University of Alberta

Scramble competition promiscuity: the behavioural and genetic mating system of North American red squirrels (*Tamiasciurus hudsonicus*).

by

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ABSTRACT

The primary mammalian subjects for most studies of mating systems and male reproductive success have been large species with pronounced sexual dimorphism. The obvious sexually selected traits in these species have ostensibly made them amenable to studies of this sort. This focus, however, has lead to an unbalanced view of mammal mating systems and the correlates of male reproductive success in general. I characterized the behavioural and genetic mating system, measured the extent of variation in male reproductive success, and outlined the correlates of this variation for a population of North American red squirrels (*Tamiasciurus hudsonicus*). The mating system was a form of scramble competition, whereby males were unable to defend reproductive access to females and searched for reproductive opportunity. Females exhibited among the highest levels of multi-male mating (MMM) of any squirrel species studied to date. They did not, however, realize either a cryptic direct, or genetic benefit to these high levels. Further, females were not shown to bias paternity of their litters to favour more genetically dissimilar males. Rather, female MMM was positively correlated to the number of males attending their estrous bouts, suggesting that female mating behaviour is a passive response to multi-female mating in males. During the mating season, males expanded their home ranges to, on average, 10 times the size of those measured during the non-mating season in order to locate and mate with receptive females and male reproductive success was more influenced by search ability and effort than by body size or age. Males showed evidence of being limited energetically during the breeding season and adjusted reproductive effort to correspond to current and future resource conditions. An understanding of the relationships between biological features of an organism and

reproductive success is fundamental to all attempts to measure selection and to understand the adaptive significance of these features. While advances with female defence mating systems have been made in this area, the results from my study, in combination with previous work, highlight the far richer candidate set of sexually-selected traits than has previously been appreciated.

DEDICATION

For my Gramma, Jean Quaschnick
and Grandad, James Lane

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CHAPTER 1. GENERAL INTRODUCTION

1.1 MATING SYSTEMS

The manners by which members of a population achieve reproductive success, including the number of mates acquired by individuals, the behavioural strategies employed in acquiring these mates and patterns of parental care, are collectively referred to as its mating system (Emlen & Oring 1977). A diversity of mating systems exists in nature, exhibiting variations on multiple mating by males (polygny), females (polyandry) and both sexes (promiscuity) as well as single mating by both sexes (monogamy). An evolutionary explanation for the specific mating system exhibited by a population requires an understanding of the divergent ways that members of each sex attain reproductive success.

Females, by definition, invest heavily in individual gametes but produce them in relatively low numbers. By contrast, male expend relatively little on individual gametes but produce them in vast quantities (Bell 1978). This fundamental difference sets the stage for divergent reproductive strategies. Males, generally, are not constrained by gamete production and their reproductive success should thus be limited by their number of mates (Bateman 1948; Trivers 1972). As gamete production of females is constrained, their reproductive success should not be limited by their number of mates but by the resources that they can allocate to individual offspring (Bateman 1948; Trivers 1972). In eutherian mammals, the initial asymmetries in resource allocation to individual gametes are further compounded throughout offspring development. Internal development increases the resource requirements to females, but also limits the paternity certainty of copulating males. In the majority of mammalian species females consequently provide exclusive parental care (Clutton-Brock 1991).

Constrained gamete production, internal development and extended parental care of female mammals limits their potential rate of reproduction and leads to them being the limiting sex in most mammal populations (Trivers 1972; Clutton-Brock & Vincent 1991). Males thus compete for access to receptive females and sexual selection should favour male traits that enhance their competitive ability (Clutton-Brock & Vincent 1991; Andersson 1994). The opportunity for sexual selection, a standardized measure of variation in reproductive success (Wade & Arnold 1984a), on males is typically higher than on females (Wade & Arnold 1984b), and sexual selection is thought to be responsible for the development of exaggerated morphological dimorphism seen in many mammalian species (Andersson 1994; Clutton-Brock et al. 1982; Kruuk et al. 2002).

1.2 SCRAMBLE COMPETITION

The most common mammalian mating systems are forms of polygyny, in which males do not provide parental care and attempt to mate with multiple females (Clutton-Brock 1989). The specific form of polygyny exhibited, however, depends on the defensibility of females. If females are clumped in space, and/or come into estrus asynchronously, dominant males should be able to defend access to receptive females, giving rise to a female defense mating system (Emlen & Oring 1977; Ims 1988). Conversely, if females are spatially dispersed, and/or breed synchronously, they become economically indefensible and a scramble competition mating system is more likely (Ims 1988; Murphy 1998). While female defence mating systems have been extensively studied (summarized in Clutton-Brock 1988), others, such as scramble competition, have received comparatively little attention.

In mammalian scramble competition mating systems, the spatial dispersion of females renders defence of them uneconomical (Schwagmeyer & Woontner 1986). Male mating success should thus correlate with variation in traits that enable them to efficiently locate and follow estrous females, rather than traits promoting dominance over competitors (Schwagmeyer & Woontner 1986; Schwagmeyer & Parker 1987; Schwagmeyer et al. 1998; Spritzer et al. 2005 a,b). Sexual selection acting on these traits has been proposed to explain both male-female spatial ability asymmetries (McNemar & Stone 1932; Joseph et al. 1978; Schwagmeyer et al. 1998, Spritzer et al. 2005a) and also why the home ranges of males often expand during the mating season, while those of females remain relatively consistent throughout the year (e.g. Erlinge & Sandell 1986; Stockley et al. 1994; Kappeler 1997; Edelman & Koprowski 2005).

1.3 EXCEPTIONS TO THE RULE?

1.3.1 Female Multiple-mating

The constrained maximum offspring production of females was originally thought to limit the benefits attained from multiple mating (Bateman 1948; Trivers 1972). Behavioural and genetic studies have revealed, however, widespread multi-male mating (MMM) by the females of diverse taxa, challenging the traditional portrayal of females as relatively choosey and monogamous. A number of hypotheses have been put forward to explain female MMM, the majority of which rely on direct benefits to the female, and hence, fit theoretical predictions (reviewed in Birkhead & Parker 1997). If females receive nutritional rewards or parental care from males, these resources can be allocated to developing offspring with corresponding benefits to the females' reproductive success. The lack of parental care and nuptial gifts in most mammals, however, limit the applicability of these hypotheses (Wolf & Macdonald 2004).

Hypotheses for MMM in mammals typically invoke either cryptic direct (e.g. fertilization assurance, infanticide avoidance; Stockley 2003; Wolff & Macdonald 2004) or indirect, genetic benefits (e.g. mating multiply for good and/or compatible genes; Neff & Pitcher 2005). The difficulties inherent in monitoring the natural mating behaviour of mammals, however, have resulted in most tests of these hypotheses being conducted under laboratory conditions (e.g. Wolff & Dunlap 2002; Klemme et al. 2007), while their relevance within the context the species' natural history have rarely been investigated (but see Hoogland 1998; Fisher et al. 2006).

1.3.2 Resource Limitations on Male Reproduction

As the reproductive success of male mammals has traditionally been assumed limited by reproductive opportunity, analyses of the correlates of success have tended to focus on traits providing males with a competitive advantage (Andersson 1994). Similarly, as female reproductive success has traditionally been assumed limited by the resources they can allocate to offspring, the energetic costs of gestation and lactation have been estimated for a variety of species (e.g. Loudon & Racey 1987; Sikes 1995; Huber et al. 1999; Byers et al. 2005). Where investigated, however, the costs of reproductive effort to males are often shown to be similar, or more than females (e.g. Michener & Locklear 1990; Galimberti et al. 2007). These results call into question the long-held assumption that energetic limitations to males are minimal as compared to females. Energetic assessments, however, have been primarily limited to large species with pronounced sexual dimorphism. To what extent these results may hold generally for mammalian species is currently unknown.

