Outbreeding increases offspring survival in wild greater horseshoe bats (*Rhinolophus ferrumequinum*)

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The factors influencing the survival of greater horseshoe bat (*Rhinolophus ferrumequinum*) offspring born over seven years at a maternity colony in south-west Britain were studied. The effects of a range of phenotypic and maternal variables were analysed using a historical data set. In addition, the influence of two genetic measures on mortality, individual heterozygosity and a new measure of outbreeding, termed mean d^2 , was assessed. Logistic regressions were undertaken with survival modelled as a binary response variable. Survival to two life stages was studied for each variable and all models were developed for both sexes separately and together. Only one variable, mean d^2 , was significantly associated with survival. Male offspring with high mean d^2 scores were more likely to survive to their first and second summers. The influence of mean d^2 was not due to a single locus under selection but a wider multilocus effect and probably represents heterosis as opposed to solely inbreeding depression. Therefore, the extent to which an individual is outbred may determine survival more than widely used phenotypic characteristics such as size and mass. Mean d^2 may reflect immunocompetence, which influences mortality. Protection of mating sites in order to facilitate gene flow and, therefore, outbreeding may help to promote population stability and growth.

Keywords: Chiroptera; genetic variation; microsatellite; offspring survival; mean d^2

1. INTRODUCTION

Animal population dynamics are affected by the fitness of the individuals within them and many studies have sought to identify those factors that influence individual fitness (see the examples in Clutton-Brock 1988). As one of the most fundamental measures of fitness, offspring survival determines population size, stability and growth. Therefore, an understanding of the variables affecting offspring survival can be important in effective population management, particularly for endangered species.

Studying these factors in natural conditions can present considerable practical problems. Particularly difficult is the number and complexity of potentially important determinants, which may confound each other's effects when considered separately. One solution has been to analyse long-term data sets, which include phenotypic, maternal and climatic variables, so that all sources of variation may be studied. Such large-scale ecological investigations have revealed that offspring survival can be influenced by numerous factors, including size, mass, food availability and population density, as well as maternal and abiotic factors (e.g. Clutton-Brock *et al.* 1987; Derocher & Stirling 1996).

Molecular techniques have also allowed examination of the effects of genetic variables (usually variability measured as individual multilocus heterozygosity or an individual inbreeding coefficient) on fitness traits. Genetic theory predicts that low heterozygosity may lead to inbreeding depression if the gene pool contains deleterious recessive alleles or if heterosis (overdominance) is lost in the population (reviewed by Avise 1994). Heterosis,

which results from outbreeding, describes the phenomenon whereby heterozygosity confers a fitness advantage on an individual (Shull 1948), although more distant outbreeding may lead to outbreeding depression and, thus, reduced fitness (Edmands 1999). Yet research on fitness and genetic variability has yielded inconsistent results and, while some studies' results have supported conventional expectations (e.g. Keller *et al.* 1994), others have detected a negative (see the examples in Turelli & Ginzburg 1983) or no correlation (e.g. Whitlock 1993) between genetic variability and fitness parameters. At least some variation among the findings probably stems from the problems of limited resolution associated with allozymes, which until recently were the only polymorphic markers widely available (David 1998).

Microsatellites are highly polymorphic and predominantly neutral and, being more sensitive in detecting genetic variation than allozymes, provide new opportunities for studying the genetic determinants of individual fitness (e.g. Rowe *et al.* 1999). However, fitness and heterozygosity associations may also result from the direct effects of specific alleles under selection or linked to such alleles rather than multilocus effects, as observed for juvenile survival in red deer (*Cervus elaphus*) using both allozymes (e.g. Pemberton *et al.* 1988; Coulson *et al.* 1998a) and microsatellites (e.g. Coulson *et al.* 1998a).

