LIFETIME SELECTION ON HERITABLE LIFE-HISTORY TRAITS IN A NATURAL POPULATION OF RED SQUIRRELS

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Abstract.—Despite their importance in evolutionary biology, heritability and the strength of natural selection have rarely been estimated in wild populations of iteroparous species or have usually been limited to one particular event during an organism's lifetime. Using an animal-model restricted maximum likelihood and phenotypic selection models, we estimated quantitative genetic parameters and the strength of lifetime selection on parturition date and litter size at birth in a natural population of North American red squirrels, *Tamiasciurus hudsonicus*. Litter size at birth and parturition date had low heritabilities ($h^2 = 0.15$ and 0.16, respectively). We considered potential effects of temporal environmental covariances between phenotypes and fitness and of spatial environmental heterogeneity in estimates of selection. Selection favored early breeders and females that produced litter sizes close to the population average. Stabilizing selection on litter size at birth may occur because of a trade-off between number of offspring produced per litter and offspring survival or a trade-off between a female's fecundity and her future reproductive success and survival.

Key words.—Evolutionary rates, heritability, life-history traits, lifetime fitness, selection, *Tamiasciurus hudsonicus*.

Two conditions must be met for an evolutionary response to selection to occur: the trait must be heritable and selection must act on the trait (Lande and Arnold 1983; Arnold and Wade 1984a,b; Falconer and Mackay 1996). Although understanding these two conditions is critical to understand microevolutionary mechanisms, estimates of both quantitative genetic parameters and selection in wild populations are very scarce (Merilä et al. 2001).

Estimating quantitative genetic parameters in the wild has been made possible through the application of techniques initially developed for improvement of domestic species to free-ranging populations of known pedigree (Réale et al. 1999; Kruuk et al. 2000; Milner et al. 2000; Réale and Festa-Bianchet 2000; Kruuk et al. 2001). In addition, following the work by Lande and Arnold (1983), a large array of methods has been created to measure and visualize phenotypic selection (Brodie et al. 1995; Fairbairn and Reeve 2001). Phenotypic selection methods have two advantages. They give standardized coefficients of selection that allow the comparison of selection pressures among traits or among populations or the measurement of temporal or spatial variation in selection pressures (Lande and Arnold 1983; Kingsolver et al. 2001). Moreover, this approach allows one to distinguish between direct and indirect selection on a set of correlated traits (Lande and Arnold 1983) and to test adaptive hypotheses (Fairbairn and Reeve 2001). Recent studies have applied such methods to wild populations of animals (Milner et al. 1999; Merilä et al. 2001; Kruuk et al. 2001; for reviews see

Fairbairn and Reeve 2001; Kingsolver et al. 2001). However, most previous studies that have used the procedure recommended by Lande and Arnold (1983) to analyze selection in wild populations of iteroparous species have been limited to one particular event during an organism's lifetime (Svensson 1997; Merilä et al. 1999). These studies have succeeded in showing between-year instability in selective pressures (Gibbs and Grant 1987; Milner et al. 1999; Przybylo et al. 2000), but limiting the analysis to a single life-history stage may provide a misleading understanding of the evolutionary dynamics of the trait distribution (Brodie and Janzen 1996; Fairbairn and Reeve 2001; Merilä et al. 2001). In addition, phenotypic expression of a trait may differ according to environmental conditions (Schlichting and Pigliuci 1998). A measure of a phenotypically plastic trait at one particular stage of the organism lifetime may not be representative of the average value of the trait during the whole life of that organism. Selection can affect the phenotypic expression of the trait at that particular life stage (e.g., when the trait affects current survival) or it can act on the overall value of the trait over the entire lifetime. With the exception of studies on humans (Kään and Jokela 1998; Kirk et al. 2001) and on red deer (Merilä et al. 2001), we are not aware of studies of phenotypic selection that have used lifetime fitness in mammals.

Another problem may arise in selection studies using long-term datasets on iteroparous species. Bias in the estimation of selection gradients may appear because of environmental conditions affecting both fitness and phenotypic values of traits (Mitchell-Olds and Shaw 1987; Wade and Kalisz 1990; Rausher 1992; Ferguson and Fairbairn 2000; Fairbairn and Reeve 2001). When applied to longitudinal studies of wild species, this kind of bias may occur because individuals born

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in different years may experience different environmental conditions during their lifetime, generating temporal environmental covariance between the trait and fitness. Studies of selection in iteroparous species, therefore, must consider temporal variation in environmental conditions that may affect both phenotypes and fitness.

