Heterozygosity-Fitness Correlations and Inbreeding Depression in Two Critically Endangered Mammals

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Abstract: *The relation among inbreeding, heterozygosity, and fitness has been studied primarily among outbred populations, and little is known about these phenomena in endangered populations. Most researchers conclude that the relation between coefficient of inbreeding estimated from pedigrees and fitness traits (inbreeding-fitness correlations) better reflects inbreeding depression than the relation between marker heterozygosity and fitness traits (heterozygosity-fitness correlations). However, it has been suggested recently that heterozygosity-fitness correlations should only be expected when inbreeding generates extensive identity disequilibrium (correlations in heterozygosity and homozygosity across loci throughout the genome). We tested this hypothesis in Mohor gazelle (*Gazella dama mhorr*) and Iberian lynx (*Lynx pardinus*). For Mohor gazelle, we calculated the inbreeding coefficient and measured heterozygosity at 17 microsatellite loci. For Iberian lynx, we measured heterozygosity at 36 microsatellite loci. In both species we estimated semen quality, a phenotypic trait directly related to fitness that is controlled by many loci and is affected by inbreeding depression. Both species showed evidence of extensive identity disequilibrium, and in both species heterozygosity was associated with semen quality. In the Iberian lynx the low proportion of normal sperm associated with low levels of heterozygosity was so extreme that it is likely to limit the fertility of males. In Mohor gazelle, although heterozygosity was associated with semen quality, inbreeding coefficient was not. This result suggests that when coefficient of inbreeding is calculated on the basis of a genealogy that begins after a long history of inbreeding, the coefficient of inbreeding fails to capture previous demographic information because it is a poor estimator of accumulated individual inbreeding. We conclude that among highly endangered species with extensive identity disequilibrium, examination of heterozygosity-fitness correlations may be an effective way to detect inbreeding depression, whereas inbreeding-fitness correlations may be poor indicators of inbreeding depression if the pedigree does not accurately reflect the history of inbreeding.*

Keywords: endangered species, *Gazella dama mhorr*, HFCs, Iberian lynx, IFCs, inbreeding, *Lynx pardinus*, Mohor gazelle, semen quality

Correlaciones Heterocigosidad- Adaptabilidad y Depresión Endogámica en Dos Especies de Mamíferos Críticamente en Peligro

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Resumen: *La relación entre endogamia, heterocigosidad y adaptabilidad ha sido estudiada en poblaciones exogámicas principalmente, y se conoce poco de estos fenómenos en poblaciones en peligro. La mayoría de los investigadores concluyen que la relación entre el coeficiente de endogamia estimado a partir de los pedigrís y los atributos de adaptabilidad (correlaciones endogamia-adaptabilidad) refleja mejor la depresión endogámica que la relación entre la heterocigosidad y los atributos de adaptabilidad (correlaciones heterocigosidad-adaptabilidad). Sin embargo, recientemente se ha sugerido que las correlaciones heterocigosidad-adaptabilidad solo podrían esperarse cuando la endogamia genera desequilibrio extensivo de identidad (correlaciones en heterocigosidad y homocigosidad en loci en todo el genoma). Probamos esta hipótesis en la gacela de Mohr (*Gazella dama mhorr*) y el lince ibérico (*Lynx pardinus*). Para la gacela de Mohr, calculamos el coeficiente de endogamia y medimos la heterocigosidad en 17 loci microsaltélites. Para el lince ibérico, medimos la heterocigosidad en 36 loci microsatélite. En ambas especies estimamos la calidad del semen, un atributo fenotípico directamente relacionado con la adaptabilidad que es controlado por muchos loci y que es afectado por la depresión endogámica. Ambas especies mostraron evidencia de un desequilibrio extensivo de identidad, y en ambas especies la heterocigosidad fue asociada con la calidad del semen. En el lince ibérico, la baja proporción de esperma normal asociada con bajos niveles de heterocigosidad fue tan extrema que es probable que limite la fertilidad de machos. En la gacela de Mohr, aunque la heterocigosidad fue asociada con la calidad del semen, el coeficiente de endogamia no lo fue. Este resultado sugiere que cuando el coeficiente de endogamia es calculado con base en una genealogía que comienza después de una larga historia de endogamia, el coeficiente de endogamia falla en la captura de información demográfica previa porque es un estimador pobre de la endogamia individual acumulada. Concluimos que en especies críticamente en peligro con desequilibrio extensivo de identidad, el examen de las correlaciones heterocigosidad-adaptabilidad puede ser una forma efectiva para detectar la depresión por endogamia, mientras que la correlaciones endogamia-adaptabilidad pueden ser indicadores pobres de la depresión por endogamia si el pedigrí no refleja con precisión la historia de la endogamia.*

