



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 ($p < 0.01$, $r^2 > 44\%$). 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 nontreated 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

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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 ($p < 0.01$, $r^2 > 44\%$). 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 multi-locus 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 (*Lycan 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 mborr*), 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.spataleecology.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 km²), 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 $2\Delta\text{AICc}$ 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^2^a | |
|---------------------|----------|-----------|-----------|-----|-----------|----------|----------|-------|
| 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: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

^aExplained deviance—Proportion of variation explained by the model.

^bFinal generalized linear model included neither IR nor MLH.

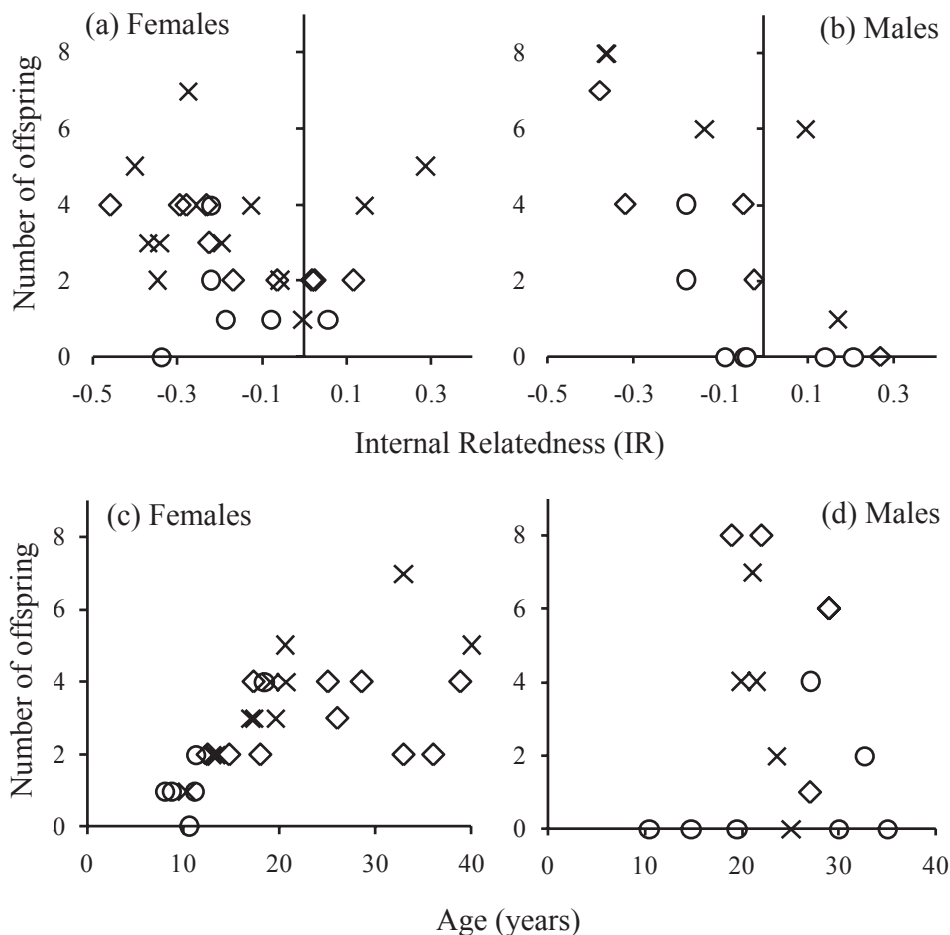


Figure 1. Relationship between the number of offspring produced and (a, b) heterozygosity (internal relatedness [IR]) and (c, d) age for (a, c) female and (b, d) male black rhinoceros from 3 reserves in Kenya: Mugie Rhino Sanctuary (circles), Lewa Wildlife Conservancy (diamonds), and Ol Pejeta Conservancy (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 ($\ln[\text{OFFs}]$) and standardized home range size ($\ln[\text{HOMs}]$) were $\ln(\text{OFFs}) = -1.885 + 0.473$, $p = 0.001$, $R^2 = 0.499$ and $\ln(\text{HOMs}) = -1.960 + 0.267$, $p = 0.004$, $R^2 = 0.433$.