In this paper, we estimated quantitative genetic parameters and selection on parturition date and litter size at birth, using a long-term dataset from a wild population of North American red squirrels (*Tamiasciurus hudsonicus*). Many bird and mammal populations show seasonal variation in offspring survival in which offspring survival prospects decline with birth or hatching date (Lack 1968; Drent and Daan 1980; Boutin 1990). As such, any changes in the environment affecting offspring survival should induce new selection pressures and result in microevolutionary changes in breeding date. Several studies have previously demonstrated significant heritabilities of breeding phenology in birds (van Noordwijk et al. 1981; Findlay and Cooke 1982; Wiggins 1991; Svensson 1997). Comparable information on the heritability of parturition date in wild mammals is lacking. Studies measuring phenotypic selection in birds have shown selection favoring survival of offspring from early layers (Price et al. 1988; Wiggins 1991; Svensson 1997). As far as we know, no such a study has estimated selection on parturition date in a wild mammal.

This study focused on four issues. First, we showed that life-history traits such as parturition date and litter size are heritable and therefore have the potential to evolve. We estimated narrow-sense heritabilities and (co)variance components of reproductive traits using a restricted maximum likelihood (REML) animal model (Meyer 1989). Second, we examined the shape and the strength of selection on parturition date and litter size at birth. Our selection study was performed using relative lifetime fitness and mean lifetime trait values, rather than relying on an index of fitness from a single selection episode. By doing this, we avoided the potential bias of temporal environmental covariances between phenotypes and fitness, we avoided pseudoreplication (only one value is used per individual), and we limited the number of tests performed (reduction of the chance of Type I errors).

MATERIALS AND METHODS

Study Population and Study Area

We studied North American red squirrels on a 130-ha site near Kluane Lake, Yukon, Canada (61°N, 138°W). The general habitat of this area was open boreal forest with white spruce (*Picea glauca*) as the dominant canopy tree. All squirrels on the study area were permanently marked with metal ear tags soon after birth and were followed longitudinally throughout their life. Approximately 325 squirrels were monitored each year (1989–2001) from March to late August (Berteaux and Boutin 2000). This study area is not isolated and is surrounded by other territories, although most recruitment occurs from within the population (S. Boutin, unpubl. data). The reproductive activity of females was monitored each year from March to late August over a 10-year period (1989–1998; see Berteaux and Boutin 2000). Parturition

dates were estimated from the trapping records of the female and the size of the young when the nest was entered (Boutin and Larsen 1993). Litter size at birth (referred to as ''litter size'') was estimated as the number of offspring when the nest was entered. Females usually breed in late winter (February–April) and give birth in early spring (March–May) after a gestation period of 38 days. Offspring first emerge from the natal nest between 35 and 45 days of age but are not fully weaned until 60 to 70 days following parturition (May–July; Humphries and Boutin 1996; S. Boutin, unpubl. data). In the boreal forest, red squirrels defend individual territories year-round. At the end of summer, squirrels store unopened conifer cones on hoarding sites (middens) located centrally on the territory. Cones stored in autumn of a given year are an important source of food for reproductive females the following spring, and any squirrel without a midden cannot survive the winter (S. Boutin, unpubl. data).

Quantitative Genetics of Reproductive Traits

We estimated heritabilities with an animal model by derivative free restricted maximum likelihood (DFREML 3.1; Meyer 1989, 1997), on 568 females. This method is particularly robust to unbalanced designs, uses all the available kin relationships within a pedigree, and constrains heritability to between zero and one (Meyer 1989). Pedigrees were built based on known mother-daughter relationships. In the absence of maternal effects, matrilineal analyses provide unbiased, although less efficient, estimates of heritability (Konigsberg and Cheverud 1992). Because sires were unknown, fathers were coded as zero in the pedigrees for the analyses. A total of 1059 records for parturition date and 884 records for litter size were used in this analysis. Between one and six records (one record per year) were available for each individual. Records were available for 568 females, including 372 base animals, 180 dams with progeny records, and 72 grand-dams with progeny records.

