Sex-Biased Inbreeding Effects on Reproductive Success and Home Range Size of the Critically Endangered Black Rhinoceros

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Abstract: *A central premise of conservation biology is that small populations suffer reduced viability through loss of genetic diversity and inbreeding. However, there is little evidence that variation in inbreeding impacts individual reproductive success within remnant populations of threatened taxa, largely due to problems associated with obtaining comprehensive pedigree information to estimate inbreeding. In the critically endangered black rhinoceros, a species that experienced severe demographic reductions, we used model selection to identify factors associated with variation in reproductive success (number of offspring). Factors examined as predictors of reproductive success were age, home range size, number of nearby mates, reserve location, and multilocus heterozygosity (a proxy for inbreeding). Multilocus heterozygosity predicted male reproductive success (p < 0.001, explained deviance >58%) and correlated with male home range size (p < 0.01, r² > 44%). Such effects were not apparent in females, where reproductive success was determined by age (p < 0.01, explained deviance 34%) as females raise calves alone and choose between, rather than compete for, mates. This first report of a 3-way association between an individual male's heterozygosity, reproductive output, and territory size in a large vertebrate is consistent with an asymmetry in the level of intrasexual competition and highlights the relevance of sex-biased inbreeding for the management of many conservation-priority species. Our results contrast with the idea that wild populations of threatened taxa may possess some inherent difference from most nonthreatened populations that necessitates the use of detailed pedigrees to study inbreeding effects. Despite substantial variance in male reproductive success, the increased fitness of more heterozygous males limits the loss of heterozygosity. Understanding how individual differences in genetic diversity mediate the outcome of intrasexual competition will be essential for effective management, particularly in enclosed populations, where individuals have restricted choice about home range location and where the reproductive impact of translocated animals will depend upon the background distribution in individual heterozygosity.*

Keywords: fitness, heterozygosity–fitness correlation, intrasexual competition, reproductive behavior, wildlife management

Efectos de la Endogamia Sesgada por el Sexo sobre el Éxito Reproductivo y el Rango del Tamaño de Hábitat del Rinoceronte Negro, Especie en Peligro Crítico

Resumen: *Una premisa central de la biología de la conservación es que las poblaciones pequeñas padecen de viabilidad reducida por medio de la pérdida de la diversidad genética y la endogamia. Sin embargo hay poca evidencia de que la variación en la endogamia impacta el éxito reproductivo individual dentro de las poblaciones remanentes de un taxón amenazado, principalmente debido a los problemas asociados con la obtención de información integral del linaje para estimar la endogamia. Con el rinoceronte negro, especie en peligro crítico que sufrió reducciones demográficas severas, usamos un modelo de selección para identificar factores asociados con el éxito reproductivo (número de descendientes). Los factores que se examinaron como indicadores del éxito reproductivo fueron la edad, el tamaño del hábitat, el número de parejas cercanas, ubicación en la reserva y heterocigocidad multilocus (un indicador de endogamia). La heterocigocidad multilocus predijo el éxito reproductivo de los machos (p < 0.01, desviación explicada >58%) y tuvo correlación con el tamaño de hábitat de los machos (p < 0.01, r² > 44%). Tales efectos no fueron aparentes con las hembras, donde el éxito reproductivo estuvo determinado por la edad (p < 0.01, desviación explicada 34%), ya que las hembras crían solas a los becerros y escogen a su pareja, en lugar de luchar por ella. El primer reporte de una asociación de tres vías entre la heterocigocidad de un macho individual, la salida reproductiva y el tamaño del territorio en un vertebrado de gran tamaño es consistente con una asimetría en el nivel de competencia intrasexual y resalta la relevancia de la endogamia con sesgo de sexo para el manejo de muchas especies con prioridad de conservación. Nuestros resultados contrastan con la idea de que las poblaciones silvestres de un taxón amenazado pueden poseer alguna diferencia inherente de la mayoría de las poblaciones no amenazadas que exige el uso de linajes detallados para estudiar los efectos de la endogamia. A pesar de la varianza sustancial en el éxito reproductivo de los machos, la aptitud incrementada de más machos heterocigotos limita la pérdida de la heterocigocidad. Entender cómo las diferencias individuales en la diversidad genética moderan el resultado de la competencia intrasexual será esencial para un manejo efectivo, particularmente en poblaciones adjuntas, donde los individuos tienen opciones restringidas de tamaño de hábitat y donde el impacto reproductivo de animales translocados dependerá del trasfondo de la distribución en la heterocigocidad individual.*

Palabras Clave: aptitud, competencia intrasexual, comportamiento reproductivo, correlación entre adecuación y heterocigocidad, manejo de vida silvestre

Introduction

Small populations are susceptible to extinction through a feedback between demographic stochasticity (Lande 1988) and accelerated loss of genetic diversity and inbreeding (Saccheri et al. 1998; Keller & Waller 2002). Although there is ample evidence for inbreeding depression in wild animal populations (Keller & Waller 2002; O'Grady et al. 2006), such effects are rarely quantified in remnant populations of threatened taxa. Reasons for this lack of data are manifold but are principally associated with the difficulty in obtaining adequate samples over enough generations to establish pedigrees that would be sufficiently deep to provide accurate inbreeding coefficients (Balloux et al. 2004; Slate et al. 2004).

An alternative proxy for the level of inbreeding is multilocus heterozygosity (MLH), the number of heterozygous loci within an individual (Szulkin et al. 2010). Inbred individuals will have increased homozygosity (decreased heterozygosity) over their genomes compared with more outbred individuals in the same population, and this can generate correlations in heterozygosity (and homozygosity) among loci throughout a genome (defined as "identity disequilibrium" by Weir & Cockerham 1973). The corollary is that a positive correlation between MLH and one or more components of fitness (a heterozygosity–fitness correlation [HFC]) could indicate inbreeding depression

(Hansson & Westerberg 2002; Szulkin et al. 2010). Many studies of wild populations have followed this approach and reported significant HFCs (Chapman et al. 2009).

A general criticism directed at HFCs is their typically weak effect size (Chapman et al. 2009) that largely reflects the expected poor correlation between estimates of MLH derived from a small panel of loci and the level of inbreeding (Balloux et al. 2004; Slate et al. 2004; Grueber et al. 2011). Although there can be considerable variation in heterozygosity among loci within individual genomes (Slate et al. 2004; Väli et al. 2008), the reasons for this are not fully understood. Nonetheless, Ljungqvist et al. (2010) emphasize that any correlation between estimates of MLH and genome-wide diversity requires the presence of identity disequilibrium. Under such conditions more polymorphic markers such as microsatellites can have greater power to predict genome-wide diversity for a given number of loci compared with less variable markers such as single nucleotide polymorphisms (Slate et al. 2004; Ljungqvist et al. 2010). Another issue is whether any HFC is a result of an indirect "local effect" of linkage disequilibrium between a neutral marker and a specific fitness locus (Hansson & Westerberg 2002; Tiira et al. 2006). Although the pattern of diversity at specific loci can be driven by selection for certain genotypes rather than by inbreeding, any correlation between a genetic marker and a fitness locus implies some inbreeding (Szulkin et al. 2010). Interpreting any HFC thus requires

an examination for identity disequilibrium and for undue influence of one or few loci.