Palabras Clave: calidad del semen, endogamia, especies en peligro, *Gazella dama mhorr*, gacela Mohr, HFCs, IFCs, lince ibérico, *Lynx pardinus*

Introduction

Decreases in abundance and increases in isolation of animal populations may lead to widespread inbreeding and loss of genetic variability. Inbreeding sensu lato (i.e., increased genetic homozygosity due to mating between relatives or to genetic drift) (Charlesworth & Charlesworth 1999), leads to a reduction in fitness (i.e., inbreeding depression) in both captive (Ralls et al. 1979; Gomendio et al. 2000) and wild populations (Crnokrak & Roff 1999; Keller & Waller 2002).

The traditional approach to analyzing the effects of inbreeding on fitness has been to calculate the coefficient of inbreeding, *f* (Wright 1922), when pedigree information was available (Keller & Waller 2002). However, reconstructing pedigrees accurately over several generations in natural populations is difficult, so an alternative approach, which recognizes that inbreeding reduces heterozygosity, is to use molecular measures of heterozygosity as surrogates of the inbreeding coefficient. Inbred individuals have a higher probability of homozygosity at all loci across the genome relative to outbred individuals. Inbreeding can thus generate correlations in heterozygosity and homozygosity across loci throughout the genome, a phenomenon termed identity disequilibrium (Weir & Cockerham 1973; David 1998; Szulkin et al. 2010). Therefore, on the basis of inbreeding theory it has been assumed that positive and significant correlations between heterozygosity and traits associated with fitness (i.e., heterozygosity-fitness correlations, hereafter HFCs) were indicative of inbreeding depression (Hansson & Westerberg 2002; Chapman et al. 2009). Heterozygosity-fitness correlations also occur among outbred populations, but they are generally weak, and heterozygosity tends to explain a low percentage of variance in fitness (Balloux et al. 2004; Slate et al. 2004). An alternative explanation is that HFCs are caused by linkage disequilibrium (i.e., nonrandom association of alleles at 2 loci in the gamete) between the marker loci and the fitness-trait loci (David 1998; Hansson & Westerberg 2002; Balloux et al. 2004). However, it has been suggested recently that although linkage disequilibrium can increase HFCs, linkage disequilibrium is not enough to generate HFCs because the effect of linked genes on fitness is small compared with the effect of the whole genome on fitness (Szulkin et al. 2010).

The first step in understanding how HFCs are generated is determining whether inbreeding coefficient and heterozygosity are correlated (Balloux et al. 2004; Pemberton 2004; Slate et al. 2004). Limited evidence shows that whereas among outbred natural populations the association between pedigree inbreeding coefficients and individual heterozygosity is weak (Markert et al. 2004; Slate et al. 2004; Overall et al. 2005), higher correlations are found among some endangered populations (Hedrick et al. 2001). This is probably because endangered

populations tend to have higher levels and greater variance of inbreeding than outbred populations (Ruiz-López et al. 2009).

The second step is to determine whether inbreeding coefficient and heterozygosity correlate with the same fitness traits (inbreeding-fitness correlations [IFCs] and HFCs). Although the conditions under which HFCs are expected to be associated with genome-wide inbreeding are thought to be rather restrictive (Balloux et al. 2004), greater mean and variance in f among endangered species may make HFCs easier to detect (Grueber et al. 2008). Results of a recent meta-analysis show no evidence that populations likely to have higher inbreeding variance have higher HFCs (Chapman et al. 2009), but this may be due to the use of inaccurate measures of demographic structure to infer levels of inbreeding. Authors of some studies on threatened species have also concluded that HFCs do not accurately reflect inbreeding depression (Grueber et al. 2011; Spiering et al. 2011).