We fit univariate and bivariate models to the data with:

$$y = Xb + Z_1a + Z_2c + e, \qquad (1)$$

where y is the trait record; $b, a,$ and c are respectively the vectors of fixed, random additive, and permanent environmental effects; $X, Z_1,$ and Z_2 are the corresponding incidence matrices relating the effects to y; and e is the vector of residuals (see Meyer 1997). The fixed part of the model included age and log of cone abundance the year preceding the reproductive event as a covariable. Year was included as a second random effect and represented common environmental effects other than cone abundance on parturition date or litter size for that year. Permanent environmental effects corresponded to effects due to the history of each individual (i.e., nonheritable effects). The convergence criterion was set to 10^{-8}. The program estimates phenotypic variance (V_P) and variances due to additive genetic (V_A), common environmental (V_C), permanent environmental (V_{PE}), and residual effects (V_E). Heritability was estimated as $h^2 = V_A/V_P$, common environmental effects as $c^2 = V_C/V_P$, and permanent environmental effects as $pe^2 = V_{PE}/V_P$. Approximate standard errors estimated by the quadratic approximation of the likelihood (Meyer 1989) allow one to test the significance of

h^2 with a Student's t-test. The difference between likelihood functions (-2 [log likelihood model 1 $-$ log likelihood model 2]) of the models with and without permanent environmental effects was assumed to follow a chi-squared distribution with degrees of freedom equal to the difference in parameters between the two models (here df $= 1$). For both traits, likelihood-ratio tests did not detect difference between the two models ($P > 0.05$). Models without permanent environmental effects are therefore presented. In some years, a few females managed to produce two litters during the summer. For these analyses we only considered data from the first litter. DFREML estimates obtained with the entire dataset (not shown here), however, did not differ from those based on the first litter. We calculated coefficients of additive genetic ($CV_A = 100\sqrt{V_A}/\bar{x}$) and of residual ($CV_R = 100\sqrt{(V_P - V_A)}/\bar{x}$) variation (Houle 1992).

Lifetime Fitness and Trait Values

We examined lifetime selection on all adult female squirrels. All females who survived to 1 year of age were included in the analysis, so survival from weaning to 1 year of age was not accounted for.

Two ways of considering fitness are available to study selection: (1) assignment of early offspring survival to maternal fitness (i.e., fitness index incorporates the survival of an individual's offspring); or (2) assignment of early offspring survival to offspring fitness (e.g., fitness index is limited to an individual's characters such as longevity or fertility, and maternal selection is included in the selection analysis; Wolf and Wade 2001). Both methods may suffer from potential shortcomings (Wolf and Wade 2001). We chose the total number of offspring weaned by a female as an index of fitness (i.e., assignment of early offspring survival to the maternal fitness). Because of the absence of sex-biased survival and growth in red squirrel (Boutin and Larsen 1993; McAdam et al. 2002) offspring of both sexes were pooled. One condition that justifies the assignment of early offspring survival to the maternal fitness is when maternal effects strongly affect offspring phenotypes (Wolf and Wade 2001). A strong maternal influence on offspring characters at weaning has been found in this population; maternal effects (mainly parturition date and litter size) are responsible for more than 80% of the variance in offspring growth (McAdam et al. 2002). More importantly, assignment of offspring fitness to the offspring would lead to biased estimates of selection in our case, because it is virtually impossible to account for the hidden part of maternal selection on parturition date and litter size in males (Wolf and Wade 2001). Moreover, analysis of maternal selection (parturition date and litter size) on survival to breeding age (i.e., assignment of offspring fitness to the offspring) shows weaker but comparable estimate of selection on parturition date and on litter size, although this analysis did not consider selection acting on the two life-history traits after their expression (A. G. McAdam and S. Boutin, unpubl. ms.).