Of particular relevance for conservation is the recent notion that the HFC approach hinders an analysis of inbreeding in wild populations of threatened taxa. This idea follows several studies that found marker heterozygosity an imprecise estimator of a pedigree-based inbreeding coefficient in small, inbred populations (Bensch et al. 2006; Grueber et al. 2008, 2011; Spiering et al. 2011), despite prior empirical (Hedrick et al. 2001) and theoretical (Balloux et al. 2004; Slate et al. 2004) support for a stronger correlation between heterozygosity and inbreeding under such demographic circumstances. Hence, no significant association between heterozygosity and fitness traits were found in populations of takahe (*Porphyrio hochstetteri*) (Grueber et al. 2008, 2011) or African wild dogs (*Lycaon pictus*) (Spiering et al. 2011). As a counterargument, Ruiz-Lopez et al. (2012) suggest that inbreeding coefficients derived from pedigrees can be imprecise when the genealogy begins after a demographic reduction and thus overlooks prior inbreeding. Indeed, Ruiz-Lopez et al. uncovered a significant correlation between sperm quality and heterozygosity, but not a pedigree-derived metric of inbreeding in Mohor gazelle (*Gazella dama mhorr*), and they uncovered a HFC among wild-caught Iberian lynx (*Lynx pardinus*). Given the conflicting outcomes from the few HFC studies of threatened taxa it remains unclear whether estimates of heterozygosity can be used to study inbreeding depression in wild populations of conservation-priority species. Without convincing evidence that inbreeding affects individual viability and behavior, genetic management of threatened taxa is typically limited, for example, to attempts at maintaining general levels of genetic diversity by establishing corridors or translocating animals to promote reproduction between areas (McCullough 1996; Spiering et al. 2011).

The critically endangered eastern black rhinoceros (*Diceros bicornis michaeli*) in Kenya is thought to have undergone significant inbreeding due to demographic reductions as a result of hunting following colonial settlement and the severe (∼98%) decline in numbers caused by the more recent intense poaching, where over 20,000 animals were reduced to just 380 between 1970 and 1987 (Okita-Okuma et al. 2007). Commencing in 1984, black rhinos from across Kenya were translocated into protected, fenced reserves to counter the poaching threat, and by 2005 Kenya had some 84% of the total eastern black rhino population (Okita-Ouma et al. 2007). The Kenyan black rhinoceros program represents a typical crisis strategy, where the urgent need to prevent extinction necessitated that animals from formally allopatric sites, and thus different inbreeding histories, were mixed within protected areas. Extensive monitoring of Kenyan black rhinos provides details about individual home range locations and sizes (Amin et al. 2001), which exhibit substantial variation among individuals in other areas (Con-way & Goodman 1989; Lent & Fike 2003). The relevance of this variation in home range size, if any, is not known. However, inbreeding can negatively affect social status and competitive ability in other vertebrates (Meagher et al. 2000; Tiira et al. 2006; Välimäki et al. 2007) and this could have important fitness implications as acquisition of resources or mates through territoriality is a key behavior exhibited by many vertebrates (Huntingford & Turner 1987). Individual black rhinos also differ in their reproductive success (Garnier et al. 2001), although the reasons for this variation are not known. With many in situ and ex situ programs for rhinoceros taxa reporting poor population growth rates due to low or declining reproduction (Mills et al. 2006), understanding the drivers of reproductive success is a widespread problem and important for sustaining their recovery. However, the long generation time of black rhinos (∼12 years) (Conway & Goodman 1989) limits the ability of a pedigree analysis to accurately capture the inbreeding coefficients.

Using the black rhinoceros as a model conservation species, we examined factors that determine individual variation in reproductive success, with an emphasis on whether any reproductive significance can be attached to variation in home range size and a HFC can be used to identify inbreeding effects.

Methods

Sample Collection and Population Data

Between 2004 and 2009, genetic material was obtained from 107 black rhinoceros from 3 Kenyan black rhinoceros sanctuaries: Lewa Wildlife Conservancy (Lewa; 361 km²), Mugie Rhino Sanctuary (Mugie; 0°74'N, 36°65'E; 93 km²), and Ol Pejeta Conservancy (Pejeta; 36°55'E, 00°02'N; 365 km²) (full details in Supporting Information). Our genetic material was feces ($n = 65$), tissue ($n = 22$), and serum ($n = 20$) from individually identified animals (Supporting Information), and these samples represented 93% ($n = 39$), 96% ($n = 27$), and 92% ($n = 41$) of the Lewa, Mugie, and Pejeta populations as of the 2006 census (Okita-Ouma et al. 2007).

All black rhinoceros within Kenyan sanctuaries can be individually identified and are monitored daily (Okita-Ouma et al. 2007). The Kenya Wildlife Service black rhinoceros database (Amin et al. 2001) provided information on animal age (AGE), mother–calf pairings, and locations and was used to identify the mature males (5 years or older at the time of calf's conception) (Garnier et al. 2001) to be included as candidate fathers during parentage analysis. Home range sizes (HOM) were calculated from global positioning system co-ordinates (collected by monitoring patrols) using 95% fixed kernels and smoothed, cross validation in Geospatial Modelling Environment (version 0.6.0.0)

(http://www.spatialecology.com/). For every individual, we used ArcGIS (version 10.0) to calculate the number of immediately available mates (MAT), defined as the number of other sex whose home ranges overlapped with the focal animal.

Genotyping, Estimates of Heterozygosity, and Parentage Analysis

Every sample was genotyped at 10 microsatellite loci (Brown et al. 1999; Cunningham et al. 1999) (Supporting Information), with the replicate extractions of the low copy material genotyped at least 6 times to ensure accuracy (Supporting Information). Details about tests for null alleles, linkage disequilibrium, departures from expected Hardy–Weinberg equilibrium conditions and metrics of genetic diversity are provided in Supporting Information.

The program IRMacroN4 (www.zoo.cam.ac.uk/zoostaff/meg/amos.htm#ComputerPrograms) was used to calculate 2 estimators of multilocus heterozygosity: internal relatedness (IR) and multilocus heterozygosity (MLH). MLH is the number of heterozygous loci within an individual that is not corrected for differences in numbers or frequencies of alleles. IR is an estimate of parental relatedness according to the extent of allele sharing weighted by allele frequency that has been suggested to be a suitable indicator of Wright's (1922) inbreeding coefficient f (Slate et al. 2004); however, MLH can be used to derive parameters that quantify the impact of any inbreeding detected from a HFC (Szulkin et al. 2010) (Supporting Information).

We determined the number of offspring produced by each mature black rhino by parentage analysis that first examined the 62 observations of mother–calf pairings and then assigned the fathers. All parentage assignments were accepted at 95% confidence as determined by Cervus (version 3.0.3) (Marshall et al. 1998) (Supporting Information).

Predictors of Reproductive Success

We used generalized linear models (GLMs) (see Zuur et al. 2009) to identify the predictors that explained the greatest proportion of variance in the number of offspring produced by black rhinos; models were fitted for total number of offspring (OFF) as the response variable (Poisson distribution) and also for the number of offspring standardized by the population average (OFFs) to correct for any variation in reproductive output among reserves (Szulkin et al. 2010). Variables assessed as predictors of offspring production were age (age, in years), home range size (HOM, in km^2), number of potential mates with overlapping home ranges (MAT), and heterozygosity (IR or MLH), with the reserve (fRES) included as a random factor when offspring number was not standardized for any variation among reserves.

Model selection was run for males and females separately. Selection of terms in the models was based on minimizing corrected Akaike's information criterion (AICc) using the dredge function within the package MuMIn (Barton 2011) in R (version 2.12.1) (R Development Core Team 2010). We selected the model with the fewest predictors that was within 2ΔAICc of the model with the lowest overall AICc (Burnham & Anderson 1998). Explained deviance (R^2) of the final GLMs was calculated as (null deviance − residual deviance)/null deviance (Zuur et al. 2009).