In some populations heterozygosity at a few loci is poorly correlated with inbreeding or fitness traits, but this finding should not be used to dismiss inbreeding as a source of HFCs (Szulkin et al. 2010). Instead, it should be recognized that HFCs arise only in particular contexts, such as frequent consanguineous matings, genetic drift in small populations, or recent bottlenecks. These conditions represent inbreeding sensu lato and generate identity disequilibrium, which is the fundamental cause of HFCs (Szulkin et al. 2010). Identity disequilibrium can be estimated by a parameter (g_2) that can be calculated using multilocus genotypes and depends only on the mean and variance of inbreeding in the population and not on locus-specific characteristics (David et al. 2007). Without variance in inbreeding (i.e., when $g_2 = 0$) HFCs cannot arise (Szulkin et al. 2010). Estimating identity disequilibrium may allow one to identify the effects of inbreeding depression in endangered populations with genetic markers. To our knowledge, we are the first to use g_2 to estimate identity disequilibrium in populations of mammals.

Our primary aim was to test whether significant heterozygosity-fitness correlations can be detected in threatened populations that exhibit extensive identity disequilibrium that may stem from a history of inbreeding. We examined sperm quality in populations of 2 critically endangered taxa: Mohor gazelle (*Gazella dama mhorr*) and Iberian lynx (*Lynx pardinus*) (IUCN 2011). We selected them because it seemed reasonable to assume they would exhibit HFCs: their abundances have decreased substantially, their wild populations went through severe bottlenecks, and because current populations are small, consanguineous matings are frequent. To understand whether HFCs could be due to inbreeding, we estimated the levels of identity disequilibrium (g_2). For both species, we chose the same fitness trait, semen quality, which is often affected by inbreeding depression (Roldan et al. 1998; Gomendio et al. 2000) and has a substantial effect on male fitness (Malo et al. 2005). For the Mohor gazelle, we also quantified the relation between pedigree inbreeding coefficient and semen quality to compare IFCs and HFCs in the same species.

Methods

Study Populations

The Mohor gazelle is a subspecies of *Gazella dama*, which is also considered critically endangered (IUCN 2011). A captive-breeding program for Mohor gazelle was established at the Parque de Rescate de Fauna Sahariana (EEZA-CSIC) in Almeria (Spain) in 1971. At the time the captive-breeding program started, no animals had been reported in the wild since 1968 (Barbosa & Espeso 2005). The program started with animals that were captured in 1958 from the Hagunia area (Morocco) (Valverde 2004). By 1963 the group had 1 male, 2 females, and 3 young. In 1970 the group consisted of 12 individuals and was divided into 2 subgroups. In 1971 the first subgroup (6 females, 1 male, and 1 individual that died) was brought to Almeria to start the breeding program. Five years later the second subgroup had increased from 4 to 10 and was integrated into the captive-breeding program in Almeria. Thus, these 2 groups, which previously had been considered 2 different founding groups, came from the same captive group. At the time the captive breeding program started, 2 females from the same geographical area were captured in the wild and incorporated in the program, but no other founding events took place throughout the program because there were no animals in the wild. Accordingly, the actual number of founders from which living individuals descended was probably fewer than stated in the pedigree (11 founders, 9 females and 2 males).

The Iberian lynx is endemic to the Iberian Peninsula (Ferrer & Negro 2004), and it is considered critically endangered (Von Arx & Breitenmoser-Wursten 2008; IUCN 2011). Substantial decreases in abundance occurred in the last decades in response to habitat loss and decreases in abundance of its principal prey, the European rabbit (*Oryctolagus cuniculus*) (Rodríguez & Delibes 1992; Palomares et al. 2002). At present 2 reproductively viable populations remain in southern Spain: Doñana and Sierra Morena (Palomares 2009). In 2002 population size was estimated to be 38 adults in Sierra Morena and 32 in Doñana (Simon et al. 2009). Results of genetic analyses show that the 2 populations are highly differentiated, which indicates a lack of recent gene flow (Johnson et al. 2004; Godoy et al. 2009). The Doñana population is thought to have become isolated before 1950 (Rodríguez & Delibes 1992), and migration between the 2 populations is considered highly unlikely due to the large distance (approximately 240 km) and the many topographic