Temporal heterogeneity in environmental conditions may affect the covariance between traits and fitness and may bias estimates of selection gradients (see above). In the Kluane population, environmental conditions experienced by a fe-

male during her lifetime may affect the number of offspring she weaned. The covariance between a trait and fitness may also be affected by potential effects of age on fitness values (fecundity and maternal experience may increase with age). To account for these biases we calculated relative lifetime fitness:

$$W'_i = \left[\sum_{l=1}^{n} (w_{i,a,l}/\bar{w}_{a,l})\right]\Big/ \bar{W}, \qquad (2)$$

where $w_{i,a,l}$ is the fitness value for the ith female at the ath age at a particular year l, $\bar{w}_{a,l}$ is the mean fitness of females of the same age for the same year, n is the number of reproductive events of the female i during her lifetime, and $\bar{W}$ is the average relative lifetime fitness value in the population. For each female, we calculated the standardized lifetime value of the trait:

$$z_i = \frac{1}{n} \sum_{l=1}^{n} [(x_{i,a,l} - \bar{x}_{a,l})/s_{x_{a,l}}], \qquad (3)$$

where the $x_{i,a,l}$ is the trait value of ith female at the ath age at a particular year l, and $\bar{x}_{a,l}$ and $s_{x,a,l}$ are respectively the mean value and the standard deviation of the trait for female of the ath age at the lth year in the population.

Analysis of Lifetime Selection

To avoid sampling bias caused by individuals with incomplete data, we limited our analysis to females that died before 2000 and for which data throughout the entire lifetime were available ($N = 303$).

To account for potential environmental covariance caused by spatial heterogeneity (see above), we considered in our selection analyses the number of food caches per female territory (number of middens) as an index of territory quality, although this index can also be influenced by characteristics of females (e.g., ability to defend a large or good territory). We also considered the average number of spruce cones available during a female's reproductive lifetime. We assessed annual cone production using an index of the number of cones on the top 3 m of 296–315 trees every August, on three zones in the population (Humphries and Boutin 2000). Average number of cones increased throughout the study period (Réale et al. 2003). To examine the relationship between number of cones and trait values independent of the cohort, we calculated the residuals of average number of cones as a function of cohort.

Standardized selection differentials, a measure of both direct selection acting on a trait and indirect selection on correlated traits, were estimated as the covariance between the standardized phenotypic value of the trait and relative lifetime fitness (Lande and Arnold 1983). Multiple selection analyses allow one to estimate direct linear and nonlinear selection gradients as a measure of direct selection acting on the trait, while holding the effects of other traits constant (Lande and Arnold 1983; Arnold and Wade 1984a,b; Fairbairn and Preziosi 1996). Selection gradients were estimated using the model:

$$w = \alpha + \sum_{i=1}^{n} \beta_i z_i + \frac{1}{2} \sum_{i=1}^{n} \gamma_{ii} z_i^2 + \sum_{i=1}^{n} \sum_{i<j}^{n} \gamma_{ij} z_i z_j \qquad (4)$$

TABLE 1. Estimates of phenotypic (V_P), additive genetic (V_A), common environmental (V_C), and residual (V_E) variances, and heritability ($\pm$SE), mean ($\pm$SD), coefficients of additive genetic (CV_A) and residual (CV_R) variations, and genetic correlation between parturition date and litter size at birth in red squirrels at Kluane, Yukon. Estimates were obtained with an animal-model restricted maximum likelihood (REML). The probability that a heritability estimate differs from zero (one-tailed tests) was determined by t-test.

	Parturition date	Litter size at birth
Mean $\pm$ SD	117.31 $\pm$ 25.12	2.10 $\pm$ 0.82
V_P	334.033	0.683
V_A	53.541	0.102
V_C	89.818	0.073
V_E	190.670	0.508
$h^2 \pm$ SE	0.16 $\pm$ 0.03	0.15 $\pm$ 0.04
P	<0.001	<0.001
CV_A	4.27	15.21
CV_R	14.28	36.30
Genetic correlation	—	0.06 $\pm$ 0.26

(Lande and Arnold 1983; Phillips and Arnold 1989). Linear gradients (β_i) were obtained from the multiple regression of relative fitness on the traits, including the two territory characteristics and their interactions with the traits. Nonlinear (stabilizing or disruptive selection: γ_{ii}) and correlational (γ_{ij}) selection gradients were then estimated from a multiple regression including the traits and all their quadratic terms, the two territory characteristics and their interactions with the traits, and their quadratic terms.

