

## VARIATION IN VIABILITY SELECTION AMONG COHORTS OF JUVENILE RED SQUIRRELS (*TAMIASCIURUS HUDSONICUS*)

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**Abstract.**—Selection will result in observable changes in traits only if it acts consistently in space and time, but few estimates of selection in natural populations have been temporally replicated. Here we estimate viability selection on nestling growth rates for 13 cohorts (1989–2001) of red squirrels (*Tamiasciurus hudsonicus*) from a natural population located in southwestern Yukon, Canada. Directional selection on nestling growth rates varied in magnitude and direction from one cohort to the next. The magnitude of directional selection was relatively weak in most years (median  $\beta' = 0.24$ ), but there were episodes of very strong viability selection ( $\beta' > 0.5$ ) in some cohorts. We found no evidence of significant stabilizing or disruptive selection on this trait. Examination of viability selection episodes over shorter time periods suggested that the strength of selection on juveniles in this population was positively related to the time scale over which selection was measured. Viability selection from birth to emergence from the natal nest (50 days of age) and from emergence to successful recruitment (100 days of age) were positively correlated, but were both independent of selection on nestling growth rates from recruitment to potential breeding age (one year). The strength of directional selection on growth rates prior to recruitment was negatively correlated with spring temperature whereas selection from recruitment to breeding was positively correlated with the abundance of spruce cones produced in the previous fall. Episodes of strong directional selection from birth to breeding age appear to be due to potentially rare combinations of environmental conditions. As a result, predicting the occurrence of very strong episodes of selection will be extremely difficult, but predicting the microevolutionary responses to observed selection on individual cohorts remains feasible.

**Key words.**—Directional selection, growth rate, litter size, maternal selection, parturition date, selection gradients.

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Since the development of methods for quantifying natural selection on multiple traits (e.g., Lande and Arnold 1983), the number of estimates of the strength of selection in natural populations has grown steadily (Endler 1986; Hoekstra et al. 2001; Kingsolver et al. 2001). Most episodes of directional selection are relatively weak, but in some circumstances very strong selection can occur (Endler 1986; Kingsolver et al. 2001). Even weak episodes of directional selection, however, are capable of causing large changes in the mean value of traits. Hoekstra et al. (2001) calculated that as few as 16 generations of “typical” selection ( $\beta' = 0.15$ ) could change the mean of a heritable trait by one standard deviation. Despite the apparent ability of typical levels of selection to cause large changes in traits with typical levels of additive genetic variation (Mousseau and Roff 1987), documented microevolutionary responses to selection in natural populations are anything but typical (Merilä et al. 2001).

One explanation for the general lack of response to documented selection is that selection measured at one point in time may not persist across generations or even across life stages within generations (Merilä et al. 2001). Relatively few studies have replicated estimates of selection either in space or time (temporal replicates reviewed by Kingsolver et al. 2001: range = 1–10; median = 1; spatial replicates: range = 1–12; median = 1, but see also Boyce and Perrins 1987; Przybylo et al. 2000; Sinervo et al. 2000; Kruuk et al. 2001; Grant and Grant 2002). A negative relationship between the strength of viability selection and the time period over which this selection is measured also suggests that strong episodes of viability selection are often followed by periods of stasis or reversal in the direction of selection within generations (Hoekstra et al. 2001).

The response of traits across multiple generations will be very difficult to predict if selection varies in space or time, particularly in populations with overlapping generations (Merilä et al. 2001). However, most natural populations experience large variability in environmental conditions (e.g., temperature, precipitation) or in the abundance of food, predators, or competitors. It, therefore, seems unlikely that estimates of a single episode of selection in natural populations will adequately predict responses of traits beyond the very narrow window within which selection was measured.

Previous estimates of genetic variation in nestling growth rates of red squirrels (*Tamiasciurus hudsonicus*) from a natural population revealed large heritable maternal effects and a large positive covariance between direct and maternal genetic effects on this life history trait (McAdam et al. 2002). Positive genetic covariances are thought to be rapidly eroded by consistent directional selection (Roff 1997), suggesting that selection on nestling growth rates in this natural population might instead vary either in space or time (McAdam et al. 2002). Red squirrels are long lived (maximum age, 9 years) and have a relatively short generation time (2.7 years) resulting in several generations (1–3) of females breeding within the same season (S. Boutin, unpubl. data). Red squirrels in this population rely almost exclusively on the cones of white spruce (*Picea glauca*) for food. The production of spruce cones varies dramatically from one year to the next and this annual variation in the abundance of food is thought to have important implications for many aspects of red squirrel life history (Berteaux and Boutin 2000; Humphries and Boutin 2000; Réale et al. 2003a). This combination of overlapping generations and the annual variation in food abundance provide both the theoretical (Ellner and Hairston 1994)

and ecological basis for our hypothesis that this large, positive direct-maternal covariance has been maintained in this population by temporally varying selection on nestling growth rates.

Here we report on an analysis of viability selection for 13 cohorts (1989–2001) of nestling red squirrels based on survival to potential breeding age. We were most interested in selection on nestling growth rates but also quantified maternal selection acting via litter size and parturition date. We examined these 13 cohorts of squirrels for evidence of temporal and spatial variation in selection using generalized linear models including temporal and spatial variables as well as their interactions with the juvenile and maternal traits. We also examined viability selection over two shorter time scales: survival to first emergence from the natal nest (50 days of age) and successful recruitment into the adult population (100 days). These measures of viability selection together with the estimates based on survival to one year of age allowed us to look for evidence of temporal variation in selection on nestling growth rates within cohorts and to evaluate the relationship between the time scale over which selection was measured and the strength of selection within a single population. Finally we attempted to generate hypotheses regarding the causes of variation in viability selection on growth rates in this population by correlating the strength of selection with a variety of ecological variables.

#### MATERIALS AND METHODS

A natural population of red squirrels in the southwest Yukon (61°N, 138°W) was monitored from 1989 to 2002. All individuals in each of the five trapping areas (total census population size: 400–600) were permanently marked with small metal ear tags and were regularly live-trapped throughout the breeding season to assess reproductive activity. Because of their territorial and diurnal activity patterns, we were able to census all squirrels within our population in both May and August of each year, although regular trapping often began in March. All offspring born in the population were enumerated within days of birth and were permanently marked prior to first emergence from their natal nest. Offspring are weaned at approximately 70 days of age at which point they must acquire a territory in order to survive the upcoming winter (for more details, see Berteaux and Boutin 2000). Red squirrels in this population do not breed as young-of-the-year, so survival through the first winter indicates survival to potential breeding age. The number of offspring produced in the population varies from year to year but often greatly exceeds the number of vacant territories, thus in most years the probability of survival to breeding age is low and the opportunity for selection on offspring traits is high. Because of the timing of breeding in this population and our trapping procedures, we assessed potential breeding age as 200 days. Any squirrel that survived to 200 days of age had successfully survived their first winter and could have initiated breeding. Hereafter, survival to 200 days of age will be referred to as survival to potential breeding age or survival to one year of age.

