

QUANTITATIVE GENETICS, SELECTION, MATE CHOICE AND RED SQUIRREL
BEHAVIOR IN A FLUCTUATING ENVIRONMENT

by

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ABSTRACT

QUANTITATIVE GENETICS, SELECTION, MATE CHOICE AND RED SQUIRREL BEHAVIOR IN A FLUCTUATING ENVIRONMENT

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Consistent individual differences in behaviour, and behavioural correlations within and across contexts, are referred to as animal personalities. These patterns of variation have been identified in many animal taxa and are likely to have important ecological and evolutionary consequences. My dissertation focuses on understanding how individual behavioral variation in free-living North american red squirrels (*Tamiasciurus hudsonicus*) might be maintained in the face of natural selective pressures. I investigated the proximate sources of variation in red squirrel behavior by estimating sources of genetic, maternal and environmental variation, and the ultimate effect of behavior on reproductive success. I used a Bayesian animal model approach to estimate genetic parameters for aggression, activity and docility and found support for low heritabilities (0.08-0.12), and cohort effects (0.07-0.09), as well as low to moderate maternal effects (0.07-0.15) and permanent environmental effects (0.08-0.16). I also found evidence of a substantial positive genetic correlation (0.68) and maternal effects correlation (0.58) between activity and aggression, providing evidence of genetically based behavioral correlations in red squirrels. These results provide evidence for the presence of heritable variation in red squirrel behavior, but also emphasize the role of other sources of variation, including maternal effects, in shaping patterns of variation and covariation in behavioral traits. I then tested the hypothesis that ecological changes through time lead to fluctuating selection, which maintains variation in behavioral traits. Linear selection gradients on red squirrel dam aggression and activity significantly fluctuated in sign across years depending on the level of competition among juveniles. Selection on aggression and activity also differed among components of fitness, between the sexes and included nonlinear components between and within traits that also changed through time. These results suggest that repeatable and heritable individual dif-

ferences in red squirrel behavior could be maintained by complex fluctuations in natural and sexual selection. Finally, I tested the hypotheses that mating chases provide the opportunity for both female and male red squirrels to select for context-dependent good genes and complementary genes for their offspring's recruitment. Specifically, I predicted that aggressive mates would be preferred in high-juvenile competition years, but disfavored in low competition years, and that mate choice for docility would be disassortative with low-docility squirrels preferring high-docility mates and vice-versa. I did not find support for adaptive context-dependent mate choice by females, but I did find support for male mate choice for complementary genes that was mediated through which female mating chase males attended. Male red squirrels attended mating chases disassortatively by docility, which would enhance fitness due to stabilizing selection on docility during juvenile recruitment. I also found evidence for post-copulatory selection for less aggressive males. These results highlight that even in systems with very high operational sex ratios, male choice is a factor that needs to be considered. In this dissertation I have shown that behavioral traits in red squirrels are important with strong evolutionary implications. Red squirrel behavior is heritable, subject to maternal effects and under strong selection that varies with environmental fluctuation and across components of reproductive success. This research has provided strong evidence that fluctuating selection could maintain variation in behavior. These findings provide a foundation for future work to elucidate mechanisms and continue exploring how and why behavioral variation is maintained.

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Chapter 1

GENERAL INTRODUCTION

Darwinian natural selection presents a paradox that if only the fittest survive, then why do less fit genotypes persist, or why does selection not erode all genetic variation? The evolutionary maintenance of variation in fitness-related traits has been a major topic in evolutionary biology since it was first popularized by Fisher (Fisher, 1930; Barton and Turelli, 1989; Houle, 1992; Merilä and Sheldon, 2000; Kruuk et al., 2008). A number of recent meta-analysis have provided some insights (*e.g.* linear selection on survival is generally weaker than on fecundity; Kingsolver et al. 2012) but some expected generalized patterns have not appeared or are subject to interpretation. For example, Siepielski et al. (2011) found that temporal fluctuations in selection was prevalent, but Morrissey and Hadfield (2012) disagree and argue that most of the temporal variation can be explained by measurement error. Additionally, relatively few studies have examined environmental correlates of selection, which often requires selection measured over multiple years, or selection on behavior (Kingsolver et al., 2012). More detailed analysis of the interaction between ecology and evolution will be necessary to understand how evolution maintains genetic variation.

Behavioral ecologists are increasingly recognizing that individual animals display consistently different behavioral tendencies across time and contexts. The study of individual behavioral consistency is not new per se, and is a major area of research in a number of behavioral fields (*e.g.*, personality in humans and domesticated animals or behavioral genetics; Fraser et al. 2001; Gosling and John 1999; Henderson 1986; Boissy 1995). However, until recently most studies in behavioral ecology did not address variation at an individual level or across multiple contexts, instead comparing population means within a single context (Sih and Bell, 2008). Behavior has been shown to be under selection in a variety of taxa (reviewed in Smith and Blumstein (2008)) and can be considered a trait closely related to fitness.

1.1 What is animal personality?

The study of individual animal behavior from an evolutionary ecology perspective started to gain momentum around the turn of the millennium while being promoted by a number influential reviews (Wilson et al., 1994; Gosling, 2001; Sih et al., 2004*b*; Réale et al., 2007, 2010*a*). As a result, there has been a rapid increase in research quantifying and comparing behavioral variation both within and between individuals, but this progress has also produced a daunting array of terminology and concepts (Figure 1.1). A number of recent articles have provided useful synopses of terminology (*e.g.* Réale et al. 2007, 2010*a*; Sih et al. 2004*b*; Stamps and Groothuis 2010; Sih and Bell 2008), but inconsistent use and overlap remains.

The terms ‘personality trait’, ‘behavioral syndrome’, and ‘animal personality’ are the most common terms but are used interchangeably, and not consistently in the literature. For example, ‘behavioral syndrome’ was first used (Stamps, 1991) and is most often used to describe a correlation between two distinct behavioral traits (*e.g.* aggression and boldness), but in a major review Sih et al. (2004*b*) defined behavioral syndrome as a “a suite of correlated behaviors across multiple (two or more) observations”; and clarified in Sih and Bell (2008) that this includes the same behavior across multiple contexts (*e.g.* activity in low-resource environments and activity in high-resource environments). Thus ‘behavioral syndrome’ can be used to describe a very broad array of behavioral phenomena. Similarly, ‘behavior’ describes a very broad array of quantifiable phenomena, and the distinction between one behavior and another is not always clear (*i.e.* is aggression towards a predator “aggression” or “boldness”?).

In any field of study consistent understanding and application of terms and the concepts that they refer to is necessary for clear scientific communication and collaboration. In new fields of biology, there may be multiple terms used for the same concept because researchers do not have a reliable framework of terminology to consult. For example, when defining “adaptation” there was great confusion regarding different conceptual meanings of the term: meanings that applied to either historical evolution or to current processes (Reeve and Sherman, 1993). The consistent application of a concept is important because it clarifies the explicit assumptions that underlie the

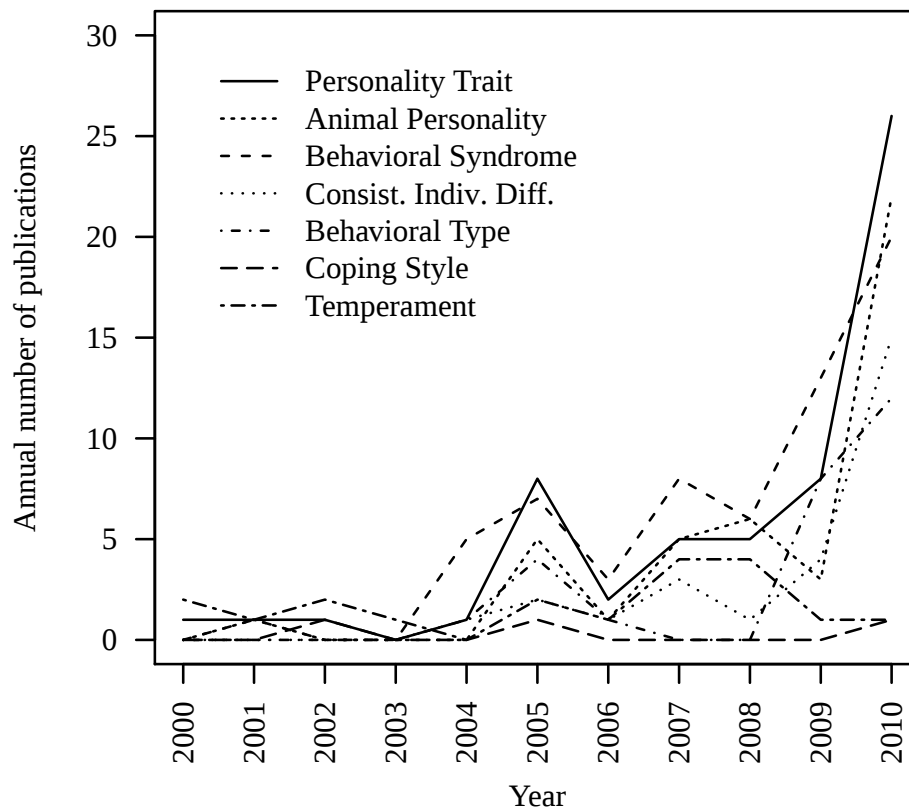


Figure 1.1: Terminology used in publications on animal personality from 2000 to 2010. Plotted are the annual number of publications containing each keyword in the title or abstract. Plural and American spelling were included in the search. I performed a search of the title, abstract and keywords of publications in behavioral journals in the Web of Science, or Science Citation Index Expanded, using the terms ‘personality trait’, ‘animal personality’, ‘behavioral syndrome’, ‘consistent individual differences’, ‘behavioral type’, ‘coping style’, and ‘temperament’. The journals searched were: Animal Behavior, Behavioral Ecology, Behavioral Ecology and Sociobiology, Behavior, Behavioral Processes, American Naturalist, Biology Letters, Ecology, Ecology Letters, Evolution, Functional Ecology, Journal of Animal Ecology, Evolution, Functional Ecology, Journal of Animal Ecology, Journal of Evolutionary Biology, Proceedings of the Academy of Sciences of the United States of America, Proceedings of the Royal Society B, Trends in Ecology and Evolution, Quarterly Review of Biology, Biological Reviews and Philosophical Transactions of the Royal Society B.

ideas (Fauth et al., 1996). The current state of terminology use in the animal personality literature is in danger of confusing those outside of the field and newcomers who rely on the terminology to organize the behavioral phenomenon being studied. For these reasons I use these terms sparingly and instead focus on describing the behavioral phenomenon that was quantified.

The fundamental principal of all personality related concepts is that individual differences in behavior exist and are quantifiable. Behavioral traits can then be treated as any other trait and all the tools evolutionary biologists have developed can be applied. Furthermore behavioral traits can be used to study many of the broader questions in ecological and evolutionary biology.

Section Fluctuating environments

Environmental heterogeneity on temporal and spatial scales is a widespread if not universal factor the evolutionary history of populations (Bell, 2010). Due to climactic, ecological and social variation individuals encounter many different environments and situations throughout their life. Evolutionary theory predicts that individuals should exhibit optimal behaviors in each situation and therefore should be behaviorally flexible when the optimal behavior varies across the situations they encounter (Stearns, 1989). However, evidence shows that individuals do not adjust behaviorally as much as we would expect indicating that behavioral plasticity can be limited (Sih et al., 2004b; Réale et al., 2007; Bell et al., 2009). Perhaps one of the more puzzling facets of behavioral consistency is that consistency may be maladaptive. For example, aggressiveness towards competitors may benefit an individual via access to resources while a similar level of aggressiveness towards predators might increase chance of death. In a high resource year active individuals may be able to consume more resources than those that are less active, but a consistently active and energetic individual is more likely to starve when resources are scarce than an individual that can tailor its behavior to the amount of resources available. The fact that variation in behavioral consistency is maintained in populations makes it a particularly interesting trait with which to study the maintenance of variation.

1.2 North American red squirrels (*Tamiasciurus hudsonicus*)

The recent finding of individual variation in activity, aggressiveness and docility in red squirrels (Boon et al., 2007, 2008) provides an excellent opportunity to study the proximate (*i.e.* genetic) and ultimate factors of behavioral variation in a species for which there exists strong ecological variation. Boon et al. showed that activity (measured via open-field trials) was correlated with nestling growth rates (grams per day) and that aggressiveness (measured via mirror-image stimulation) was correlated with pup survival in nest and overwinter (Boon et al., 2007). These results show that personality is associated with other phenotypes and affects fitness. Importantly, the magnitude and direction of these correlations varied across years (Boon et al., 2007).

Northern populations of red squirrels experience a high degree of annual fluctuation in available resources due to the synchronization of white spruce (*Picea glauca*) cone production. Spruce trees typically mast once every 4-6 years and drive population density fluctuations in squirrels with low density preceding the mast and peak density following the mast. The combination of low density and high resources during a mast event results in weak selection for individual quality traits in juvenile squirrels (*e.g.* parturition date, growth rate), but in the year following a mast selection for quality traits is high due to high densities and scarce resources (Andrew McAdam, in prep). Additionally, this variation in environmental conditions experienced by squirrels in early life has been shown to have long lasting fitness consequences (Descamps et al., 2008). These cohort effects may result in differences in residual reproductive value across years that may select for different personalities (*sensu* Wolf et al. 2007). For instance the likelihood that an individual born during a mast year will survive to reproduce in a future mast year is lower than individuals born in the year after a mast. The combination of detailed life-history data (collected since 1988; McAdam et al. 2007), personalities associated with fitness and a variable and quantifiable environment make northern red squirrels an excellent model system in which to study the generation and maintenance of behavioral variation.

1.3 Outline

In this dissertation I investigate the proximate sources of variation in red squirrel behavior and the ultimate effect of behavior on reproductive success. I use the same behavioral methods as Boon et al. (described in detail in Chapter 2). In Chapter 2 I leverage the extensive pedigree that has been assembled for Kluane red squirrels to characterize the quantitative genetic source of behavioral variation in a wild population of red squirrels. I used a Bayesian animal model approach to estimate additive genetic, maternal, cohort and permanent environmental sources of (co)variation in individual behavior and showed that red squirrel behaviors are heritable and have maternal, cohort and environmental effects. Additionally, I showed that aggression and activity are genetically and maternally correlated.

I then investigated whether fluctuating selection could maintain variation in behavior (Chapter 3). I first showed that there was a significant effect of year on selection for female aggression and activity. I then estimated linear and non-linear selection gradients for annual reproductive success separately for each of 8 years to show that selection for female aggression and activity fluctuated significantly in sign. I performed a canonical rotation of the non-linear selection gradients to reveal nonlinear multivariate relationships on fitness, which exposed nonlinear stabilizing selection contrasting sire activity with docility in high juvenile-competition years. Finally, I used generalized linear mixed models to partition selection on behavior through annual reproductive success into selection through fecundity (or for males, siring success) and offspring overwinter survival. Selection on dam behavioral traits acted entirely through offspring overwinter survival, but selection on sire traits acted through siring success and offspring overwinter survival.

In my final chapter (4), I addressed a specific hypothesis that red squirrel mating chases are opportunities for female squirrels to select good genes for their offspring, and that attendance to mating chases is an opportunity for male squirrels to select good genes (Chapter 4). I did not find evidence of female mate choice, but I did find that males attended mating chases disassortatively by docility, which would enhance fitness due to stabilizing selection on docility during juvenile recruitment.

1.4 A note about writing style

Chapter 2 has been published and is reprinted in this dissertation with only changes to American English from the original British English. The title of chapter 2 remains in British English. I use “we” throughout this dissertation to recognize that this work results from collaboration with my co-authors.

Chapter 2

LOW HERITABILITIES, BUT GENETIC AND MATERNAL CORRELATIONS BETWEEN RED SQUIRREL BEHAVIOURS

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Author Contributions

R. W. Taylor and A. G. McAdam developed the concept of the paper. R. W. Taylor, A. K. Boon, and B. Dantzer collected behavioral data. All authors contributed to the collection of pedigree data. J. C. Gorrell and D. W. Coltman performed the paternity assignment. R. W. Taylor performed all other analyses and wrote the paper. All other authors provided intellectual insight and comments.

2.1 Introduction

Consistent individual differences in behavior and behavioral correlations within and across contexts have been identified in many animal taxa (Sih et al., 2004a; Réale et al., 2010a). The persistence of individual differences in behavior has stimulated much research (Gosling, 2001; Sih et al., 2004b,a; Réale et al., 2007, 2010a), because behavior was previously thought to be very labile, and have been increasingly accepted as important traits with ecological and evolutionary consequences (Réale et al., 2010a). Animal personalities have been shown to have important fitness effects in a number of systems (Dingemanse and Réale, 2005; Smith and Blumstein, 2008) and balancing selection has been proposed as a mechanism that could maintain these individual differences (Dingemanse et al., 2004; Boon et al., 2007; Wolf et al., 2007). For example, Dingemanse et al. (2004) found that adult great tit (*Parsus major*) overwinter survival was related to exploratory

behavior and that this relationship fluctuated in direction across years. Understanding how natural selection shapes patterns of variation in animal personalities, however, requires an understanding of the underlying genetic structure of these important traits.

The genetic and environmental sources of (co)variation in specific repeatable behaviors, or personality traits, have rarely been characterized for wild populations (Dochtermann and Roff, 2010), despite the widely recognized importance of genetic variation for evolutionary processes (Roff, 1997; Lynch and Walsh, 1998). A long history of study in captive animals has established that heritable variation in behavior is common in laboratory populations (Stirling et al., 2002). However, because these populations have been bred in captivity for many generations and are not exposed to the degree of environmental variation found in the wild, these heritability estimates may not be representative of wild populations (Weigensberg and Roff, 1996; Hoffmann and Merilä, 1999; Hoffmann, 2000; Conner et al., 2003). The number of studies that have measured heritability of personality traits in wild populations is small, but growing (Table 2.1), yet more estimates will be necessary before broad patterns emerge. For example, it is unclear whether certain personality traits or suits of correlated behaviors are more heritable than others, and which sources of variation in personality traits are more sensitive to changes in environmental circumstances (*i.e.*, genotype $\times$ environment, maternal $\times$ environment interactions).

Table 2.1: Heritability of behavior measured in wild populations, including those from entirely wild populations (w) and those estimated from offspring raised in captivity (c). (—) Parameter was not estimated; NS: parameter was estimated but found not significant; S: a significant genetic correlation was found between this trait and another behavioral trait.

Species	Behavior	h^2	m^2	r_G	Reference
<i>Ovis canadensis</i> (Sheep) (w)	Boldness	0.21	—	—	Réale et al. 2000
<i>Parus major</i> (Tits) (w)	Exploration	0.22 to 0.41	—	—	Dingemanse et al. 2002
<i>Gasterosteus aculeatus</i> (Sticklebacks) (c)	Activity	0.05	—	S	Bell 2005
Population 1	Aggression	0.01	—	S	
	Boldness	0.04	—	S	
Population 2	Activity	0.16	—	S	
	Aggression	0.14	—	S	Sinn et al. 2006
	Boldness	0.002	—	S	
<i>Euprymna tasmanica</i> (Squids) (c)	Boldness	0.21	NS	—	
Antipredator context	Activity	0.67	NS	—	
	Reactivity	0.89	NS	—	Dingemanse et al. 2009
Feeding context	Boldness	NS	NS	—	
	Activity	NS	NS	—	
	Reactivity	NS	NS	—	
<i>Gasterosteus aculeatus</i> (Sticklebacks) (c)	Multiple traits	0.06 to 0.27	—	S	Dingemanse et al. 2009
Antipredator context	Multiple traits	0.15 to 0.32	—	S	
<i>Sialia mexicana</i> (Bluebirds) (w)	Aggression	0.34	NS	S	Duckworth and Kruuk 2009
	Dispersal	0.52	NS	S	
<i>Ovis canadensis</i> (Sheep) (w)	Docility	0.65	NS	S	Réale et al. 2009
	Boldness	0.39	NS	S	
<i>Parus major</i> (Tits) (w)	Exploration	0.23	—	—	Quinn et al. 2009
<i>Marmota flaviventris</i> (Marmots) (w)	Vigilance	0.08	NS	NS	Blumstein et al. 2010
	Sprint speed	0.21	NS	NS	
<i>Thamnophis ordinoides</i> (Garter Snakes) (c)	Anti-predator	0.54 to 0.65	—	S	Brodie 1993

Correlated behaviors (*sensu* Sih et al. 2004*a,b*) are an important component of animal personalities and quantifying genetic correlations between behaviors will provide important insights into the functional integration of behaviors (Cheverud, 1996) and potential constraints on behavioral evolution. A genetic behavioral correlation would suggest that independent adaptation of the behavioral traits involved could be constrained (Roff and Fairbairn 2007; but see Blows and Hoffmann 2005). Most previous studies have quantified only phenotypic correlations between behavioral traits (Dochtermann and Roff, 2010), but the degree or sign of a phenotypic correlation may not match the underlying genetic correlation (Roff, 1997; Kruuk et al., 2008). So far, there have been few estimates of genetic correlations among personality traits in wild populations but those that have estimated genetic correlations have generally found results consistent with patterns of variation observed at the phenotypic level (Bell, 2005; Réale et al., 2009; Blumstein et al., 2010). For example, a number of studies have found positive phenotypic correlations between aggression and boldness (Koolhaas et al., 1999; Sih et al., 2004*a*; Carere et al., 2005; Boon et al., 2007; Sih and Bell, 2008; Réale et al., 2009) and, where examined, positive genetic correlations between boldness and aggression (Bell, 2005; Dingemanse et al., 2007; Réale et al., 2009). Additionally, in a meta-analysis Dochtermann (2011) found a strong correlation between phenotypic and genetic correlations for behavioral traits. However, the aggression-boldness syndrome is not ubiquitous and has been shown to depend on environmental context (Bell, 2005; Dingemanse et al., 2007). Genetic correlations may be the result of pleiotropic relationships (Conner and Via, 1993; Blows and Hoffmann, 2005). Alternatively, natural selection for optimal trait combinations (correlational selection) may produce genetic correlations through linkage disequilibrium (Sinervo and Svensson, 2002), as hypothesized for correlated behaviors by Wolf et al. (2007); Stamps (2007); Biro and Stamps (2008); Duckworth and Kruuk (2009); Houston (2010).

