



Microsatellites reveal heterosis in red deer

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The fitness consequences of inbreeding and outbreeding are poorly understood in natural populations. We explore two microsatellite-based variables, individual heterozygosity (likely to correlate with recent inbreeding) and a new individual-specific internal distance measure, mean d^2 (focusing on events deeper in the pedigree), in relation to two measures of fitness expressed early in life, birth weight and neonatal survival, in 670 red deer calves (*Cervus elaphus*) born on the Isle of Rum between 1982 and 1996. For comparison, we also analyse inbreeding coefficients derived from pedigrees in which paternity was inferred by molecular methods.

Only 14 out of 231 calves (6.1%) had non-zero inbreeding coefficients, and neither inbreeding coefficient nor individual heterozygosity was consistently related to birth weight or neonatal survival. However, mean d^2 was consistently related to both fitness measures. Low mean d^2 was associated with low birth weight, especially following cold Aprils, in which foetal growth is reduced. Low mean d^2 was also associated with low neonatal survival, but this effect was probably mediated by birth weight because fitting birth weight to the neonatal survival model displaced mean d^2 as an explanatory variable.

We conclude that in the deer population fitness measures expressed early in life do not show evidence of inbreeding depression, but they do show evidence of heterosis, possibly as a result of population mixing. We also demonstrate the practical problems of estimating inbreeding via pedigrees compared with a direct marker-based estimate of individual heterozygosity. We suggest that, together, individual heterozygosity and mean d^2 , estimated using microsatellites, are useful tools for exploring inbreeding and outbreeding in natural populations.

Keywords: red deer; microsatellites; stepwise mutation; inbreeding depression; heterosis; birth weight

1. INTRODUCTION

In diploid organisms the genetic relationship between two parents may vary in ways that affect offspring fitness. Inbreeding, matings between close kin, increases the proportion of polymorphic loci at which an individual is homozygous (Wright 1921), and if deleterious recessive alleles are segregating in a population, will reduce fitness (East & Jones 1919). In addition, if heterozygote advantage, or overdominance, is a general phenomenon across the genome, increased homozygosity will also reduce fitness (Crow 1948). The relative importance of these two processes is still a matter of research (Charlesworth & Charlesworth 1987), but together they are known as inbreeding depression. Inbreeding depression may have shaped many aspects of mating systems in nature, especially self-incompatibility in plants and sex-biased dispersal in both plants and animals, although evidence is often lacking in this area (Charlesworth & Charlesworth 1987).

Matings between distantly related individuals can also have fitness consequences for the offspring, but with

diverse outcomes. In the context of animal and plant breeding, crosses between diverged inbred lines can result in hybrid vigour or heterosis (Shull 1948), presumably due to increased heterozygosity for loci with overdominance and reduced homozygosity for loci with deleterious recessives. However, more extreme outbreeding, for example in the context of naturally forming hybrid zones, often reduces the fitness of hybrid offspring (so called 'outbreeding depression'), which is attributed to disruption of local adaptation or co-adapted groups of genes or both (Templeton 1986).

The genetic relationship between the parents of an individual can thus be viewed as a continuum along which there may be changing fitness consequences for the offspring (Waser 1993). Hitherto, measuring offspring fitness consequences along parts of this continuum in natural populations has proved difficult. For example, estimating the inbreeding coefficient of large numbers of individuals in natural populations is made difficult by the problems of establishing multigeneration pedigrees. Even so, there is now a small number of studies showing inbreeding depression in natural populations, for example Greenwood *et al.* (1978) and Keller *et al.* (1994), both studies of wild passerines. An alternative approach,

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avoiding pedigrees, is to use individual average heterozygosity (hereafter referred to as individual heterozygosity), measured across several marker loci, as the independent variable against which to test fitness. There is an extensive literature on this topic using allozyme variation. Overall, reviews tend to favour a positive association between individual heterozygosity and measures of fitness (e.g. Allendorf & Leary 1986; Ledig 1986; Mitton 1993) and favour overdominance as well as dominance as the mechanisms involved. However, non-significant results are probably underreported. A general problem with these studies is the relatively small number of polymorphic loci studied and the rather small number of alleles segregating, so that most loci are homozygous in the absence of inbreeding. At the other end of the continuum, in some hybrid zones, marker-based measures of hybridism have been correlated with reduction in fitness (e.g. Sage *et al.* 1986).