#### *Measurement of Traits*

We examined viability selection on juvenile squirrels based on survival to potential breeding age. The offspring trait we were most interested in was nestling growth rates, which were measured as the increase in mass of nestling squirrels from soon after birth to approximately one month of age (just prior to first emergence from the natal nest). Growth over this time period is approximately linear (see McAdam et al. 2002). Offspring with initial weight measurements greater than 50 g, final weight measurements greater than 100 g, or less than five days between weight measurements were excluded from the analysis. Offspring involved in food supplementation or litter size manipulation experiments (e.g., Humphries and Boutin 2000) were not included in these analyses. We also measured the effects of birth date (Julian date) and litter size on offspring viability, because these two traits have been found to have strong maternal effects on offspring growth (McAdam et al. 2002). Females in this population rarely attempt a second litter late in the breeding season (Réale et al. 2003b). We excluded offspring from second litters in this study to be consistent with previous estimates of selection on litter size and parturition date (Réale et al. 2003b). Results of analyses including offspring from second litters are similar to those presented here.

In the 13 cohorts between 1989 and 2001, we estimated viability selection on a total of 1623 nestling red squirrels based on survival to potential breeding age. Sample sizes within a given cohort varied from 31 to 304 but often exceeded (median, 109) the sample size of most previously published estimates of selection in natural populations (median, 134; Kingsolver et al. 2001).

#### *Temporal and Spatial Variation*

We used generalized linear models (binomial response; McCullagh and Nelder 1989, Venables and Ripley 2002) to assess the significance of nestling growth rate, litter size, and parturition date to offspring viability. Sex was included in the model to account for differences between males and females in their probability of survival to one year of age. The significance of spatial and temporal variation in selection were assessed by the comparison of four separate a priori models using analysis of deviance (McCullagh and Nelder 1989; Venables and Ripley 2002). The basic model (1) included only the three juvenile traits and *SEX* as predictors of survival to one year of age. The small-scale spatial model (2) included the basic model and the specific spatial location of the natal territory of each offspring within the trapping areas (*X* and *Y* grid coordinates  $\pm 3$  m), their cross-product (*XY*) and interactions with juvenile traits. The medium-scale spatial model (3) included the basic model as well as a dummy variable representing one of the five 40 ha trapping areas (*GRID*; average distance between grids is approximately 1.3 km), and interactions between *GRID* and the juvenile traits. The temporal model (4) included the basic model, cohort (*C*), and interactions of cohort with juvenile traits. In models 2–4, significant main effects represented spatial or temporal variation in the probability of survival to one year of age whereas significant interactions with juvenile traits repre-

sented spatial or temporal variation in selection on these traits. In model 2, the small-scale spatial effect was considered to be the combined effects of the three spatial variables (two coordinates and their cross-product). As a result, the deviances explained by each of these three variables were summed and tested against a chi-square distribution with 3 df (analysis of deviance; McCullagh and Nelder 1989; Venables and Ripley 2002). Interactions between the small-scale spatial variable and each of the three traits were interpreted in the same way. Data for two cohorts (1989 and 1990) and two trapping grids were excluded from this analysis because of small sample sizes relative to the other years and grids.

#### *Estimation of Viability Selection*

Linear selection gradients were estimated for the entire dataset using a linear model that included grid, sex, litter size, parturition date, and nestling growth rate as predictors (Lande and Arnold 1983). Trait values were standardized and relative fitness was calculated within each cohort to avoid confounding selection gradients with covariances between environmental conditions, trait values, and fitness. Quadratic and correlational selection gradients were estimated in a separate linear model that included all linear, quadratic and pairwise cross-products of traits.

Linear, quadratic, and correlational selection gradients were also estimated for each cohort, using separate linear models. For the cohort analysis, traits were adjusted to a mean of zero prior to analysis and selection gradients were subsequently standardized to avoid rounding errors (Endler 1986). Standardized selection gradients ( $\beta'_i$ ,  $\gamma'_{ii}$  and  $\gamma'_{ij}$ ) are presented  $\pm$  one standard error (SE). Standard errors were generated by jackknifing. No significant nonlinearities in fitness surfaces within cohorts were found using generalized additive models fit with cubic splines, in which the smoothing parameter ( $\lambda$ ) was obtained by generalized cross-validation (Mathsoft 1999a, b).

There was no sign of any temporal autocorrelation in the strength of selection on nestling growth rates (one-year lag:  $r = 0.03$ ,  $n = 12$ ,  $P = 0.93$ ; two-year lag:  $r = -0.01$ ,  $n = 11$ ,  $P = 0.97$ ; three-year lag:  $r = -0.07$ ,  $n = 10$ ,  $P = 0.85$ ; Legendre and Legendre 1998), thus selection gradients for each cohort were assumed to be independent tests of the null hypothesis of no selection. As a result, we corrected for multiple comparisons of selection gradients (3 traits  $\times$  3 gradients) only within cohorts using a sequential Bonferroni correction (Rice 1989). Gradients significantly different from zero at an uncorrected alpha of 0.05 as well as those significantly different from zero based on the sequential Bonferroni correction are indicated in the tables and figure.

#### *Time Scale of Viability Selection*

To examine the effect of the length of time over which viability selection was measured we also estimated linear selection gradients for nestling growth rates for each of the 13 cohorts based on survival to 50 days of age (emergence from the natal nest) as well as survival to 100 days of age (recruitment into the adult population). Survival to 50 days, 100 days, and one year of age correspond roughly to the three temporal categories of days, months, and years outlined by

Hoekstra et al. (<31 days; 31–364 days; >365 days; Hoekstra et al. 2001). It is unlikely that growth rates could be measured in squirrels that survive for less than 20 days, thus selection on nestling growth rates based on survival to 50 days of age represents a very short time period. The magnitude of directional selection on nestling growth rates (absolute value) was compared among time periods for the 13 cohorts of squirrels using a linear mixed-effects model in which the time period was included as a fixed effect and the year was included as a random (blocking) factor ( $n = 39$  estimates of selection gradients). The strength of viability selection on nestling growth rates from birth to one year of age was decomposed into three separate selection episodes (birth to emergence, emergence to recruitment, recruitment to breeding) corresponding to the time periods described above, based on the additive nature of selection gradients across successive selection episodes (Arnold and Wade 1984).