Environmental conditions during an individual's development can also have consistent long lasting effects (Lindstrom, 1999). These cohort effects can be important sources of trait variation especially when the environment substantially fluctuates on an annual basis. Environmental effects shared by relatives may also play a role in shaping variation in personalities and, if not accounted

for, may bias estimates of heritabilities (Kruuk and Hadfield, 2007). Parental effects are a special case in which the environment provided by one or both parents results in similar offspring phenotypes (Mousseau and Fox, 1998; Mousseau et al., 2009), which can cause an overestimation of additive genetic effects when the effects of a common parental environment are not considered (Falconer and Mackay, 1996; Kruuk and Hadfield, 2007). These effects can be especially strong in species where parents provide extended care to their offspring, as in the case of mammals where mothers typically nurse their young for extended periods (Reinhold, 2002). Although documented in captivity (Forstmeier et al., 2004), maternal (or parental) effects have rarely been estimated for personality traits in wild populations and have never been shown to account for a substantial proportion of the variation in those traits (Table 2.1). The potential for maternal effects to shape evolutionary processes has received a lot of recent attention (Mousseau et al., 2009) and they may play an important role in the evolution of personality traits, but this has yet to be thoroughly examined.

Personality traits and a behavioral syndrome were recently identified in a population of red squirrels (*Tamiasciurus hudsonicus* Erxleben; Boon et al. 2007), for which an extensive pedigree has been established (e.g., Réale et al. 2003b; McFarlane et al. 2011). Significant phenotypic correlations between aggressive behavior towards a mirror-image, activity in an open-field arena and activity in response to handling (*i.e.* docility) indicated the presence of a behavioral syndrome where aggressive squirrels tended to be more active and less docile (Boon et al., 2008), which is similar to the aggressive-boldness syndrome that has been identified in many taxa (Tulley and Huntingford, 1988; Koolhaas et al., 1999; Careau et al., 2010).

Boon et al. (2007) also found that natural selection on activity and aggression fluctuated in direction and magnitude across years, a mechanism by which genetic and phenotypic variation in red squirrel personality traits could be maintained. Understanding how patterns of behavioral variation respond to natural selection, however, depends also on the levels of genetic variation and covariation in these traits. We, therefore, estimated heritabilities, maternal effects and sources of environment variation (e.g., cohort, permanent environmental and residual effects) in aggression,

activity and docility, as well as genetic correlations among these traits for this population of red squirrels using a Bayesian animal model approach (Hadfield, 2010).

2.2 Methods

A population of wild red squirrels has been monitored since 1989 in the southwest Yukon (61°N, 138°W), and a detailed description of the population and general methods can be found in McAdam et al. (2007). *T. hudsonicus* is a small (150 – 250 g), diurnal, semi-arboreal rodent in the family Sciuridae that is present in much of forested North America (Steele, 1998). Individuals of both sexes defend exclusive year-round territories (Smith, 1968). This territoriality allows for complete enumeration of the study population through targeted trapping and behavioral observations. Though trappability is related to red squirrel personality traits (Boon et al., 2008), the ability to target individuals minimizes sampling bias by ensuring that all individuals in the population are sampled (Biro and Dingemanse, 2009). Each squirrel in the study area was uniquely marked with numbered ear-tags as nestlings or at first capture after emergence from the natal nest, and followed from birth until death. Nests of lactating females were entered once immediately after parturition, then again when pups were approximately 25 days age, to tag pups and collect tissue samples for paternity analysis (Lane et al., 2008b).

We measured personality traits of individuals from three study areas. The Kloo and Sulphur study areas have been monitored continuously since 1989. In contrast, the Agnes study area has been monitored only recently. Since the autumn of 2004, *ad libitum* peanut butter has been experimentally provided to every squirrel in the Agnes study area between October and May of each year.

Following Boon et al. (2007), we used three behavioral tests to measure red squirrel personality traits. The first test, an open field (OF) test, was used to measure an individual's activity, exploration and behavioral stress response in a novel environment (Walsh and Cummins, 1976; Martin and Réale, 2008). The second test was a mirror-image stimulation (MIS) that measured aggression in response to the individual's reflection (Svendsen and Armitage, 1973). The third test, conducted

during routine handling events, measured docility as the struggle rate of an individual confined in a mesh handling bag. We performed 556 OF and MIS trials on 183 female and 183 male red squirrels and 3122 struggle rate tests on 291 female and 301 male squirrels over a four-year period (2005 and 2008 – 2010). The mean number of OF and MIS trials per individual was 1.4 (range 1 to 5) with a mean interval of 261 days (range 12 to 1435) and the mean date of trials was July 2 (range April 29 to September 24). The mean number of struggle rate trials per individual was 6.2 (range 1 to 44) with a mean interval of 56 days (range 0 to 1436 days) and the mean date of trials was June 22 (range April 29 to September 24). Individual squirrels were tested within one hour of being trapped on their territory. To measure docility, the captured squirrel was immediately transferred from the trap into a handling bag and placed onto dry ground and the proportion of time the squirrel spent struggling was measured over 30 seconds. If the ground was wet or snowy a foam or cloth barrier was positioned between the squirrel and the ground. After the docility test was performed, ear-tag numbers, mass, and reproductive status were recorded. If the squirrel was to be tested in the OF or MIS test, the squirrel was then transferred into the arena through a sliding door to begin the OF trial. The testing arena for the OF and MIS tests was a $60 \times 80 \times 50$ cm white corrugated-plastic box with a clear acrylic lid through which the behavior of the squirrel was recorded with a digital video camera. Four blind holes were placed in the floor to provide the focal squirrel with the opportunity to explore. A 45×30 cm mirror at one end of the arena was exposed during the MIS portion of the session. OF and MIS trials were both performed in the same testing session. OF performance was tested first so that it would also serve as a habituation period for the MIS trial. After 7.5 minutes the mirror was exposed to start the 5-minute MIS trial. At the conclusion of the session the squirrel was released where it was trapped, the number of feces deposited in the arena counted and the arena was cleaned with 70% ethanol. These procedures were the same as those performed by Boon et al. (2007).

We quantified each squirrel's behavior during the trials by scoring the videotaped trials using JWatcher Video 1.0 (Blumstein and Daniel, 2007) and the same ethogram as described in Boon et al. (2007). During the OF trial, we recorded latencies, rates and proportions of time the squirrel

spent engaged in a variety of activities that we did not consider to be unique behaviors or traits, but which we hoped would collectively represent behaviors such as activity or aggression. These behavioral measurements included the proportion of time spent walking, sniffing, chewing (gnawing at arena sides), rearing, grooming, scanning (a clear movement of the head in a side to side manner), and still. These measurements were mutually exclusive. We also recorded the proportion of time spent hanging from the arena lid, which is mutually exclusive with walking, rearing, grooming, and still but not mutually exclusive to sniffing, chewing, or scanning. In addition to these proportions, we recorded rates of jumping and rates of interactions with the false holes. The measurements recorded during MIS trials were proportions of time spent in the third of the arena closest to the mirror (front), and farthest from the mirror (back), and the proportion of time spent stretching towards the mirror. We also recorded the rate of aggressive contact with the mirror (attacks), the rate of grunting vocalizations, the rate of crouches (tail positioned over head with hairs erect) and the latency in seconds until the first attack and first approach towards the mirror. A. K. Boon performed and analyzed all trials in 2005 (study 1). Trials from 2008, 2009, and 2010 (study 2) were performed by R. W. Taylor and 4 assistants and analyzed by R. W. Taylor and 5 assistants. We tested the inter-observer reliability of our measurements by calculating the correlation between two observers' scores of the same trial (56 trials were scored by multiple observers). We removed measurements with a reliability of less than 0.7 (see Appendix Table A.1; Martin and Bateson 1993).

Maternity has been determined with certainty by ear-tagging juveniles prior to first emergence from their natal nest since 1989 on the Kloo and Sulphur study areas and since 2002 on the Agnes study area; adoption in red squirrels is extremely rare (Gorrell et al., 2010). Tissue samples for paternity analysis have been collected since 2003. Paternity was assigned based on 16 microsatellite loci using CERVUS 3.0 (Kalinowski et al., 2007) with matches accepted at 95% or greater probability and no more than 1 mismatch (detailed in Gunn et al. 2005; Lane et al. 2008*b*). The complete pedigree included 7086 individuals, 819 of which were informative for the docility phenotype, and 451 were informative for OF and MIS phenotypes (Appendix Table A.2).

2.3 Statistical Analysis

We did not consider each of our measurements from the behavioral trials to be unique behaviors and given the intercorrelation that necessarily results from mutually exclusive scores, we did not attempt to interpret them as unique behaviors. Instead our goal was to collect many measurements that we hoped would provide a reliable overall assessment of the behavior of squirrels under these conditions that have previously been found to have important ecological and evolutionary consequences (Boon et al., 2007). We, therefore, used principal component analysis to reduce the redundancy among our measurements and to identify the dominant axes of behavioral variation in the OF and MIS trials. Principal components were calculated separately for the OF and MIS measurements using a correlation matrix (Table 2.2; results). We obtained very similar loadings to Boon et al. (2007), confirming that the correlation matrices are consistent across years so principal components were calculated for all years combined. All further analyses used the scores calculated from the first principal component loadings for each trial and will be referred to as OF PC1 and MIS PC1.

We estimated the variance components for the squirrel struggle rate, and their first principal components scores for the OF and MIS trials (interpreted as docility, activity and aggressiveness, respectively; see results and Boon et al. 2007) using a mixed-effect ‘animal model’, which allows for variance structures associated with pedigrees (Henderson, 1984; Lynch and Walsh, 1998; Kruuk, 2004; Wilson et al., 2009). We fitted the animal models using a Markov Chain Monte Carlo for Generalized Linear Mixed Models (MCMCglmm) analysis in the R statistical package (Hadfield, 2010; R Development Core Team, 2011). We assumed that all study areas functioned as a single population and, therefore, estimated common variance components across all populations in all analyses. Phenotypic (V_P), additive genetic (V_A), maternal (V_M), permanent environmental (V_I) and cohort (V_C) variances and their covariances were estimated using a trivariate MCMCglmm model with individual, additive genetic, dam and birth year as random effects. Variance components were estimated as the mode of the posterior distribution and 95% credible intervals are given. Covariances were rescaled as correlations ($r = Cov_{a,b} / \sqrt{V_a V_b}$). The variances for aggression and

activity were not transformed into coefficients of variation because they were estimated from principal component scores and so had mean values of 0. Heritabilities, maternal effects, permanent environmental effects were calculated for each MCMC sample by dividing the relevant variance component by the total phenotypic variance ($V_A + V_M + V_I + V_C$) and the mode and 95% credible intervals of these posterior distributions are reported. Repeatabilities were estimated as the mode of a posterior distribution generated by dividing the among-individual variance (genetic and non-genetic) by the sum of among- and within-individual variances (Lessells and Boag, 1987) for each MCMC sample.

Covariances were supported when 95% credible intervals excluded zero. This is not applicable to variances because they are bounded above zero, so we determined support of variances by comparing deviance information criterion (DIC) values of the fitted models (DIC values for each model are provided in Appendix Tables A.5, A.6 & A.7). DIC can be viewed as the Bayesian equivalent to the Akaike information criterion (AIC) and the rules of thumb developed for AIC (Burnham and Anderson, 1998) appear to also work well for DIC (Spiegelhalter et al., 2002). In all cases the most complete models were within 2 DIC of the best model (Appendix Tables A.5, A.6 & A.7) so we report values from the models that include all random effects to avoid biased parameter estimates.

Because missing values are not allowed in random effects, unique dams were generated for individuals whose dam was unknown and treated as founders in the pedigree. This generation of dams allowed us to use all the information available in our estimates of the variance components, but assumes that all individuals with unknown dams were unrelated.

To account for the effects of habituation we included as covariates for the OF and MIS scores a fixed effect term for lifetime and yearly trial number. For struggle rate we included lifetime and yearly handling event, which included handling events where no struggle test was performed, because handling for routine data collection is similar to the struggle rate trial. We also included a quadratic term for trial numbers to account for a non-linear response to repeated trials. To control for effects of seasonality and study area we included day of year and study area as continuous and

categorical fixed effects, respectively. Finally, we included observer as a fixed effect in the docility models. Following Wilson (2008) we only attempted to control for methodological variation (measurement error) through the inclusion of fixed effects in our models, and did not attempt to account for other biological sources of variation (*e.g.* age, sex, birth year, reproductive status or mass) to avoid removing phenotypic variation that might be relevant to natural selection.

Priors for the reported models were slightly informative and generated by partitioning the variance in phenotype evenly among each random term and were given a low degree of belief ($V = \text{diag}(n) * V_P / r$, $\nu = 0.2$; Hadfield and Hadfield 2011). Altering the priors so that V_P was not evenly distributed had no effect on the results. We evaluated convergence by visually inspecting time series plots of the model parameters and assessing autocorrelation values (all were < 0.1 for reported results). The posterior distribution of the animal models was sampled every 500 iterations after a burn-in period of 50,000 iterations for a total of 2,000 samples.

2.4 Results

Red squirrel responses to the open field arena varied from active to sedentary behavior and the major axis of behavioral variation (Table 2.2) was best described as activity in a novel environment. After release into the arena active individuals immediately began walking, sniffing, jumping, hanging and chewing while sedentary individuals remained still with longer latency until first movement (Table 2.2). This variation in activity was captured by the first principal component (OF PC1) and explained 32% of the behavioral variation in the open field test (Table 2.2). Many individuals were able to hang from the top corners of the arena by clinging to seams in the walls of the arena. This hanging was often accompanied by chewing directed at the walls and corner of the arena. The second principal component for the open field test separated individuals that spent much of the trial hanging and chewing from those who did not and explained 16% of the behavioral variation (Appendix Tables A.3, A.4 for all PC loadings). Because the second principal component explained relatively little variation and its biological relevance is not readily apparent we did not perform any further analyses on this axis of variation.

Table 2.2: First principle component loadings for behaviors from an open field arena test (OF PC1) and a mirror-image stimulation test (MIS PC1) in North American red squirrels. Behaviors were measured as percentage of time unless otherwise noted. Latencies were log transformed prior to principle component analysis. Additional principle component axis are provided in Appendix Table A.3

Behavior	OF PC1	Behavior	MIS PC1
Walk	0.49	Front	0.49
Jump rate	0.44	Attack rate	0.37
Hole rate	0.31	Back	-0.41
No. Pellets	0.29	Attack latency	-0.47
Hang	0.25	Approach latency	-0.48
Chew	0.24		
Groom	-0.06		
Still	-0.52		
SD	1.71		1.67
% Total variance	0.36		0.56

Red squirrel behavior in the arena immediately and noticeably changed upon exposure to their mirror image. Individuals varied in their response with some immediately approaching and attacking the mirror and others retreating to the opposite end of the arena and adopting a passive posture. This variation in behavior was reflected in the first principal component for the mirror image stimulation trial (MIS PC1) which differentiated aggressive interactions directed at the mirror (*e.g.* approaching and attacking the mirror) from avoidance (*e.g.* retreating from the mirror and staying in the back of the arena) and explained 56% of the total variation in behavioral response to the mirror image (Table 2.2).

Red squirrels varied in their response to restraint in the mesh handling bag with some individuals struggling for the entire 30 seconds of the test and others remaining entirely still. We have interpreted this behavior as a measure of docility.

The complete univariate models for struggle rate, which included permanent environment, additive genetic, dam and cohort as random effects, had the lowest DIC score and the complete models for OF PC1 and MIS PC1 were within 2 DIC of the best models (Appendix Tables A.5 & A.6). The most complete trivariate model also had the lowest DIC score (Appendix Table A.7).

Because our focus was on achieving the best estimates for each (co)variance we evaluated the most complete trivariate model to avoid estimates that may be confounded in the reduced models.

For all three behavioral measures (OF PC1; MIS PC1; struggle rate) the addition of identity as a random effect greatly improved the fit of the model (Appendix Tables A.5 & A.6). Along with substantial repeatabilities (Table 2.4), this demonstrates that consistent individual differences in behavior exist for all three of these variables that we have interpreted as representing activity, aggression and docility (see above). Red squirrels were less active with repeated lifetime trials and less docile with repeated yearly and lifetime handling events, however there was no effect of trial number on aggression (Table 2.3). There was a quadratic component to the effect of handling events on docility such that the effect of each successive handling event diminished. There was a small positive effect of day of year on activity and docility. Red squirrels on the Kloo and Sulphur study areas were more active, and squirrels on the Sulphur study area were more aggressive than squirrels on the Agnes study area.

Table 2.3: Effects of habituation, day of year and study area on red squirrel docility, aggression and activity. Year trial number is the trial number counted from the start of each year, while life trial number is the trial number counted over each individual's entire life. Trial numbers for docility include all handling events, even those in which docility was not measured. 95% credible intervals are given in parentheses and those that exclude zero are indicated in bold. The effects of the Kloo and Sulphur study areas are assessed relative to the Agnes study area.

	Docility	Aggression	Activity
Intercept	15.494 (13.614 to 17.944)	-0.187 (-1.666 to 0.943)	0.784 (-0.588 to 1.834)
Life Trial No.	-0.057 (-0.088 to -0.011)	0.650 (-0.285 to 1.705)	-0.841 (-1.929 to -0.100)
Life Trial No. ²	0.00043 (0.00005 to 0.00078)	-0.101 (-0.357 to 0.108)	0.148 (-0.050 to 0.377)
Year Trial No.	-0.204 (-0.312 to -0.138)	-0.118 (-1.894 to 1.852)	-0.962 (-3.045 to 0.405)
Year Trial No. ²	0.004 (0.002 to 0.005)	-0.012 (-0.651 to 0.383)	0.234 (-0.237 to 0.749)
Day of Year	0.010 (0.004 to 0.018)	-0.002 (-0.007 to 0.003)	0.005 (0.001 to 0.010)
Kloo	1.484 (0.201 to 2.503)	0.295 (-0.124 to 0.840)	0.565 (0.102 to 0.972)
Sulphur	0.784 (-0.506 to 2.227)	0.617 (0.046 to 1.019)	1.020 (0.563 to 1.467)

Table 2.4: Heritability ($h^2 = V_A/V_P$), maternal effects ($m^2 = V_M/V_P$), permanent environmental effects ($PE = V_{PE}/V_P$), cohort effects ($C = V_C/V_P$), repeatability ($[V_A + V_M + V_{PE} + V_C]/V_P$), and the mean trait value. Variances were estimated using a trivariate model. Variances are calculated as the mode of the posterior distribution with 95% credible intervals in parentheses and are bound above zero. Coefficients of variation ($CV = 100 \times \text{standard deviation} / \text{mean}$) are given for docility. Because aggression and activity are scores from a principal component analysis using a correlation matrix the trait means are 0 and coefficients of variation cannot be calculated.

	h^2	m^2	PE	C	Repeatability
Docility	0.09 (0.05 to 0.19)	0.07 (0.03 to 0.10)	0.16 (0.08 to 0.21)	0.07 (0.03 to 0.17)	0.41 (0.36 to 0.49)
Aggression	0.12 (0.03 to 0.22)	0.09 (0.03 to 0.18)	0.07 (0.03 to 0.20)	0.07 (0.03 to 0.23)	0.44 (0.33 to 0.56)
Activity	0.08 (0.03 to 0.19)	0.15 (0.05 to 0.26)	0.08 (0.04 to 0.21)	0.09 (0.04 to 0.27)	0.51 (0.40 to 0.63)
	CV_A	CV_M	CV_{PE}	CV_C	Mean
Docility	15.54 (9.37 to 18.54)	10.67 (7.21 to 13.81)	16.99 (12.78 to 19.97)	10.24 (6.75 to 18.89)	18.65

Heritabilities were low for all traits (0.09 – 0.12) as were cohort effects (0.07 – 0.09), maternal effects on aggression and docility (0.07 – 0.09) and permanent environmental effects on activity and aggression (0.07 – 0.08). However, maternal effects on activity (0.15) and permanent environmental effects on docility (0.16) were nearly twice as strong as the other effects (Table 2.4).