barriers separating them. The implementation of in situ conservation programs in recent years has increased lynx abundance in Sierra Morena (approximately 95 adults in 2008). Lynx abundance in Doñana has remained stable over time, but the sex ratio has changed due to a decrease in the number of adult males in response to an outbreak of feline leukemia in 2007 (Simon et al. 2009). A captive-breeding program and a genome resource bank were established in 2003 (Roldan et al. 2009) with wild lynx from the Doñana and Sierra Morena areas.

Sample Collection

Animal handling (trapping, anaesthetization, and electroejaculations) were performed in accordance with the Spanish Animal Protection Regulation, RD1201/2005, which conforms to European Union Regulation 2003/65/CE. Animals were anaesthetized and blood (5 mL) and semen were collected. We collected sperm through electroejaculation. From 2001 through 2005, we took samples from 22 male Mohor gazelles in the captive-breeding program at the Parque de Rescate de Fauna Sahariana. We collected samples from 2005 through 2008 from 20 male Iberian lynx (14 from the Sierra Morena population and 6 from Doñana). At the time of sampling, 15 of these males were captive. Except for 1 individual, all captive male lynx were born in the wild. There were no differences in semen quality between captive and wild Iberian lynx (Gañan et al. 2010).

Semen and Molecular Analyses

We evaluated sperm morphology as described previously (gazelle, Garde et al. [2003]; lynx, Gañan et al. [2010]). We assessed the percentage of sperm with normal morphology as 100 minus the percentage of head, midpiece, and principal piece plus terminal piece abnormalities. Mohor gazelle males sampled were different from the ones included in previous studies (Roldan et al. 1998; Gomendio et al. 2000). We focused on percentage of normal spermatozoa because this value directly affects male fertility and fitness (Gomendio et al. 2007) and is affected by inbreeding depression (Roldan et al. 1998; Gomendio et al. 2000).

We placed blood in tubes containing ethylenediaminetetraacetic acid (EDTA) and held the tubes at −80 °C. We performed DNA extractions as described in Ruiz-López et al. (2009). The genotypes of individuals were assessed at 17 microsatellite loci that were originally developed for several ungulate species and amplify successfully in Mohor gazelles (Ruiz-López et al. 2009) (Supporting Information). Fluorescent microsatellites products were amplified with standard polymerase chain reactions (PCR) (Supporting Information). We analyzed the PCR products on an ABI PRISM 3700 (Applied Biosystems, Carlsbad, California) and scored alleles with GeneMapper (version 3.1, Applied Biosystems).

Immediately after collection, we mixed 1 volume of Iberian lynx blood with 4 volumes of lysis buffer (0.1 M Tris-HCl, pH 8.0; 0.1 M Na-EDTA; 0.01 M NaCl; 0.5% SDS) and stored the samples at 4 °C. We extracted DNA with standard phenol-chloroform methods (Sambrook et al. 1989). We assessed the genotype of each individual at 36 microsatellite loci, originally developed for the domestic cat (*Felis catus*), bobcat (*Lynx rufus*), and Canadian lynx (*Lynx canadensis*), that had been successfully amplified in Iberian lynx (Johnson et al. 2004) (Supporting Information). Loci with fluorescent labels were amplified in standard PCR reactions (Johnson et al. 2004) and analyzed in an ABI 3130xl Genetic Analyzer (Applied Biosystems). We scored alleles with (GeneMapper, version 3.7).

For both species, we tested whether genotype frequencies deviated from those expected under Hardy-Weinberg equilibrium and whether there was linkage disequilibrium between all pairs of loci with the program Genepop version 3.4 (Raymond & Rousset 1995). We used a Bonferroni correction to control for multiple tests. We calculated the mean number of alleles and mean expected and observed heterozygosity values with the program Arlequin 3.1 (Excoffier et al. 2006). We estimated the probability of null alleles with Microchecker 2.2.3 (van Oosterhout et al. 2004).

