University of Alberta

Maternal effects and the microevolution of juvenile growth rates in red squirrels (*Tamiasciurus hudsonicus*).

by

Andrew G. McAdam

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

Environmental Biology and Ecology

Department of Biological Sciences

Edmonton, Alberta

Fall 2003

National Library
of Canada

Acquisitions and
Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale
du Canada

Acquisisitons et
services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 0-612-88019-2
Our file Notre référence
ISBN: 0-612-88019-2

Canada

University of Alberta

Library Release Form

Name of Author: Andrew Graham McAdam

Title of Thesis: Maternal effects and the microevolution of juvenile growth rates in red squirrels (*Tamiasciurus hudsonicus*).

Degree: Doctor of Philosophy

Year this Degree Granted: 2003

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

June 3, 2003

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Maternal effects and the microevolution of juvenile growth rates in red squirrels (*Tamiasciurus hudsonicus*)" submitted by Andrew G. McAdam in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Environmental Biology and Ecology.

St

May 30th, 2003

"*None of biology makes sense except in the light of evolution*"

- Theodosius Dobzhansky

"*The rodents are very low in the scale of intelligence, but the red squirrel ranks high in its class. It is gifted with a burning curiosity, which, tempered by prudence and aided by agility, is an excellent start on the road to knowledge.*"

- Ernest T. Seton, 1909

ABSTRACT

Maternal effects are widespread and are predicted to result in unusual and sometimes counterintuitive evolutionary dynamics if they are themselves heritable. However, estimates of the genetic basis to maternal effects have previously been restricted to laboratory or captive bred animals, so their relevance to evolution in natural populations was not known. I used cross-fostering techniques and a long-term study of a natural population of red squirrels, *Tamiasciurus hudsonicus*, in the southwest Yukon, Canada, to estimate both direct (heritability) and indirect (maternal) influences on the evolutionary dynamics of juvenile growth rates.

The growth in body mass of juvenile red squirrels raised in both high and low food conditions had significant amounts of direct genetic variation, but also experienced large, heritable maternal effects. The consideration of these indirect genetic effects revealed a greater than three-fold increase in the potential for evolution of growth rates relative to that predicted by heritability alone. Directional selection on growth rates, based on the survival to potential breeding age of juveniles born between 1989 and 2001, varied in magnitude and direction from one cohort to the next. Directional selection was relatively weak in most years, but there were episodes of very strong viability selection in some cohorts, which were some of the strongest yet recorded for a natural population.

Observed responses of juvenile growth rates across one generation of selection (1989 – 2000) supported both of the general predictions of models of maternal effect evolution. First, observed responses to selection were nearly 5 times greater than predictions based on direct genetic effects alone, but were consistent with an estimate including maternal effects. Second, the response to selection in the current generation was determined not only by the strength of selection in the current generation but also by selection in the previous generation, providing empirical evidence of evolutionary momentum. Finally, maximum likelihood estimates of realised maternal effect parameters closely matched estimates from the cross-fostering experiments. The results of this study, therefore, provided both qualitative and quantitative evidence for the importance of maternal effects to the evolutionary dynamics of this juvenile trait in a natural population of red squirrels.

ACKNOWLEDGEMENT

This research would not have been possible without the hard work of many people who have collected field data over the past 16 years. In particular, thanks to Joe Litke, Tamie Hucal, Jordan de Groot, Ainsley Sykes, Janine Arnold, Shannon Ripley, Helen Jewell, Liz Anderson, Simone Runyan, Steve Henry, Mike Melnychuk, Erin Burrell, Debora Ko, Elin Boalt and Magdalena Nystrand for their help in the 1999 and 2000 field seasons.

Research funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC), a Circumpolar/Boreal Alberta Research Grant, the Northern Scientific Training Program of the Department of Indian Affairs and Northern Development (DIAND) with the Canadian Circumpolar Institute at the University of Alberta, and through grants-in-aid of research from the American Society of Mammalogists and the Arctic Institute of North America. Personal support was provided by NSERC, the Killam Trust, the University of Alberta and the American Society of Mammalogists. Field work was conducted under license of the Yukon – Canada Scientists' and Explorers' Act with the permission of Agnes Moose of the Champagne-Aishihik First Nation.

Jan Murie, Francis Yeh, Barb Thomas, Rich Moses, Dominique Berteaux, Derek Roff and Tim Mousseau have provided me with helpful comments and encouragement on this project since its inception. Thanks to Colleen Cassady St. Clair, Jack Millar and Tony Sinclair for the loan of field equipment and to Steve Osborne of Yukon College for the use of computer facilities in the Yukon. Thanks to Jens Roland for the introduction to generalized linear models and SPLUS. Ainsley Sykes was a wonderful help in preparing

for work in the field and in sorting out duplications, omissions and errors in the long-term data, but I also thank her for her friendly smiles and conversation.

During my time at the University of Alberta I have benefited from formal and informal discussions with many wonderful researchers and friends. I have learned many things from them and appreciate their laughter, encouragement and support. In particular, Stan Boutin has been a terrific supervisor and mentor throughout my graduate program. His enthusiasm and industry inspired me to dig a little deeper, to reach a bit higher and to give those manuscripts one more kick at the cat. Whether in the lab, at Squirrel Camp or on Sunday morning rides our discussions always left me motivated and a little bit wiser.

My family also continues to provide me with the many skills and the strength needed to make it through life's challenges, academic and otherwise, and I thank them all for their maternal and paternal effects. Finally, I would like to thank Merritt for being such a wonderful colleague, partner and friend. This thesis would not have been possible without her love and support.

TABLE OF CONTENTS

CHAPTER 4. TEMPORAL VARIATION IN VIABILITY SELECTION

CHAPTER 5. MATERNAL EFFECTS AND THE RESPONSE TO SELECTION

CHAPTER 6. GENERAL DISCUSSION AND CONCLUSIONS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF SYMBOLS

In addition to standard statistical symbols, I have used a number of symbols throughout the thesis that I have compiled here. I have also defined each symbol at first use within each chapter.

Symbol	Name
V_P	Total phenotypic variance
V_{AO}	Direct additive genetic effects
V_{EM}	Environmental maternal effects
V_{DO}	Dominance variance in offspring trait
h^2	Heritability (direct genetic effects only)
V_{AM}	Indirect additive genetic effects
m	Maternal effect coefficient
h_m^2	Heritability of maternal effects (indirect genetic effects)
$Cov(A_O, A_M)$	Covariance between direct and maternal genetic effects
r_{mo}	Direct-maternal genetic correlation
$Cov(E_O,E_M)$	Covariance between direct and maternal environmental effects
$Cov(D_O,D_M)$	Covariance between direct and maternal dominance effects
h_t^2	Total heritability (includes indirect genetic effects)
σ^2_D	Variance due to dam
σ^2_N	Variance due to nurse
σ^2_{DN}	Variance due to dam by nurse interaction

σ^2_{error}	Error variance
cov_{ij}	Covariance between the growth rate of offspring raised in environment i and mothers raised in environment j
r_x	Genetic correlation across environments
CV	Coefficient of variation (CV = standard deviation / mean)
I	Opportunity for selection
β_i	Directional selection gradient on trait i
β_i'	Standardized directional selection gradient (sd units)
S	Directional selection differential ($S = \beta * V_p$)
γ_{ii}	Quadratic (stabilising or disruptive) selection gradient on trait i
γ_{ij}	Correlational selection gradient between traits i and j

1.0 GENERAL INTRODUCTION

1.1 MICROEVOLUTION

Evolution by natural selection is the process by which differential survival or reproduction among individuals results in genetically based changes in a population across generations. As such, an understanding of microevolutionary processes (evolutionary changes within or between populations; Hendry & Kinnison 2001) requires knowledge of both selection within generations (section 1.1.1), and the transmission of variation across generations (heritability; section 1.1.2).

1.1.1 *Selection*

Natural selection is a sorting process by which some individuals, as a result of particular characters or combinations of characters, contribute more progeny to subsequent generations than other individuals. Several techniques have been developed to quantify selection on individual traits or sets of traits based on a particular mode of selection (e.g., directional, stabilizing or disruptive selection; Brodie *et al.* 1995). Directional selection is characterized by a linear relationship between trait values and fitness such that either increases or decreases in trait values are consistently favoured (Figure 1.1A). Disruptive and stabilizing selection, on the other hand, favour extreme and intermediate trait values, represented by positive (Figure 1.1B) and negative quadratic functions (Figure 1.1C), respectively. True associations between fitness and trait variation, however, are unlikely to be restricted to linear and quadratic functions. The irregular shape of particular fitness surfaces can be visualized using nonparametric regression techniques, where combinations of traits result in unique fitness values that are not constrained to any particular parametric function (see Figure 1.2; Schluter 1988, Schluter & Nychka 1994). While extremely useful for identifying unusual nonlinearities, such as local fitness peaks, these nonparametric approaches to visualizing fitness surfaces do not provide parameters that are easily interpreted or applied to quantitative genetic equations. As a result, fitness functions are most frequently approximated by linear and quadratic terms representing directional and nonlinear selection (stabilizing or disruptive), respectively. The development of techniques for quantifying natural selection on multiple traits (e.g., Lande & Arnold 1983), has resulted in a large increase in the number of estimates of the strength of selection in natural populations (Endler 1986, Hoekstra *et al.* 2001, Kingsolver *et al.* 2001).

Most episodes of directional selection have been found to be 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 result in change of one standard deviation in the mean of trait with a reasonable heritability.

1.1.2 *Heritability*

The proportion of the total phenotypic variance (V_{PO}) that is due to additive genetic variance (V_{AO}) is referred to as narrow sense heritability (h^2; Falconer & Mackay 1996).

$$h^2 = V_{AO} / V_{PO}$$

There are several techniques for estimating heritabilities (see Lynch & Walsh 1998), but all involve examining the degree of phenotypic resemblance among individuals of known relatedness. Estimating the genetic basis to variation in quantitative traits allows 1) the inference of the strength of previous selection episodes, and 2) the prediction of future responses to episodes of selection (Boake 1994).

Heritability estimates are thought to reflect the strength of previous selection because the loss of genetic variation, through the fixation of high-fitness alleles, should be greater in traits subject to strong directional or stabilizing selection than traits experiencing weaker, less consistent or disruptive selection (Roff 1997). In general, traits more closely associated with fitness, such as life history traits, have been found to have lower heritabilities than traits with weaker associations to fitness (typically morphological traits; Gustafsson 1986, Mousseau & Roff 1987, Kruuk *et al.* 2000, Merilä & Sheldon 2000). Examination of the specific contributions of genetic and environmental variation to these heritabilities, however, suggests that life history traits actually have higher levels of genetic variation, but this is outweighed by increased environmental variation (Houle 1992). Higher levels of environmental and genetic variation in life history traits may reflect their increased ecological and genetic complexity, respectively. Many life history traits are influenced by a number of component traits (i.e., are ecologically complex). For example, fecundity may be influenced by several less complex traits such as body size, body condition, foraging ability or basal metabolic rate. Each component trait is influenced partly by environmental sources, but environmental variation in complex traits is compounded across all component traits (Price and Schluter 1991). The increased ecologically complexity of life history traits, therefore, provides an explanation for their increased environmental variances (Price & Schluter 1991). Life history traits may have higher genetic variances because of their increased genetic complexity (Houle *et al.* 1996). Genetic variation is generated by recombination and random mutations at each locus, so traits influenced by more loci represent larger overall targets for mutation. Life history traits appear to have higher overall mutation rates than morphological traits, suggesting that they are influenced by a larger number of loci (Houle *et al.* 1996). Inferences about selection history based on heritability differences between traits may, therefore, be confounded by differences in ecological and genetic architecture (Merilä & Sheldon 1999).

The influence of selection history on genetic variation and heritability may be better represented by the expression of the same trait in different environments. For example, if selection eliminates low fitness alleles more efficiently in common favourable environments, then less frequent unfavourable conditions should maintain higher levels of genetic variation (Holloway *et al.* 1990). However, several other hypotheses, providing complementary and contradictory predictions, have also been proposed regarding the expression of genetic variation in favourable and unfavourable conditions (Hoffmann & Merilä 1999) and empirical generalizations regarding the effects of environmental quality on heritable variation in natural populations have been slow to emerge. One general trend has been a tendency for decreased heritabilities of size-related traits in natural populations of birds experiencing unfavourable conditions (see Merilä 1997, Hoffmann & Merilä 1999), but there are many other studies that have demonstrated an increase in genetic variation or no consistent response across traits in unfavourable conditions (e.g., Gebhardt-Henrich & van Noordwijk 1991, Merilä *et al.* 1999, Coltman *et al.* 2001, Kause & Morin 2001, see also Hoffmann & Merilä 1999). More empirical estimates of the effects of environmental conditions on sources of variation are needed before the validity of these various hypotheses (see Hoffmann & Merilä 1999) can be assessed adequately.

1.2 RESPONSE TO SELECTION

The second reason for estimating heritabilities is that they can be used to predict the evolutionary response across generations when the strength of selection is known. The predicted change in the mean phenotype (R) of a single trait can be quantified by the breeders' equation,

$$R = h^2 S,$$

as the product of the heritability (h^2) and the strength of selection (S) acting on that trait (Falconer & Mackay 1996). Despite ample evidence of significant levels of both genetic variation (e.g., Weigensberg & Roff 1996), and directional selection (Endler 1986, Hoekstra *et al.* 2001, Kingsolver *et al.* 2001), studies documenting contemporary responses to selection in the wild are relatively rare (Merilä *et al.* 2001; but see Hairston & Walton 1986, Reznick *et al.* 1997, Merilä *et al.* 2001, Grant & Grant 2002, Réale *et al.* 2003).

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 & Perrins 1987, Przybylo *et al.* 2000, Sinervo *et al.* 2000, Kruuk *et al.* 2001, Grant & 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).

A second explanation for the lack of documented responses to selection is that estimates of heritability may be biased (Merilä *et al.* 2001). Of the 165 heritabilities compiled by Weigensberg & Roff (1996) from natural populations, 116 were based on parent-offspring regressions, which are potentially confounded by environmental covariances, and paternal or maternal effects (Lynch & Walsh 1998; see Milner *et al.* 2000 for an example). In addition to confounding estimates of heritability, genetically based maternal effects may also contribute to an evolutionary response to selection (Kirkpatrick & Lande 1989, Wolf *et al.* 1998).

1.3 MATERNAL EFFECTS

Maternal effects occur when the phenotype of a mother, or the environment she experiences, causes phenotypic effects in her offspring (Mousseau & Fox 1998). Mothers can influence both the phenotype and fitness of their offspring in many ways including their choice of a breeding territory, mate and nest site, as well as through the degree of maternal care and the number of offspring among which this care must be shared (Mousseau & Fox 1998). Maternal effects have now been documented for a variety of taxa (Roach & Wulff 1987, Rossiter 1996, Bernardo 1996, Mousseau & Fox 1998), but are thought to be particularly strong in mammals because of their long period of maternal dependence (Roff 1997). Recent interest in maternal effects (Mousseau & Fox 1998) has not only resulted from their ubiquity but also from their potentially important implications for evolutionary dynamics (Kirkpatrick & Lande 1989, Wolf *et al.* 1998).

Models of maternal effect evolution suggest that maternal effects can greatly accelerate or retard the evolutionary response to selection and are predicted to introduce an evolutionary time lag, in which the response to selection in the current generation also depends on the strength of selection in the previous generation (Kirkpatrick & Lande 1989, Lande & Kirkpatrick 1990, Wolf *et al.* 1998). As a result, populations may continue to evolve after selection has ceased and evolution may also temporarily proceed in a direction opposite to the direction of selection (Kirkpatrick & Lande 1989). For example, the predicted response (Kirkpatrick & Lande 1989, Kirkpatrick & Lande 1992) of an offspring trait to 10 generations of directional selection are shown in Figure 1.3 for models including positive ($m = 0.8$), negative ($m = - 0.8$) and no maternal effects ($m = 0$). In the absence of maternal effects (solid dots), the offspring trait is predicted to change consistently throughout the period of selection (following the breeders' equation above) and then remain constant once selection is relaxed. The presence of positive or negative maternal effects, however, is predicted to result in accelerated and retarded overall responses to selection, respectively. Furthermore, in the presence of maternal effects the response to selection is not consistent throughout the period of selection, but instead is influenced by the strength of selection in the previous generation (Figure 1.3). In some cases the response of the offspring trait can even be in the opposite direction of selection (Figure 1.3, open dots; Kirkpatrick & Lande 1989).

These dramatic and sometimes counterintuitive predictions of maternal effects models, however, depend on the presence of a genetic basis to maternal variation (Kirkpatrick & Lande 1989, Wolf *et al.* 1998). While the genetic basis to maternal effects has been estimated frequently in populations subject to generations of artificial selection or benign laboratory conditions (e.g., see Roff 1997, Cheverud 1984), indirect genetic effects in nondomestic species (Hunt & Simmons 2002, Rauter & Moore 2002, Agrawal *et al.* 2001) and under natural field conditions have been investigated only recently (Byers *et al.* 1997, Thiede 1998). As a result, the importance of maternal effects to evolutionary dynamics in natural populations is not yet known.

1.4 CROSS-FOSTERING

Cross-fostering experiments provide a useful tool for partitioning the contribution of genetic, maternal and environmental variation to overall phenotypic variation. In a reciprocal cross-fostering experiment half the offspring from one brood are exchanged with an equal number of offspring from a paired brood. This design, therefore, separates additive genetic variance and maternal variance within a single statistical model (Rutledge *et al.* 1972). Cross-fostering experiments have been used to estimate heritabilities in laboratory populations of mice (Rutledge *et al.* 1972; Atchley & Rutledge

1980; Cheverud *et al.* 1983; Cheverud 1984; Leamy & Cheverud 1984; Riska *et al.* 1984; Riska *et al.* 1985) and in some natural populations of birds (see Roff 1997 Table 7.16, Merilä & Sheldon 2001). In most mammals, however, offspring are inaccessible or mothers have highly developed mechanisms to discriminate between related and unrelated juveniles. These difficulties associated with cross-fostering most mammals in the wild (but see Murie *et al.* 1998) have thus far prevented the use of this technique to quantify heritability and maternal effects in natural populations of mammals.

1.5 RED SQUIRRELS

North American red squirrels (*Tamiasciurus hudsonicus*) provide a unique opportunity to examine the influence of direct and maternal genetic variation on dynamics of offspring traits in a natural population of mammals. A population of red squirrels near Kluane Lake in the southwest Yukon, Canada, have been studied consistently since 1987, so there exists both an extensive pedigree of marked individual with known matrilines, and a comprehensive understanding of the ecology of this system (e.g., Boutin & Larsen 1993, Boutin *et al.* 1993, Stuart-Smith & Boutin 1995, Humphries & Boutin 1996, Humphries & Boutin 2000, Berteaux & Boutin 2000, Boutin *et al.* 2000, Anderson & Boutin 2002, Réale *et al.* 2003). In addition, this population is relatively dense (approximately 2.5 squirrels / ha) so data can be collected on a large number of juveniles within a single breeding season. Finally, it is possible to cross-foster offspring between litters (Humphries & Boutin 1996), which enabled me to experimentally partition genetic, environmental and maternal effects.

Red squirrels in this population feed almost exclusively on the seeds of white spruce (*Picea glauca*) cones. Spruce cones are harvested from trees in late summer and are stored for future consumption in centrally located caches (middens; Hatt 1929), within exclusively defended year-round territories (Smith 1968). The production of spruce cones has been measured in the study area since 1986 and varies dramatically from one year to the next (Humphries & Boutin 2000). This annual variation in the abundance of food is thought to have important implications for many aspects of red squirrel life history (Berteaux & Boutin 2000, Humphries & Boutin 2000, Réale *et al.* 2003).

Individual variation in early growth is known to affect future survival and reproduction in many birds and mammals (Lindstrom 1999). Growth rates have been measured on nestling red squirrels in the Kluane population since 1987. Increased juvenile growth in body mass has been associated with increased survival prior to weaning (Boutin & Larsen 1993), and decreased age at first reproduction in red squirrels (Becker *et al.* 1998) suggesting that this is an important life history trait, but the strength of selection on this juvenile trait has not yet been measured.

1.6 OBJECTIVE

The objective of this study was to quantify the genetic basis of maternal effects on juvenile growth in body mass and to determine their implications for evolutionary dynamics in a natural population of red squirrels. To meet this objective, I measured both the magnitude of direct and maternal (indirect) genetic effects on nestling growth rates, and the strength of selection on this juvenile trait. Given the importance of spruce cones as a food item for red squirrels and the large annual variation in the availability of cones, I investigated the influence of the abundance of spruce cones on both the strength of selection and the potential for evolution in this trait. The quantification of both the strength of selection and direct and indirect sources of genetic variation in juvenile growth rates allowed me to predict evolutionary changes in growth rates using a model of maternal effect evolution (Kirkpatrick & Lande 1989). Qualitative and quantitative predictions of the maternal effect model were tested using observed changes in juvenile growth rates over 12 years to determine the importance of maternal effects to the evolutionary dynamics of this juvenile trait.

1.7 THESIS OUTLINE

The magnitude of maternal effects and their contribution to the potential for evolution of juvenile growth rates was quantified in Chapter 2 using a combination of a cross-fostering experiment in 1999 and existing long-term data for this population (Réale *et al. in press*). The objective of this chapter was to highlight the potential importance of maternal effects to the potential for evolution in a natural population.

The cross-fostering experiment in Chapter 2, however, was performed in the spring following the highest production of spruce cones yet recorded for this population. Conditions for growth in 1999, therefore, were very good (Chapter 2). Theoretical and empirical results, however, indicate that both environmental and genetic sources of variation can be influenced by the environmental conditions experienced during the expression of the trait (Hoffmann & Merilä 1999). As a result, I repeated the cross-fostering experiment in 2000 under conditions of very low food. Some cross-fostered litters were also supplemented with food in 2000 to experimentally improve conditions for growth. The effects of food abundance on heritable variation in growth rates was examined in Chapter 3 using results from the cross-fostering experiments in 1999 and 2000, as well as growth rates measured on mothers and their offspring in this population over the past 16 years (1987 - 2002).

In chapter 4, I quantified the strength of selection on juvenile growth rates by examining the survival of 1623 juveniles, born between 1989 and 2001, from birth to potential breeding age. In particular, I looked for evidence of temporal and spatial variation in the strength and direction of selection and decomposed viability selection into three separate episodes (birth to emergence, emergence to recruitment and recruitment to one year of age) to test the hypothesis that episodes of selection are frequently followed by period of stasis or reversals (Hoekstra *et al.* 2001). I also examined the influence of several ecological variables (e.g., population density, cone abundance) on the strength of selection on juvenile growth rates.

Chapters 2 through 4 quantified the necessary and sufficient conditions for evolution by natural selection. In Chapter 5, I examined observed changes in growth rates from prior to selection to one generation after selection for each of 12 cohorts (1989 – 2000) and compared these to predictions from a maternal effect model of evolution. Two general predictions of the maternal effect model were tested using these observed responses to selection and the strength of selection in the current and previous generation, while realised maternal effect parameters were quantified using maximum likelihood techniques.

Together these 4 chapters provide a comprehensive quantitative genetic investigation of the evolution of juvenile growth rates in red squirrels within the context of large annual fluctuations in food abundance. The combination of short-term manipulations of relatedness, together with a 16-year pedigree of individuals followed throughout their entire lifetime, has provided a rare opportunity to examine the influence of maternal effects on evolutionary dynamics in a natural population.

1.8 REFERENCES

Agrawal, A. F., Brodie, E. D., III & Brown, J. 2001. Parent-offspring coadaptation and the dual genetic control of maternal care. *Science,* **292**: 1710-1712.

Anderson, E. M. & Boutin, S. 2002. Edge effects on survival and behaviour of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Canadian Journal of Zoology,* **80**: 1038-1046.

Atchley, W. R. & Rutledge, J. J. 1980. Genetic components of size and shape. I. Dynamics of components of phenotypic variability and covariability during ontogeny in the laboratory rat. *Evolution,* **34**: 1161-1173.

Becker, C. D., Boutin, S. & Larsen, K. W. 1998. Constraints on first reproduction in North American red squirrels. *Oikos,* **81**: 81-92.

Bernardo, J. 1996. Maternal effects in animal ecology. *American Zoologist,* **36**: 83-105.

Berteaux, D. and S. Boutin. 2000. Breeding dispersal in female North American red squirrels. *Ecology,* **81**: 1311-1326.

Boake, C. R. B. 1994. *Quantitative Genetic Studies of Behavioral Evolution.* The University of Chicago Press, Chicago.

Boutin, S. & Larsen, K. W. 1993. Does food availability affect growth and survival of males and females differently in a promiscuous small mammal, *Tamiasciurus hudsonicus*? *Journal of Animal Ecology,* **62**: 364-370.

Boutin, S., Larsen, K. W. & Berteaux, D. 2000. Anticipatory parental care: acquiring resources for offspring prior to conception. *Proceedings of the Royal Society of London, Series B,* **267**: 2081-2085.

Boutin, S., Tooze, Z. & Price, K. 1993. Post-breeding dispersal by female red squirrels (*Tamiasciurus hudsonicus*): the effect of local vacancies. *Behavioral Ecology,* **4**: 151-155.