Evidence for Genomewide or Local Effects

We assessed the relative importance of a potential local effect at one or few loci compared with a general genomewide reduction in heterozygosity (Hansson & Westerberg 2002; Tiira et al. 2006) with an F-ratio test. Briefly, we used R software to compare a single and a multiple regression (i.e., MLH versus single locus heterozygosities expressed as 0 or 1) of heterozygosity against offspring number (Szulkin et al. 2010). Estimates of identity disequilibrium (g_2) were calculated using RMES (David et al. 2007) and 10,000 randomizations.

Effect of a HFC upon Reproductive Success

We used the formulas provided by Szulkin et al. (2010) that use the basic descriptors of the HFC to estimate the inbreeding load in male black rhinoceros (Supporting Information). The inbreeding load is the decline in fitness with inbreeding (f) due to exposure of deleterious alleles in inbred individuals and is typically represented as the number of lethal equivalents per gamete (Keller & Waller 2002); doubling the inbreeding load thus estimates the number of lethal equivalents in a diploid individual.

Effect of Mating System upon Offspring Heterozygosity

To quantify the effect of mating behavior upon average offspring heterozygosity, we simulated offspring genotypes that would have been produced by each female rhino under 3 conditions: selecting the males identified as parents for all offspring; selecting a father at random for every offspring; randomly selecting a male for each female but then using his genotype to sire an appropriate number of full-siblings. If females had mated with several males, we selected additional males at random and simulated the appropriate offspring. This third mating system represents random mate choice with realistic variance in reproductive success (see Results). For each of these mating conditions, males were selected from the same population as females. We then used Ploc (version 1.0) (Matson et al. 2008) to generate 61 offspring genotypes, using the actual parental genotype data but from the

simulated parent pairs; the procedure was repeated 100 times.

Results

Genotyping

Comparisons between samples that had complementary tissue and faecal samples ($n = 21$) indicated a low genotyping error rate (mean = 0.13%; range = 0.0–0.24%). Most (>99%) discrepancies were due to allelic dropouts in one of the genotyping rounds. Ambiguous genotypes were resolved by 2 or more additional PCRs. Null alleles were detected at one locus (DB44) that was excluded from the analyses. There were no significant deviations from expected Hardy-Weinberg equilibrium conditions ($p > 0.05$) for the remaining microsatellite loci and no evidence for significant ($p > 0.05$) linkage disequilibrium among any pair of loci, except the single comparison of Br17 and Br4 in Mugie (Supporting Information).

Parentage Analysis

Our data represented the reproductive activity of 27 females and 18 males between 1990 and 2006. Maternity analysis confirmed the field observations (Amin et al. 2001) of all mother–offspring pairs at Mugie and Lewa and all but 2 pairs at Pejeta; maternity of these 2 calves was resolved using the most likely father (>95% critical LOD score) as a known parent and then selecting between the available females of reproductive age (>2 years) at the time of conception. Paternity was assigned at 95% confidence (critical delta) to 61 of the 62 offspring with the one unassigned offspring from Lewa (<80% critical delta) likely sired by a male present in the early 1990s but who was subsequently removed (i.e., unsampled).

Variance in numbers of offspring was significantly greater among males than females (variance in offspring production: female = 2.44, male = 11.42; $K^2 = 11.96$, df = 1, $p = 5.44 \times 10^{-4}$, Bartlett test of homogeneity of variance).

Predictors of Reproductive Success

Both estimators of heterozygosity (MLH and IR) were significantly correlated in our data ($r = -0.912$, df = 43, $p = 2.2 \times 10^{-16}$) and yielded comparable results (Table 1). Reproductive success was determined by different factors in male and female rhinoceros. In females the only significant predictor of reproductive success was age. Older females produced more calves (Fig. 1c) and age accounted for a third of the variation among animals (Table 1). Reproductive output was more variable in older females (Fig. 1c) and reflected reproduction prior to monitoring in reserves or senescence in the oldest

females. A GLM based on younger (<30 years) female rhinos improved explanatory power (OFF = –0.252 + 0.069age, $p = 0.003$, $R^2 = 0.520$). Heterozygosity had little impact on female reproduction (Fig. 1a), even in a GLM with all other factors excluded (OFF = 0.962 – 0.524IR, $p = 0.415$, $R^2 = 0.028$) (Supporting Information). The opposite was true for males: individual differences in heterozygosity correlated with male reproductive success (Table 1, Fig. 1b), whereas age had no impact on the number of offspring sired (OFF = 1.245 – 0.000age, $p = 0.987$, $R^2 = 0.000$) (Fig. 1d). Heterozygosity was always retained during model selection, and reserve was a significant factor when MLH, but not IR, was used as the estimator (Table 1).

Potential environmental and genetic differences among reserves did not create an apparent HFC because more heterozygous males produced more offspring in every reserve (Fig 1b) and there were no significant differences between reserves in average heterozygosity (analysis of variance [ANOVA]: female IR – $F = 0.012$, $p = 0.912$; male IR – $F = 0.955$, $p = 0.343$; female MLH – $F = 3.234$, $p = 0.084$; male MLH – $F = 0.000$, $p = 0.976$) or age (ANOVA: female – $F = 2.254$, $p = 0.146$; male – $F = 0.165$, $p = 0.690$) (see Supporting Information for diversity metrics). Moreover, our analysis of reproductive success of black rhinos from Zimbabwe (Garnier et al. 2001) revealed a significant ($p < 0.001$ for males only) sex-biased HFC (Supporting Information). Standardizing the variation in offspring among reserves returned heterozygosity as the single predictor of male reproductive success that accounted for 61% of the variation in the number of calves produced (Table 1). Final model residuals did not exhibit significant ($p > 0.05$) departures from normality (Shapiro–Wilk test) or homoscedasticity (Breusch–Pagan test), except for standardized female age (BP = 4.693, $p = 0.030$) (Supporting Information).

There was a significantly positive correlation between heterozygosity ($r = -0.740$, df = 15, $p = 6.86 \times 10^{-4}$ for IR; $r = 0.664$, df = 15, $p = 0.0036$ for MLH) and home range size in male rhinos (Fig. 2b). A male's home range thus predicted his reproductive success (OFF = 0.082 + 0.072HOM, $p = 2.75 \times 10^{-5}$, $R^2 = 0.264$). Female home range size was not correlated with heterozygosity ($r = 0.064$, df = 25, $p = 0.752$ for IR; $r = -0.003$, df = 25, $P = 0.988$ for MLH) (Fig. 2a), and home range size had no impact upon female calf production (OFF = 0.975 + 0.005HOM, $p = 0.788$, $R^2 = 0.003$).

Evidence for Genome-wide or Local Effects

There was no support that the male HFC was generated by linkage disequilibrium between a microsatellite marker and a specific fitness locus (F-ratio test; $F = 0.842$, df = 16,8, $p > 0.05$). Significant identity disequilibrium was detected for all adult rhinos ($g_2 = 0.012$, $p = 0.027$) and for the sample of adult males ($g_2 = 0.018$, $p = 0.047$).

Table 1. Generalized linear models (GLMs) of the best predictors of the total number of offspring (OFF) or the standardized number of offspring (OFFs) produced by black rhinoceros from 3 reserves in Kenya.