#### *Correlates of Viability Selection*

We looked for potential external and internal ecological correlates of viability selection on the growth rates of juvenile red squirrels acting from birth to recruitment and from recruitment to breeding. External variables were the abundance of cones produced in the current and previous year and the average spring temperature. An index of the abundance of spruce cones produced each year was measured by averaging the number of visible cones on the top 3 m of each of 190 designated trees among the study areas (for details, see Humphries and Boutin 2000). Cones are harvested by the squirrels in August and are stored for subsequent consumption over the following year or more. Because our index of spruce cone production varied annually over three orders of magnitude (Humphries and Boutin 2000), we log transformed these values prior to analysis. Weather data were collected from the Burwash weather station located approximately 50 km from the study area and spring temperatures were calculated as the average of April, May, and June mean monthly temperatures, following Réale et al. (2003a). The only internal variable examined was spring population density, measured as the number of adults owning territories within the core 12-ha area of each of the study areas. Spring population density has been found to be positively correlated with the previous year's cone production (S. Boutin, unpubl. data), so the residuals of the relationship between population density and the previous year's cone production were included as an internal ecological variable here. There was no sign of any temporal autocorrelation in the strength of selection on nestling growth rates (see above) so the selection gradient from each cohort was used as an independent replicate in this analysis. These four ecological variables were correlated with each of the selection gradients ( $n =$  number of cohorts) using ordinary least squares multiple regression. As a result of the small sample size in this analysis, nonsignificant variables ( $P < 0.05$ ) in each multiple regression were removed in a step-wise manner to arrive at a single reduced model. Statistical results from the entire model are presented for variables removed in the step-wise procedure, while results for significant variables are presented from the final reduced model.

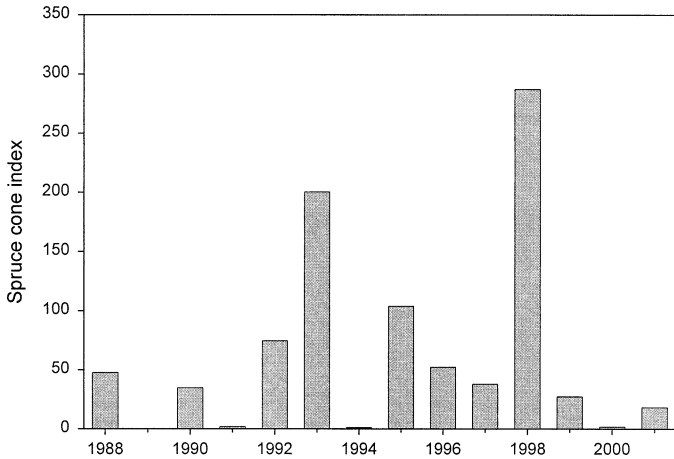


FIG. 1. Annual variation in the production of spruce cones from 1988 to 2001. Values represent an index of the average spruce cone production on three study areas in the southwest Yukon.

RESULTS

The fates of 1623 offspring were followed from 13 different cohorts from 1989 to 2001. The production of spruce cones varied dramatically from one year to the next (Fig. 1). In some years there was a cone crop failure (e.g., 1989, 1991, 1994, 2000), while in other mast years (e.g., 1993, 1998) there was a very large number of cones produced. Nestling growth rates varied among years (Table 1) and were positively correlated with the abundance of spruce cones produced in the previous year (slope =  $0.23 \pm 0.04 \text{ g} \times \text{day}^{-1} \times \text{cones}^{-1}$ ,  $n = 13$ ,  $r^2 = 0.74$ ,  $P < 0.001$ ). The opportunity for selection on offspring varied among years but in general was very high (median, 5; see Table 2). Variation among cohorts in the opportunity for selection was positively correlated with both the abundance of spruce cones produced in the previous fall (slope =  $5.5 \pm 2.0$ ,  $t_{10} = 2.7$ ,  $P = 0.02$ ) and the relative spring population density (slope =  $9.0 \pm 2.5$ ,  $t_{10} = 3.6$ ,  $P = 0.004$ ).

The overall model of viability selection indicated that there

TABLE 2. Probability of survival from birth to 50 days, 100 days, and one year of age and the opportunity for selection (*I*) based on survival to one year of age for red squirrels born between 1989 and 2001. See Table 1 for sample sizes (number of juveniles) within each cohort.

	50 days	100 days	One year	Opportunity for Selection ( <i>I</i> )
1989	0.90	0.58	0.13	7.0
1990	0.61	0.30	0.21	3.8
1991	0.76	0.44	0.38	1.7
1992	0.53	0.26	0.17	5.0
1993	0.88	0.71	0.65	0.5
1994	0.80	0.33	0.05	17.7
1995	0.63	0.50	0.48	1.1
1996	0.61	0.25	0.13	6.8
1997	0.75	0.43	0.36	1.8
1998	0.67	0.58	0.50	1.0
1999	0.52	0.14	0.03	32.9
2000	0.48	0.12	0.09	10.2
2001	0.47	0.21	0.13	6.8

was significant selection for increased nestling growth rates ( $\beta' = 0.34 \pm 0.10$ ,  $t_{1617} = 3.53$ ,  $P = 0.0004$ ; Fig.2) and selection for earlier parturition dates ( $\beta' = -0.20 \pm 0.07$ ,  $t_{1617} = 3.13$ ,  $P = 0.002$ ; Table 3). There was no significant directional selection on litter size ( $\beta' = 0.04 \pm 0.07$ ,  $t_{1617} = 0.54$ ,  $P = 0.59$ ; Table 3) or nonlinear selection on growth rate, parturition date or litter size (growth rate  $\gamma' = 0.02 \pm 0.06$ ,  $t_{1611} = 0.43$ ,  $P = 0.67$ ; parturition date  $\gamma' = -0.04 \pm 0.04$ ,  $t_{1611} = 0.99$ ,  $P = 0.32$ ; litter size  $\gamma' = -0.09 \pm 0.05$ ,  $t_{1611} = 1.76$ ,  $P = 0.08$ ; Table 4). Correlational selection gradients from the overall model were also not significantly different from zero ( $t_{1611} < 1.84$ ,  $P > 0.06$ ; Table 4).

Temporal and Spatial Variation

The basic model of nestling survival to one year of age explained only 5.2 percent of the overall deviance (Table 5). Sex (females had a higher probability of survival than males; analysis of deviance:  $\chi^2_1 = 27.8$ ,  $P < 0.001$ ), growth rate ( $\chi^2_1 = 6.9$ ,  $P = 0.01$ ), and parturition date ( $\chi^2_1 = 52.1$ ,  $P <$

TABLE 1. Phenotypic means ( $\pm$  one standard deviation) and covariances between nestling growth rates (GR; g/day), litter size (LS), and parturition date (PD; Julian date) before selection from 1989 to 2001. Sample sizes (number of juveniles) for each cohort are reported at the right of each row.