Because the distribution of fitness traits may not support the normality assumption of parametric models, we used a bootstrap procedure (Manly 1997) to estimate standard errors associated with each partial coefficient of the regression model. To do this we performed regression models on 2000 random samplings, with replacement, of the datasets. We also used the same procedure to estimate standard errors associated with selection differentials by calculating the covariance between standardized values of each trait and relative lifetime fitness. Bootstrap estimates of selection coefficients were calculated as the mean of the selection coefficients for the 2000 regression (or covariance) models, and the standard errors were obtained as the standard deviation of the bootstrap (Manly 1997). Rather than using Bonferroni adjustment, which is not appropriate for correlated variables (Rice 1989), we preferred to report probability values associated with the bootstrap estimates. Because parametric models may not be able to detect nonlinear, nonquadratic relationships between a phenotype and fitness, we calculated the cubic spline estimates (Schluter 1988) of fitness as a function of parturition date and litter size at birth, using glmsWIN1.0 program (Schluter 2000).

RESULTS

Quantitative Genetics of Reproductive Traits

Heritabilities of parturition date and litter size were low but were significantly different from zero (Table 1). The genetic correlation between parturition date and litter size was low, positive, and not significantly different from zero (Table 1). A low but nonsignificant phenotypic correlation was found between parturition date and litter size ($r = 0.06$, $N = 303$, $P = 0.27$). Common year effects had a moderate effect on the phenotypic variance in parturition date and litter size. Cone abundance was negatively related to parturition date (-20.29 days/cone index), indicating that females experiencing high food availability bred earlier.

Number of middens was neither correlated with lifetime parturition date ($r = -0.02$, $N = 303$, $P = 0.73$) nor lifetime litter size ($r = 0.04$, $N = 303$, $P = 0.44$). Average number of cones (residual value) was not correlated with lifetime parturition date ($r = -0.04$, $N = 303$, $P = 0.48$) and was negatively correlated with lifetime litter size ($r = -0.13$, $N = 303$, $P = 0.03$).

Selection Analyses

Estimates of selection differentials suggest that earlier parturition dates were associated with increased fitness (Table 2). The multiple linear regression model of relative lifetime fitness on parturition date and litter size, including number of middens and average number of cones and their interactions with the traits, was significant ($F_{8,294} = 3.024$, $P = 0.003$, $R^2 = 0.08$), indicating that one of the traits was significantly related to fitness. Interactions between territory characteristics and the traits were not significant (all $P > 0.11$), and thus were removed from the model. Direct linear selection on parturition date was negative and significant, but the linear selection gradient was positive and not significant for litter size (Table 2). Lifetime relative fitness significantly increased with the number of middens per territory (partial coefficient of regression: $b = 0.26 \pm 0.09$, $P = 0.0039$), but was not significantly related to average number of cones ($b = -0.0016 \pm 0.0011$, $P = 0.146$).

The multiple nonlinear regression model of relative lifetime fitness on parturition date and litter size and their quadratic terms, including territory characteristics, and their interactions with the traits was significant ($F_{15,287} = 2.3299$, $P = 0.004$, $R^2 = 0.11$), indicating that one of the traits was

TABLE 2. Standardized selection differential ($S_i' \pm$ SE), linear ($\beta_i \pm$ SE), nonlinear ($\gamma_{ii} \pm$ SE), and correlational ($\gamma_{ij} \pm$ SE) selection gradients on parturition date and litter size at birth in female red squirrels. To account for potential environmental covariance between the trait and fitness two environmental parameters, number of middens per territory and number of cones, were included in the analyses (see Results). Standard errors were obtained by a bootstrap procedure. Number of females used in the analyses, $N = 303$. Probability values were estimated using a t-test, where t equals the parameter divided by its standard error.