Coulson *et al.* (1998b) recently proposed a new microsatellite-based measure of outbreeding, mean d^2 , based on the population genetic distance statistic D^2 (Goldstein *et al.* 1995) assuming a stepwise model of microsatellite mutation (Valdes *et al.* 1993). Mean d^2 is the average of the squared distances between pairs of microsatellite alleles calculated over several loci in an individual. This measure denotes the divergence level between gametes,

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thus providing more detailed information on genetic mixing than heterozygosity, which only reflects recent inbreeding (see Coulson *et al.* 1998b). Significant positive trends have been detected between mean d^2 and both neonate weight and survival in *C. elaphus* calves (Coulson *et al.* 1998b) and harbour seal pups (*Phoca vitulina*) (Coltman *et al.* 1998), while comparable results using individual heterozygosity were weaker or non-significant. Subsequent analysis has since revealed opposing sex-specific effects of mean d^2 on *C. elaphus* calf survival (males with low mean d^2 values survive more), which were attributable to physiological differences underlying sexual dimorphism (Coulson *et al.* 1999). Although these studies have demonstrated both the importance of outbreeding effects on fitness and the usefulness of mean d^2 in identifying apparent heterosis (reviewed by Pemberton *et al.* 1999), further investigations are clearly needed in order to assess the universality of such trends.

Bats are interesting mammalian subjects for the study of fitness for several reasons. Like deer and seals, they are long lived with low fecundity and, thus, compared to most other small mammals, offspring survival probably has a larger impact on their population dynamics since it represents a fundamental component of reproductive success. However, in contrast to most larger mammals, marked sexual size dimorphism is usually absent or slight. The greater horseshoe bat *Rhinolophus ferrumequinum* is typical in these respects. Endangered over most of its European range, *R. ferrumequinum* females form discrete maternity colonies, while the slightly smaller adult males are more solitary, often occupying underground sites throughout the year (Ransome 1991). Permanent movements between populations are rare (Ransome 1991) and gene flow probably stems mainly from mating during autumn and winter, when territorial males are visited by females from different colonies (Rossiter *et al.* 2000a,b).

Perinatal mortality rates for *R. ferrumequinum* offspring are low (*ca.* 1–2% annually), but rise rapidly during early foraging and peak at *ca.* 45–55 days old at weaning (Ransome 1990, 1991). Ransome (1989) showed that the numbers of first-year *R. ferrumequinum* reaching hibernacula was negatively related to mean birth date and suggested that the mortality of late-born offspring was related to incomplete skeletal growth prior to climatic deterioration. In turn, birth date is critically related to climate in April and May, with higher temperatures leading to earlier births (Ransome & McOwat 1994). Survival at weaning and through hibernation is thus closely related to climate. Prolonged cold winters also result in above-average mortality in all age classes, particularly in late spring when bats are vulnerable to starvation (Ransome 1991). Consequently, depending largely on climatic conditions, the number of bats alive one year after birth may represent between 0% (in 1986) and 52% (in 1995 and 1996) of the original cohort (Ransome 1990, 1998). Yet, since females may breed up to 29 years of age (Ransome 1995), early survival is likely to be a critical factor in determining population stability and growth.

The present study aimed to extend previous research on *R. ferrumequinum* offspring survival by examining the influence of a wide range of non-genetic variables relating to both offspring and mother. In addition, we studied the importance of two genetic measures derived from micro-

satellite data, i.e. individual heterozygosity and mean d^2 . We predicted that survival would correlate with both phenotypic and genetic variables in both sexes and vary between years depending on climate.

2. METHODS

(a) Study site and population

The Woodchester Mansion maternity colony of *R. ferrumequinum* (Gloucestershire, UK) has been researched for over 40 years and is the most intensively studied bat population worldwide (see Ransome 1989). All bats born at this site are ringed soon after birth (Ransome 1990). Females are strongly philopatric and return to the roost each year to give birth (Ransome 1995). Immature males also return to the natal colony in their second summer, but are less philopatric thereafter, generally dispersing to nearby caves (Ransome 1990). Presence and absence can thus be used to infer the survival and mortality of females and juveniles accurately, while winter surveys of hibernacula may provide more limited data on the survival of some males (Ransome 1990). For this study, the factors potentially influencing survival in 154 individuals born between 1991 and 1997 inclusive, for which sex and survival data were recorded, were studied. In addition, where possible, newborn offspring were measured and weighed and sampled for genetic analysis. Full details of the specific measurements taken are given in table 1.