Individual Heterozygosity and Inbreeding Coefficient

To measure individual genetic diversity, we calculated the standardized multilocus heterozygosity (sMLH) as the proportion of analyzed loci that were heterozygous for that individual, divided by the average heterozygosity of the loci that were successfully genotyped for each individual (Coltman et al. 1998). We used Excel macro IRmacroN4 (W. Amos [available from http://www.zoo.cam.ac.uk/zoostaff/meg/amos.htm#ComputerPrograms]) to calculate these values.

For the Mohor gazelle, we calculated the inbreeding coefficient (f) on the basis of pedigree information from the international studbook (Barbosa & Espeso 2005) with the Stevens-Boyce algorithm (Boyce 1983) implemented in PEDSYS software (Southwest Foundation for Biomedical Research, San Antonio, Texas). Inbreeding coefficients for the Mohor gazelle have been underestimated in previous studies that erroneously assumed founders were unrelated and not inbred (Ruiz-López et al. 2009). We used a more realistic coefficient of inbreeding (for further details see Ruiz-López et al. [2009]). Inbreeding coefficient and heterozygosity are not associated in the Mohor gazelle (Ruiz-López et al. 2009).

Estimation of Identity Disequilibrium

Levels and statistical significance of identity disequilibrium can be estimated by calculating the covariance in heterozygosity for 2 loci standardized by their average

heterozygosity (David et al. 2007). When individuals are inbred, this measure equals g_2, which is constant for whatever pair of loci are being analyzed and therefore depends only on the mean and variance of inbreeding in the population. The g_2 for 2 loci is calculated as follows: $\mathrm{cov}(h_i, h_j)/\bar{h}_i\bar{h}_j = [E(h_i h_j) - \bar{h}_i\bar{h}_j]/\bar{h}_i\bar{h}_j$, where h_i is heterozygosity at locus i ($h_i = 1$ for heterozygotes, $h_i = 0$ for homozygotes), h_j is the heterozygosity at locus j, and $E(h_i h_j)$ is the mean of $h_i h_j$. For each of the Iberian lynx populations and for Mohor gazelle we combined all loci to compute a single estimate of g_2 in REMS2009 software (calculation details in David et al. [2007]). We tested whether g_2 differed significantly from zero by resampling genotypes (random reassortment of single-locus heterozygosities among individuals within the population). If g_2 differs significantly from zero there is variance in inbreeding that will increase the probability of detection of HFCs due to identity disequilibrium (Szulkin et al. 2010).

Statistical Analyses

We used additive general linear models without interactions, which minimized the probability of type I error associated with small sample size, to analyze the relation between semen quality and inbreeding coefficient and heterozygosity. We applied general linear models to each of the species separately. For the Iberian lynx, we conducted 2 separate analyses. First, we analyzed Sierra Morena and Doñana populations jointly and included population in the model as a fixed factor to control for its effects. However, because it is important to consider population structure (Slate & Pemberton 2006; Luquet et al. 2011), we repeated the analyses for each population separately. For all models we tested whether the residuals fit a normal distribution. If they did not, we conducted Monte-Carlo simulations to test whether the observed trends could be due to chance. We randomized the data 1000 times to generate a new distribution to estimate the level of significance (Gotelli & Ellison 2004). The statistical analyses were conducted in STATISTICA 10.0 (Statsoft, Tulsa, Oklahoma) and the Monte Carlo simulations in Matlab 7.0 (The Mathworks, Natick, Massachusetts). In addition, for Mohor gazelle, we used the HFC to calculate the decrease in sperm quality per unit of inbreeding following Szulkin et al. (2010) (Supporting Information).

