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Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*)

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Evolutionary and conservation biologists have a long-standing interest in the consequences of inbreeding. It is generally recognized that inbred individuals may experience reduced fitness or inbreeding depression. By the same token, relatively outbred individuals can have greater than average fitness, i.e. heterosis. However, nearly all of the empirical evidence for inbreeding depression comes from laboratory or domestic species. Inbreeding depression and heterosis are difficult to detect in natural populations due to the difficulties in establishing pedigrees. An alternative method is to correlate heterozygosity, which is measured using genetic markers, with a trait related to fitness. The typically studied traits, such as juvenile survival and growth rates, either cover only early life or are weakly correlated with lifetime breeding success (LBS). In this paper we show that heterozygosity is positively associated with male and female adult LBS in a wild population of red deer (*Cervus elaphus*) on the Isle of Rum, Scotland. To the authors' knowledge, this is the first time that inbreeding depression and/or heterosis have been detected for a trait highly correlated with overall fitness in both sexes in a wild population.

Keywords: mean d^2; microsatellite; lifetime reproductive success; dominance variance; natural population

1. INTRODUCTION

One of the paradigms of evolutionary biology (Charlesworth & Charlesworth 1987) and conservation biology (Ralls *et al*. 1986) is that inbred individuals (those resulting from matings between kin) are expected to be less fit than outbred individuals, a phenomenon known as inbreeding depression. Inbreeding depression arises because closely inbred individuals have increased homozygosity at the genetic loci influencing fitness. This increased homozygosity will result in reduced fitness under either of the main proposed mechanisms for inbreeding depression, i.e. the overdominance and partial dominance hypotheses (Charlesworth & Charlesworth 1987). Similarly, heterosis occurs when inbred lines or distantly related individuals are crossed and heterozygosity at the loci in question is restored, leading to an increase in fitness or hybrid vigour (Falconer 1989). Thus, fitness should increase as the degree of inbreeding decreases.

There are numerous studies demonstrating inbreeding depression in laboratory and domestic species (reviewed in Charlesworth & Charlesworth 1987; Falconer 1989; Lynch & Walsh 1998) but relatively few in wild populations (see chapters 9–17 in Thornhill (1993) and Keller (1998)). However, a recent meta-analysis suggested that inbreeding depression may be greatest in the wild (Crnokrak & Roff 1999), possibly due to the harsher environmental conditions. Those studies that have been carried out in wild populations have focused on traits that are either expressed in early life, such as juvenile survival or are not necessarily strongly correlated with overall fitness, such as growth rates. Thus, it is unclear whether they demonstrate inbreeding depression for overall fitness. We know of only one study documenting inbreeding depression for overall fitness in a wild population, i.e. Keller's (1998) demonstration that inbred female song sparrows (*Melospiza melodia*) on Mandarte Island, Canada, had a reduced lifetime reproductive success.

Demonstrating inbreeding depression for overall fitness in a wild population is problematic for two reasons. First, if the study species is long lived then populations have to be intensively studied over a period of many years in order to measure their fitness. Second, estimating the relatedness of an individual's parents and, hence, its inbreeding coefficient requires extensive knowledge of the pedigree of a population across several generations. An alternative approach is to exploit the fact that inbreeding reduces heterozygosity (Hartl & Clark 1997). Hence, inbreeding depression in the wild can be detected by correlating the multilocus marker heterozygosity of individuals with a trait that is presumed to be associated with overall fitness (reviewed in Allendorf & Leary 1986; Roff 1997). The general consensus is that associations between multilocus heterozygosity and the components of fitness are common (Britten 1996), but that the statistical power for detecting these associations is usually low and negative results often go unreported (Whitlock 1993).