Hence, there was a strong correlation between MLH and inbreeding ($r^2_{H,f} = 0.401$). 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

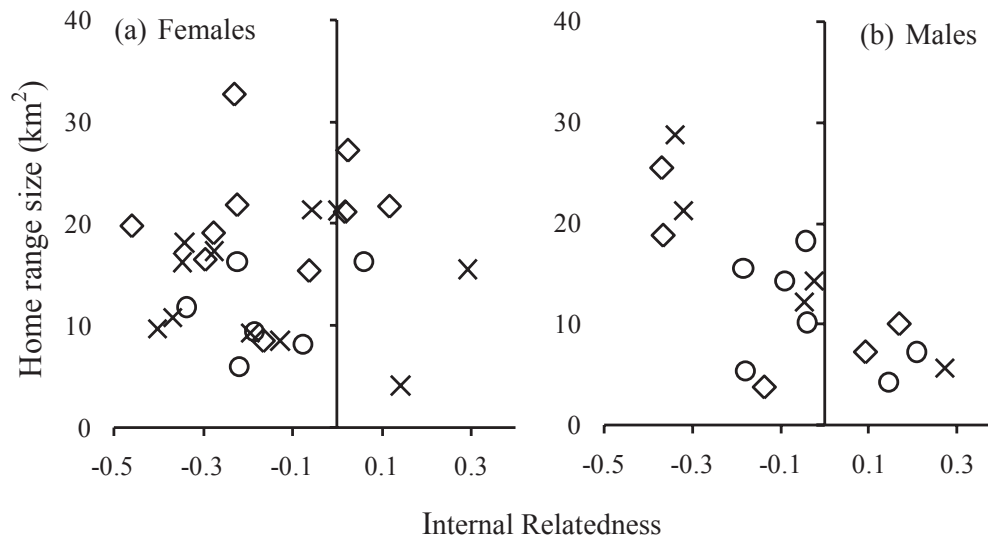


Figure 2. Variation between home range size and heterozygosity (internal relatedness) in (a) female and (b) male black rhinoceros from 3 reserves in Kenya: Mugie Rhino Sanctuary (circles), Lewa Wildlife Conservancy (diamonds), and Ol Pejeta Conservancy (crosses).

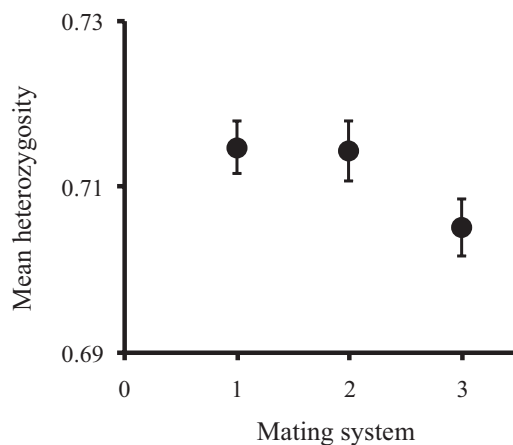


Figure 3. Simulated average heterozygosity at 9 microsatellite loci of 61 black rhinoceros offspring produced by females under 3 different mating systems: (1) selecting the actual males that were identified as fathers; (2) selecting a sire at random for every offspring; and (3) selecting males at random 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.

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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.