Boyce, M. S. & Perrins, C. M. 1987. Optimizing great tit clutch size in a fluctuating environment. *Ecology,* **68**: 142-153.

Brodie, E. D., III, Moore, A. J. & Janzen, F. J. 1995. Visualizing and quantifying natural selection. *Trends in Ecology and Evolution,* **10**: 313-318.

Byers, D. L., Platenkamp, G. A. J. & Shaw, R. G. 1997. Variation in seed characters in *Nemophila menziesii*: evidence of a genetic basis for maternal effects. *Evolution,* **51**: 1445-1456.

Cheverud, J. M. 1984. Evolution by kin selection: a quantitative genetic model illustrated by maternal performance in mice. *Evolution,* **38**: 766-777.

Cheverud, J. M., Rutledge, J. J. & Atchley, W. R. 1983. Quantitative genetics of development: genetic correlations among age-specific trait values and the evolution of ontogeny. *Evolution,* **37**: 895-905.

Coltman, D. W., Pilkington, J., Kruuk, L. E. B., Wilson, K. & Pemberton, J. M. 2001. Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. *Evolution,* **55**: 2116-2125.

Endler, J. A. 1986. *Natural Selection in the Wild.* Princeton University Press, Princeton, New Jersey.

Falconer, D. S. 1952. The problem of environment and selection. *American Naturalist,* **86**: 293-298.

Falconer, D. S. and Mackay, T. F. C. 1996. *Introduction to Quantitative Genetics.* 4th edn. Longman, Harlow, UK.

Gebhardt-Henrich, S. G. & van Noordwijk, A. J. 1991. Nestling growth in teh great tit I. Heritability estimates under different environmental conditions. *Journal of Evolutionary Biology,* **4**: 341-362.

Grant, P. R. & Grant, R. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science,* **296**: 707-711.

Gustafsson, L. 1986. Lifetime reproductive success and heritability: Empirical support for Fisher's fundamental theorem. *American Naturalist,* **128**: 761-764.

Hairston, N. G. Jr. & Walton, W. E. 1986. Rapid evolution of a life history trait.

Proceedings of the National Academy of Science, USA, **83**: 4831-4833.

Hatt, R. T. 1929. The red squirrel: its life history and habits, with special reference to the Adirondacks of New York and the Harvard Forest. *Roosevelt Wildlife Annals,* **2**: 11-146.

Hendry, A. P. & Kinnison, M. T. 2001. An introduction to microevolution: rate, pattern, process. *Genetica,* **112-113**: 1-8.

Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hoang, A., Hill, C. E., Beerli, P. & Kingsolver, J. G. 2001. Strength and tempo of directional selection in the wild. *Proceedings of the National Academy of Science, USA,* **98**: 9157-9160.

Hoffmann, A. A. & Merilä, J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution,* **14**: 96-101.

Holloway, G. J., Povey, S. R. & Sibly, R. M. 1990. The effect of new environment on adapted genetic architecture. *Heredity,* **64**: 323-330.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics,* **130**: 195-204.

Houle, D., Morikawa, B. & Lynch, M. 1996. Comparing mutational variabilities. *Genetics,* **143**: 1467-1483.

Humphries, M. M. & Boutin, S. 1996. Reproductive demands and mass gains: a paradox in female red squirrels (*Tamiasciurus hudsonicus*). *Journal of Animal Ecology,* **65**: 332-338.

Humphries, M. M. & Boutin, S. 2000. The determinants of optimal litter size in free-ranging red squirrels. *Ecology,* **81**: 2867-2877.

Hunt, J. & Simmons, L.W. 2002. The genetics of maternal care: Direct and indirect genetic effects on phenotype in the dung beetle *Onthophagus taurus. Proceedings of the National Academy of Science, USA* **99**, 6828-6832.

Kause, A. & Morin, J.-P. 2001. Seasonality and genetic architecture of development time and body size of the birch feeding sawfly *Priophorus pallipes. Genetical Research,* **78**: 31-40.

Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hill, C. E., Hoang, A., Gibert, P. & Beerli, P. 2001. The strength of phenotypic selection in natural populations. *American Naturalist,* **157**: 245-261.

Kirkpatrick, M. & Lande, R. 1989. The evolution of maternal characters. *Evolution,* **43**: 485-503.

Kirkpatrick, M. & Lande, R. 1992. The evolution of maternal characters: Errata. *Evolution,* **46**: 284.

Kruuk, L. E. B., Clutton-Brock, T. H., Slate, J., Pemberton, J. M., Brotherstone, S. & Guinness, F. E. 2000. Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Science, USA,* **97**: 698-703.

Kruuk, L. E. B., Merilä, J. & Sheldon, B. C. 2001. Phenotypic selection on a heritable size trait revisited. *American Naturalist,* **158**: 557-571.

Lande, R. & Arnold, S. J. 1983. The measurement of selection on correlated characters. *Evolution,* **37**: 1210-1226.

Lande, R. & Kirkpatrick, M. 1990. Selection response in traits with maternal inheritance. *Genetical Research,* **55**: 189-197.

Leamy, L. & Cheverud, J. M. 1984. Quantitative genetics and the evolution of ontogeny. II. Genetic and environmental correlations among age-specific characters in randombred mice. *Growth,* **48**: 339-353.

Lindstrom, J. 1999. Early development and fitness in birds and mammals. *Trends in Ecology and Evolution,* **14**: 343-348.

Lynch, M. and B. Walsh. 1998. *Genetics and Analysis of Quantitative Traits.* Sinauer Associates, Sunderland, Massachusetts.

Merilä, J. 1997. Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. *Evolution,* **51**: 526-536.

Merilä, J., Kruuk, L. E. B. & Sheldon, B. C. 2001. Cryptic evolution in a wild bird population. *Nature,* **412**: 76-79.

Merilä, J., Przybylo, R. & Sheldon, B. C. 1999. Genetic variation and natural selection on blue tit body condition in different environments. *Genetical Research,* **73**: 165-176.

Merilä, J. & Sheldon, B. C. 1999. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity,* **83**: 103-109.

Merilä, J. & Sheldon, B. C. 2000. Lifetime reproductive success and heritability in nature. *American Naturalist,* **155**: 301-310.

Merilä, J. & Sheldon, B. C. 2001. Avian quantitative genetics. *Current Ornithology,* **16**: 179-255.

Merilä, J., Sheldon, B. C. & Kruuk, L. E. B. 2001. Explaining stasis: microevolutionary studies in natural populations. *Genetica,* **112-113**: 199-222.

Milner, J. M., Pemberton, J. M., Brotherstone, S. & Albon, S. D. 2000. Estimating variance components and heritabilities in the wild: a case study using the 'animal model' approach. *Journal of Evolutionary Biology,* **13**: 804-813.

Mousseau, T. A. and C. W. Fox. 1998. *Maternal Effects as Adaptations.* Oxford University Press, New York.

Mousseau, T. A. & Roff, D. A. 1987. Natural selection and the heritability of fitness components. *Heredity,* **59**: 181-197.

Murie, J. O., Stevens, S. D. & Leoppky, B. 1998. Survival of captive-born cross-fostered juvenile Columbian ground squirrels in the field. *Journal of Mammalogy,* **79**: 1152-1160.

Price, T. & Schluter, D. 1991. On the low heritability of life-history traits. *Evolution,* **45**: 853-861.

Przybylo, R., Sheldon, B. C. & Merilä, J. 2000. Patterns of natural selection on morphology of male and female collared flycatchers (*Ficedula albicollis*).

Biological Journal of the Linnean Society, **69**: 213-232.

Rauter, C. M. & Moore, A. J. 2002. Evolutionary importance of parental care performance, food resources, and direct and indirect genetic effects in a burying beetle. *Journal of Evolutionary Biology,* **15**: 407-417.

Réale, D., Berteaux, D., McAdam, A. G. & Boutin, S. *in press*. Lifetime selection on heritable life history traits in a natural population of red squirrels. *Evolution.*

Réale, D., McAdam, A. G., Boutin, S. & Berteaux, D. 2003. Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London, Series B,* **270**: 591-596.

Reznick, D. N., Shaw, F. H., Rodd, F. H. & Shaw, R. G. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science,* **275**: 1934-1937.

Riska, B., Atchley, W. R. & Rutledge, J. J. 1984. A genetic analysis of targeted growth in mice. *Genetics,* **107**: 79-101.

Riska, B., Rutledge, J. J. & Atchley, W. R. 1985. Covariance between direct and maternal genetic effects in mice, with a model of persistent environmental influences. *Genetical Research,* **45**: 287-297.

Roach, D. A. & Wulff, R. D. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics,* **18**: 209-235.

Roff, D. A. 1997. *Evolutionary Quantitative Genetics.* Chapman & Hall, New York.

Rossiter, M. 1996. Incidence and consequences of inherited environmental effects. *Annual Review of Ecology and Systematics,* **27**: 451-476.

Rutledge, J. J., Robinson, O. W., Eisen, E. J. & Legates, J. E. 1972. Dynamics of genetic and maternal effects in mice. *Journal of Animal Science,* **35**: 911-918.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution,* **42**: 849-861.

Schluter, D. & Nychka, D. 1994. Exploring fitness surfaces. *Evolution,* **143**: 597-616.

Sinervo, B., Svensson, E. & Comendant, T. 2000. Density cycles and an offspring quantity and quality game driven by natural selection. *Nature,* **406**: 985-988.

Smith, C. C. 1968. The adaptive nature of social organization in the genus of tree squirrels *Tamiasciurus. Ecological Monographs,* **38**: 31-63.

Stuart-Smith, A. K. & Boutin, S. 1995. Predation on red squirrels during a snowshoe hare decline. *Canadian Journal of Zoology,* **73**: 713-722.

Thiede, D. A. 1998. Maternal inheritance and its effect on adaptive evolution: a quantitative genetic analysis of maternal effects in a natural plant population. *Evolution,* **52**: 998-1015.

Weigensberg, I. & Roff, D. A. 1996. Natural heritabilities: can they be reliably estimated in the laboratory? *Evolution,* **50**: 2149-2157.

Wolf, J. B., Brodie, E. D., III, Cheverud, J. M., Moore, A. J. & Wade, M. J. 1998. Evolutionary consequences of indirect genetic effects. *Trends in Ecology and Evolution,* **13**: 64-69.

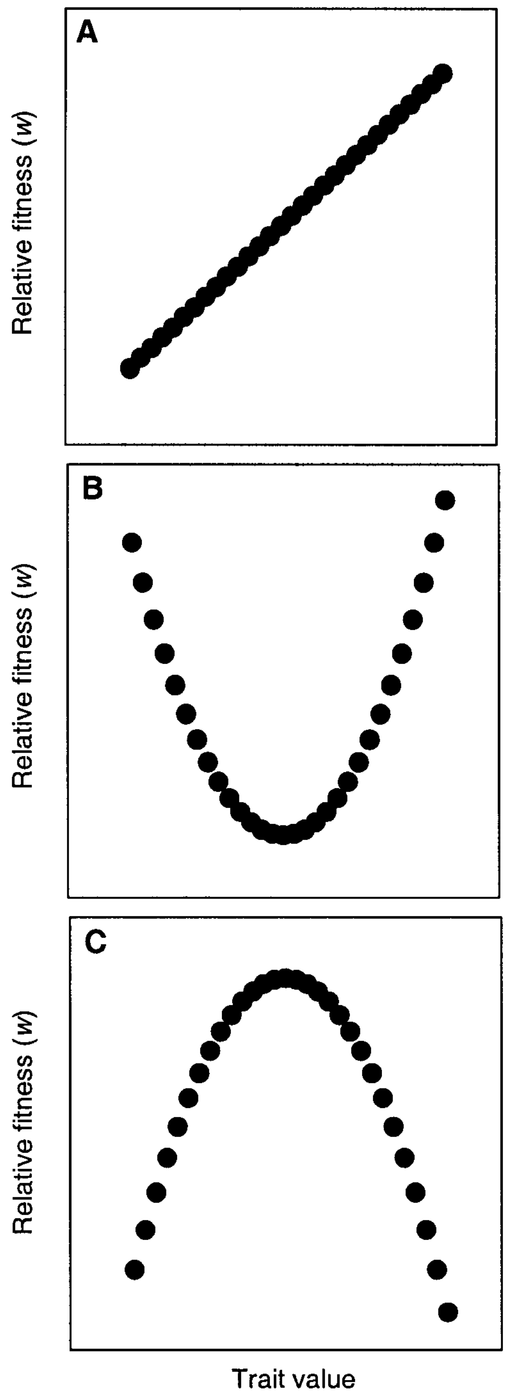

Figure 1.1 Linear (A) and quadratic (B, C) fitness functions representing directional (A), disruptive (B) and stabilizing (C) modes of selection on a hypothetical trait.

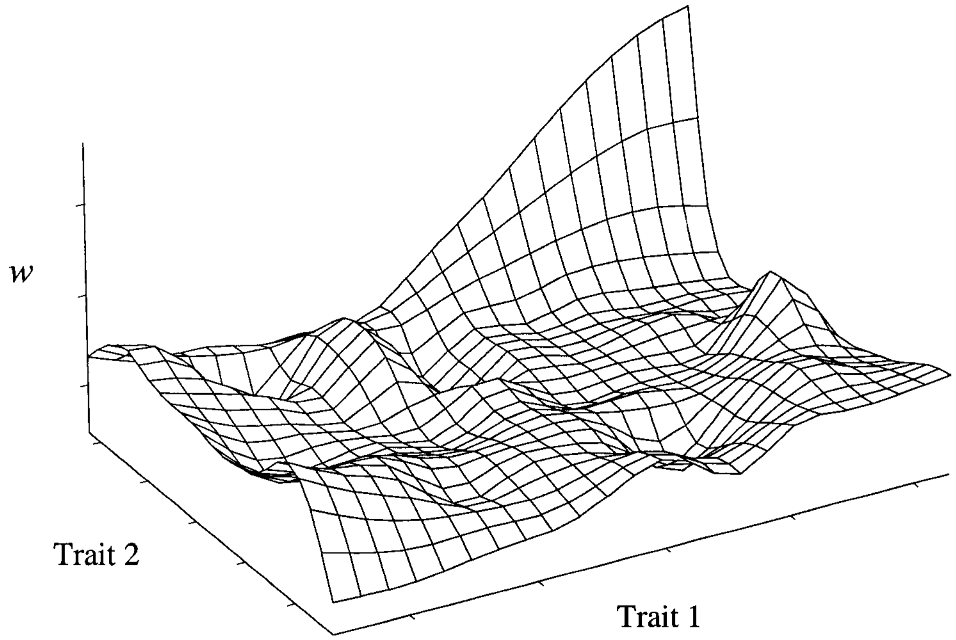

Figure 1.2. Nonlinear fitness surface of two hypothetical traits (x and y axes), depicting the irregular relationship between combinations of these traits and relative fitness (*w*; z axis). The surface is fit using a localized regression technique (cubic splines).

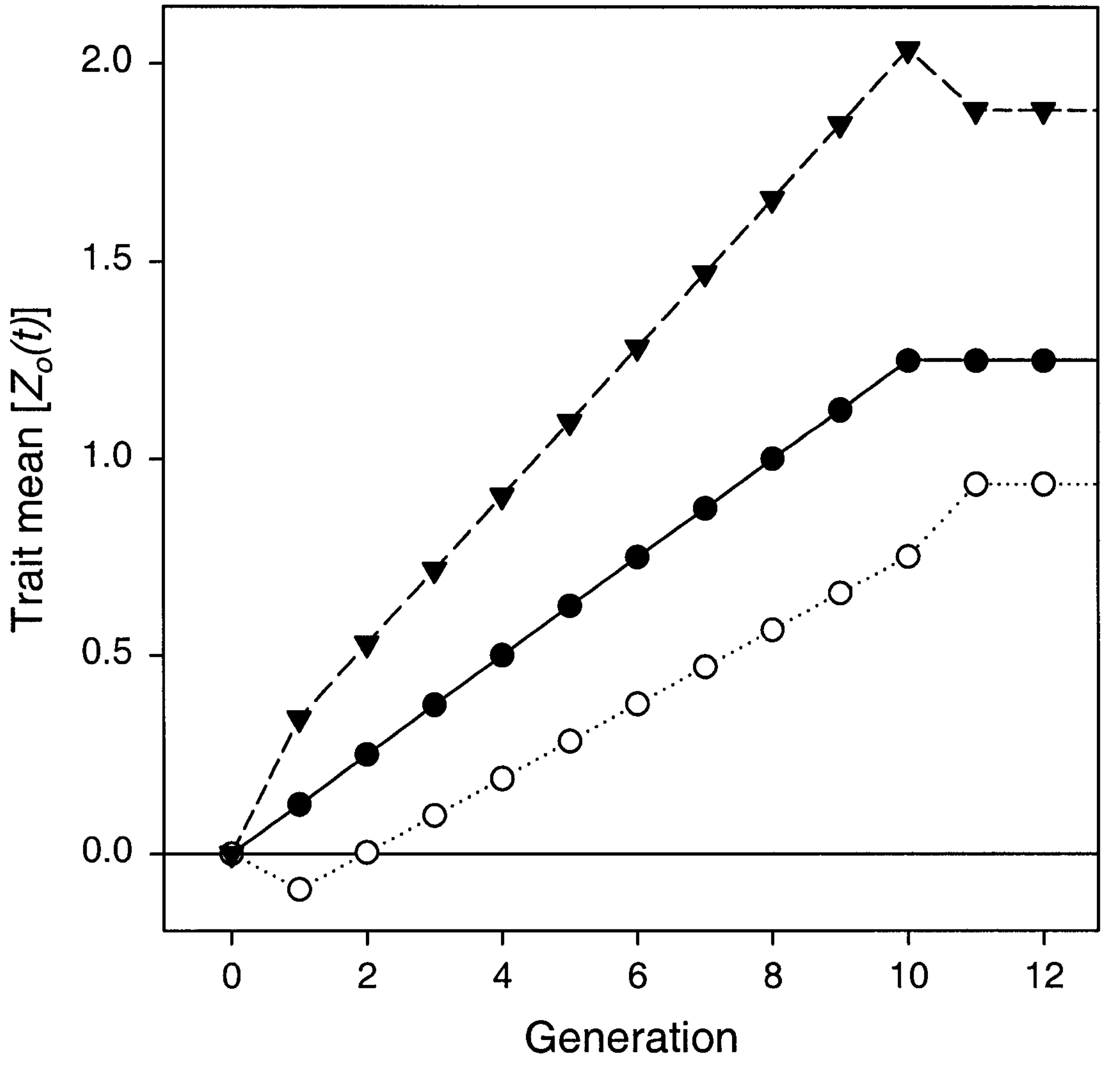

Figure 1.3. Predicted responses of a juvenile trait to 10 generations of selection for increased values of the trait ($\beta = 0.5$; following Kirkpatrick & Lande 1989, Kirkpatrick & Lande 1992). Three evolutionary trajectories are shown corresponding to no maternal effects ($m = 0$; solid dot), positive maternal effects ($m = 0.8$; solid triangle) and negative maternal effects ($m = -0.8$; open dot). Direct genetic variance (V_{AO}), indirect genetic variance (V_{AM}) and their correlation (r_{mo}) were set to 0.25, 0.1 and 0.5, respectively.

2.0 MATERNAL EFFECTS AND THE POTENTIAL FOR EVOLUTION.[1]

2.1 INTRODUCTION

Evolution by natural selection pervades all aspects of biology, but an evolutionary response to selection can occur only if the trait under selection has a genetic basis. Most studies of natural populations have estimated only direct genetic effects (heritability; Weigensberg & Roff 1996, Hoffmann 2000), but recent theoretical and laboratory work suggests that heritable maternal effects can have important indirect influences on the potential for evolution (Wolf *et al.* 1998). Maternal effects occur when the phenotype of a mother or the environment she experiences has a phenotypic effect on her offspring (Mousseau & Fox 1998). When maternal effects exist, a response to selection depends not only on direct genetic effects, but also on indirect genetic effects (heritable maternal effects), which can result in accelerated, dampened or even non-intuitive responses to selection (Wolf *et al.* 1998). Dickerson (1947) expanded the simple definition of heritability ($h^2 = V_{AO} / V_{PO}$), to include not just direct genetic effects (V_{AO}), but also indirect genetic effects (V_{AM}) and the direct-indirect genetic covariance [$Cov\ (A_O, A_M)$] as a proportion of the total phenotypic variation (V_{PO}) in the offspring trait (h^2_t represents total heritability).

$$h^2_t = (V_{AO} + 1/2\ V_{AM} + 3/2\ Cov\ (A_O, A_M))\ /\ V_{PO}$$

---

[1] A version of this chapter has been published. McAdam *et al.* 2002. *Evolution*, 56: 846-851.

Large maternal effects have been found in many natural populations of animals (Mousseau & Fox 1998). In collared flycatchers (*Ficedula albicollis*), over 25% of variation in clutch size was attributed to maternal effects (Schluter & Gustafsson 1993, Price 1998, but see Merilä *et al.* 2001), and significant maternal effects have also been reported for a range of life history traits in red deer (*Cervus elaphus*) including total fitness (Kruuk *et al.* 2000). Our ability to quantify the genetic basis of maternal effects (i.e. indirect genetic effects) and the direct-indirect genetic covariance, however, has been limited to captive animals (Roff 1997, Shaw & Byers 1998, Thiede 1998). In plants, controlled breeding designs under combined greenhouse and field conditions suggest that indirect genetic effects can either accelerate or constrain the potential response to selection (Thiede 1998, Byers *et al.* 1997). The influence of indirect genetic effects on the potential for evolution in a natural population of animals is not known.

In this chapter I measured the potential for evolution of nestling growth rates in a natural population of North American red squirrels (*Tamiasciurus hudsonicus*). In this model system I had both the ability to cross-foster newborn squirrels and 11 previous years of data that together allowed me to estimate both direct and indirect genetic contributions to the potential for evolution in a natural environment. Studies of natural populations are particularly important because it is within these variable environments that natural selection shapes both the direct and indirect sources of genetic variation and, consequently, the potential for future evolutionary change.

2.1 METHODS

2.1.1 *Red squirrel population and general field techniques*

A natural population of red squirrels (*Tamiasciurus hudsonicus*) has been monitored annually from March to August since 1987. The 130 ha study area, located near Kluane National Park in the southwestern Yukon, Canada (61° N, 138° W), is dominated by white spruce (*Picea glauca*) with a willow (*Salix* spp.) understory. Red squirrels in this area feed almost exclusively on the seeds of white spruce cones. Spruce cones are harvested from trees in late summer and are stored in centrally located caches (middens) for future consumption (Hatt 1929).

Tomahawk live-traps baited with peanut butter were set on each squirrel's midden or in the immediate vicinity. Individuals captured for the first time were given a permanent 5 digit metal ear tag (Monel #1) in each ear. In addition, body mass, sex and reproductive status were recorded at each capture. Female reproductive status was determined by the size and colour of her nipples and through abdominal palpations for pregnancy. Lactation or a steady mass gain followed by a dramatic loss of mass indicated parturition. Once a female was determined to have given birth, her nest was located through a combination of radio-telemetry and behavioural observations. Juveniles were temporarily removed from their natal nest to determine litter size and were weighed using a Pesola spring balance (grams) and sexed. At approximately 25-30 days postpartum, juveniles were removed from the nest for a second time to be weighed and given permanent metal ear tags. As a result, maternity of nestlings is known with certainty.

Juveniles first emerge from the natal nest at approximately 42 days of age. Known nest locations were visited within a female's territory at the timing of juvenile emergence to record which juveniles had successfully emerged. Offspring are weaned at

70 days of age and must acquire their own territory. In some cases, females bequeath the natal territory to offspring or share it with them (Berteaux & Boutin 2000, Boutin *et al.* 2000). In May and August of each year (1987 – 2002), the owner of every midden on the study area was determined. Ownership was determined primarily by live-trapping, territorial calls and behavioural observations. Any newly established middens were recorded and monitored in all future censuses. Squirrels that do not own a midden do not survive through the winter (Boutin, unpublished data). While there is some immigration into the area, the fate of most squirrels is known from birth to death.

2.2.2 *Cross-fostering design*

Red squirrels in the Kluane population were monitored from March through August 1999. Litters of squirrels were paired as closely as possible based on parturition dates. Offspring from 33 pairs of litters were reciprocally cross-fostered so that roughly half the offspring of one litter was exchanged with an equal number of offspring from the paired litter. Growth of nestling red squirrels was monitored from the time of cross-fostering to approximately 25 days of age, which was just prior to first emergence from the natal nest. Body mass was measured (± 0.1 g) using portable electronic balances and the width of the zygomatic arch (± 0.1 mm) was used as an index of overall body size (Becker 1992). Non-linear growth trajectories were assessed using differential equations involving specific growth rates (Kaufmann 1981).