(b) Genetic measurements

Biopsy punches (3 mm) were taken from the tail membranes of 140 individuals (90.9% of bats studied) and stored in NaCl/DMSO buffer. Following DNA extraction, 138 bats were typed at seven polymorphic microsatellite loci (see Rossiter *et al.* (1999) for details of the sequences and genetic variation), from which values of individual heterozygosity and mean d^2 were calculated. The number of alleles per locus present in this sample ranged from three to eight. One locus was not typed for two bats due to inconsistent banding patterns and so values were averaged over six loci. In order to calculate individual heterozygosity, homozygous loci were scored as zero and heterozygous loci as unity. These scores were then averaged to give a mean value for each bat. Mean d^2 was calculated as follows:

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

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 (i.e. six or seven).

(c) Statistical analysis

(i) Modelling genetic variables

In order to assess the influence of individual heterozygosity and mean d^2 on survival, simple logistic regression models were developed (Collett 1991). Survival was modelled as a binary response variable, with zero representing death and unity representing survival. Two levels of survival were examined: survival to the second summer, which equates to survival of the first winter and survival to the third summer, which represents the first year that early breeders may produce offspring (Ransome 1995). Where survival to both stages was significant, a third binary logistic regression was undertaken in order to compare bats that survived the first winter but died before the third summer to bats that survived to this stage. We assessed the

Table 1. A summary of the number of individuals for which genetic and phenotypic measurements have been collected

(Sample sizes are provided for males, females and both sexes for each variable and expressed as a percentage of the total sampled).

variable type	variable	females (<i>n</i> = 88)	% females	males (<i>n</i> = 66)	% males	total (<i>n</i> = 154)	% total
birth variables	year of birth	88	100.0	66	100.0	154	100.0
	day of birth	88	100.0	66	100.0	154	100.0
	birth sequence	83	94.3	62	93.9	145	94.2
	incubator present	87	98.9	13	19.7	100	64.9
survival variables	first winter survival	88	100.0	66	100.0	154	100.0
	survival to breeding age	88	100.0	66	100.0	154	100.0
genetic variables	heterozygosity	74	84.1	66	100.0	140	90.9
	mean d^2	74	84.1	66	100.0	140	90.9
phenotypic variables	sex	88	100.0	66	100.0	154	100.0
	growth rate	86	97.7	66	100.0	152	98.7
	mass	86	97.7	56	84.8	142	92.2
	final forearm length	80	90.9	56	84.8	136	88.3
	length of fifth digit	69	78.4	41	62.1	110	71.4
data on mother	age of mother	72	81.8	50	75.8	122	79.2
	final forearm length	58	65.9	42	63.6	100	64.9
	length of fifth digit of mother	51	58.0	42	63.6	93	60.4

change in 'deviance' ($-2 \log$ likelihood) of the current model compared with a null model containing only a constant for all models. Deviance has a χ^2 -distribution with $n-p$ degrees of freedom, where n is the number of cases and p is the number of regression coefficients. The smaller the deviance, the better the model fits the data and, therefore, the change in deviance associated with the addition of a variable can be used for assessing model improvement.

Models were undertaken using the data from males and females separately and in combination. In addition, we refitted the best survival model with d^2 scores based on single loci and reduced mean d^2 scores, which were calculated in turn by omitting the data from one of the seven loci. This was performed in order to ensure that any association observed between survival and mean d^2 was not due to the influence of a single locus, but a genome-wide effect.

(ii) Modelling non-genetic terms

In addition to genetic terms, further bivariate logistic regressions were undertaken in order to investigate whether survival in *R. ferrumequinum* offspring is associated with a range of non-genetic variables, as listed below.

- Year of birth: this is a categorical variable with seven levels (years 1991–1997 inclusive). This may be important if the climate varied widely between years.
- Day of birth: this is a continuous variable, where 01 represents 1 June. Late birth dates have previously been found to correlate negatively with numbers of individuals reaching hibernation (Ransome 1989) and subsequent population growth (Ransome & McOwat 1994).
- Birth sequence: this is a continuous variable that denotes the number of pups that a female has produced, including the current one. This may be important if offspring survival is influenced by maternal experience.
- Incubator present: this is a categorical variable with two levels (present and absent). In 1994 an incubator was installed into a section of the roost in order to encourage

rapid growth of offspring as part of an ongoing management programme (Ransome 1998).