Activity and aggression were positively phenotypically correlated ($r_P = 0.40$; 0.26 to 0.50), while docility was negatively correlated with both activity ($r_P = -0.20$; -0.30 to -0.08) and aggression ($r_P = -0.12$; -0.25 to -0.01), confirming the presence of a behavioral correlation (Table 2.5). The genetic correlations were in the same direction as the phenotypic correlations but varied in strength. There was a strong positive genetic correlation between aggression and activity ($r_G = 0.68$; 0.12 to 0.87), and a moderate negative correlation between aggression and docility ($r_G = -0.49$; -0.81 to 0.07) and between activity and docility ($r_G = -0.45$; -0.71 to 0.20), though the credible intervals between docility and the other traits overlapped with zero (Table 2.5). There were also a maternal effect correlations ($r_M = 0.58$; 0.01 to 0.81) and a permanent environmental effect correlation ($r_{PE} = 0.61$; 0.03 to 0.83) between activity and aggression and a permanent environmental effect correlation between activity and docility ($r_{PE} = -0.45$; -0.74 to 0.01) that overlapped with zero (Table 2.6). We did not find any support for cohort effects correlations (Table 2.6).

2.5 Discussion

Theoretical studies of the evolution or maintenance of animal personalities often make the assumption that personalities are heritable (reviewed in Dingemanse and Wolf 2010). For instance Wolf et al. (2007) proposed that correlational selection between life-history and personality traits can give rise to suites of personality traits maintained by frequency-dependent selection. Similarly, Stamps and Biro (Stamps, 2007; Biro and Stamps, 2008) hypothesized that selection will favor certain combinations of productivity (*e.g.* growth rates, or fecundity) and behavior leading to stable personalities. If variable selection is to contribute to the generation and maintenance of

Table 2.5: Additive genetic and phenotypic variances, covariances and correlations (G-matrix and P-matrix) of red squirrel personality traits. Variances are indicated along the diagonal, the upper triangle contains correlations and the lower triangle covariances. Variances are calculated as the mode of the posterior distribution with 95% credible intervals in parentheses and are bounded above zero. Correlations and covariances that were different from zero (based on 95% credible intervals) are indicated in bold.

	Genetic		
	Docility	Aggression	Activity
Docility	8.40 (3.05 to 11.95)	-0.49 (-0.81 to 0.07)	-0.45 (-0.71 to 0.20)
Aggression	-0.50 (-1.71 to 0.21)	0.35 (0.11 to 0.74)	0.68 (0.12 to 0.87)
Activity	-0.45 (-1.39 to 0.27)	0.11 (0.01 to 0.47)	0.23 (0.09 to 0.57)
	Phenotypic		
	Docility	Aggression	Activity
Docility	63.35 (58.02 to 72.33)	-0.12 (-0.25 to -0.01)	-0.20 (-0.30 to -0.08)
Aggression	-1.61 (-3.68 to -0.13)	3.25 (2.72 to 3.94)	0.40 (0.26 to 0.50)
Activity	-2.48 (-4.34 to -0.96)	1.31 (0.77 to 1.63)	2.89 (2.43 to 3.63)

personalities, then the personality traits on which selection acts must be heritable.

We found low heritabilities that were smaller than typically found for behavioral traits (mean $h^2 = 0.30 \pm 0.02$ reviewed by Mousseau and Roff 1987; Stirling et al. 2002) and substantially lower than our estimates of repeatability, which sets the upper bound for heritability (Hoffmann, 2000; Dohm, 2002; Bell et al., 2009). Small heritabilities may be the consequence of stabilizing or directional selection eroding additive genetic variation or due to large environmental variances (Barton and Turelli, 1989; Roff, 2007).

We found support for habituation in activity and docility, but not for aggression. The novelty of the open-field arena is an important component of the testing environment, which is altered with repeated exposure and may explain why individuals grew less active with subsequent trials (Martin and Réale, 2008; Archer, 1973). Red squirrels also grew less docile with increased handling events both within year and over their lifetime showing that the intensity of their reaction to handling decreased with experience.

Red squirrels experience large yearly fluctuations in their main food source, seeds from white spruce cones (*Picea glauca*; McAdam and Boutin 2003a; Boutin et al. 2006; LaMontagne et al.

Table 2.6: Maternal, permanent environmental and cohort (birth year) variances, covariances and correlations of red squirrel personality traits. Variances are indicated along the diagonal, the upper triangle contains correlations and the lower triangle covariances. Variances are calculated as the mode of the posterior distribution with 95% credible intervals in parentheses and are bound above zero. Correlations that were different from zero (based on 95% credible intervals) are indicated in bold.

	Maternal		
	Docility	Aggression	Activity
Docility	3.96 (1.81 to 6.63)	-0.22 (-0.65 to 0.25)	-0.31 (-0.67 to 0.16)
Aggression	-0.24 (-0.91 to 0.33)	0.24 (0.10 to 0.59)	0.58 (0.01 to 0.81)
Activity	-0.41 (-1.13 to 0.21)	0.16 (-0.03 to 0.43)	0.47 (0.16 to 0.78)
	Permanent Environmental		
	Docility	Aggression	Activity
Docility	10.3 (5.68 to 13.86)	-0.26 (-0.71 to 0.19)	-0.45 (-0.74 to 0.01)
Aggression	-0.3 (-1.53 to 0.44)	0.29 (0.10 to 0.70)	0.61 (0.03 to 0.83)
Activity	-0.4 (-1.66 to 0.06)	0.05 (-0.05 to 0.44)	0.34 (0.12 to 0.64)
	Cohort Effect		
	Docility	Aggression	Activity
Docility	3.64 (1.59 to 2.41)	-0.02 (-0.65 to 0.58)	-0.16 (-0.70 to 0.49)
Aggression	-0.02 (-1.40 to 1.09)	0.20 (0.09 to 0.89)	0.16 (-0.55 to 0.73)
Activity	-0.07 (-1.59 to 0.97)	0.01 (-0.32 to 0.38)	0.30 (0.09 to 0.95)

2005), and fluctuations in these resources have long lasting cohort effects on red squirrel fitness and life-history traits (Descamps et al., 2008). We found support for small cohort effects (approximately equal to V_A) on all three personality traits. Permanent environmental effects represented a larger proportion of individual variation in docility (approximately twice V_A) but low amounts of variation in activity and aggression (approximately equal to V_A). Permanent environmental effects represent the effects of an individual's environment that are consistent over the individual's lifetime (Roff, 1997). Individual red squirrels typically maintain a consistent territory over their lifetime (Smith 1968; but see Boutin et al. 1993; Price and Boutin 1993) and the environmental effect of the quality of their territory could contribute to permanent environmental effects on behavior. However, because red squirrels rarely change territories (Berteaux and Boutin, 2000) 'individual' and 'territory' are too confounded to reliably distinguish in the analysis of these personality data.

Determining whether phenotypic correlations adequately reflect underlying genetic correlations requires large sample size (Kruuk, 2004) and consequently the credible intervals around our estimates were wide. The phenotypic correlations between docility and aggression ($r_P = -0.12$) and docility and activity ($r_P = -0.20$) were low and the 95% credible intervals for genetic, maternal, permanent environmental and cohort correlations overlapped with zero. However, the overlap was small for the genetic correlation between docility and aggression ($r_G = -0.49$) and the permanent environmental effect correlation between docility and activity ($r_G = -0.45$). We were able to detect a more strongly supported genetic correlation ($r_G = 0.68$), a maternal effects correlation ($r_M = 0.58$) and a permanent environmental correlation ($r_{PE} = 0.61$) between aggression and activity. Together these results show that the degree to which genetic and other correlations can be inferred from phenotypes alone can depend on the particular traits being considered even within a single class of traits within a single species (Kruuk and Hadfield, 2007). Furthermore, other sources of covariation such as maternal and permanent environmental covariation may be important components of observed phenotypic correlations between behaviors. Here we were unable to assess whether these genetic correlations were due to pleiotropy or linkage disequilibrium, but are currently performing selection analyses to determine whether contemporary patterns of correlational selection are consistent with the strong positive genetic correlations that we found among activity and aggression.

We found stronger maternal effects for activity than docility and aggression and maternal effects on activity and aggression were correlated. The maternal effects correlation shows that the effect of maternal environment on activity is strongly correlated with the effect on aggression, but as maternal effects were weak the magnitude of these correlations may be misleading as other sources of variation play a large role in determining the phenotypes of offspring. Maternal effects have been found for personality traits in captive populations (Forstmeier et al., 2004; van Oers et al., 2004) and have been examined in a few wild populations (Table 2.1), however, to our knowledge, this is the first documentation of maternal effects on a personality trait in a wild population. We did not have the power to further separate the maternal effects into maternal environmental and

maternal genetic effects so can not reject the possibility that part of the maternal variance estimated here is of genetic origin (Wilson and Réale, 2006).

Maternal hormonal responses to environmental variation and consequent differential early hormone exposure of offspring (*i.e.* hormone-mediated maternal effects) could have persistent consequences on personality traits and generate the correlations between personality traits that we observed. For example, increased early exposure to androgens can increase aggression (Dloniak et al., 2006; Eising et al., 2006; Mann and Svare, 1983) or simultaneously increase both aggression and activity (Pasterski et al., 2007). In contrast, heightened early exposure to glucocorticoids may decrease activity (Koolhaas et al., 1999; Wilcoxon and Redei, 2007). The effects of early exposure to heightened androgens or glucocorticoids on personality traits can persist into adulthood (Eising et al., 2006) and perhaps across generations through epigenetic programming of neuroendocrine traits (Champagne, 2008; Meaney, 2001; Weinstock, 2008). We are currently investigating the hormonal responses of female red squirrels to environmental variation and associations between maternal hormone concentrations and the behavioral attributes of their offspring.

The persistence of consistent individual variation and covariation in behavior across a wide range of taxa has led to many adaptive hypotheses that explicitly or implicitly assume sufficient underlying genetic variation for these personalities to evolve or be maintained (*e.g.* (Stamps, 2007; Wolf et al., 2007; Biro and Stamps, 2008; Houston, 2010)). Here we have not only provided evidence of the genetic basis to personality in red squirrels, but have also identified maternal effects as a potentially important source of variation in the personality of a wild vertebrate. Further studies of the inheritance of personality traits in a variety of wild organisms are needed before general patterns will emerge regarding differences in the magnitude of genetic, maternal and environmental sources of variation among personality traits. These might also shed further light on the differences that appear to exist between sources of variation in behaviors in the wild (Stirling et al., 2002) and other traits typically studied from a quantitative genetic perspective (Houle, 1992). Such studies are needed if we are to evaluate the potential for current and future models to describe the evolution of persistent individual differences in behavior of wild animals.

Chapter 3

FLUCTUATING AND NONLINEAR SELECTION ON BEHAVIOR IN A WILD POPULATION OF RED SQUIRRELS

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Author Contributions

R. W. Taylor and A. G. McAdam developed the concept of the paper. R. W. Taylor collected behavioral data. All authors contributed to the collection of fitness data. J. C. Gorrell and D. W. Coltman performed the paternity assignment. R. W. Taylor performed all other analyses and wrote the paper. All other authors provided intellectual insight and comments.

3.1 Introduction

Fluctuating selection has long been proposed as a mechanism by which genetic variation might be maintained (Levins, 1968; Ellner and Hairston Jr, 1994; Ellner and Sasaki, 1996) despite strong directional selection being common (Kingsolver et al., 2001). If selection fluctuates strongly in direction over time then overall directional selection may be reduced, limiting any erosion of genetic variation (Bell, 2010). Fluctuating selection has been documented in a few systems (Bell, 2010), and enough longitudinal studies of selection have been undertaken that Siepielski et al. (2009) were able to conduct a meta-analysis documenting the prevalence of variation in selection (but see Morrissey and Hadfield 2012). However, new longitudinal studies of the ecological basis of variation in selection will be necessary for a better understanding of fluctuating selection.

Although behavior was previously thought to be very labile, the persistence of individual differences in behavior has stimulated much recent research (Gosling, 2001; Sih et al., 2004*a,b*; Réale et al., 2007, 2010*a*), with persistent behavioral differences increasingly accepted as important traits with ecological and evolutionary consequences (Sih et al., 2004*a,b*; Réale et al., 2010*a*). Individual differences in behavior have been shown to have important fitness effects in a number of systems (Dingemanse and Réale, 2005; Smith and Blumstein, 2008) and fluctuating selection has been proposed as a mechanism that might maintain these individual differences (Dingemanse et al., 2004; Boon et al., 2007; Dingemanse and Wolf, 2010).

Behavioral aggression and activity are often positively correlated, both phenotypically and genetically, as part of a ‘proactive-reactive’ behavioral syndrome (Koolhaas et al., 1999; Réale et al., 2010*b*). At the ‘proactive’ end of the continuum individuals are more aggressive, active, have a lower stress response and explore superficially. Recently, Réale et al. (2010*b*) advocated for the integration of behavior into the pace-of-life syndrome concept, which has its roots in the concepts of *r*- and *K*-selection (MacArthur and Wilson, 1967). Réale et al. posit that ‘proactive’ (Koolhaas et al., 1999) individuals will outperform ‘reactive’ individuals in *r*-selected low-competition environments with the opposite in *K*-selected high-competition environments. As a result, the direction of selection on pace-of-life strategies, and associated behavioral traits, may fluctuate in responses to changes in important environmental conditions.

Consistent individual differences in behavior have been previously documented in a population of North American red squirrels (*Tamiasciurus hudsonicus*) near Kluane national park, Canada (Boon et al., 2007; Taylor et al., 2012). Furthermore, northern populations of red squirrels experience large fluctuations in available resources due to the synchronization of white spruce (*Picea glauca*) cone production (Boutin et al., 2006). Additionally Boon et al. (2007) found evidence that the relationship between dam behavior and juvenile growth rates and survival was not consistent across years. This presents an ideal system for a comprehensive analysis of fluctuating selection on behavior. We hypothesized that ecological changes in resource production through time led to fluctuating selection, which could potentially maintain the variation in behavioral traits that we

have previously documented.

Individual differences in red squirrel behavior have been quantified using open-field trials, a mirror-image stimulation test and handling tests (Boon et al., 2007; Taylor et al., 2012) and have been interpreted as measurements of activity, aggression and docility. Activity characterizes motor movements in response to a novel environment and has been considered a measure of exploration in other systems (*e.g.* Dingemanse et al. 2002; Careau et al. 2008; Walsh and Cummins 1976). In Kluge red squirrels aggression and activity are positively genetically correlated, which is consistent with a ‘proactive-reactive’ behavioral syndrome (Taylor et al., 2012). Docility is negatively phenotypically correlated with aggression and activity (Boon et al., 2007; Taylor et al., 2012), but is not simply the opposite of either and is best thought of as the response to stressful confinement. Docile individuals would fall on the reactive end of the spectrum (Réale et al., 2010*b*).

Spruce masting results in large fluctuations in available cones, the main food source for northern red squirrels (Fletcher et al. in prep.), that can vary annually across 3 orders of magnitude (Boutin et al., 2006). McAdam et al. (in prep) have shown that these resource pulses have strong effects on juvenile competition for recruitment, which results in episodic alternating *r*- and *K*-selected environments. Thus, the general pace-of-life syndrome model proposed by Réale et al. (2010*b*) would predict that high activity, high aggression and low docility are favored in *r*-selected, high resource, ‘fast’ pace-of-life environments, with the opposite phenotypes favored in ‘slow’ *K*-selected environments.

However, the biology of the red squirrel system necessitates an alternate prediction for aggression. Because red squirrels are highly territorial (Smith, 1968), and a territory is required for overwinter survival (Larsen and Boutin, 1994), juvenile recruitment depends on the availability of vacant territories and not food resource availability per se. After emergence from their nest, juveniles search for territory vacancies, a process that often involves interactions with other juvenile squirrels. It is not clear how red squirrels resolve conflicts, as physical contact and fighting is very rarely observed (Dantzer et al., 2012), but it is reasonable to suspect that more aggressive individuals will be better able to win these contests over vacant territories. When competition for vacant

territories is high in low resource environments there are many juveniles for every vacancy, which increases the probability of intense and direct competition among juveniles for limited vacancies. Therefore, we predict that high-competition, low-resource, environments will favor aggressive and docile individuals but select against active individuals.

3.2 Methods

A population of wild red squirrels was monitored since 1989 in the southwest Yukon (61°N , 138°W), and a detailed description of the population and general methods can be found in McAdam et al. (2007). Red squirrels are small (150 – 250 g), diurnal, semi-arboreal rodents that inhabit much of forested North America (Steele, 1998). White spruce cone production has been quantified, using visual cone counts since 1989 (LaMontagne et al., 2005). Individuals of both sexes defend exclusive year-round territories (Smith, 1968). All squirrels in each of two study areas were uniquely marked with numbered ear-tags as nestlings or at first capture after emergence from the natal nest, and followed from birth until death. Nests of lactating females were entered immediately after parturition when young were counted and weighed, then again when young were approximately 25 days age to tag and re-weigh the young for the estimation of growth rates (grams per day; (McAdam and Boutin, 2003a)). Parturition dates were calculated based on weight loss of the mother recorded during trapping or from the weight of young during the first nest entry.

We used annual reproductive success (ARS), calculated as the number of offspring that survived overwinter to the following spring (March 1st), as our measure of fitness. The study areas are large relative to the dispersal distance of juvenile squirrels (Berteaux and Boutin, 2000) and previous comparisons of the survival of juveniles on the edge of our study areas compared to survival of juveniles in the core supports our assumption that disappearance represents death (Kerr et al., 2007; McAdam et al., 2007). We also investigated selection for two components of annual reproductive success: fecundity (or siring success for males) and offspring overwinter survival (OWS). Fecundity was calculated as the total number of offspring produced by a female each season; similarly, siring success was the total number of offspring sired by a male each season.

OWS was calculated as the proportion of offspring produced by an individual that survived to the following spring, and therefore could not be determined for individuals that did not produce any offspring. Maternity was assigned during nest entries (see above) and paternity was assigned based on 16 microsatellite loci using CERVUS 3.0 (Kalinowski et al., 2007) with matches accepted at 95% or greater probability and no more than one mismatch (detailed in Gunn et al. 2005; Lane et al. 2008b).

3.2.1 Behavioral Traits

We measured behavioral traits of individuals from two study areas that have been monitored continuously since 1989. Following Boon et al. (2007); Taylor et al. (2012), we used three behavioral trials to measure red squirrel behavioral traits. Here we give a brief description of the behavioral methods, but full details can be found in Boon et al. (2007); Taylor et al. (2012). The first test, an open field (OF) trial in a portable arena, was used to measure an individual's activity, exploration and stress response in a novel environment (Walsh and Cummins, 1976; Martin and Réale, 2008). The second test was a mirror-image stimulation (MIS) that measured sociability and aggression (Svendsen and Armitage, 1973). The testing arena for the OF and MIS tests was a $60 \times 80 \times 50$ cm white corrugated-plastic box with a clear acrylic lid through which the behavior of the squirrel was recorded with a digital video camera. Four blind holes were placed in the floor to provide the focal squirrel with the opportunity to explore. A 45×30 cm mirror at one end of the arena was exposed during the MIS portion of the session. OF and MIS trials were both performed in the same testing session (trial). OF performance was tested first so that it would also serve as a habituation period for the MIS trial. Individual squirrels were tested within one hour of being trapped on their territory. The third test, conducted during routine handling events, measured docility as the struggle rate of individuals confined in a mesh handling bag. To measure docility, squirrels were immediately transferred from the trap into a handling bag and placed onto dry ground and the proportion of time the squirrel spent struggling was measured over 30 seconds. The exclusive territoriality of red squirrel allowed for complete enumeration of the study population through targeted trapping and

behavioral observations. Importantly the ability to target individuals allows us to avoid sampling bias (Biro and Stamps, 2008; Boon et al., 2008). In 2005, 2008, 2009 and 2010 we performed 359 OF and MIS trials on 105 female and 130 male red squirrels and 2266 docility tests on 141 female and 209 male squirrels for which we had fitness data.

We quantified each squirrel's behavior by scoring the rate or proportion of time each squirrel spent performing specific behaviors from the videotaped trials using JWatcher Video 1.0 (Blumstein and Daniel, 2007) and the same ethogram as Boon et al. (2007); Taylor et al. (2012). We did not consider each of our measurements from the behavioral trials to be unique behaviors, and given the intercorrelation that necessarily results from mutually exclusive scores, we did not attempt to interpret them as unique behaviors. Instead our goal was to collect many measurements that we hoped would provide a reliable overall assessment of the behavior of squirrels under these conditions that have previously been found to have important ecological and evolutionary consequences (Boon et al., 2007; Taylor et al., 2012). We used the principal component loadings from Taylor et al. (2012) to calculate behavioral scores for each trial. All further analyses used the scores calculated from the first principal component loadings for each trial and will be referred to as activity (OF principal component 1) and aggression (MIS principal component 1.)