Literature Cited

- Amin, R., B. Okita, and M. Mulama. 2001. Kenya implementing a new black rhino information management system. *Pachyderm* **30**:96–97.
- Balloux, F., W. Amos, and T. Coulson. 2004. Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology* **13**:3021–3031.
- Barton, K. 2011. MuMIn: Multi-model inference. R package version 1.3.10. <http://CRAN.R-project.org/package=MuMIn>.
- Bateson, M., and S. D. Healy. 2005. Comparative evaluation and its implications for mate choice. *Trends in Ecology and Evolution* **20**:659–664.
- Bensch, S., H. Andren, B. Hansson, H. C. Pedersen, H. Sand, D. Sejberg, P. Wabakken, M. Akesson, and O. Liberg. 2006. Selection for heterozygosity gives hope to a wild population of inbred wolves. *PLoS ONE* **1**:e72.
- Berger, J., and C. Cunningham. 1998. Natural variation in horn size and social dominance and their importance to the conservation of black rhinoceros. *Conservation Biology* **12**:708–711.
- Brown, S. M., and B. A. Houlden. 1999. Isolation and characterization of microsatellite markers in the black rhinoceros (*Diceros bicornis*). *Molecular Ecology* **8**:1559–1561.
- Burnham, K. P., and D. R. Anderson. 1998. Model selection and multimodal inference. A practical information-theoretic approach. New York, Springer.
- Chapman, J. R., S. Nakagawa, D. W. Coltman, J. Slate, and B. C. Sheldon. 2009. A quantitative review of heterozygosity–fitness correlations in animal populations. *Molecular Ecology* **18**:2746–2765.
- Conway, A. J., and P. S. Goodman. 1989. Population characteristics and management of black rhinoceros *Diceros bicornis minor* and white rhinoceros *Ceratotherium simum simum* in Ndumugame reserve, South Africa. *Biological Conservation* **47**:109–122.
- Cunningham, J., E. H. Harley, and C. O’Ryan. 1999. Isolation and characterization of microsatellite loci in black rhinoceros (*Diceros bicornis*). *Electrophoresis* **20**:1778–1780.
- David, P., B. Pujol, F. Viard, V. Castella, and J. Goudet. 2007. Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology* **16**:2474–2487.
- Garnier, J. N., M. W. Bruford, and B. Goossens. 2001. Mating system and reproductive skew in the black rhinoceros. *Molecular Ecology* **10**:2031–2041.
- Grueber, C. E., G. P. Wallis, and I. G. Jamieson. 2008. Heterozygosity–fitness correlations and their relevance to studies on inbreeding depression in threatened species. *Molecular Ecology* **17**:3978–3984.
- Grueber, C. E., J. M. Waters, and I. G. Jamieson. 2011. The imprecision of heterozygosity–fitness correlations hinders the detection of inbreeding and inbreeding depression in a threatened species. *Molecular Ecology* **20**:67–79.
- Hansson, B., and L. Westerberg. 2002. On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology* **11**:2467–2474.
- Hedrick, P. W., R. Fredrickson, and H. Ellegren. 2001. Evaluation of d^2 , a microsatellite measure of inbreeding and outbreeding, in grey wolves with a known pedigree. *Evolution* **55**:1256–1260.
- Hoffman, J. I., J. Forcada, P. N. Trathan, and W. Amos. 2007. Female fur seals show active choice for males that are heterozygous and unrelated. *Nature* **445**:912–914.
- Höglund, J., S. B. Piertney, R. V. Alatalo, J. Lindell, A. Lundberg, and P. T. Rintamaki. 2002. Inbreeding depression and male fitness in black grouse. *Proceedings of the Royal Society of London B* **269**:711–715.
- Huntingford, F. A., and A. Turner. 1987. Animal conflict. Chapman & Hall, London.

- Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* **17**:230–241.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* **241**:1455–1460.
- Lent, P. C., and B. Fike. 2003. Home ranges, movements and spatial relationships in an expanding population of black rhinoceros in the Great Fish River Reserve, South Africa. *South African Journal of Wildlife Research* **33**:109–118.
- Linklater, W. K., K. Adcock, P. du Preez, R. R. Swaisgood, P. R. Law, M. H. Knight, J. V. Gedir, and G. I. H. Kerley. 2011. Guidelines for large herbivore translocation simplified: black rhinoceros case study. *Journal of Applied Ecology* **48**:493–502.
- Linklater, W. L., and I. R. Hutchesson. 2010. Black rhinoceros are slow to colonize a harvested neighbour's range. *South African Journal of Wildlife Research* **40**:58–63.
- Linklater, W. L., and R. R. Swaisgood. 2008. Reserve size, conspecific density, and translocation success for black rhinoceros. *Journal of Wildlife Management* **72**:1059–1068.
- Ljungqvist, M., M. Åkesson, and B. Hansson. 2010. Do microsatellites reflect genome-wide genetic diversity in natural populations? A comment on Väli et al. (2008). *Molecular Ecology* **19**:851–855.
- Mainguy, J., S. Côté, and D. W. Coltman. 2009. Multilocus heterozygosity, parental relatedness and individual fitness components in a wild mountain goat, *Oreamnos americanus* population. *Molecular Ecology* **18**:2297–2306.
- Mallet, M. A., and A. K. Chippendale. 2011. Inbreeding reveals stronger net selection on *Drosophila melanogaster* males: implications for mutation load and the fitness of sexual females. *Heredity* **106**:994–1002.
- Marshall, T. C., J. Slate, L. E. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**:639–655.
- Matson, S. E., M. D. Camara, W. Eichert, and M. A. Banks. 2008. P-LOCI: a computer program for choosing the most efficient set of loci for parentage assignment. *Molecular Ecology Resources* **8**:765–768.
- McCullough, D. R. 1996. *Metapopulations and wildlife conservation*. Island Press, Washington DC.
- Meagher, S., D. J. Penn, and W. K. Potts. 2000. Male-male competition magnifies inbreeding depression in wild house mice. *Proceedings of the National Academy of Sciences USA* **97**:3324–3329.
- Mills, A., P. Morkel, A. Amiyo, V. Runyoro, M. Borner, and S. Thirgood. 2006. Managing small populations in practice: black rhino *Diceros bicornis michaeli* in the Ngorongoro Crater, Tanzania. *Oryx* **40**:319–323.
- Mitton, J. B., W. S. F. Schuster, E. G. Cothran, and J. C. Defries. 1993. Correlation between the individual heterozygosity of parents and their offspring. *Heredity* **71**:59–63.
- O'Grady, J. J., B. W. Brook, D. H. Reed, J. D. Ballou, D. W. Tonkyn, and R. Frankham. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation* **133**:42–51.
- Okita-Ouma, B., R. Aman, and R. Kock. 2007. Conservation and management strategy for the black rhino (*Diceros bicornis michaeli*) and management guidelines for the white rhino (*Ceratotherium simum simum*) in Kenya (2007–2011). Pages 1–157 in Kenyan Wildlife Service, editors. 3rd edition. Kenyan Wildlife Service, Nairobi.
- R Development Core Team. 2010. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria.
- Ralls, K., J. D. Ballou, and A. Templeton. 1998. Estimates of lethal equivalents and the cost on inbreeding in mammals. *Conservation Biology* **2**:185–193.
- Ruiz-Lopez, M. J., N. Ganan, J. A. Godoy, A. Del Olmo, J. Garde, G. Espeso, A. Vargas, F. Martinez, E. R. S. Roldan, and M. Gomendio. 2012. Heterozygosity-fitness correlations and inbreeding depression in two critically endangered mammals. *Conservation Biology* **26**:1121–1129.
- Saccheri, I. J., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**:491–494.
- Seddon, N., W. Amos, R. A. Mulder, and J. A. Tobias. 2004. Male heterozygosity predicts territory size, song structure and reproductive success in a cooperatively breeding bird. *Proceedings of the Royal Society of London B* **271**:1823–1829.
- Slate, J., P. David, K. G. Dodds, B. A. Veenvliet, B. C. Glass, T. E. Broad, and J. C. McEwan. 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* **93**:255–265.
- Spiering, P. A., M. Szykman Gunther, M. J. Somers, D. E. Wildt, M. Walters, A. S. Wilson, and J. Maldonado. 2011. Inbreeding, heterozygosity and fitness in a reintroduced population of endangered African wild dogs (*Lycaon pictus*). *Conservation Genetics* **12**:401–412.
- Szulkin, M., N. Bierne, and P. David. 2010. Heterozygosity-fitness correlations: a time for reappraisal. *Evolution* **64**:1202–1217.
- Tiira, K., A. Laurila, K. Enberg, J. Piironen, A. Aikio, E. Ranta, and C. R. Primmer. 2006. Do dominants have higher heterozygosity? Social status and genetic variation in brown trout, *Salmo trutta*. *Behavioural Ecology and Sociobiology* **59**:657–665.
- Väli, U., A. Einarsson, L. Waits, and H. Ellegren. 2008. To what extent to microsatellite markers reflect genome-wide genetic diversity in natural populations? *Molecular Ecology* **17**:3808–3817.
- Välimäki, K., G. Hinten, and I. Hanski. 2007. Inbreeding and competitive ability in the common shrew (*Sorex araneus*). *Behavioural Ecology and Sociobiology* **61**:997–1005.
- Weir, B., and C. Cockerham. 1973. Mixed self and random mating at two loci. *Genetical Research* **21**:247–262.
- Wright, S. 1922. Coefficients of inbreeding and relationship. *The American Naturalist* **56**:330–338.
- Zuur, A. F., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. *Mixed effects models and extensions in ecology with R*. Springer, New York.