1.4 RED SQUIRRELS

North American red squirrels (*Tamiasciurus hudsonicus*, hereafter red squirrel) are small (ca. 250 g), semi-arboreal mammals with a diurnal activity cycle. Populations of red squirrels are found throughout North America, and their distribution roughly parallels the distribution of coniferous forests (Steele 1998). In northern coniferous forests individuals maintain year-round exclusive territories, centered on a food cache of conifer cones (midden; Smith 1968). The possession of a territory, with its associated midden, is thought to be necessary for over-winter survival (Rusch & Reeder 1978; McAdam & Boutin 2003).

Attributes of the natural history of red squirrels allowed for a rare opportunity to conduct a comprehensive examination of the mating system of a free-ranging population. All activity, including mating, takes place during the day. This enabled the direct observation of individual estrous females as well as attending and copulating males. Additionally, the majority of young are born in easily accessible arboreal grass nests, allowing immediate access after parturition. It was thus possible to access litters to collect tissue for genetic analysis and take measurements (e.g. offspring mass, litter size) at the earliest possible stage. The long-term territory occupancy of individuals both permits the repeated capture of individuals to measure morphological and physiological characters (e.g. mass, testis size, energy expenditure) and also allows for an accurate census of population size. Finally, the primary food resource, seeds of white spruce (*Picea glauca*), is easily quantified using established protocols (e.g. Humphries & Boutin 2000; McAdam & Boutin 2003). Populations of red squirrels have been monitored at my study site since 1986 and detailed information is now available on the basic ecology, life history and behaviour of all individuals (e.g. Berteaux & Boutin 2000; Humphries & Boutin 2000; Reale et al. 2003; McAdam et al. 2007).

1.5 OBJECTIVES AND THESIS STRUCTURE

As a first step in characterizing the mating system of red squirrels, I documented the levels of female multiple mating and paternity in Chapter 2. I employed detailed behavioural observations on estrous females with genetic paternity analysis of resultant offspring to quantify the natural levels of multiple mating and paternity and tested four hypotheses for multiple mating in mammals (fertilization assurance, infanticide avoidance, genetic quality and genetic diversity).

I used the genotypes of resultant offspring and paternity patterns established for Chapter 2 to test the hypothesis that genetic relatedness of mates predicts patterns of parentage in Chapter 3. The detrimental effects of inbreeding, long-studied under laboratory conditions, have recently been shown to have equivalent, or enhanced, effects in the wild. Multiple mating females of a number of taxa have been shown to bias paternity of their offspring to favour genetically-dissimilar males. Thus, post-copulatory inbreeding avoidance represents a plausible hypothesis for multiple mating. I tested this hypothesis, both by comparing observed to predicted parentage patterns based on genetic relatedness, and also by investigating the performance and fate of offspring with parents varying in genetic relatedness.

In Chapter 4, I established the second component of the mating system for this population: patterns of male reproductive behaviour and success. Red squirrels exhibited a scramble competition mating system, in which male reproductive success should be more limited by their ability to locate and follow, than by their ability to defend access to, receptive females. I tested this hypothesis and calculated standardized sexual selection gradients on both mate search ability and effort.

I quantified the daily energy expenditure (DEE) of males during the breeding season in Chapter 5. In Chapter 4, male reproductive success was shown to be limited by search ability and effort, therefore I measured one hypothesized influence on search effort, energetic cost. I measured the DEE of males in two breeding seasons characterized by varying fitness payoffs and across two varying food conditions to test whether males are limited by current resources available for reproduction and/or vary resource allocation when faced with varying fitness returns.

Together these four chapters provide a comprehensive examination of the mating system of a free-ranging population. The combination of detailed behavioural observations on a tractable study system, genetic analyses of paternity, physiological measures of effort and measurement of the performance and fate of resultant juveniles provided me with the rare opportunity to examine both the influences on, and consequences of, observed reproductive patterns. Further, by studying the natural mating system of a free-ranging population, I was able to investigate the influence of ecological variables (e.g. mating season duration, fluctuating food resources) that would not have been possible with a laboratory-based study.

1.6 REFERENCES

Andersson, M. B. 1994. *Sexual Selection*. Princeton: Princeton University Press.

Arnold, S. J. & Wade, M. J. 1984a. On the measurement of natural and sexual selection: theory. *Evolution*, **38**, 709-719.

Arnold, S. J. & Wade, M. J. 1984b. On the measurement of natural and sexual selection: applications. *Evolution*, **38**, 720-734.

Bateman, A. J. 1948. Intra-sexual selection in drosophila. *Heredity*, **2**, 349-368.

Birkhead, T. R. & Parker, G. A. 1997. Sperm Competition and Mating Systems. In: *Behavioural Ecology: An Evolutionary Approach*. 4th edn. (Ed. by J. R. Krebs & N. B. Davies), pp. 121-148. Oxford: Blackwell Scientific.

Bell, G. 1978. The evolution of anisogamy. *Journal of Theoretical Biology* **73**: 247-270.

Clutton-Brock, T. H. 1988. *Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems*. Chicago: University of Chicago Press.

Clutton-Brock, T. H. 1989. Mammalian mating systems. *Proceedings of the Royal Society of London, Series B*, **236**, 339-372.

Clutton-Brock, T.H. 1991. *The Evolution of Parental Care*. Princeton: Princeton University Press.

Clutton-Brock, T. H., Guinness, F. E. & Albon, S. D. 1982. *Red deer: Behavior and Ecology of Two Sexes*. Chicago: University of Chicago Press.

Clutton-Brock, T. H. & Vincent, A. C. J. 1991. Sexual selection and potential reproductive rates of males and females. *Nature*, **351**, 58-60

Edelman, A. J. & Koprowski, J. L. 2006. Seasonal changes in home ranges of Abert's squirrels: Impact of mating season. *Canadian Journal of Zoology*, **84**, 404-411.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and evolution of mating systems. *Science*, **197**, 215-223.

Erlinge, S. & Sandell, M. 1986. Seasonal changes in the social organization of male stoats, *mustela erminea*: An effect of shifts between 2 decisive resources. *Oikos*, **47**, 57-62.

Ims, R. A. 1988. Spatial clumping of sexually receptive females induces space sharing among male voles. *Nature*, **335**, 541-543.

Joseph, R., Hess, S. & Birecree, E. 1978. Effects of hormone manipulations and exploration on sex-differences in maze-learning. *Behavioral Biology*, **24**, 364-377.

Kappeler, P. M. 1997. Intrasexual selection in *Mirza coquereli*: evidence for scramble competition polygyny in a solitary primate. *Behavioral Ecology and Sociobiology*, **41**, 115-127.

Klemme, I., Eccard, J. A. & Ylönen, H. 2007. Why do female bank voles, *Clethrionomys glareolus*, mate multiply? *Animal Behaviour*, **73**, 623-628.

Kruuk, L. E. B., Slate, J., Pemberton, J. M., Brotherstone, S., Guinness, F. & Clutton-Brock, T. 2002. Antler size in red deer: heritability and selection but no evolution. *Evolution*, **56**, 1683-1695.

McNemar, Q. & Stone, C. P. 1932. Sex difference in rats on three learning tasks. *Journal of Comparative Psychology*, **14**, 171-180.