Year	Growth Rate (g/day)	Litter Size <sup>1</sup>	Parturition Date (Julian) <sup>1</sup>	Covariance (GR, LS)	Covariance (GR, PD)	Covariance (LS, PD)	<i>n</i>
1989	1.94 $\pm$ 0.51	2.84 $\pm$ 0.69	108.7 $\pm$ 4.4	-0.046	-0.017	-0.343	31
1990	1.58 $\pm$ 0.51	2.82 $\pm$ 0.58	137.8 $\pm$ 10.5	0.043	-1.295	2.966	33
1991	1.95 $\pm$ 0.38	2.78 $\pm$ 0.58	125.6 $\pm$ 11.6	-0.068	0.409	-1.930	98
1992	1.35 $\pm$ 0.41	3.38 $\pm$ 0.80	149.9 $\pm$ 17.0	-0.234	-1.170	1.941	120
1993	1.98 $\pm$ 0.49	3.33 $\pm$ 0.77	116.0 $\pm$ 27.5	-0.147	1.104	0.003	131
1994	2.03 $\pm$ 0.51	3.25 $\pm$ 0.65	98.3 $\pm$ 12.0	-0.035	-1.768	1.376	186
1995	1.68 $\pm$ 0.53	3.57 $\pm$ 0.99	155.8 $\pm$ 19.1	-0.053	2.128	6.850	150
1996	1.92 $\pm$ 0.42	3.46 $\pm$ 0.65	103.2 $\pm$ 20.2	-0.027	-2.196	-8.001	69
1997	1.83 $\pm$ 0.42	3.10 $\pm$ 0.85	112.6 $\pm$ 17.2	-0.059	-0.565	-1.210	153
1998	1.96 $\pm$ 0.62	3.53 $\pm$ 0.88	128.9 $\pm$ 28.7	-0.122	-5.307	2.361	88
1999	2.00 $\pm$ 0.45	3.14 $\pm$ 0.67	94.1 $\pm$ 10.2	-0.077	-0.665	-0.614	304
2000	1.67 $\pm$ 0.44	2.74 $\pm$ 0.59	119.0 $\pm$ 14.9	-0.089	-2.786	2.350	89
2001	1.52 $\pm$ 0.39	3.56 $\pm$ 0.72	125.3 $\pm$ 9.7	-0.135	-0.278	-1.396	171
All years	1.82 $\pm$ 0.51	3.24 $\pm$ 0.79	117.9 $\pm$ 25.9	-0.105	-3.930	2.313	1623

<sup>1</sup> Mean litter sizes and parturition dates for each year refer to the mean value experienced by juvenile red squirrels, therefore these values will differ from those in which litters were used as the unit of replication (e.g., Humphries and Boutin 2000).

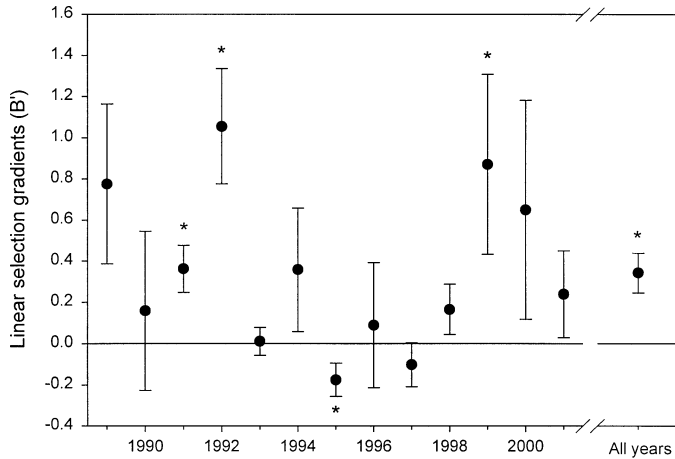


FIG. 2. Variation among cohorts in the standardized directional selection gradient ( $\beta'$ ) on nestling growth rates based on survival to one year of age in juvenile red squirrels. Gradients are plotted  $\pm$  SE. Gradients indicated by an asterisk were significantly different from zero ( $P < 0.05$ ). Gradients in 1995 and 1999 were not significant after sequential Bonferroni correction.

0.001) were all significant predictors of survival to one year of age. The addition of either the small- or the medium-scale spatial parameters to the model increased the amount of explained deviance in juvenile survival (small-scale 6.9% of overall deviance explained,  $\chi^2_{12} = 26.6$ ,  $P = 0.01$ ; medium-scale 6.3% of overall deviance explained,  $\chi^2_8 = 17.9$ ,  $P = 0.02$ ), but much of the increase in deviance explained by these models was due to spatial main effects on survival (small-scale  $\chi^2_3 = 15.5$ ,  $P = 0.001$ ; medium-scale  $\chi^2_2 = 10.8$ ,  $P = 0.005$ ) and there was no evidence of a significant interaction between the spatial variables and nestling growth rates for either model (small-scale  $\chi^2_3 = 4.1$ ,  $P = 0.25$ ; medium-scale  $\chi^2_2 = 0.6$ ,  $P = 0.76$ ). The addition of cohort and its interaction with the three juvenile traits greatly improved the amount of deviance explained (29.5% of overall deviance explained,  $\chi^2_{40} = 401.1$ ,  $P < 0.0001$ ; Table 5) and there was significant variation among cohorts in the strength of selec-

TABLE 3. Standardized maternal selection gradients ( $\pm$  SE) on litter size and parturition date (Julian) prior to the expression of these adult traits. Selection gradients were based on juvenile survival to one year of age of red squirrels born between 1989 and 2001. Boldface gradients were significantly different from zero based on SE generated by jackknifing ( $P < 0.05$ ). Selection gradients for litter size in 1989 and parturition date in 1992 were not significant after sequential Bonferroni correction. See Table 1 for sample sizes (number of juveniles) within each cohort.