In recent years microsatellites have become the marker of choice in many population genetic studies, owing to their abundance and high heterozygosity in most eukaryote genomes (Queller *et al.* 1993; Jarne & Lagoda 1996). For these reasons alone they are likely to be better than allozymes for measuring variation in individual heterozygosity due to inbreeding. In parallel, several microsatellite-specific population distance measures have been developed which incorporate the mutation dynamics of microsatellites (Slatkin 1995; Goldstein *et al.* 1995). Together these developments suggested to us a novel use of microsatellites to measure an individual-specific internal distance, which potentially captures variation in the relationship between the parents of an individual ranging from recent inbreeding through to population mixing.

Microsatellites consist of tandem repeats of a simple DNA motif, one of the commonest mammalian motifs being CA (Moore *et al.* 1991). The high level of polymorphism observed at many microsatellite loci is generated by a relatively high mutation rate, *ca.* 10^{-3} (Gyapay *et al.* 1994), to new length variants with different numbers of repeats. Most observed mutations consist of an increase or decrease of one repeat (Weber & Wong 1993; Di Rienzo *et al.* 1994), which has led to the stepwise model of microsatellite evolution (Valdes *et al.* 1993) under which, because new alleles are most likely to have been derived from alleles one repeat unit different from themselves, allele lengths contain historical information. It follows that the distance D (in repeat units) between two alleles at a locus is related to their time since coalescence. Working at the population level, Goldstein *et al.* (1995) have shown that this distance squared (D^2), averaged over many loci (mean D^2), is linearly correlated with the time since two populations diverged.

Using the same logic we propose that a similar measure can be estimated for each individual, which we denote as mean d^2 , using lower case to indicate that it applies to an individual. Mean d^2 is estimated from the two alleles each individual has at a locus averaged over loci and is a measure of the genetic distance between the gametes that formed the individual. It is useful to contrast what individual heterozygosity and individual mean d^2 (hereafter referred to as mean d^2) measure when calculated from the same microsatellite data. Individual heterozygosity measures

the proportion of loci which are heterozygous versus homozygous and should be inversely correlated with the extent of recent inbreeding in the pedigree. Mean d^2 records homozygosity, but in microsatellites with several alleles, the contribution of homozygosity may be swamped by the contribution of allele length variation. In general, therefore, mean d^2 focuses on events deeper in the pedigree than individual heterozygosity. In an isolated population, allele length variation, and hence variation in mean d^2 , will reflect the founding alleles and mutations since founding, but in a population subject to immigration, mean d^2 will include differences in allele length between populations due to stepwise mutation since coalescence. We hypothesize that the probability that loci throughout the genome accumulate mutations responsible for heterosis and/or inbreeding depression will be correlated with time since microsatellite allele coalescence.

In this paper we investigate inbreeding coefficient, individual heterozygosity and mean d^2 in relation to two fitness measures expressed early in life in individually monitored red deer calves (*Cervus elaphus*) on the Isle of Rum, Scotland. Birth weight has been extensively studied in the population and is positively associated with several fitness components including neonatal survival, first winter survival, and several aspects of female reproductive success (Clutton-Brock *et al.* 1982; Albon *et al.* 1987; Clutton-Brock & Albon 1989). Neonatal survival has been extensively investigated in relation to inbreeding in captive non-domesticated ruminants and frequently shows inbreeding depression (e.g. Ralls *et al.* 1979).

2. MATERIALS AND METHODS

(a) *Study area and population*

All data were collected in the North Block of the Isle of Rum, Scotland (57° 01'N, 06° 17'W, NM-402996), where the red deer are the subject of a long-term individual-based study (Clutton-Brock *et al.* 1982; Clutton-Brock & Albon 1989). The population was founded by introduction starting in 1845 and was sourced from at least four different British mainland populations, of which the most recent introduction was from Eastern Scotland in the 1970s.