Murphy, C. G. 1998. Interaction-independent sexual selection and the mechanisms of sexual selection. *Evolution*, **52**, 8-18.

Neff, B. D. & Pitcher, T. E. 2005. Genetic quality and sexual selection: An integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19-38.

Schwagmeyer, P. L. & Parker, G. A. 1987. Queuing for mates in 13-lined ground-squirrels. *Animal Behaviour*, **35**, 1015-1025.

Schwagmeyer, P. L., Parker, G. A. & Mock, D. W. 1998. Information asymmetries among males: Implications for fertilization success in the thirteen-lined ground squirrel. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 1861-1865.

Schwagmeyer, P. L. & Woontner, S. J. 1986. Scramble competition polygyny in 13-lined ground-squirrels: the relative contributions of overt conflict and competitive mate searching. *Behavioral Ecology and Sociobiology*, **19**, 359-364.

Spritzer, M. D., Solomon, N. G. & Meikle, D. B. 2005a. Influence of scramble competition for mates upon the spatial ability of male meadow voles. *Animal Behaviour*, **69**, 375-386.

Spritzer, M. D., Meikle, D. B. & Solomon, N. G. 2005b. Female choice based on male spatial ability and aggressiveness among meadow voles. *Animal Behaviour*, **69**, 1121-1130.

Stockley, P. 2003. Female multiple mating behaviour, early reproductive failure and litter size variation in mammals. *Proceedings of the Royal Society of London, Series B*, 270, 271-278.

Stockley, P., Searle, J. B., Macdonald, D. W. & Jones, C. S. 1994. Alternative reproductive tactics in male common shrews: relationships between mate-searching behavior, sperm production, and reproductive success as revealed by DNA-fingerprinting. *Behavioral Ecology and Sociobiology*, **34**, 71-78.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man, 1871-1971* (Ed. by Campbell, B. G.), pp. 136-179. Chicago: Aldine.

Wolff, J. O. & Macdonald, D. W. 2004. Promiscuous females protect their offspring. *Trends in Ecology and Evolution*, **19**, 127-134.

Wolff, J. O. & Dunlap, A. S. 2002. Multi-male mating, probability of conception, and litter size in the prairie vole (*Microtus ochrogaster*). *Behavioural Processes*, **58**, 105-110.

CHAPTER 2. FEMALE MULTIPLE MATING AND PATERNITY IN FREE-RANGING NORTH AMERICAN RED SQUIRRELS[1]

2.1 INTRODUCTION

The selective advantage to males of mating with multiple females is well established (i.e. elevated reproductive success) but the constrained maximum offspring production of females was originally thought to limit their corresponding benefits (Bateman 1948; Trivers 1972). Females have thus been historically portrayed as relatively choosey and monogamous with multi-male mating (MMM) arising indirectly as a consequence of selection for multi-female mating in males (reviews in Petrie et al. 1992; Schwagmeyer 1990; Wolff & Macdonald 2004). Behavioural and genetic studies have revealed, however, widespread MMM by the females of diverse taxa, leading many authors to suggest that females may also realize a selective benefit to MMM (reviewed in Birkhead & Møller 1998).

While many hypotheses have been put forward in an attempt to explain MMM (reviewed in Birkhead & Parker 1997), the vast majority of the theory underlying them, as well as the empirical data used to test them, has come from birds and invertebrates (Simmons 2005). In these taxa, direct (e.g. paternal care and nuptial gifts; Reynolds 1996) and/or indirect, genetic, benefits (Jennions & Petrie 2000) are thought to outweigh the potential (although frequently unmeasured; but see Daly 1978; Magnhagen 1991) costs to MMM. The cost/benefit structure in other taxa, however, is for the most part unknown. For example, in mammals, the prevalence of paternal care and/or nuptial gifts is exceptionally low, thereby negating many of the hypothesized direct benefits and

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[1] A version of this chapter has been accepted for publication in *Animal Behaviour*

potentially some of the costs of MMM (Andersson 1994). The loss of paternal care in species with strictly maternal care clearly is irrelevant, as is the potential for social mate retaliation. While the social structures exhibited by mammals vary (Clutton-Brock 1989; Wolff & Sherman 2007), in few cases would females risk being evicted from a breeding territory as a consequence of mating with multiple males. Indeed, female mammals may realize little cost to MMM, thereby requiring little offsetting benefit.

The difficulties inherent in monitoring the natural mating behaviour of free-ranging mammals have necessitated most studies of MMM being conducted under controlled laboratory settings (e.g. Berteaux et al. 1999; Thom et al. 2004; Klemme et al. 2007). Under these conditions, experimental females are typically mated to single or multiple males and the hypothesized benefits to MMM are compared between the two experimental groups. To my knowledge, only two mammalian studies have directly investigated these benefits under ecologically relevant conditions. By releasing offspring born to female brown antechinuses (*Antechinus stuartii*) experimentally mated to single or multiple males into the wild, Fisher et al. (2006a) showed that MMM increased offspring survival, thereby providing support for genetic benefit hypotheses. On the other hand, the benefits to Gunnison's prairie dogs (*Cynomys gunnisoni*) arose through a cryptic direct benefit (i.e. fertility assurance; Hoogland 1998). Focal females having at least three mates in this study experienced elevated pregnancy rates and litter sizes relative to those females having only one or two. Additional work, however, is necessary to determine how relevant these findings may be for mammalian mating systems in general. For example, while the semelparous life history of *Antechinus* proved useful in controlling for potential confounding variables (e.g. maternal effects; Fisher et al. 2006a), it negated examination of other hypothesized benefits (e.g. infanticide avoidance; sensu Hrdy 1979).

I studied the behavioural and genetic mating system of North American red squirrels (*Tamiasciurus hudsonicus*; hereafter red squirrel) and measured the quality, quantity, and fate of resultant offspring to test the relevant hypotheses of MMM for a free-ranging mammal (Table 2.1). Attributes of the natural history of red squirrels allowed me the rare opportunity to examine the influences on, and consequences of, MMM in a free-ranging population. Specifically, all activity, including mating, takes place during the day. This enabled me to directly observe individual estrous females and enumerate their mates. Additionally, the majority of young are born in easily accessible arboreal grass nests, allowing me immediate access after parturition. I was thus able to measure attributes of litters (e.g. offspring mass, litter size) at the earliest possible stage. I, further, estimated the realized costs to MMM. In the absence of obvious direct costs, such as social mate retaliation, loss of paternal care or eviction from the breeding territory, females may still pay a cost to MMM in the form of increased energy expenditure, vulnerability to predation, or acquisition of a sexually transmitted disease (Daly 1978, Sheldon 1993). For these costs to have biological relevance they should manifest as either reduced survivorship or reproductive output of the female. Finally, to determine whether females experienced a selective benefit (or cost) from MMM, I estimated the strength of selection on MMM, using standardized selection gradients (Lande and Arnold 1983).

2.2 METHODS

2.2.1 Study Area and Population

I studied a free-ranging population of red squirrels near Kluane National Park in the southwest Yukon (61° N, 138° W) from just prior to the mating season (late-January to mid-February) through to the completion of juvenile settlement (mid-September). Red squirrels were resident on two 40 ha study grids bisected by the Alaska Highway. Details of the study population and landscape have been reported previously (e.g. Humphries & Boutin 2000; Boutin et al. 2006; McAdam et al. 2007). Briefly, the landscape is boreal forest with a willow (*Salix* spp.) understory and a white spruce (*Picea glauca*) dominated canopy, the seeds of which provide the primary food source for red squirrels at my study site. Red squirrels clip the new spruce cones in the autumn (August-October) of each year and cache them in a larder hoard (midden; Smith 1968). These middens form the center of individual territories (0.2-0.5 ha; LaMontagne 2007), which are defended against members of both sexes year round (Smith 1968). Population densities range from ca. 1.2-5.3 squirrels•ha^{-1} in response to food availability. The three years of this study were conducted during low food conditions and squirrel densities remained below 2.2 squirrels•ha^{-1} (LaMontagne 2007; S. Boutin, unpublished data).