Previous studies of the unmanaged red deer (*Cervus elaphus*) population on the Isle of Rum, Scotland, have detected inbreeding depression and/or heterosis for juvenile traits (Coulson *et al*. 1998, 1999). Microsatellite markers were used to calculate two variables believed to

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reflect the relative degree of individual inbreeding or outbreeding. The first is multilocus heterozygosity and the second a recently introduced measure specific to microsatellites, mean d^2 (Coltman *et al*. 1998; Coulson *et al*. 1998). Mean d^2 is calculated from the length difference (in repeat units) between the two alleles at a microsatellite locus averaged across all typed loci. Assuming that microsatellites evolve under the stepwise mutation model (Valdes *et al*. 1993), d^2 at a locus should be related to the time since allelic coalescence. Hence, mean d^2 averaged across several loci provides a measure of the genetic distance between the parental gametes (Coulson *et al*. 1998). Mean d^2 is considered to focus on events deeper in an individual's ancestry than heterozygosity and is perhaps a tool more suited to detecting heterosis rather than inbreeding depression (Pemberton *et al*. 1999).

Calves with a high mean d^2 were on average born heavier and, hence, had greater neonatal survival than their low mean d^2 counterparts (Coulson *et al*. 1998). A subsequent analysis of first year over-winter survival found that mean d^2 was positively associated with female survival but negatively associated with male winter survival (Coulson *et al*. 1999). It was suggested that outbred male calves have high levels of androgens that are responsible for muscle growth and, thus, grow muscle rather than fat, leading to reduced survival in poor winters. However, outbred surviving calves might be expected to reach a large adult body size and obtain a high lifetime breeding success (LBS), i.e. the number of offspring produced in an individual's lifetime. Thus, it was predicted that mean d^2 or heterozygosity will be positively associated with LBS in males (Coulson *et al*. 1999). None of the juvenile traits discussed were associated with heterozygosity, leading to the suggestion that heterosis rather than inbreeding depression may be operating (Pemberton *et al*. 1999). Here, we consider whether inbreeding depression and/or heterosis influence LBS in the Isle of Rum study population. Earlier analyses of the study population have demonstrated that outbred calves tend to be born heavier (Coulson *et al*. 1998) and that heavier male calves have a greater LBS (Kruuk *et al*. 1999). Thus, we also investigated whether any associations between inbreeding and male LBS were independent of the role of inbreeding on birth weight. We focused only on animals that survived to breeding age as the role of inbreeding depression and/or heterosis on juvenile traits is already documented (see above).

2. MATERIAL AND METHODS

(a) *Study area and population*

The data were collected in the north block of the Isle of Rum, Scotland (57°0′ N, 6°20′ W), where the red deer population has been intensively monitored since 1971 (Clutton-Brock *et al*. 1982). Life-history data collected up to 1998 were used. Culling in the study area ceased in 1973 and the population density has fluctuated around its carrying capacity since 1982. All study area deer were individually recognized and their life-history data were accumulated from censuses performed five times per month (daily during the rutting and calving seasons). Routine genetic sampling of calves began in 1982, but almost 300 animals born prior to this date were sampled by chemical immobilization or from postmortem tissue.

The analyses presented in this paper considered individuals that survived to breeding age (three years old in females and five years old in males) and died of natural causes (primarily starvation during winter). Males born after 1986 and females born after 1985 were not considered, as estimates of their LBS from surviving individuals born into later cohorts were likely to be inaccurate. The sample sizes were 96 males and 113 females, of which 12 males and eight females were still alive by 1998. However, 95% of male LBS is achieved by age 12 years (Marshall 1998), while female fecundity declines after age 12 years (Clutton-Brock *et al*. 1982) with few calves born to females aged 14 years or more. All females included in the data set that were still alive in 1998 were aged at least 14 years.

(b) *Measuring lifetime breeding success*

A female's LBS was calculated as the number of calves born to a female during her lifetime. Female LBS was calculated from census data as calves remain with their mother for at least one year. Subsequent genetic analyses revealed no cases of misassigned maternity inferred by this method (Marshall *et al*. 1998). Male LBS was also determined using behavioural data. The conception date of a calf was estimated to have occurred in the period 230–240 days before birth (Clutton-Brock *et al*. 1982). Paternity was assigned to a male if (i) the calf's mother was observed to be in oestrus while in his harem, or (ii) she spent longer in his harem than in any other male's harem during this '11 day window'. If the mother was in the harem of rival males for an equal period of time, paternity was assigned to one candidate at random. While the resolution of correctly assigning paternity may be compromised by this approach, estimates of the relative LBS between males should still be accurate (Pemberton *et al*. 1992). The paternity of a number of calves was also previously determined by genetic methods (see Marshall *et al*. 1998). However, routine genetic sampling of the calves did not begin until 1982 and so the LBS of males born into earlier cohorts could not be accurately estimated using genetic approaches. The correlation between genetically and behaviourally derived estimates of LBS was 0.86 for males born after 1982 (Marshall 1998).