Results

We did not find evidence of null alleles or deviations from Hardy-Weinberg equilibrium at any locus after Bonferroni correction for Mohor gazelle or Iberian lynx. None of the possible primer pairs in any population exhibited statistically significant linkage disequilibrium after Bonferroni correction. The 22 Mohor gazelle males in the HFC analyzes were genotyped at least at 16 markers, and 21 individuals were genotyped for all 17 markers. Individual lynx included in the HFC analyzes were genotyped for a minimum of 34 loci, and the full set of 36 markers was assessed for 17 of 20 lynx. Levels of heterozygosity and inbreeding for the subsample of males were representative of the entire population (Table 1). For Mohor gazelle, inbreeding coefficient and standardized multilocus heterozygosity (sMLH) were not correlated. These results are consistent with those of Ruiz-López et al. (2009).

The molecular estimate of g_2 differed significantly from zero in the Mohor gazelle ($g_2 = 0.096$ [SD 0.062], $p < 0.005$) and in the 2 Iberian lynx populations ($g_{2\text{Doñana}} = 0.022$ [SD 0.010], $p < 0.001$; $g_{2\text{SierraMorena}} = 0.010$ [SD 0.007], $p < 0.001$). Thus, we found identity disequilibrium, which probably occurs as a result of inbreeding, and there may be a significant correlation between sMLH and semen quality if this trait is affected by inbreeding.

For both species, an increase in sMLH was associated with an increase in the percentage of normal spermatozoa (Table 2 & Fig. 1). However, among Mohor gazelle f was unrelated to the percentage of normal spermatozoa (Table 2 & Supporting Information). On the basis of the HFCs, we estimated that the percentage of normal spermatozoa decreased 21% per unit of inbreeding (Supporting Information).

For the Iberian lynx, sMLH and percentage of normal spermatozoa were significantly associated when Sierra Morena and Doñana were analyzed jointly, and population of origin did not have a significant effect on this trait (Table 2 & Fig. 1b). When populations were analyzed separately, results were consistent, suggesting that

Table 1. Mean and standard deviation (σ) of the pedigree inbreeding coefficient (f) and heterozygosity (H) and standardized multilocus heterozygosity (sMLH) for the males in the analyses of heterozygosity-fitness correlations for Mohor gazelles ($n = 22$) and the 2 populations (Doñana and Sierra Morena) of Iberian lynx (pooled and for each population) ($n = 20$).*

	Mean f	σ (f)	*Mean* H	σ (H)	*Mean* sMLH	σ (sMLH)
Mohor gazelle	0.301 (0.303)	0.027 (0.028)	0.466 (0.476)	0.098 (0.110)	0.980 (0.968)	0.205 (0.226)
Iberian lynx (total)			0.420 (0.388)	0.111 (0.112)	0.919 (0.948)	0.242 (0.274)
Doñana	–	–	0.333 (0.339)	0.124 (0.102)	0.730 (0.828)	0.272 (0.250)
Sierra Morena	–	–	0.454 (0.460)	0.087 (0.085)	0.995 (1.120)	0.180 (0.207)

**Values in parentheses are from a larger sample that includes males and females (Mohor gazelles, n = 111; Iberian lynx, Doñana, n = 141; Sierra Morena, n = 98).*

Table 2. General linear model for the relation between the percentage of normal sperm, standardized multilocus heterozygosity (sMLH), and inbreeding coefficient (f) for Mohor gazelles and Iberian lynx.

Species	*Parameter*	*df*	β *(SE)*[a]	*Lower 95% CI*[b]	*Upper 95% CI*	p
Mohor gazelle	f	1,19	0.280 (0.16)	−0.055	0.611	0.097
	sMLH	1,19	0.724 (0.16)	0.392	1.058	0.000
Iberian lynx	sMLH	1,17	0.632 (0.22)	0.177	1.087	0.009
	population[b]	1,17	0.014 (0.22)	−0.440	0.470	0.948

[a] *Standardized parameter coefficient.*
[b] *Sierra Morena and Doñana populations are analyzed together and the population variable is included as a factor.*

the HFCs observed were not due to population structure. For the Sierra Morena population, the same relation was found: individuals with higher standardized multilocus heterozygosity (sMLH) had a higher percentage of normal spermatozoa ($F_{1,12} = 8.34$, $p = 0.01$, $R^2 = 0.41$) (Fig. 1b). For the Doñana population, the percentage of normal spermatozoa increased when standardized multilocus heterozygosity (sMLH) increased, but this relation was not statistically significant. Lack of significance may have reflected the small sample size ($n = 6$).