I captured individuals in live traps (Tomahawk Live Trap, Tomahawk, Wisconsin) placed on, or in the immediate vicinity of, their middens. Most individuals in the study population were originally handled in their natal nest, and had there received unique alphanumeric ear tags. Ages of such focal females ($N = 45$) were known with certainty. Any immigrating adults received eartags on first capture, and for these focal females ($N = 18$), I treat age as a minimum estimate (i.e. age = 1 in year of first capture).

Restricting my analyses to include only females of known age did not influence any of my main conclusions. Each individual was also given a unique combination of one or two coloured wires, threaded through their eartags, on first capture of the season to allow for identification from a distance. Females were outfitted with radio-collars (model PD-2C, 4 g, Holohil Systems Limited, Carp, Ontario) to facilitate behavioural observations during the mating season and aid in the location of nests. Female body mass was recorded at each capture, and the mean of all measurements preceding individual mating chases (range = 1-7) was taken to represent pre-estrous body mass.

2.2.2 Mating Season Observations

The mating season for this population usually commences in mid-late winter (late-January to mid-February; Lane et al. 2007; S. Boutin, unpublished data) with selection favouring early breeding (Réale et al. 2003). Females typically produce a single litter, each year, after a 35-day gestation period but will occasionally attempt a second litter after litter loss and, rarely, following a successful litter (Boutin et al. 2006). Behavioural estrus commences with the receptive female emerging from the nest in the morning and continues until the female retires to the nest in the evening, thereby encompassing the full activity period for one day. During the ensuing 'mating chase' females relax their regular territorial behaviour. In this scramble-competition mating system (sensu Schwagmeyer & Woontner 1986), males search for reproductive opportunity (Chapter 4). Upon locating an estrous female, males congregate on the female's territory, chase and attempt to copulate with her and utter distinctive vocalizations ('mating buzzes'). All resident females were monitored daily for reproductive activity in 2003-2005. In 2003 I monitored 46 mating chases, through to completion. In 2004 and 2005, due to logistical constraints, I monitored a smaller sample (N = 23 (2004), 16 (2005)), through to completion.

For mating chase observations, I employed a combination of focal-animal sampling of the estrous female, scan sampling for attending males and all-occurrence sampling of mating behavior (Altmann 1974; Martin & Bateson 1986). This protocol allowed me to identify and enumerate both attending and copulating males and quantify the total number of copulations for each female. Copulating males were identified as mating with the female and attending males were identified as chasing and attempting to copulate with the female. All estrous females and 91 ± 1% of males in each mating chase were individually identifiable. Because a large number of copulations occur underground, I judged these copulations as occurring when a female was followed underground by a male and the pair remained there for a minimum of 60 s or if copulatory vocalizations could be heard, regardless of the time spent underground. Copulations of tree squirrels generally last less than 60 s (Koprowski 2007) and this duration is adequate for fertilization in red squirrels (J. E. Lane and S. Boutin, unpublished data). This criterion has been used previously in this (Lane et al. 2007) and other (Waterman 1998) systems. I also noted whether the female was in or out of sight every 10 min. I then calculated standardized indices of MMM, and number of attendees and copulations, by dividing the number of observed copulatory partners, attending males and total copulations, respectively, by the product of the total observation time and the proportion of 'in-sight' intervals.

2.2.3 Offspring Measurements

I checked nests soon after parturition to sample offspring for genetic analyses, measure individual offspring mass (N = 215 offspring), and determine litter size (N = 76 litters). A small tissue biopsy from the ear was collected with sterile scissors and preserved in 70% ethanol. I checked nests a second time at approximately one month post-parturition to determine juvenile growth rates (N = 138 offspring), and tag all offspring with unique alphanumeric eartags. Growth rates during this period follow a linear trajectory (Boutin & Larsen 1993; McAdam et al. 2002; McAdam & Boutin 2003a, b) and were calculated following McAdam & Boutin (2003b). Briefly, offspring mass at both nest checks was measured with a portable electronic balance (± 0.1 g) and any offspring with an initial mass greater than 50 g (ca. 25 days old), a final mass greater than 100 g (ca. 57 days old), or less than five days between measurements were excluded from analyses to ensure linearity. Neonatal masses for nest checks occurring after the date of parturition (195 of 215) were calculated following Lane et al. (2007). For individuals surviving to the second nest check (N = 118), I used the individual's growth rate and known parturition date to estimate neonatal mass. For those individuals (N = 77) that did not survive to the second nest check, I used the average annual growth rate of the population to estimate neonatal mass.

Litter fate was determined by recording whether any juveniles emerged from the nest, which occurs between 42 and 50 days post-parturition (Berteaux & Boutin 2000). During this period I employed regular focal observations at the natal nest and focused trapping on, and in the immediate vicinity of, the natal midden. Population-wide census trapping during the winter and spring following birth was used to determine offspring and female survival to reproductive age. The ability to document over-winter survival with confidence is enhanced by the territorial social structure of red squirrels, the size of the study area (80 ha) relative to the natal dispersal distance of red squirrels (Mean ± S.E. = 96 ± 94 m; Berteaux & Boutin 2000) and the relatively poor quality of the surrounding habitat. The over-winter survival of offspring is an established measure of fitness in this study system (e.g. McAdam & Boutin 2003a) and dispersal has been shown to not bias fitness calculations (Kerr et al. 2007). Females were also monitored in the year following mating season observations to determine whether or not they mated. In 2004 and 2005, this was achieved through behavioural observation, described above. In 2006 this was achieved through regular trapping and checking of nipple status and palpations for pregnancy using established protocols (e.g. McAdam et al. 2007). The Biosciences Animal Policy and Welfare Committee at the University of Alberta approved all protocols.

2.2.4 Molecular Analyses and Paternity Assignment

DNA was extracted from ear tissue using an ammonium acetate-alcohol precipitation protocol (Bruford et al. 1998) or DNeasy Tissue extraction kits (Qiagen, Venlo, The Netherlands). Extracted DNA was quantified using a fluorometer (Fluostar Optima, BMG Labtechnologies, Aylesbury, United Kingdom) and was normalized to a standard concentration of 10 ng/μl. Polymerase chain reaction (PCR) amplification was performed for a panel of 16 microsatellite loci (for details of microsatellite loci used, including the number, and size range of alleles at each locus, as well as observed (H_O) and expected (H_E) heterozygosities see Lane et al. (2007)). PCR amplification was performed on a

DNA Engine Tetrad thermocycler (MJ Research, Waltham, Massachusetts) using 10 μl reactions. Each 10 μl reaction contained 1 μl genomic DNA, 1 μM of each forward and reverse primer, 0.05 μl BioTaq DNA polymerase (Bioline, London, United Kingdom), 1 μl of 10x reaction buffer (20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl pH 9.0, 0.01% (w/v) Tween), 1.5 mM $MgCl_2$, 4.65 μl H_2O and 1 μl dNTPs (0.2 mM of each dNTP). Details of PCR conditions and dye labeling of forward primers have been described previously (Gunn et al. 2005). Microsatellite allele length was estimated using an ABI Prism 3730 (Applied Biosystems, Foster City, California), and genotypes were scored using GENEMAPPER 3.5 software (Applied Biosystems, Foster City, California). The average proportion of loci genotyped, across individuals, was 0.95.