- Growth rate (mm day^{-1}): this is a continuous variable. The logarithm of the growth rate was calculated from forearm measurements taken between the ages of 4 and 14 days (Ransome 1998).
- Birth mass (g): this is a continuous variable. All offspring were weighed within two days of birth and many were caught within several hours of birth, as characterized by attached umbilical cords (Ransome 1998).
- Forearm (radius) length (mm): this is a continuous variable representing a standard measure of body size for bats. Final forearm growth in *R. ferrumequinum* is achieved at ca. 45 days (Ransome 1998) and only these data were used.
- Fifth digit length (mm): as for forearm length. The fifth digit may increase in length until ca. 60 days and, therefore, continues to grow for a longer time-span than the radius. Consequently, this measure may be a more sensitive indicator of early foraging success and climate deterioration in late summer rather than simply reflecting the quality of maternal care (Ransome 1989).
- Mother's age (years): this is a continuous variable that is fitted as a linear function (a plot of mother's age against a continuous measure of survival revealed no quadratic relationship).
- Mother's forearm length (mm): this is used to determine the importance of a mother's size on her offspring's fitness.
- Mother's fifth digit length (mm): as for mother's forearm length.

As before, both survival models were undertaken for each variable for the sexes combined as well as separately. We aimed to enter all significant terms (both genetic and non-genetic) into a multiple logistic regression in order to determine whether individual variables remained significant after the influence of other variables was included.

Table 2. *Simple binary logistic regression models of offspring survival to their third summer fitted with genetic variables*

(The effect of fitting individual mean heterozygosity and mean d^2 to separate null models of offspring survival shown for males, females and both sexes. The regression coefficient (b), degrees of freedom associated with the term (d.f.), model deviance obtained by fitting the term and resulting change in deviance are given for each model (null model deviance = total deviance + change in deviance). Significant values are indicated with asterisks (* $p < 0.05$, ** $p < 0.01$).)

	term	
	mean heterozygosity	mean d^2
males		
n	66	66
d.f.	1	1
b	0.88	0.37
total deviance	80.46	74.56
Δ deviance	0.34 (0.42%)	6.42* (8.62%)
females		
n	74	74
d.f.	1	1
b	1.83	0.16
total deviance	100.18	100.54
Δ deviance	1.77 (1.77%)	1.84 (1.83%)
both		
n	140	140
d.f.	1	1
b	1.39	0.25
total deviance	188.44	182.76
Δ deviance	2.10 (1.11%)	7.85** (4.30%)

3. RESULTS

Twenty-nine to fifty-five percent of the offspring survived to their second summer in the seven years studied, while 25–45% survived to their third summer. Individual heterozygosity values ranged from 0.14 to 1.0 (mean = 0.66) and were normally distributed (Kolmogorov–Smirnov goodness-of-fit test, $p > 0.05$). The values of mean d^2 ranged from 0.71 to 136.19 (mean = 36.65) and were strongly skewed to the right (Kolmogorov–Smirnov goodness-of-fit test, $p < 0.01$). Following square-root transformation, the mean d^2 data approximated to a normal distribution (Kolmogorov–Smirnov goodness-of-fit test, $p > 0.05$) ranging from 0.84 to 11.67 (mean = 5.71) and were therefore suitable for regression analysis. Henceforth, mean d^2 refers to its square root.

(a) *Modelling genetic variables*

(i) *Sexes combined*

Individual heterozygosity and mean d^2 had different effects when fitted separately in simple logistic regressions. Individual heterozygosity did not significantly improve the null model of offspring survival to either the second ($\chi^2 = 2.93$, d.f. = 1 and $p > 0.05$) or third summers ($\chi^2 = 2.10$, d.f. = 1 and $p > 0.05$). In contrast, fitting mean d^2 resulted in significant reductions in model deviance and was positively associated with survival to the second ($\chi^2 = 6.90$, d.f. = 1 and $p < 0.01$) and third summers ($\chi^2 = 7.85$, d.f. = 1 and $p < 0.01$). The difference in the

deviance values suggested that mean d^2 affects survival during the intervening period, although a comparison of bats that died between their second and third summers with survivors showed that this was not significant ($\chi^2 = 1.24$, d.f. = 1, $p > 0.05$ and $n = 70$). Details of the model of offspring survival to the third summer fitted separately with these genetic variables are shown in table 2.