Year	Maternal Selection Gradients ( $\beta'$ )	
	Litter Size	Parturition Date
1989	<b>-1.13 <math>\pm</math> 0.52</b>	0.24 $\pm$ 0.43
1990	0.16 $\pm$ 0.54	-0.83 $\pm$ 0.53
1991	-0.01 $\pm$ 0.13	-0.15 $\pm$ 0.12
1992	0.46 $\pm$ 0.28	<b>-0.50 <math>\pm</math> 0.18</b>
1993	0.02 $\pm$ 0.07	-0.05 $\pm$ 0.07
1994	-0.08 $\pm$ 0.21	0.03 $\pm$ 0.25
1995	<b>-0.29 <math>\pm</math> 0.08</b>	0.09 $\pm$ 0.09
1996	0.07 $\pm$ 0.58	-0.40 $\pm$ 0.49
1997	-0.17 $\pm$ 0.11	-0.03 $\pm$ 0.11
1998	0.06 $\pm$ 0.12	-0.14 $\pm$ 0.12
1999	0.31 $\pm$ 0.25	-0.51 $\pm$ 0.27
2000	0.51 $\pm$ 0.29	-0.15 $\pm$ 0.27
2001	0.13 $\pm$ 0.07	0.11 $\pm$ 0.15
All years	0.04 $\pm$ 0.07	<b>-0.20 <math>\pm</math> 0.07</b>

tion on both nestling growth rates (growth rate, cohort interaction:  $\chi^2_{10} = 30.0$ ,  $P = 0.001$ ) and litter size (litter size, cohort interaction:  $\chi^2_{10} = 20.2$ ,  $P = 0.03$ ).

*Estimation of Viability Selection*

Viability selection on nestling growth rates varied among cohorts in both direction and magnitude (Fig. 2). In most years selection favored increased nestling growth rates, however, in 1995 and 1997 decreased nestling growth rates were favored. The magnitude of selection on nestling growth rates was also generally weak (median  $|\beta'| = 0.24$ ), but there were episodes of very strong directional selection (e.g., 1989, 1992, 1999, and 2000). In general standard errors associated with these selection gradients were quite large and most selection gradients were not significantly different from zero.

TABLE 4. Standardized quadratic and correlational selection gradients ( $\pm$  SE) on nestling growth rates (GR; g/day), litter size (LS), and parturition date (PD; Julian) based on survival to one year of age of red squirrels born between 1989 and 2001. Bold gradients were significantly different from zero based on SE generated by jackknifing ( $P < 0.05$ ). Only correlational selection gradients for GR  $\times$  LS and LS  $\times$  PD in 1989 were significant after sequential Bonferroni correction. See Table 1 for the number of juveniles within each cohort.

Year	Quadratic selection gradients ( $\gamma_{ii}$ )			Correlational selection gradients ( $\gamma_{ij}$ )		
	GR	LS	PD	GR $\times$ LS	GR $\times$ PD	LS $\times$ PD
1989	0.18 $\pm$ 0.19	-0.40 $\pm$ 0.41	-0.72 $\pm$ 0.44	<b>-1.65 <math>\pm</math> 0.51</b>	<b>1.48 <math>\pm</math> 0.50</b>	<b>-2.28 <math>\pm</math> 0.74</b>
1990	-0.11 $\pm$ 0.35	0.82 $\pm$ 1.33	0.09 $\pm$ 0.74	-1.02 $\pm$ 1.14	-0.30 $\pm$ 0.60	-0.93 $\pm$ 1.93
1991	0.06 $\pm$ 0.09	-0.13 $\pm$ 0.11	0.02 $\pm$ 0.14	0.05 $\pm$ 0.14	-0.08 $\pm$ 0.12	0.12 $\pm$ 0.19
1992	-0.01 $\pm$ 0.45	0.14 $\pm$ 0.42	0.00 $\pm$ 0.23	-0.10 $\pm$ 0.80	-0.25 $\pm$ 0.34	-0.11 $\pm$ 0.37
1993	0.01 $\pm$ 0.04	0.06 $\pm$ 0.09	-0.03 $\pm$ 0.08	0.05 $\pm$ 0.13	-0.09 $\pm$ 0.13	-0.10 $\pm$ 0.11
1994	0.11 $\pm$ 0.15	-0.33 $\pm$ 0.20	-0.30 $\pm$ 0.19	0.08 $\pm$ 0.15	-0.20 $\pm$ 0.42	0.31 $\pm$ 0.32
1995	-0.14 $\pm$ 0.08	-0.11 $\pm$ 0.07	0.06 $\pm$ 0.11	-0.19 $\pm$ 0.12	0.03 $\pm$ 0.12	-0.08 $\pm$ 0.14
1996	-0.11 $\pm$ 0.45	0.32 $\pm$ 0.84	0.40 $\pm$ 0.59	-0.03 $\pm$ 1.37	0.09 $\pm$ 0.80	1.87 $\pm$ 1.50
1997	0.06 $\pm$ 0.10	0.00 $\pm$ 0.07	-0.11 $\pm$ 0.07	<b>-0.33 <math>\pm</math> 0.12</b>	0.09 $\pm$ 0.10	-0.16 $\pm$ 0.15
1998	-0.14 $\pm$ 0.09	-0.06 $\pm$ 0.18	0.07 $\pm$ 0.15	0.24 $\pm$ 0.20	-0.16 $\pm$ 0.12	0.04 $\pm$ 0.15
1999	0.14 $\pm$ 0.33	-0.47 $\pm$ 0.26	0.24 $\pm$ 0.19	0.26 $\pm$ 0.25	-0.58 $\pm$ 0.39	-0.20 $\pm$ 0.34
2000	0.28 $\pm$ 0.49	-0.31 $\pm$ 0.22	-0.21 $\pm$ 0.28	0.10 $\pm$ 0.46	0.16 $\pm$ 0.54	0.20 $\pm$ 0.30
2001	-0.11 $\pm$ 0.18	-0.03 $\pm$ 0.25	<b>-0.21 <math>\pm</math> 0.07</b>	-0.20 $\pm$ 0.44	0.01 $\pm$ 0.16	-0.07 $\pm$ 0.23
All years	0.02 $\pm$ 0.06	-0.09 $\pm$ 0.05	-0.04 $\pm$ 0.04	-0.01 $\pm$ 0.06	-0.11 $\pm$ 0.06	-0.04 $\pm$ 0.07

TABLE 5. Four candidate models representing spatial (at two scales) and temporal variation in selection on nestling growth rates (GR), litter size (LS), and parturition date (PD). Models are generalized linear models of survival to one year of age (binomial response) for 1509 juvenile red squirrels born between 1991 and 2001. The basic model considers only the gender of the juvenile (SEX) and the three offspring traits. Spatial and temporal models include both the spatial (trapping grid: GRID, or x and y coordinates of the natal nest and diagonal: X, Y, XY) or temporal (cohort: C) variables and interaction effects. Null deviance, 1653.5 and null df, 1508. Significance of each model was assessed against the basic model using analysis of deviance.