Sex		Response	Intercept	Age	HET	fRES$_p$	fRES$_l$	R^{2a}
Female[b]		OFF	0.328	0.033**				0.342
		OFFs	−1.451*	0.072**				0.255
Male	IR	OFF	0.878***		−3.336***			0.589
		OFFs	−0.856		−9.439***			0.609
	MLH	OFF	−3.075***		0.419***	1.604***	1.618***	0.693
		OFFs	−9.345***		1.367***			0.614

Note: Significant predictors after model selection included age in years of parent, heterozygosity (HET) (internal relatedness [IR] or multilocus heterozygosity [MLH]) and reserve site as a random factor (fRES) (fRES$_p$ and fRES$_l$ are the factor values for Pejeta and Lewa reserves, respectively). Significance: *p < 0.05, **p < 0.01, ***p < 0.001.
[a]Explained deviance—Proportion of variation explained by the model.
[b]Final generalized linear model included neither IR nor MLH.
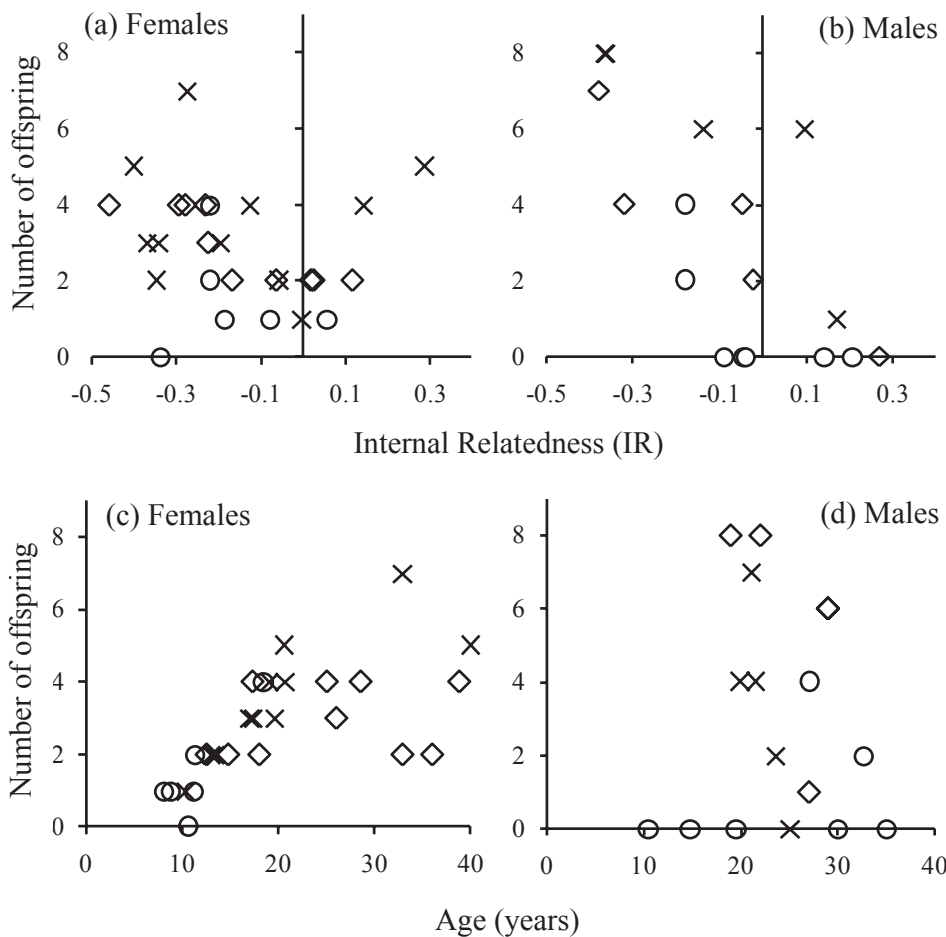
Figure 1. Relationship between the number of offspring produced and (a, b) heterozygosity (internal relatedness [IR]) and (c, d) age for (a, c) female and (b, d) male black rhinoceros from 3 reserves in Kenya: Mugie Rhino Sanctuary (circles), Lewa Wildlife Conservancy (diamonds), and Ol Pejeta Conservancy (crosses). More heterozygous animals have lower values of IR.

We used the latter estimate of g_2 to calculate inbreeding parameters.

Effect of a HFC upon Reproductive Success

For male rhinos, the HFCs based on MLH and the logarithms of standardized number of offspring (ln[OFFs]) and standardized home range size (ln[HOMs]) were ln(OFFs) = −1.885 + 0.473, p = 0.001, R^2 = 0.499 and ln(HOMs) = −1.960 + 0.267, p = 0.004, R^2 = 0.433.

Hence, there was a strong correlation between MLH and inbreeding (r^2_{Hf} = 0.401). The inbreeding load for male offspring production and home range size was estimated to be −8.06 and −4.55, respectively.

Effect of Mating System upon Offspring Heterozygosity

Average heterozygosity of simulated offspring (Fig. 3) was significantly lower when there was variance in reproductive success among randomly selected males
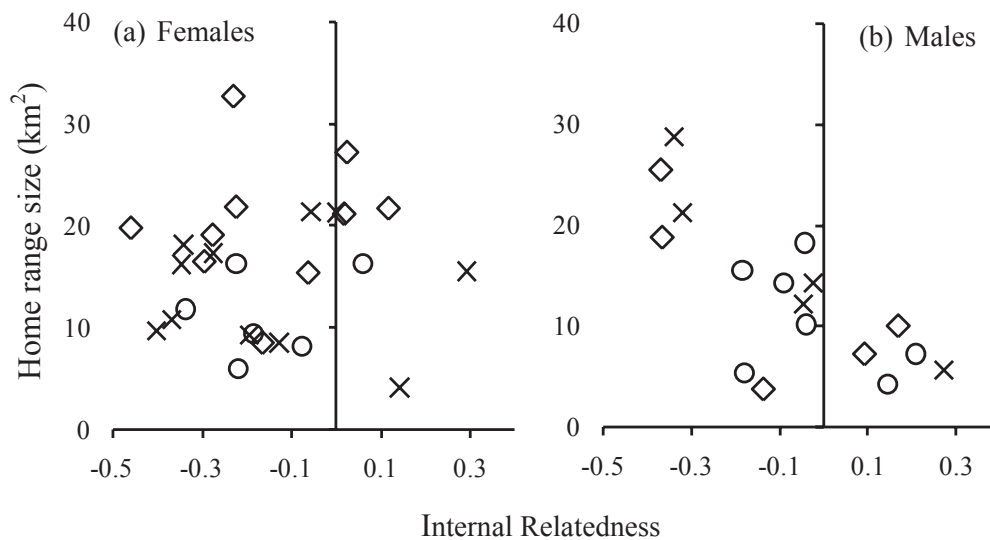
Figure 2. Variation between home range size and heterozygosity (internal relatedness) in (a) female and (b) male black rhinoceros from 3 reserves in Kenya: Mugie Rhino Sanctuary (circles), Lewa Wildlife Conservancy (diamonds), and Ol Pejeta Conservancy (crosses).
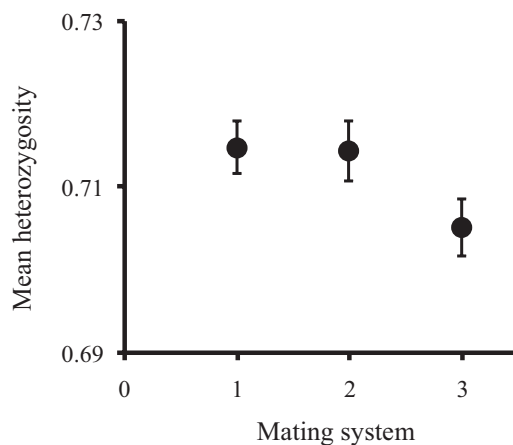

Figure 3. Simulated average heterozygosity at 9 microsatellite loci of 61 black rhinoceros offspring produced by females under 3 different mating systems: (1) selecting the actual males that were identified as fathers; (2) selecting a sire at random for every offspring; and (3) selecting males at random but maintaining the observed variance in reproductive success displayed by animals from the 3 Kenyan reserves (i.e., simulating number of full and half siblings identified for every female).