We studied all calves born into the study population between 1982 (when routine genetic sampling began) and 1996. Date of birth was known for 93% of calves, and of these approximately 70% were caught within a few days of birth. Calves were weighed, sexed, tagged and sampled for genetic analysis by taking blood samples or ear plugs. Birth weight was estimated by regressing capture weight against capture age (Guinness *et al.* 1978a). As the residuals about the common regression line were randomly distributed, the residual of each individual from the fitted line

$$\text{capture weight (kg)} = 6.483 + 0.0154 * \text{capture age (h)} + s$$

was added to the predicted weight at age zero. s is a sex-related constant equal to zero for females and 0.38 for males.

Neonatal mortality was defined as death between birth (median 8 June) and 1 October. However, most neonatal mortality occurred in the first few days after birth (Guinness *et al.* 1978b). Neonatal calf mortality averaged 18% and did not vary significantly between years. In most cases (76% of deaths) the calf disappeared and the cause of mortality was unknown;

where identified, the causes of mortality included calving complications, accidents, and predation by golden eagles (*Aquila chrysaetos*).

(b) *Microsatellites, individual heterozygosity and mean d^2*

Individual calves were typed at nine dinucleotide repeat microsatellite loci, OarFCB193, OarFCB304, CelJP15, CelJP27, CelJP38, MAF109, MAF35, OarCP26 and TGLA94 (Marshall *et al.* 1998). Loci were originally selected for paternity inference and had between six (CelJP27) and 13 alleles (OarCP26). Not all individuals were successfully scored at all loci. Individuals that had been scored at fewer than seven loci were omitted from heterozygosity and mean d^2 analyses.

Individual heterozygosity was calculated across all scored loci. If an individual was heterozygous at a locus it was scored as a '1' and if homozygous as a '0'; the mean across all loci scored was then taken.

Mean d^2 was calculated as the squared distance in repeat units between the two alleles an individual had at a microsatellite locus, averaged over all loci at which an individual was scored, i.e.

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

where i_a and i_b are the lengths in repeat units of alleles a and b at locus i , and n is the total number of loci at which an individual was scored (seven, eight or nine).

(c) *Paternity analysis and inbreeding coefficient*

We have undertaken a likelihood-based analysis of paternity in the study population using the method described in detail in Marshall *et al.* (1998). Briefly, using genotypes for the nine microsatellite loci named above and three allozyme loci (mannose phosphate isomerase, isocitrate dehydrogenase and transferrin, see Pemberton *et al.* (1988)), and in some cases DNA fingerprints (Pemberton *et al.* 1992) we inferred paternity for 231 study calves at 95% confidence. We used these inferred paternities, paternities inferred for pre-1982 calves and known maternal identities to calculate individual inbreeding coefficients (f) using the program FASTINB (Boyce 1983, Freeware). Note that the minimum pedigree required to record a non-zero inbreeding coefficient includes at least one grandparent, in our case usually a grandmother. For each calf with an inferred paternity we had this minimal pedigree, but in many cases we had little more than this minimum.

(d) *Statistical analysis*

We investigated the influence of inbreeding coefficient, individual heterozygosity and mean d^2 on two traits expressed early in life: birth weight and neonatal survival. All analyses were done using generalized linear models in the package Genstat (Genstat 5 Committee 1995).

(i) *Birth weight*

Birth weight is normally distributed and was analysed using bivariate regression models or general linear models with a normal error structure (Sokal & Rohlf 1995). Initial simple models examined the relation between birth weight and inbreeding coefficient (alone), individual heterozygosity (alone) or mean d^2 (alone).

Previous studies have documented several variables affecting birth weight (Guinness *et al.* 1978a; Albon *et al.* 1983, 1987), so more complex general linear models were developed to investigate the influence of inbreeding coefficient, individual heterozygosity and mean d^2 after other known sources of variation had been removed. In all cases the significance of terms was obtained by dropping them from the model, and terms only remained in the full model if significant at $p < 0.05$. The influence of inbreeding coefficient, individual heterozygosity and mean d^2 was explored by fitting them to the full model and checking that all other terms remained significant. Associations between birth weight and two-way interactions between fitted variables were also explored. The following variables were investigated.