2.2.3 *Sources of variation*

Sources of variation in growth in body mass and growth in body size were estimated using two separate ANOVAs. In each analysis, total phenotypic variation within pairs of cross-fostered litters was partitioned using a two-way nested ANOVA in which dam, nurse and their interaction were all nested within cross-fostered pairs (Riska *et al.* 1985), represented by the linear model

$$Y_{hijk} = \mu + P_h + D_{i(h)} + N_{j(h)} + DN_{ij(h)} + e_{hijk},$$

where Y_{hijk} is the growth rate of the k^{th} squirrel raised by nurse j and born to dam i in the h^{th} pair. μ is the grand mean growth rate, and $D_{i(h)}$ and $N_{j(h)}$ are the effects of the i^{th} dam and j^{th} nurse within the h^{th} pair, respectively. $DN_{ij(h)}$ is the interaction effect and e_{hijk} is the residual deviation of the k^{th} squirrel. All effects except μ were assumed to be random independent variables with zero means and variances equal to σ^2_P, σ^2_D, σ^2_N, σ^2_{DN} and σ^2_e. Variance due to the dam (σ^2_D), nurse (σ^2_N) and dam by nurse interaction (σ^2_{DN}) represented genetic, maternal and genotype by environment interaction effects, respectively. Variance components were estimated using Restricted Maximum Likelihood (REML) in the VARCOMP procedure in S-Plus (Mathsoft 1999b, Mathsoft 1999a).

Genetic expectations of variance components from the linear model above based on full-sib litters (but see below) following Willham (1963) are:

$$\sigma^2_D = 1/2V_{AO} + 1/4V_{DO}$$

$$\sigma^2_N = V_{AM} + V_{DM} + V_{EM}$$

$$\sigma^2_{DN} = 0$$

$$\sigma^2_e = 1/2V_{AO} + 3/4V_{DO} + V_E.$$

Rutledge *et al.* (1972) initially estimated the direct-maternal genetic covariance [$cov(A_O, A_M)$] from the dam by nurse interaction variance (σ^2_{DN}; see also Lynch & Walsh 1998, Rauter & Moore 2002). This expectation assumes that the interaction variance (σ^2_{DN}) represents the covariance among full sibs raised by their own mother minus both σ^2_D and σ^2_N (Lynch & Walsh 1998), which is true if nurse by dam combinations (cells in the 2 x 2 design) represented full sibs raised by their own mothers as it does in a North Carolina II design (see Lynch & Walsh 1998). However, in a cross-fostering design offspring raised by an unrelated female experience maternal effects independent of direct genetic effects. As a result, $cov(A_O, A_M)$ is not included in the phenotypic variance of fostered offspring and σ^2_{DN} has no genetic expectation (see genetic expectations above; Riska *et al.* 1985). Therefore, I estimated $cov(A_O, A_M)$ from two separate ANOVA's as the difference between the among-litter variance of offspring that remained in their natal nest and the among-litter variance of cross-fostered offspring (Ahlschwede & Robison 1971, Riska *et al.* 1985). In addition, I calculated total phenotypic variance (V_P) as the total variance within cross-fostered pairs ($\sigma^2_D + \sigma^2_N + \sigma^2_{DN} + \sigma^2_e$) plus $cov(A_O, A_M)$ following Riska *et al.* (1985).

2.2.4 *Maternal effects*

The cross-fostering design did not allow me to determine the genetic basis to maternal effects (V_{AM}) directly, but the composite term maternal performance is simply the combined effect of several maternal characteristics for which I may derive heritabilities individually (Wolf *et al.* 1998). I used multiple regression analysis to determine which maternal characteristics were correlated with maternal performance for offspring growth. Since offspring were distributed roughly equally between mothers within a cross-fostered pair, differences between these mothers in the average growth rate of all nursed offspring (genetic and foster) represented differences in maternal performance independent of direct genetic effects. As a result, differences in maternal characteristics within pairs were correlated with differences in maternal performance (sample size: $n = 26$ complete dyads). Maternal characteristics included in the multiple regression were: litter size, parturition date, age, body size, maternal mass at parturition and weaning, territory size and the azimuth of the natal nest. Means for each characteristic were substituted for missing data, except for parturition date. Parturition dates could not be estimated accurately for six pairs so these were deleted in a pair-wise manner ($n = 20$ dyads).

Of these nine maternal characteristics only litter size and parturition date were significantly correlated with maternal performance. The exclusive contributions of these two maternal characteristics to overall maternal performance were calculated using partial linear regression (Legendre & Legendre 1998). The contributions of litter size and parturition date to the heritability of maternal performance (h^2_m) were calculated as the product of the proportion of maternal performance explained by that trait (R^2_i) and its heritability (h^2_i). The heritability of maternal performance then, was calculated as the sum of these products for litter size and parturition date ($h^2_m = R^2_{litter\ size}h^2_{litter\ size} + R^2_{parturition\ date}h^2_{parturition\ date}$). The product of variation in nestling growth due to maternal performance (σ^2_N) and the heritability of maternal performance was used as an estimate of indirect genetic effects (V_{AM}).

2.2.5 *Heritability*

The heritability of growth in body mass and body size were calculated from the cross-fostering experiment as the proportion of total phenotypic variation within cross-fostered pairs that was due to additive genetic variation. Heritability estimates for litter size and parturition date were previously calculated from existing long-term data for this population (1988-1998; Réale *et al. in press*) using Derivative Free Restricted Maximum Likelihood techniques (DFREML 3.0; Meyer 1989). Total heritabilities were calculated following Dickerson (1947).

2.2.6 *Environmental effects*

Estimates of $Cov\ (A_O, A_M)$ derived from cross-fostering experiments are potentially confounded by persistent maternal environmental effects [bVE_M (Lynch & Walsh 1998) or m_m (Riska *et al.* 1985)] or direct-maternal environmental covariances [$Cov\ (E_O, E_M)$]. A direct-maternal environmental covariance will arise, for example, if environmental conditions early in life (e.g. food abundance) affect both a female's growth rate and her subsequent maternal performance for offspring growth (e.g. litter size). Persistent maternal environmental effects will occur if environmental conditions affect not only a mother's performance, but also the maternal performance of her daughter. Measures of maternal characteristics for the same mother in multiple years, suggest that persistent environmental effects on litter size and parturition date are both small (as percentages of total variance: litter size 0.02 ± 0.02%, parturition date 0.00%) and the DFREML models including persistent environmental effects were not distinguishable from the previous models excluding these environmental effects (log-likelihood ratio test: litter size $G_1 = 0.000004$, $p = 0.99$; parturition date $G_1 = 0.0014$, $p = 0.97$; Réale *et al. in press*). I estimated the magnitude of $Cov\ (E_O, E_M)$ and $Cov\ (D_O, D_M)$ for growth in body mass by performing both a mother-offspring ($n = 604$) and a maternal grandmother-grandoffspring regression ($n = 195$) for all available combinations in the existing long-term dataset (1990-1999). The difference between the mother-offspring covariance and twice the maternal grandmother-grandoffspring covariance estimates $Cov\ (E_O, E_M) + Cov\ (D_O, D_M) - bVE_M$ (Lynch & Walsh 1998).

2.2.7 *Paternity and dominance variance*

Levels of multiple paternity within litters are not known in red squirrels and dominance variance cannot be adequately estimated with my experimental design. I initially assumed that offspring within litters were full sibs ($V_{AO} = 2\ \sigma^2_D$), and that dominance variance was negligible, but later relaxed these assumptions and examined a range of possible values of relatedness and dominance variance. Variance components from the two-way nested ANOVA (σ^2_D and σ^2_{error}) were used to calculate the minimum relatedness (r) and maximum levels of dominance variance (V_{DO}). In the ANOVA design $\sigma^2_D = r\ V_{AO} + 0.25\ V_{DO}$ and $\sigma^2_{error} = (1 - r)\ V_{AO} + 0.75\ V_{DO} + e$ (Riska *et al.* 1985). I examined values of the average degree of relatedness within natal litters (r) ranging from single paternity (0.5) to complete multiple paternity (0.25), and values of V_{DO} from 0 to 0.05. Values of r and V_{DO} resulting in a negative value of e are not possible.

Values are presented throughout the text as means ± one standard error (SE). Standard errors for population parameters [e.g. V_{AO}, $Cov\ (A_O, A_M)$, h^2_t] were calculated by jack-knifing at the level of the cross-fostered pair.

2.3 RESULTS

Growth in nestling body mass averaged 1.91 g/day and appeared linear over the time period measured. There was, however, a positive correlation between linear growth rate (g/day) and the first mass measurement ($r^2 = 0.03$, $n = 177$, $P = 0.02$), so the residuals of this relationship were used for subsequent analyses (mean = -0.00073 g/day). The width of the zygomatic arch grew by an average of 0.30 mm/day but followed a Gompertz curve, where specific growth rates decreased with the geometric mean of the initial and final body size measurements (ln transformed). As a result, adjusted specific growth rates were used for subsequent analyses (mean = 0.017 day^{-1}). Growth in body mass and body size did not differ between male and female offspring (mass: $t_{175} = -0.89$, $P = 0.38$; size: $t_{175} = 0.52$, $P = 0.60$) so both sexes were examined together.

There were strong genetic and maternal effects on both growth in body mass (dam: $F_{33,26} = 2.08$, $P = 0.007$; nurse: $F_{27,26} = 16.74$, $P < 0.0001$) and body size (dam: $F_{33,26} = 2.06$, $P = 0.008$; nurse: $F_{27,26} = 4.25$, $P < 0.0001$), but no indication of genotype by environment interactions (mass: $F_{26,58} = 0.82$, $P = 0.71$; size: $F_{26,58} = 1.03$, $P = 0.44$; see Figure 2.1). The heritability of nestling growth, calculated as the proportion of total phenotypic variance within dyads (V_{PO}) that was due to direct additive genetic variance (V_{AO}), was low for body mass (0.09 ± 0.04) and moderate for body size (0.33 ± 0.28; see Table 2.1).

The cross-fostering design revealed that 81% of the total phenotypic variation in growth in body mass was due to maternal performance (see Figure 2.1), which is the sum of both genetic (V_{AM}) and environmental (V_{EM}) maternal effects. Both litter size at birth and parturition date were related negatively to maternal performance; females who gave birth to fewer offspring earlier in the season raised faster growing offspring. These two maternal characters together explained 69% of the variation in maternal performance (paired multiple regression; $R^2 = 0.69$, $n = 20$, $P < 0.0001$). The heritabilities of litter size and parturition date, as calculated by the DFREML model, were 0.15 ± 0.04 and 0.16 ± 0.03, respectively (Réale *et al.* 2003). If we assume that the remaining 30% of unexplained variation in maternal performance has no genetic basis, we can estimate the heritability of maternal performance (h_m^2) conservatively as 0.11 (see Table 2.2). The cross-fostering design also revealed a large positive $Cov\ (A_O, A_M)$ for growth in body mass (0.02 ± 0.04), suggesting that genes for fast offspring growth in body mass were associated positively with genes for maternal performance leading to fast offspring growth (smaller litter sizes and earlier parturition dates).

The total heritability (h_t^2) of growth in body mass including indirect effects was calculated as 0.31 ± 0.33. This value represents a greater than three-fold increase in the potential response to a given amount of selection relative to that predicted by direct genetic effects alone (0.09).

For growth in body size, 43% of the total phenotypic variation within dyads was due to maternal effects. Litter size and parturition date remained the best predictors of maternal performance and explained 60% of the variation in maternal performance for growth in body size ($R^2 = 0.60$, $n = 20$, $P < 0.001$), but the heritability of maternal performance for growth in body size dropped slightly to 0.10. In addition, the $Cov\ (A_O, A_M)$ of growth in body size was very small ($2.2 \times 10^{-8} \pm 190.0 \times 10^{-8}$). As a result, direct genetic effects alone (0.33) adequately estimated the total heritability of growth in body size (0.35 ± 0.51).

I examined the magnitude of direct-maternal environmental and dominance covariances as possible sources of bias in my $Cov\ (A_O, A_M)$ estimate for growth in body mass. The difference between the mother-offspring covariance (0.033) and twice the maternal grandmother-grandoffspring covariance (0.083) was substantially negative (-0.05), suggesting that combined effects of $Cov\ (E_O, E_M)$ and $Cov\ (D_O, D_M)$ were negative and not positive. Environmental covariances, therefore, did not inflate my estimate of the direct-maternal genetic covariance.

The effective partitioning of variance in growth in body mass allowed me to estimate maximum levels of paternity and dominance variance. Values of $r < 0.29$ or $V_{DO} > 0.024$ resulted in negative values of e and are, therefore, not possible.

2.4 DISCUSSION

In this chapter, I quantified the contribution of both direct and indirect genetic effects to the potential for evolution in a natural population of animals. The heritability of nestling growth was low for body mass (0.09) and moderate for body size (0.33), but both estimates were similar to previously reported values for life history traits (Mousseau & Roff 1987). Growth in body size, but particularly growth in body mass were subject to large maternal effects, which accounted for over 80% of the total phenotypic variation in growth in body mass. These large maternal effects were correlated with litter size and parturition date, which were themselves heritable ($h^2 = 0.15$ and 0.16, respectively; Réale *et al. in press*). The combination of these two maternal traits resulted in a heritability of maternal performance (0.11), which is lower than most previous estimates of h^2_m from laboratory animals (see Cheverud 1984), but provides evidence for a potential indirect contribution of maternal performance to the evolution of offspring traits in a natural population of animals. The consideration of both direct and indirect genetic effects revealed a greater than three-fold increase in the potential for evolution of growth in body mass relative to that predicted by direct genetic effects alone. As with previous heritability estimates for single traits, this represents the potential response to selection, which may not be realized if there is opposing selection on either genetically correlated offspring traits or maternal traits such as litter size and parturition date.

The large increase in the potential for evolution of body mass was the result of both a heritable basis to maternal performance and a large positive $Cov\ (A_O, A_M)$ (13% of V_{PO}) that was greater than most previous estimates for mice in the laboratory (Riska *et al.* 1985, Moore *et al.* 1970, Cheverud 1984). This covariance corresponds to a direct-maternal genetic correlation that is greater than one (1.39) under the assumptions of single paternity and negligible dominance variance, but which is less than one for several possible values of these variables. There was no evidence to suggest that my estimate of $Cov\ (A_O, A_M)$ was inflated by either persistent maternal effects or maternal-offspring environmental covariances. Prenatal maternal effects lasting to 25 days of age could have confounded my estimate of additive genetic variation and $Cov\ (A_O, A_M)$, if prenatal and postnatal maternal effects were correlated positively. Prenatal maternal effects on postnatal growth rates in laboratory rodents are often thought to be relatively small (Roff

1997, Riska *et al.* 1984, but see Desai & Hales 1997, Rhees *et al.* 1999) and independent of postnatal maternal effects (Rhees *et al.* 1999), but their influence in natural populations is not known. My analysis of the residuals of the relationship between growth in body mass and the initial weight measurement should have controlled for genetic and maternal effects acting prior to cross-fostering.

Estimates of V_{AO} depend on the degree of relatedness among siblings and in cross-fostering experiments the dam term includes ¼ of the dominance variance (V_{DO}; Riska *et al.* 1985). The effective partitioning of phenotypic variation by the cross-fostering design, however, allowed me to infer minimum levels of relatedness among siblings and the maximum amount of V_{DO} for growth in body mass in this population of squirrels. The small amount of error variance in growth in body mass (see Figure 2.1) allowed me to estimate the minimum relatedness among siblings as 0.29, which suggests that complete multiple paternity within litters is not common in this population. This contrasts with behavioural observations of female red squirrels mating with many males (range = 4 to 16, mean = 7.4, *n* = 16; Currie and Boutin, unpublished data). The maximum level of dominance variance in growth in body mass was estimated as 0.024, or 16% of the total phenotypic variation. My estimates of V_{AO}, therefore, were not biased substantially by V_{DO}, and dominance variance does not appear to be a likely mechanism by which genetic variation in growth in body mass of red squirrels can be maintained in the presence of strong selection (Crnokrak & Roff 1995).

A common extension of Fisher's fundamental theorem of natural selection (Fisher 1930) suggests that traits more closely associated with fitness will have lower heritabilities than traits more distantly related to fitness (Kruuk *et al.* 2000, Mousseau &

Roff 1987, Merilä & Sheldon 2000) given similar genetic architecture (Merilä & Sheldon 1999). The associations between fitness and growth in body size and growth in body mass in red squirrels are not known, but the consideration of indirect genetic effects eliminated what originally appeared to be a very large difference between the simple heritabilities (h^2) of these two traits. These data suggest that the relationship between the heritabilities of traits and their association with fitness can depend on whether indirect genetic effects are considered.

Positive covariances between direct and indirect genetic effects may arise from selection for offspring phenotypes that correspond to the environment that their mother can provide (Wade 1998). Positive correlations between traits subject to the same directional selection, however, are thought to be fixed rapidly so negative correlations should predominate (Roff 1997). In fact, most previous estimates of $Cov\ (A_O, A_M)$ from captive animals raised in controlled environments have been negative (Roff 1997, Cheverud 1984). The large positive $Cov\ (A_O, A_M)$ and the small amount of dominance variation reported here for growth in body mass, suggest that this trait may be particularly susceptible to allelic fixation under consistent directional selection. The occurrence of reasonable amounts of direct and indirect additive genetic variation suggest that selection on growth in body mass may instead vary spatially or temporally in this population of red squirrels. Periodic fluctuations in spruce cone production (Humphries & Boutin 2000) and the strong philopatry of red squirrels (Larsen & Boutin 1994) provide an ecological framework within which a positive $Cov\ (A_O, A_M)$ could be maintained through temporal or spatial variation in food resources.

The ability to predict evolutionary responses to selection and to infer previous levels of selection depends on the quantification of all sources of additive genetic variation. My results clearly demonstrate the importance of indirect genetic effects on potential evolutionary trajectories in a natural population of animals. Furthermore, positive relationships between direct and indirect sources of genetic variation, which are maintained in inherently variable natural systems, may not be represented adequately by estimates derived from captive animals under controlled conditions.

2.5 REFERENCES

Ahlschwede, W. T. & Robison, O. W. 1971. Prenatal and postnatal influences on growth and backfat in swine. *Journal of Animal Science,* **32**: 10-16.

Becker, C. D. 1992. Proximate factors influencing the timing and occurrence of reproduction in red squirrels (*Tamiasciurus hudsonicus*). Ph.D. dissertation, University of Alberta, Edmonton, AB.

Berteaux, D. and S. Boutin. 2000. Breeding dispersal in female North American red squirrels. *Ecology,* **81**: 1311-1326.

Boutin, S., Larsen, K. W. & Berteaux, D. 2000. Anticipatory parental care: acquiring resources for offspring prior to conception. *Proceedings of the Royal Society of London, Series B,* **267**: 2081-2085.

Byers, D. L., Platenkamp, G. A. J. & Shaw, R. G. 1997. Variation in seed characters in *Nemophila menziesii* : evidence of a genetic basis for maternal effects. *Evolution,* **51**: 1445-1456.

Cheverud, J. M. 1984. Evolution by kin selection: a quantitative genetic model illustrated by maternal performance in mice. *Evolution,* **38**: 766-777.

Crnokrak, P. & Roff, D. A. 1995. Dominance variance: associations with selection and fitness. *Heredity,* **75**: 530-540.

Desai, M. & Hales, C. N. 1997. Role of fetal and infant growth in programming metabolism in later life. *Biological Reviews,* **72**: 329-348.

Dickerson, G. E. 1947. Composition of hog carcasses as influenced by heritable differences in rate and economy of gain. *Research Bulletin Iowa Agricultural Experiment Station,* **354**: 489-524.

Fisher, R. A. 1930. *The Genetical Theory of Natural Selection.* Clarendon Press, Oxford.

Hatt, R. T. 1929. The red squirrel: its life history and habits, with special reference to the Adirondacks of New York and the Harvard Forest. *Roosevelt Wildlife Annals,* **2**: 11-146.

Hoffmann, A. A. 2000. Laboratory and field heritabilities: some lessons from *Drosophila.* in Mousseau, T. A., B. Sinervo and J. Endler (eds) *Adaptive Genetic Variation in the Wild.* Oxford University Press, New York.

Humphries, M. M. & Boutin, S. 2000. The determinants of optimal litter size in free-ranging red squirrels. *Ecology,* **81**: 2867-2877.

Kaufmann, K. W. 1981. Fitting and using growth curves. *Oecologia,* **49**: 293-299.

Kruuk, L. E. B., Clutton-Brock, T. H., Slate, J., Pemberton, J. M., Brotherstone, S. &

Guinness, F. E. 2000. Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Science, USA,* **97**: 698-703.

Larsen, K. W. & Boutin, S. 1994. Movements, survival, and settlement of red squirrel (*Tamiasciurus hudsonicus*) offspring. *Ecology,* **75**: 214-223.

Legendre, P. and L. Legendre. 1998. *Numerical Ecology.* Elsevier, Amsterdam.

Lynch, M. and B. Walsh. 1998. *Genetics and Analysis of Quantitative Traits.* Sinauer Associates, Sunderland, Massachusetts.

Mathsoft. 1999a. *S-PLUS 2000 Guide to Statistics, Volume 1.* Data Analysis Products Division, Mathsoft, Seattle, WA.

Mathsoft. 1999b. *S-PLUS 2000 User's Guide.* Data Analysis Products Division, Mathsoft, Seattle, WA.

Merilä, J., Kruuk, L. E. B. & Sheldon, B. C. 2001. Natural selection on the genetical component of variance in body condition in a wild bird population. *Journal of Evolutionary Biology,* **14**: 918-929.

Merilä, J. & Sheldon, B. C. 1999. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity,* **83**: 103-109.

Merilä, J. & Sheldon, B. C. 2000. Lifetime reproductive success and heritability in nature. *American Naturalist,* **155**: 301-310.

Meyer, K. 1989. Restricted maximum likelihood to estimate variance components for animal models with several random effects using a derivative-free algorithm.

Genetics Selection and Evolution, **21**: 317-340.

Moore, R. W., Eisen, E. J. & Ulberg, L. C. 1970. Prenatal and postnatal maternal influences on growth in mice selected for body weight. *Genetics,* **64**: 59-68.

Mousseau, T. A. and C. W. Fox. 1998. *Maternal Effects as Adaptations.* Oxford University Press, New York.

Mousseau, T. A. & Roff, D. A. 1987. Natural selection and the heritability of fitness components. *Heredity,* **59**: 181-197.

Price, T. 1998. Maternal and paternal effects in birds. in Mousseau, T. A. and C. W. Fox (eds) *Maternal Effects as Adaptations.* Oxford University Press, New York.

Rauter, C. M. & Moore, A. J. 2002. Evolutionary importance of parental care performance, food resources, and direct and indirect genetic effects in a burying beetle. *Journal of Evolutionary Biology,* **15**: 407-417.

Réale, D., Berteaux, D., McAdam, A. G. & Boutin, S. *in press.* Lifetime selection on heritable life history traits in a natural population of red squirrels. *Evolution.*

Rhees, B. K., Ernst, C. A., Miao, C. H. & Atchley, W. R. 1999. Uterine and postnatal maternal effects in mice selected for differential rate of early development. *Genetics,* **153**: 905-917.

Riska, B., Atchley, W. R. & Rutledge, J. J. 1984. A genetic analysis of targeted growth in mice. *Genetics,* **107**: 79-101.

Riska, B., Rutledge, J. J. & Atchley, W. R. 1985. Covariance between direct and maternal genetic effects in mice, with a model of persistent environmental influences. *Genetical Research,* **45**: 287-297.

Roff, D. A. 1997. *Evolutionary Quantitative Genetics.* Chapman & Hall, New York.

Rutledge, J. J., Robinson, O. W., Eisen, E. J. & Legates, J. E. 1972. Dynamics of genetic and maternal effects in mice. *Journal of Animal Science,* **35**: 911-918.

Schluter, D. & Gustafsson, L. 1993. Maternal inheritance of condition and clutch size in the collared flycatcher. *Evolution,* **47**: 658-667.

Shaw, R. G. and D. L. Byers. 1998. Genetics of maternal and paternal effects. in Mousseau, T. A. and C. W. Fox (eds) *Maternal Effects as Adaptations.* Oxford University Press, New York.

Smith, C. C. 1968. The adaptive nature of social organization in the genus of tree squirrels *Tamiasciurus. Ecological Monographs,* **38**: 31-63.

Thiede, D. A. 1998. Maternal inheritance and its effect on adaptive evolution: a quantitative genetic analysis of maternal effects in a natural plant population. *Evolution,* **52**: 998-1015.

Wade, M. J. 1998. The evolutionary genetics of maternal effects in Mousseau, T. A. and C. W. Fox (eds) *Maternal Effects as Adaptations.* Oxford University Press, New York.

Weigensberg, I. & Roff, D. A. 1996. Natural heritabilities: can they be reliably estimated in the laboratory? *Evolution,* **50**: 2149-2157.

Willham, R. L. 1963. The covariance between relatives for characters composed of components contributed by related individuals. *Biometrics,* **19**: 18-27.

Wolf, J. B., Brodie, E. D., III, Cheverud, J. M., Moore, A. J. & Wade, M. J. 1998. Evolutionary consequences of indirect genetic effects. *Trends in Ecology and Evolution,* **13**: 64-69.