Model	Model df	Residual Deviance	% Null Deviance Explained	Analysis of Deviance
Basic	4	1566.7	5.2	—
Survival ~ SEX + GR + LS + PD				
Spatial—small Scale	16	1540.1	6.9	$\chi^2_{12} = 26.6$ $P = 0.01$
~Basic + X + Y + XY + X:GR + X:LS + X:PD + Y:GR + Y:LS + Y:PD + XY:GR + XY:LS + XY:PD				
Spatial—medium scale	12	1548.8	6.3	$\chi^2_8 = 17.9$ $P = 0.02$
~Basic + GRID + GRID:GR + GRID:LS + GRID:PD				
Temporal	44	1165.6	29.5	$\chi^2_{40} = 401.1$ $P < 0.0001$
~Basic + C + C:GR + C:LS + C:PD				

Viability selection on litter size and parturition date were weak (median  $|\beta'| = 0.16$  and  $0.15$ , respectively). In most years, offspring born earlier in the season had a higher probability of survival (but see 1989, 1994, 1995 and 2001), but there was no consistent direction of selection on litter size among cohorts (Table 3). There was no evidence of consistent stabilizing or disruptive selection for any of the traits examined here. Quadratic selection gradients for all three traits were weak in most years (median  $|\gamma'|$  growth rate,  $0.11$ ; litter size,  $0.14$ ; parturition date,  $0.11$ ) and were not consistently positive or negative among years (Table 4).

The median absolute value of correlational selection was weak for the three combinations of traits ( $GR \times LS = 0.19$ ;  $GR \times PD = 0.16$ ;  $LS \times PD = 0.16$ ) and varied in direction from one cohort to the next. In general there was little evidence of significant or consistent correlational selection between pairs of traits. However, in 1989 there was significant negative correlational selection between both growth rate and

litter size and litter size and parturition date, suggesting that fast growing offspring from small litters born later in the season had a higher probability of survival (Table 4).

*Time Scale of Viability Selection*

There were significant differences among the three time periods over which directional selection on nestling growth was estimated ( $F_{2,24} = 8.0, P = 0.002$ ), but these differences were not in the direction we might have predicted. The strength of viability selection on nestling growth increased with increases in the time-period over which selection was measured (Fig. 3). In particular, there was a positive correlation between the strength of selection from birth to emergence (50 days) and from emergence to recruitment (50 to 100 days;  $r = 0.71, n = 13, P = 0.007$ ; Fig. 4a), indicating that selection over these time periods was similar in direction and magnitude. Selection on nestling growth rates from 100 days of age to one year of age was independent of selection prior to recruitment ( $r = 0.04, n = 13, P = 0.90$ ; Fig. 4b). All of the cohorts experiencing significant ( $P < 0.05$ ) directional selection (1991, 1992, 1995 and 1999) were the result of a consistent direction of selection both before and after 100 days of age.

*Correlates of Viability Selection*

Selection acting on nestling growth rates prior to recruitment (100 days of age) was significantly negatively correlated with spring temperature (slope =  $-0.11 \pm 0.05, t_{11} = -2.4, P = 0.04$ ), but was not related to relative spring density ( $t_8 = -0.04, P = 0.97$ ), the abundance of cones in the current year ( $t_8 = -0.71, P = 0.50$ ), or the abundance of cones in the previous year ( $t_8 = -0.99, P = 0.35$ ). In contrast, there was a positive relationship between the strength of selection on nestling growth rates from recruitment to breeding age and the abundance of cones produced in the previous fall (slope =  $0.16 \pm 0.071, t_{11} = 2.05, P = 0.06$ ), but not the abundance of cones in the current year ( $t_8 = -1.15, P = 0.28$ ), relative density ( $t_8 = 0.87, P = 0.41$ ), or spring temperature ( $t_8 = -0.66, P = 0.53$ ).

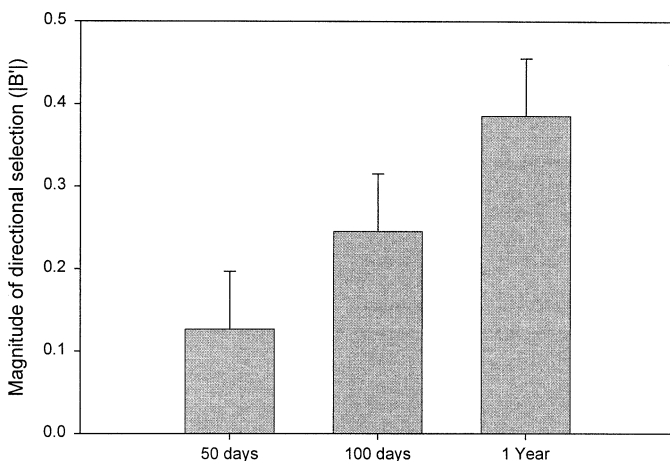


FIG. 3. Effects of the length of time over which viability selection was measured on the strength of directional selection on nestling growth rates. The time periods of days, months, and years correspond to viability based on survival to 50 days, 100 days, and one year, respectively. Differences among the three time periods were assessed using a linear mixed effects model in which year was included as a random blocking factor (13 years, 39 gradients). Values are presented as cell means + SE.

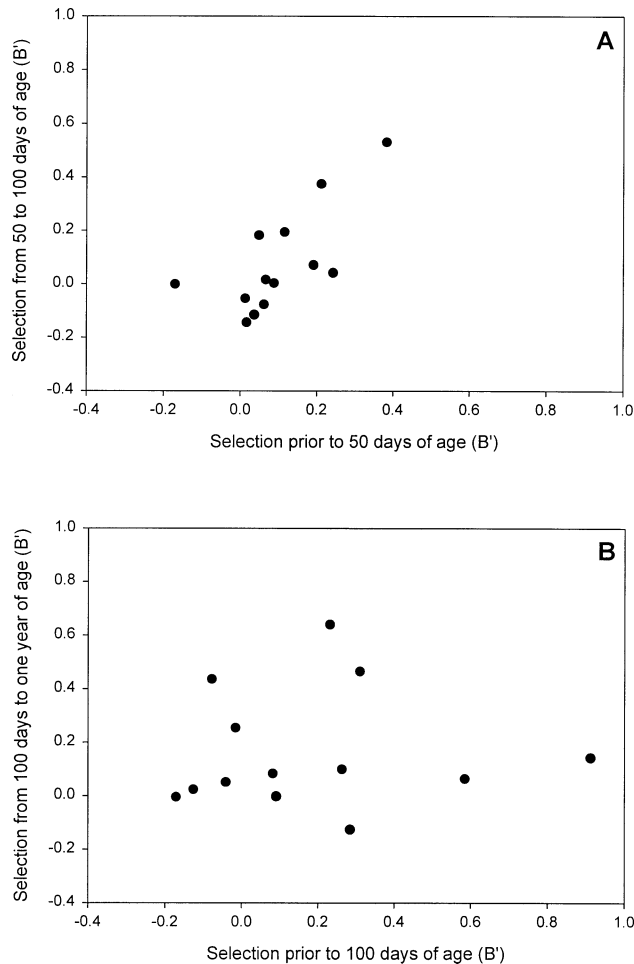


FIG. 4. Correlations between the strength of directional viability selection ( $\beta'$ ) acting prior to emergence (50 days of age) and from emergence to recruitment (100 days of age; A), and prior to recruitment and from recruitment to potential breeding age (one year; B).