(c) *Molecular markers and genetic variables*

The animals were typed at up to nine highly variable microsatellite markers (see Coulson *et al*. 1998; Marshall *et al*. 1998). An animal was only included in an analysis if it was typed at six or more loci. Four genetic variables were calculated from the microsatellite data.

(i) Multilocus heterozygosity. The proportion of typed loci for which an individual was heterozygous.

(ii) Standardized multilocus heterozygosity. The ratio of the heterozygosity of an individual to the mean heterozygosity of those loci at which the individual was typed. This measure avoids any potential bias that may be introduced by individuals being untyped at particular loci (Coltman *et al*. 1999).

(iii) Mean d^2. The squared difference in repeat units between the two alleles at a locus averaged over all loci at which an individual was scored (see Coulson *et al*. 1998; Pemberton *et al*. 1999).

$$\text{Mean } d^2 = \sum_{i=1}^{n} \frac{(i_a - i_b)^2}{n}, \qquad (1)$$

Table 1. *Summary statistics for the genetic variables*

(All of the genetic variables were significantly correlated with each other. Note that the two heterozygosity measures were highly correlated with each other while the two mean d^2 measures were only weakly correlated with one another and with the heterozygosity measures. All of the correlations were significant at $p < 0.001$. The sample size was 209 (96 males and 113 females).)

		correlation with		
variable	mean (s.d.)	standardized heterozygosity	mean d^2	standardized mean d^2
heterozygosity	0.78 (0.14)	0.942	0.338	0.425
standardized heterozygosity	1.03 (0.18)	—	0.363	0.385
mean d^2	37.63 (19.74)	—	—	0.276
standardized mean d^2	0.045 (0.027)	—	—	—

Table 2. *GLMs of LBS*

(Full models for male and female LBS are shown. The change in deviance when each term is dropped yields a χ^2-statistic with 1 d.f. The proportion deviance refers to the proportion of the null model deviance explained by each term in the model. Population density in the year of birth was calculated as the number of females observed in the study area in at least 10% of the censuses performed in that year. Spring temperature was the mean maximum daily temperature in April and May of the year of birth. In females heterozygosity had a marginally higher χ^2-statistic than the standardized heterozygosity. However, the standardized measure is shown for consistency with the male model and also because it is an unbiased measure (see § 2). For males $n = 96$, the null model deviance = 109.5 and the proportion explained = 4.9%, and for females $n = 113$, the null model deviance = 149.1 and the proportion explained = 16.7%.)

term	χ^2	proportion deviance (%)	p	coefficient (s.e.)
males				
standardized heterozygosity	5.33	4.9	0.021	2.21 (0.87)
females				
density	13.74	9.2	< 0.001	− 0.0041 (0.0011)
standardized heterozygosity	5.29	3.5	0.021	0.48 (0.21)
spring temperature	3.73	2.5	0.053	0.094 (0.049)

where i_a and i_b are the lengths in repeat units of alleles a and b at locus i and n is the number of typed loci.

(iv) Standardized mean d^2. Calculated as above, but d^2 at each locus is scaled by the variance in d^2 at that locus before summing across loci. Standardizing mean d^2 in this way is expected to reduce the influence of highly polymorphic loci on the overall measure (Pemberton *et al*. 1999).