TABLE 2.1. The potential for evolution and sources of variation in growth in body mass and growth in body size of nestling red squirrels (± SE) based on direct genetic effects alone (h^2) and including maternal genetic effects (h_t^2). Sources of variation for growth in body mass and size are reported as $(g/day)^2$ and x 10^{-6} $(1/day)^2$, respectively. V_{AM} was calculated as the product of the phenotypic variance due to maternal performance from the cross-fostering experiment (V_M) and the heritability of maternal performance (h^2_m; see Table 2.2). h^2_t was calculated following Dickerson (1947).

	Growth in Body Mass	Growth in Body Size
Total phenotypic variance (V_{PO})	0.171 ± 0.066	5.34 ± 2.80
Direct effects		
Direct genetic variance (V_{AO})	0.016 ± 0.005	1.77 ± 0.76
Heritability (h^2)	0.09 ± 0.04	0.33 ± 0.28
Maternal effects		
Maternal variance (V_M)	0.123 ± 0.036	2.30 ± 1.13
Maternal genetic variance (V_{AM})	0.013 ± 0.004	0.21 ± 0.10
Direct-Maternal Genetic Covariance [$Cov(A_O, A_M)$]	0.020 ± 0.039	0.02 ± 1.89
Total heritability (h_t^2)	0.31 ± 0.33	0.35 ± 0.51

TABLE 2.2. Maternal characters as components of maternal performance for growth in body mass. The heritability of maternal performance (h^2_m) was calculated as the sum of maternal character heritabilities weighted by their relative contribution to maternal performance. Heritabilities (h_i^2) were estimated from an eleven-year pedigree for this population (1988-1998) using DFREML (Réale *et al. in press*). Unexplained variation in maternal performance is assumed to have a heritability of zero.

Maternal characters (*i*)	R^2_i	h^2_i	$R^2_i\, h^2_i$
Litter Size	0.43	0.16	0.07
Parturition date	0.26	0.15	0.04
Unexplained	0.30	0.00^3	0.00
Total	1.00		$h_m^2 = 0.11$

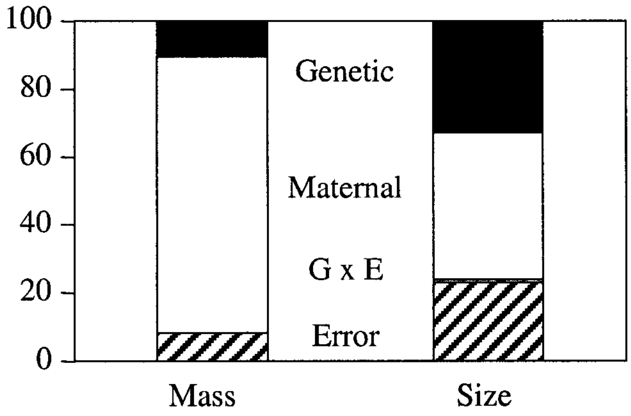

FIGURE 2.1. Sources of variation as percentages of phenotypic variation in growth in body mass and body size in cross-fostered red squirrels. The black, white, grey and hatched areas within each bar represent the percentage of total phenotypic variation within pairs that was due to genetic (V_{AO} assuming single paternity), maternal (V_M), interaction (genotype by environment) and error variances, respectively. Total phenotypic variation in growth of body mass and body size were 0.151 (g/day)^2 and 5.32 x 10^{-6} (1/day)^2, respectively.

3.0 EFFECTS OF FOOD ABUNDANCE ON GENETIC AND MATERNAL VARIATION.[2]

3.1 INTRODUCTION

The heritable transmission of trait variation from parents to offspring is a necessary condition for evolution by natural selection. As a result, one of the central goals of quantitative genetics has been to determine the degree to which trait variation is determined by genetic and environmental sources. The narrow-sense heritability (h^2), defined as the proportion of total phenotypic variation (V_P) that is due to additive genetic variation (V_{AO}), provides a useful estimate of the potential for a population to respond to selection. Heritability estimates, however, are characteristic only of the particular circumstances under which they are measured and as such can vary both among populations and over time within a given population as a result of selection history or in response to changes in environmental conditions (Falconer & Mackay 1996). The inherent temporal and spatial variability in food resources, competitors and predators in natural systems may make heritability estimates from natural populations particularly susceptible to change.

Since heritabilities are ratios, differences in heritability estimates between environments can be due to changes in additive genetic variation, environmental variation or both. Several hypotheses have been proposed to explain how sources of trait variation should respond to different environmental conditions (see Hoffmann & Merilä 1999 for a review). For example, selection is thought to eliminate low fitness alleles more

---

[2] A version of this chapter has been accepted for publication in the *Journal of Evolutionary Biology*.

efficiently in common favourable environments, suggesting that less frequent unfavourable conditions should maintain higher levels of genetic variation (Holloway *et al.* 1990). Falconer (1952) recognised that traits expressed in different environments could be considered separate traits linked by a genetic correlation across environments. In the presence of a genetic correlation across environments, selection in one environment will result in both a correlated response of the trait mean and a corresponding elimination of low fitness alleles in the alternate environment. As a result, the opportunity for both independent adaptation and differences in genetic variance between two environments is influenced by the strength of the genetic correlation across environments. Differences in genetic variation between environments due to differential selection history, therefore, are expected to be associated with weak genetic correlations across environments (Hoffmann & Merilä 1999).

Most of the hypotheses reviewed by Hoffmann & Merilä (1999) concern the effects of environmental conditions on direct genetic and residual variation. However, maternal effects are now widely recognised as important components of total phenotypic variation (Mousseau & Fox 1998), particularly in mammals and other organisms with long periods of maternal dependence (Roff 1997). Maternal effects are experienced as an environmental effect by offspring, but like any other trait they are composed of both genetic and environmental components (e.g., Dickerson 1947, Wolf *et al.* 1998). As a result, indirect maternal genetic effects can have important influences on the potential evolutionary response to selection (Wolf *et al.* 1998, McAdam *et al.* 2002, Chapter 2). Responses of maternal effect variation to environmental conditions, therefore, may exacerbate or compensate for differences in direct genetic variation between favourable and unfavourable environments. Some of the same hypotheses that have been proposed regarding the expression of genetic variation in other traits (Hoffmann & Merilä 1999) can also be applied to maternal effects. However, since maternal effects are sheltered from selection on males, they are expected to maintain higher levels of additive genetic variation in the presence of selection than direct genetic effects (Wade 1998). Differences in selection history between favourable and unfavourable environments should, therefore, lead to greater differences in direct genetic variation than maternal genetic variation.

Generalisations regarding the effects of environmental quality on heritable variation in natural populations have been slow to emerge. For example, there has been a general trend towards decreased heritability of size-related traits in natural populations of birds experiencing unfavourable conditions (see Merilä 1997, Hoffmann & Merilä 1999). This was presumed to be due to decreased additive genetic variation and increased residual variation in stressful conditions although common environmental effects or genetic correlations across environments may have influenced these results (Hoffmann & Merilä 1999). However, there are many other studies that have demonstrated an increase in genetic variation or no consistent response across traits in unfavourable conditions (e.g., Gebhardt-Henrich & van Noordwijk 1991, Merilä *et al.* 1999, Coltman *et al.* 2001, Kause & Morin 2001, see also Hoffmann & Merilä 1999). Empirical generalisations may be hindered by ambiguity in the specific conditions under which variance components were measured (Hoffmann & Merilä 1999). If predictions of the various hypotheses are to be adequately assessed across systems, authors must be clear in their definition of favourable and unfavourable conditions. These terms have been used to differentiate effects on phenotypes, the rarity of these conditions as well as the expected strength of selection. A comprehensive understanding of the natural history of an organism will, therefore, be needed to properly define favourable and unfavourable conditions and interpret quantitative genetic comparisons across environments.

North American red squirrels (*Tamiasciurus hudsonicus*) provide a unique mammalian system within which the effects of environmental variability on sources of variation can be examined. In the northern parts of their geographic distribution, red squirrels feed almost exclusively on the seeds of white spruce cones (*Picea glauca*). The production of cones by white spruce trees is spatially synchronous but temporally variable. Annual variation in the production of spruce cones in the southwest Yukon spans three orders of magnitude (McAdam & Boutin *in press*; Chapter 4). The abundance of spruce cones, therefore, provides a relatively simple and repeatable measure of environmental quality that has been found to have important implications for the life history of red squirrels (Berteaux & Boutin 2000, Humphries & Boutin 2000, Réale *et al.* 2003). Furthermore, the combination of existing long-term data for a population in the southwest Yukon, Canada and the ability to cross-foster newborn red squirrels provide valuable tools for the quantification of sources of variation in life history traits (McAdam *et al.* 2002, Réale *et al.* 2003, Réale *et al. in press*, Chapter 2).

In this study I used 16 years of data (1987 – 2002) for a single population of red squirrels to examine the effects of environmental conditions on mother-offspring resemblance in juvenile growth in body mass using mother-offspring regressions. Conditions for growth were defined based on the availability of spruce cones and were confirmed by differences in trait means. I estimated mother-offspring covariances in each of 4 combinations of good and poor maternal and offspring environments for growth, which allowed me to compare environmental effects on the resemblance of mothers and offspring raised in the same conditions and to estimate the genetic correlation of growth in body mass between good and poor environments.

Mother-offspring covariance estimates, however, are confounded by maternal effects (Lynch & Walsh 1998), which are known to account for a large proportion of total phenotypic variation in growth in body mass (McAdam *et al.* 2002, Chapter 2). As such, differences in mother-offspring resemblance between good and poor environments may reflect changes in direct additive genetic variance, maternal genetic variance or both. To further investigate changes in these components of variation in response to food availability, I performed cross-fostering experiments in both 1999 and 2000. The breeding seasons of 1999 and 2000 followed the highest and one of the lowest years of spruce cone production in this study, respectively. As a result, data from 1999 corresponded to very good conditions while 2000 corresponded to poor conditions for juvenile growth. Finally, I performed a food supplementation experiment in 2000, in which some females were supplied with ad lib access to sunflower seeds to experimentally improve the conditions for growth. Sources of variation including direct genetic, maternal, genotype by environment and residual variation were examined in both the natural environmental conditions as well as the experimental environment to determine the effects of food availability on sources of variation in nestling growth rates.

3.2 METHODS

A natural population of red squirrels (*Tamiasciurus hudsonicus*) has been monitored in the southwest Yukon (61 °N, 138 °W) since 1987. This population of squirrels has been extensively studied and details on the population and general live-trapping and census techniques are given elsewhere (Berteaux & Boutin 2000, Humphries & Boutin 2000). In this study I was interested in the growth of body mass from soon after birth to approximately one month of age. In most cases, natal nests were located soon after birth and offspring were briefly removed from the nest and weighed to the nearest gram using a Pesola spring balance. Second mass measurements were taken in a similar manner just prior to first emergence from the natal nest (approximately 30 days of age) when offspring were permanently identified with metal ear tags. Over the 16 years of this study there were several cases in which mass measurements were missed or were not taken at the appropriate time. As a result, I restricted the data to include only offspring with initial mass measurements less than 50g, final mass measurements less than 100g, and more than 5 days between first and second mass measurements. Offspring subjected to food or litter size manipulations were excluded from the analysis.

3.2.1 *Conditions for growth*

Offspring were classified as having been raised in good or poor conditions for growth based on the availability of spruce cones. The number of spruce cones in the top 3 m (viewed from one side) of each of approximately 250 trees spaced systematically throughout the study areas has been recorded each year since 1986. The average number of cones counted per tree within a year provides an index (*cone index*) of the availability of food for that year. Annual cone counts vary over three orders of magnitude (1's, 10's or 100's of cones counted per tree) and so it is often useful to log transform these annual indices prior to analysis (*log cone index*).

Mature spruce cones are harvested in late summer and early fall by red squirrels and are stored in centrally located caches of cones (middens) for future consumption. Red squirrels depend on stored cones for survival over winter and for reproduction the following spring. The availability of cones produced in the previous fall has been correlated with variation in several life history traits (Berteaux & Boutin 2000, Humphries & Boutin 2000, Réale *et al.* 2003), including the growth in body mass of offspring born in the first litters of the season (McAdam & Boutin *in press*, Chapter 4). However, in addition to caching current year's cones, squirrels also consume new cones and new cones may help to meet current reproductive demands late in the breeding season.

3.2.2 *Cone effects on growth*

I briefly investigated the influence of current year's cone production on the growth rates of juvenile red squirrels. The growth in body mass of 1912 juveniles born between 1987 and 2002 were correlated with both the abundance of spruce cones produced in the previous fall and the abundance of cones produced in the current year using multiple regression techniques. In order to determine how the partial correlation coefficients for previous and current year's cone production changed across the breeding season, I repeated this overall analysis with progressively smaller subsets of the data corresponding to offspring born later in the breeding season. Specifically, each repetition of the analysis excluded an additional 10 days of the breeding season starting with the earliest born offspring; those born prior to March 22nd. Years in which the cone index was greater than 50 were classified as being good years (1992, 1993, 1995, 1996 and 1998), while those with a cones index less than 50 were classified as poor years (1987, 1988, 1989, 1990, 1991, 1994, 1997, 1999, 2000, 2001 and 2002).

3.2.3 *Mother-offspring covariances*

Mother-offspring covariances were estimated separately for each of the four possible combinations of environmental conditions for mothers (good or poor) and offspring (good or poor). The growth rates of all offspring born to each dam were averaged within each environmental category so that each female and her offspring contributed only one data point to each covariance estimate. The genetic correlations across environments (r_x) was, therefore, calculated as

$$r_x = (cov_{gp} + cov_{pg})/2\sqrt{[(cov_{gg})(cov_{pp})]},$$

where cov_{ij} represents the mother-offspring covariance for offspring raised in environment i (either good g or poor p) born to mothers that were raised in environment j (Falconer & Mackay 1996, Roff 1997). There were no differences between mother and offspring variances in any of the four environment combinations ($F < 1.6$, $P > 0.20$). In addition to estimating additive genetic effects (1/2 V_{AO}), mother-offspring covariance estimates are also confounded by maternal effects (Lynch & Walsh 1998). The relative contribution of direct genetic effects and maternal effects to differences in mother-offspring covariances between good and poor environments was explored further using cross-fostering experiments.

3.2.4 *Cross-fostering experiments*

Cross-fostering experiments were performed in 1999 and 2000 to partition direct and maternal components of variation in growth in body mass in both good and poor conditions for growth. In 1998, spruce trees in this area produced more cones than in any of the past 13 years (mean *cone index* = 286.7 ± 17.2, n = 248 trees; see also McAdam & Boutin *in press*, Chapter 4). As a result, conditions for growth of juveniles born in the spring of 1999 were very good. Conversely, very few cones were produced in the fall of 1999 (*cone index* = 27.6 ± 3.9, n = 248 trees), so conditions for growth in the spring of 2000 were poor.

All females in each of the study areas (approximately 40 ha each) were monitored for reproductive activity through regular live-trapping starting early in the spring of 1999 and 2000. Changes in female body mass and external morphology allowed me to precisely estimate the timing of parturition. Litters of squirrels were paired as closely as possible based on the timing of parturition and offspring were reciprocally cross-fostered between paired litters such that half the offspring from one litter were exchanged with an equal number of offspring in the paired litter. As a result, natal litter sizes were not changed by the cross-fostering technique. Females appear not to discriminate between genetic and foster offspring and there do not appear to be any detrimental effects of the cross-fostering technique on offspring growth rates (Humphries & Boutin 1996, McAdam *et al.* 2002, Chapter 2). All measurements of offspring from the cross-fostering experiments were made on the same day within pairs.

Seven of the 29 pairs of litters cross-fostered in 2000 were supplemented with food to experimentally improve conditions for growth within the 2000 breeding season. Pairs of cross-fostered litters from two of the four study areas were randomly assigned to either control or food supplemented treatments. The other two areas could not be supplemented with food because of the ongoing long-term study of these populations, but there do not appear to be any differences between these pairs of study areas that would have biased the results of this study. Females within food-supplemented pairs received ad lib access to sunflower seeds (*Helianthus* sp.) from the day on which offspring were cross-fostered until the time when the second mass measurements were collected. Sunflower seeds were provided in a single plastic feeder located on the central midden in each supplemented female's territory. Feeders were checked and replenished with new seed regularly to ensure that supplemented females had ad lib access to seeds.

3.2.5 *Growth measurement in 1999 and 2000*

In 1999 and 2000, I examined the growth in body mass of red squirrels from soon after birth (3.07 ± 0.07 days) to approximately 25 days of age. Body mass was measured (± 0.1 g) using portable electronic balances. Growth in body mass over this time period is linear. Previous analyses (McAdam *et al.* 2002, Chapter 2) have corrected these linear growth measurements for correlations with the initial mass measurement. While this correction eliminates potentially confounding effects of persistent prenatal maternal effects on postnatal growth it may also result in overly conservative estimates of direct genetic variation if prenatal and postnatal growth rates are genetically correlated. I feel that the likelihood of a positive genetic correlation between prenatal and postnatal growth is higher than that of persistent prenatal maternal effects and so I have not corrected linear growth rates for initial mass measurements in this study. As such, the results presented in this study will differ slightly from those previously reported (McAdam *et al.* 2002, Chapter 2).

3.2.6 *Statistical analysis*

Variation among nestling growth rates was partitioned using a two-way nested ANOVA where dam (*D*) and nurse (*N*) effects and their interaction (*DN*) were all nested within cross-fostered pairs (see Chapter 2 for details). Variances were estimated from these linear models using restricted maximum likelihood (REML) in the VARCOMP procedure in S-PLUS (Insightful 2001a). Genetic expectations of variance components from the two-way nested ANOVA above following Willham (1963) are:

$$\sigma^2_D = 1/2V_{AO} + 1/4V_{DO}$$

$$\sigma^2_N = V_{AM} + V_{DM} + V_{EM}$$

$$\sigma^2_{DN} = 0$$

$$\sigma^2_e = 1/2V_{AO} + 3/4V_{DO} + V_E.$$

I assumed that offspring within genetic litters were fullsibs (see also McAdam *et al.* 2002, Chapter 2). Twice the dam variance, therefore, estimates the direct genetic variance (V_{AO}), but also includes dominance variance (½ V_{DO}). I have previously estimated V_{DO} to be low for this trait (McAdam *et al.* 2002, Chapter 2) and do not further consider the potential confounding effects of V_{DO} here. Maternal variance represents the sum of maternal genetic (V_{AM}), dominance (V_{DM}) and environmental (V_{EM}) variances.

The direct-indirect genetic covariance [$cov\ (A_O, A_M)$] was estimated from two separate analyses as the difference between the among litter variance of offspring who remained in their natal nest and the among litter variance of cross-fostered offspring (Riska *et al.* 1985). In addition, I calculated total phenotypic variance (V_P) as the total variance within cross-fostered pairs ($\sigma^2_D + \sigma^2_N + \sigma^2_{DN} + \sigma^2_e$) plus $cov(A_O, A_M)$ following Riska *et al.* (1985).

Standard errors for the variance components were estimated by jackknifing at the level of the cross-fostered pair. Variance components were standardised as coefficients of variation (*CV*) for comparisons across environments to avoid bias due to changes in mean growth rates ($CV = sd\ /\ mean$).

3.2.7 *Components of maternal performance for growth*

I attempted to decompose maternal effects on offspring growth into the contributions of various maternal characteristics. Since offspring were distributed roughly evenly between foster mothers within a cross-fostered pair, I used the difference between pairs of mothers in the average growth rate of all offspring raised (foster and genetic) as a measure of maternal performance for offspring growth independent of direct genetic effects (see also McAdam *et al.* 2002, Chapter 2). I then attempted to correlate differences in various maternal characteristics between mothers within a pair with differences in maternal performance for offspring growth. The maternal characteristics examined here were: litter size at birth, parturition date, body size, territory size, age, maternal mass at parturition and offspring emergence, and the azimuth of the natal nest. Litter size and parturition date were found to be significant predictors of maternal performance for offspring growth in 1999 (McAdam *et al.* 2002, Chapter 2). In this study I was interested in determining whether components of maternal performance differed between good and poor years for offspring growth. As a result, I analysed maternal performance data for both 1999 and 2000 in a single general linear model. In addition to the maternal characteristics listed above, I also included their interactions with *Year* to test for any differences between good and poor years for growth. I started with a simple model that included only litter size and parturition date and attempted to add additional characteristics and interaction variables. Terms were added sequentially to the model based on changes in the likelihood version of Mallows' *Cp* statistic (Insightful 2001a).

All statistical analyses were performed using STATISTICA (StatSoft Inc. 2000) and SPLUS 6.1 (Insightful 2001b, Insightful 2001a). Values are presented as means ± one standard error.

3.3 RESULTS

There was a strong positive correlation between the rate of nestling growth in body mass and the *log cone index* in the previous fall (slope = 0.261 ± 0.015). The partial correlation between growth and previous year's cone production, however, declined as the season progressed, while the partial correlation between growth rates and the current year's cone production increased (Figure 3.1). In particular, for offspring born after May 30th (Julian date = 150), current year's cone production was a better predictor of juvenile growth rates than previous year's cone production. As a result, I considered the environment for growth to be determined by previous year's cone production for those offspring born on or before May 30th and by current year's cone production for those offspring born after May 30th.

3.3.1 *Mother-offspring covariances*

Average growth rate of juveniles raised in good years (1.91 ± 0.015 g/day) was significantly higher than the average rate of growth in body mass of juveniles raised in poor years (1.66 ± 0.017 g/day; $t_{1910} = 10.87$, $P < 0.0001$; Figure 3.2). There was a significant positive covariance between the growth rate of mothers and their offspring in good years [$cov(O_{good},M_{good}) = 0.061 \pm 0.022$, $t_{85} = 2.77$, $P = 0.007$; Table 3.1]. The covariance between offspring and maternal growth rates in poor environments [$cov(O_{poor},M_{poor}) = 0.113 \pm 0.074$; Table 3.1] was more than 80% greater than in good environments but was not significant as a result of the smaller sample size ($t_{28} = 1.53$, $P = 0.14$). Mother-offspring covariances measured across environments were much lower and were not significantly different from zero [$cov(O_{poor},M_{good}) = 0.038 \pm 0.033$, $t_{37} = 1.15$, $P = 0.26$; $cov(O_{good},M_{poor}) = - 0.014 \pm 0.033$, $t_{41} = 0.42$, $P = 0.68$; Table 3.1]. As a result, the genetic correlation across environments was calculated as 0.143 ± 0.281 and was not significantly different from zero ($t_{154} = 0.51$, $P = 0.61$), but was significantly lower than one ($t_{154} = 3.05$, $P = 0.003$).

3.3.2 *Cross-fostering*

The rate of growth in body mass of nestling red squirrels was significantly slower in 2000 (1.66 ± 0.05 g/day) than 1999 (1.91 ± 0.03 g/day, $t_{246} = 4.71$, $P < 0.0001$, Figure 3.2). Growth rates in 1999 and 2000 were typical of growth rates recorded in this population over the past 16 years in good (1.91 ± 0.02 g/day) and poor (1.66 ± 0.02 g/day) years for growth, respectively (Figure 3.2). In addition, survival of offspring from birth to the time of the second set of measurements was much higher in 1999 than 2000. In 1999, 177 of the 201 (88%) cross-fostered offspring survived to roughly one month of age, while in 2000 only 72 of 122 (59%) cross-fostered offspring that did not receive the food supplement were alive at the time of the second set of measurements.

Squirrels that were supplemented with food in 2000 had significantly higher rates of growth in body mass (1.98 ± 0.05 g/day) than controls within the 2000 season ($t_{102} = 4.06$, $P < 0.0001$) and were not significantly different from growth rates in 1999 ($t_{206} = 1.14$, $P = 0.26$, Figure 3.2). Food supplementation also improved juvenile survival in 2000. Thirty-two of the 41 (78%) offspring whose mothers were supplemented with food in 2000 survived to one month of age.

3.3.3 *Sources of variation in growth*

The cross-fostering design revealed significant dam and nurse effects on growth in body mass in 1999 (Table 3.2). In 2000, there was a significant nurse effect but the dam effect was no longer significant. The dam by nurse interaction was not significant in either 1999 or 2000. The coefficient of total phenotypic variation for growth in body mass was 26% higher in poor conditions for growth (2000) than in high food conditions

(1999; Figure 3.3). This resulted from a large increase in the coefficient of direct additive genetic variation (26%) and a smaller increase in maternal variation and interaction variation. Residual variation decreased slightly. In addition the direct-maternal genetic covariance was much larger in 2000 (0.079 ± 0.060) than 1999 (0.043 ± 0.035).

There were no significant dam or nurse effects on growth rates in food supplemented dyads (Tables 3.2). This was due in part to the small number of cross-fostered dyads that received supplemental food, but the addition of food also greatly reduced maternal effects (Figure 3.3) and the large, positive direct-maternal genetic covariance (- 0.005 ± 0.057) compared to control dyads. Other components of total phenotypic variation in growth in body mass were very similar between food supplemented and control dyads in 2000 (Figure 3.3).