Maternity was determined by behavioural observation. Because tissue samples were collected from juvenile squirrels prior to their emergence from the natal nest, mother-offspring relationships are robust. Some mothers did not have DNA samples available. For the purposes of paternity assignment, the offspring of those individuals were treated as having an unknown mother. Paternity was assigned at 95% confidence using CERVUS 2.0 (Marshall et al. 1998). Separate analyses were conducted for each of the years 2003, 2004 and 2005. The input parameters for the simulation step of CERVUS were as follows: 10,000 cycles, 10 candidate fathers, 90% of the population sampled, 95% of all loci genotyped and 3 relatives with a relatedness coefficient of 0.25 among the candidate fathers. The high percentage of relatives included among the candidate parents was based on the philopatric nature of squirrel dispersal (Larsen & Boutin 1994; Berteaux & Boutin 2000). Paternities assigned by CERVUS were only accepted if there were two or less mismatches between offspring and both parents. The genotyping error rate based on known mother-offspring pair mismatches was approximately 2%.

I calculated two measurements of litter genetic diversity (*N* = 63 polytocous litters): litter allelic diversity and multiple paternity. Litter allelic diversity was quantified as the number of different alleles represented in the litter, divided by the number of total alleles (to standardize across varying litter sizes and genotype success rate). Levels of multiple paternity were calculated in polytocous litters as the number of sires assigned paternity to individuals in the litter. Individual offspring within a litter for which I was unable to assign a sire were assumed to have been sired by an un-sampled male (*N* = 25 litters). If I was unable to assign a sire to more than one offspring within a litter (*N* = 7 litters) I calculated the pairwise genetic relatedness of the unassigned offspring (Wang 2002) in the program SPAGeDi 1.2 (Hardy & Vekemans 2002). This relatedness coefficient estimates the degree of genetic similarity of two individuals by using the proportion of shared alleles between the individuals weighted by the allele frequencies in the whole population. If relatedness values for two offspring fell within the range of known values for first order relatives (estimated from mother-offspring pairs: 0.24-0.94; Lane et al. 2007) they were assumed to be full-siblings and therefore sired by the same un-sampled male (*N* = 4 litters). If values fell below this level they were treated as half-siblings with different sires (*N* = 3 litters).

2.2.5 Statistical Analysis

I used a general linear model (LM) to determine whether traits of the female (pre-estrous mass, minimum age, and (minimum age)^2) and/or traits of the mating chase (date and number of attending males index) influenced MMM. The quadratic term for female age was included due to a senescent decline in female quality with age (McAdam et al. 2007). Date, in all analyses, was calculated as the linear deviation of the female's mating chase date from the annual population average date. To ensure independence I randomly selected one record from each female for the analysis. Year (2003, 2004, or 2005) was tested as a three-level categorical explanatory term in the model.

To investigate the hypothesized benefits of MMM I used either LMs, generalized linear models (GLMs) or linear mixed effects models (LMEs). In all cases, year was tested as a three-level categorical variable. For hypotheses in which multiple mating by the same male may confer a benefit (e.g. fertility assurance) I fitted both the MMM and the number of copulations indices as fixed effects. Whether multiple mating influenced the conception success and/or litter size of females (i.e. the fertility assurance hypothesis) was tested using GLMs with binomial and Poisson error structures, respectively. In both cases the MMM and number of copulations indices were fitted as fixed effects and only one record from each female was used in the analysis to ensure independence. To test the prediction that females engaging in higher levels of MMM should have a lower probability of nest loss (i.e. the infanticide avoidance hypothesis), I fitted the MMM index as a fixed effect in a GLM with the emergence fate of litters as a binary variable (1 = at least one offspring emerged; 0 = no offspring emerged). Female minimum age, (minimum age)^2, pre-estrous body mass and mating chase date were fitted as covariates in the above three models. Litter size was also included as a covariate in the GLM of litter fate.

Two separate LMEs were used to determine the effect of MMM on two offspring quality traits: neonatal mass and growth rate (i.e. the genetic quality hypothesis). An LM was used to determine whether MMM explained patterns of allelic diversity and GLM with a Poisson error structure was used to determine whether MMM explained patterns of multiple paternity (i.e. the genetic diversity hypothesis). In all models, the MMM index was fitted as a fixed effect and the potential covariates included in the models were: female minimum age, $(\text{minimum age})^2$, pre-estrous body mass, mating chase date and litter size. As multiple offspring from individual females do not represent independent data points, litter identity was fitted as a random effect in the LMEs.

The realized costs to multiple mating were assessed with a GLM. Over-winter survivorship of the female, coded as a binary variable (1= survived; 0 = did not survive) represented the dependent variable. I did not analyze whether MMM influenced the subsequent year's mating success because all females that survived over-winter were observed to mate. I fitted the MMM and number of copulations indices as fixed effects and the potential covariates included in the model were: female minimum age, $(\text{minimum age})^2$, pre-estrous body mass, mating chase date and litter size.

I obtained the standardized linear selection gradients (β'_i) from the multiple regression of my fitness metric (number of offspring surviving over-winter) on the MMM index, female minimum age and pre-estrous body mass (Lande and Arnold 1983). Trait values were standardized to a mean of zero, and relative fitness was calculated within each year to avoid confounding selection gradients with covariances between environmental conditions, trait values and fitness. The standard errors were generated by jackknifing. Due to my limited sample size, I did not attempt to estimate the quadratic selection (i.e. stabilizing or disruptive) gradient.

All analyses were implemented in R (ver. 2.4.1; R Core Development Team), and, unless otherwise noted, values are presented as mean ± 1 S.E.. Sample sizes vary for analyses due to suitability of records and therefore are included in the statistical output for analyses. For example, if I observed a female's mating chase, but she died prior to her estimated parturition date, she would be included in all analyses of MMM influences, but not in analyses relevant to hypothesized consequences.

2.3 RESULTS

I followed 62 individual females for an average of 9.5 ± 0.3 h during a total of 85 mating chases ($N = 46$ (2003), 23 (2004), 16 (2005)). Five females contributed records in all three years, 12 females contributed records in two years and one female, who lost her litter and re-cycled in 2003, contributed two records for that year. During the 85 mating chases, females were observed to copulate with an average of 5.9 ± 0.3 different males and the mean number of total copulations per female was 22.8 ± 1.9. This resulted in a calculated mean MMM index and number of copulations index of 1.0 ± 0.1 and 3.6 ± 0.2, respectively. To evaluate the validity of my MMM index I selected 29 'high quality' records and compared the actual number of observed mates with my calculated MMM index. For these mating chases, I observed the female for the majority of the day (10.4 ± 0.3 h), had a high percentage of recordings as 'in sight' (74.6 ± 2.8%) and observed all males later identified as sires copulating. The MMM index correlated well with the actual observed number of mates (LM: $F_{1,27} = 16.43$, $r = 0.62$, $P < 0.001$). Using the resultant regression equation (Number of mates = 3.21 + 3.68•(MMM Index)), I then estimated the mean number of mates per estrous female as 6.9 ± 0.2.