Discussion

Our results show that in endangered populations of Mohor gazelle and Iberian lynx, there is a significant association between levels of heterozygosity and semen quality. Both species showed extensive identity disequilibrium, which is typically the result of extensive inbreeding in a population (Weir & Cockerham 1973). Therefore, HFCs for semen quality in these populations seem to provide evidence of inbreeding depression. Thus, our results show that given certain demographic conditions, such as a long history of intense inbreeding and severe bottlenecks that generate extensive identity disequilibrium, HFCs can detect inbreeding depression. Our results contrast with previous studies that showed heterozygosity is frequently a poor proxy for inbreeding. The results of the few studies that tested for identity disequilibrium showed that g_2 did not differ from zero. This suggests that the markers used were not representative of individual inbreeding coefficients (Grueber et al. 2011) and would explain why such studies found weak evidence of HFCs.

Whereas Mohor gazelle heterozygosity was significantly associated with sperm morphology, inbreeding coefficient was not. This result seems to contradict results of previous studies that showed relations between inbreeding coefficient and semen quality in other species of gazelles (Roldan et al. 1998; Gomendio et al. 2000). This discrepancy may have arisen because pedigree coefficient of inbreeding is not a good estimator of real levels of inbreeding for this population of Mohor gazelle, whereas in other species it is. In the population of Mohor gazelles we examined, the lack of association between inbreeding coefficient and molecular metrics probably reflects that the founding population was formed by related individuals and that abundance decreased and inbreeding was prevalent before the captive breeding program—and

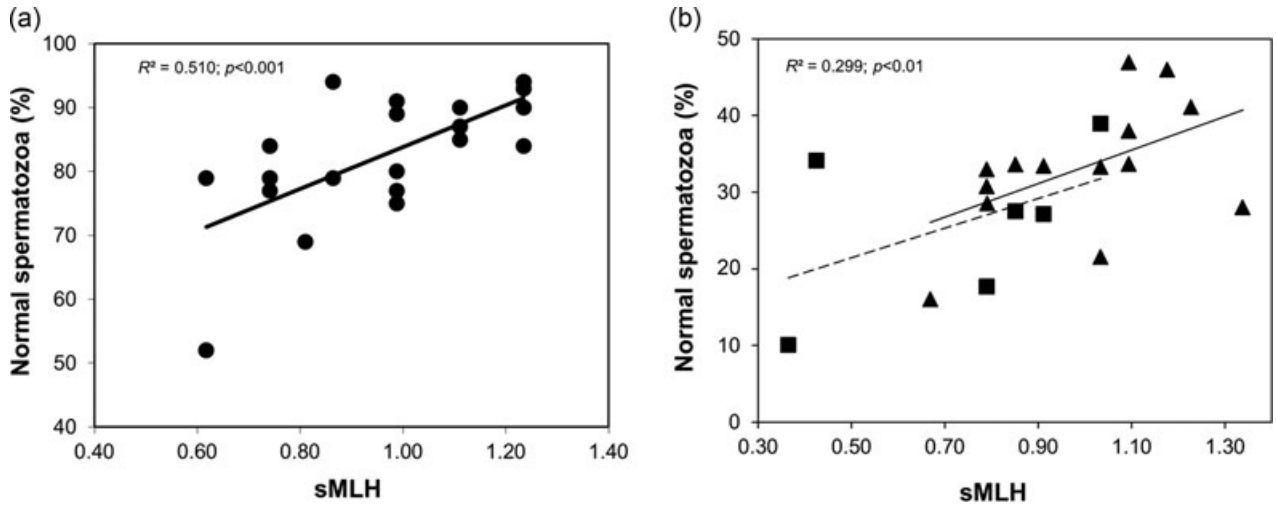

Figure 1. Relation between standardized multilocus heterozygosity (sMLH) and percentage of normal spermatozoa for (a) Mohor gazelles and (b) Iberian lynx from Sierra Morena (triangles and continuous regression line) and Doñana, Spain (squares and dashed regression line), populations.