3.3.4 *Components of maternal performance for growth*

The initial model of maternal performance including only litter size and parturition date explained 43% of the total variation in maternal performance for offspring growth ($F_{2,30} = 11.35$, $P = 0.0002$). None of the other single maternal characteristics improved the fit of the model, but the interaction of litter size and year as well as the interaction of parturition date and year entered into the final model ($N = 33$ pairs, $R^2 = 0.53$, $F_{4,28} = 7.9$, $P = 0.0002$). Litter size and parturition date both represented negative maternal effects on offspring growth (*litter size*: coefficient ± SE = - 0.29 ± 0.078; *parturition date*: - 0.19 ± 0.047) suggesting that offspring from larger litters born later in the season had lower growth rates. Both the *Litter Size x Year* and *Parturition*

Date x Year terms were positively correlated with maternal performance (*litter size x year*: 0.13 ± 0.081; *parturition date x year*: 0.09 ± 0.046) indicating that the strength of the negative effect of litter size and parturition date on juvenile growth rates was weaker in 2000 than 1999.

3.4 DISCUSSION

Natural populations of red squirrels experience large annual fluctuations in the abundance of spruce cones, which have important implications for many aspects of their life history (Berteaux & Boutin 2000, Humphries & Boutin 2000, Réale *et al.* 2003). Understanding the evolution of life history traits in the presence of such large fluctuations in environmental conditions requires an understanding of the influence of food abundance on the potential for an evolutionary response to selection. Furthermore, general insights into the ways in which environmental variability influences the evolution of natural populations of animals may be gained through the examination of populations, such as this, in which parents and offspring are frequently raised in conditions that differ remarkably from one another.

My classification in this study of good and poor environments for growth based on the abundance of spruce cones was reflected in large differences between mean phenotypes; offspring born into good conditions had much higher growth rates than offspring born into poor conditions. Cone availability and nestling growth rates in 1999 and 2000, when the cross-fostering experiments took place, were typical of good and poor conditions, respectively. As a result, I am comfortable that the conditions during which the cross-fostering experiments were performed reflected conditions experienced by this population during the last 16 years.

Results of both the cross-fostering experiments and the analysis of long-term data for this same population indicated that sources of resemblance between mothers and offspring were greater during poor conditions for growth. Most importantly the coefficient of direct additive genetic variance increased by 26%, but there were also increases in the coefficient of maternal (nurse) variance, and dam by nurse interaction variance and a decrease in the coefficient of residual variance. Together with an increase in the direct-maternal genetic covariance, these results indicate that there is a greater potential for evolutionary change in response to selection in red squirrels in poor years ($h_t^2 = 0.70$) than in good years for offspring growth ($h_t^2 = 0.52$; following Dickerson 1947; $h_m^2 = 0.11$ from Chapter 2). These changes in variance components estimated from the cross-fostering experiment were quantitatively consistent with observed mother-offspring covariances from the long-term data. Predicted mother-offspring covariances based on genetic expectations presented by Lynch & Walsh (1998) and results from the cross-fostering experiment (Table 3.3) were not significantly different from observed mother-offspring covariances (Table 3.1), providing further evidence that the results of the cross-fostering experiment may be representative of good and poor years in general.

The documented increase in mother-offspring resemblance in poor conditions in red squirrels is opposite to most previous studies of natural populations of birds, which have suggested that heritabilities tend to decrease in poor conditions (see Hoffmann & Merilä 1999). Hoffmann & Parsons (1997a) hypothesized that this trend in the bird literature might be due to unique characteristics of nutritional stress. The results of this study, however, indicate that nutritional stress can also result in an increase in additive genetic variance similar to other stress agents (Hoffmann & Parsons 1997b). Indirect maternal genetic effects can have a large influence on the response of traits to a given amount of selection (Wolf *et al.* 1998, McAdam *et al.* 2002, Chapter 2) and so maternal effects may make a significant contribution to the change in evolutionary potential between favourable and unfavourable environments. Here, I documented an increase in maternal effects in poor conditions, but this increase was much smaller than the increase in direct genetic effects. Coltman *et al.* (2001) also reported increases in both direct and maternal genetic variation in female Soay sheep (*Ovis aries*) in spring when the energetic demands of reproduction are thought to be stressful (but see Réale *et al.* 1999). Poor conditions have also been shown to increase maternal variation in great tits (*Parus major*; Gebhardt-Henrich & van Noordwijk 1991) and prolong the expression of maternal effects in the annual plant *Erigeron annuus* (Stratton 1989). Clearly more studies need to be done on the effects of environmental quality on direct genetic and maternal variation in natural populations before generalisations emerge.

The observed increase in heritable variation in poor conditions in this study is consistent with the hypothesis that stronger and more frequent selection in good years has eroded direct genetic variation for nestling growth rates more than in poor years. In the red squirrel system, years of low cone production are more common than years of high cone production (10 of the last 16 years had low cone production; see methods for definitions), but many more offspring are produced in years of high cone production, such that 59% of offspring born since 1987 were born in good conditions. In addition, adult over-winter survival is higher following years of high cone production (Boutin, unpublished data) so that juvenile squirrels born in years of high food abundance experience intense competition for few vacant territories. More specifically, viability selection on juvenile growth rates from recruitment to breeding age over the past 13 years was positively correlated with the abundance of spruce cones in the previous year, but there was no effect of cones on selection acting during the period of maternal dependence (McAdam & Boutin *in press*, Chapter 4). It is likely, however, that most effects of selection history on contemporary variation were largely due to selection history prior to the start of this long-term study.

The observed weak genetic correlation between good and poor environments suggests that selection in one environment will have a limited effect on genetic variation in the opposite environment. The presence of this weak genetic correlation between environments provides the opportunity for differences in selective history to be reflected in differences in genetic variation and suggests the potential for independent adaptation of nestling growth rates to both good and poor environments in this system. As I have mentioned earlier, my estimate of the genetic correlation across environments confounds maternal effects and direct genetic effects (Lynch & Walsh 1998). I have shown previously that maternal effects on offspring growth are themselves heritable and have the potential to contribute to a response to selection on growth rates (McAdam *et al.* 2002, Chapter 2). Environmentally dependent expression of maternal effects is a possible explanation for the independence of heritable variation across environments and provides a possible mechanism for the maintenance of the observed large positive covariance between direct and maternal genetic effects (McAdam *et al.* 2002, Chapter 2). I found evidence of interactions between year and both litter size and parturition date suggesting that the strength of these maternal effects differs in good and poor conditions. In both cases the negative maternal effect was stronger in good conditions than poor conditions. These results for litter size, including the increased effect of litter size in good years, are consistent with experimental effects of litter size augmentation on juvenile growth rates (Humphries & Boutin, 2000). There remained, however, a large proportion of unexplained variation in maternal performance that was not correlated with any of the other maternal characters examined here.

The experimental addition of food in 2000 maintained high rates of growth similar to those observed in favourable years. However, the effects of the experimental food addition on sources of variation in nestling growth rates differed from the comparative control results in two important ways. The addition of food greatly reduced both the large maternal effects and the large positive $cov(A_O, A_M)$ for nestling growth rates observed in 1999 and 2000. The experimental addition of food reduced maternal effects by over 70%, suggesting that most of the variation in maternal performance for offspring growth is related to the ability of females to secure food resources for their offspring even in years of high food abundance. The ability of females to acquire resources for their offspring may frequently represent a strong maternal effect on offspring traits in natural populations where food resources often vary in both space and time. Estimates of the strength of maternal effects and $cov(A_O, A_M)$ from animals raised either in the laboratory or captive populations where ad lib food is provided may, therefore, underestimate the importance of maternal effects to variation in offspring traits in natural populations.

Finally, in these analyses I have assumed that offspring born to the same dam were full-sibs. However, female red squirrels mate with multiple males and so there is likely some degree of multiple paternity. An interesting alternative explanation for the increase in variance due to dams in unfavourable conditions could be due to food effects on the degree of multiple paternity. For example, an increase in the degree of multiple paternity in good conditions would appear as a decrease in variance among dams in the present cross-fostering design since σ^2_D would more appropriately estimate ¼ V_{AO} rather than ½ V_{AO}. The decrease in error variance in growth in body mass in 2000 and the high CV_{AO} for growth in body mass of supplemented squirrels in 2000 is also consistent with this hypothesis although standard errors for CV_{AO} are very large. Future studies examining patterns of multiple paternity in this system should address this hypothesis further.

3.5 REFERENCES

Berteaux, D. and S. Boutin. 2000. Breeding dispersal in female North American red squirrels. *Ecology,* **81**: 1311-1326.

Coltman, D. W., Pilkington, J., Kruuk, L. E. B., Wilson, K. & Pemberton, J. M. 2001. Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. *Evolution,* **55**: 2116-2125.

Dickerson, G. E. 1947. Composition of hog carcasses as influenced by heritable differences in rate and economy of gain. *Research Bulletin Iowa Agricultural Experiment Station,* **354**: 489-524.

Falconer, D. S. 1952. The problem of environment and selection. *American Naturalist,* **86**: 293-298.

Falconer, D. S. and Mackay, T. F. C. 1996. *Introduction to Quantitative Genetics*, 4th edn. Longman, Harlow, UK.

Gebhardt-Henrich, S. G. & van Noordwijk, A. J. 1991. Nestling growth in the great tit I. Heritability estimates under different environmental conditions. *Journal of Evolutionary Biology,* **4**: 341-362.

Hoffmann, A. A. & Merilä, J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution,* **14**: 96-101.

Hoffmann, A. A. & Parsons, P. A. 1997a. Consistent heritability changes under poor growth conditions. *Trends in Ecology and Evolution,* **12**: 460-461.

Hoffmann, A. A. and P. A. Parsons. 1997b. *Extreme Environmental Change and Evolution.* Cambridge University Press, Cambridge.

Holloway, G. J., Povey, S. R. & Sibly, R. M. 1990. The effect of new environment on adapted genetic architecture. *Heredity,* **64**: 323-330.

Humphries, M. M. & Boutin, S. 1996. Reproductive demands and mass gains: a paradox in female red squirrels (*Tamiasciurus hudsonicus*). *Journal of Animal Ecology,* **65**: 332-338.

Humphries, M. M. & Boutin, S. 2000. The determinants of optimal litter size in free-ranging red squirrels. *Ecology,* **81**: 2867-2877.

Insightful. 2001a. *S-PLUS 6 for Windows Guide to Statistics, Volume 1.* Insightful Corporation, Seattle, WA.

Insightful. 2001b. *S-PLUS 6 for Windows User's Guide.* Insightful Corporation, Seattle, WA.

Kause, A. & Morin, J.-P. 2001. Seasonality and genetic architecture of development time and body size of the birch feeding sawfly *Priophorus pallipes*. *Genetical Research,* **78**: 31-40.

Lynch, M. and B. Walsh. 1998. *Genetics and Analysis of Quantitative Traits.* Sinauer Associates, Sunderland, Massachusetts.

McAdam, A. G. & Boutin, S. *in press.* Variation in viability selection among cohorts of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Evolution.*

McAdam, A. G., Boutin, S., Réale, D. & Berteaux, D. 2002. Maternal effects and the potential for evolution in a natural population of animals. *Evolution,* **56**: 846-851.

Merilä, J. 1997. Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. *Evolution,* **51**: 526-536.

Merilä, J., Przybylo, R. & Sheldon, B. C. 1999. Genetic variation and natural selection on blue tit body condition in different environments. *Genetical Research,* **73**: 165-176.

Mousseau, T. A. and C. W. Fox. 1998. *Maternal Effects as Adaptations.* Oxford University Press, New York.

Réale, D., Berteaux, D., McAdam, A. G. & Boutin, S. *in press*. Lifetime selection on heritable life history traits in a natural population of red squirrels. *Evolution.*

Réale, D., Festa-Bianchet, M. & Jorgenson, J. T. 1999. Heritability of body mass varies with age and season in wild bighorn sheep. *Heredity,* **83**: 526-532.

Réale, D., McAdam, A. G., Boutin, S. & Berteaux, D. 2003. Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London, Series B,* **270**: 591-596.

Riska, B., Rutledge, J. J. & Atchley, W. R. 1985. Covariance between direct and maternal genetic effects in mice, with a model of persistent environmental influences. *Genetical Research,* **45**: 287-297.

Roff, D. A. 1997. *Evolutionary Quantitative Genetics.* Chapman & Hall, New York.

StatSoft Inc. 2000. *STATISTICA for Windows.* StatSoft, Inc., Tulsa, OK.

Stratton, D. A. 1989. Competition prolongs expression of maternal effects in seedlings of *Erigeron annuus* (Asteraceae). *American Journal of Botany,* **76**: 1646-1653.

Wade, M. J. 1998. The evolutionary genetics of maternal effects in Mousseau, T. A. and C. W. Fox (eds) *Maternal Effects as Adaptations.* Oxford University Press, New York.

Willham, R. L. 1963. The covariance between relatives for characters composed of components contributed by related individuals. *Biometrics,* **19**: 18-27.

Wolf, J. B., Brodie, E. D., III, Cheverud, J. M., Moore, A. J. & Wade, M. J. 1998.

Evolutionary consequences of indirect genetic effects. *Trends in Ecology and Evolution,* **13**: 64-69.

Table 3.1. Mother-offspring covariances (± SE) within and between favourable (good) and unfavourable (poor) conditions for nestling growth in body mass. Sample size (*n*) represents the number of families. Standard errors were generated by jackknifing.

	Maternal environment			
Juvenile environment	Good	*n*	Poor	*n*
Good	0.061 ± 0.022*	86	- 0.014 ± 0.033	42
Poor	0.038 ± 0.033	38	0.113 ± 0.074	29

* $P < 0.05$

Table 3.2. Results of model II ANOVA (type III SS) used to partition variation in nestling growth rates of body mass into direct genetic, maternal, interaction and residual components. Separate models are presented for 1999, 2000 and food supplemented red squirrels (*Tamiasciurus hudsonicus*).

		1999			*2000*			*Food*	
Source	df	MS	F	df	MS	F	df	MS	F
Pair	32	0.314		19	0.361		5	0.297	
Dam (Pair)	33	0.051	2.83**	19	0.054	2.05	6	0.076	1.90
Nurse (Pair)	26	0.318	17.67***	8	0.290	10.74**	5	0.061	1.53
Nurse x Dam (Pair)	26	0.018	0.82	7	0.027	1.93	5	0.040	2.35
Residual	58	0.022		18	0.014		10	0.017	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 3.3. Components of mother-offspring covariance in good and poor years for offspring growth. Variance components were estimated from the cross-fostering experiment and V_{AM} is based on a heritability of maternal performance of 0.11 (Chapter 2).

		Component		
Conditions	$\frac{1}{2}V_{AO}$	$5/4\mathrm{Cov}(A_O,A_M)$	$\frac{1}{2}V_{AM}$	Predicted covariance
Good	0.011	0.054	0.006	0.071
Poor	0.013	0.098	0.005	0.116

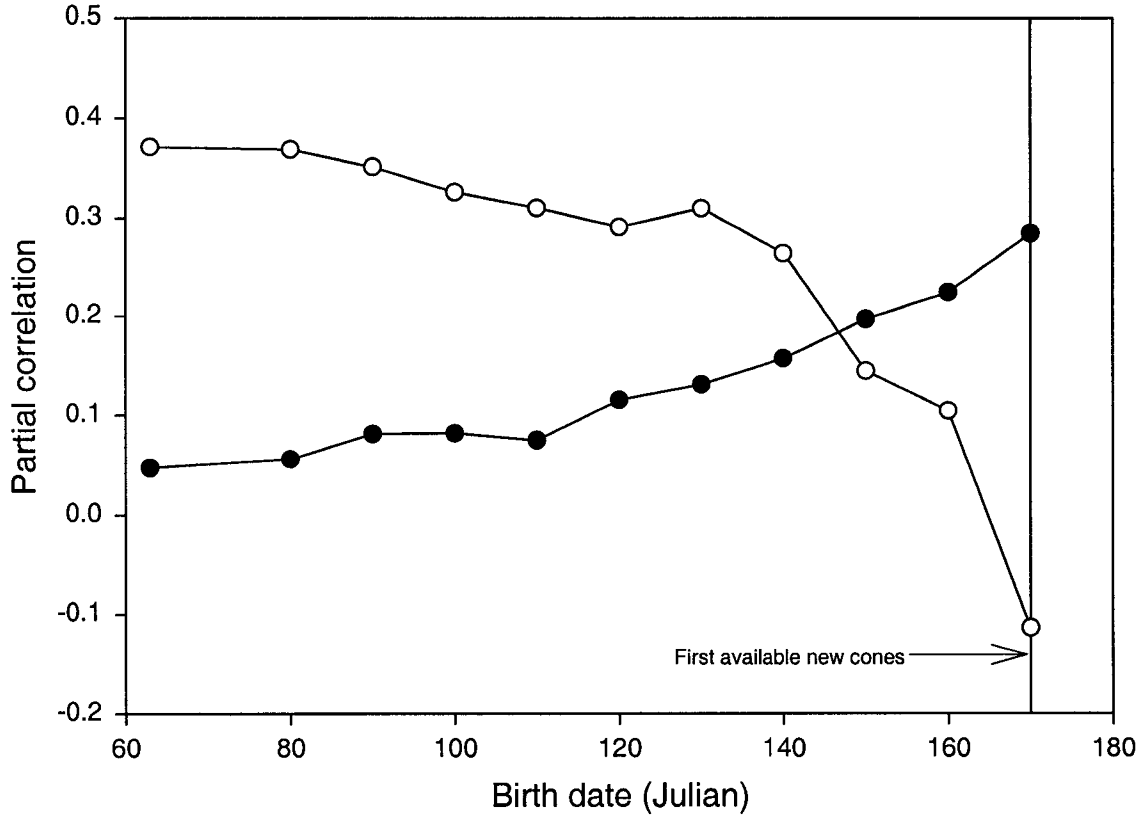

Figure 3.1. Partial correlation coefficients between the rate of growth in body mass of nestling red squirrels (*Tamiasciurus hudsonicus*) and the current year's *log cone index* (solid dot) and the previous year's *log cone index* (empty dot) throughout the breeding season. Growth rates were measured on a total of 1912 offspring born in this study area since 1987. Each pair of data points represents the partial correlation coefficients for a decreasing subset of all growth rates throughout the breeding season. The timing of the first availability of current year's cones for consumption is also indicated (S. Boutin, A. Sykes & A. McAdam, unpublished data)

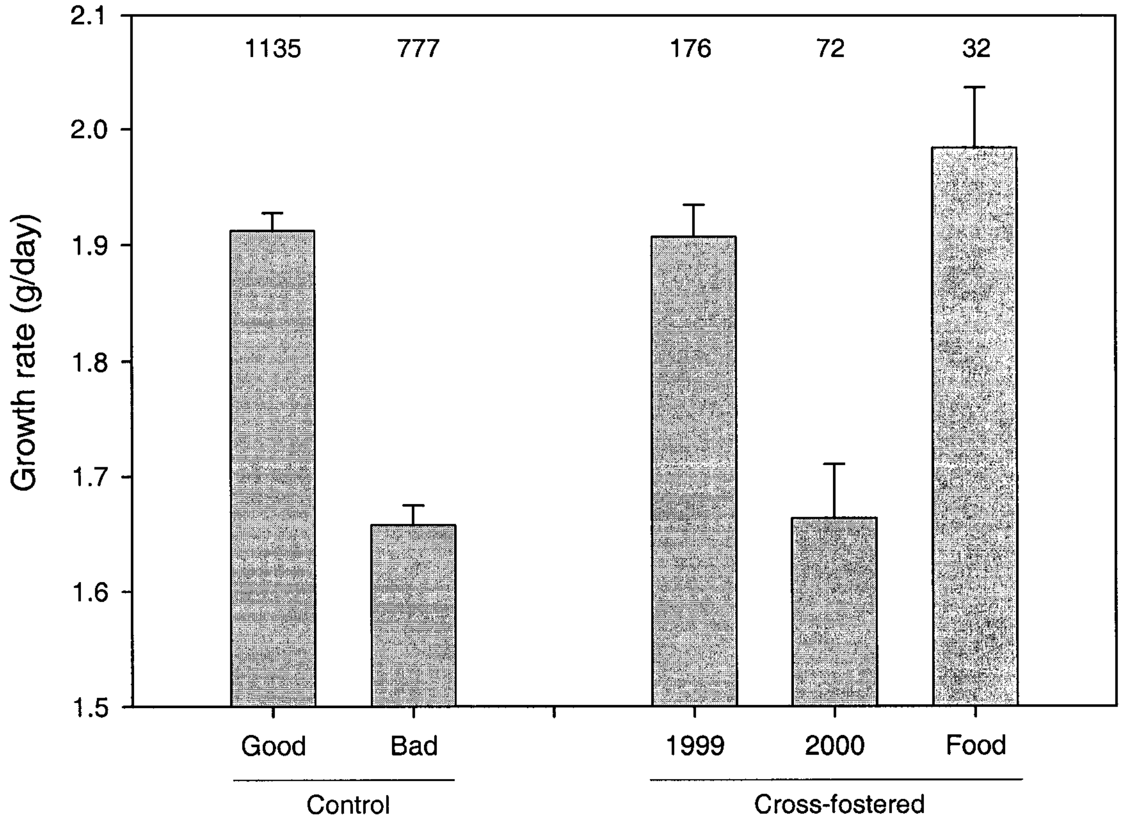

Figure 3.2. Rates of growth in body mass (g/day) of nestling red squirrels (*Tamiasciurus hudsonicus*) in good and poor conditions for growth. Control growth rates were measured on nestling squirrels born in this population over the past 16 years (1987 – 2002). Growth rates of cross-fostered offspring in 1999 and 2000 correspond to good and poor conditions for growth, respectively. Some cross-fostered offspring received a food supplementation (*Food*) in 2000. Values are presented as means ± one standard error. Sample sizes (number of juveniles) are indicated above each bar.

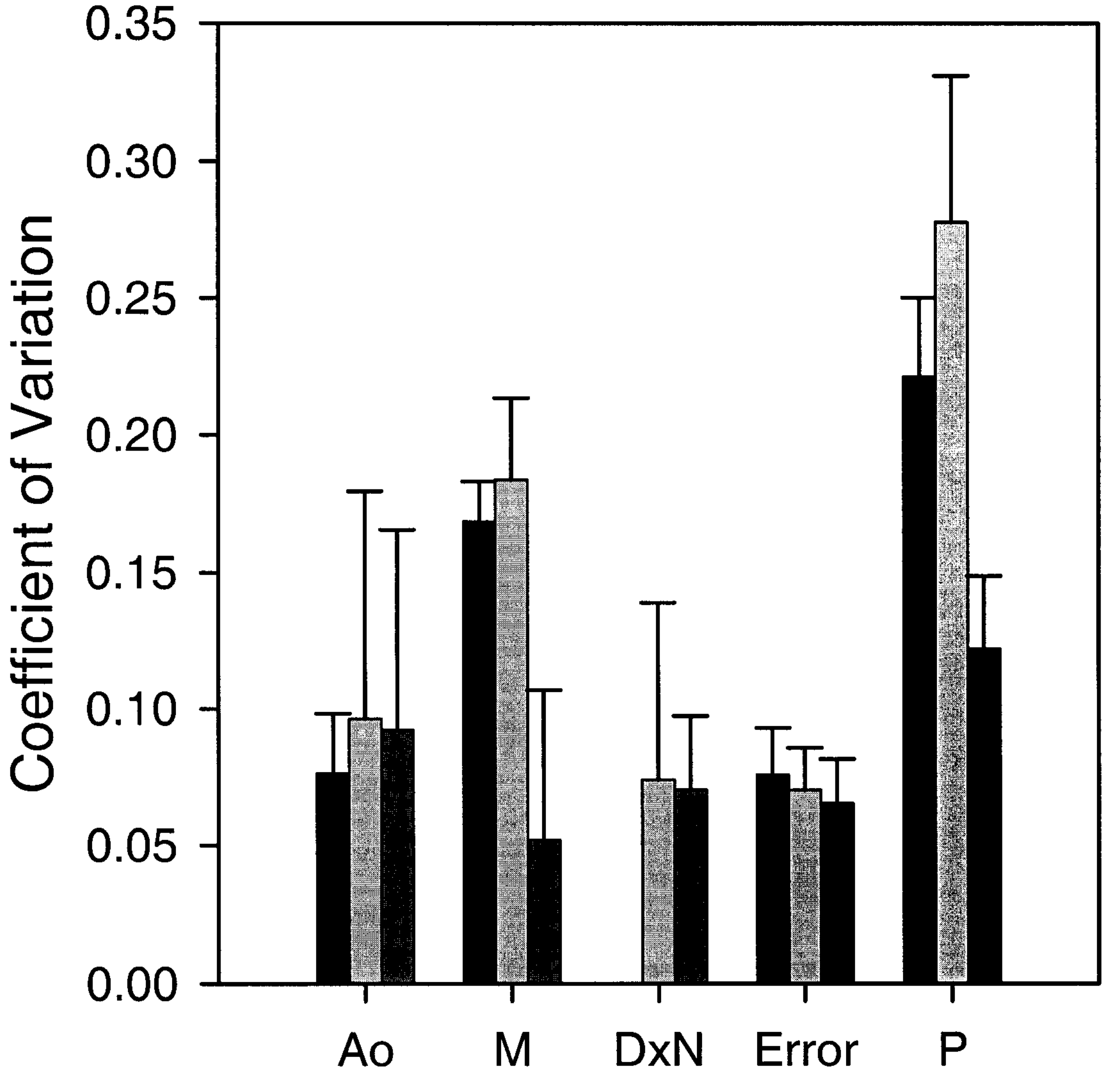

Figure 3.3. Coefficients of variation (*CV* = *sd* / *mean*) for components of growth in body mass of nestlings born in good (black bars), poor (light grey), and experimentally enriched (dark grey) environments. Components of total phenotypic variation (*P*) include coefficients of direct genetic (*Ao*), maternal (*M*), dam by nurse interaction (*DxN*), and residual (*Error*) variation. Standard errors were calculated by jackknifing.