3.2.2 Selection

We controlled for a number of methodological sources of variation in behavior prior to evaluating selection on the behavioral traits by extracting best linear unbiased predictors (BLUPs) from linear mixed-effects models with individual as the random effect (Pinheiro and Bates, 2000). To account for effects of habituation we included as covariates, for the activity and aggression scores, fixed effect terms for lifetime and yearly trial number. For struggle rate we included lifetime and yearly handling event, which included handling events where no struggle test was performed, because handling for routine data collection is similar to the struggle rate trial. We also included a quadratic term for trial numbers to account for a nonlinear response to repeated trials. To control for effects of seasonality and study area we included day of year as a continuous fixed effect and

study area as a categorical fixed effect. Finally, we included observer as a fixed effect in the docility models. Following (Wilson, 2008), we only attempted to control for methodological variation (measurement error) through the inclusion of fixed effects in our models, and did not attempt to account for other biological sources of variation (*e.g.* age, sex, birth year, reproductive status or mass) to avoid removing phenotypic variation that might be relevant to selection. Results from these models are presented and discussed further by Taylor et al. (2012). The behavioral traits analyzed here are, therefore, equivalent to those studied by Taylor et al. (2012).

To test whether selection on behavior fluctuated across years, we fitted generalized linear mixed-models of male or female ARS that included the three behavioral traits (activity, aggression and docility) and year as a fixed factor. To test for temporal variation in selection we included interactions between year and the behavioral traits. We modeled selection for each sex separately. To account for pseudoreplication (Hurlbert, 1984) we fitted the models with study area as a fixed effect and individual as a random effect. We tested the significance of the interactions between year and the behavioral traits using a type II analysis of deviance. All generalized linear mixed-models for annual reproductive success were fitted assuming a Poisson error distribution using a log link function (Bolker et al., 2008).

We estimated linear selection differentials and gradients (Lande and Arnold, 1983) using linear regression models of relative fitness separately for each year. Relative fitness was calculated and traits were standardized for each study area-year combination, but standardized data from multiple study areas were combined for phenotypic selection analyses within each year. Included as covariates in the selection gradient models were the three behavioral traits. We also estimated selection gradients using a model that additionally included three life-history traits (offspring growth rate, litter size and parturition date) that have been shown to affect fitness in red squirrels (McAdam and Boutin, 2003a; Réale et al., 2003a). The inclusion of the life-history traits did not substantially affect the selection gradients estimated from just the behavioral traits, so to reduce the number of terms in the model we only included the behavioral traits. Standard errors for selection gradients were generated using a delete-one jackknife procedure (Mitchell-Olds and Shaw, 1987).

We calculated summary statistics describing yearly selection gradients to facilitate comparison of our results to recent meta-analysis of variation in selection (Kingsolver and Diamond, 2011; Morrissey and Hadfield, 2012; Siepielski et al., 2011). If the ratio ‘mean of absolute values of selection gradients’ : ‘absolute value of the mean of selection gradients’ is high, then fluctuating selection reduced overall directional selection (sensu Kingsolver and Diamond 2011). A large standard deviation among selection gradients relative to the mean of their standard errors indicates that variation in selection was not due to sampling error (sensu Morrissey and Hadfield 2012). The frequency of sign changes was calculated as the number of changes in direction between successive years relative to $n - 1$, where n is the total number of years (sensu Siepielski et al. 2011).

We assessed how selection on the three behavioral traits was affected by the amount of competition among juveniles for vacant territories. Competition was measured for each year as the total number of offspring produced in the population divided by the total number that recruited into the population (the sum of each dam’s ARS). We first quantified the effects of this putative agent of selection for dams and sires separately by examining the relationship between linear selection on the behavioral traits and competition using generalized linear mixed models (GLMMs). ARS was used as the dependent variable, and the three behavioral traits, competition, and all trait interactions with competition were included as covariates. Individual identity was included as a random effect. We found strong and significant interactions between competition and linear selection for dam behavioral traits (Appendix B.1). Our sample sizes were not sufficient to include additional interactions between nonlinear selection and competition in the GLMMs without overfitting the models. So, we continued our analysis of linear and nonlinear selection separately for high and low competition environments, using data pooled for each sex across high and low-competition years. We defined high-competition years to be those with juvenile competition greater than the median, whereas we considered years with juvenile competition lower than the median to be low-competition years.

To better understand how behavior could affect ARS we performed a path analysis for each sex in each environment. We included direct effects of parental behavior on ARS and indirect effects

through fecundity (or siring-success) and OWS. Coefficients for each path model were estimated using linear regression of standardized trait values on relative fitness (Kingsolver and Schemske, 1991). We did not evaluate the significance of the path models, but instead used them as a guide for further analysis using GLMMs. We evaluated selection separately for high- and low-competition years using GLMMs that included all three behavioral traits, quadratic terms for each behavioral trait and pairwise interactions between the behavioral traits. These models were reduced in a step-wise manner by removing the least significant term until further removal altered the significance of the remaining terms. Non-significant second-order interaction terms were removed prior to any first-order terms. We estimated linear and nonlinear selection gradients separately (Lande and Arnold, 1983) for each sex from data pooled across all high-competition years and data pooled across all low-competition years. Quadratic selection coefficients were doubled to give quadratic selection gradients (Lande and Arnold, 1983; Stinchcombe et al., 2008). To further investigate the mechanism of selection we analyzed the effect of behavior on two components of ARS: fecundity (or siring success) and offspring overwinter survival (OWS). We used GLMMs with a Poisson error distribution to estimate the effect of behavior on dam fecundity and male siring success. To estimate the effect of behavior on OWS we used GLMMs with a binary error distribution weighted by fecundity.

To further examine multivariate selection we performed a canonical rotation of the γ -matrix to reveal nonlinear multivariate relationships on fitness (Phillips and Arnold, 1989; Blows and Brooks, 2003). The resulting eigenvalues represent the nonlinearity of new combinations of the original traits and the eigenvectors represent the contribution of the original traits to the eigenvalues. Higher magnitude eigenvalues indicate greater curvature along the axis of selection and negative eigenvalues indicate a concave surface while positive values indicate a convex surface (Phillips and Arnold, 1989). The significance of the canonical axes was estimated by entering the eigenvectors into a quadratic regression on the original fitness values (Blows and Brooks, 2003). Surfaces were visualized using thin-plate splines and the smoothing parameter was chosen to minimize general cross-validation scores (Phillips and Arnold, 1989; Brodie III et al., 1995). Unless

Table 3.1: The effect of year on selection for dam and sire behavioral traits through annual reproductive success. Significance was calculated with Wald χ^2 tests from an analysis of deviance. GLMMs were fitted with squirrel identity as a random effect and assumed a Poisson error distribution.

	Dams			Sires		
	χ^2	Df	P	χ^2	Df	P
Year	52.89	7	< 0.0001	41.85	6	< 0.0001
Study Area	2.04	1	0.15	0.00	1	0.98
Activity	0.80	1	0.37	1.71	1	0.19
Aggression	0.05	1	0.82	0.32	1	0.57
Docility	0.11	1	0.74	0.03	1	0.87
Year $\times$ Activity	24.31	7	< 0.005	11.60	6	0.07
Year $\times$ Aggression	25.79	7	< 0.001	14.22	6	0.03
Year $\times$ Docility	8.75	7	0.27	5.76	6	0.45

otherwise noted all analysis were performed using R 2.15.0 (R Development Core Team, 2012). Generalized linear mixed models were fitted using the lme4 package (Bates et al., 2012), type II anova was performed using the car package (Fox and Weisberg, 2011), fitness surfaces were visualized using the mgcv package (Wood, 2003, 2006) and the lattice package (Sarkar, 2008); other figures were plotted using the ggplot2 package (Wickham, 2009).

3.3 Results

3.3.1 Temporal variation and annual selection

The addition of pairwise interactions between year and the three behavioral traits significantly improved the models of dam and sire ARS showing that selection was not consistent across the eight years that we studied (Table 3.3.1).

Therefore, we estimated linear selection gradients separately for each year. Standardized selection gradients for dam behavioral traits (Table 3.2 & Figure 3.1) fluctuated in sign across years and were significantly positive for activity in 2004 ($\beta' = 0.65$, CI = 0.05 to 1.25) but negative in 2009 ($\beta' = -0.97$, CI = -1.78 to -0.16).

Table 3.2: Standardized linear selection gradients $\pm$ standard errors for dam and sire behavioral traits on annual reproductive success. Significance was based on standard errors generated by jackknifing. The sample size (n) indicates the number of individuals.

Year	Dams				Sires			
	Aggression	Activity	Docility	n	Aggression	Activity	Docility	n
2003	-0.13 ± 0.59	0.82 ± 0.53	-0.11 ± 0.33	18				
2004	$-0.70 \pm 0.31^*$	$0.65 \pm 0.29^*$	0.35 ± 0.25	26	0.01 ± 0.50	$-1.18 \pm 0.64^\dagger$	0.09 ± 0.41	53
2005	$-0.42 \pm 0.19^*$	0.30 ± 0.23	0.15 ± 0.17	50	0.17 ± 0.38	-0.58 ± 0.39	0.12 ± 0.26	68
2006	$0.62 \pm 0.26^*$	-0.14 ± 0.39	-0.37 ± 0.27	48	-0.30 ± 0.47	0.22 ± 0.33	0.11 ± 0.31	97
2007	-0.10 ± 0.25	0.23 ± 0.33	0.09 ± 0.27	40	-0.12 ± 0.24	0.12 ± 0.26	0.27 ± 0.20	93
2008	$0.78 \pm 0.35^*$	-0.48 ± 0.35	-0.22 ± 0.40	45	1.06 ± 0.65	$-1.09 \pm 0.51^*$	0.19 ± 0.41	75
2009	$0.52 \pm 0.29^\dagger$	$-0.97 \pm 0.40^*$	$-0.51 \pm 0.20^*$	35	1.06 ± 1.66	0.20 ± 0.96	0.30 ± 0.30	109
2010	0.19 ± 0.24	0.10 ± 0.21	0.03 ± 0.13	34	0.00 ± 0.21	-0.20 ± 0.25	$-0.35 \pm 0.21^\dagger$	84

* $P < 0.05$, $^\dagger P < 0.01$

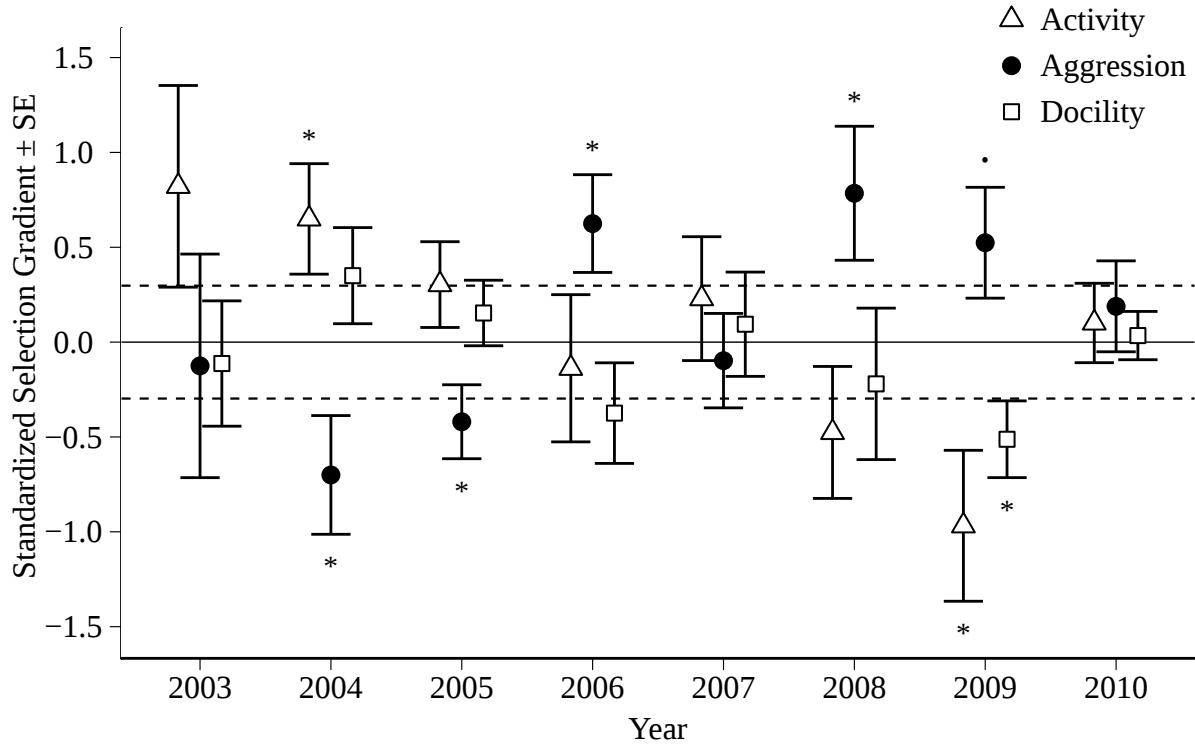


Figure 3.1: Directional selection gradients $\pm$ standard errors of annual reproductive success for dam aggression (MIS), activity (OF) and struggle rate (ST). 75th percentiles are given for directional selection gradient magnitudes compiled by Kingsolver et al. (2001). * $P < 0.05$, • $P < 0.1$.

Selection for aggression was positive in 2006 ($\beta' = 0.62$, CI = 0.11 to 1.14) and 2008 ($\beta' = 0.78$, CI = 0.07 to 1.50), but negative in 2004 ($\beta' = -0.70$, CI = -1.34 to -0.06) and 2005 ($\beta' = -0.42$, CI = -0.81 to -0.03). Selection for docility was never significantly positive, but was significantly negative in 2009 ($\beta' = -0.51$, CI = -0.92 to -0.10). Selection on activity and selection on docility occurred in the same direction in each of the eight years (correlation for all years, $\gamma = 0.76$, CI = 0.12 to 0.95), but selection on activity was in the opposite direction of aggression for six of those years (2004 to 2009; correlation between activity and aggression for all years, $\gamma = -0.79$, CI = -0.96 to -0.20). Linear selection gradients for sire behavioral traits also fluctuated in sign across years but were only significant for activity in 2008 ($\beta' = -1.09$, CI = -2.11 to -0.08; Table 3.2). Sire linear selection gradients for the three behavioral traits based on annual reproductive success as our measure of fitness were not correlated with each other or

with dam selection gradients (but see Sexual conflict below).

Table 3.3: Reduced models of selection on behavioral traits for dam annual reproductive success, fecundity and offspring overwinter survival. The full GLMM models included linear, pairwise interaction and quadratic terms and were reduced by removing the least significant term until all remaining terms were significant. Interaction and quadratic terms were removed prior to linear terms starting with the least significant. Study-area-year combination was a random effect and a Poisson error distribution was assumed. Random effect grid-year variances for high-competition years: ARS = 0.34, Fecundity = 0, OWS = 0.84, and for low-competition years: ARS = 0.11, Fecundity = 0.11, OWS = 0.

Fitness Component	Term	High Competition			Low Competition		
		Estimate	Z	P	Estimate	Z	P
Annual Reproductive Success	Intercept	-1.13 ± 0.26	-4.28	< 0.001	0.19 ± 0.14	1.34	0.182
	Aggression	0.48 ± 0.18	2.68	0.007	-0.22 ± 0.11	-2.08	0.038
	Activity	-0.32 ± 0.18	-1.77	0.076	0.23 ± 0.11	2.14	0.032
	Docility	-0.26 ± 0.15	-1.77	0.077			
Fecundity*	Intercept	1.22 ± 0.04	27.24	< 0.001	1.44 ± 0.12	11.62	< 0.001
Offspring Overwinter Survival	Intercept	-2.42 ± 0.42	-5.79	< 0.001	-0.95 ± 0.12	-7.93	< 0.001
	Aggression	0.61 ± 0.23	2.68	0.007	-0.44 ± 0.18	-2.42	0.016
	Activity	-0.45 ± 0.24	-1.88	0.061	0.39 ± 0.18	2.19	0.028
	Docility	-0.41 ± 0.21	-1.93	0.054			
	Docility ²	-0.20 ± 0.15	-1.35	0.178			
	Aggression $\times$ Activity	0.41 ± 0.19	2.16	0.031			

* No terms significantly explained fecundity

Table 3.4: Reduced models of selection on behavioral traits for sire annual reproductive success, sire success and offspring overwinter survival. The full GLMM models included linear, pairwise interaction and quadratic terms and were reduced by removing the least significant term until all remaining terms were significant. Interaction and quadratic terms were removed prior to linear terms starting with the least significant. Study-area-year combination was a random effect and a Poisson error distribution was assumed.

Fitness Component	Term	High Competition			Low Competition		
		Estimate	Z	P	Estimate	Z	P
Annual Reproductive Success	Intercept	-2.65 ± 0.34	-7.71	< 0.001	-1.14 ± 0.22	-5.25	< 0.001
	Aggression	-0.08 ± 0.32	-0.25	0.803	0.45 ± 0.32	1.39	0.164
	Activity	0.10 ± 0.32	0.32	0.752	-0.56 ± 0.26	-2.16	0.031
	Docility	0.37 ± 0.31	1.19	0.235			
	Aggression ²	0.36 ± 0.19	1.87	0.062	-0.46 ± 0.24	-1.93	0.054
	Activity ²	-0.35 ± 0.34	-1.02	0.306			
	Docility ²	-0.19 ± 0.23	-0.83	0.406			
	Aggression × Activity	-0.48 ± 0.27	-1.77	0.076			
Siring Success	Intercept	-0.93 ± 0.16	-5.73	< 0.001	-0.42 ± 0.30	-1.41	0.158
	Aggression	-0.35 ± 0.19	-1.85	0.064	0.40 ± 0.26	1.56	0.118
	Activity	0.01 ± 0.17	0.06	0.953	-0.61 ± 0.22	-2.78	0.006
	Docility	0.26 ± 0.13	1.90	0.058	-0.08 ± 0.15	-0.56	0.578
	Aggression ²	0.32 ± 0.11	2.79	0.005	-0.21 ± 0.16	-1.33	0.184
	Aggression × Activity	-0.40 ± 0.16	-2.51	0.012			
Offspring Overwinter Survival	Intercept	-1.67 ± 0.21	-7.79	< 0.001	-0.70 ± 0.13	-5.35	< 0.001
	Aggression	0.45 ± 0.18	2.53	0.011			
	Docility	0.30 ± 0.22	1.33	0.182			
	Docility ²	-0.30 ± 0.15	-1.99	0.046			

Table 3.5: Vector of standardized directional selection gradients (β') for annual reproductive success, and the matrix of standardized quadratic and correlational selection gradients (γ). Linear and quadratic selection gradients were estimated in separate regressions. Quadratic selection coefficients were doubled to give quadratic selection gradients (Stinchcombe et al. 2008). Significance was based on standard errors generated by jackknifing.

	High Competition							
	Dams				Sires			
	β'	Aggression	Activity	Docility	β'	Aggression	Activity	Docility
Aggression	$0.55 \pm 0.14^*$	0.13 ± 0.41			0.43 ± 0.48	1.67 ± 1.20		
Activity	$-0.36 \pm 0.20^\dagger$	0.14 ± 0.35	0.20 ± 0.50		-0.08 ± 0.29	-0.66 ± 0.51	-0.48 ± 0.47	
Docility	$-0.31 \pm 0.14^*$	-0.10 ± 0.23	-0.02 ± 0.27	-0.22 ± 0.27	$0.27 \pm 0.16^\dagger$	0.10 ± 0.40	0.23 ± 0.25	-0.40 ± 0.29
	Low Competition							
	Dams				Sires			
	β'	Aggression	Activity	Docility	β'	Aggression	Activity	Docility
Aggression	$-0.28 \pm 0.12^*$	0.13 ± 0.26			0.02 ± 0.18	$-0.72 \pm 0.28^*$		
Activity	$0.30 \pm 0.12^*$	-0.14 ± 0.20	0.31 ± 0.29		$-0.60 \pm 0.24^*$	-0.14 ± 0.24	$0.74 \pm 0.43^\dagger$	
Docility	0.12 ± 0.10	-0.14 ± 0.15	0.20 ± 0.18	0.16 ± 0.18	-0.11 ± 0.15	0.08 ± 0.24	0.03 ± 0.32	0.10 ± 0.22

* $P < 0.05$, $^\dagger P < 0.01$

3.3.2 Interactions with Competition and Path Analysis

We found strong and significant interactions between juvenile competition and linear selection for dam behavioral traits (Appendix B.1). This finding prompted us to split the dataset into high and low-competition years to investigate selection on different components of fitness using path analysis (see above). The path diagrams presented in figure 3.2

illustrate the direct and indirect linear effects of aggression, activity and docility on ARS. Dam behavior affected ARS indirectly through OWS, whereas sire behavior affected ARS both through siring-success and OWS. The only direct effect of behavior on ARS was from sire aggression in high-competition years. The proportion of variation in ARS explained by the models for each sex in each competitive environment was high ($R^2 = 0.7$ to 0.9) because fecundity/siring-success and OWS are, not surprisingly, large linear components of ARS. However, behavior explained little of the variation in fecundity/siring-success or OWS ($R^2 = 0.02$ to 0.06). The path analysis does not model nonlinear or multivariate selection, but we used it as a blueprint for further analyses that included nonlinear selection.