(mating system 3) than in a random-mating population (mating system 2; $t = 3.578$, df $= 12,187$, $p = 0.0003$) or when the simulated offspring genotypes were selected from the actual male–female pairs (mating system 1; $t = 3.999$, df $= 12,101$, $p = 6.407 \times 10^{-5}$). There was no significant difference ($t = 0.202$, df $= 12,025$, $p = 0.840$) in offspring average heterozygosity produced under ran-

dom mating and under simulated natural mating behavior (Fig. 3).

Discussion

In the absence of deterministic factors, such as poaching or loss of habitat, behind a population decline, the genetic characteristics and recovery of threatened populations is mediated by success of any reintroductions and subsequent reproductive behavior. We found a contrast in the factors associated with reproductive success in male and female black rhinoceros; a male's ability to maintain a large home range and his reproductive success was determined by his heterozygosity, whereas female reproductive output was a function of age. This first report of a significant HFC for reproductive output in an endangered species demonstrates a crucial role for individual differences in genetic diversity upon conservation management.

Greater variation in reproductive success among males than females is typical of a polygynous mating system associated with resource defense and underlines the widespread importance of territoriality (Huntingford & Turner 1987). Reproductive skew and polygyny in black rhinos has been noted (Garnier et al. 2001), but these data indicate that male variance in reproductive success is mediated by differences in heterozygosity (Fig. 1b) that are manifest also as variation in home range size (Fig. 2b). Although this is the first report of a direct association between heterozygosity, territory size, and reproductive success per se among individual vertebrates, there is emerging evidence that inbreeding affects individual ability to establish a territory; For example, the level of

inbreeding affects social status in fish (Tiira et al. 2006), competitive ability in shrews (Välimäki et al. 2007), and the probability of acquiring a breeding territory in some birds, either during competition for a lek (Höglund et al. 2002) or as a group effect of heterozygosity upon territory size and reproductive success in a cooperative breeder (Seddon et al. 2004).

Intrasexual competition is particularly relevant for conservation because inbreeding depression is more pronounced under conditions of increased stress (Keller & Waller 2002) and animals in enclosed reserves will be relatively restricted in their choice of home range characteristics and ability to avoid conflict with conspecifics. Smaller reserve size is associated with increased mortality rates in black rhinos (Linklater & Swaisgood 2008). Given the fitness benefits of obtaining a good territory, male–male competition for home range location will be intense. This competition is particularly evident for rhinos where fights are frequently fatal (Berger & Cunningham 1998; Linklater & Swaisgood 2008; Linklater et al. 2011), and the home range of every mature adult male in our samples did not overlap with the home range of any other male. By contrast, female rhinos experience lower intrasexual competition than males, for example, because males are generally available for mating because they make little paternal investment in their young beyond the provision of sperm and because female reproductive success apparently does not depend upon territory size. Although the nonsignificant effect of heterozygosity upon female rhino reproductive success contrasts with other studies of large vertebrates (e.g., Mainguy et al. 2009), a sex-biased HFC is consistent with an asymmetry in the level of intrasexual competition (Meagher et al. 2000; Mallet & Chippendale 2011). An indication of the level of variance in reproductive success among male rhinos could be inferred from data on home range sizes and thus be made without the need to use genetic techniques to identify the paternity of calves.

Because female black rhinos are almost exclusively dominant during intersexual encounters (Berger & Cunningham 1998), models of rhino mating behavior should incorporate an aspect of female choice for more heterozygous males (Hoffman et al. 2007). The signal for male genetic quality is unknown but presumably is olfactory as rhinos have poor eyesight. The association between male home range size and reproductive success indicates that the area over which scent is broadcasted may be important. The crucial issue for rhino breeding programs is whether mating decisions are based upon comparative evaluation among individuals or if there is a threshold value in quality (e.g., in heterozygosity, home range size) below which reproduction fails (Bateson & Healy 2005).

Genetic erosion is a concern for the health of small populations, and identifying factors that maintain genetic diversity remains important. It is therefore interesting that greater reproductive success of more heterozygous males limited the rate of loss of heterozygosity (Fig. 3), presumably either through parent–offspring correlations in heterozygosity (Mitton et al. 1993) or selection (Bensch et al. 2006). Anthropogenic strategies to maximize diversity within a group of enclosed reserves or isolated areas include actively translocating animals or establishing corridors to form a connected network. Such meta-population management is seen as an important tool to sustain genetic diversity in otherwise isolated populations (McCullough 1996; Linklater et al. 2011; Spiering et al. 2011). Rather few clear-cut ecological and demographic reasons behind success or failure of black rhinoceros translocations have been identified, and current best advice is that restocking may not be as complicated as generally believed (Linklater et al. 2011). Our data indicate that the reproductive success (cf. survival) of translocated males will depend upon the spatial distribution of heterozygosity. Individual-level measures of genetic diversity should be incorporated into any active management attempts to sustain representative genetic variation.

Significant identify disequilibrium and lack of evidence for an indirect local effect indicate that male black rhinoceros experience a fitness cost associated with a genome-wide loss of heterozygosity (Mainguy et al. 2009; Szulkin et al. 2010), presumably through spatial variation in drift and inbreeding. Quantitative estimates of inbreeding depression in vertebrate captive-breeding programs provide a median effect of 3.1 lethal equivalents per individual for juvenile survival (Ralls et al. 1998). In wild vertebrate populations, estimates of inbreeding depression vary between 2.5 and 8.1 diploid lethal equivalents for fecundity and from ∼1 up to >13.4 diploid lethal equivalents for traits affecting first year survival and survival to sexual maturity (O'Grady et al. 2006). The impact of inbreeding upon male wild black rhinoceros fitness traits is underpinned by an estimated 16 and 9 diploid lethal equivalents for offspring production and home range size, respectively. These data could be used to predict the consequences of inbreeding upon male fitness traits For example, in these populations mating between first cousins could lead to a 0.28 km^2 reduction in male home range size (Supporting Information).

The significant associations between marker heterozygosity and either reproductive output (black rhinoceros [this study]) or semen quality (Mohor gazelle, Iberian lynx, [Ruiz-Lopez et al. 2012]) represent a contrast with the few other studies on endangered animals that failed to detect inbreeding effects with a HFC (Grueber et al. 2008, 2011; Spiering et al. 2011). This difference highlights the lack of empirical data on inbreeding depression in wild populations of threatened species and that International Union for Conservation of Nature classification per se is less important than the context under which HFCs develop (Balloux et al. 2004; Slate et al.

2004; Szulkin et al. 2010). For example, statistical power may be limited through low marker polymorphism because of the type of locus (Ljungqvist et al. 2010) or prolonged inbreeding (Grueber et al. 2011). In black rhinos, reasonably high levels of marker polymorphism (Supporting Information) may be a consequence of the greater reproductive success of more heterozygous males (Fig. 3) and fairly recent demographic reductions. Moreover, any HFC requires individual variance in the level of inbreeding (Slate et al. 2004), which will be created through spatial differences in the severity of any bottlenecks as well as by naturally occurring population structure (Balloux et al. 2004) that will occur in philopatric species like rhinos (Linklater & Hutcheson 2010). Our black rhino study populations were a mixture of animals from formerly allopatric populations, most prominently from the south of Kenya and from the central highlands (B.C., A.B.W, & B.O., unpublished data). This mixing of animals with varied genetic backgrounds and intrasexual competition presumably act in synergy to expose a strong HFC. Bearing in mind that the extent of correlation between heterozygosity and the inbreeding coefficient is context dependent (e.g., Hedrick et al. 2001; Slate et al. 2004; Ruiz-Lopez et al. 2012), there is no a priori reason to dismiss HFCs as a means of studying inbreeding effects in wild populations of threatened taxa, particularly in cases where obtaining deep and accurate pedigrees is challenging.