4.0 TEMPORAL VARIATION IN VIABILITY SELECTION.[3]

4.1 INTRODUCTION

Since the development of methods for quantifying natural selection on multiple traits (e.g., Lande & 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 & 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 & Perrins 1987, Przybylo *et al.* 2000, Sinervo *et al.* 2000, Kruuk *et al.* 2001, Grant & Grant 2002). A negative relationship between the strength of viability

---

[3] A version of this chapter has been accepted for publication in *Evolution.*

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). Most natural populations, however, 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, Chapter 2). 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, Chapter 2). 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 (Boutin, unpublished 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 & Boutin 2000, Humphries & Boutin 2000, Réale *et al.* 2003). This combination of overlapping generations and the annual variation in food abundance, provide both the theoretical (Ellner & 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 I report on an analysis of viability selection for 13 cohorts (1989 – 2001) of nestling red squirrels based on survival to potential breeding age. I was most interested in selection on nestling growth rates but also quantified maternal selection acting via litter size and parturition date. I 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. I 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 me 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 I 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.

4.2 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 the 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 (see Berteaux & Boutin 2000 for more details). Red squirrels can initiate breeding at one year of age 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, so in most years the probability of survival to breeding age is low and as a consequence 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.

4.2.1 *Measurement of traits*

I examined viability selection on juvenile squirrels based on survival to potential breeding age. The offspring trait I was 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, Chapter 2). Offspring with initial weight measurements greater than 50 g, final weight measurements greater than 100 g, or less than 5 days between weight measurements were excluded from the analysis. Offspring involved in food supplementation or litter size manipulation experiments (e.g., Humphries & Boutin 2000) were not included in these analyses. I 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, Chapter 2). Females in this population rarely attempt a second litter late in the breeding season (Réale *et al. in press*). I 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. in press*). Results of analyses including offspring from second litters are similar to those presented here.

In the 13 cohorts between 1989 and 2001, I 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).

4.2.2 *Temporal and spatial variation*

I used generalized linear models (binomial response; McCullagh & Nelder 1989, Venables & 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 4 separate *a priori* models using analysis of deviance (McCullagh & Nelder 1989, Venables & 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 co-ordinates ± 3m), 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 while significant interactions with juvenile traits represented 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 3 spatial variables (2 co-ordinates 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 degrees of freedom (analysis of deviance; McCullagh & Nelder 1989; Venables &

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.

4.2.3 *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 & 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 (β_i', γ_{ii}' and γ_{ij}') are presented ± 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 (λ) was obtained by generalized cross-validation (Mathsoft 1999b, Mathsoft 1999a).

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), so selection gradients for each cohort were assumed to be independent tests of the null hypothesis of no selection. As a result, I corrected for multiple comparisons of selection gradients (3 traits x 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.

4.2.4 *Time scale of viability selection*

To examine the effect of the length of time over which viability selection was measured I 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, so selection on nestling growth rates based on survival to 50 days of age approaches the shortest time period over which selection on this trait can be measured. 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 time period was included as a fixed effect and 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 & Wade 1984).

4.2.5 *Correlates of viability selection*

I 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 (see Humphries and Boutin 2000 for details). Cones are harvested by the squirrels in August and are stored for subsequent consumption over the following year or more. Since the index of spruce cone production varied annually over three orders of magnitude (Humphries & Boutin 2000), I 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.* (2003). 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 (Boutin, unpublished 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, non-significant 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.

4.3 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 (Figure 4.1). In some years there was a cone crop failure (e.g., 1989, 1991, 1994, 2000), while in other mast years (e.g., 1993, 1998) a very large number of cones was produced. Nestling growth rates varied among years (Table 4.1) and were positively correlated with the abundance of spruce cones produced in the previous year (slope = 0.23 ± 0.04 $g*day^{-1}*cones^{-1}$, $N = 13$, $r^2 = 0.74$, $P < 0.001$). The opportunity for selection on offspring varied among years but in general was very high (median = 5; see Table 4.2). 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 ± 2.0, $t_{10} = 2.7$, $P = 0.02$) and the relative spring population density (slope = 9.0 ± 2.5, $t_{10} = 3.6$, $P = 0.004$).

The overall model of viability selection indicated that there was significant selection for increased nestling growth rates ($\beta' = 0.34 \pm 0.10$, $t_{1617} = 3.53$, $P = 0.0004$; Figure 4.2) and selection for earlier parturition dates ($\beta' = -0.20 \pm 0.07$, $t_{1617} = 3.13$, $P = 0.002$; Table 4.3). There was no significant directional selection on litter size ($\beta' = 0.04 \pm 0.07$, $t_{1617} = 0.54$, $P = 0.59$; Table 4.3) 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 4.4). Correlational selection gradients from the overall model were also not significantly different from zero ($t_{1611} < 1.84$, $P > 0.06$; Table 4.4).

4.3.1 *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 4.5). 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 ($\chi^2_1 = 52.1$, $P < 0.001$) 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 selection on both nestling growth rates (growth rate, cohort interaction: $\chi^2_{10} = 30.0$, $P = 0.001$) and litter size (litter size, cohort interaction: $\chi^2_{10} = 20.2$, $P = 0.03$).

4.3.2 *Estimation of viability selection*

Viability selection on nestling growth rates varied among cohorts in both direction and magnitude (Figure 4.2). In most years selection favoured increased nestling growth rates; however, in 1995 and 1997 decreased nestling growth rates were favoured. The magnitude of selection on nestling growth rates was often weak (median $|\beta'| = 0.24$), but there were episodes of strong directional selection (e.g., 1989, 1992, 1999 and 2000). Most standard errors associated with these selection gradients were large and most selection gradients were not significantly different from zero.

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 4.3). 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 4.4).

The median absolute value of correlational selection was weak for the three combinations of traits (GR x LS = 0.19; GR x PD = 0.16; LS x 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. In 1989, however, 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 4.4).

4.3.3 *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 I would have predicted. The strength of viability selection on nestling growth increased with increases in the time-period over which selection was measured (Figure 4.3). 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; $r = 0.71$, $N = 13$, $P = 0.007$; Figure 4.4a), 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 ($r = 0.04$, $N = 13$, $P = 0.90$; Figure 4.4b). 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.

4.3.4 *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 ± 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, $P = 0.35$). On the other hand, 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 = 0.146 ± 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, $P = 0.53$).

4.4 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 (Boutin, unpublished 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 & Grant 1981) in 6 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 & 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 (Boutin, unpublished 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. This opportunity for selection, however, 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 4 of the 13 cohorts had selection gradients that 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, Chapter 2). This large positive covariance is incompatible with consistent directional selection on this trait and I have previously suggested that selection on this trait might instead vary either in space or time (McAdam *et al.* 2002, Chapter 2). I 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, I 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, Boutin, unpublished data) are sufficient to maintain the observed genetic variation and covariation for this trait (Ellner & Hairston 1994).

The direction and magnitude of maternal selection on litter size varied among cohorts, while maternal selection on parturition date was negative (favouring 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. in press*). My estimates of maternal selection on litter size and parturition date correspond to maternal selection differentials (calculated following Kirkpatrick & Lande 1989, Kirkpatrick & Lande 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 & Reeve 2001).

There was very little evidence of either stabilizing or disruptive 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, Chapter 2) 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. I 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 when the estimate of the direct-maternal covariance was made (1999; McAdam *et al.* 2002, Chapter 2), but correlational selection was not common.

I 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 my 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 to one year 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 & Boutin 1995b). After emergence, juvenile mortality is due almost exclusively to predation (Stuart-Smith & Boutin 1995b, Anderson & Boutin 2002), but the risk of predation is likely mediated by food abundance, individual condition and behaviour (Stuart-Smith & Boutin 1995a, Stuart-Smith & Boutin 1995b, Anderson & 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 & 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 behaviourally mediate predation risk (Stuart-Smith & Boutin 1995a, Anderson & 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 (Boutin, unpublished 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. Episodes of very strong directional selection, therefore, 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 & Grant 2002) extremely difficult. 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, Chapter 2, Chapter 3), however, provide the necessary and sufficient conditions for predicting the responses of individual cohorts to observed episodes of viability selection.

4.5 REFERENCES

Anderson, E. M. and S. Boutin. 2002. Edge effects on survival and behaviour of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Canadian Journal of Zoology* **80**: 1038-

1046.

Arnold, S. J. & Wade, M. J. 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. & Grant, P. R. 1981. Intense natural selection in a population of Darwin's finches (*Geospizinae*) in the Galápagos. *Science,* **214**: 82-85.

Boutin, S., Larsen, K. W. & Berteaux, D. 2000. Anticipatory parental care: acquiring resources for offspring prior to conception. *Proceedings of the Royal Society of London, Series B,* **267**: 2081-2085.

Boyce, M. S. & Perrins, C. M. 1987. Optimizing great tit clutch size in a fluctuating environment. *Ecology,* **68**: 142-153.

Brodie, E. D., III, Moore, A. J. & Janzen, F. J. 1995. Visualizing and quantifying natural selection. *Trends in Ecology and Evolution,* **10**: 313-318.

Ellner, S. & Hairston, N. G. Jr. 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *American Naturalist,* **143**: 403-417.

Endler, J. A. 1986. *Natural Selection in the Wild.* Princeton University Press, Princeton, New Jersey.

Fairbairn, D. J. and J. P. Reeve. 2001. Natural selection. in Fox, C. W., D. A. Roff and D.

J. Fairbairn (eds) *Evolutionary ecology: concepts and case studies.* Oxford University Press, New York.

Grant, P. R. & Grant, R. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science,* **296**: 707-711.

Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hoang, A., Hill, C. E., Beerli, P. & Kingsolver, J. G. 2001. Strength and tempo of directional selection in the wild. *Proceedings of the National Academy of Science, USA,* **98**: 9157-9160.

Humphries, M. M. & Boutin, S. 2000. The determinants of optimal litter size in free-ranging red squirrels. *Ecology,* **81**: 2867-2877.

Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hill, C. E., Hoang, A., Gibert, P. & Beerli, P. 2001. The strength of phenotypic selection in natural populations. *American Naturalist,* **157**: 245-261.

Kirkpatrick, M. & Lande, R. 1989. The evolution of maternal characters. *Evolution,* **43**: 485-503.

Kirkpatrick, M. & Lande, R. 1992. The evolution of maternal characters: Errata. *Evolution,* **46**: 284.

Kruuk, L. E. B., Merilä, J. & Sheldon, B. C. 2001. Phenotypic selection on a heritable size trait revisited. *American Naturalist,* **158**: 557-571.

Lande, R. & Arnold, S. J. 1983. The measurement of selection on correlated characters. *Evolution,* **37**: 1210-1226.

Mathsoft. 1999a. *S-PLUS 2000 Guide to Statistics, Volume 1.* Data Analysis Products Division, Mathsoft, Seattle, WA.

Mathsoft. 1999b. *S-PLUS 2000 User's Guide.* Data Analysis Products Division, Mathsoft, Seattle, WA.

McAdam, A. G., Boutin, S., Réale, D. & Berteaux, D. 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.*, 2nd edn. Chapman and Hall, New York.

Merilä, J., Sheldon, B. C. & Kruuk, L. E. B. 2001. Explaining stasis: microevolutionary studies in natural populations. *Genetica,* **112-113**: 199-222.

Mousseau, T. A. & Roff, D. A. 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., Sheldon, B. C. & Merilä, J. 2000. Patterns of natural selection on morphology of male and female collared flycatchers (*Ficedula albicollis*). *Biological Journal of the Linnean Society,* **69**: 213-232.

Réale, D., Berteaux, D., McAdam, A. G. & Boutin, S. *in press.* Lifetime selection on heritable life history traits in a natural population of red squirrels. *Evolution.*

Réale, D., McAdam, A. G., Boutin, S. & Berteaux, D. 2003. Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London, Series B,* **270**: 591-596.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution,* **43**: 223-225.

Roff, D. A. 1997. *Evolutionary Quantitative Genetics.* Chapman & Hall, New York.

Sinervo, B., Svensson, E. & Comendant, T. 2000. Density cycles and an offspring quantity and quality game driven by natural selection. *Nature,* **406**: 985-988.

Stuart-Smith, A. K. & Boutin, S. 1995a. Behavioural differences between surviving and depredated juvenile red squirrels. *Écoscience,* **2**: 34-40.

Stuart-Smith, A. K. & Boutin, S. 1995b. Predation on red squirrels during a snowshoe hare decline. *Canadian Journal of Zoology,* **73**: 713-722.

Venables, W. N. and B. D. Ripley. 2002. *Modern applied statistics with S.*, 4th edn. Springer-Verlag, New York.

Table 4.1. Phenotypic means (± 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 (*N*; number of juveniles) for each cohort are reported at the right of each row.

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

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

Table 4.2. 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 4.1 for sample sizes (number of juveniles) within each cohort.

	50 days	100 days	One year	Opportunity for Selection (*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

Table 4.3. Standardized maternal selection gradients (± 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. Underlined gradients were significantly different from zero based on SE's 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 4.1 for sample sizes (number of juveniles) within each cohort.

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

Table 4.4. Standardized quadratic and correlational selection gradients (± 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. Underlined gradients were significantly different from zero based on SE's generated by jackknifing ($P < 0.05$). Only correlational selection gradients for GR x LS and LS x PD in 1989 were significant after sequential Bonferroni correction. See Table 4.1 for the number of juveniles within each cohort.

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

Table 4.5. 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 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 Survival ~ SEX + GR + LS + PD	4	1566.7	5.2	------
Spatial - small Scale ~ Basic + X + Y + XY + X:GR + X:LS + X:PD + Y:GR +	16	1540.1	6.9	$\chi^2_{12} = 26.6$ $P = 0.01$
Spatial - medium scale ~ Basic + GRID + GRID:GR + GRID:LS + GRID:PD	12	1548.8	6.3	$\chi^2_{8} = 17.9$ $P = 0.02$
Temporal ~ Basic + C + C:GR + C:LS + C:PD	44	1165.6	29.5	$\chi^2_{40} = 401.1$ $P < 0.0001$

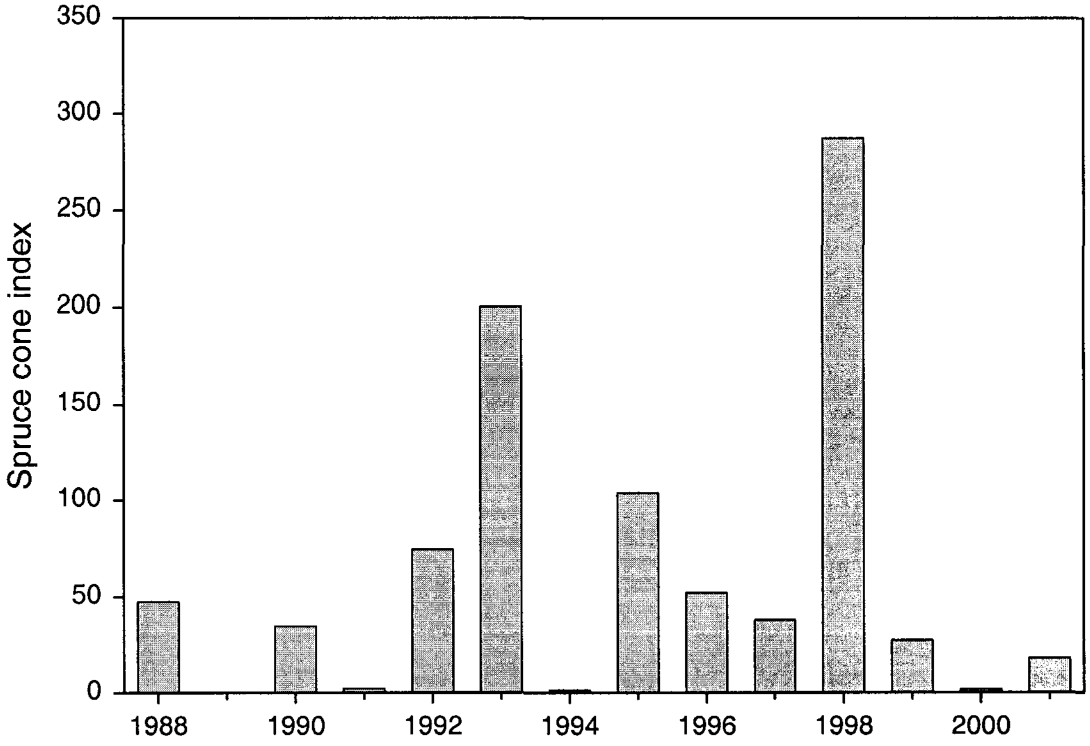

Figure 4.1. Annual variation in the production of spruce cones from 1989 to 2001. Values represent an index of the average spruce cone production on three study areas in the southwest Yukon.

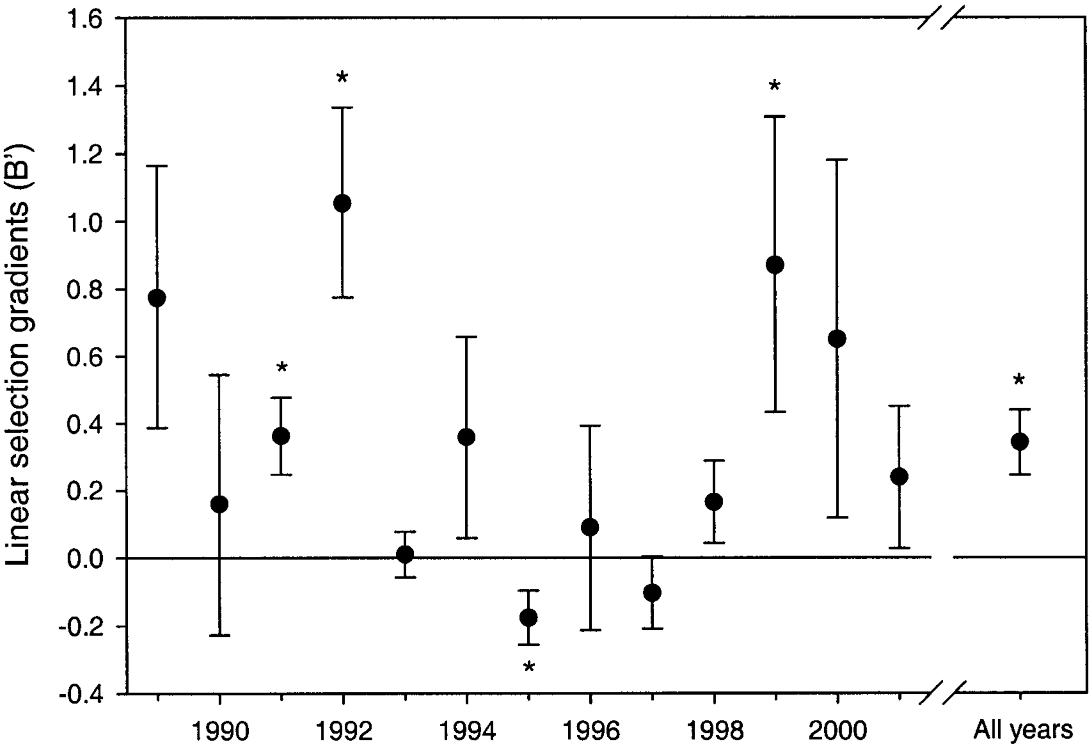

Figure 4.2. Variation among cohorts in the standardized directional selection gradient (β') on nestling growth rates based on survival to one year of age in juvenile red squirrels. Gradients are plotted ± SE. Gradients indicated by an asterisk were significantly different from zero ($P < 0.05$). Gradients in 1995 and 1999 were not significant after sequential Bonferroni correction.

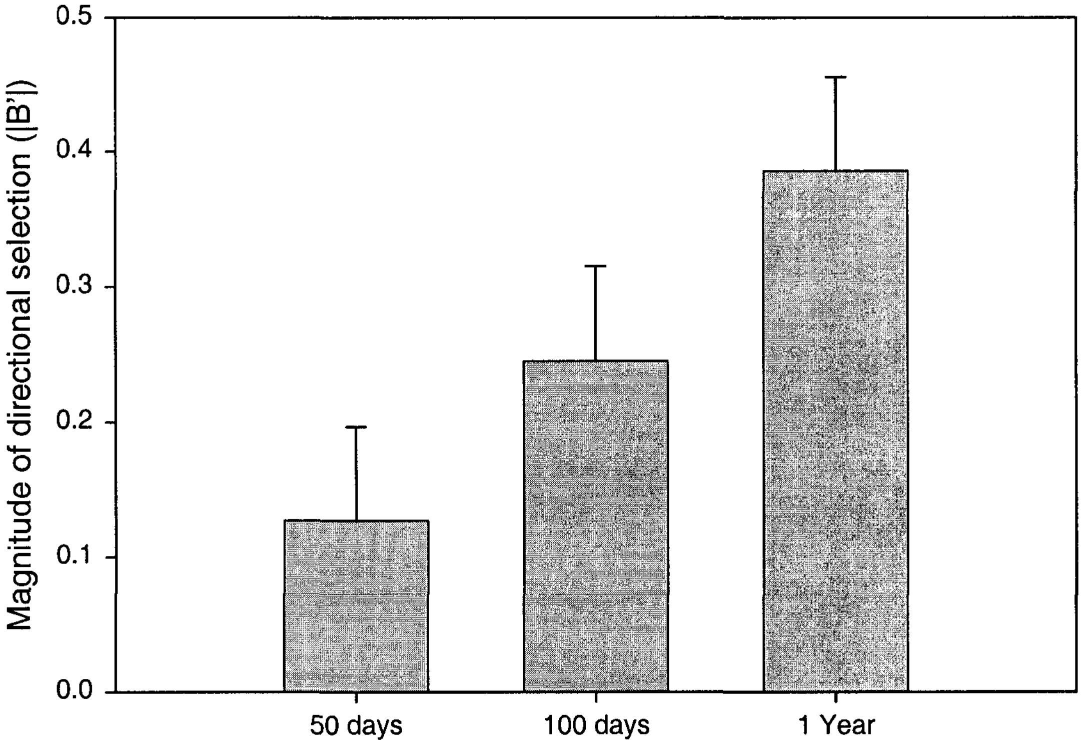

Figure 4.3. Effects of the length of time over which viability selection was measured on the strength of directional selection on nestling growth rates. The time periods of days, months and years correspond to viability based on survival to 50 days, 100 days and one year, respectively. Differences among 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.

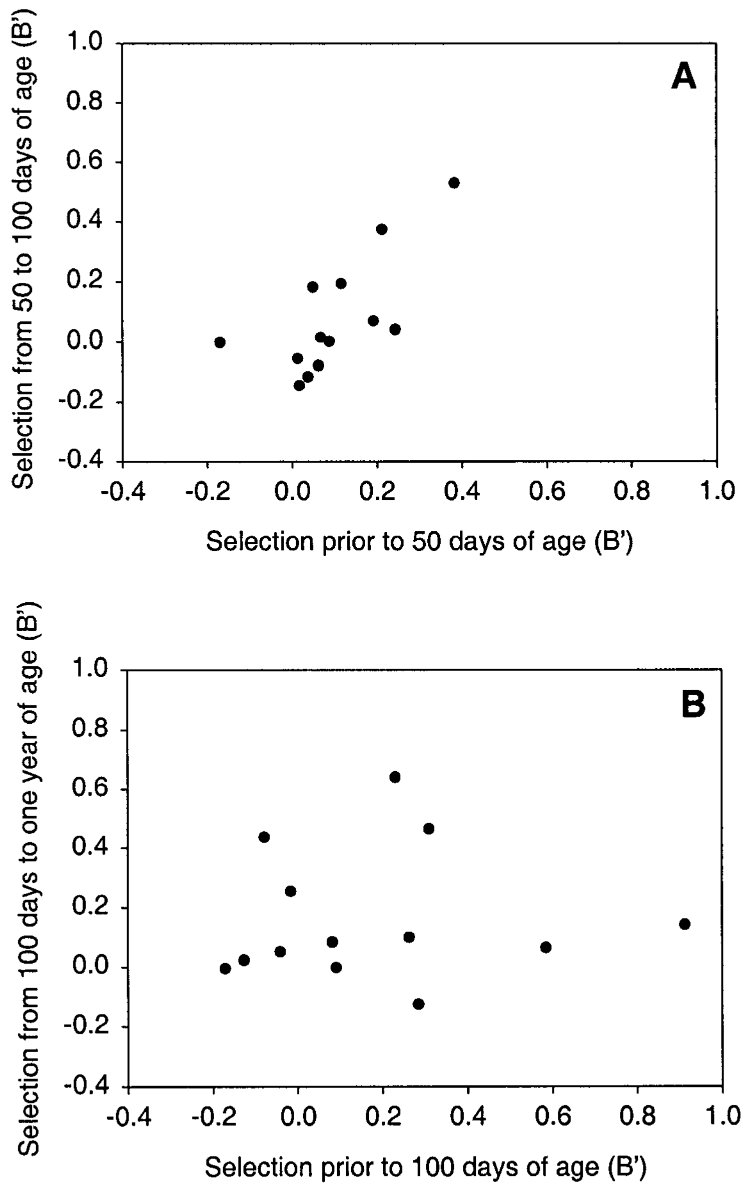

Figure 4.4. Correlations between the strength of directional viability selection (β') acting prior to emergence (50 days of age) and from emergence to recruitment (100 days of age; A), and prior to recruitment and from recruitment to potential breeding age (one year; B).