3.3.3 Linear and nonlinear selection on ARS

We evaluated selection on dam and sire behavior separately for high and low-competition environments using GLMMs (Tables 3.4 and 3.5). In high-competition years there was selection for increased dam aggression ($b = 0.48 \pm 0.18$, $Z = 2.68$, $P = 0.007$; Table 3.4; Figure 3.3) but selection for reduced activity ($b = -0.32 \pm 0.18$, $Z = -2.04$, $P = 0.042$) and docility ($b = -0.26 \pm 0.13$, $Z = -1.99$, $P = 0.047$). In low-competition years selection on dam aggression ($b = -0.24 \pm 0.11$, $Z = -2.10$, $P = 0.035$) and activity ($b = 0.23 \pm 0.11$, $Z = 2.17$, $P = 0.03$) was reversed.

We investigated the effect of competition on nonlinear selection for dam and sire behavior through ARS separately for high and low-competition environments using GLMMs (Tables 3.3 and 3.4). The addition of quadratic and correlational interaction terms uncovered selection through sire ARS (Table 3.4; γ -matrix in table 3.5) that was not evident from linear interactions with com-

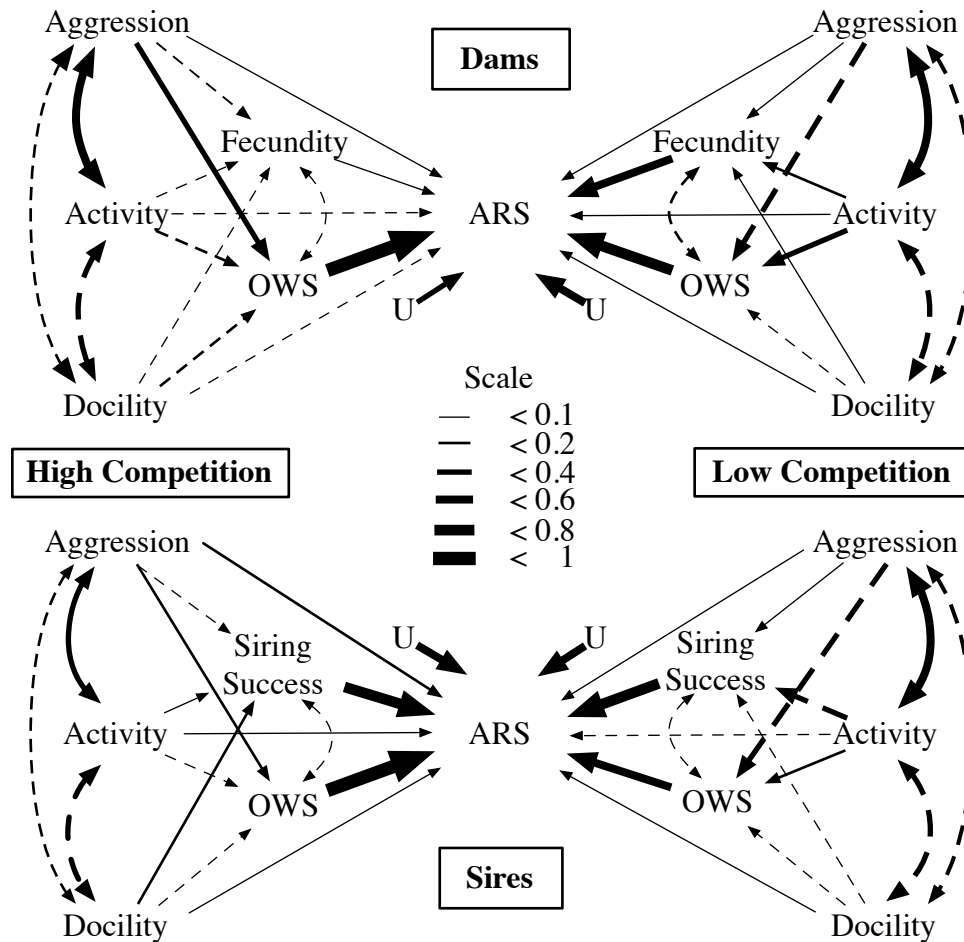


Figure 3.2: Path models depicting direct and indirect effects of parental behavior on annual reproductive success (ARS) in high and low-competition environments. Indirect effects of behavior are mediated through fecundity (or siring-success) and offspring overwinter survival (OWS). Variation due to error for fecundity, siring-success and OWS was high ($U = 0.97$ to 0.99) and omitted for simplicity. Dashed lines depict negative coefficients, and line-width is proportional to the standardized coefficients (see scale).

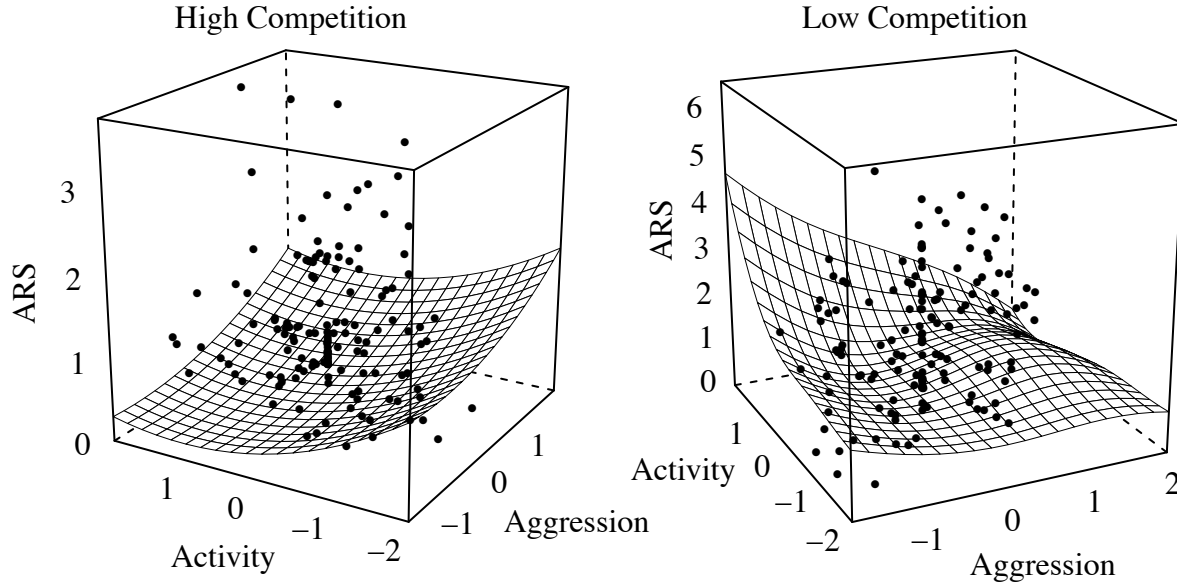


Figure 3.3: Fitness surfaces of ARS for dam aggression and activity in high and low-competition years. Selection favored aggression ($\beta' = 0.55 \pm 0.14$, $P < 0.05$) and disfavored activity ($\beta' = -0.36 \pm 0.20$, $P < 0.1$) in high-competition years. In low-competition years selection favored activity ($\beta' = 0.30 \pm 0.12$, $P < 0.05$), but disfavored aggression ($\beta' = -0.28 \pm 0.12$, $P < 0.05$). Surfaces were visualized using thin plate splines. Surfaces and data points are conditioned on docility.

petition (Table 3.3) or the path analysis (Figure 3.2). In high-competition years we found negative correlational selection between activity and aggression ($b = -0.35 \pm 0.16$, $Z = -2.15$, $P = 0.032$). That is, sires with low activity and high aggression had higher fitness while sires with high activity and low aggression had lower fitness (Figure 3.4).

In addition, we found evidence for disruptive quadratic selection for aggression ($b = 0.31 \pm 0.12$, $Z = 2.56$, $P = 0.01$) and stabilizing selection for docility ($b = -0.34 \pm 0.17$, $Z = -2.02$, $P = 0.043$) in high-competition years. A quadratic term for stabilizing selection on activity ($b = -0.42 \pm 0.24$, $Z = -1.77$, $P = 0.076$) in high-competition years was also marginally significant. In low-competition years low activity was favored ($b = -0.60 \pm 0.16$, $Z = -3.71$, $P < 0.001$) and we found stabilizing selection for aggression ($b = -0.43 \pm 0.15$, $Z = -2.83$, $P = 0.005$).

We estimated linear and nonlinear selection gradients for dam and sire behavior separately in

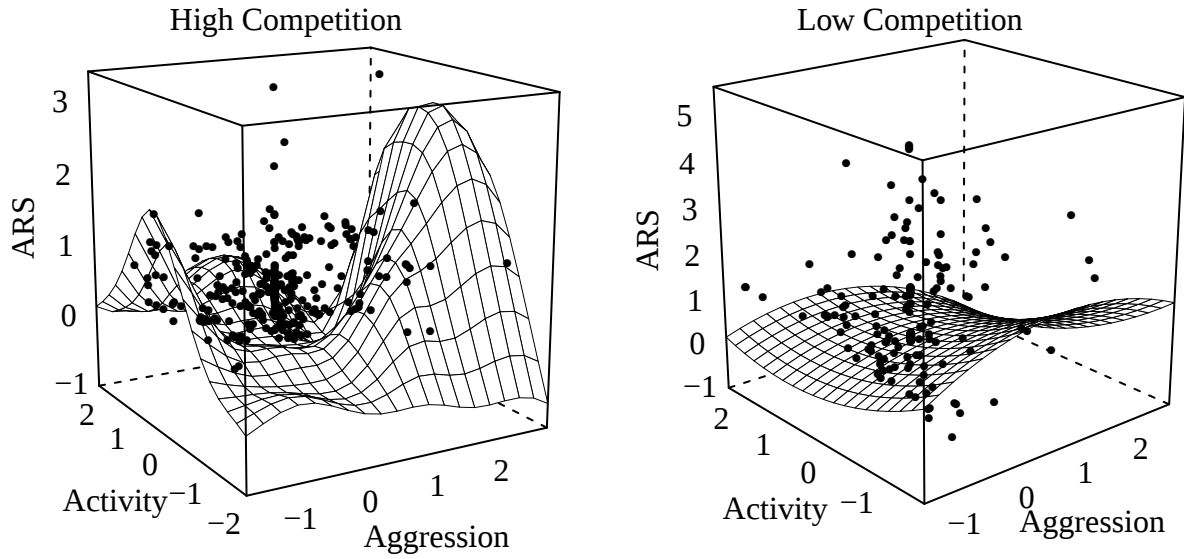


Figure 3.4: Fitness surfaces of ARS for sire aggression and activity in high and low-competition years. Selection on aggression was disruptive in high-competition years ($\gamma_{ii} = 1.67 \pm 1.20$, $P > 0.1$), but stabilizing in low-competition years ($\gamma_{ii} = -0.72 \pm 0.28$, $P < 0.05$). Selection on activity fluctuated in the opposite direction and was stabilizing in high-competition years ($\gamma_{ii} = -0.48 \pm 0.47$, $P > 0.1$), but disruptive in low-competition years ($\gamma_{ii} = 0.72 \pm 0.28$, $P < 0.05$). Surfaces were visualized using thin plate splines. Surfaces and data points are conditioned on docility.

high and low-competition environments (Table 3.5). The direction and shape of selection was consistent with GLMMs (above). Visual inspection of the fitness surfaces (Figure 3.3) showed that the positive quadratic selection on dam activity in low-competition environments was not strictly disruptive (*sensu* Mitchell-Olds and Shaw 1987) because only one stationary maximum appeared to exist.

3.3.4 Components of fitness

We continued investigating selection on the components of fitness separately for high and low-competition environments using GLMMs (Tables 3.3 and 3.4). We did not find any effect of behavior on dam fecundity in either high or low-competition environments. Selection on behavior for dam offspring OWS was very similar to selection for dam ARS (Table 3.3) with the addition of

significant correlational selection between activity and aggression ($b = 0.32 \pm 0.16$, $Z = 2.00$, $P = 0.046$) in high-competition years. These results suggest that selection on dam behavior acts through offspring OWS and not fecundity (Figure 3.2).

In low-competition environments there was no selection on sire behavior through offspring OWS, so selection through ARS acted entirely through selection for siring-success (Table 3.3). This contrasted with selection for sire behavior in high-competition environments. We found negative correlational selection between aggression and activity ($b = -0.24 \pm 0.06$, $Z = -4.11$, $P < 0.001$), meaning that sires with dissimilar aggression and activity had higher siring-success. We also found disruptive selection on aggression ($b = 0.23 \pm 0.04$, $Z = 5.38$, $P < 0.001$) and positive linear selection on docility for siring-success ($b = 0.24 \pm 0.05$, $Z = 4.39$, $P < 0.001$). Selection on aggression for offspring OWS was positive ($b = 0.45 \pm 0.18$, $Z = 2.53$, $P = 0.011$), and we found stabilizing selection for docility ($b = -0.30 \pm 0.15$, $Z = -1.99$, $P = 0.046$). Selection gradients for dam and sire OWS and fecundity or siring-success are presented in Appendix B.

3.3.5 Sexual Conflict

Selection on dam aggression through ARS was negatively correlated with selection on sire aggression through siring success ($r = 0.77$, $CI = -0.96$ to -0.04 ; Figure 3.5a). In contrast, we found a positive correlation between selection for dam aggression through dam ARS and sire aggression through OWS, though it was not significant ($r = 0.62$, $CI = -0.25$ to 0.94 ; Figure 3.5b).

3.3.6 Fitness surface estimation

We performed a canonical rotation of the γ matrix to investigate multivariate fitness surfaces (Phillips and Arnold, 1989; Blows and Brooks, 2003). The M matrix of eigenvectors and their associated eigenvalues are presented in Table 3.6. Eigenvectors for dam and sire behavioral traits in the high-competition years and for sire traits in low-competition years had both positive (disruptive) and negative (stabilizing) eigenvalues suggesting that the fitness surfaces contained at least one saddle and that no stationary point exists (Blows et al., 2003; Phillips and Arnold, 1989). In

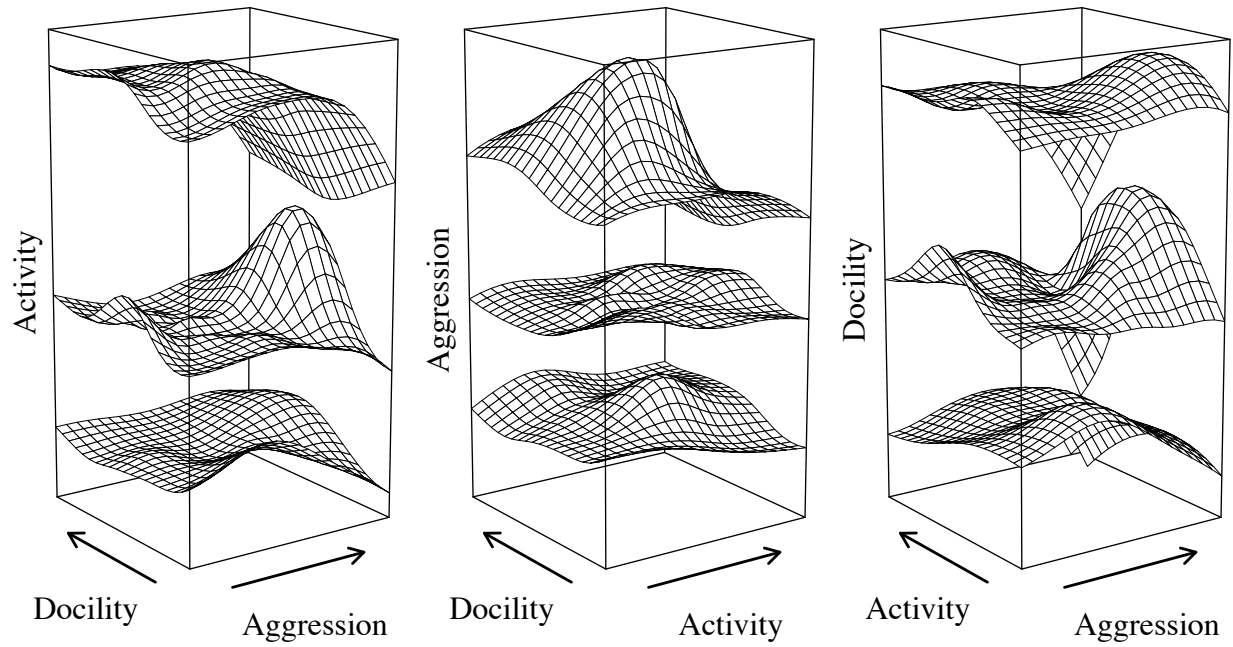


Figure 3.5: Fitness surfaces for sire behavioral traits in high competition years. Fitness surfaces are plotted for 1st, 50th and 99th percentiles of the third behavioral trait. Surfaces were visualized using thin plate splines

low-competition years the eigenvalues for dam traits were all positive suggesting that alternative maxima may exist on the fitness surface. We found significant nonlinear selection on sire traits in high and low-competition years so we will interpret these selection surfaces in more detail. The $\mathbf{m}_1$ eigenvector for sires in high-competition years represented strong convex selection on aggression, which can be readily visualized in Figures 3.4 and 3.5. The remaining eigenvectors represent convex positive correlational selection ($\mathbf{m}_2$) and negative correlational selection ($\mathbf{m}_3$) between activity and docility. Plotted apart these surfaces would be saddles with peaks at the +, + corners in the case of $\mathbf{m}_2$, and peaks at the +, - corners for $\mathbf{m}_2$. However, they interact in complex ways and are best interpreted through visualization (Figure 3.5). The $\mathbf{m}_3$ eigenvector for sire low-competition years almost entirely reflects aggression and contrasts with a non-significant, positive eigenvector ($\mathbf{m}_1$) in high-competition years. This pattern of selection reflects the fluctuating positive and negative γ_{ii} gradients for aggression from the quadratic regression and does not reflect any correlational selection.

Table 3.6: The **M** matrix of eigenvectors from the canonical analysis of γ for annual reproductive success. The eigenvalue (λ) of each eigenvector (**m**) is given in the first column.

High Competition								
Dams					Sires			
	λ	Aggression	Activity	Docility	λ	Aggression	Activity	Docility
m₁	0.247	−0.528	−0.846	0.076	1.717	0.989	−0.147	0.016
m₂	0.095	0.837	−0.534	−0.122	−0.325	0.061	0.504	0.862
m₃	−0.228	0.144	−0.001	0.990	−0.605*	−0.135	−0.851	0.507

Low Competition								
Dams					Sires			
	λ	Aggression	Activity	Docility	λ	Aggression	Activity	Docility
m₁	0.395	0.342	−0.822	−0.456	0.743 [†]	0.048	−0.999	−0.018
m₂	0.134	−0.603	−0.564	0.564	0.097	0.050	−0.016	0.999
m₃	0.077	0.720	−0.082	0.689	−0.728*	0.998	0.049	−0.049

* $P < 0.05$, [†] $P < 0.01$

3.4 Discussion

3.4.1 Fluctuating selection

We found strong temporal fluctuations in selection on dam aggression and activity that involved significant changes in the direction of selection across years. These results confirm and add support to Boon et al. (2007) conclusion that selection for activity and aggression fluctuates across years. Moreover, the magnitude of linear selection gradients for dam aggression and activity repeatedly exceeded 95th percentile of selection gradients assembled by Kingsolver et al. (2001). In a meta-analysis of a large dataset, Siepielski et al. (2009) found that selection commonly varies from year to year, but they were quick to acknowledge that measurement error may contribute substantially to this variation. Morrissey and Hadfield (2012) re-analyzed a portion of Siepielski et al.’s dataset to show that the majority of variation in selection could be explained by measurement error, which led to their conclusion that selection was actually “remarkably consistent in time” (Morrissey and Hadfield, 2012). Nevertheless, fluctuating selection clearly plays a strong role in a number of natural systems (*e.g.* Darwin’s finches; Grant and Grant 2002; see also Bell 2010) and remains

an intuitive and plausible mechanism for the maintenance of genetic variation. Our results, along with a previous study showing that red squirrel behavioral traits are heritable (Taylor et al., 2012) suggest that temporally fluctuating selection could play a large role in maintaining variation in red squirrel behavior despite strong associations with fitness, a result that is consistent with the oligogenic rather than infinitesimal model of selection (Bell, 2010).

Linear selection on sire aggression and activity did not fluctuate significantly through ARS as it did for dam traits, but the mean frequency of changes in sign was the same for dam and sire aggression and activity (0.5; Table 3.7) and the standard deviation of the selection coefficients were very similar for dams (0.56) and sires (0.58). However, the mean of standard errors for sire selection coefficients (0.53) were higher than those for dams (0.32), which may be due to selection acting on multiple components of ARS in sires and nonlinear selection (see below).

Table 3.7: Summary statistics for standardized linear selection gradients (β'). A high ratio of mean of absolute values of selection gradients to the absolute value of the mean of selection gradients indicates that fluctuations in selection reduced overall directional selection (*sensu* Kingsolver and Diamond 2011). Large standard deviations among selection gradients relative to the mean of their standard errors indicates, along with significant changes in sign (Table 2 and Figure 1), that variation in selection was not due to sampling error (*sensu* Morrissey and Hadfield 2012). The frequency of sign changes was calculated as the number of changes in direction between successive years relative to $n - 1$, where n is the total number of years (*sensu* Siepielski et al. 2011).