Individual levels of MMM were correlated with extrinsic aspects of the mating chase and were unrelated to either measure of female quality (final LM: $F_{3,58} = 50.49$, $R^2 = 0.72$, $P < 0.001$, $N = 62$ independent mating chases). Levels of MMM increased with the number of attending males index (LM: $F_{1,58} = 121.68$, $P < 0.001$; Fig. 2.1) and decreased with advancing date (LM: $F_{1,58} = 23.82$, $P < 0.001$; Fig. 2.1). In contrast, female minimum age (LM: $F_{1,53} = 0.21$, $P = 0.65$), (minimum age)^2 (LM: $F_{1,54} = 0.35$, $P = 0.56$) and pre-estrous body mass (LM: $F_{1,55} = 0.45$, $P = 0.51$) were unrelated to the MMM index.

Of the 85 mating chases, 76 resulted in the female conceiving a litter (89.4%), with a mean litter size of 3.0 ± 0.1 offspring. Two females died during gestation (one female was killed by an avian predator, 30 days after her mating chase and one died as a result of a radio-collar complication, 29 days after her mating chase). Of the remaining records ($N = 60$ independent mating chases), neither the probability of conception, nor litter size were influenced by the MMM index (probability of conception: GLM: $\chi^2_1 = 0.25$, $P = 0.62$; litter size: GLM: $\chi^2_1 = 0.03$, $P = 0.86$; Fig. 2.2) or the number of copulations index (probability of conception: GLM: $\chi^2_1 = 1.63$, $P = 0.20$; litter size: GLM: $\chi^2_1 = 0.53$, $P = 0.47$). Of the seven females that did not produce a litter following their mating chase, three came back into estrus (42.9%). Additionally, 12 of the 22 (54.5%) females that lost their litter post-parturition, but survived to the estimated litter emergence date, re-cycled. The probability that a female successfully had one offspring emerge from the nest ($N = 52$ independent litters) was unrelated to her MMM index

(GLM: $\chi^2_1 = 0.21$, $P = 0.65$; Fig. 2.3). The only covariate retained as significant in the final model was year (GLM: $\chi^2_1 = 4.71$, $P = 0.03$). In 2003 and 2004, nearly three quarters of observed females (72.4 and 72.7%, respectively) had at least one offspring emerge. In 2005, however, fewer than half of the observed females (41.7%) had at least one offspring emerge. Neither neonatal mass ($N = 169$ offspring from 55 independent litters), nor growth rate ($N = 123$ offspring from 42 independent litters) were related to the MMM index (neonatal mass: LME: $F_{1,45} = 0.02$, $P = 0.89$; growth rate: LME: $F_{1,33} = 0.02$, $P = 0.88$; Fig. 2.4).

Over the course of this study I was able to assign paternity to at least one offspring in the litters resulting from 68 mating chases. The mean number of different alleles represented in polytocous litters ($N = 63$) and the mean litter allelic diversity was 41.8 ± 0.7 and 0.47 ± 0.1 respectively. Litter allelic diversity was unrelated to the MMM index (LM: $F_{1,46} = 1.60$, $P = 0.21$, $N = 48$ independent mating chases; Fig. 2.5). The majority of the polytocous litters were multiply sired (52/63 = 82.5%), with a mean number of sires represented in litters as 2.3 ± 0.1 (Fig. 2.5). For 42 litters, 38 of which were polytocous, I was able to assign paternity to all offspring. The majority of these polytocous litters were multiply sired (27/38 = 71.0%), with a mean number of sires represented of 2.0 ± 0.1. Levels of multiple paternity were unrelated, however, to females' MMM index, whether I restrict my analysis to litters for which I was able to assign all sires (GLM: $\chi^2_1 < 0.01$, $P = 0.96$, $N = 31$ independent litters), or if I include the entire data set (GLM: $\chi^2_1 = 0.15$, $P = 0.70$, $N = 48$ independent litters).

In addition to realizing no observable benefit to MMM, females were not observed to incur any realized cost. The mean proportion of females that survived over-winter was 49.2 ± 9.8% and whether or not a female ($N = 62$ females) survived was unrelated to her MMM index (GLM: $\chi^2_1 = 0.09$, $P = 0.77$; Fig. 2.6). Additionally, all females that survived over-winter mated the following year. The combination of both a lack of observed benefits and costs yielded MMM selectively neutral in free-ranging red squirrels. The standardized linear selection gradient was -0.37 ± 2.87, and was not significantly different from 0 (one-sample t test: $t_{62} = 0.13$, $P = 0.90$).

2.4 DISCUSSION

My investigations of a free-ranging population of red squirrels support the original explanations of MMM occurring as a result of indirect selection for multi-female mating on males (Bateman 1948; Trivers 1972). During their single annual day of estrus all females, but one, were observed to mate multiple times and with multiple males, and the mean number of males mated with was high. Females were directly observed to copulate with 5.9 ± 0.3 (range: 1-12) males, and I estimated that they may have copulated with 6.9 ± 0.2 (range: 3-13). In contrast to many laboratory studies, however, these high levels of MMM resulted in neither detectable direct (fertilization assurance or infanticide avoidance) nor indirect (genetic quality or diversity) benefits.

2.4.1 Fertilization Assurance

While the hypothesized fertilization assurance benefits of MMM have been supported through interspecific comparison (Stockley 2003), evidence from intraspecific comparison has been more ambiguous. Wolff & Dunlap (2002) revealed no difference in the litter size or pregnancy rate among female prairie voles (*Microtus ochrogaster*) mating with one to three males. While Hoogland (1998) showed an increase in pregnancy rate and litter size with MMM in Gunnison's prairie dogs, these analyses did not control for multiple mating by the same male and Klemme et al. (2007) showed that, although female bank voles (*Clethrionomys glareolus*) mating with two males experienced elevated pregnancy rates relative to singly-mated females, the same benefits could be achieved by mating twice with the same male. Under natural conditions I did not detect an increase in either pregnancy rate or litter size with MMM in red squirrels. Multi-male mating as a bet-hedging strategy against sperm-depleted males may be unnecessary in this species as sperm depletion is likely a relatively rare event. Unlike many ground squirrel species, which restrict mating to as little as one to two weeks following emergence from hibernation (Murie & Michener 1984), the mean duration of the mating season over the three years of this study was 12.3 ± 1.7 weeks. This temporally dispersed mating season results in relatively fewer estrous females per day, and should therefore place less of a drain on males' spermatogenesis abilities.

2.4.2 Infanticide Avoidance

Red squirrels fit two of Wolff and Macdonald's (2004) key criteria for mammal species most likely to use MMM as a guard against infanticide: estrus cycling is induced following litter loss and females give birth to altricial young. I was unable, however, to detect an effect of MMM on rates of litter loss. Admittedly, absolute litter loss represents an indirect metric and the three years of this study were conducted during relatively low food conditions when litter loss due to energetic limitations may have obscured patterns due to infanticide. To control for inter-individual and inter-annual differences in energetic limitations, I included female minimum age, (minimum age)^2 and pre-estrous body mass and year, respectively, as covariates in my analyses. The role of energy availability is potentially revealed by 2005 having the highest rates of litter loss of all three years. This year followed six years of food shortage and was the last preceding a mast year of high food abundance. It is likely that females had depleted all of their food cache and were extremely limited energetically. An explicit demonstration of the role of infanticide is likely to require a more direct estimate involving focused behavioural observations of nests and/or potentially infanticidal males. Because the act of copulation with any female can inhibit infanticide, a test of the infanticide avoidance hypothesis would then involve observing whether males that do not receive any copulations during the mating season kill any pups in the population.