CHAPTER 5. MATERNAL EFFECTS AND THE RESPONSE TO SELECTION.

5.1 INTRODUCTION

Maternal effects are now recognised as having widespread phenotypic effects in a variety of taxa (Roach & Wulff 1987, Rossiter 1996, Mousseau & Fox 1998). These maternal contributions are experienced as environmental effects by offspring but may be genetically based and, therefore, can contribute to an evolutionary response to selection (Wolf *et al.* 1998). Models of maternal effect evolution suggest that genetically based maternal effects can result in unusual evolutionary responses to selection (Kirkpatrick & Lande 1989, Lande & Kirkpatrick 1990, Kirkpatrick & Lande 1992, Wolf *et al.* 1998). Specifically, heritable maternal effects (indirect genetic effects; Wolf *et al.* 1998) can greatly accelerate or retard the evolutionary response to selection and are predicted to introduce an evolutionary time lag, in which the response to selection in the current generation also depends on the strength of selection in the previous generation (Kirkpatrick & Lande 1989). As a result, populations may continue to evolve after selection has ceased and evolution may also temporarily proceed in a direction opposite to the direction of selection (Kirkpatrick & Lande 1989).

These dramatic and sometimes counterintuitive predictions of maternal effects models, however, depend on the presence of a genetic basis to maternal variation (Kirkpatrick & Lande 1989, Wolf *et al.* 1998). While the genetic basis to maternal effects has been estimated frequently in populations subject to generations of artificial selection or benign laboratory conditions (e.g., see Roff 1997), indirect genetic effects in nondomestic species (Hunt & Simmons 2002, Rauter & Moore 2002, Agrawal *et al.*

2001) and under natural field conditions have been investigated only recently (Byers *et al.* 1997, Thiede 1998, McAdam *et al.* 2002). As a result, the importance of maternal effects to evolutionary dynamics in natural populations is not yet known.

Here I used 14 years of data (1989 – 2002) from a population of North American red squirrels (*Tamiasciurus hudsonicus*) to examine the importance of genetically based maternal effects to evolutionary dynamics in a natural population. Juvenile growth in body mass of red squirrels is heritable ($h^2 = 0.10$; McAdam *et al.* 2002, Chapter 2), but also experiences large heritable maternal effects and a large positive covariance between direct and maternal genetic effects, resulting in a much greater potential response to selection than would have been predicted from direct genetic effects alone ($h_t^2 = 0.36$; McAdam *et al.* 2002, Chapter 2). In this study, I quantified changes in juvenile growth rates from prior to selection to one generation after selection (Falconer & Mackay 1996, Grant & Grant 2002) for each of 12 cohorts of squirrels born since 1989. These observed responses, in conjunction with previous estimates of the strength of directional selection on this trait (McAdam & Boutin *in press*, Chapter 4), allowed me to estimate the realised contribution of maternal effects to the evolutionary dynamics of this juvenile trait.

5.2 METHODS

5.2.1 *Red squirrel population.*

Squirrels within this population of approximately 325 adults have been monitored since 1987. Details of the population are given elsewhere (Berteaux & Boutin 2000, Humphries & Boutin 2000, Réale *et al.* 2003, Chapter 2). Juveniles were permanently ear-tagged prior to first emergence from their natal nest, so that maternity was certain. In addition, the diurnal territorial nature of red squirrels allowed us to census the entire population in both spring and fall of each year.

5.2.2 *Juvenile growth rates.*

Growth rates were defined as the linear increase in mass from soon after birth to just prior to first emergence from the natal nest (approximately 30 days; see also Chapter 4). Over the course of this study there were some cases in which mass measurements were missed or were not taken at the appropriate time. As a result, I restricted the data to include only offspring with initial mass measurements less than 50g, final mass measurements less than 100g, and more than 5 days between first and second mass measurements. I examined only the growth rates of offspring born in first litters of the season to be consistent with previous estimates of selection on this trait (McAdam & Boutin *in press*, Chapter 4). Cross-fostered offspring and those subjected to food or litter size manipulations were excluded from this analysis.

5.2.3 *Environmental effects on growth.*

Red squirrels in this population feed almost exclusively on the seeds of white spruce cones (*Picea glauca*). Spruce cones are harvested by red squirrels in late summer and are stored for subsequent consumption over the following year or more. 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 distributed systematically throughout the study areas (see Humphries & Boutin 2000 for details). These annual values were log transformed prior to analysis.

The average growth rate of juveniles born in the spring is positively correlated with the abundance of spruce cones in the previous fall (McAdam & Boutin *in press*, Chapter 3, Chapter 4), so I corrected juvenile growth rates for environmental effects prior to estimating the response to selection. Growth rates were corrected by examining the differences among the growth rates of offspring raised by mothers that bred in multiple years. Specifically, the raw residual (r_{ij}) of the average growth rate of offspring raised by mother i in year j from the average of all litters raised by mother i in her lifetime was used as a measure of the environmental effect of that year on offspring growth for female i. These growth rate residuals, therefore, represented the combined effects of all annual environmental sources of variation. Females breeding in a greater number of years experienced a wider range of environmental conditions, so a weighted average of residuals across females within each year was used to calculate the environmental effect for each year (e_j) according to the equation

$$e_j = \frac{\sum_{i=1}^{n} r_{ij} \times y_i}{\sum_{i=1}^{n} y_i}, \qquad (5.1)$$

where growth rate residuals were weighted by the number of years in which each female bred (y_i). Positive values of e_j, therefore, indicated that females in year j raised offspring that grew faster than normal, while negative values of e_j represented a poor year for offspring growth. All juvenile growth rates were corrected for environmental effects experienced in the year of their birth (e_j) prior to estimating the response to selection.

I attempted to determine the ecological variables responsible for annual variation in environmental effects (e_j) using ordinary least squares multiple regression ($n = 12$ years). Predictors of annual environmental effects included the abundance of spruce cones (log transformed) produced in the previous and current year, the relative spring population density, and average spring temperature. Spring population density, measured as the number of adults owning territories within the core 12 ha area of each of the study areas, has been found to be positively correlated with the previous year's cone production (Boutin, unpublished data), so the residuals of the relationship between population density and the previous year's cone production were considered here. Temperature 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.* (2003). Non-significant variables ($P < 0.05$) in the 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.

5.2.4 *Response to Selection.*

The observed response to selection was measured as the change in mean juvenile growth rates from prior to selection to one generation after selection (Falconer & Mackay 1996; Grant & Grant 2002). Since the strength and direction of selection on juvenile growth varied among cohorts of squirrels (McAdam & Boutin *in press*, Chapter 4), the response to selection was calculated separately for each cohort. Therefore, for each cohort, growth rates prior to selection represent the average growth rate of all offspring born in that year, while growth rates after selection represent the average growth rate of all offspring raised by mothers born in that cohort.

Observed responses to selection have been used to estimate the heritability of traits from episodes of selection with known strength, where the realised heritability (h_r^2) equals the slope of the regression of the response to selection (Δz_t) on directional selection differentials (S_t; Falconer & Mackay 1996). Models of maternal effect evolution, however, predict that the response to selection in the current generation (Δz_t) also depends on the strength of selection in the previous generation (S_{t-1}; Kirkpatrick & Lande 1989, Lande & Kirkpatrick 1990). I, therefore, included both the strength of selection in the current generation (S_t) and the strength of selection in the previous generation (S_{t-1}) in a multiple regression of the observed response to selection (Δz_t).

5.2.5 *Maternal effects model.*

I used a simple maternal effect model that considered litter size as the only maternal trait (from Kirkpatrick & Lande 1989, Lande & Kirkpatrick 1990, Kirkpatrick & Lande 1992)

$$
\begin{aligned}
\Delta \bar{z}_{o(t)} &= [\mathrm{cov}(A_O, A_M)/2 + mV_{AM}]\beta_{m(t)} + [V_{AO} + m\,\mathrm{cov}(A_O, A_M)/2]\beta_{o(t)} \\
&+ m[\mathrm{cov}(A_O, A_M) + mV_{AM}/2]\beta_{o(t-1)} + [m\,\mathrm{cov}(P_O, P_M)_t]\beta_{o(t)} \\
&- [m\,\mathrm{cov}(P_O, P_M)_{(t-1)}]\beta_{o(t-1)}
\end{aligned}
\tag{5.2}
$$

where $\Delta Z_{o(t)}$ is the predicted change in the mean growth rate of offspring at time *t*. Litter size in this population is under stabilizing selection (Réale *et al. in press*) so directional selection on litter size ($\beta_{m(t)}$) was set to 0. The strength of selection on growth rates in the current generation ($\beta_{o(t)}$) was previously estimated for each cohort based on viability selection of juveniles from birth to potential breeding age (McAdam & Boutin *in press*, Chapter 4) and are also shown in Table 5.1. The strength of selection in the previous generation ($\beta_{o(t-1)}$) was estimated for each cohort as the average strength of juvenile viability selection experienced by mothers of that cohort. Viability selection was not measured prior to 1989, so I assumed that all mothers born prior to 1989 experienced as juveniles the overall strength of directional selection on growth rates from 1989 to 2001 ($\beta_o = 0.725$; McAdam & Boutin *in press*, Chapter 4). My estimate of the strength of selection in the previous generation for cohorts born early in the study (1989 –1991) may, therefore, be less accurate than for later cohorts. Additive genetic variance in litter size (V_{AM}) has been previously calculated as 0.102 (Réale *et al. in press*). Direct additive genetic variance (V_{AO}) was calculated separately for each cohort based on the total phenotypic variance in growth rates for that cohort and a direct genetic heritability of 0.10 (McAdam *et al.* 2002). I calculated the phenotypic covariance between growth rate and litter size, $cov(P_O,P_M)$, separately for each cohort (McAdam & Boutin *in press*, Chapter 4) and used the average $cov(P_O,P_M)$ experienced by mothers as an estimate of $cov(P_O,P_M)_{(t-1)}$, as above. Cross-fostering experiments have revealed both a negative maternal effect coefficient for litter size (m) and a large negative genetic correlation between growth rate and litter size [r_{mo}, where $r_{mo} = cov(A_O,A_M) / \sqrt{(V_{AO} * V_{AM})}$; McAdam *et al.* 2002, Chapter 2, Chapter 3]. However, here I estimated the realised values of m and r_{mo} using likelihood techniques. Likelihoods were calculated for values of r_{mo} between –1 and 1 and m between 0 and – 0.9 using a systematic grid scan. Maximum likelihood estimates on the other hand were found using an iterative procedure that was not limited to this parameter space. Observational uncertainty in the response to selection was assumed to be normally distributed with an unknown standard deviation. Predicted responses to selection for each of the 12 cohorts were generated from the maternal effect model above. Models based on parameter combinations of m and r_{mo} were tested against the null model, which excluded maternal effects ($m = 0$). All likelihoods for combinations of m and r_{mo} were scaled relative to the likelihood of this null model for ease of comparison. The significance of each combination of parameters was assessed using the likelihood ratio test, where twice the reduction in the negative log-likelihood from the null model was assumed to follow a chi-square distribution with one degree of freedom (Hilborn & Mangel 1997). Combinations of m and r_{mo} resulting in a significant improvement in the fit of the observed data compared to the null model are indicated. Predicted responses to selection based on maximum likelihood estimates of m and r_{mo} were compared against observed responses to selection using ordinary least squares regression ($n = 12$ cohorts).

5.3 RESULTS

As expected, annual environmental effects were positively correlated with the abundance of spruce cones produced in the previous fall (slope = 0.163 ± 0.047, $t_9 = 3.44$, $P = 0.007$; Figure 5.1), but they were also negatively correlated with the current year's cone production (slope = - 0.106 ± 0.045, $t_9 = - 2.38$, $P = 0.04$). Relative spring population density and average spring temperature were not significantly correlated with environmental effects on growth (relative density: $t_9 = - 1.06$, $P = 0.33$; spring temperature: $t_9 = -1.00$, $P = 0.35$). The abundance of spruce cones produced in the previous and current year together explained 72% of the variation in annual environmental effects ($F_{2,9} = 11.73$, $P = 0.003$).

The responses of juvenile growth rates to selection differed in direction and magnitude among cohorts (Table 5.1). Some cohorts exhibited a significant increase in growth rates in response to selection (e.g., 1992), while others exhibited a significant decrease in growth (e.g., 1997, 1998). Surprisingly, 1992 was the only cohort that exhibited a significant response to significant directional selection. Three other cohorts that experienced significant directional selection on growth (1991, 1995 and 1999) did not exhibit significant responses to selection, and more surprisingly, two cohorts exhibited significant responses to very weak selection (1997 and 1998).

There was a strong positive correlation between the observed change in juvenile growth rates and the strength of selection in the current generation (slope = 0.556 ± 0.169; $t_9 = 3.3$, $P = 0.009$; Figure 5.2A), but there was also a positive effect of the strength of selection in the previous generation (slope = 0.684 ± 0.306; $t_9 = 2.2$, $P = 0.052$; overall model $N = 12$, $R^2 = 0.65$, $P = 0.009$; Figure 5.2B). The removal of cohorts from early in the study (1989 - 1991), for which I did not have adequate data on selection in the previous generation, improved the fit of the overall relationship ($N = 9$, $R^2 = 0.78$, $P = 0.01$) but did not change the coefficients for either selection in the current generation (slope = 0.594 ± 0.160; $t_6 = 3.7$, $P = 0.01$) or the previous generation (slope = 0.893 ± 0.324; $t_6 = 2.8$, $P = 0.03$).

Maximum likelihood estimates of the maternal effect coefficient (m) and the direct-maternal genetic correlation (r_{mo}), based on observed changes in growth rates and predictions from a maternal effect model including litter size as the only maternal effect were – 0.30 and –3.1, respectively. Combinations of a direct-maternal genetic correlation less than zero and a maternal effect coefficient between – 0.25 and – 0.55 resulted in a significant improvement in the fit of the observed data over a model with no maternal effects (i.e., m = 0; $\chi^2 > 3.94$, $P < 0.05$; Figure 5.3).

Predictions from this simple maternal effect model, using the maximum likelihood estimates of m and r_{mo}, explained 57% of the variation in observed responses to selection across these 12 cohorts ($n = 12$, $R^2 = 0.57$, $P = 0.004$). The relationship between predicted and observed responses to selection had a slope and intercept that did not differ from one (1.37 ± 0.37; $t_{10} = 0.98$, $P = 0.35$) and zero (- 0.06 ± 0.04, $t_{10} = - 1.56$, $P = 0.15$), respectively.

5.4 Discussion

In the absence of maternal effects, the response to selection (R) can be predicted as the product of heritability (h^2) and selection (S) using the breeders' equation ($R = h^2 S$; Falconer & Mackay 1996). Genetic responses to selection, however, may not be reflected in phenotypic changes across generations if environmental conditions change over this same period (Grant & Grant 1995, Merilä *et al.* 2001). As a result, phenotypic responses to selection can only be expected to reflect underlying genetic changes if environmental sources of variation are controlled or corrected across generations. The growth rates of juvenile red squirrels experience large environmental effects, that are due mostly to the abundance of spruce cones produced in the previous fall (McAdam & Boutin *in press*, Chapter 3, Chapter 4). As a result, annual variation in growth rates is largely the result of annual variation in cone production (McAdam & Boutin *in press*, Chapter 4). However, juvenile growth rates have also been subject to episodes of strong directional selection (McAdam & Boutin *in press*, Chapter 4), so genetic responses to selection may also contribute to the dynamics of this trait. There were significant changes in growth rates (corrected for environmental effects) from prior to selection to one generation after selection in three of the 12 cohorts examined in this study (Table 5.1), but, these were not always associated with significant episodes of selection in the current generation, as one would predict from the breeders' equation.

In fact, juvenile growth rates in red squirrels are influenced by large, genetically based maternal effects (McAdam *et al.* 2002, Chapter 2, Chapter 3), so the predicted response to selection is no longer simply the product of direct genetic effects and the strength of selection in the current generation. Instead, models of maternal effect evolution predict that the response to selection will also depend on indirect genetic effects and the strength of selection in the previous generation (Kirkpatrick & Lande 1989, Lande & Kirkpatrick 1990). Observed responses to selection of these 12 cohorts (1989 – 2000) supported both of the general predictions of models of maternal effect evolution (Lande & Kirkpatrick 1990). First, maternal effects resulted in an accelerated response to selection. The regression coefficient between the observed change in juvenile growth rates and the strength of selection in the current generation corresponded to a realised heritability ($h^2_r = 0.56 \pm 0.17$) that was six times greater than my previous estimate of heritability for this trait considering direct genetic effects alone ($h^2 = 0.09$; Chapter 2). This realised heritability also exceeded a previous estimate of heritability including indirect genetic effects ($h^2_t = 0.33$;, Chapter 2), although not significantly.

Second, there was a positive correlation between the response of juvenile growth rates in the current generation and the strength of selection in the previous generation, providing empirical evidence of evolutionary momentum, as predicted by models of maternal evolution (Kirkpatrick & Lande 1989, Lande & Kirkpatrick 1990). The removal of cohorts from early in the study (1989 - 1991), for which I did not have adequate data on the strength of selection in the previous generation, only improved the fit of the overall relationship.

The underestimation of the realised responses to selection in the current generation suggests that my total heritability estimate (h^2_t) might in fact be a conservative estimate of the potential for evolution in this offspring trait. This estimate of the indirect genetic contribution of litter size to the evolution of juvenile growth rates was made in a year of very high food abundance (1999; McAdam *et al.* 2002, Chapter 2). Theoretical and empirical studies have shown that the potential response to selection can depend on the environmental context within which genetic variation is expressed (see Hoffmann & Merilä 1999). Results from cross-fostering experiments in poor food conditions (2000) suggested that direct and indirect genetic effects and their positive covariance in this population were greater in poor than in high food conditions (Chapter 3). Furthermore, this initial estimate of the potential response of growth rates to selection corrected linear growth rates for correlation with the initial mass measurement (McAdam *et al.* 2002, Chapter 2) to avoid potential confounding effects of persistent prenatal maternal effects. This technique, however, may be overly conservative if prenatal and postnatal growth rates are positively genetically correlated. Estimates of the potential response to selection from uncorrected linear growth rates (h^2_t = 0.53 – 0.70; Chapter 3) were more similar to the realised heritability of this trait (h^2_r = 0.56).

In addition to providing support for the two general predictions of a maternal effects model, the observed responses to selection also allowed me to estimate realised maternal effect parameters. I used a simple model in which litter size was included as the only maternal effect (Kirkpatrick & Lande 1989, Kirkpatrick & Lande 1992) to calculate maximum likelihood estimates of the maternal effect coefficient and the direct-maternal genetic correlation based on observed responses to selection of these 12 cohorts. The maximum likelihood estimate of the maternal effect coefficient (m = - 0.30) was similar to both a previous estimate of this maternal parameter from previous cross-fostering experiments (m = - 0.29; Chapter 3) and the effects of experimental litter size manipulations in this population (m = - 0.21 to – 0.27; Humphries & Boutin 2000). Somewhat surprisingly, the maximum likelihood estimate of the realised genetic correlation between direct and maternal genetic effects on offspring growth far exceeded one (r_{mo} = - 3.1). Previous estimates of the direct-maternal genetic correlation from cross-fostering experiments have also exceeded one (McAdam *et al.* 2002, Chapter 2, Chapter 3) and may reflect the contribution of additional maternal effects that also contribute positively to the evolution of this offspring trait. Combinations of a direct-maternal genetic correlation less than zero and a maternal effect coefficient between – 0.25 and – 0.55 resulted in a significant improvement in the fit of the observed data over a model with no maternal effects (Figure 5.3), suggesting that maternal effects made a significant contribution to the response of this juvenile trait to selection. Predictions from this simple maternal effect model, using the maximum likelihood estimates of m and r_{mo}, explained 57% of the variation in observed responses to selection across these 12 cohorts and captured the overall direction and magnitude of the observed response to selection of this juvenile trait. Litter size explains only 43% of the maternal variation in juvenile growth rates (McAdam *et al.* 2002, Chapter 2), so there are clearly other maternal traits that influence the response of growth rates to selection. Despite its simplicity this model did an adequate job of capturing the dynamics of juvenile growth rates over this 12-year period.

Maternal effects have generated a great deal of interest in recent years as a result of both their widespread phenotypic influences and their potentially unusual and important implications for evolutionary dynamics. Juvenile red squirrels experience large genetically based maternal effects. Here I have shown that these indirect genetic effects have important influences on the evolutionary dynamics of this juvenile trait in a natural population.

5.5 REFERENCES

Agrawal, A. F., Brodie, E. D., III & Brown, J. 2001. Parent-offspring coadaptation and the dual genetic control of maternal care. *Science,* **292**: 1710-1712.

Berteaux, D. and S. Boutin. 2000. Breeding dispersal in female North American red squirrels. *Ecology,* **81**: 1311-1326.

Byers, D. L., Platenkamp, G. A. J. & Shaw, R. G. 1997. Variation in seed characters in *Nemophila menziesii* : evidence of a genetic basis for maternal effects. *Evolution,* **51**: 1445-1456.

Falconer, D. S. and Mackay, T. F. C. 1996. *Introduction to Quantitative Genetics.*, 4th edn. Longman, Harlow, UK.

Grant, P. R. & Grant, B. R. 1995. Predicting microevolutionary response to directional selection on heritable variation. *Evolution,* **49**: 241-251.

Grant, P. R. & Grant, R. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science,* **296**: 707-711.

Hilborn, R. and M. Mangel. 1997. *The Ecological Detective: confronting models with data.* Princeton University Press, Princeton, NJ.

Hoffmann, A. A. & Merilä, J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution,* **14**: 96-101.

Humphries, M. M. & Boutin, S. 2000. The determinants of optimal litter size in free-ranging red squirrels. *Ecology,* **81**: 2867-2877.

Hunt, J. & Simmons, L.W. 2002. The genetics of maternal care: Direct and indirect genetic effects on phenotype in the dung beetle *Onthophagus taurus. Proceedings of the National Academy of Science, USA* **99**, 6828-6832.

Kirkpatrick, M. & Lande, R. 1989. The evolution of maternal characters. *Evolution,* **43**: 485-503.

Kirkpatrick, M. & Lande, R. 1992. The evolution of maternal characters: Errata. *Evolution,* **46**: 284.

Lande, R. & Arnold, S. J. 1983. The measurement of selection on correlated characters.

Evolution, **37**: 1210-1226.

Lande, R. & Kirkpatrick, M. 1990. Selection response in traits with maternal inheritance. *Genetical Research,* **55**: 189-197.

McAdam, A. G. & Boutin, S. *in press.* Variation in viability selection among cohorts of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Evolution.*

McAdam, A. G., Boutin, S., Réale, D. & Berteaux, D. 2002. Maternal effects and the potential for evolution in a natural population of animals. *Evolution,* **56**: 846-851.

Merilä, J., Kruuk, L. E. B. & Sheldon, B. C. 2001. Cryptic evolution in a wild bird population. *Nature,* **412**: 76-79.

Mousseau, T. A. and C. W. Fox. 1998. *Maternal Effects as Adaptations.* Oxford University Press, New York.

Rauter, C. M. & Moore, A. J. 2002. Evolutionary importance of parental care performance, food resources, and direct and indirect genetic effects in a burying beetle. *Journal of Evolutionary Biology,* **15**: 407-417.

Réale, D., Berteaux, D., McAdam, A. G. & Boutin, S. *in press.* Lifetime selection on heritable life history traits in a natural population of red squirrels. *Evolution.*

Réale, D., McAdam, A. G., Boutin, S. & Berteaux, D. 2003. Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London, Series B,* **270**: 591-596.

Roach, D. A. & Wulff, R. D. 1987. Maternal effects in plants. *Annual Review of Ecology* *and Systematics,* **18**: 209-235.

Roff, D. A. 1997. *Evolutionary Quantitative Genetics.* Chapman & Hall, New York.

Rossiter, M. 1996. Incidence and consequences of inherited environmental effects. *Annual Review of Ecology and Systematics,* **27**: 451-476.

Thiede, D. A. 1998. Maternal inheritance and its effect on adaptive evolution: a quantitative genetic analysis of maternal effects in a natural plant population. *Evolution,* **52**: 998-1015.