The strength and direction of the association between two variables in simple logistic models are typically described by the exponential of the regression coefficient or odds ratio, which gives the change in the odds of an event happening (i.e. surviving) for a unit change in the independent variable. Thus, in the case of fitting mean d^2 to the data on survival to third year, the resulting odds ratio of 1.28 (95% confidence limits of 1.07–1.53) indicated that, for a 1.0 unit change in mean d^2 , bats were 1.28 times more likely to survive to this stage. In the equivalent model fitted with heterozygosity, the odds ratio was larger (4.02) due to the smaller range of the heterozygosity values, but did not differ significantly from unity (95% confidence limits of 0.60–26.9) and, therefore, did not reveal any influence of heterozygosity on survival.

(ii) *Males and females*

The models developed separately for males and females showed positive relationships between both genetic variables and offspring survival to the second (data not shown) and third summers (see table 2), with odds ratios consistently greater than unity. However, as with the regressions based on the combined sex data, the associations observed between individual heterozygosity and mortality were not significant (table 2). Significant deviance reduction was only obtained by fitting mean d^2 to models of male survival to the second ($\chi^2 = 6.02$, d.f. = 1 and $p < 0.05$) and third summers ($\chi^2 = 6.42$, d.f. = 1 and $p < 0.05$) (see table 2). The degree of outbreeding may therefore be more important in determining the survival of males rather than females.

(iii) *Decomposition of mean d^2*

The values of d^2 derived from single loci and the mean d^2 values calculated by omitting individual loci were normalized by square-root transformation and fitted to the best survival model (survival to third summer using the combined sex data), in turn replacing the full mean d^2 values. Out of the seven models developed for assessing the relationship between survival to the third summer and d^2 (each based on a different locus), six revealed positive associations, although only one was significant (locus Rferr11) ($\chi^2 = 4.83$, d.f. = 1 and $p < 0.05$) (table 3). Positive relationships, six of which were significant, were detected between survival to breeding age in all seven sets of reduced mean d^2 scores, with deviance reduction ranging from 2.97 (d.f. = 1 and $p > 0.05$) when locus Rferr11 was excluded to 11.17 (d.f. = 1 and $p < 0.01$) when locus Rferr03 was excluded (table 3). Accordingly, the associated odds values were all significantly larger than unity indicating that, for a single unit increase in the reduced mean d^2 , bats are up to 1.32 times more likely to survive to breeding age.

Table 3. Model improvement (survival to third summer using combined sex data) obtained by fitting two sets of decomposed genetic variables derived from mean d^2 values

(The first seven terms are mean d^2 scores based on each individual locus. The second set of terms are reduced mean d^2 scores that were calculated by omitting a single locus. See table 2 for an explanation of the reported values.)

genetic term	d.f.	<i>b</i>	deviance	Δ deviance	<i>p</i>
d^2 (Rferr06)	1	0.06	186.2	3.31 (1.78%)	n.s.
d^2 (Rferr12)	1	0.08	188.96	0.56 (0.30%)	n.s.
d^2 (Rferr11)	1	0.07	184.68	4.83 (2.62%)	< 0.05
d^2 (Rferr01)	1	0.02	189.32	0.19 (0.10%)	n.s.
d^2 (Rferr03)	1	-0.03	188.94	0.58 (0.31%)	n.s.
d^2 (Rferr08)	1	0.02	189.44	0.06 (0.03%)	n.s.
d^2 (Rferr09)	1	0.04	185.44	0.77 (0.40%)	n.s.
mean d^2 (-Rferr06)	1	0.20	185.92	4.07 (2.19%)	< 0.05
mean d^2 (-Rferr12)	1	0.21	185.44	6.80 (3.67%)	< 0.01
mean d^2 (-Rferr11)	1	0.16	182.70	2.97 (1.63%)	n.s.
mean d^2 (-Rferr01)	1	0.21	186.54	6.53 (3.50%)	< 0.05
mean d^2 (-Rferr03)	1	0.27	182.98	11.17 (6.10%)	< 0.01
mean d^2 (-Rferr08)	1	0.21	178.34	6.91 (3.87%)	< 0.01
mean d^2 (-Rferr09)	1	0.20	182.60	5.97 (3.27%)	< 0.05