#### DISCUSSION

In this population of red squirrels, the number of offspring produced in a given year exceeds the number of territories vacated by adult mortalities (S. Boutin, unpubl. data). As a result, the opportunity for viability selection on juveniles was often high, but there was a large amount of annual variation in the opportunity for selection. In all years the opportunity for selection on juvenile red squirrels was greater than that of Bumpus' sparrows ( $I = 0.90$ ; calculated from O'Donald 1973), and exceeded selection due to the drought of the late 1970's for Darwin's finches ( $I = 6.56$ ; calculated from Boag and Grant 1981) in six of the 13 cohorts. It is not surprising that the opportunity for selection was correlated with both the previous fall's cone production and spring population density. In this territorial system, juveniles usually acquire a territory through adult mortality (but see Berteaux and Boutin 2000; Boutin et al. 2000) and spring population density represents the inverse of territory vacancy. In this population, juveniles that do not acquire a territory do not survive the winter. In addition, more juveniles are produced in years

following high cone production (S. Boutin, unpubl. data), so that juveniles born in springs with high population density following years of high cone production will experience severe competition for very few vacant territories. However, this opportunity for selection only represents the maximum potential for natural selection (Brodie et al. 1995). The strength of this selection episode also depends on the covariance between relative fitness and juvenile traits.

Viability selection on nestling growth rates varied in both magnitude and direction from one cohort to the next. In most years, selection on nestling growth rates was weak but there were several episodes of very strong ( $\beta' > 0.5$ ; Kingsolver et al. 2001) directional selection on this trait (1989, 1992, 1999, and 2000). In particular, 1989, 1992, and 1999 all fell within the top 5% of published selection gradients (Kingsolver et al. 2001). Standard errors were in general quite large and only four of the 13 cohorts had selection gradients which were significantly different from zero based on  $\alpha = 0.05$  (1991, 1992, 1995, and 1999).

Variation in nestling growth rates is caused by both direct and maternal genetic variation as well as a large positive covariance between direct and maternal genetic effects (McAdam et al. 2002). This large positive covariance is incompatible with consistent directional selection on this trait and we have previously suggested that selection on this trait might instead vary either in space or time (McAdam et al. 2002). We found no evidence to support the hypothesis that selection varies over the two spatial scales examined in this study, but it remains possible that selection varies at larger spatial scales than those encompassed by our trapping grids. On the other hand, we found strong evidence that selection on nestling growth rates varies in both direction and magnitude from one cohort to the next. It is not known whether the magnitude of the reported temporal variation in selection reported here and the degree of overlap in generations ( $G = 2.7$  years; A. McAdam and S. Boutin, unpubl. data) are sufficient to maintain the observed genetic variation and covariation for this trait (Ellner and Hairston 1994).

The direction and magnitude of maternal selection on litter size varied among cohorts, whereas maternal selection on parturition date was negative (favoring earlier breeding) for most cohorts. Previous estimates of adult lifetime selection for this population suggest that litter size has been under stabilizing selection ( $\gamma' = -0.63 \pm 0.20$ ) while parturition date has experienced consistent directional selection for earlier breeding ( $\beta' = -0.53 \pm 0.15$ ; Réale et al. 2003b). Our estimates of maternal selection on litter size and parturition date correspond to maternal selection differentials (calculated following Kirkpatrick and Lande 1989, 1992;  $h^2_{\text{litter size}} = 0.15$ ,  $h^2_{\text{parturition date}} = 0.16$ , genetic correlation = 0.06, Réale et al., in press) ranging from  $-0.58$  to  $0.003$  offspring (median =  $-0.03$ ) and  $-4.08$  to  $0.25$  days (median =  $-1.05$ ) for litter size and parturition date, respectively. These indicate that nearly all cohorts experienced a reduction in litter size and advancement in parturition date prior to the expression of these maternal traits as a result of differential early viability. These episodes of selection at early life stages may have important influences on the evolution of adult traits in this population (Fairbairn and Reeve 2001).

There was very little evidence of either stabilizing or dis-

ruptive selection on any of the three traits examined. There was evidence of correlational selection between growth and litter size and litter size and parturition date in 1989, but none of the other cohorts experienced significant correlational selection. The large positive covariance between direct and indirect genetic effects on nestling growth rates for this population (McAdam et al. 2002) is the result of negative genetic correlations between growth rate and both litter size and parturition date, and negative phenotypic effects of both litter size and parturition date on growth rates. We had expected then to find some evidence of strong negative correlational selection between growth rate and parturition date or litter size. There was evidence of negative correlational selection between growth rate and litter size in 1997, which represented the dominant breeding cohort in 1999 when the estimate of the direct-maternal covariance was made (McAdam et al. 2002), but correlational selection was not common.

We found no evidence to support the hypothesis that estimates of viability selection from longer time periods are associated with weaker selection than estimates over shorter time periods (Hoekstra et al. 2001). In fact our data provide evidence to the contrary. Estimates of viability selection based on survival to one year of age were greater than estimates based on either 50 or 100 days of age. In addition, selection on nestling growth rates from birth to 50 days of age and from 50 to 100 days of age were positively correlated, suggesting that the same selective forces may be acting at both of these biologically distinct time periods (birth to emergence from the natal nest and emergence to recruitment). There was no correlation between selection on nestling growth rates prior to 100 days of age and from 100 days of age.

Prior to emergence, the proximate causes of mortality in red squirrels are exposure, malnourishment, disease and maternal mortality; there has been no evidence of predation of juveniles within the natal nest (Stuart-Smith and Boutin 1995a). After emergence, juvenile mortality is due almost exclusively to predation (Stuart-Smith and Boutin 1995a; Anderson and Boutin 2002), but the risk of predation is likely mediated by food abundance, individual condition, and behavior (Stuart-Smith and Boutin 1995a, b; Anderson and Boutin 2002). The close correspondence between selection on nestling growth rates prior to emergence and from emergence to recruitment is surprising given the two distinct agents of mortality during these two time periods.