Wolf, J. B., Brodie, E. D., III, Cheverud, J. M., Moore, A. J. & Wade, M. J. 1998. Evolutionary consequences of indirect genetic effects. *Trends in Ecology and Evolution,* **13**: 64-69.

Table 5.1. Observed responses of juvenile growth rates (g/day) to one generation of selection for cohorts of red squirrels born between 1989 and 2000. Growth rates prior to selection is the average (± SE) of all offspring born in each cohort, while the growth rate after selection is the average of offspring born to mothers from each cohort. The standardised directional selection gradient (β'; Lande & Arnold 1983) for each cohort is also indicated (from Chapter 4). Significant selection gradients are indicated by asterisks.

	Prior to selection		After selection		Response			Selection
Cohort	*n*	*mean* ± *se*	*n*	*mean* ± *se*		*t*	*P*	β'
1989	32	1.49 ± 0.09	69	1.68 ± 0.05	0.195	1.9	0.06	0.78
1990	33	1.76 ± 0.09	53	1.93 ± 0.06	0.166	1.5	0.13	0.16
1991	98	1.76 ± 0.04	165	1.71 ± 0.04	- 0.048	- 0.9	0.36	0.36*
1992	117	1.56 ± 0.04	103	1.91 ± 0.06	0.351	4.8	< 0.001	1.06*
1993	131	1.97 ± 0.04	251	1.90 ± 0.03	- 0.064	- 1.2	0.22	0.01
1994	160	1.88 ± 0.04	66	2.00 ± 0.07	0.126	1.6	0.10	0.36
1995	151	1.89 ± 0.04	180	1.87 ± 0.03	- 0.019	- 0.3	0.74	- 0.18*
1996	70	1.80 ± 0.05	54	1.77 ± 0.05	- 0.031	- 0.4	0.66	0.09
1997	155	1.92 ± 0.03	190	1.75 ± 0.04	- 0.163	- 3.3	0.001	- 0.10
1998	88	1.88 ± 0.07	237	1.72 ± 0.03	- 0.165	- 2.3	0.02	0.17
1999	211	1.81 ± 0.03	30	1.86 ± 0.09	0.054	0.6	0.58	0.87*
2000	57	1.65 ± 0.06	11	1.62 ± 0.14	- 0.026	- 0.2	0.87	0.65

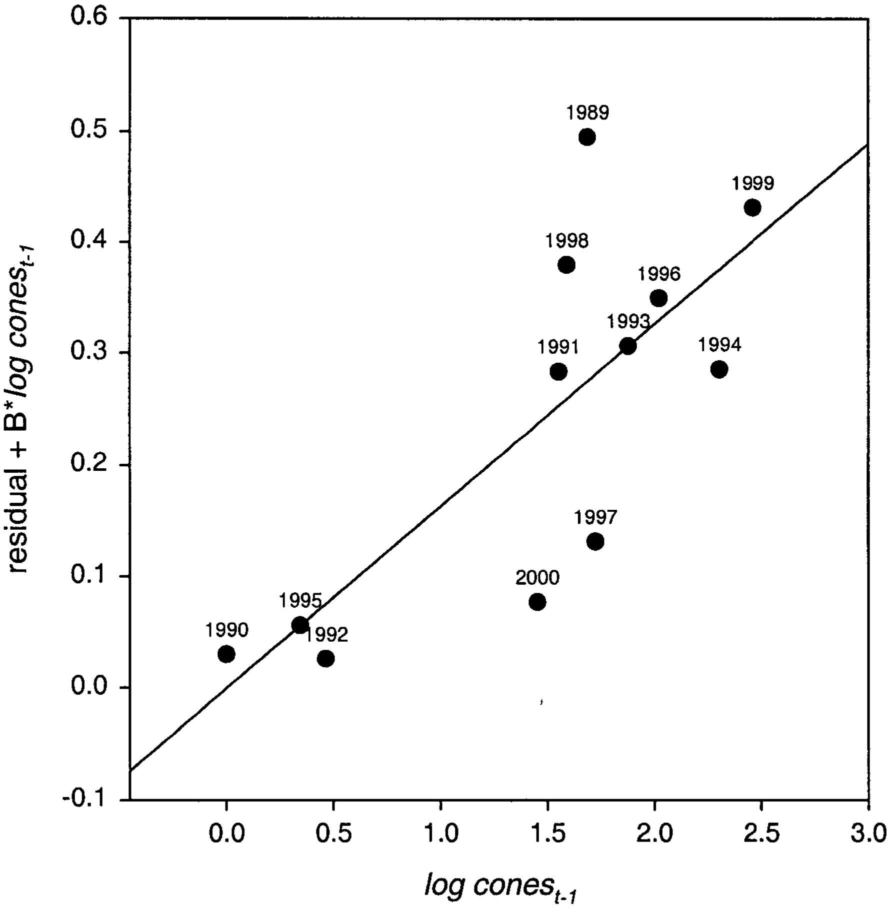

Figure 5.1. Partial regression on the effects of the abundance of spruce cones produced in the previous fall (*log cones*$_{t-1}$) on the environmental deviations of juvenile growth rates the following spring. The overall regression model also included the effect of the abundance of cones produced in the current year (*log cones*$_t$; $n = 12$ years, $R^2 = 0.72$, $P = 0.003$).

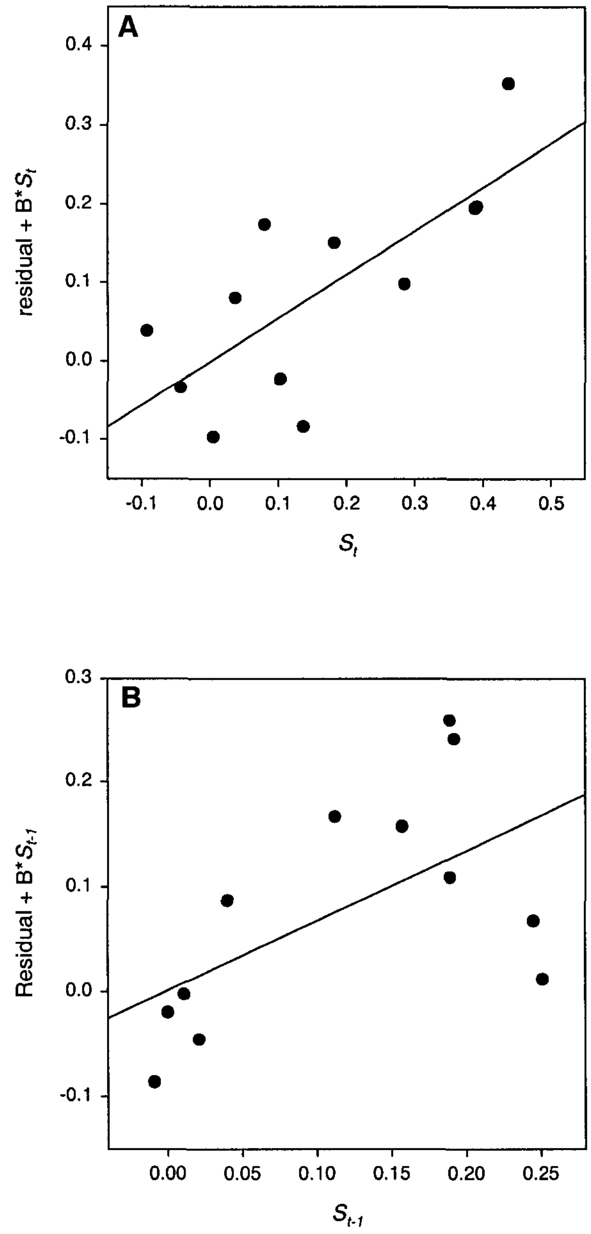

Figure 5.2. Partial regression plots of the effects of the strength of directional selection in the current generation (A) and the strength of selection in the previous generation (B) on the evolutionary response of juvenile growth rates in red squirrels.

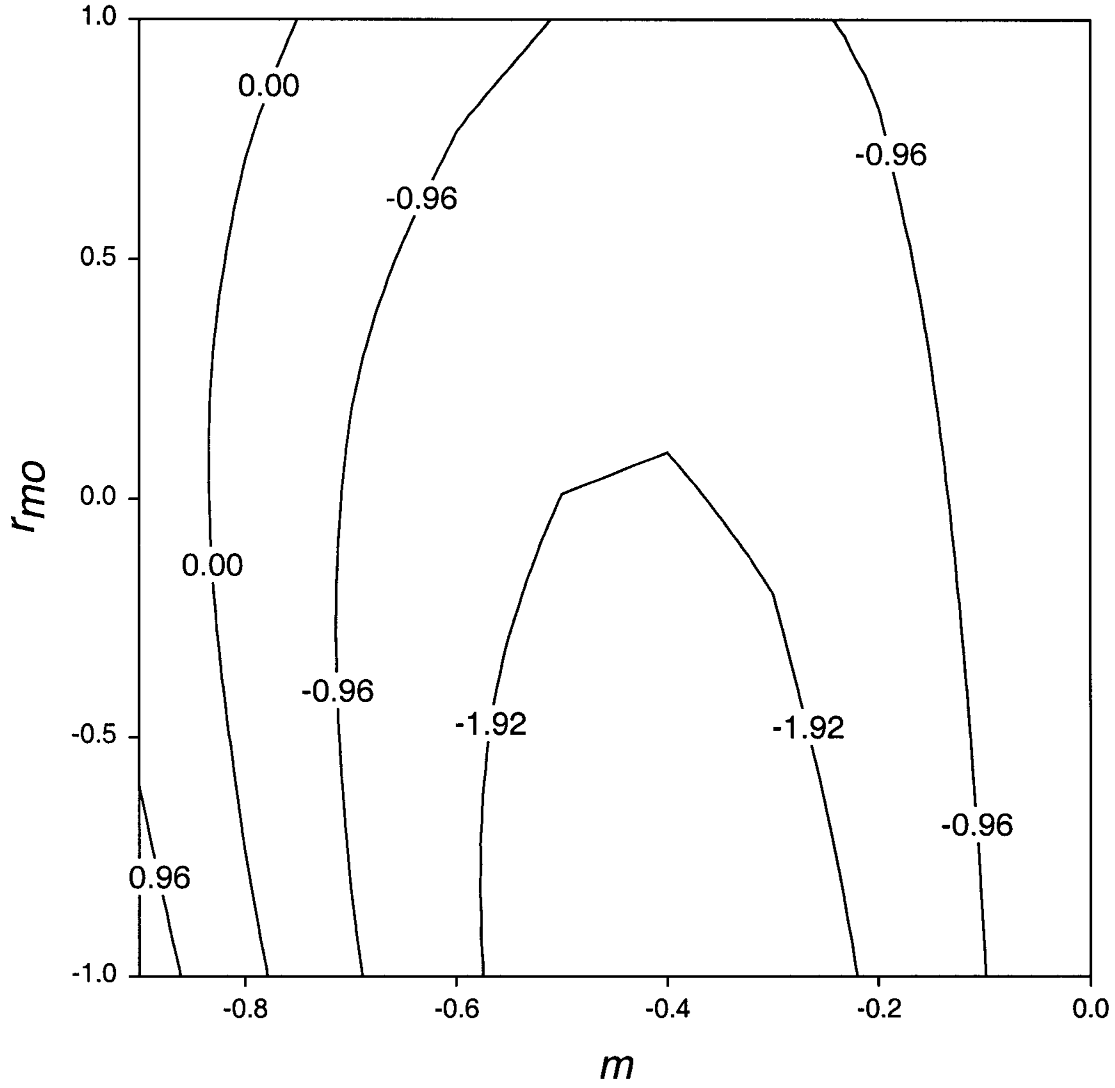

Figure 5.3. Likelihood surface of the strength of the maternal effect coefficient (m) and the direct-maternal genetic correlation (r_{mo}). Contours represent changes in negative log-likelihoods relative to the model in which maternal effects are absent ($m = 0$), where increasingly negative contours represent more likely combinations of parameters. Values of m and r_{mo} resulting in a significant improvement in the fit of predicted responses to selection over predicted responses in the absence of maternal effects fall within the – 1.92 region (i.e., $\chi^2 > 3.84$, $P < 0.05$).

6.0 GENERAL DISCUSSION AND CONCLUSIONS

6.1 MATERNAL EFFECTS AND MICROEVOLUTION

Maternal effects are widespread (Mousseau & Fox 1998) and have been predicted to result in dramatic and sometimes counterintuitive evolutionary dynamics if they are themselves heritable (Kirkpatrick & Lande 1989). While the genetic basis to maternal effects has been measured frequently in the laboratory or in agricultural populations subject to artificial selection (see Roff 1997), there have been very few estimates of indirect genetic effects in natural populations. As a result, the influence of maternal effects on evolutionary dynamics in natural populations has not been determined previously.

In this study, I provided a comprehensive examination of the influence of genetically based maternal effects on the dynamics of a juvenile trait in the wild. Growth rates of juvenile red squirrels were found to be heritable ($h^2 = 0.09$), but also experienced large genetically based maternal effects that together result in a three to five-fold increase in the potential response to selection compared to direct genetic effects alone (Chapter 2, Chapter 3). Estimates of direct genetic and maternal variation, as well as the covariance between direct and maternal genetic effects were larger in low food conditions than when ample food was available (Chapter 3). These direct and indirect sources of genetic variation resulted in a greater overall potential for evolution in poor food conditions than in good conditions for offspring growth (Chapter 3).

In addition to possessing significant levels of direct and indirect genetic variation, this offspring trait has also been subject to episodes of directional selection (Chapter 4) that were among the strongest yet recorded for a natural population (Kingsolver *et al.* 2001). Strong directional selection on juvenile growth rates was the result of potentially rare combinations of environmental conditions acting during the period of maternal dependence and after weaning. As a result, the prediction of future selection events (see also Grant & Grant 2002) will be extremely difficult. However, the quantification of both the sources of genetic variation in nestling growth rates (Chapter 2, Chapter 3) and the strength of directional selection on this trait (Chapter 4) provided the necessary and sufficient conditions for predicting the responses to observed selection on growth rates. The strong temporal variation in selection, combined with the high degree of generation overlap in this population made omnibus predictions of the response to selection across multiple generations impossible. Instead, I made a series of stepwise single generation predictions for each of 12 cohorts.

In the absence of maternal effects, the response to selection (R) can be predicted as the product of heritability (h^2) and selection (S) using the breeders' equation ($R = h^2 S$; Falconer & Mackay 1996). Three of the 12 cohorts of squirrels examined in this study exhibited significant changes in growth rates across one generation of selection, but significant responses were not always associated with significant selection as would be expected from the breeders' equation. In the presence of genetically based maternal effects, however, the response to selection in the current generation also depends on the strength of selection in the previous generation (Kirkpatrick & Lande 1989, Lande & Kirkpatrick 1990). These observed responses to selection qualitatively supported both of the general predictions of a model of maternal effect evolution (Kirkpatrick & Lande 1989, Lande & Kirkpatrick 1990). First, positive, genetically based maternal effects resulted in an accelerated response to selection; observed responses were more than five times greater than predictions based on direct genetic effects alone. Second, there was a positive correlation between the response of growth rates in the current generation and the strength of selection in the previous generation, providing empirical evidence of evolutionary momentum, as predicted by models of maternal evolution (Kirkpatrick & Lande 1989, Lande & Kirkpatrick 1990). Finally, maximum likelihood estimates of maternal effect parameters were significantly different from zero providing quantitative evidence that maternal effects made a significant contribution to the response of this juvenile trait to selection.

6.2 EVOLUTIONARY STASIS

Despite ample evidence of significant levels of both genetic variation (e.g., Weigensberg & Roff 1996), and directional selection (Endler 1986, Hoekstra *et al.* 2001, Kingsolver *et al.* 2001), studies have rarely documented contemporary responses to selection in the wild (Merilä *et al.* 2001b, but see Hairston & Walton 1986, Reznick *et al.* 1997, Merilä *et al.* 2001a, Grant & Grant 2002, Réale *et al.* 2003). The paucity of observed responses that are consistent with predictions of the breeders' equation may be due in part to biased estimates of heritability (Merilä *et al.* 2001b). By far, the most common technique for estimating heritabilities in the wild is parent-offspring regression, which potentially confounds heritabilities with environmental covariances, and maternal effects (Lynch & Walsh 1998). For example, positive environmental covariances between parent and offspring rearing locations were found to have inflated the heritability of lay date in great tits (*Parus major*) in Wytham Wood, United Kingdom by 50% (van der Jeugd & McCleery 2002).

The effect of environmental autocorrelation on parent-offspring covariances also has potentially important implications for the estimation of maternal effects. For species without paternal care, maternal effects are frequently estimated as the difference between mother-offspring and father-offspring covariances (see Lynch & Walsh 1998). However, in species with male-biased dispersal (e.g., most mammals; Pusey 1987), positive spatial autocorrelation in environmental effects will result in environmentally inflated mother-offspring covariances and the spurious appearance of maternal effects. Cross-fostering designs experimentally disrupt environmental covariances between parents and offspring and estimates of maternal effects from cross-fostering experiments are not derived from the difference between mother-offspring and father-offspring covariances. Estimates of heritability and maternal effects from cross-fostering experiments, therefore, are less likely to be confounded by environmental autocorrelation.

Levels of both genetic and environmental variation also may differ between favourable and unfavourable environmental conditions (Hoffmann & Merilä 1999). As a result, heritabilities estimated in one environment may not be appropriate for estimating the response to selection in a different environment. In red squirrels, years in which spruce cones were scarce had increased direct and indirect contributions to the potential for evolution (Chapter 3); however, there did not appear to be any difference in the response to selection between cohorts experiencing high or low food.

A third 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.* 2001b). Relatively few studies have replicated estimates of selection either in space or time (see Kingsolver *et al.* 2001) and a negative relationship between the strength of viability selection and the time period over which this selection is measured suggests that strong episodes of viability selection are often followed by periods of stasis or reversal in the direction of selection (Hoekstra *et al.* 2001). In this study, there was clear evidence of temporal variation in the strength and direction of selection across the 13 years of data for this same population (Chapter 4). The quantification of selection over only 3 or 4 seasons, typical of a granting cycle or PhD program, might have led to very different conclusions about the strength or direction of selection on this juvenile trait. The unpredictability of selection in natural populations (see also Grant & Grant 2002) underscores the importance of long-term population monitoring to studies of microevolution in the wild.

Finally, significant genetic responses to selection may be masked by concomitant changes in environmental conditions (Grant & Grant 1995, Merilä *et al.* 2001a). As a result, phenotypic responses to selection can only be expected to reflect underlying genetic changes if environmental sources of variation are controlled or corrected across generations. Most female red squirrels breed in several seasons and the abundance of spruce cones varies annually over three orders of magnitude (Figure 4.1). I, therefore, used multiple observations per female to correct for environmental effects on growth. As expected, these annual environmental effects were due mostly to the abundance of spruce cones produced in the previous fall (Figure 5.1). It is likely that these large environmental effects of spruce cone abundance would have masked the response of uncorrected growth rates to selection.

6.3 THE IMPORTANCE OF STUDYING NATURAL POPULATIONS

Great advances have been made in the field of quantitative genetics through the study of both model organisms in the laboratory, and agricultural species. Captive populations, however, are characterized by consistent selection, either directly through generations of artificial selection in agricultural systems, or indirectly through highly controlled laboratory environments. The red squirrel system clearly illustrates how environmental conditions, selection and genetic variation can interact in a natural evolutionary context. Red squirrels in this population feed almost exclusively on the seeds of white spruce cones, which vary annually in abundance over three orders of magnitude (Humphries & Boutin 2000; Figure 4.1). This large temporal variation in the abundance of food has been found to have important implications for the life history of red squirrels (Berteaux & Boutin 2000, Humphries & Boutin 2000, Réale *et al.* 2003). Here I have also shown that estimates of both the potential for evolution (Chapter 3) and the strength and direction of selection on juvenile growth rates (Chapter 4) are related to the production of spruce cones.

In agricultural systems, direct-maternal genetic covariances have been typically found to be negative (Roff 1997), as would be expected of traits subject to consistent directional selection (Roff 1997). In red squirrels, however, the direct-maternal genetic covariance for growth in body mass was clearly positive and is likely maintained by temporal variation in selection related to food abundance. In addition, the supplementation of squirrels with *ab libitum* food resulted in both a large reduction in maternal effects on growth and the complete elimination of this large positive direct-maternal genetic covariance. The contributions of maternal effects to the potential for evolution, particularly those related to the acquisition of food resources, may be greatly underestimated under controlled conditions of captivity.

Natural selection and sources of genetic variation are not static properties of populations. The strength and mode of selection influence levels of direct and indirect genetic variation through the fixation of high-fitness alleles and the loss of low-fitness alleles from the population. Conversely, direct and indirect sources of genetic variation together produce the phenotypic variation on which natural selection acts. As a result, the contemporary genetic architecture of natural populations reflects many generations of interactions between heritability and selection. Studies of natural populations, therefore, are essential for understanding the ways in which heritable variation and natural selection interact with one another and together result in evolutionary changes across generations.

6.4 CONCLUSIONS

The unique ability to cross-foster newborn red squirrels together with 16 years of detailed individual-based data for the same population allowed me to perform a comprehensive analysis of the contribution of maternal effects to the evolution of a juvenile trait in the wild. Juvenile growth in body mass was found to be heritable, but also experienced large genetically based maternal effects. Both direct and indirect contributions to the potential for evolution increased in conditions of low food abundance. In addition, this trait has been subject to episodes of very strong directional selection over the past 13 years. Observed responses of growth rates across one generation of selection were much greater than would have been predicted based on direct genetic effects alone but were consistent with an additional indirect contribution of maternal effects to the evolutionary dynamics of this juvenile trait.

It is clear that not all traits will be influenced by maternal effects to the same extent. For example, juvenile growth in body size experienced much stronger direct genetic effects than growth in body mass and only a small indirect genetic contribution to the potential for evolution (Chapter 2). The influence of maternal effects on the response to selection of growth in body mass, therefore, represents an example of the potential importance of maternal effects to evolutionary dynamics in the wild. The generality of these results will only be revealed through the further investigation of the influence of maternal effects on evolutionary dynamics in natural systems.

6.5 REFERENCES

Berteaux, D. and S. Boutin. 2000. Breeding dispersal in female North American red squirrels. *Ecology,* **81**: 1311-1326.

Endler, J. A. 1986. *Natural Selection in the Wild.* Princeton University Press, Princeton, New Jersey.

Falconer, D. S. and Mackay, T. F. C. 1996. *Introduction to Quantitative Genetics.*, 4th edn. Longman, Harlow, UK.

Grant, P. R. & Grant, B. R. 1995. Predicting microevolutionary response to directional selection on heritable variation. *Evolution,* **49**: 241-251.

Grant, P. R. & Grant, R. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science,* **296**: 707-711.

Hairston, N. G. Jr. & Walton, W. E. 1986. Rapid evolution of a life history trait. *Proceedings of the National Academy of Science, USA,* **83**: 4831-4833.

Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hoang, A., Hill, C. E., Beerli, P. & Kingsolver, J. G. 2001. Strength and tempo of directional selection in the wild. *Proceedings of the National Academy of Science, USA,* **98**: 9157-9160.

Hoffmann, A. A. & Merilä, J. 1999. Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution,* **14**: 96-101.

Humphries, M. M. & Boutin, S. 2000. The determinants of optimal litter size in free-ranging red squirrels. *Ecology,* **81**: 2867-2877.

van der Jeugd, H. P. & McCleery, R. 2002. Effects of spatial autocorrelation, natal philopatry and phenotypic plasticity on the heritability of laying date. *Journal of Evolutionary Biology,* **15**: 380-387.

Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hill, C. E., Hoang, A., Gibert, P. & Beerli, P. 2001. The strength of phenotypic selection in natural populations. *American Naturalist,* **157**: 245-261.

Kirkpatrick, M. & Lande, R. 1989. The evolution of maternal characters. *Evolution,* **43**: 485-503.

Lande, R. & Kirkpatrick, M. 1990. Selection response in traits with maternal inheritance. *Genetical Research,* **55**: 189-197.

Lynch, M. and B. Walsh. 1998. *Genetics and Analysis of Quantitative Traits.* Sinauer

Associates, Sunderland, Massachusetts.

Merilä, J., Kruuk, L. E. B. & Sheldon, B. C. 2001a. Cryptic evolution in a wild bird population. *Nature,* **412**: 76-79.

Merilä, J., Sheldon, B. C. & Kruuk, L. E. B. 2001b. Explaining stasis: microevolutionary studies in natural populations. *Genetica,* **112-113**: 199-222.

Mousseau, T. A. and C. W. Fox. 1998. *Maternal Effects as Adaptations.* Oxford University Press, New York.

Pusey, A. 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology and Evolution*, **2**: 295-299.

Réale, D., McAdam, A. G., Boutin, S. & Berteaux, D. 2003. Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London, Series B,* **270**: 591-596.

Reznick, D. N., Shaw, F. H., Rodd, F. H. & Shaw, R. G. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science,* **275**: 1934-1937.

Roff, D. A. 1997. *Evolutionary Quantitative Genetics.* Chapman & Hall, New York.

Weigensberg, I. & Roff, D. A. 1996. Natural heritabilities: can they be reliably estimated in the laboratory? *Evolution,* **50**: 2149-2157.