1. Calf sex: categorical, female or male.
2. Birth date: continuous, the number of days after 1 May that a calf was born.
3. Mother's age: continuous, in years, fitted as a linear or a quadratic function.
4. Mother's status: categorical, with five levels.
 - (i) first breeder—had not bred previously;
 - (ii) true yield—did not breed in the previous year;
 - (iii) summer yield—bred in previous year, but calf died before 1 October;
 - (iv) winter yield—bred in previous year, but calf died in winter;
 - (v) milk—successfully reared a calf the previous year.
5. Population density: continuous, the number of females over one-year-old using the study area (as determined from regular censuses of the study area).
6. Weather during gestation: continuous, mean monthly temperature ($^{\circ}\text{C}$) and total monthly precipitation (mm) for January to June.

(ii) *Neonatal survival*

Neonatal survival is a binary variable and was analysed using logistic regression models with a binomial error structure (McCullagh & Nelder 1989). Initial simple models examined the relation between neonatal survival and inbreeding coefficient (alone), individual heterozygosity (alone) or mean d^2 (alone).

As for birth weight, more complex models of neonatal survival were developed to incorporate the influence of other variables likely to affect neonatal survival (Guinness *et al.* 1978b). In all cases the significance of terms was obtained by dropping them from the model, and terms only remained in the full model if significant at $p < 0.05$. Variables considered were as for birth weight, with the following two additions:

- (1) weather during gestation and the neonatal period—continuous, this was fitted as for birth weight, but values for the summer months July to September were also investigated;
- (2) birth weight: continuous, see above.

3. RESULTS

(a) *Inbreeding coefficients, individual heterozygosity and mean d^2*

Of 231 study calves for which an inbreeding coefficient could be estimated, only 14 (6.1%) had a non-zero inbreeding coefficient and among these mean f was 0.0750 (range 0.0156–0.125). Of these calves, birth weight and other variables were only available for 176 individuals. Given the extremely skewed distribution of f , in later

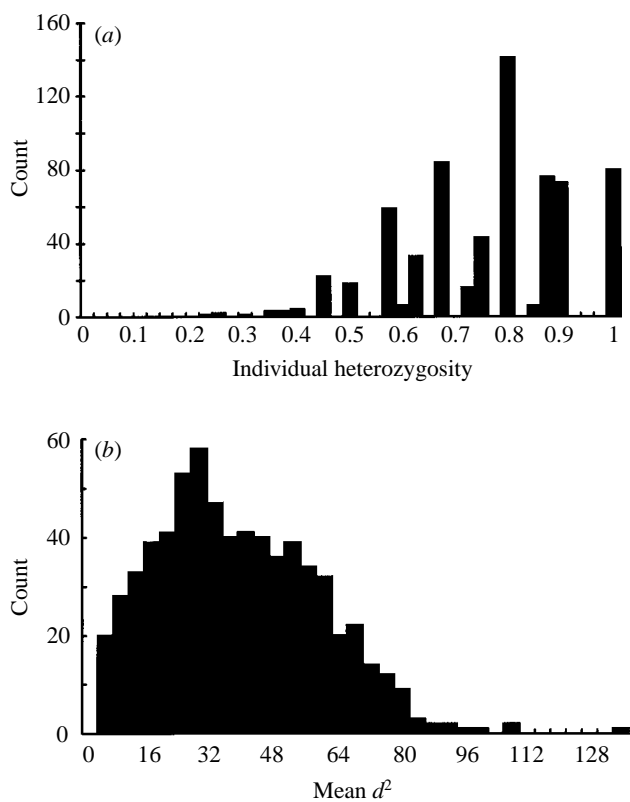


Figure 1. Distribution of (a) individual heterozygosity and (b) mean d^2 for 670 red deer calves born between 1982 and 1996 and genotyped at least seven of nine microsatellite loci (see text).

analyses (below) we fitted inbreeding coefficient as a factor with two levels, inbred ($f > 0$) and non-inbred ($f = 0$).

The distributions of individual heterozygosity and mean d^2 for 670 calves are shown in figure 1a,b. Individual heterozygosity has a spiky appearance because an integer was divided by nine to estimate it for most calves; intermediate values between the peaks resulted when only seven or eight loci had been scored in an individual. Mean d^2 is a more continuous variable because the numerator took a wide range of values. Although both measures are somewhat skewed they were not transformed for two reasons. First, generalized linear models are robust to somewhat skewed data (Genstat 5 Committee 1995). Second, the distribution of the standardized residuals about the regression between individual heterozygosity and birth weight (which below is revealed as the trait of interest) and between mean d^2 and birth weight are normal (data not shown).