Acknowledgments

Supporting Information

Descriptions of study sites, genotyping methods, and additional results (Appendix S1) and an analysis of parentage in a black rhino population in Zimbabwe (Garnier et al. 2001) (Appendix S2) 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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Sex-biased inbreeding effects impact upon reproductive success and home range size in the critically endangered black rhinoceros

Supporting Information for Online Publication Only

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Supporting Information provides a detailed description of the study sites, genotyping methods and additional results (Appendix S1) and a reanalysis of the parentage study on a black rhino population in Zimbabwe (data from Garnier et al. 2001) (Appendix S2).

Supporting Information Appendix S1

Additional Methods and Results
Site characteristics
Lewa Wildlife Conservancy (Lewa) is a 267 km^2 wildlife conservancy in Isiolo District. It was formerly a 162 km^2 private ranch, within which a 20 km^2 fenced black rhinoceros sanctuary was established in 1984 with a founding population of 4 females and 3 males from the north of Kenya and Solio Game Reserve. In 1987 another female was moved into the population from Solio Game Reserve. In 1994, the rhino protected area was expanded to include the entire ranch area, and another 4 males were moved into the population from Solio Game Reserve. LWC now includes 37 km^2 of additional land and the 57 km^2 Ngare Ndare Forest which forms a wildlife corridor between the Mount Kenya forest and the Samburu lowlands.

Mugie Rhino Sanctuary (Mugie) is a 93 km^2 part of the privately-owned Mugie Ranch located in northern Laikipia (0°74'N, 36°65'E). The ranch is 200 km^2 and subdivided into a working cattle ranch and a black rhino sanctuary, the two areas are bisected by the unpaved road which runs between Rumeruti and Maralal in Samburu District. In 2004, the sanctuary was founded by 20 black rhinos from Nairobi National Park, Lake Nakuru National Park and Solio Game Reserve.

Ol Pejeta Conservancy (Pejeta) (36°55'E, 00°02'N) is a 365 km^2 wildlife conservancy in the Laikipia District of Kenya. In 1989, 96 km^2 of land was designated as a game reserve predominantly for the conservation of 19 founding black rhinoceros that were received between 1989 and 1993 from Solio Game Reserve or Nairobi National Park, and with one male from Lewa Wildlife Conservancy.

Sample collection
The Kenya Wildlife Service (KWS) maintains a comprehensive database of all black rhinoceros within the sanctuary system with extensive training given to sanctuary personnel on the identification and monitoring of individual animals. One hundred and seven individually-identified black rhinoceros were sampled from the three sanctuaries. Faeces was the predominant source of DNA from Lewa (*n*=33) and for the new calves born in Mugie (*n*=7) with OPC sampled through either faecal samples (*n*=22) or tissue (*n*=19). Faecal sampling involved locating animals on foot and identifying individuals by either distinctive ear notches or horn shape. Once an animal was located and identified it was followed until the animal either defecated or ran away. For every positively-identified animal, two ~5 g samples of faeces were collected from the outside of the fresh dung pile. Samples were preserved with an approximately 5:1 ratio of desiccating silica:faeces and kept for up to six weeks at room temperature prior to DNA extraction. Several independent faecal sampling events occurred for most animals to ensure accurate identification. For the founder Mugie population KWS made available all of the serum samples (*n*=20) collected at the time of translocation. We also collected faecal samples from 21 animals (*n*=15 from Pejeta; *n*=6 from Lewa) and genotyped all material to assess the reliability of genotypes derived from faecal DNA extractions.

DNA extraction and genotyping
DNA was extracted from faecal samples using a QIAamp® DNA Stool Mini Kit (Qiagen) with minor modifications to the manufacturers' protocol of (1) extending the initial lysis at 55°C to overnight and (2) making two 50 µl elutions in 1xTE buffer after a 15 min incubation; three separate extractions were performed on each faecal sample. DNA extractions from tissue (Qiagen DNeasy Blood & Tissue Kit) and serum (Zymo Research Serum DNA Kit™) were performed according to the manufacturers' instructions.

For faecal DNA, the four replicate extracts with the highest DNA concentration were used to generate genotypes, with the added precaution for low-copy DNA of genotyping every sample six times at all loci. PCRs were performed in 25-µL final reaction volume containing 2 µl of faecal DNA extract (200 ng of tissue/serum DNA), 0.1µg/µl BSA, 200 µM each dNTP, 2.0-2.5 mM $MgCl_2$, 2.5 µl 10X Qiagen® PCR Buffer, 0.625U Qiagen® HotStarTaq™, and 0.5-1.0 µM each primer (forward primers were 5'-labelled with NED, PET, 6-FAM or VIC). Thermal cycling conditions were 96°C for 15 min, followed by 30 (tissue) or 40 cycles (faecal and serum) of (94°C, 1 min; T_a°C, 30 s; 72°C for 1 min), where T_a is the locus-specific annealing temperature (Brown et al. 1999; Cunningham et al. 1999; see Supporting Information Appendix S1 Table 1).

Analyses of genetic diversity
Genotype data were examined for allelic dropout and null alleles using MICROCHECKER v.2.2.3 (Van Oosterhout et al. 2004); additional estimates of allelic dropout for faecal DNA samples were derived by comparing genotypes obtained from faecal and tissue samples collected from the same animal (*n*=21). We used GENEPOP v.4.0 (Raymond & Rousset 1995; Rousset 2008) to calculate exact tests to identify whether there were any significant deviations from expected Hardy-Weinberg equilibrium conditions within each population (Markov chain parameters of 1,000 dememorisations, 100 batches and 1,000 iterations per batch) and to test for linkage disequilibrium between all pairs of microsatellite loci within populations. Because of this multiple testing, sequential Bonferroni corrections were applied to maintain a population-specific error rate of *α*=0.05 (Rice 1989).

Comparisons between samples that had complementary tissue and faecal samples (*n*=21) indicated a low genotyping error rate over all microsatellite loci (mean=0.13%; range=0.0-0.24%), with most (>99%) discrepancies due to allelic dropouts in one of the genotyping rounds. The multiple tubes genotyping approach thus allowed any ambiguous genotypes to be identified and resolved by two or more additional rounds of PCR. Independent faecal sampling events and multiple genotyping ensured that complete genotypes were derived for almost all individuals; eight loci were scored for two of the offspring at Lewa, and for 5 animals at Mugie (representing only one mature individual), and all individuals from Pejeta had complete genotypes at 9 microsatellite loci.

Null alleles were detected at one locus (DB44) that was excluded from the analyses. There was no significant deviation from expected Hardy-Weinberg Equilibrium conditions (*P*>0.05) for the nine microsatellite loci that did not suffer from null alleles (*i.e.* all loci except DB44). There was no evidence of significant (*P*>0.05) linkage disequilibrium among any pairs of loci, with the one exception of one pair of loci (Br17 & Br4) in one location (Mugie Rhino Sanctuary). Measures of genetic diversity for each reserve are provided in Supporting Information Appendix S1 Table 2.

Parentage analysis
We determined the number of offspring produced by each mature black rhino by parentage analysis. The 62 observations of mother-calf pairings were checked by parentage assignment using CERVUS v.3.0.3 (Marshall et al. 1998). Maternal candidates included all females aged 2 years or more at the time of offspring conception within the same sanctuary (Garnier et al. 2001). Critical LOD scores were determined by simulation for 100,000 offspring and a conservative (see Results) genotyping error rate of 1%. Mother-offspring pairings with >95% certainty were accepted. Paternity assignment was undertaken using the confirmed mother-calf pairings and a simulation of 100,000 offspring to determine critical delta scores, with a genotyping error rate of 1% and a conservative estimate of 80% probability of the true parent being sampled (we sampled ~92-95% of animals based on the Kenyan census). Parentage was accepted for all trio (mother-father-calf) estimates of >95% certainty. The genotype data were checked to ensure that all accepted fathers had compatible genotypes and that we had not overlooked any "next-best" fathers. Given the intensive monitoring of animals in these closed populations, the matches between genetic data and observed maternal-

calf pairings and the high probabilities obtained for parentage assignment it is unlikely our paternity data are biased by levels of genetic diversity (Wang 2010).