	Dams			Sires		
	Aggression	Activity	Docility	Aggression	Activity	Docility
Mean $ \beta' $	0.43	0.46	0.23	0.51	0.51	0.20
$ \text{Mean } (\beta') $	0.10	0.07	0.07	0.36	0.36	0.10
SD of β'	0.53	0.59	0.29	0.60	0.60	0.22
Mean SE of β'	0.31	0.34	0.25	0.48	0.48	0.30
Frequency of β' sign changes	0.43	0.57	0.71	0.67	0.67	0.17

3.4.2 Competition and the Pace-of-Life Hypothesis

The opportunity for selection through offspring viability (survival over first winter, and subsequent recruitment into the population) is strong in this system (McAdam and Boutin, 2003*b*), so we hypothesized that variation in competition among juvenile squirrels would explain variation in the direction of selection for behavioral traits. We found that selection favored aggressive dams when competition was high and active dams when competition was low. These results are consistent with our predictions that low-competition, high-resource environments would favor active individuals and high-competition environments would favor aggressive individuals. A more integrated study of the effect of competition on relationships between behavior, physiology and life-history will be necessary to understand how these results fit into the pace-of-life framework (Réale et al., 2010*b*).

This opposing selection is surprising given that aggression and activity are positively genetically correlated, but opposing linear selection does not imply negative correlational selection. In fact, GLMMs and surface-visualization indicated positive correlational selection for dam aggression and activity in high-competition years (Table 3.5 and Figure 3.3). Thus, correlational selection on dam aggression and activity within high-competition years could help to maintain the genetic correlation despite opposing patterns of linear selection. This positive correlational selection on female behavior, however, was opposed by negative correlational selection on sire aggression and activity in high-competition years (Table 3.5) indicating sexual conflict within the population (Figure 3.6; Trivers 1972; Chapman et al. 2003). These results highlight the complexity of interactions between the environment, ecology, sex and the effects of behavioral phenotypes on fitness.

3.4.3 Fitness Components

Selection on dam behavioral traits for annual reproductive success acted almost entirely through offspring overwinter survival (Table 3.3); we did not find any significant effect of dam behavior on fecundity (correlations between dam selection gradients for ARS and OWS ranged from 0.94 to 0.99 all $P < 0.001$). Selection on sire behavior acted both through both siring success and OWS. Sexual selection on sire behavior through siring success might act through a combination of

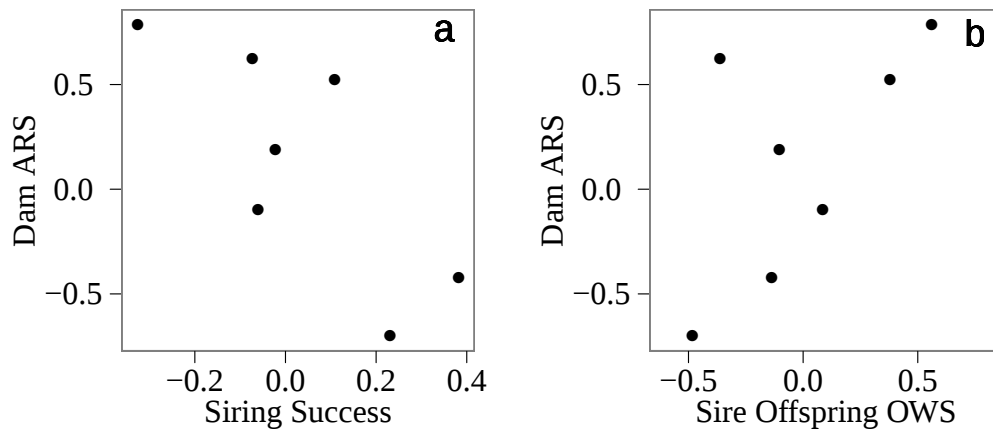


Figure 3.6: The relationships between selection gradients for dam and sire aggression. On the y-axes are selection gradients for dam aggression through annual reproductive success. On the x-axes are selection gradients for sire aggression for a) siring success ($r = -0.77$, CI = -0.96 to -0.04) and b) offspring overwinter survival ($r = 0.62$, CI = -0.25 to 0.94).

male-male competition (Lane et al., 2008a) and, potentially, also female mate-choice (see below). Parental behavioral could directly affect OWS if parents intervened in offspring competition for vacant territories or preparation for winter. However, these behaviors have not been observed, so if they occur they would be exceedingly rare. Dams occasionally bequeath their territory to a juvenile (Price and Boutin, 1993; Berteaux and Boutin, 2000), and this could be considered a direct reflection of the mother's behavior, but Boon et al. (2007) found no relationship between bequeathal and dam behavior. In this population it is more likely that parental behavior influences OWS indirectly either through correlated parental care, or through parent-offspring phenotypic resemblance.

Because sires do not provide parental care, selection on sire behavior through OWS almost surely acts through heritable behavioral components. We can test this assumption and hypothesize how selection acts on dam behavior by comparing sire and dam selection gradients across years. For selection that acts through heritable components we would expect selection through OWS to be similar for sires and dams and we find that linear selection gradients for aggression were marginally positively correlated ($r = 0.67$, CI = -0.16 to 0.95 , $P = 0.1$). This suggests that some component of selection for aggression could act through effects of heritable behaviors on

offspring survival. We also found stabilizing selection for sire docility, and marginally significant stabilizing selection for dam docility through OWS in high-competition environments. We were unable to estimate nonlinear selection gradients separately for each year, so we cannot compare those, but the similarity in selection on sire and dam docility through offspring survival also hint that selection on parental docility acts through heritable components. Finally, GLMMs did not detect any selection for sire activity through OWS and we found no correlation between sire and dam linear selection gradients for activity ($r = 0.23$, $CI = -0.57$ to 0.80 , $P = 0.6$), which suggests that selection for dam activity does not act through a heritable component and instead acts through correlated traits or maternal effects. A further piece of evidence that supports this hypothesis is that, in this population, there are stronger maternal effects on activity than aggression and that aggression is more strongly heritable than activity (Taylor et al., 2012). Ideally we would also have been able to directly measure selection on juvenile behavioral traits, but trappability of juveniles is low immediately after emergence and juvenile death prior to measurement could cause substantial bias (*e.g.* “the invisible fraction”; Grafen 1988; Hadfield 2008). The relative importance of parental behavior and offspring behavior to offspring survival could be assessed directly in future studies by measuring juvenile behavior before emergence or by looking for evidence of a genetic correlation between personality and survival using the analysis of a multigenerational pedigree (Hadfield, 2008; Sinervo and McAdam, 2008) even if phenotypic behavior are not available for offspring.

Red squirrels perform elaborate and demanding mating chases in which males compete for opportunities to mate with each female on her one day of estrus (Smith, 1968), providing opportunity for male-male competition (Lane et al., 2008a) and female mate choice. Lane et al. (2008a) characterized the mating system as scramble competition and identified home range size and the number of oestrous females found by searching males as positive predictors of siring success. Additionally, Lane et al. (2008a) hypothesized that behavior might influence search ability. Here we do not test this hypothesis directly but we do show that behavioral traits are also important predictors of siring success and that their effects on siring success vary with environmental conditions. Future analysis using detailed behavioral data collected during mating chases should directly address how

selection acts on behavior through mating chases.

Nonlinear selection on sire aggression through ARS fluctuated from convex in high-competition years to concave in low-competition years. Visual inspection of the selection surfaces (Figure 3.4) show two stationary points in high-competition years, meaning that selection was disruptive, and a ridge in low-competition years exhibiting stabilizing selection (Mitchell-Olds and Shaw, 1987). The opposite was true for sire activity with a concave surface in low-competition years and a convex surface in high-competition years. The concave surface appears to be stabilizing, but the convex surface has one stationary point so exhibits nonlinear directional selection.

Canonical rotation of the y -matrix exposed nonlinear stabilizing selection contrasting sire activity with docility in high-competition years (Table 3.6). Interpreting these values can be difficult, so we visualized the interactions between the three traits by plotting the activity by docility surface for three levels of aggression (Figure 3.5). It is clear from these graphs that the fitness surface for sire traits in high-competition years is complex. There has been some discussion of the utility of matrix diagonalization methods advocated by Blows (Blows, 2007 a,b ; Conner, 2007; Kruuk and Garant, 2007). We found that canonical rotation highlighted nonlinear selection that was not evident in the y -matrix for selection on sire behavior in high-competition environments. So, in this case it was clearly a useful tool in alerting us to multivariate-nonlinear selection (Blows, 2007 a,b). However, interpreting the resulting nonlinear selection in a biological meaningful manner remains challenging (Conner, 2007; Kruuk and Garant, 2007).

3.4.4 Conclusion

We tested the hypothesis that ecological changes through time lead to fluctuating selection, which might maintain variation in behavioral traits. Our results show that linear selection on red squirrel dam aggression and activity is sometimes very strong, but fluctuates in sign across years depending on the level of competition among juveniles for vacant territories. We also found that selection on red squirrel behavior varies among components of fitness and between the sexes, and includes nonlinear components between and within traits. These results, combined with previous work

showing that these behavioral traits are heritable (Taylor et al., 2012), suggest that behavioral variation can be maintained by complex patterns of variation in selection across years, among fitness components and between the sexes. Furthermore, congruent directional selection through offspring overwinter survival on male and female aggression and stabilizing selection on docility suggest that selection is acting on heritable components of aggression and docility in contrast to selection on activity, which seems to be affected by either correlated maternal care or maternal effects on behavior.

Chapter 4

DISASSORTATIVE MATE CHOICE ON BEHAVIOR IN RED SQUIRRELS

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Author Contributions

R. W. Taylor and A. G. McAdam developed the concept of the chapter. R. W. Taylor collected behavioral trial data. R. W. Taylor and J. E. Lane collected mating chase behavioral data. All authors contributed to the collection of fitness data. J. C. Gorrell and D. W. Coltman performed the paternity assignment. R. W. Taylor performed all other analyses and wrote the paper. A. G. McAdam provided comments.

4.1 Introduction

Sexual selection occurs when individuals vary in reproductive success (Darwin, 1871). Sexual selection can arise through intra-sexual competition or through inter-sexual mate choice (Bateson, 1983; Andersson and Iwasa, 1996; Kokko et al., 2003). With high investment in gametes and high post-fertilization care, individuals should be more selective in their mates to maximize fitness through benefits provided by mates (Darwin, 1871; Bateson, 1983; Andersson and Iwasa, 1996). Benefits can be directly beneficial, as in food, shelter or protection, or indirectly beneficial through an increase in the genetic quality of offspring. For example, female side-blotched lizards (*Uta stansburiana*) prefer mates with experimentally improved high-quality territories, presumably for the direct benefits offered by the territory (Calsbeek and Sinervo, 2002). But females also prefer-

entially fertilize male offspring with sperm from large males, presumably for the indirect benefit of the large-male genes that confer high fitness (Calsbeek and Sinervo, 2002).

The direct and indirect benefits of potential mates may be context-dependent and may also vary with environmental conditions. For example, in flagfish (*Jordanella floridae*) female preference for direct benefits of paternal care depends on salinity (Hale, 2008). Alternatively the mate preference of female side-blotched lizards (*Uta stansburiana*) for direct benefits depends on the social environment (Alonzo and Sinervo, 2001). Good genes might not only be context-dependent, but their indirect benefits may also depend on the particular genotype of the choosing individual. Mate preference for indirect, genetic benefits can be the same for all individuals in which case the benefit of good genes is independent of the receiver's genes. In such cases mate choice results in the direct inheritance of good genes, which give an additive genetic benefit to offspring (and indirectly, parental) fitness. Alternatively, the benefit of genes may depend on interactions between the male and female genomes (*i.e.* genetic compatibility), in which case different individuals might exhibit different adaptive mate choice decisions. In this scenario mate choice is for compatible genes, which could potentially operate on either a genome wide scale or at individual loci (Tregenza and Wedell, 2000). In these cases the genetic effect on fitness would be non-additive and involve dominance and/or epistasis (Neff and Pitcher, 2005). For example, side-blotched lizards exist in distinct genetic morphs that are differentiated by life-history and reproductive strategies (Sinervo and Lively, 1996; Sinervo et al., 2000). Female side-blotched lizards exhibit assortative mate choice by preferring males morphs with the same life-history strategy as their own (Bleay and Sinervo, 2007). However, after their first clutch, females of the fast life-history morph switched their preference to exhibit disassortative mate choice, suggesting that the compatibility of genes can be context dependent (Bleay and Sinervo, 2007).

Typically gametic and post-fertilization investment is greater for females than males, leading to female mate choice and strong competition among males for reproductive success (Darwin, 1871; Trivers, 1972). However, whenever there are costs or constraints that limit male reproductive output and females vary in quality, then we can expect male mate choice to evolve as well. Male costs

could accrue from parental care (Sargent et al., 1986), sperm production (Nakatsuru and Kramer, 1982) or mate searching including predation risks (Magnhagen, 1991). Male choice has been documented numerous times (*e.g.* Herdman et al. 2004; Bateman and Fleming 2006); however, as emphasized by Barry and Kokko (2010), male choice may not evolve if mating opportunities are scarce even when there is variation in female quality and high costs to reproduction.

Consistent individual differences in behavioral traits are widespread (Réale et al., 2007) and have been shown to be heritable (Taylor et al., 2012; Drent et al., 2002) and under selection in a number of species (reviewed in Smith and Blumstein 2008). Mate choice based on behavioral traits has been documented in humans and in animals (reviewed in Schuett et al. (2010), though few studies have shown inter-individual variation in mate choice based on behavior Schuett et al. (2010). In one notable exception, high-exploratory female zebra finches (*Taeniopygia guttata*) were found to prefer high-exploratory males, but low-exploratory females had no preference for male exploration (Schuett et al., 2011).

Red squirrels (*Tamiasciurus hudsonicus*) have been shown to exhibit consistent individual differences in aggression (response to a mirror image), activity in a novel environment (open field arena) and docility (time spent still while being handled; Boon et al. 2007). Additionally, these traits are heritable, experience maternal effects, and aggression and activity are positively genetically correlated in a behavioral syndrome (Taylor et al., 2012). Selection on these red squirrel behavioral traits was also found to differ among components of fitness, between the sexes and includes linear and nonlinear components between and within traits that fluctuate over time (Taylor et al. in prep; Chapter 3). Taylor et al. found that both aggressive males and intermediately docile males produced offspring with enhanced overwinter survival. Males do not provide parental care, but aggression and docility are heritable so presumably these good behavior genes were inherited by offspring, which enhanced their survival. Even if this is not the case, and other genes are the targets of selection for overwinter survival, aggression and docility could provide indirect, but reliable cues for the selection of good genes.

Fluctuations in selection on aggression have been linked to annual variation in the strength of

juvenile competition for vacant territories (Taylor et al. in prep). Red squirrels defend exclusive territories year-round (Smith, 1968) and a territory is necessary for overwinter survival (Larsen and Boutin, 1994), so juvenile squirrels born in the spring and summer must compete for territory ownership in order to survive the winter and recruit into the population. Survival to one year of age accounts for 60% of a female's contribution to population growth (McAdam et al., 2007) and as a result the opportunity for selection on this stage of life is very high (McAdam and Boutin, 2003b). Variation in juvenile competition for territory vacancies is influenced by adult overwinter survival and food availability (McAdam et al. in prep). Using the ratio of juveniles produced in a year to the number that survived overwinter as an index of competition, Taylor et al. (in prep; Chapter 3) found that selection on dam activity and aggression fluctuated significantly in sign across years depending on intensity of competition among juvenile squirrels for vacant territories. Female red squirrels can anticipate autumn abundance of their main food source, seeds from white spruce cones (*Picea glauca*), and produce more offspring in anticipation of higher offspring recruitment due to higher resource availability (Boutin et al., 2006). Male red squirrels increase energy expenditure during the mating season in advance of autumns with high cone production (Lane et al., 2009). Additionally, red squirrels adjust their behavior in response to changes in population density (Dantzer et al., 2012). The ability to anticipate autumn resource levels and assess current population density indicates that red squirrels have the capacity to anticipate upcoming juvenile competition for territory vacancies. Because male red squirrels do not provide parental care, any female mate choice should be based on indirect (genetic) benefits provided by males. Good genes should enhance offspring recruitment for the upcoming juvenile competitive environment.

Red squirrels perform elaborate and demanding mating chases where up to a dozen males vie for opportunities to mate with each female on her single day of estrus (Smith, 1968; Lane et al., 2008b). In this scramble mating system, the ability of males to locate females is a strong predictor of male siring success (Lane et al., 2008a) and energy expenditure of males during the mating season approaches that of females during lactation (Lane et al., 2009). Female red squirrels mate with many males (mean 6.9; McFarlane et al. 2011; Lane et al. 2008b) during their day of estrous.

During mating chases a dominant male follows the estrous female, only leaving her to drive away any other males that approach (Smith, 1968). How males establish dominance is unknown because contact among males is rare (Smith, 1968). During mating chases there is considerable variation in how fast and far the female travels (area covered ranges from 45 m² to 56,020 m²), indicating that females may be able to manipulate the characteristics of the mating chase to attract males or to favor certain males over others. Lane et al. (2008b) hypothesized that, if multiple mating is adaptive, then the benefits of multiple mating should outweigh its costs, but found no benefits of multiple mating on fertilization assurance, infanticide avoidance, litter allelic diversity or parental relatedness (Lane et al., 2008b). However (Lane et al., 2008b) also found no detectable costs of multiple mating for females in the form of reduced female overwinter survival. Here we consider the hypothesis that mating chases provide a mechanism that allows female mate choice for high-quality mates, and test this hypothesis with predictions about context-specific indirect benefits for offspring overwinter survival.

Here we make explicit predictions based on our prior selection analyses (Taylor et al. in prep.; Chapter 3) about patterns of male red squirrel attendance at mating chases (male mate choice) and the siring success of males that attend a female's mating chase (female choice). Parental aggression was favored in high-competition environments, but was a disadvantage in low-competition environments (Taylor et al. in prep; Chapter 3). As a result, we first predicted that, in high-competition years, both males and females should prefer more aggressive mates because of the benefits of aggressive alleles for offspring survival under those conditions. In low-competition years less aggressive mates should be favored. Second, in high-competition years intermediate values of paternal docility increased offspring survival whereas sires with very high or low docility had low offspring survival (*i.e.* stabilizing selection), but no significant selection on docility was found in low-competition environments (Taylor et al. in prep.; Chapter 3). Therefore, we predicted that preference for docility would depend on the chooser's docility. Specifically, a highly docile individual should prefer a less docile mate so that the additive effect of the mate's genes would moderate offspring docility. Conversely, a less docile individual should prefer a more docile mate,

resulting is disassortative mate choice for docility.

4.2 Methods

A population of North American red squirrels has been monitored since 1989 in the southwest Yukon (61°N, 138°W), and a detailed description of the population and general methods can be found in (McAdam et al., 2007). Red squirrels are small (150 – 250 g), diurnal, semi-arboreal rodents that inhabit much of forested North America (Steele, 1998). Individuals of both sexes defend exclusive year-round territories (Smith, 1968). Each squirrel in each of two study areas was uniquely marked with numbered ear-tags as nestlings or at first capture after emergence from the natal nest, and followed from birth until death. To allow for observational identification, unique combinations of colored wires and/or pipe-cleaners were threaded through each squirrel's ear-tags. Stakes placed every 30m in a grid allowed for spatial locations of squirrels to be recorded.

4.2.1 Siring success and parturition date

Nests of lactating females were entered and tissue samples were taken from pups immediately after parturition and again at 25 days of age for paternity analysis. Paternity was assigned based on 16 microsatellite loci using CERVUS 3.0 (Kalinowski et al., 2007) with matches accepted at 95% or greater probability and no more than one mismatch (detailed in Gunn et al. 2005; Lane et al. 2008a). We evaluated siring success both as a continuous and as a binary variable. Since we found no substantial differences in our results, we present only the results from siring success as a binary response (*i.e.* sired or did not sire offspring). Parturition was identified based on weight loss of the mother recorded during trapping and parturition dates were calculated from trapping records or from the weight of young during the first nest entry. For mating chases that were not observed we calculated the mating chase date as 35 days prior to parturition (Steele 1998; S. Boutin, personal communication).

4.2.2 Behavioral Traits

We measured behavioral traits of individuals from two study areas that have been monitored continuously since 1989. Following Boon et al. (2007); Taylor et al. (2012), we used two behavioral trials to measure aggression and docility. Here we give a brief description of the behavioral methods, but full details can be found in Boon et al. (2007); Taylor et al. (2012). The aggression test was a mirror-image stimulation (MIS) test in a portable arena that measured the aggressive response to a mirror image (Svendsen and Armitage, 1973). In the same testing session (trial), prior to the MIS test an open field (OF) test was used to measure an individual's activity in a novel environment (Walsh and Cummins, 1976; Martin and Réale, 2008), but also served as a habituation period for the MIS test. The testing arena for the OF and MIS tests was a $60 \times 80 \times 50$ cm white corrugated-plastic box with a clear acrylic lid through which the behavior of the squirrel was recorded with a digital video camera. Four blind holes were placed in the floor to provide the focal squirrel with the opportunity to explore. A 45×30 cm mirror at one end of the arena was exposed during the MIS portion of the session. Individual squirrels were tested within one hour of being trapped on their territory. The docility test, conducted during routine handling events, measured docility as the struggle rate of individuals confined in a mesh handling bag. To measure docility squirrels were immediately transferred from the trap into a handling bag and placed onto dry ground and the proportion of time the squirrel spent struggling was measured over 30 seconds. The exclusive territoriality of red squirrel allowed for complete enumeration of the study population through targeted trapping and behavioral observations. Importantly the ability to target individuals allows us to avoid sampling bias (Biro and Dingemanse, 2009; Boon et al., 2008). We performed 556 OF and MIS trials on 183 female and 183 male red squirrels and 3122 struggle rate tests on 291 female and 301 male squirrels over a four-year period (2005 and 2008 – 2010).