We examined the relationships between inbreeding coefficient, individual heterozygosity and mean d^2 . There was no difference in the heterozygosity of inbred versus non-inbred calves ($F_{1,175} = 0.01$, $p = 0.907$) and no difference in the mean d^2 of inbred versus non-inbred calves ($F_{1,175} = 0.40$, $p = 0.551$). We presume this reflects the small sample of calves with non-zero inbreeding coefficients and the inaccuracy of our estimates of inbreeding coefficients due to short pedigrees. As might be expected from the fact that homozygotes contribute to both measures, individual heterozygosity and mean d^2 were positively correlated ($r = 0.387$, $p < 0.001$).

Table 1. *Full general linear model of birth weight in red deer calves, including mean d^2 and an interaction between mean d^2 and mean April temperature*

(The interaction is illustrated in figure 2. All terms included in the model explained independent variation in birth weight. The significance of each term was determined by dropping it from the model.)

term	F	d.f.	p
sex	19.4	1,634	<0.001
mother's status	2.88	4,637	0.022
mother's age (quadratic)	7.07	2,635	<0.001
mean d^2	4.13	1,635	0.042
mean April temperature	4.67	1,635	0.031
mean d^2 *mean April temperature	7.2	1,634	0.007

(b) *Birth weight*

When fitted alone in a simple bivariate regression model, inbreeding coefficient and birth weight were not significantly correlated ($F_{1,175} = 0.00$, $p = 0.969$), heterozygosity and birth weight were not significantly correlated ($F_{1,669} = 0.2$, $p = 0.655$) but mean d^2 and birth weight were ($F_{1,669} = 4.34$, $p = 0.038$). Calves with high mean d^2 were significantly heavier than calves with low mean d^2 .

In a more complex general linear model of birth weight the following terms were included simultaneously because they each explained independent variation in birth weight: calf sex, mother's age, mother's status, population density and mean April temperature. When fitted to this model, inbreeding coefficient was not associated with birth weight ($F_{1,164} = 0.11$, $p = 0.743$), individual heterozygosity was not associated with birth weight ($F_{1,635} = 0.72$, $p = 0.398$), whereas mean d^2 was ($F_{1,635} = 4.13$, $p = 0.042$); again, calves with high mean d^2 had higher birth weights than calves with low mean d^2 . Furthermore, there was a significant interaction between mean d^2 , April temperature and birth weight ($F_{1,634} = 7.2$, $p = 0.007$) in which the prominent feature was that low mean d^2 calves born following cold Aprils had low birth weights. The full birth weight model, including the interaction between mean d^2 and April temperature, is shown in table 1 and the interaction is illustrated in figure 2.

(c) *Neonatal survival*

When fitted alone in a simple logistic regression model, inbreeding coefficient and neonatal survival were not significantly correlated ($\chi^2 = 2.1$, d.f. = 1, $p > 0.05$), but heterozygosity and neonatal survival were significantly correlated ($\chi^2 = 4.5$, d.f. = 1, $p < 0.05$) and so were mean d^2 and neonatal survival ($\chi^2 = 4.8$, d.f. = 1, $p < 0.05$). Calves with high heterozygosity or high mean d^2 were most likely to survive the neonatal period.

In a more complex logistic regression model of neonatal survival including the terms mother's age and mean June temperature, which explain independent variation in neonatal survival, inbreeding coefficient was not significantly associated with neonatal survival ($\chi^2 = 1.9$, d.f. = 1, $p > 0.05$), nor was individual heterozygosity ($\chi^2 = 0.1$, d.f. = 1, $p > 0.05$), but mean d^2 was ($\chi^2 = 4.7$, d.f. = 1, $p < 0.05$); calves with high mean d^2 were more likely to