(b) Modelling non-genetic terms

Simple models of offspring survival to the second (data not shown) and third summers (see table A1 in electronic Appendix A) were developed for all non-genetic variables, in each case examining the sexes together as well as separately. However, when fitted alone, no non-genetic term significantly reduced deviance. Furthermore, no significant interactions were detected among any variables, including the genetic terms. Since the only variable found to correlate with *R. ferrumequinum* offspring survival significantly was genetic, no multiple logistic model was developed. Consequently, the simple regression of survival to the third summer fitted with mean d^2 remained the optimal model associated with the greatest change in deviance (table 2).

4. DISCUSSION

In this study, we analysed one of the most detailed data sets collected for a wild mammal in order to identify the factors influencing offspring survival in *R. ferrumequinum*. Surprisingly, despite examining several potentially important variables, only mean d^2 was significantly associated with survival. Decomposition of mean d^2 suggested that this variable's effect was multilocus in origin and was not due to the sole influence of a single locus under selection, as was also found in *C. elaphus* and *P. vitulina* (summarized in Pemberton *et al.* 1999).

Mean d^2 was positively associated with significantly increased survival to both the second and third summers in males only, although mortality between these two stages was not significant. Comparable regression estimates for females were positive but not significant. Sex-specific responses to mean d^2 scores have also been observed in *C. elaphus* (Coulson *et al.* 1999), although our findings differed in several respects. Unlike *R. ferrumequinum*, a significant positive relationship between survival and mean d^2 was recorded in *C. elaphus* females, while a significant negative association was recorded in males. To explain this, Coulson *et al.* (1999) proposed that outbred males may undergo increased hormone-dependent

growth, leading to greater muscle but lower fat deposition and, thus, a higher probability of winter mortality. However, as *C. elaphus* is characterized by marked sexual size dimorphism and larger stags outcompete smaller ones for mates (Clutton-Brock *et al.* 1982), outbred adult males are larger and achieve higher lifetime breeding success than less outbred individuals (Slate *et al.* 2000). In contrast, *R. ferrumequinum* females are marginally larger than males (Ransome 1991) and reproductive success in males may not correlate with size (see Rossiter *et al.* 2000a). It follows that male survival is probably less affected by selective pressures for rapid early growth, which may explain the positive relationship observed between mean d^2 and survival in males.

Although careful to avoid attributing the effect of mean d^2 on *C. elaphus* calf survival to heterosis (as the same trend could result from inbreeding depression among non-survivors), Pemberton *et al.* (1999) noted that this explanation was more likely given the weaker association observed between mortality and individual heterozygosity. Coulson *et al.* (1999) tested this by comparing the results of survival models fitted with mean d^2 , individual heterozygosity and mean d^2 with scores based solely on heterozygous loci (thus removing the component of mean d^2 which stems from recent inbreeding). The significant trends found in males between survival and both mean d^2 scores indicated that mortality was affected principally by outbreeding, while female survival was probably influenced by both inbreeding and outbreeding, as suggested by the single significant result associated with the full mean d^2 . Using this approach in the present study, an equivalent model of male survival to the third summer in *R. ferrumequinum* fitted with the mean d^2 values based on heterozygous loci was significant ($\chi^2=6.63$, d.f.=1 and $p<0.05$). Together with the consistent non-significant results obtained by fitting individual heterozygosity, this indicates that the observed effects of mean d^2 in males are best explained as the result of heterosis, as originally proposed by Coulson *et al.* (1998b). It is therefore interesting to note that the non-survival of males (and females to a lesser extent) with the lowest mean d^2 scores (see

Figure 1. Percentage frequency distributions for all bats and survivors to the third summer for (a) both sexes combined, (b) males and (c) females.

figure 1) resembled purging (zero out of eight offspring with mean d^2 values scores of less than 3 survived), as associated with inbreeding depression (e.g. Keller *et al.* 1994). As these values correspond to a mixture of low and high values of individual heterozygosity and mean d^2 based on heterozygous loci, inbreeding may influence survival in some instances.