2.4.3 Genetic Quality and Diversity

While definitive conclusions regarding genetic quality hypotheses may be premature in the absence of lifetime reproductive success data on resultant offspring, my evidence suggests that MMM is unlikely to be used to enhance offspring quality. The acquisition of a territory, and associated midden, is necessary for over-winter survival of juvenile red squirrels (Smith 1968; Berteaux & Boutin 2000), thus placing strong selection on weaning early, while in good condition. In spite of this, I found no effect of MMM on either the neonatal mass or growth rate of offspring. It is currently unknown whether female red squirrels are able to assess the quality of attending males. Should they be able to do so, however, MMM may in fact prevent females from limiting mating to these males at the expense of their offspring. While evidence from laboratory studies of invertebrates (e.g. Tregenza & Weddell 2002) and one mammal (Fisher et al. 2006b) have corroborated genetic benefit hypotheses for MMM, field studies, primarily on extra-pair copulations in socially monogamous birds (e.g. Foerster et al. 2003, but see Fisher et al. 2006a), have provided mixed support. The extent to which these suggested benefits have general explanatory power thus requires further investigation of MMM in a range of species.

I also recorded no support for the prediction that female red squirrels may use MMM to enhance the genetic diversity of their litters (sensu Loman et al. 1988; Jennions & Petrie 2000; Cohas et al. 2007). While the majority of red squirrel litters were multiply sired, neither levels of litter allelic diversity nor multiple paternity were correlated to levels of MMM. Females are thus limited in their ability to manipulate the genetic diversity of their litters. Although one mechanism that could explain this constraint, sperm precedence, is not apparent in red squirrels (J. E. Lane and S. Boutin, unpublished data), other factors influencing siring success may play a role. Both testis size (Schulte-Hostedde & Millar 2004) and copulation duration (Schwagmeyer and Foltz 1990) have both been shown to influence paternity patterns in other Sciurid species and could overwhelm a female's ability to manipulate paternity patterns within her litter.

2.4.4 Why are Female Red Squirrels Promiscuous?

I have shown that female red squirrels do not accrue a detectable benefit to MMM. In addition, I was unable to show an obvious cost to MMM and my selection analysis revealed it to be selectively neutral. Despite this, female red squirrels are among the most promiscuous Sciurids studied to date (Boellstorff et al. 1994; Murie 1995). If I restrict my comparison to the number of copulating males observed in my 'high quality' mating chases, red squirrels engage in higher levels of MMM than all but one squirrel species (Boellstorff et al. 1994), and if I broaden my comparison to include the number of males estimated from my MMM index, red squirrels engage in the highest level of MMM of any squirrel species. This raises the question of what causes such high levels of MMM in red squirrels? I suggest that, rather than actively mating for selective benefit, female red squirrels mate passively, as is evidenced by MMM following a linear relationship to the number of attending males. Passive mating could arise because females either do not incur a cost to MMM or because the costs to MMM do not exceed those of evading attending males. Indeed, one cost to evading attending males, vulnerability to predation, could be greater than mating with multiple males as the former occurs above ground, while the majority of the latter occurs below ground.

Of note is that the majority of research on Sciurid mating systems has been on ground squirrels. Given equal population densities, a direct consequence of the relatively longer mating season of red squirrels will be fewer females in estrus per day and a heavily male biased daily operational sex ratio (OSR; Emlen & Oring 1977). For example, both red squirrels and thirteen-lined ground squirrels exhibit a scramble competition mating system (Schwagmeyer & Woontner 1986), yet the average daily OSR in red squirrels (25 males/females) is an order of magnitude higher than thirteen-lined ground squirrels (1.9-5.9 males/females; Schwagmeyer and Brown 1983; Foltz and Schwagmeyer 1989). Thus, relatively more males focus attention on more temporally dispersed females, and with MMM being directly related to the number of attending males, this leads to higher levels of MMM. Levels of MMM also decreased with advancing date, suggesting that males may focus greater effort on earlier mating chases because resultant offspring from these females have a greater probability of survival (Réale et al. 2003). The extrinsic influence of the mating system is further supported by mating patterns observed in Cape ground squirrels (*Xerus inauris*). Similar to red squirrels, this African species does not hibernate. Year round activity leads to a mating season that extends throughout the year and a predictably male-biased OSR and this, in turn, yields relatively high levels of MMM (Waterman 1998).

While carefully controlled laboratory experiments have undoubtedly provided important information regarding the influences on (e.g. Berteaux et al. 1999; Thom et al. 2004), and outcomes of (e.g. Fisher et al. 2006b; Klemme et al. 2007) MMM, I emphasize the continued need for evaluation of the generality of these findings under natural conditions. In Soay sheep (*Ovis aries*) a detailed understanding of the natural mating system proved necessary to understand the seemingly paradoxical result of poorer quality females having higher levels of MMM. In this system, males guard higher quality females more vigorously, while lower quality females are subject to less stable consortships and consequently copulate with more (but of lower quality) males (Preston et al. 2005). In red squirrels, a laboratory comparison of monandrous and polyandrous females would have little ecological relevance because under natural conditions such a small minority (1/85 = 1.1%) of females mate monandrously. In general, the extent to which the consequences of MMM observed under laboratory conditions manifest under natural conditions and the level to which these benefits, relative to extrinsic influences of the populations' mating system, govern female mating behaviour will require further investigations within the context of each species' natural history.

2.5 REFERENCES

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behavior*, **9**, 227-267.

Andersson, M. 1994. *Sexual Selection*. Princeton: Princeton University Press.

Baer, B. & Schmid-Hempel, P. 1999. Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. *Nature*, **397**, 151-154.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, **2**, 349-368.

Berteaux, D., Bêty, J., Rengifo, E. & Bergeron J-M. 1999. Multiple paternity in meadow voles (*Microtus pennsylvanicus*): investigating the role of the female. *Behavioral Ecology and Sociobiology*, **45**, 283-291.

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

Birkhead, T. R & Møller, A. P. 1998. *Sperm competition and sexual selection.* San Diego: Academic Press.

Birkhead, T. R. & Parker, G. A. 1997. Sperm Competition and Mating Systems. In: *Behavioural Ecology: An Evolutionary Approach.* 4th edn. (Ed. by J. R. Krebs & N. B. Davies), pp. 121-148. Oxford: Blackwell Scientific.

Boellstorff, D. E., Owings, D. H., Penedo, M. C. T. & Heresk, M. J. 1994. Reproductive behavior and multiple paternity of California ground squirrels. *Animal Behaviour*, **47**, 1057-1064.

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

Boutin, S., Wauters, L. A., McAdam, A. G., Humphries, M. M., Tosi, G & Dhondt, A. A. 2006. Anticipatory reproduction and population growth in seed predators. *Science*, **314**, 1928-1930.

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1998. Multilocus and single-locus DNA fingerprinting. In: *Molecular Genetic Analysis of Populations: A Practical Approach.* (Ed. by A. R. Hoelzel), pp. 225-269. Oxford: IRL Press.

Clutton-Brock, T. H. 1989. Mammalian mating systems. *Proceedings of the Royal Society of London, Series B*, **236**, 339-372.

Cohas, A., Yoccoz, N. G. & Allainé, D. 2007. Extra-pair paternity in alpine marmots, *Marmota marmota*: genetic quality and genetic diversity effects. *Behavioral Ecology and Sociobiology*, **61**, 1081-1092.

Daly, M. 1978. The cost of mating. *American Naturalist*, **112**, 771-774.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection and the evolution of mating systems. *Science*, **197**, 215-223.

Fisher, D. O., Double, M. C., Blomberg, S. P., Jennions, M. D. & Cockburn, A. 2006a. Post-mating sexual selection increases lifetime fitness of polyandrous females in the wild. *Nature*, **444**, 89-92.

