diversity within populations. *Genetics*, **129**, 437–447.

Clutton-Brock TH, Guinness FE, Albon SD (1982) *Red Deer: Behavior and Ecology of Two Sexes*. Edinburgh University Press, Edinburgh, UK.

Clutton-Brock TH, Albon SD, Guinness FE (1988) Reproductive success in male and female red deer. In: *Reproductive Success* (eds Clutton-Brock TH), pp. 325–343. The University of Chicago Press, Chicago & London.

Coulson TN, Pemberton JM, Albon SD *et al*. (1998) Microsatellite reveal heterosis in red deer. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 489–495.

Emery AM, Wilson IJ, Craig S, Boyle PR, Noble LR (2001) Assignment of paternity groups without access to parental genotypes: multiple mating and developmental plasticity in squid. *Molecular Ecology*, **10**, 1265–1278.

Engen S, Lande R, Saether BE (2005) Effective size of a fluctuating age-structured population. *Genetics*, **170**, 941–954.

Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131–140.

Fuimera AC, Asmussen MA (2001) Difficulties in parentage analysis: the probability that an offspring and parent have the same heterozygous genotype. *Genetical Research*, **78**, 163–170.

Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology*, **11**, 2476–2474.

Hmwe SS, Zachos FE, Sale JB, Rose HR, Hartl GB (2006) Genetic variability and differentiation in red deer (*Cervus elaphus*) from Scotland and England. *Journal of Zoology*, **270**, 479–487.

Hoffman JI, Forcada J, Trathan PN, Amos W (2007) Female fur seals show active choice for males that are heterozygous and unrelated. *Nature*, **445**, 912–914.

Houle D (1989) Allozyme-associated heterosis in *Drosophila melanogaster*. *Genetics*, **123**, 789–801.

Jones AG, Walker D, Kvarnemo C, Lindström K, Avise JC (2001) How cuckoldry can decrease the opportunity for sexual selection: data and theory from a genetic parentage analysis of the sand goby, *Pomatoschistus minitus*. *Proceedings of the National Academy of Sciences, USA*, **98**, 9151–9156.

Keaeuffer R, Coltman DW, Chapuis JL, Pontier D, Réale D (2007) Unexpected heterozygosity in an island mouflon population founded by a single pair of individuals. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 527–533.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**, 230–241.

Keller FK, Arcese P, Smith JNM, Hochachka WM, Stearns SC (1994) Selection against inbred song sparrows during a natural population bottleneck. *Nature*, **372**, 356–357.

Kempenaers B, Adriansen F, Van Noordiwijk AJ, Dhont A (1996) Genetic similarity, inbreeding and hatching failure in blue tits: are unhatched eggs infertile?. *Proceedings of the Royal Society B: Biological Sciences*, **263**, 179–185.

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics*, **49**, 725–738.

Kotiaho JS, Lebas NR, Puurtinen M, Tomkins JL (2008) On the resolution of the lek paradox. *Trends in Ecology & Evolution*, **23**, 1–3.

Kuehn R, Schroeder W, Pirchner F, Rottmann O (2003) Genetic diversity, gene flow and drift in Bavarian red deer populations (*Cervus elaphus*). *Conservation Genetics*, **4**, 157–166.

Ledig FT (1986) Heterozygosity, heterosis and fitness in outbreeding plants. In: *Conservation Biology: The Science of Scarcity and Diversity* (ed. Soulé ME), pp. 77–90. Sinauer & Associates, Sunderland, Massachusetts.

Lewontin RC (1972) The apportionments of human diversity. *Evolutionary Biology*, **6**, 381–398.

Lewontin RC, Ginzburg LR, Tuljapurkar SD (1978) Heterosis as an explanation for large amounts of genetic polymorphism. *Genetics*, **88**, 149–170.

Lowe A, Harris S, Ashton P (2004) *Ecological Genetics: Design, Analysis, and Application*. Blackwell, Oxford.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Martínez JG, Carranza J, Fernández-García JL, Sánchez-Prieto CB (2002) Genetic variation of red deer populations under hunting exploitation in southwest Spain. *Journal of Wildlife Management*, **66**, 1273–1282.