(d) *Statistical analysis*

Associations between genetic variables and LBS were examined using generalized linear models (GLMs) (McCullagh & Nelder 1989). As male LBS was positively skewed, it was analysed assuming a negative binomial error structure (see Kruuk *et al*. 1999). Female LBS was modelled with a Poisson error structure, and a log-link function was used for both traits. An earlier analysis by Kruuk *et al*. (1999) identified factors influencing LBS in each sex in the study population and, so, models were constructed both with and without these additional terms fitted. The fitted terms were birth weight for males and spring temperature and population density in the year of birth for females. All predictor variables and first-order interaction terms were initially fitted in a full model. Each term was then dropped from the full model unless doing so significantly reduced the amount of deviance explained. The change in deviance between two models was distributed as a χ^2-statistic with the degrees of freedom equal to the difference in the degrees of freedom between the models. Only one genetic variable was tested in any model. In addition, non-parametric correlations were performed in order to ensure that significant results obtained from the GLMs could not be attributable to data points of high leverage. A significance level of $p < 0.05$ was used for all statistical tests. All statistical analyses were performed using SPLUS (v. 4.5, MathSoft, Inc., 101 Main Street, Cambridge, MA 02142-1521, USA).

3. RESULTS

(a) *Summary statistics*

The means and standard deviations for LBS were 6.28 (s.d. = 2.77) and 5.84 (s.d. = 8.27) for females and males, respectively. The means, standard deviations and correlations (Pearson) with the other genetic variables for each genetic variable are given in table 1. Standardized mean d^2 was positively skewed and was square-root transformed prior to GLM construction.

(b) *Male lifetime breeding success*

Simple models tested whether any of the genetic variables fitted alone explained significant variation in male LBS. Standardized heterozygosity ($\chi^2_1 = 5.33$ and $p = 0.021$) (table 2) and standardized mean d^2 ($\chi^2_1 = 4.35$ and $p = 0.037$) explained a significant proportion of the variation in male LBS, while unstandardized heterozygosity ($\chi^2_1 = 3.15$ and $p = 0.076$) and unstandardized

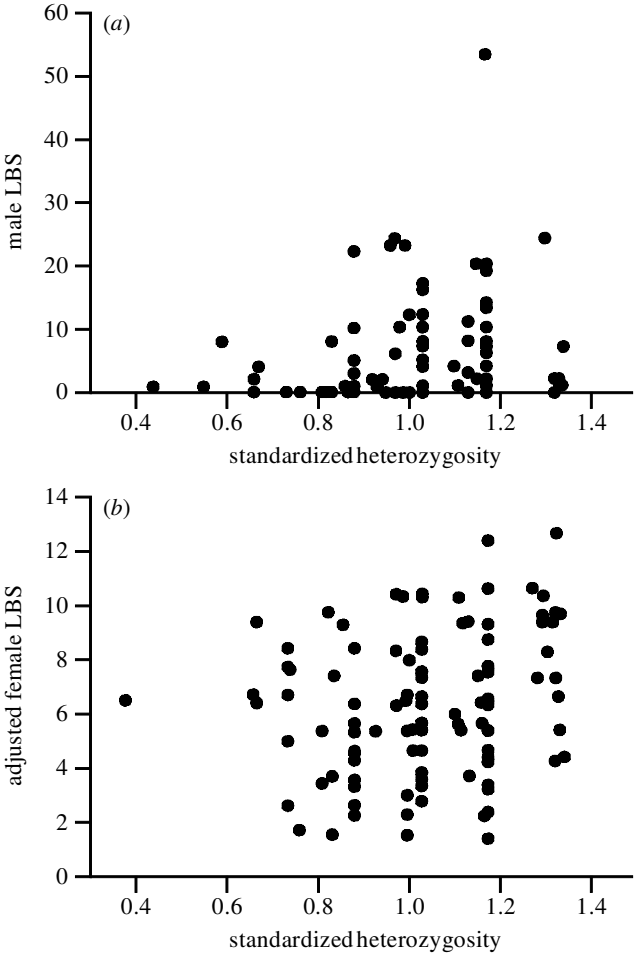

Figure 1. LBS plotted against standardized heterozygosity for (*a*) males and (*b*) females. Female LBS was adjusted for the effects of density and spring temperature in the year of birth. The relationship between the standardized heterozygosity and LBS is significant for both sexes ($p = 0.021$ for each sex).

mean d^2 ($\chi^2_1 = 3.50$ and $p = 0.061$) did not. Relatively outbred males had greater LBS (see figure 1*a*). All significant results were supported by a significant ranked correlation between male LBS and the genetic variable of interest, suggesting that significant terms in the parametric models cannot be attributed to points of high leverage.