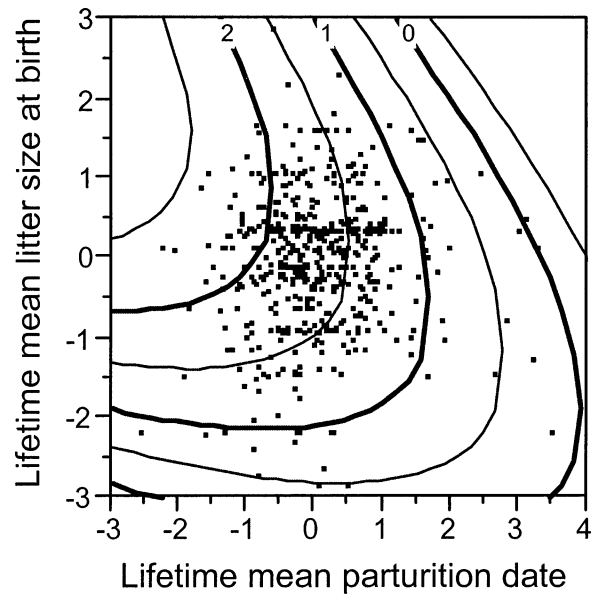| Traits	S_i'	P	β_i	P	γ_{ii}	P	γ_{ij}	P
Parturition date	-0.17 ± 0.05	0.0006	-0.24 ± 0.07	0.0006	-0.38 ± 0.20	0.058	-0.16 ± 0.07	0.022
Litter size	0.10 ± 0.06	0.095	0.12 ± 0.07	0.088	-0.56 ± 0.18	0.0019		

FIG. 2. Contour plot of fitness surface (lifetime relative fitness) on parturition date and litter size in red squirrels (*Tamiasciurus hudsonicus*) studied at Kluane, Yukon, Canada. The regression includes the traits, all their quadratic terms, and two measures of territory quality (number of middens and average number of cones; see Materials and Methods). Dots represent individual females (*N* = 303).

FIG. 1. Cubic spline visualization of selection (using relative lifetime fitness) on parturition date and litter size in red squirrels (*Tamiasciurus hudsonicus*) studied at Kluane, Yukon, Canada. Data were corrected for age effects. Data on parturition date and litter size at birth were standardized on a yearly basis, and the mean value was calculated for each female. Relative lifetime fitness is the sum of relative yearly fitness values for each female. $\lambda = 0.9$ for parturition date and $\lambda = 2.0$ for litter size at birth. Standard errors (dotted lines) were obtained by bootstrapping.

significantly related to fitness. Interactions between the traits and territory characteristics were not significant (all $P > 0.16$) and were removed from the model. Nonlinear selection gradients on both parturition date and litter size were negative, although this gradient was only significant for litter size (Table 2). The effects of the territory characteristics on lifetime relative fitness were similar to the linear model. The probability associated with stabilizing selection on parturition date was close to the 5% level with the parametric method (Table 2), and a cubic spline showed the presence of an optimum on the left of the population average (Fig. 1A). The decrease in fitness to the left of the optimum, revealed by the nonparametric analysis, is likely due to a small number of very precocious individuals. Stabilizing selection on litter size at birth was confirmed by the graphical visualization of

fitness as a function of litter size (Fig. 1B). The optimum litter size fit well with the average litter size in the population. Fitness was constant for a large range of values around the mean (mean $\pm$ 1 SD), and then decreased for extreme litter sizes. Significant correlational selection was found between parturition date and litter size (Table 2). Fitness was high for early breeders with large litters, but declined strongly with parturition date, whereas fitness was low and constant for females producing small litters, whatever their parturition date (Fig. 2).

DISCUSSION

The analysis of 10 years of data for the Kluane population of red squirrels revealed significant levels of additive genetic variation for parturition date and litter size as well as significant selection on these two life-history traits. Our selection analysis using lifetime fitness and average lifetime values of traits suggested that selection differed in form between the two traits examined. Directional selection favored earlier parturition, and stabilizing selection maintained litter size close to the population average. Our results indicate the presence of a weak stabilizing selection on parturition date caused by the lower fitness of a small number of females at the extreme left of the distribution (Table 2, Fig. 1A). Correlational selection between parturition date and litter size seemed to play a role on lifetime fitness, with the strength of the relationship between fitness and parturition date increasing with litter size (Fig. 2). Our estimates of directional selection on parturition date and stabilizing selection on litter size were higher than the median values of directional (0.08) and stabilizing (0.10) selection gradients reported for life-history traits in natural systems (Kingsolver et al. 2001). This

indicates that selection on both parturition date and litter size was relatively strong in the Kluane population during the study period.

Unlike an episode-based approach of selection (Arnold and Wade 1984b), our approach using mean lifetime trait values and lifetime relative fitness cannot allow us to provide information on age-related changes in selection on the traits, but it shows that selection can be measured on the overall value of the trait over lifetime. Our main goal was to provide estimates of variance and of selection that could help us understand the evolutionary dynamics of the trait under study.