Best predictors of the numbers of offspring
To determine that multicollinearity among predictors did not affect our analyses we calculated variance inflation factors (VIFs) using the CORVIF function in the AED package (Zuur *et al*. 2009), where VIF>3 indicates a potential problem with multicollinearity (Zuur *et al*. 2009).

All VIFs for the female predictors were less than 3 therefore were retained for model selection; the correlations between all pairs of predictors were less than +/-0.3, except between home range size (HOM) and the number of females with overlapping home ranges (FOH) (r=0.763, df=25, p=3.77x10^{-6}). VIFs varied between 1.06 and 2.33 for the male predictors, with low correlations (r=+/-0.4 or less) between all pairs of variables except between IR and HOM (R=-0.740, df=15, p=6.86x10^{-4}) and MLH and HOM (R=0.664, df=15, p=0.0036).

Residuals of the final models did not exhibit a significant departure from normality (Shapiro-Wilk test; for female AGE – W=0.978, p=0.807; for male IR - W=0.948, p=0.392; for male MLH - W=0.948, p=0.401) or nor did the show any significant homoscedasticity (Breusch-Pagan test; for male IR - BP=0.007, p=0.932; for male MLH - BP=1.066, p=0.302). A similar outcome is obtained when examining the residuals for the final models that are based on standardised offspring number (Shapiro-Wilk test; for female AGE – W=0.964, p=0.444; for male IR - W=0.946, p=0.365; for male MLH - W=0.933, p=0.223) or homoscedasticity (Breusch-Pagan test; for female AGE – BP=1.533, p=0.216; for male IR - BP=0.0136, p=0.907; for male MLH - BP=0.817, p=0.775), with the exception that the final model for standardised female age depart from heteroscedasticity (Breusch-Pagan test; for female AGE – BP=4.693, p=0.030), although this test would not be significant if a sequential Bonferroni test for $k{\geq}2$ multiple tests of model residuals was applied (Rice 1989).

Potential effect of a HFC upon male fitness
To provide wider context to any HFC, an estimate of the inbreeding load can be derived using (1) an estimate of identity disequilibrium [g_2] and the basic descriptors of the HFC itself: (2) the mean [H] and (3) variance [$\sigma^2(H)$] of the estimate of MLH, (4) the regression slope ($\beta_{W,H}$) and (5) the coefficient of determination ($r^2_{W,H}$) of the relationship between MLH and the logarithm of the fitness measure (see Szulkin et al. 2010 for details). The potential impact of inbreeding upon the reproductive success of male black rhinoceros was estimated using the equations provided by Szulkin et al. (2010) that calculate the following:

(1) the squared correlation between the number of offspring and inbreeding ($r^2_{W,f}$),
$$r^2_{W,f} = (r^2_{W,H} / g_2) \text{ x } (\sigma^2(H) / H^2),$$

(2) the squared correlation between heterozygosity and inbreeding ($r^2_{H,f}$)
$$r^2_{H,f} = r^2_{W,H} / r^2_{W,f} \text{ , and}$$

(3) the potential inbreeding load ($\beta_{W,f}$)
$$\beta_{W,f} = \beta_{W,H} / \beta_{f,H},$$
$$\text{where } \beta_{f,H} \text{ is } [-H g_2 (1\text{-}f)] / [\sigma^2(H)].$$

Note that the inbreeding load is the slope of the regression of the (logarithm of) fitness trait on inbreeding and thus requires an estimate of the inbreeding coefficient (f). For black rhinoceros, we assumed a negligible average inbreeding coefficient (*i.e*. f=0), which generates a reasonable estimate of the inbreeding load $\beta_{W,f}$ unless there is particularly high inbreeding (Szulkin et al. 2010).

It is difficult to determine whether any inbreeding depression is the result of a few major mutations (*i.e.* lethals or semilethals) or a consequence of many mutations that have rather smaller detrimental effects. Thus the inbreeding load $\beta_{W,f}$ is typically referred to as an estimate of the number of lethal equivalents (LEs) that represent the reduction in fitness due to deleterious alleles that are exposed in inbred individuals per gamete (Keller & Waller 2002); doubling $\beta_{W,f}$ thus estimates the number of LEs per diploid individual.

For male rhinos, the HFCs based on MLH and the logarithms of standardised number of offspring (ln[OFFs]) and standardised home range size (ln[HOMs]) were: ln(OFFs)=-1.885+0.473, $p=0.001$, $R^2=0.499$ and ln(HOMs)=-1.960+0.267, $p=0.004$, $R^2=0.433$. Hence, there is a strong correlation between MLH and inbreeding ($r^2_{H,f}=0.401$) and variation in inbreeding apparently explains all ($r^2_{W,f}=1.245$) of the variance in males offspring production, with the high r^2 reflecting the error associated with the parameters used to derive $r^2_{W,f}$ (P. David, pers. comm.). The inbreeding load ($\beta_{W,f}$) for male offspring production is -8.06, which represents 18 lethal equivalents per diploid individual. There was a comparably strong effect of variation in inbreeding upon male home range size ($r^2_{W,f}=1.082$) the lead to an estimated inbreeding load of $\beta_{W,f}=-4.55$ and a concomitant estimate of 9 lethal equivalents per diploid individual.

Given that the inbreeding load ($\beta_{W,f}$) is the decline in fitness with inbreeding (*f*) (Keller & Waller 2002; Szulkin et al. 2010), the potentially deleterious effect of mating between relatives upon fitness traits can be illustrated. For example, reproduction between half-sibs and first cousins produces offspring with inbreeding coefficients of *f*=0.125 and *f*=0.0625 respectively. Such inbred males are predicted to experience a reduction in home range size of ~0.57-0.28 km^2 (*i.e.* 4.55x0.125 and 4.55x0.0625) and a reduction in reproductive success of between 1 and "0.5" calves. Of course, the actual impact would depend upon the background genetic characteristics (heterozygosity) of the animals within other populations.

Analysis of potential female HFC
Because of the qualitative relationship between IR and OFF for females we examined the potential HFC effect for females in more detail. No HFC was detected using all data (OFF=0.962-0.524IR, $p=0.415$, $R^2=0.028$). Rerunning the model selection procedure by excluding the "outlier" females with low heterozygosity (high IR of >0.1 in Fig. 1 main text) and high numbers of offspring (*n*=4 and 5) returns a final model with age as the sole, best explanatory variable for both IR and MLH. GLMs that incorporate an interaction between age and heterozygosity to predict offspring number are not significant ($p>0.05$ for both IR and MLH). Examining the reduced data set with heterozygosity as the single predictor of offspring numbers returns a significant HFC for when IR us used as the estimator of genetic diversity (OFF=0.634-1.778IR, $p=0.033$, $R^2=0.211$) but not with MLH (OFF=0.055-0.130MLH, $p=0.251$, $R^2=0.060$).

An analysis of a separate black rhino data (Garnier et al. 2001) set also failed to uncover a significant HFC in females (Supporting Information Appendix S2 Table 3). Thus, selective removal of data reveals a potential HFC that should be monitored, but the effect at present is weak as almost all females get to breed.