Martínez M, Rodríguez-Vigal C, Jones OR, Coulson T, San Miguel A (2005) Different hunting strategies select for different weights in red deer. *Biology Letters*, **1**, 353–356.

Mays LM, Hill GE (2004) Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology & Evolution*, **19**, 554–559.

Meagher S, Penn DJ, Potts WK (2000) Male-male competition magnifies inbreeding depression in wild house mice. *Proceedings of the National Academy of Sciences, USA*, **97**, 3324–3329.

Millar SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Research*, **16**, 1215.

Mitton JB (1993) Theory and data pertinent to the relationship between heterozygosity and fitness. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (ed. Thornhill NW), pp. 17–35. University of Chicago Press, Chicago, Illinois.

Mitton JB, Grant MC (1984) Associations among protein heterozygosity, growth rate, and developmental homeostasis. *Annual Reviews in Ecology and Systematics*, **15**, 479–499.

Møller AP, Cuervo JJ (2004) Sexual selection, germline mutation rate and sperm competition. *BMC Evolutionary Biology*, **3**, 1–11.

Møller AP, Garamszegi LZ, Spottiswoode CN (2008) Genetic similarity, breeding distribution range and sexual selection. *Journal of Evolutionary Biology*, **21**, 213–225.

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA*, **70**, 3321–3323.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Nunney L (1991) The influence of age structure and fecundity on effective population size. *Proceedings of the Royal Society B: Biological Sciences*, **246**, 71–76.

Nunney L (1993) The influence of mating system and overlapping generations on effective population size. *Evolution*, **53**, 1–10.

Nunney L, Elam DR (1994) Estimating the effective size of conserved populations. *Conservation Biology*, **8**, 175–184.

Penn DJ, Damjanovich K, Potts WK (2002) MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proceedings of the National Academy of Sciences, USA*, **99**, 11260–11264.

Petrie M, Roberts G (2007) Sexual selection and the evolution of evolvability. *Heredity*, **98**, 198–205.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Robertson A (1961) Inbreeding in artificial selection programmes. *Genetical Research*, **2**, 189–194.

Rose KE, Clutton-Brock TH, Guinness FE (1998) Cohort variation in male survival and mating success in red deer. *Journal of Animal Ecology*, **67**, 979–986.

Santiago E, Caballero A (1995) Effective size of populations under selection. *Genetics*, **139**, 1013–1030.

Shuster SM, Wade MJ (2003) *Mating Systems and Strategies*. Princeton University Press, Princeton, New Jersey.

Simpson EH (1949) Measurement of diversity. *Nature*, **163**, 688.

Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH (2000) Inbreeding depression influences lifetime breeding success in a wild population of red deer. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 1657–1662.

Slate J, David P, Dodds KG *et al*. (2004) Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity*, **93**, 255–254.

Storz JF, Bhat HR, Kunz H (2001) Genetic consequences of polygyny and social structure in an Indian fruit bat, *Cynopterus sphinx*, II. Variance in male mating success and effective population size. *Evolution*, **55**, 1224–1232.

Wahlund S (1928) Zusammersetung von populationen und korrelation-sercheinungen von standpunkt der verebungslehre aus betrachtet. *Hereditas*, **11**, 65–106.

Wenink PW, Groen AF, Roelke-Parker ME, Prins HHT (1998) African buffalo maintain high genetic diversity in the major histocompatibility complex in spite of historically known population bottleneck. *Molecular Ecology*, **7**, 1315–1322.

Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.

Wright S (1938) Size of population and breeding structure in relation to evolution. *Science*, **87**, 1417–1429.

Young AG, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation in plants. *Trends in Ecology & Evolution*, **11**, 413–418.

This study is part of the doctoral thesis of Javier Pérez-González, conducted under the supervision of Juan Carranza with the collaboration of Concha Mateos. All three authors are interested in mating systems and sexual selection, including the use of molecular genetic tools to understand behavioural processes and their evolutionary consequences. They are included in a group that devotes much of its research on Iberian red deer and other wild ungulates in Mediterranean ecosystems in Spain.

Supporting information

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

Appendix S1 Genetic characteristics of study populations; Genetically connected areas; Parentage analysis and polygyny degree

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