The reason why outbred *R. ferrumequinum* male offspring are more likely to survive their first and second winters is not known, particularly as no mediation through (or interaction with) phenotype was detected. Indeed, while the effect of mean d^2 on *C. elaphus* calf mortality is probably mediated by birth weight and growth (Coulson *et al.* 1998b, 1999), no correlation was found between mean d^2 and any maternal or offspring phenotypic variables in *R. ferrumequinum* (data not shown). Perhaps surprisingly, non-significant results were obtained by fitting day of birth and year, despite previous evidence that mortality rates rise following late births and cold springs (Ransome 1989; Ransome & McOwat 1994). However, earlier studies included data from 1986, when the spring and summer were exceptionally cold, resulting in a major population crash (see Ransome 1990) and were also undertaken before a heater was installed in the roost. Comparatively, more stable climatic conditions were associated with the present study, both within and outside the roost, perhaps also masking the importance of other variables (if phenotype interacts with climate to affect survival in harsh winters). For example, while survival in Soay sheep lambs (*Ovis aries*) is positively associated with body weight (Clutton-Brock *et al.* 1991) and negatively with adverse weather (Milner *et al.* 1999a), selection for body weight is almost absent in years when survival is high (Milner *et al.* 1999b).

So what are the advantages associated with high mean d^2 that improves the chances of survival in *R. ferrumequinum*? To explain the positive trend with survival (independent of birth weight) in *P. vitulina*, Coltman *et al.* (1998) suggested that pups with high mean d^2 scores are better at avoiding sharks, the main cause of juvenile mortality in the population. In comparison, most *R. ferrumequinum* offspring deaths result from starvation, usually at weaning and predation is rare (Ransome 1991). One possibility is that mean d^2 reflects immunocompetence, which in turn

influences mortality. Associations between genetic variability (including overdominance), immune function and mortality have been reported previously (e.g. Clayton & Price 1994; Coltman *et al.* 1999). Furthermore, immunocompetence may be adversely affected by environmental stress, such as overcrowding or food shortages (e.g. Oppliger *et al.* 1998), potentially leading to greater mortality in cold weather. Characterization of major histocompatibility complex loci may provide a suitable test of this hypothesis.

Following a dramatic population decline last century, *R. ferrumequinum* is now confined to south-west England and Wales. Intercolony gene flow varies and probably depends on the extent of isolation (Rossiter *et al.* 2000b). Woodchester is genetically differentiated from those neighbouring colonies studied (Rossiter *et al.* 2000b) and females at this site breed with males originating from Woodchester as well as other colonies (Rossiter *et al.* 2000a). Females may therefore be able to vary the extent to which they outbreed, although the overall opportunity for outbreeding is probably limited by the distribution of mating sites (Rossiter *et al.* 2000b). By demonstrating a link between fitness and outbreeding, this study has further highlighted the potential importance of mating site protection as part of the conservation management of this species. The facilitation of genetic mixing will not only counter genetic differentiation, but may contribute to elevated offspring survival and, therefore, population growth.

Heterosis occurring in wild mammal populations has previously been demonstrated in just a handful of studies. *R. ferrumequinum* is only the third organism for which associations between mean d^2 and fitness traits have been reported. This study provides an advance over the investigations of *C. elaphus* and *P. vitulina*, since the former involved an island population where outbreeding was limited (Coulson *et al.* 1998b, 1999) and the latter involved a population for which gene flow levels were not known (Coltman *et al.* 1998). Additional work should help to characterize further the relationship between mean d^2 values, fitness, levels of interpopulation differentiation and gene flow. However, bats, deer and seals are all long lived, slowly reproducing mammals. More studies of mean d^2 are needed in order to establish how general

these trends are. Of particular interest would be comparisons with rapidly breeding short-lived mammals such as rodents, in which the consequences of mean d^2 score on population growth could also be more easily studied, as well as more distantly related taxa, in order to determine the wider applicability of this measure.

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