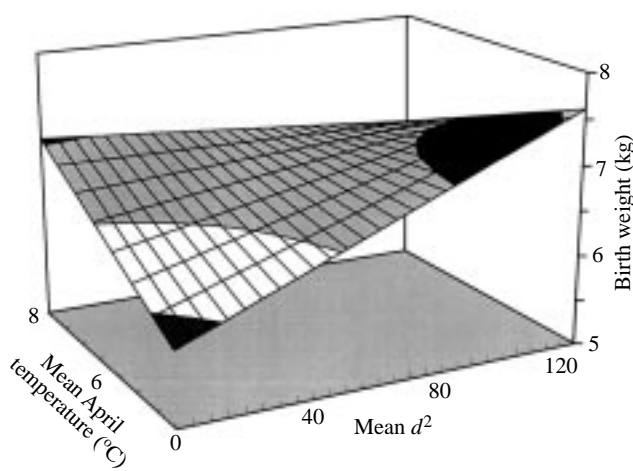


Figure 2. Surface relating calf birth weight, mean April temperature and mean d^2 , from the complex model of birth weight shown in table 1. In years when mean April temperature is low, calves with low mean d^2 were likely to be born at lower birth weights than calves with high mean d^2 . In years of higher mean April temperature, there was no association between mean d^2 and calf birth weight.

survive the neonatal period than calves with low mean d^2 . However, when fitted to this model, birth weight was strongly associated with neonatal survival, with low birth weight calves more likely to die ($\chi^2=12.9$, d.f.=1, $p<0.001$), the interaction between birth weight and mean June temperature was also significant ($\chi^2=8.5$, d.f.=1, $p<0.01$) (low birth weight calves were more likely to die in cold Junes), but mean d^2 was no longer significant ($\chi^2=0.5$, d.f.=1, $p>0.05$). These models of neonatal survival are shown in table 2*a,b*. In summary, the best model of neonatal survival, in terms of variance explained, does not incorporate mean d^2 , but it does incorporate birth weight, which mean d^2 appears to influence.

4. DISCUSSION

Our analyses indicate that close inbreeding is relatively rare in the Rum deer and that inbreeding depression in birth weight or neonatal survival is weak or undetectable. Only 14 out of 231 calves (6.1%) had non-zero inbreeding coefficients, and the highest detected value, of which we only found one case, was 0.125, resulting from a mating between half sibs (of course, these values can only rise with improving pedigree information). There was no association between inbreeding coefficient and either fitness measure, nor between individual heterozygosity and birth weight. A weak association between individual heterozygosity and neonatal survival was not upheld in the complex model of this trait. Either inbreeding depression for the traits studied is weak or, perhaps more likely, the number of study individuals which were inbred was so small that the power of these analyses was low.

In contrast, our analysis of mean d^2 suggests that there is heterosis for birth weight in the deer population, which affects the probability of neonatal survival. Mean d^2 is strongly associated with birth weight, and through birth weight is associated with neonatal survival. Given the lack of significant results with individual heterozygosity (above) we infer that the differences in allele lengths

Table 2. Full logistic regression models of neonatal survival in red deer calves

((a) Model including mean d^2 but not birth weight. (b) Model including birth weight, and an interaction between birth weight and mean June temperature. In each model all terms included in the model explained independent variation in neonatal survival. The significance of each term was determined by dropping it from the model. The association between mean d^2 and neonatal survival shown in (a) is not significant once birth weight is fitted as in (b) ($\chi^2=0.5$, d.f.=1, $p>0.05$). However, birth weight itself is associated with mean d^2 (table 1 and figure 2). Taken together, these analyses suggest the association between mean d^2 and neonatal survival in (a) is mediated by the associations between mean d^2 and birth weight. There is no significant correlation between mother's age and birth weight in model (b) ($r<0.00001$).

(a)

term	χ^2	d.f.	p
mother's age (linear)	5.6	1	<0.05
mean June temperature	5.1	1	<0.05
mean d^2	4.7	1	<0.05

(b)

term	χ^2	d.f.	p
mother's age (linear)	5.1	1	<0.05
mean June temperature	6.5	1	<0.05
birth weight	12.9	1	<0.01
mean June temperature *birth weight	8.5	1	<0.01

among heterozygotes are generating the significant effects with mean d^2 . As outlined in §1, we suggest that this indicates heterosis resulting from mixing of populations which have diverged to some degree, not only at their microsatellite loci, but also at other loci affecting fitness across the genome.