We quantified each squirrel's MIS behavior by scoring the rate or proportion of time each squirrel spent performing specific behaviors (*e.g.* attacks, time spent in the closest 1/3 of the arena to the mirror) from the videotaped trials using JWatcher Video 1.0 (Blumstein and Daniel, 2007) and the same ethogram as Boon et al. (2007); Taylor et al. (2012). We did not consider each of our

measurements from the behavioral trials to be unique behaviors and given the intercorrelation that necessarily results from mutually exclusive scores, we did not attempt to interpret them as unique behaviors. Instead our goal was to collect many measurements that we hoped would provide a reliable overall assessment of the behavior of squirrels under these conditions that have previously been found to have important ecological and evolutionary consequences (Boon et al., 2007; Taylor et al., 2012). We used the principal component loadings from (Taylor et al., 2012) to calculate behavioral scores for each trial. All further analyses used the scores calculated from the first principal component loadings for each trial, which were interpreted as the aggressive response to a mirror image and referred to as aggression.

We controlled for a number of methodological sources of variation in behavior prior to evaluating mate choice through the behavioral traits by extracting best linear unbiased predictors (BLUPs) from linear mixed-effects models with individual as the random effect (Pinheiro and Bates, 2000). To account for effects of habituation we included as covariates for the docility and aggression scores fixed effects terms for lifetime and yearly trial number. For struggle rate we included lifetime and yearly handling event, which included handling events where no struggle test was performed, because handling for routine data collection is similar to the struggle rate trial. We also included a quadratic term for trial numbers to account for a nonlinear response to repeated trials. To control for effects of seasonality and study area we included day of year as a continuous fixed effect and study area as a categorical fixed effect. Finally, we included observer as a fixed effect in the docility models. Following (Wilson et al., 2009), we only attempted to control for methodological variation (measurement error) through the inclusion of fixed effects in our models, and did not attempt to account for other biological sources of variation (*e.g.* age, sex, birth year, reproductive status or mass) to avoid removing phenotypic variation that might be relevant to selection. Results from these models are presented and discussed further by Taylor et al. (2012). The behavioral traits analyzed here are, therefore, equivalent to those studied by Taylor et al. (2012).

4.2.3 Mating Chase Observations

In order to assess courtship behavior and copulation success we observed red squirrel mating in 2003, 2004, 2005 and 2008. See (Lane et al., 2008a) for additional methodology. A red squirrel mating chase begins when the female emerges in estrus from her nest early in the day, and continues until she returns to her nest at the end of the day. Male red squirrels actively search out estrous females during the breeding season by visiting female territories and assessing olfactory, visual and vocal cues for signs of estrus (Smith, 1968; Lane et al., 2008a). Upon locating a receptive female, males compete for access to the female, which often leads to a congregation of 2 to 7 males vying for access to the female at once. At any time one male is ‘dominant’ to all others and attempts to monopolize access to the female by chasing off other males, yet male squirrels rarely contact each other (R. Taylor, personal observation) and conspicuous wounding is not prevalent during the mating season (Smith, 1968).

To identify mating chases we located each female twice daily before noon using radio-telemetry. Mating chases were identified by the existence of male squirrels on a female’s territory and her tolerance of their presence. To quantify mating chase behavior we used radio-telemetry to follow the female squirrel throughout the chase and employed a combination of scan sampling for attending males and all-occurrence sampling of mating behavior (Altmann, 1974; Martin and Bateson, 1993). We recorded all dominant and subordinate males, mounting, attempted mounting, chasing, periods out of site or underground, vocalizations, and other noteworthy events as well as spatial locations. Copulations were judged to have occurred when male squirrels successfully mounted a female or when he accompanied the female underground for a minimum of 60s. Copulations of tree squirrels generally last less than 60 seconds (Koprowski, 2007), which is adequate for fertilization in red squirrels (J. E. Lane & S. Boutin, unpublished data). This criterion has been used previously in this (*e.g.* Lane et al. 2008b,a) and other (Waterman, 1998) systems.

Table 4.1: Sample sizes for each study area-year. Total number of individuals living on the study area, and the number of individuals for which we had behavioral data are given. Behavior was measured in 2005 and 2008.

Study Area	Year	Total number of individuals		Number of individuals with behavior		Proportion with behavior	
		Females	Males	Females	Males	Females	Males
Kloo	2003	23	34	4	8	0.17	0.24
Kloo	2004	10	27	3	10	0.30	0.37
Kloo	2005*	8	22	6	16	0.75	0.73
Kloo	2008*	21	47	20	46	0.95	0.98
Sulphur	2003	18	28	10	5	0.56	0.18
Sulphur	2004	12	19	9	7	0.75	0.37
Sulphur	2005*	12	16	12	12	1.00	0.75

4.2.4 Components of siring success

We evaluated four potential components of siring success. Each of these components is also a mechanism by which mate choice might occur. Attendance was assessed as whether a male squirrel was present during a female's mating chase. We assessed both copulation success (binary: yes or no) and copulation number (the number of discrete copulations). Copulation order was the order in which successive males copulated with a female. Multiple successive copulations by the same male were counted as a single copulation event when calculating the copulation order. When a male copulated with a female in more than one session (*i.e.* copulation events separated by the female's copulation with another male) his copulation order was determined by his earliest copulation.

4.2.5 Mate choice

In order to assess mate choice we generated a dataset consisting of all parental combinations for each grid-year consisting of individuals for which we had behavioral data (See Table 4.1 for sample sizes and Appendix Table C.1 for data structure).

For example the data subset for the study area Kloo in 2010 consisted of 840 rows, one row

for each possible combination of male (40 individuals) and female (21 individuals). This data structure allowed us to test, using generalized linear mixed models (GLMMs), for effects of male and female behavior as well as interactions between male and female behavior on siring success and components of siring success. In all models we included the distance between the male and female territories as a fixed effect. To account for pseudoreplication (Hurlbert, 1984), we fitted the models with mating chase (analogous to female identity) and male identity as random effects. Models for male attendance, copulation, and binary siring success were fitted assuming a binomial error distribution (logit link function). Models for copulation order, number of copulations and siring success, measured as the number of offspring sired, were fitted assuming a Poisson error distribution (log link function). All traits were standardized to a mean of zero and unit variance prior to analysis. GLMMs were reduced using backward reduction. All analysis were performed using R 2.15.0 (R Development Core Team, 2012). Generalized linear mixed models were fitted using the lme4 package (Bates et al., 2012),

We tested for mate choice on siring success and the four components of siring success: mating chase attendance, copulation success, copulation order and siring success (Lane et al., 2008a). To test our prediction that aggression would be favored in high-competition years we included aggression, competition and an interaction between competition and aggression in the models. To test our prediction that individuals would prefer mates with opposite docility in high-competition years we included an interaction between male and female docility and a three-way interaction with male and female docility and the degree of juvenile competition for vacant territories in that year. A positive interaction between male and female docility would indicate assortative mating, whereas a negative interaction would indicate disassortative mating. We included the three-way interaction between male docility, female docility and competition, because we expected to see disassortative mating (male docility $\times$ female docility interaction) only in high competition years (*i.e.* three-way interaction: male docility $\times$ female docility $\times$ competition).

For the analysis of attendance, copulation success, copulation order and copulation number we subsetting the dataset to only include females whose mating chases we followed. For the models

of copulation success we subsetting the data to only include males that attended a chase, which modeled copulation success given attendance at the chase. For the models of copulation order and copulation number we subsetting the data to only include males who successfully copulated, which modeled the effect of copulation order and number after controlling for copulation success. For the model of attendance we included the Julian date of the mating chase to control for seasonal variation in the probability of a male attending a mating chase (Lane et al., 2008a). In this model only, we included female aggression because attendance represents male mate-choice. In all other models we included male aggression because we were modeling female choice. Models for male and female choice included both male and female docility because we expected an interaction between the chooser's docility and their mate preference.

4.3 Results

4.3.1 Distance

Males that lived farther from females were less likely to attend her mating chase ($b = -2.03 \pm 0.14$, $Z = -14.51$, $P < 0.001$; Table 4.2).

Table 4.2: Reduced models of male attendance, siring success and copulation success of males that attended a mating chase, and siring success of males that copulated. The full GLMM models are described in the methods. The least significant terms were removed, starting with the highest order. Male identity and mating chase identity were included as random effects. A binary error distribution was assumed.

Response	Term	Estimate	Z	P	Random Variances	
					Male id.	Chase id.
Male attendance	Intercept	-1.96 ± 0.44	-4.48	< 0.001	0.952	0
	Julian day of chase	0.19 ± 0.08	2.23	0.026		
	Distance	-2.03 ± 0.14	-14.51	< 0.001		
	Female docility	-0.12 ± 0.34	-0.34	0.732		
	Male docility	-0.40 ± 0.44	-0.90	0.37		
	Competition	-0.10 ± 0.05	-1.83	0.067		
	Competition $\times$ Female docility	0.03 ± 0.04	0.65	0.514		
	Competition $\times$ Male docility	0.07 ± 0.06	1.18	0.239		
	Male Docility $\times$ Female docility	-0.90 ± 0.37	-2.45	0.014		
	Competition $\times$ Male docility $\times$ Female docility	0.12 ± 0.04	2.70	0.007		
Siring success of attending males	Intercept	-2.51 ± 0.17	-15.04	< 0.001	0.885	0
	Number of sires	0.72 ± 0.18	4.07	< 0.001		
	Distance	-0.45 ± 0.14	-3.09	0.002		
Copulation success of attending males	Intercept	1.51 ± 0.32	4.67	< 0.001	0	0.764
	Number of copulatory partners	-0.29 ± 0.09	-3.35	< 0.001		
	Distance	-0.10 ± 0.05	-2.08	0.038		
Siring success of copulating males	Intercept	-1.85 ± 0.22	-8.52	< 0.001	0.467	0
	Number of sires	-0.33 ± 0.21	-1.60	0.111		
	Male aggression	-0.88 ± 0.31	-2.81	0.005		

Table 4.3: Reduced models of male copulation order , siring success after controlling for copulation order, copulation number and siring success after controlling for copulation number. The full GLMM models are described in the methods. The least significant terms were removed, starting with the highest order. Male identity and mating chase identity were included as random effects. A binary error distribution was assumed for the siring success models, and a Poisson error distribution was assumed for the copulation order and copulation number models.

Response	Term	Estimate	Z	P	Random Variances	
					Male id.	Chase id.
Copulation order	Intercept	1.31 ± 0.06	22.34	< 0.001	0.051	0.073
	Docility	-0.10 ± 0.05	-1.98	0.048		
Siring success controlled for copulation order	Intercept	-2.02 ± 0.24	-8.39	< 0.001	0.165	0
	Copulation order	-0.84 ± 0.24	-3.48	< 0.001		
	Number of sires	0.59 ± 0.23	2.59	0.009		
	Male aggression	-1.05 ± 0.34	-3.11	0.002		
Copulation number	Intercept	0.83 ± 0.16	5.23	< 0.001	0.051	0.147
	Number of partners	0.05 ± 0.02	2.34	0.019		
Siring success controlled for copulation number	Intercept	-1.96 ± 0.24	-8.24	< 0.001	0.388	0
	Number of sires	0.61 ± 0.23	2.70	0.007		
	Male aggression	-1.03 ± 0.33	-3.10	0.002		

The effect of distance persisted among males that attended a mating chase; males that lived farther from females had lower copulation success ($b = -0.1 \pm 0.05$, $Z = -2.08$, $P < 0.038$) and lower siring success given attendance ($b = -0.40 \pm 0.15$, $Z = -2.79$, $P = 0.005$). After controlling for copulation success there was no longer any effect of distance on siring success ($b = -0.25 \pm 0.25$, $Z = -0.98$, $P = 0.3$). An interaction effect between distance and juvenile competition on attendance was marginally significant, so was removed during the backwards model selection procedure. To explore this effect more directly, we fitted a separate GLMM of male attendance with only competition, distance and the interaction between juvenile competition and distance as predictors, and found a significant negative interaction between distance and juvenile competition such that the effect of distance was stronger in years with high juvenile competition (Figure 4.2; Intercept = -1.97 ± 0.27 , $Z = -7.39$, $P < 0.001$; distance = -1.18 ± 0.20 , $P < 0.001$, $t = -6.01$; competition = -0.14 ± 0.04 , $P < 0.001$, $t = -3.53$; distance $\times$ competition = -0.13 ± 0.03 , $t = -3.91$, $P < 0.001$; Random effect variance: Male identity = 1.69 ± 1.30 , mating chase identity = 0.10 ± 0.32).

4.3.2 Male Mate Choice

We found a significant positive three-way interaction between juvenile competition, male docility and female docility ($b = 0.12 \pm 0.04$, $Z = 2.71$, $P = 0.007$; Table 4.2; Figure 4.1) indicating that males attended mating chases disassortatively based on docility and that the disassortative attendance was strongest in low juvenile competition years. After removing the three-way interaction term, we no longer found a significant negative interaction between male and female docility on attendance. We also found that males were more likely to attend mating chases that occurred later in the season ($b = 0.19 \pm 0.08$, $Z = 2.26$, $P = 0.03$).

4.3.3 Female Mate Choice

We found no effect of male aggression or any interaction between male docility and female docility on the siring success (continuous or binary) of males that attended a mating chase (stats). After

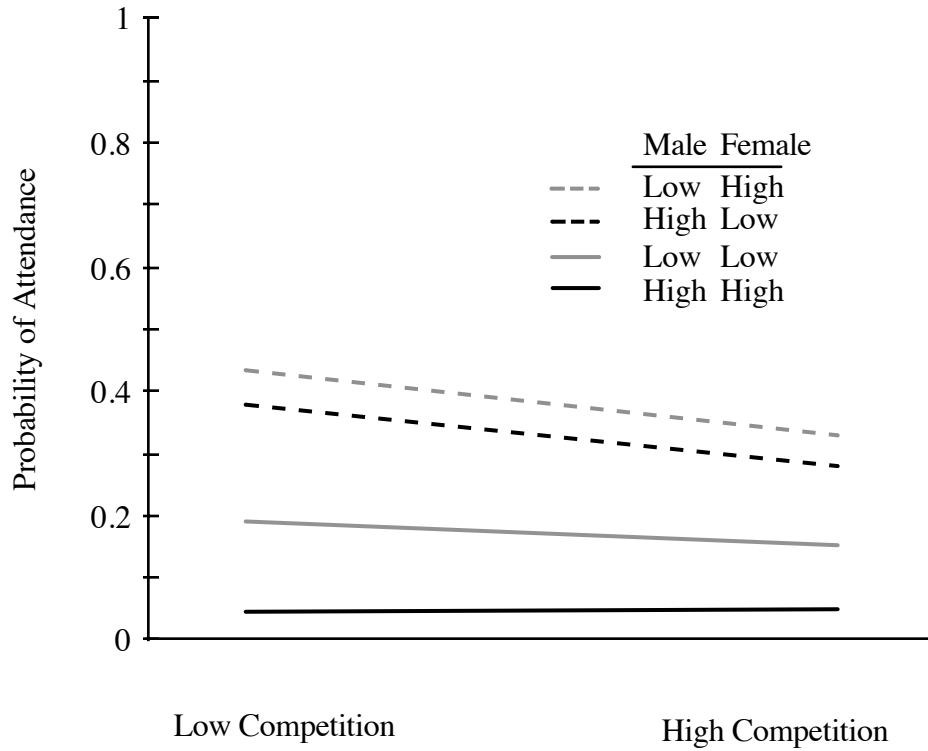


Figure 4.1: Three-way interaction plot of the interaction between male docility, female docility and competition on male attendance.

sub-setting the data to only include males that copulated we found that more aggressive males had lower siring success ($b = -0.87 \pm 0.31$, $Z = -2.83$, $P = 0.005$), but this effect did not interact with the degree of juvenile competition. This negative effect of aggression persisted after controlling for copulation order and after controlling for number of copulations. Docile males copulated earlier in order than less docile males ($b = -0.13 \pm 0.05$, $Z = -2.46$, $P = 0.01$; Table 4.2) and we found a negative effect of copulation order on siring success ($b = -0.84 \pm 0.24$, $Z = -3.48$, $P < 0.001$) meaning that males that mated with a female earlier in her day of estrus were more likely to sire some of her offspring.

4.4 Discussion

Despite strong associations between sire personality and offspring survival (Taylor et al. in prep; Chapter 3), we did not find support for our hypothesis that mating chases are mechanisms by which

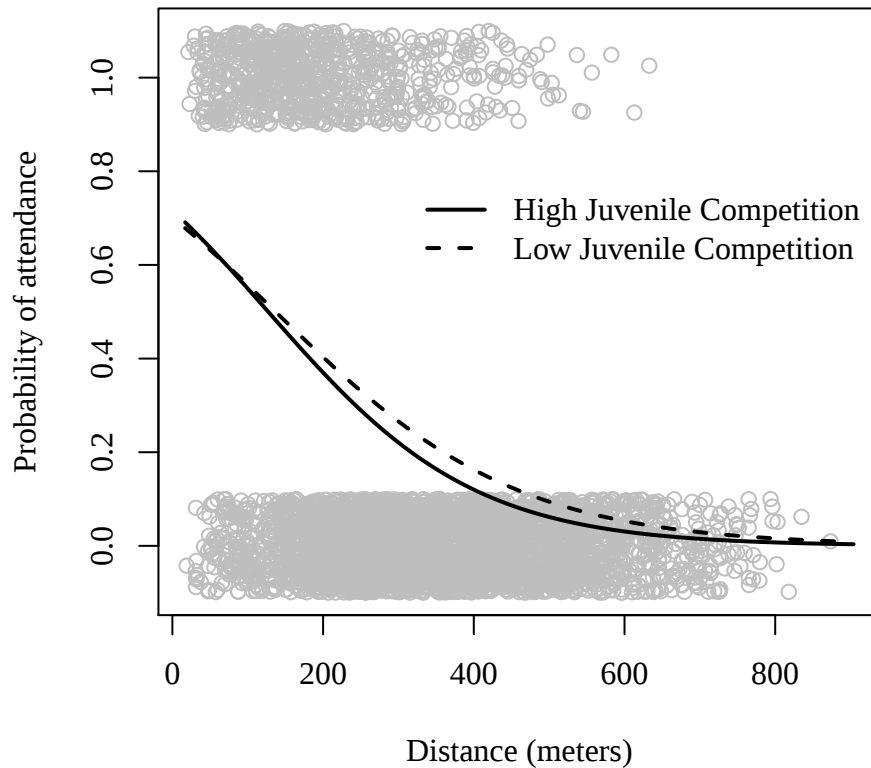


Figure 4.2: The effect distance on male attendance to a mating chase. The dashed line indicates predicted probability of attendance at -1 , and the solid line at $+1$, standard deviation of juvenile competition. In high juvenile-competition years the effect of distance is stronger.

female squirrels can acquire good genes for offspring overwinter survival. In fact we found no evidence for female mate choice, but our analysis was narrowly targeted to specific predictions based on selection results from Taylor et al. (in prep; Chapter 3) rather than a survey for any evidence of female mate choice. There may be traits other than aggression or docility, behavioral or otherwise (*e.g.* energetic or life-history), that females are choosing. It is also possible that male-male competition may swamp our ability to detect female mate choice. We did, however find evidence for male mate choice based on docility via mating chase attendance. Taylor et al. (in prep; Chapter 3) found that males with intermediate levels of docility sired offspring with higher overwinter survival. Thus we inferred, because males do not provide paternal care that these offspring are

avored because they inherited good genes from their sires. Following this line of reasoning we predicted disassortative mating for docility whereby individuals would prefer a mate with opposite docility in order to produce offspring with moderate docility. We found a negative interaction between male docility and female docility on attendance, showing that males attended mating chases disassortatively based on docility. Because stabilizing selection for docility was only present in high-competition environments we additionally predicted that disassortative mate choice for docility should be stronger in high-competition years. However, we found a positive three-way interaction between male docility, female docility and competition. This interaction can be interpreted as disassortative mating based on docility that is mediated by the third variable, competition, and indicates that the two-way interaction decreased in strength with competition, the opposite of our prediction. Why should male red squirrels be picky and not take advantage of every mating opportunity? There are costs in energy, time, territorial defense and predation to attending mating chases and to identifying when a female is in estrus. Male red squirrels must travel to each female's territory in order to ascertain her reproductive status in advance of her day of estrus. These costs are clearly represented by the effect of distance on mating chase attendance, but it is surprising that males that live close to a female do not always attend her chase. Approximately one third of mating chases were concurrent with at least one other mating chase (*i.e.* on the same day) and, because sperm precedence is important, the benefits of attending a second mating chase in one day may be very small. Male squirrels were also more likely to attend a particular mating chase later in the season when the number of females that had not had their mating chase was lower. There may be a point at which the cost of keeping track of additional females outweighs any benefits of attending an additional mating chase at which point males could prioritize which female's chases have the highest benefit. Males attend more mating chases in low-competition years, which may be why we find stronger mate choice in those years. Males also increase their energetic expenditure during the mating season (Lane et al., 2009) in anticipation of elevated autumn resources and associated high juvenile recruitment. High juvenile competition years are the result of high spring (adult) density and low autumn resources (McAdam et al. in prep). There may be higher

costs to assessing the reproductive status of distant females when population densities are high due to potentially increased intruder pressure and fewer gaps between territories in which squirrels could travel unmolested. In fact we do find that in high competition years there is a stronger negative effect of distance on male attendance (Figure 4.2). Our finding that disassortative mating is stronger in low juvenile-competition years may be due not to a larger benefit of mate choice, but to a greater opportunity to be selective. We did not find any evidence of mate-choice based on aggression. We had predicted that aggressive mates would be preferred in high-competition environments because Taylor et al. (in prep; Chapter 3) found positive selection for parental aggression in high-competition years. We did find significant effects of aggression on siring success after controlling separately for copulation success, copulation order and copulation number, but there was no significant interaction between aggression and competition, and the effect of aggression on siring success was negative. A negative relationship between aggression and sperm competition has been reported in a number of fish species (Alonzo and Warner, 2000; Le Comber et al., 2003; Spence and Smith, 2005; Rezucha and Smith, 2012), and Koyama and Kamimura (2000) found a negative relationship between aggression and sperm motility in mice. Therefore our results may represent a trade-off between aggression and sperm quality. Conclusion We tested the hypotheses that mating chases and male attendance at mating chases are mechanisms by which red squirrels can acquire context dependent good and complementary genes for their offspring's recruitment. We did not find support for the mating chase hypothesis, but we did find support for mate choice through attendance by male red squirrels for complementary genes. Male red squirrels attended mating chases disassortatively by docility, presumably because stabilizing selection for recruitment favored juveniles with moderate docility. However, disassortative attendance was stronger in low juvenile-competition years, which did not match our prediction. We also surprisingly identified post-copulatory selection for less aggressive males, which may be due to a trade-off between aggression and sperm quality. The role female red squirrels play in mating chases remains a mystery that we hope future studies will uncover.