the pedigree records—started (Ruiz-López et al. 2009). On the basis of this information, we suggest that in this case the coefficient of inbreeding calculated from the start of the captive breeding programme does not account for previous demographic history of the population, which would make it a poor estimator of inbreeding depression. However, although we recalculated the coefficient of inbreeding there is also the possibility that molecular information is accurately representing recent inbreeding, whereas the coefficient of inbreeding is failing to do so due to the erroneous founder assumptions. In both cases, such uncertainties may not affect HFCs because molecular variation is a product of the complete demographic history of a population and as such does not require knowledge or assumptions about the levels of inbreeding and degree of relatedness among founders.

For the Iberian lynx, the decrease in abundance and increase in isolation over the last century have resulted in low genetic diversity and high inbreeding (Johnson et al. 2004; Godoy et al. 2009). We found that decreases in heterozygosity were also associated with decreases in the proportion of normal sperm. We believe this association probably reflects inbreeding depression for 2 reasons. First, the HFCs were present when we analyzed the Sierra Morena and Doñana populations together and when we analyzed only the Sierra Morena population (a larger population than Doñana). Second, g_2 was significantly different from zero in both populations, suggesting there is significant identity disequilibrium. Our results therefore are consistent with the expectation that HFCs are more likely to be detected when heterozygosity explains a large proportion of the variance in fitness within the population (Szulkin et al. 2010).

The detection of HFCs as a consequence of inbreeding depression may have also been facilitated by the choice of trait because correlations between fitness and heterozygosity are only expected to be high for traits with a direct effect on fitness that are influenced by a large number of loci (Chapman et al. 2009; Szulkin et al. 2010). Semen quality in general and the proportion of normal sperm in particular determine male fertility and are therefore directly linked to male fitness (Malo et al. 2005; Gomendio et al. 2007). In addition, the proportion of abnormalities in the head, midpiece, and principal piece of spermatozoa are likely to be under the control of many loci (Gómez Montoto et al. 2011).

The effect of heterozygosity on sperm quality was strong for both species. On the basis of HFCs, the estimated inbreeding load for the Mohor gazelle decreased 21% per unit increase of inbreeding, which is an extremely high percentage (population mean is 83%). For example, compared with an outbred individual ($f = 0$), an inbred male ($f = 0.25$) will have on average 5.25% (21/4) fewer normal spermatozoa, which will have a strong effect on the fertility of the inbred individual. The proportion of normal sperm was lower for Iberian lynx relative to Mohor gazelles of similar heterozygosity. Results of previous studies show that Iberian lynx have a relatively high proportion of abnormal sperm, as is common among other felids (Roldan et al. 2009; Gañan et al. 2010). Results of experimental studies show the percentage of normal sperm in Iberian lynx is associated with the fertility of males (Gañan et al. 2009). These findings strongly suggest that Iberian lynx males with low levels of heterozygosity that have as low as 10% normal sperm may have low fertility rates, which implies that the effective population size may be lower than the actual number of reproductively mature males.

We believe our results can inform management of inbred species. In species that have inbred intensively over a long period, levels of heterozygosity may be reliable predictors of levels of inbreeding. Assumptions about the degree of relatedness that are estimated on the basis of shallow pedigrees built under the assumption that founders are unrelated and not inbred may be erroneous (Ruiz-López et al. 2009). Males with low levels of heterozygosity are likely to have low fertility and may therefore limit the reproduction of females they mate with. Preservation in genome resource banks of semen from as many males as possible (from captive and wild populations) may allow maximization of genetic diversity and promotion of gene flow among populations.

In our 2 case studies of critically endangered species, HFCs successfully indicated inbreeding depression. We suggest this occurred because the populations analyzed exhibited extensive levels of identity disequilibrium, probably due to extreme bottlenecks and a long history of inbreeding. Therefore, our results indicate HFCs can be used to detect inbreeding in highly endangered species.

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Supporting Information

Microsatellite data (Appendix S1), details of calculations on the decrease of sperm quality per unit of inbreeding (Appendix S2), and the relation between coefficient of inbreeding and percentage of normal spermatozoa (Appendix S3) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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