The contrast in outcome between individual heterozygosity and mean d^2 thus offers a route to investigating a class of effects, those due to outbreeding via population mixing, which have not yet been investigated to any extent in natural populations, and which on the basis of our results may be important in determining variation in individual fitness. Note that our approach does not allow discrimination of the underlying genetic mechanism; the effects detected could be due to deleterious recessive alleles or to overdominance at loci affecting fitness throughout the genome.

Mean d^2 and individual heterozygosity can be measured from a single sample from the relevant individual. We included inbreeding coefficient alongside individual heterozygosity throughout our analyses to estimate the frequency of inbred matings directly (see above), and to contrast the practical utility of these two ways of estimating the strength of recent inbreeding. Despite a substantial molecular analysis of paternity (investigating all 670 calves in the study cohorts and 350 other individuals), pedigrees for the calculation of inbreeding coefficients were established for only 231 calves born

between 1982 and 1996. The pedigrees that were established were generally very short, so only a proportion of recent inbreeding events will have been captured and the distribution of inbreeding coefficients was statistically inconvenient (217/231 were zero). The factors contributing to these problems are not unique to this study and will apply to many organisms for which it is possible to make field estimates of fitness. Around 35% of paternities are not resolvable due to the fact that 35% of rutting stags immigrate to the study area solely for the rut and are difficult to catch and sample, and the number and polymorphism of the microsatellites used were not powerful enough to resolve all the remaining 65% of paternities at high levels of confidence (Marshall *et al.* 1998). The red deer generation time is long (*ca.* 7 years), so on average only two generations have elapsed since genetic sampling of the population began (in 1982) and many fathers are immigrants to the study area, with unknown parents. In contrast, individual heterozygosity could be calculated for all 670 calves which had been scored for seven loci or more and had a much more convenient distribution for statistical analysis. Furthermore, when measured, as here, using multi-allelic microsatellites, individual heterozygosity should reflect inbreeding more accurately than previous estimates using allozyme variation.

The most significant result in our analysis of mean d^2 involves an interaction with an environmental variable (figure 2) and lends support to the idea that genetic differences in fitness are often conditional. In common with several analyses of inbreeding depression (Heschel & Paige 1995; Pray *et al.* 1994) and previous analyses of genotypic and phenotypic variation in red deer living on Rum (Pemberton *et al.* 1988, 1991; Coulson *et al.* 1998) and Soay sheep living on St Kilda (Gulland *et al.* 1993; Bancroft *et al.* 1995; Moorcroft *et al.* 1996), variation in fitness (in this case measured by birth weight) was revealed under adverse environmental conditions. Mean d^2 was most strongly associated with low birth weight following a cold April, when poor grass growth probably suppresses foetal growth (Albon *et al.* 1983) and a low birth weight was most disadvantageous for neonatal survival during a cold June, presumably due to direct chilling of the newborn calf or again through poor maternal nutrition during early lactation. Nevertheless, note that had birth weight not been available to us, we would still have concluded that mean d^2 was associated with neonatal survival.

Clearly, the measure mean d^2 requires further exploration, in several directions. First, is our explanatory hypothesis correct? An alternative is that particular microsatellite alleles are linked to specific alleles at loci affecting fitness. It seems likely that the rate of recombination is too high relative to the rate of microsatellite mutation for this idea to work, but modelling is required to address this issue. Second, if fitness correlations with mean d^2 do reflect population divergence followed by mixing, then the Rum deer population, which was formed relatively recently from several sources, is perhaps a promising scenario in which to detect such effects. However, a recent study of a harbour seal (*Phoca vitulina*) population subject to natural migration showed independent associations between mean d^2 and birth weight and between mean d^2 and neonatal survival (Coltman *et al.*

1998), suggesting our observations for deer are not necessarily an artefact of human activity. In general, one would predict little association between mean d^2 and fitness in populations which have been isolated for many generations or in populations which, although geographically separate, have regularly exchanged members over many generations and thus show little genetic divergence. Modelling studies are required to estimate the time-scale over which our proposed effect decays, and more empirical studies to check these predictions. Third, it would be useful to investigate the choice of loci for estimating mean d^2 and the number of loci used. In general, it seems likely that the most polymorphic loci will be most informative, but a very high mutation rate may be disadvantageous. Finally, it would be interesting to investigate associations between mean d^2 and aspects of fitness expressed later in the lifetime of the individual and in traits subject to sexual selection.

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