Variance in Parturition Date and Litter Size

Parturition date and litter size showed moderate to low, but significant, heritabilities that are consistent with heritabilities generally found for life-history traits (Mousseau and Roff 1987). It should be noted that because of unknown paternity, we were not able to estimate maternal effects on parturition date or litter size. Maternal effects might affect heritability estimates upward or downward (Falconer and Mackay 1996). Maternal effects have been found to be strong for juvenile traits in squirrels (McAdam et al. 2002), but are generally thought to decrease with age, and evidence for strong maternal effects on adult traits are rare (Cheverud and Moore 1994; but see Kruuk et al. 2000; Milner et al. 2000). Strong environmental effects (e.g., climate, food availability) on parturition date and litter size may also be partly responsible for these low heritability estimates (Price and Schluter 1991; Houle 1992). There was a small positive genetic correlation between litter size and parturition date, indicating limited constraints on the independent evolution of these two traits (Lande 1979).

Selection Patterns on Parturition Date and Litter Size

Our results and another study on the Kluane population (O'Donoghue and Boutin 1995) show selection favoring early breeders. Two main functional mechanisms could be at the origin of the selective advantage of early breeders relative to late breeders: early breeders may have a higher average reproductive success or may have a higher reproductive longevity than late breeders. Early breeders may also be able to produce more litters per year than late breeders. Second litters, however, are rare (8.5% of the 1677 female-year observations) and cannot explain the negative relationship between parturition date and relative lifetime fitness. O'Donoghue and Boutin (1995) reported a negative relationship between parturition date and survival of offspring from the first litter in this same population. In addition, strong viability selection favoring earlier born offspring has been found, both during the period of complete maternal dependence and from emergence to weaning (A. G. McAdam and S. Boutin, unpubl. data). The fact that selection is associated with offspring survival in the nest indicates that differential survival of young born at different times may be related to maternal expenditure. It is possible that the correlation between parturition date and fitness was caused by the correlation between parturition date and some unmeasured components of maternal care. Early parturient females defend their litter more strongly than late parturient females (Price

et al. 1990). In contrast, there is no relationship between parturition date and the amount of time lactating mothers spent in the nest, foraging, and resting (Humphries and Boutin 2000) or their tendency to bequeath or to share a territory with offspring (Berteaux and Boutin 2000). To date we have no evidence that the correlation between parturition date and fitness is caused by the covariation between parturition date and maternal care among females.

Stabilizing selection (using lifetime fitness) on litter size suggests the presence of trade-offs between fecundity and other traits related to fitness. This result supports the adaptationist hypotheses about the evolution of clutch size in animals (Lack 1968; Roff 1992; Stearns 1992), and highlights the importance of lifetime selection studies to our understanding of the evolution of traits in nature. Theoretical models on the evolution of clutch size in nature predict an optimal clutch size, and the occurrence of stabilizing selection on clutch size (reviewed in Roff 1992). Interestingly, we found that the optimum lifetime value of litter size in the Kluane population corresponds to a plateau around the mean $\pm$ 1 SD (Fig. 1B). Stabilizing selection on litter size may occur because of a trade-off between a female's fecundity and her future survival (Roff 1992; Stearns 1992). However, no evidence for survival or reproductive cost of reproduction could be detected in a short-term, litter manipulation experiment (Humphries and Boutin 2000). Stabilizing selection on litter size may also result from a trade-off between number of offspring produced per litter and offspring survival (Roff 1992; Stearns 1992). At Kluane, offspring born in larger litters have lower growth rate (McAdam et al. 2002) and lower survival in most years (Humphries and Boutin 2000; A. G. McAdam and S. Boutin, unpubl. ms).

Evolutionary Dynamics of Parturition Date and Litter Size

Additive genetic variation and selection on parturition date and litter size provide the necessary and sufficient conditions for the evolution of these two life-history traits in a natural population of mammals. Directional selection favored early breeding, and we would, therefore, expect to see advancement in parturition date across generations. Parturition date has indeed advanced of 6 days per generation, over four generations (Réale et al. 2003). Application of quantitative genetic models (the breeder's equation and its multivariate formula; Lande 1979) using our estimates of heritability ($h^2 = 0.16$) and selection differential ($S'_i = -0.17$) indicates that part of the phenotypic change in parturition date (microevolutionary response: $R = 0.6$ days/generation) observed during the study period was caused by microevolutionary change (Réale et al. 2003). These results were confirmed by significant changes in the mean estimated breeding values (0.8 days/generation) across generations (Réale et al. 2003). These results differ from those of studies on birds that have documented significant selection on laying date or clutch size and high heritability estimates for these traits, but no change in these traits across generations (Lack 1968; Price et al. 1988; Price and Liou 1989; Cooke et al. 1990; Roff 1992).