A subset of 60 males were typed for at least six loci and also weighed at birth. In this relatively small sample, birth weight ($\chi^2_1 = 1.41$ and $p = 0.225$) did not explain significant variation in male LBS. Mean d^2 was the only genetic variable to explain significant variation in male LBS ($\chi^2_1 = 4.12$ and $p = 0.042$). However, birth weight was retained in the model and each genetic term then fitted to test whether they explained variation in the male LBS independently of any effect they may have had on birth weight. None of the genetic terms were significant (heterozygosity, $\chi^2_1 = 0.86$ and $p = 0.35$; standardized heterozygosity, $\chi^2_1 = 1.64$ and $p = 0.20$; mean d^2, $\chi^2_1 = 2.80$ and $p = 0.094$, and standardized mean d^2, $\chi^2_1 = 1.60$ and $p = 0.20$). However, ranked correlations between LBS (corrected for birth weight) and each genetic variable were at or close to significance, suggesting that the degree of inbreeding or outbreeding did have an effect on male

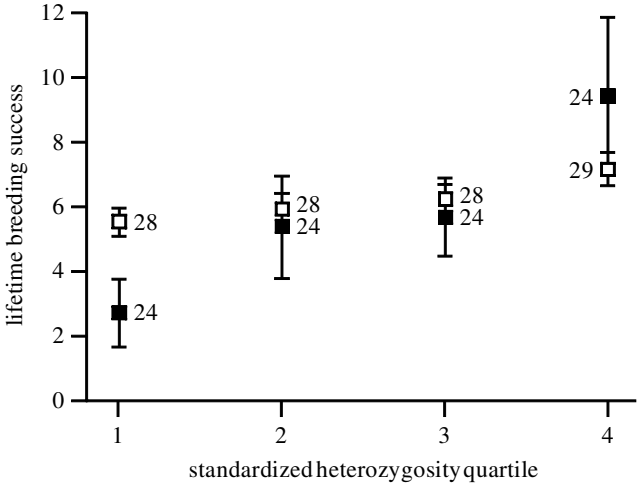

Figure 2. LBS of males and females in relation to standard ized heterozygosity quartiles. The female LBS was adjusted for the effect of density and spring temperature in the year of birth. The relationship between the standardized heterozygosity and LBS appears to be stronger in males (filled squares) than females (open squares).The numbers indicate the sample size in each quartile and the error bars represent one standard error of the mean.

LBS, regardless of any effect on birth weight. The correlations between the birth-weight-corrected LBS and each genetic variable were as follows: heterozygosity Spearman's $r_s = 0.183$ and $p = 0.162$, standardized heterozygosity Spearman's $r_s = 0.232$ and $p = 0.075$, mean d^2 Spearman's $r_s = 0.266$ and $p = 0.040$ and standardized mean d^2 Spearman's $r_s = 0.228$ and $p = 0.080$.

(c) *Female lifetime breeding success*

When fitted alone, heterozygosity ($\chi^2_1 = 4.97$ and $p = 0.026$) and standardized heterozygosity ($\chi^2_1 = 4.60$ and $p = 0.032$) explained significant variation in female LBS. Mean d^2 ($\chi^2_1 = 0.00$ and $p = 0.97$) did not explain variation in female LBS, but standardized mean d^2 approached significance ($\chi^2_1 = 3.72$ and $p = 0.054$). Females with high heterozygosity had greater LBS. Models were constructed in which spring temperature and density in the year of birth were also fitted (see Kruuk *et al.* 1999). Again, heterozygosity ($\chi^2_1 = 5.90$ and $p = 0.015$) and standardized heterozygosity ($\chi^2_1 = 5.29$ and $p = 0.021$) (see table 2 and figure 1*b*) were significant terms but mean d^2 ($\chi^2_1 = 0.01$ and $p = 0.93$) and standardized mean d^2 ($\chi^2_1 = 0.87$ and $p = 0.35$) were not. Note that spring temperature was not quite significant ($p = 0.053$), but was retained in the full model as this term explained a highly significant amount of variation in female LBS in the analysis by Kruuk *et al.* (1999). The full model is shown in table 2.