Selection on nestling growth rates prior to recruitment was correlated negatively with average spring temperatures, indicating that selection for increased nestling growth rates was associated with cooler spring temperatures. Ambient temperatures have large energetic implications for adult female squirrels (Humphries and Boutin 2000), and are likely also an important factor in the susceptibility of juveniles to exposure or starvation prior to emergence. Energetic demands may also influence the ability of juveniles to behaviorally mediate predation risk (Stuart-Smith and Boutin 1995b; Anderson and Boutin 2002). In both cases, increased nestling growth rates may provide an energetic buffer, improving the ability of juveniles to both withstand periods of thermal stress in the nest as well as allowing for reduced foraging activity and therefore reduced predation risk following emergence.

Selection acting after 100 days of age was positively correlated with the abundance of spruce cones produced in the previous fall. High spruce cone production is associated with both high adult survival over winter and high reproductive output the following spring (S. Boutin, unpubl. data). As a result, years following high cone production have intense competition among juveniles for vacant territories. Offspring with higher nestling growth rates may be more successful at competing for vacant territories or may be better able to persist in suboptimal habitat until a territory vacancy appears. These correlations between both spring temperature and the abundance of spruce cones and the strength of selection on nestling growth rates could be verified through experimental manipulation of nest thermal characteristics and food abundance, respectively.

In some cohorts these two distinct episodes of selection were complementary and resulted in very strong viability selection on juvenile red squirrels. In other cohorts these episodes acted antagonistically, reducing the overall strength of selection on nestling growth rates. Therefore, episodes of very strong directional selection may represent unique and possibly rare combinations of environmental conditions. This combined effect of multiple environmental conditions on the overall strength of selection within cohorts will make the prediction of future selection events (Grant and Grant 2002) extremely difficult. However, the quantification of the strength of selection on nestling growth rates as well as sources of genetic variation in this offspring trait (McAdam et al. 2002), provide the necessary and sufficient conditions for predicting the responses of individual cohorts to observed episodes of viability selection.

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#### LITERATURE CITED

- Anderson, E. M., and Boutin, S. 2002. Edge effects on survival and behaviour of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Can. J. Zool.* 80:1038–1046.
- Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: theory. *Evolution* 38:709–719.
- Berteaux, D., and S. Boutin. 2000. Breeding dispersal in female North American red squirrels. *Ecology* 81:1311–1326.
- Boag, P. T., and P. R. Grant. 1981. Intense natural selection in a population of Darwin's finches (*Geospizinae*) in the Galápagos. *Science* 214:82–85.
- Boutin, S., K. W. Larsen, and D. Berteaux. 2000. Anticipatory parental care: acquiring resources for offspring prior to conception. *Proc. R. Soc. Lond. B* 267:2081–2085.
- Boyce, M. S., and C. M. Perrins. 1987. Optimizing great tit clutch size in a fluctuating environment. *Ecology* 68:142–153.
- Brodie, E. D. III, A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. *Trends Ecol. Evol.* 10: 313–318.
- Ellner, S., and N. G. Hairston, Jr. 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *Am. Nat.* 143:403–417.



- Endler, J. A. 1986. *Natural Selection in the Wild*. Princeton Univ. Press, Princeton, NJ.
- Fairbairn, D. J., and J. P. Reeve. 2001. Natural selection. Pp. 29–43 in C. W. Fox, D. A. Roff, and D. J. Fairbairn, eds. *Evolutionary ecology: concepts and case studies*. Oxford Univ. Press, New York.
- Grant, P. R. and R. Grant. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296:707–711.
- Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli, and J. G. Kingsolver. 2001. Strength and tempo of directional selection in the wild. *Proc. Natl. Acad. Sci. USA* 98:9157–9160.
- Humphries, M. M., and S. Boutin. 2000. The determinants of optimal litter size in free-ranging red squirrels. *Ecology* 81:2867–2877.
- Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. *Am. Nat.* 157:245–261.
- Kirkpatrick, M., and R. Lande. 1989. The evolution of maternal characters. *Evolution* 43:485–503.
- . 1992. The evolution of maternal characters: errata. *Evolution* 46:284.
- Kruuk, L. E. B., J. Merilä, and B. C. Sheldon. 2001. Phenotypic selection on a heritable size trait revisited. *Am. Nat.* 158:557–571.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Legendre, P., and L. Legendre. 1998. *Numerical ecology*. 2nd ed. Elsevier, Amsterdam.
- Mathsoft 1999a. *S-PLUS 2000 guide to statistics*. Vol. 1. Data analysis products products Division, Mathsoft, Seattle, WA.
- . 1999b. *S-PLUS 2000 user's guide*. Data Analysis Products Division, Mathsoft, Seattle, WA.
- McAdam, A. G., S. Boutin, D. Réale, and D. Berteaux. 2002. Maternal effects and the potential for evolution in a natural population of animals. *Evolution* 56:846–851.
- McCullagh, P., and J. A. Nelder. 1989. *Generalized linear models*. 2d ed. Chapman and Hall, New York.
- Merilä, J., B. C. Sheldon, and L. E. B. Kruuk. 2001. Explaining stasis: microevolutionary studies in natural populations. *Genetica* 112–113:199–222.
- Mousseau, T. A. and D. A. Roff. 1987. Natural selection and the heritability of fitness components. *Heredity* 59:181–197.
- O'Donald, P. 1973. A further analysis of Bumpus' data: the intensity of natural selection. *Evolution* 27:398–404.
- Przybylo, R., B. C. Sheldon, and J. Merilä. 2000. Patterns of natural selection on morphology of male and female collared flycatchers (*Ficedula albicollis*). *Biol. J. Linn. Soc.* 69:213–232.
- Réale, D., McAdam, A. G., Boutin, S. and Berteaux, D. 2003a. Genetic and plastic responses of a northern mammal to climate change. *Proc. R. Soc. Lond. B* 270:591–596.
- Réale, D., Berteaux, D., McAdam, A. G. and Boutin, S. 2003b. Lifetime selection on heritable life-history traits in a natural population of red squirrels. *Evolution* 57: *In press*.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- Roff, D. A. 1997. *Evolutionary quantitative genetics*. Chapman and Hall, New York.
- Sinervo, B., Svensson, E., and Comendant, T. 2000. Density cycles and an offspring quantity and quality game driven by natural selection. *Nature* 406:985–988.
- Stuart-Smith, A. K., and S. Boutin. 1995a. Predation on red squirrels during a snowshoe hare decline. *Can. J. Zool.* 73:713–722.
- , and S. Boutin. 1995b. Behavioural differences between surviving and depredated juvenile red squirrels. *Écoscience* 2: 34–40.
- Venables, W. N. and B. D. Ripley. 2002. *Modern applied statistics with S*. 4th ed. Springer-Verlag, New York.

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