Supporting Information Table 1. Information about ten microsatellite loci (originally characterised by Brown et al. 1999; Cunningham et al. 1999) used to genotype black rhinoceros in there Kenyan reserves. Motif, longest published stretch of uninterrupted repeats; Dye, 5' fluorophore (Applied Bioystems) used for to label primers; T_a, PCR annealing temperature (ºC); $MgCl_2$, magnesium chloride concentration in PCR; Size, size range (in base pairs) of alleles; N_a, number of alleles.

Locus	BR17	DB5	DB1	DB66	BR4	BR6	DB52	DB23	DB44	DB14
Motif	$(GT)_{18}$	$(CA)_{13}$	$(CA)_{14}$	$(CA)_{16}$	$(CA)_{19}$	$(CA)_{15}$	$(CA)_{21}$	$(CA)_{12}$	$(CA)_{16}$	$(CA)_{13}$
Dye	PET	PET	NED	VIC	VIC	6-FAM	VIC	6-FAM	PET	6-FAM
T_a (ºC)	59	59	59	57	46	50	63	55	64	60
$MgCl_2$ (mM)	2.5	2	2	2	2	2	2	1.5	2	2.5
Size (bp)	127-137	187-209	118-130	182-208	117-147	139-145	209-225	174-185	172-192	283-289
N_a	6	10	6	8	13	4	9	4	6	3

Supporting Information Table 2. Sample sizes and mean levels of genetic diversity (with standard errors in parentheses) for three populations of black rhinoceros in Kenya. *N,* total number of animals sampled; N_m, number of male samples; N_f, number of female samples; N_a, number of alleles; A_r, allelic richness; IR, internal relatedness separately for males and females and for the entire population; MLH, multilocus heterozygosity for males and females and for the entire population.

Population	N	N_m	N_f	N_a	A_r	IR Male	IR Female	IR Total	MLH Male	MLH Female	MLH Total
Lewa	39	17	22	4.56 (0.50)	3.88 (0.43)	-0.047 (0.067)	-0.121 (0.046)	-0.089 (0.039)	0.671 (0.041)	0.710 (0.029)	0.693 (0.024)
Mugie	27	14	13	5.44 (0.58)	4.52 (0.35)	-0.040 (0.053)	-0.031 (0.062)	-0.036 (0.038)	0.759 (0.041)	0.762 (0.047)	0.761 (0.029)
Pejeta	41	18	23	5.22 (0.60)	4.38 (0.44)	-0.041 (0.049)	-0.043 (0.041)	-0.041 (0.031)	0.731 (0.036)	0.735 (0.033)	0.737 (0.024)
Total	**107**	**49**	**58**	**5.07 (0.32)**	**4.26 (0.23)**	**-0.043 (0.032)**	**-0.065 (0.027)**	**-0.057 (0.021)**	**0.718 (0.023)**	**0.731 (0.019)**	**0.730 (0.015)**

Supporting Information Appendix S2

**Re-analysis of Garnier et al.'s (2001) black rhinoceros data for an effect of HFC**
Garnier et al. (2001) provided an analysis of the mating system of a community of 35 black rhinoceros (17 males, 15 females, three of unknown sex and 19 offspring) in the Save Valley Conservancy (20°E, 31°S) in Zimbabwe. The animals were monitored over a four year period (August 1995 and August 1999) and exhibit substantial variance in reproductive success in males. There was no attempt to estimate a HFC in the study by Garnier et al. (2001). Using their genotype data (the same panel of ten microsatellite loci that were used in our study; Brown et al. 1999; Cunningham et al. 1999) and the results of the parentage analyses, we first calculated MLH and IR using IRMacroN4 (www.zoo.cam.ac.uk/zoostaff/meg/amos.htm#ComputerPrograms) (Amos et al. 2001) and then ran GLMs in *R* (R Development Core Team 2010) that used these estimators of heterozygosity as predictors of the numbers of offspring produced. One male was excluded from the analysis as he was one of the offspring that had matured sufficiently to sire just one offspring. No data on home range size or age were available for these animals.

Garnier et al.'s (2001) data reveal the same pattern reported for our 3 Kenyan reserves. Heterozygosity (both IR and MLH) are both significant predictors of the number of offspring produced by male rhinoceros, explaining about 60-70% of the variance between males (Supporting Information Appendix S2 Table 3). Heterozygosity has no significant effect upon offspring production by female black rhinos (Supporting Information Appendix S2 Table 3, Fig. 1).

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Supporting Information Table 3. Results of GLMs that examine the ability of heterozygosity (IR or MLH) to explain variation in the number of offspring (OFF) produced by male and female black rhinoceros from the Save Valley Conservancy, Zimbabwe. R^2 is the proportion of variation (explained deviance) explained by the final model (Zuur et al. 2009). Data are taken from Garnier et al. (2001).

		intercept	HET	R^2
Females	IR	0.923***	0.260	0.018
	MLH	1.217	-0.046	0.021
Males	IR	-0.856	-5.772***	0.714
	MLH	-7.494**	1.049**	0.620

*p<0.05, **p<0.01, ***p<0.001
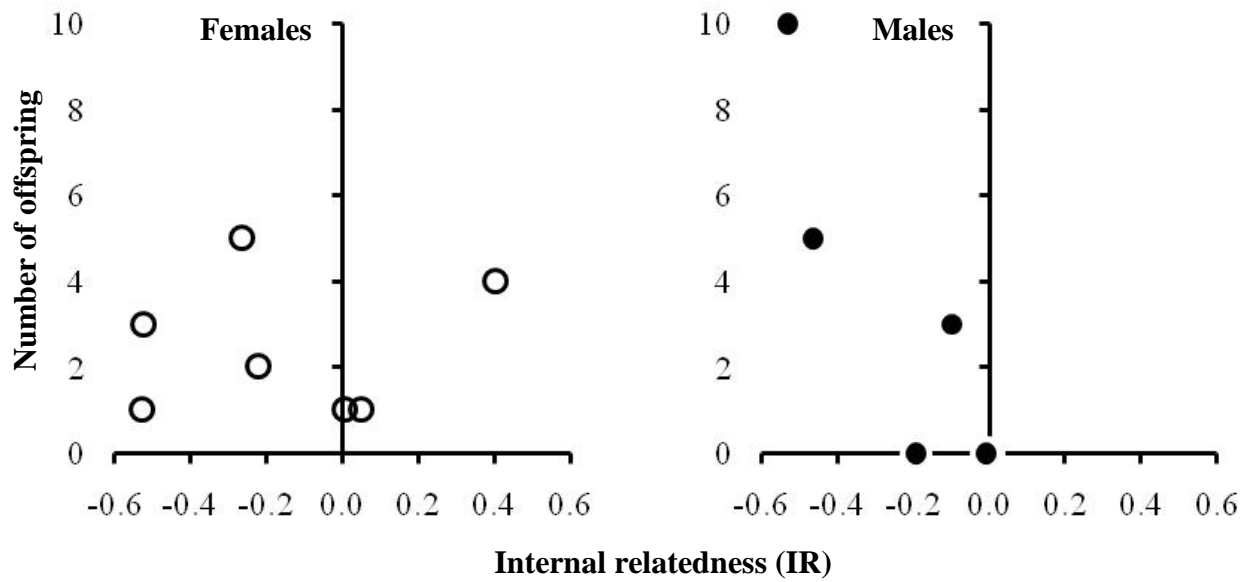
Supporting Information Figure 1. Variation in the numbers of offspring produced by male and female black rhinoceros at Save Valley Conservancy (20°E, 31°S) in Zimbabwe as a function of internal relatedness (a measure of heterozygosity, with more heterozygous individuals having lower values of IR). Data taken from Garnier et al. (2001).

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