4. DISCUSSION

In this paper, we have demonstrated that LBS increases with multilocus heterozygosity or related variables in both female and, in particular, male red deer (figure 2). This suggests that relatively outbred red deer have greater LBS than their more inbred counterparts. To the authors' knowledge, this is the first time that inbreeding depression

and/or heterosis for LBS have been demonstrated for both sexes in a wild population. The observation that heterozygosity explained more variance in the male LBS supports recent evidence that male–male competition can exacerbate inbreeding depression in the wild (Meagher *et al*. 2000).

An earlier study (Coulson *et al*. 1998) demonstrated that relatively outbred calves had greater birth weights than inbred calves. Subsequently, Kruuk *et al*. (1999) showed that birth weight was positively associated with male LBS. These two findings suggest that any association between LBS and inbreeding may be due to the effects of birth weight. In this study only 60 calves were weighed and genotyped, but even with this limited data set it appears that the relationship between the degree of inbreeding and male LBS is independent of any influence of birth weight. This study also confirms Coulson *et al*.'s (1999) prediction that, although outbred males suffer reduced juvenile survival, outbred survivors have greater LBS.

In these analyses, heterozygosity (standardized or not) tended to be more frequently associated with LBS than either mean d^2 measure. This was in contrast to the juvenile traits where all associations were with mean d^2 (Coulson *et al*. 1998, 1999). It is unclear why heterozygosity was associated with some traits in this population while mean d^2 was associated with others. It is possible that heterosis (as detected by mean d^2) influenced the juvenile traits whereas inbreeding depression (as detected by heterozygosity) influenced the adult traits, although there is no concrete evidence for this. Alternatively, the lack of association for some genetic variable–trait combinations may simply reflect a lack of statistical power in detecting what are only subtle effects. The standardized measures were more often significant terms than their unstandardized equivalents. In models of female LBS standardized mean d^2 approached significance, while the unstandardized measure explained no deviance in the model. However, in the full model for female LBS neither term was significant. The two mean d^2 measures were only weakly correlated with each other and with heterozygosity, while the two heterozygosity measures were strongly correlated. Until the microsatellite mutation process is fully understood it is impossible to know exactly what mean d^2 is measuring. It may be possible to understand the relationship between the different genetic variables better using data from studies that use larger panels of markers, such as quantitative trait loci analyses.

A common interpretation of Fisher's (1930) fundamental theorem of natural selection is that the traits most closely related to overall fitness have a relatively low heritability, as favourable alleles will be selected to fixation (for empirical evidence see Gustafsson 1986; Mousseau & Roff 1987; Falconer 1989; Kruuk *et al*. 2000; Merilä & Sheldon 2000). There is some debate as to whether the modest heritability of life-history traits is due to low levels of additive genetic variance or simply because these traits have a large environmental variance component (Houle 1992; Kruuk *et al*. 2000; Merilä & Sheldon 2000). It has been argued (Crnokrak & Roff 1995; Roff 1997) that, if the former is true, a greater proportion of the genetic and phenotypic variation in life-history traits should be due to non-additive genetic variance such as dominance variance. Therefore, inbreeding depression, which can only occur when dominance variance is present, should be greatest for fitness traits under strong directional selection. In this population, heterozygosity and/or mean d^2 explained *ca*. 1–2% of the deviance in the models of juvenile survival (Coulson *et al*. 1998, 1999) but 4–5% of the deviance in the models for LBS, lending support to the idea that dominance variance explains a greater proportion of the overall variation in traits closely related to fitness. Future work will seek to estimate dominance variance across a suite of morphometric and life-history traits in this population.

Finally, although the implications of inbreeding depression have long been considered in conservation biology (Ralls *et al*. 1986), there is rather limited evidence that it actually influences the viability of natural populations. In one notable study, Saccheri *et al*. (1998) demonstrated that Finnish populations of the Glanville fritillary butterfly (*Melitaea cinxia*) were more likely to become extinct if they were inbred. If the results presented in this paper are general to other vertebrate species, it seems likely that both reintroduced and small, isolated populations may experience a risk of extinction due to the effects of inbreeding depression on LBS.

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