Price et al. (1988) have suggested that strong apparent selection on laying date may result from the strong influence of environmental effects on traits, such as body condition or

territory, that are responsible for most of the phenotypic variation in laying date (Price et al. 1988). In our study, the negative covariance between parturition date and fitness cannot be explained by a higher fecundity (litter size) or a higher territory quality (number of middens and average number of cones) of early breeders relative to late breeders. Female body mass at parturition, a surrogate for body condition, was available for a subset of females ($N = 210$). Including body mass in selection analyses did not change the estimates of selection gradients on parturition date and litter size (results not shown). Furthermore, body mass was not significantly correlated with parturition date ($r = -0.10$, $P = 0.11$) and weakly correlated with litter size at birth ($r = 0.14$, $P = 0.04$). These results indicate that parturition date was mostly the target of selection in the Kluane population. The absence of microevolutionary change in clutch size in birds may also be due to incorrect estimation of selection restricted to one life-history stage (Brodie and Janzen 1996; Fairbairn and Reeve 2001; Merilä et al. 2001). Studies of selection on clutch size in birds generally have focused on yearly analyses of selection, and therefore may have missed some important patterns of selection, which could only be detected with lifetime study of selection. For instance, yearly viability selection on juvenile squirrels at Kluane suggests weak directional maternal selection on litter size (A. G. McAdam and S. Boutin, unpubl. data), whereas stabilizing selection has been clearly shown with the lifetime selection analysis. Finally, there is now evidence that undetectable evolutionary changes in a heritable trait subject to selection can result from antagonistic environmental effects on the phenotype (Merilä et al. 2001). In our study, environmental and microevolutionary effects both led to an advancement in parturition date (Réale et al. 2003).

How fast did the Kluane population evolve? We calculated phenotypic and genetic rates of evolution (Hendry and Kinnison 1999) over four successive generations ($N = 289$ females), using mean lifetime values of parturition date (standardized for age effects) and estimated breeding values provided by the animal model (Réale et al. 2003). Data were ln-transformed prior to running the analyses (Hendry and Kinnison 1999). We regressed the standardized change in parturition date (i.e., the mean value of the trait for each generation divided by the pooled standard deviation; Hendry and Kinnison 1999) as a function of generation. Phenotypic ($h_p = -0.394$ haldanes; 95% confidence interval: -0.040, -0.748), and genetic ($h_g = -0.161$ haldanes; 95% confidence interval: 0.006, -0.328) rates of evolution were both higher than the average evolutionary rates reported by Kinnison and Hendry (2001) for that time interval (i.e., $h = 0.140$ haldanes). The higher phenotypic haldane compared to the genetic haldane support the idea that phenotypic plasticity can play an important role in microevolutionary rates (Hendry and Kinnison 1999; see also Réale et al. 2003).

Selection patterns appear to be related to environmental changes that occurred in the Kluane population during the study period (Réale et al. 2003). The increase in cone abundance during the study period was accompanied by an increase in average spring temperature (Réale et al. 2003). Maternal expenditure may be affected by seasonal variation in food availability or quality. Cone abundance significantly affected breeding date within individual females (Réale et al. 2003) and experimental addition of cones in middens has led to the same effect (Larsen et al. 1997). Contrary to many bird species that have to adjust their breeding phenology to peaks of prey abundance (van Noordwijk et al. 1995), red squirrels rely on spruce cones hoarded during the previous autumn for their lactation needs, so the amount of cones hoarded on a female's territory continuously decreases from winter to summer. Early breeders may thus benefit from a higher abundance or higher quality of cones to satisfy the energetic needs of lactation than may late breeders. Currently the mechanism that relates climatic changes with food quality in the Kluane population is not known and additional studies need to be done.

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Corresponding Editor: J. Cheverud