Chapter 5

CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Conclusions

In this dissertation I investigated the proximate sources of variation in red squirrel behavior by estimating sources of genetic, maternal and environmental variation, and the ultimate effect of behavior on reproductive success. In Chapter 2, I used a Bayesian animal model approach to estimate genetic parameters for aggression, activity and docility and found support for low heritabilities ($V_A/V_P = 0.08 - 0.12$), and cohort effects ($V_{cohort}/V_P = 0.07 - 0.09$), as well as low to moderate maternal effects ($V_M/V_P = 0.07 - 0.15$) and permanent environmental effects ($V_{PE}/V_P = 0.08 - 0.16$). I also found evidence of a substantial positive genetic correlation (0.68) and maternal effects correlation (0.58) between activity and aggression, providing evidence of genetically based behavioral correlations in red squirrels. These results provide evidence for the presence of heritable variation in red squirrel behavior, but also emphasize the role of other sources of variation, including maternal effects, in shaping patterns of variation and covariation in behavioral traits. I then tested the hypothesis that ecological changes through time lead to fluctuating selection, which maintains variation in behavioral traits (Chapter 3). Linear selection gradients on red squirrel dam aggression and activity significantly fluctuated in sign across years depending on the level of competition among juveniles. Selection on aggression and activity also differed among components of fitness, between the sexes and included nonlinear components between and within traits that also changed through time. These results suggest that repeatable and heritable individual differences in red squirrel behavior could be maintained by complex fluctuations in natural and sexual selection. Finally, I tested the hypotheses that mating chases provide the opportunity for both female and male red squirrels to select for context-dependent good genes and complementary genes for their offspring's recruitment (Chapter 4). Specifically, I predicted that aggressive mates would be preferred

in high-juvenile competition years, but disfavored in low competition years, and that mate choice for docility would be disassortative with low-docility squirrels preferring high-docility mates and vice-versa. I did not find support for adaptive context-dependent mate choice by females, but I did find support for male mate choice for complementary genes that was mediated through which female mating chase males attended. Male red squirrels attended mating chases disassortatively by docility, which would enhance fitness due to stabilizing selection on docility during juvenile recruitment. I also found evidence for post-copulatory selection for less aggressive males. These results highlight that even in systems with very high operational sex ratios, male choice is a factor that needs to be considered. In this dissertation I have shown that behavioral traits in red squirrels are important with strong evolutionary implications. Red squirrel behavior is heritable, subject to maternal effects and under strong selection that varies with environmental fluctuation and across components of reproductive success. This research has provided strong evidence that fluctuating selection could maintain variation in behavior (Chapter 2). Incidentally, while also showing that mate choice may reduce variation (Chapter 3). These findings provide a foundation for future work to elucidate mechanisms and continue exploring how and why behavioral variation is maintained. There are major areas of research regarding red squirrel personality that have yet to be investigated (some of which are outlined below), but these results also expose targeted questions that can be asked (as in Chapter 3).

5.2 Future Directions

5.2.1 Are there consistent individual differences in behavioral plasticity?

There is increasing evidence that individuals differ in their behavioral responsiveness to environmental variation (reviewed in Dingemanse et al. 2010) and that behavioral plasticity is linked with fitness (e.g. Betini and Norris 2011). Red squirrels have evolved in a variable environment and plasticity in response to environmental variation has been documented for life-history traits (McAdam and Boutin 2003*b*; Boutin et al. 2006; Dantzer et al. in prep). In Chapter 2 I found

evidence of behavioral plasticity in aggression and docility in the form of habituation as well as in response to seasonal change (i.e. day of year). Future studies should test whether behavioral plasticity occurs in response to variation in density and resources. Additionally, using individual behavioral reaction norms (Dingemanse et al., 2010) future studies should investigate whether individuals vary in their plasticity and if that variation is under selection.

5.2.2 Investigation of links between behavior and life-history, energetics and hormone levels.

There has been growing interest in how behavior co-evolves with life-history, energetics and hormones. A number of hypotheses positing explanations for consistent individual differences rely on life-history variation as the source of behavioral variation (Wolf et al., 2007; Biro and Stamps, 2008; Dingemanse and Wolf, 2010). Similarly, links between energetics and personality have also been proposed (Careau et al., 2008). Recently Réale et al. (2010*b*) integrated these hypotheses into the pace-of-life syndrome (POLS) framework (Ricklefs and Wikelski, 2002). Whether mediated by proximate or ultimate mechanisms these hypotheses posit that behavioral variation may arise through selection for optimal combinations of behavior and life-history, energetic or hormonal traits. Studies of this fundamental component of behavioral theory are sorely needed as it has rarely been tested in the wild (but see Réale et al. 2009). The Kluane red squirrel system provides an excellent opportunity to study these questions due to the multi-disciplinary approach already underway.

APPENDICES

Appendix A

Table A.1: Between-observer reliability, means and variances of specific behaviors recorded during open field and mirror-image stimulation tests. Correlations were calculated for each behavior between two observers' scores for the same trial. Behaviors with a reliability greater than 0.7 were used in further analysis. Mirror image stimulation behaviors are indicated above, open field behaviors are below.

Behavior	Reliability (r^2)	Kept	Mean	Variance
Crouch Rate	0.50		0.45	0.77
Front	0.86	Y	26.13	1023.99
Attack Rate	0.97	Y	0.66	2.15
Grunt Rate	0.00 *		0.93	15.99
Stretch	0.32		0.81	3.12
Back	0.99	Y	42.59	1605.63
Attack Latency	1.00	Y	217.76	13472.92
Approach Latency	0.94	Y	138.71	16610.15
Hole Rate	0.90	Y	1.90	2.41
Jump Rate	1.00	Y	4.26	19.82
Chew	0.80	Y	5.68	109.33
Still	0.85	Y	31.75	705.5
Hang	0.99	Y	14.10	251.44
Groom	0.94	Y	3.70	17.17
Rear	0.13		8.94	99.28
Scan	0.67		12.70	217.98
Walk	0.71	Y	16.79	133.26
Sniff	0.39		4.93	18.00

* Grunt rate was not consistently recorded

Table A.2: Summary statistics for the entire multi-generational red squirrel pedigree, pedigree of individuals informative for docility and pedigree of individuals informative for activity and aggression.

	Entire Pedigree	Docility Pedigree	OF & MIS Pedigree
Records	7086	819	451
Maternities	5620	444	247
Paternities	1549	311	146
Full sibs	1056	61	21
Maternal sibs	27501	486	194
Maternal half sibs	26445	425	173
Paternal sibs	5051	255	90
Paternal half sibs	3995	194	69
Maternal grandmothers	3543	258	135
Maternal grandfathers	1080	154	63
Paternal grandmothers	458	119	53
Paternal grandfathers	342	88	28
Maximum pedigree depth	8	8	7
Founders	1442	361	193
Mean maternal sibship size	6.862	2.166	1.871
Mean paternal sibship size	3.688	1.968	1.780
Mean pairwise relatedness	0.001	0.003	0.004

Table A.3: Principle component loadings for behaviors from an open field arena test in North American red squirrels. Behaviors were measured as percentage of time unless otherwise noted.

Open Field Behavior	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Walk	0.490	-0.259	0.119	0.063	-0.045	-0.199	-0.576	-0.549
Jump Rate	0.436	-0.057	0.106	0.298	-0.138	-0.587	0.580	0.089
Hole Rate	0.307	-0.495	0.131	0.080	0.452	0.561	0.339	-0.051
No. Pellets	0.288	-0.089	0.023	-0.678	-0.601	0.219	0.199	-0.012
Hang	0.253	0.634	-0.227	0.333	-0.144	0.400	0.175	-0.401
Chew	0.240	0.381	-0.107	-0.563	0.626	-0.249	0.077	-0.094
Groom	-0.061	-0.344	-0.914	-0.011	-0.011	-0.114	0.089	-0.144
Still	-0.517	-0.109	0.241	-0.118	-0.004	-0.134	0.365	-0.705
Std. Deviation	1.706	1.130	0.993	0.964	0.875	0.774	0.619	0.389
Prop. Variance	0.364	0.160	0.123	0.116	0.096	0.075	0.048	0.019

Table A.4: Principle component loadings for behaviors from an mirror-image stimulation test in North American red squirrels. Behaviors were measured as percentage of time unless otherwise noted. Latencies were log transformed prior to principle component analysis.

MIS Behavior	PC1	PC2	PC3	PC4	PC5
Front	0.491	-0.268	0.161	0.813	-0.006
Attack Rate	0.373	0.622	0.606	-0.143	-0.292
Back	-0.407	0.612	-0.369	0.519	-0.235
Attack Latency	-0.469	-0.400	0.387	0.070	-0.683
Approach Latency	-0.484	0.081	0.567	0.211	0.627
Std. Deviation	1.668	0.948	0.789	0.611	0.567
Prop. Variance	0.557	0.180	0.124	0.075	0.064

Table A.5: Candidate univariate models of sources of variation in behavior in North American red squirrels. Fixed effects (FE) are trial number, quadratics for trial number, study area, day of year and observer. Random effects are individual identity (V_{PE}), additive genetic (V_A), maternal effects (V_M) and cohort effects (V_C). Deviance Information Criterion (DIC) estimates are given for each model. Models within 2 DIC of the best model were considered to have equal support (bold).

Open field (OF)	DIC	Δ DIC
OF~FE+ V_{PE} + V_M + V_C	1916.2	0
OF~FE+ V_{PE} + V_A + V_M + V_C	1916.4	0.2
OF~FE+ V_{PE} + V_M	1919.7	3.5
OF~FE+ V_{PE} + V_A + V_M	1920.6	4.4
OF~FE+ V_{PE} + V_C	1931.9	15.7
OF~FE+ V_{PE} + V_A + V_C	1932.9	16.7
OF~FE+ V_{PE}	1935.7	19.5
OF~FE+ V_{PE} + V_A	1936.3	20.1
OF~FE+ V_C	2028.9	112.7
OF~FE	2037.9	121.7
OF~ 1	2110.7	194.5
Mirror image (MIS)	DIC	Δ DIC
MIS~FE+ V_{PE} + V_A + V_C	2018.6	0
MIS~FE+ V_{PE} + V_A	2019.7	1.1
MIS~FE+ V_{PE} + V_C	2019.7	1.1
MIS~FE+ V_{PE}	2019.9	1.3
MIS~FE+ V_{PE} + V_A + V_M + V_C	2020.4	1.8
MIS~FE+ V_{PE} + V_M + V_A	2021.3	2.8
MIS~FE+ V_{PE} + V_M	2022.8	4.2
MIS~FE+ V_{PE} + V_M + V_C	2023.0	4.5
MIS~FE+ V_C	2079.3	60.7
MIS~FE	2082.1	63.5
MIS~FE	2084.5	65.9

Table A.6: Candidate univariate models of sources of variation in behavior in North American red squirrels. Fixed effects (FE) are trial number, quadratics for trial number, study area, day of year and observer. Random effects are individual identity (V_{PE}), additive genetic (V_A), maternal effects (V_M) and cohort effects (V_C). Deviance Information Criterion (DIC) estimates are given for each model. Models within 2 DIC of the best model were considered to have equal support (bold).

Struggle rate (SR)	DIC	Δ DIC
SR~FE+ V_{PE} + V_A + V_M + V_C	25140.6	0
SR~FE+ V_{PE} + V_A + V_M	25141.4	0.8
SR~FE+ V_{PE} + V_A + V_C	25142.2	1.6
SR~FE+ V_{PE} + V_A	25142.8	2.2
SR~FE+ V_{PE} + V_M + V_C	25143.2	2.7
SR~FE+ V_{PE} + V_M	25145.2	4.6
SR~FE+ V_{PE} + V_C	25149.2	8.7
SR~FE+ V_{PE}	25151.1	10.5
SR~FE+ V_C	26452.0	1311.4
SR~FE	26515.0	1374.4
SR~FE	26856.6	1716.0

Table A.7: Candidate trivariate animal models of sources of (co)variation of the behavioral traits aggression, activity and docility. Fixed effects (FE) are trial number, quadratics for trial number, study area, day of year and observer. Random effects are individual identity (V_{PE}), additive genetic (V_A), maternal effects (V_M) and cohort effects (V_C). Deviance Information Criterion (DIC) estimates are given for each model. Models within 2 DIC of the best model were considered to have equal support.

Model	DIC	Δ DIC
(OF, MIS, SR)~FE+ V_{PE} + V_A + V_M + V_C	29220.86	0
(OF, MIS, SR)~FE+ V_{PE} + V_M + V_C	29228.14	7.28
(OF, MIS, SR)~FE+ V_{PE} + V_A + V_M	29234.71	13.85
(OF, MIS, SR)~FE+ V_{PE} + V_A + V_C	29235.83	14.97
(OF, MIS, SR)~FE+ V_{PE} + V_M	29240.49	19.63
(OF, MIS, SR)~FE+ V_{PE} + V_C	29250.52	29.66
(OF, MIS, SR)~FE+ V_{PE} + V_A	29254.60	33.74
(OF, MIS, SR)~FE+ V_{PE}	29267.78	46.92

Appendix B

Table B.1: The effect of competition on linear selection for dam and sire behavioral traits through annual reproductive success. Coefficients are from a GLMM with ARS as the response and each study-area-year combination as a random effect and a Poisson error distribution.

Term	Dams			Sires		
	Estimate	Z	P	Estimate	Z	P
Intercept	-1.33 ± 0.23	-5.66	< 0.001	-2.05 ± 0.17	-11.75	< 0.001
Competition	-4.06 ± 0.70	-5.83	< 0.001	-0.63 ± 0.16	-3.88	< 0.001
Aggression	0.75 ± 0.23	3.28	0.001	0.02 ± 0.20	0.08	0.94
Activity	-0.30 ± 0.20	-1.51	0.13	-0.22 ± 0.18	-1.22	0.22
Docility	-0.30 ± 0.16	-1.88	0.06	-0.02 ± 0.15	-0.15	0.88
Competition×Aggression	2.55 ± 0.69	3.69	< 0.001	-0.10 ± 0.17	-0.59	0.56
Competition×Activity	-1.30 ± 0.60	-2.17	0.03	0.07 ± 0.15	0.45	0.65
Competition×Docility	-1.02 ± 0.44	-2.30	0.02	0.01 ± 0.14	0.05	0.96

Random effect grid-year variances: Dam = 0.035, Sire = 0.129

Table B.2: Vector of standardized directional selection gradients(β') for fecundity or siring success, and the matrix of standardized quadratic and correlational selection gradients (γ). Linear and quadratic selection gradients were estimated in separate regressions. Quadratic selection coefficients were doubled to give quadratic selection gradients (Stinchcombe et al. 2008)

<u>High Competition</u>								
	Dams				Sires			
	β'	Aggression	Activity	Docility	β'	Aggression	Activity	Docility
Aggression	$-0.08 \pm 0.04^\dagger$	$0.20 \pm 0.08^*$			-0.07 ± 0.18	0.67 ± 0.41		
Activity	-0.01 ± 0.04	-0.03 ± 0.06	0.03 ± 0.09		0.05 ± 0.14	-0.33 ± 0.20	-0.10 ± 0.21	
Docility	-0.02 ± 0.04	-0.09 ± 0.07	$0.14 \pm 0.07^\dagger$	0.04 ± 0.07	$0.26 \pm 0.09^*$	-0.20 ± 0.17	0.11 ± 0.13	0.07 ± 0.15
<u>Low Competition</u>								
	Dams				Sires			
	β'	Aggression	Activity	Docility	β'	Aggression	Activity	Docility
Aggression	-0.02 ± 0.05	0.04 ± 0.11			0.15 ± 0.17	$-0.63 \pm 0.21^*$		
Activity	$0.09 \pm 0.05^\dagger$	-0.01 ± 0.08	-0.05 ± 0.11		$-0.48 \pm 0.16^*$	-0.21 ± 0.20	0.33 ± 0.30	
Docility	$0.10 \pm 0.04^*$	-0.09 ± 0.07	0.06 ± 0.07	-0.01 ± 0.06	0.01 ± 0.13	-0.04 ± 0.18	0.00 ± 0.20	0.15 ± 0.20

* $P < 0.05$, $^\dagger P < 0.1$

Table B.3: Vector of standardized directional selection gradients(β') for offspring overwinter survival, and the matrix of standardized quadratic and correlational selection gradients (γ). Linear and quadratic selection gradients were estimated in separate regressions.

<u>High Competition</u>								
	Dams				Sires			
	β'	Aggression	Activity	Docility	β'	Aggression	Activity	Docility
Aggression	$0.53 \pm 0.14^*$	0.02 ± 0.37			0.20 ± 0.22	0.07 ± 0.68		
Activity	-0.28 ± 0.19	0.28 ± 0.28	0.06 ± 0.46		-0.14 ± 0.19	-0.18 ± 0.26	$-0.77 \pm 0.34^*$	
Docility	$-0.29 \pm 0.14^*$	0.00 ± 0.20	-0.09 ± 0.26	-0.22 ± 0.28	-0.07 ± 0.16	-0.06 ± 0.23	$0.52 \pm 0.26^*$	-0.39 ± 0.25
<u>Low Competition</u>								
	Dams				Sires			
	β'	Aggression	Activity	Docility	β'	Aggression	Activity	Docility
Aggression	$-0.31 \pm 0.12^*$	0.12 ± 0.29			-0.20 ± 0.21	0.38 ± 0.46		
Activity	$0.24 \pm 0.12^\dagger$	-0.13 ± 0.22	0.27 ± 0.28		0.12 ± 0.19	0.06 ± 0.33	0.27 ± 0.53	
Docility	0.00 ± 0.09	-0.07 ± 0.15	0.07 ± 0.16	0.09 ± 0.16	-0.07 ± 0.12	0.42 ± 0.35	-0.03 ± 0.26	0.07 ± 0.27

* $P < 0.05$, $\dagger P < 0.1$

Appendix C

Table C.1: Reduced models of male attendance, siring success and copulation success of males that attended a mating chase, and siring success of males that copulated. The full GLMM models are described in the methods. The least significant terms were removed, starting with the highest order. Male identity and mating chase identity were included as random effects. A binary error distribution was assumed.

Record	Grid Year	Competition	F ID	Julian	Attendees	Partners	Sires	F Agg.	F Act.	F Doc.
1	KL2005	3.3	4329	93	9	7	3	−0.5	−0.6	3.0
2	KL2005	3.3	4329	93	9	7	3	−0.5	−0.6	3.0
3	KL2005	3.3	4706	103	12	2	2	−0.2	−0.2	7.7
Record	M ID	M Agg.	M Act.	M Doc.	SS	BS	Attend	Copulate	Cop. Order	No. Cops.
1	4559	−0.6	−0.5	3.2	0	0	Y	Y	3	2
2	4029	−0.2	−1.1	4.2	1	1	N	N	NA	NA
3	4559	−0.6	−0.5	3.2	0	0	NA	NA	NA	NA
Record	M-F Distance (m)									
1	289.3									
2	114.0									
3	162.6									

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LITERATURE CITED

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