Fisher, D. O., Double, M. C. & Moore, B. D. 2006b. Number of mates and timing of mating affect offspring growth in the small marsupial *Antechinus agilis*. *Animal Behaviour*, **71**, 289-297.

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J.T. & Kempenaers, B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, **425**, 714-717.

Foltz, D. W. & Schwagmeyer, P. L. 1989. Sperm competition in the thirteen-lined ground squirrel: differential fertilization success under field conditions. *American Naturalist*, **133**, 257-265.

Gunn, M. R., Dawson, D. A., Leviston, A., Hartnup, K., Davis, C. S., Strobeck, C., Slate, J. & Coltman, D. W. 2005. Isolation of 18 polymorphic microsatellite loci from the North American red squirrel, *Tamiasciurus hudsonicus* (Sciuridae, Rodentia), and their cross-utility in other species. *Molecular Ecology Notes*, **5**, 650-653.

Hardy, O. J. & Vekemans, X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618-620.

Hoogland, J. L. 1998. Why do female Gunnison's prairie dogs copulate with more than one male? *Animal Behaviour*, **55**, 351-359.

Hrdy, S. B. 1979. Infanticide among animals: a review, classification, and examination of the implications for the reproductive strategies of females. *Ethology and Sociobiology*, **1**, 13-40.

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

Jennions, M. D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews of the Cambridge Philosophical Society*, **75**, 21-64.

Keil, A., Epplen, J. T. & Sacher, N. 1999. Reproductive success of males in the promiscuous-mating yellow-toothed cavy (*Galea musteloides*). *Journal of Mammalogy*, **80**, 1257-1263.

Kerr, T. D., Boutin, S., LaMontagne, J. L., McAdam, A. G. & Humphries, M. M. 2007. Persistent maternal effects on juvenile survival in North American red squirrels. *Biology Letters*, **3**, 289-291.

Klemme, I., Eccard, J. A. & Ylönen, H. 2007. Why do female bank voles, *Clethrionomys glareolus*, mate multiply? *Animal Behaviour*, **73**, 623-628.

Koprowski, J. L. 2007. Alternative reproductive tactics and strategies of tree squirrels. In: Rodent Societies: An Ecological and Evolutionary Perspective. (Ed. by J. O. Wolff & P. W. Sherman), pp. 86-95. Chicago: University of Chicago Press.

LaMontagne, J. M. 2007. Spatial and temporal variability in white spruce (*Picea glauca*) cone production: individual and population responses of North American red squirrels (*Tamiasciurus hudsonicus*). Ph.D. Thesis, University of Alberta.

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

Lane, J. E., Boutin, S., Gunn, M. R., Slate, J. & Coltman, D. 2007. Genetic relatedness of mates does not predict patterns of parentage in North American red squirrels. *Animal Behaviour*, **74**, 611-619.

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

Loman, J., Madsen, T. & Håkansson, T. 1988. Increased fitness from multiple matings, and genetic heterogeneity: a model of a possible mechanism. *Oikos*, **52**, 69-72.

Magnhagen, C. 1991. Predation risk as a cost of reproduction. *Trends in Ecology and Evolution*, 6, 183-186.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural conditions. *Molecular Ecology*, 7, 639-655.

Martin, P. & Bateson, P. 1986. *Measuring behavior: an introductory guide.* Cambridge: Cambridge University Press.

McAdam, A. G. & Boutin, S. 2003a. Variation in viability selection among cohorts of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Evolution*, **57**, 1689-1697.

McAdam, A. G. & Boutin, S. 2003b. Effects of food abundance on genetic and maternal variation in the growth rate of juvenile red squirrels. *Journal of Evolutionary Biology*, **16**, 1249-1256.

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

McAdam, A. G., Boutin, S., Sykes, A. & Humphries, M.M. 2007. Life histories of female red squirrels and their contributions to population growth and lifetime fitness. *Écoscience*, **14**, 362-369.

Murie, J. O. 1995. Mating behavior of Columbian ground squirrels. I. Multiple mating by females and multiple paternity. *Canadian Journal of Zoology*, **73**, 1819-1826.

Murie, J. O. & Michener G. R. 1984. *The Biology of Ground-dwelling Squirrels.* Nebraska: University of Nebraska Press.

Neff, B. D. & Pitcher, T. E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19-38.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelström, H. 1996. Sperm selection by females. *Nature*, **383**, 585.

Petrie, M., Hall, M., Halliday, T., Budgey, H. & Pierpoint, C. 1992. Multiple mating in a lekking bird: why do peahens mate with more than one male and with the same male more than once? *Behavioral Ecology Sociobiology*, **31**, 349-358.

Preston, B. T., Stevenson, I. R., Pemberton, J. M. & Wilson, K. 2001. Dominant rams lose out by sperm depletion. *Nature*, **409**, 681-682.

Preston, B. T., Stevenson, I. R., Pemberton, J. M., Coltman, D. W. & Wilson, K. 2005. Male mate choice influences female promiscuity in Soay sheep. *Proceedings of the Royal Society of London, Series B*, **272**, 365-373.

Réale, D., Berteaux, D., McAdam, A. G. & Boutin, S. 2003. Lifetime selection on heritable life-history traits in a natural population of red squirrels. *Evolution*, **57**, 2416-2423.

Reynolds, J. D. 1996. Animal breeding systems. *Trends in Ecology and Evolution*, **11**, 68-72.

Schulte-Hostedde, A. I. & Millar, J. S. 2004. Intraspecific variation in testis size and sperm length in the yellow-pine chipmunk (*Tamias amoenus*): implications for sperm competition and reproductive success. *Behavioral Ecology and Sociobiology*, **55**, 272-277.

Schwagmeyer, P. L. 1990. Ground squirrel reproductive behavior and mating competition: a comparative perspective. In: *Contemporary Issues in Comparative Psychology* (Ed. By D. Dewsbury), pp. 175-196. Massachusetts: Sinauer.

Schwagmeyer, P. L. & Brown, C. H. 1983. Factors affecting male-male competition in thirteen-lined ground squirrels. *Behavioral Ecology and Sociobiology*, **13**, 1-6.

Schwagmeyer, P. L. & Foltz, D. W. 1990. Factors affecting the outcome of sperm competition in thirteen-lined ground squirrels. *Animal Behaviour*, **39**, 156-162.

Schwagmeyer, P. L. & Woontner, S. J. 1986. Scramble competition polygyny in thirteen-lined ground squirrels: the relative contributions of overt conflict and competitive mate searching. *Behavioral Ecology Sociobiology*, **19**, 359-364.

Sheldon, B. C. 1993. Sexually transmitted disease in birds: occurrence and evolutionary significance. *Philosophical Transactions of the Royal Society of London, Series B*, **339**, 491-497.

Simmons, L. W. 2005. The evolution of polyandry: sperm competition, sperm selection and offspring viability. *Annual Review of Ecology, Evolution and Systematics*, **36**, 125-146.

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

Stockley, P. 2003. Female multiple mating behaviour, early reproductive failure and litter size variation in mammals. *Proceedings of the Royal Society of London, Series B*, 270, 271-278.

Stockley, P., Searle, J. B., Macdonald, D. W. & Jones, C. S. 1993. Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. *Proceedings of the Royal Society of London, Series B*, 254, 173-179.

Thom, M. D., Macdonald, D. W., Mason, G. J., Pedersen, V. & Johnson, P. J. 2004. Female American mink, *Mustela vison*, mate multiply in a free-choice environment. *Animal Behaviour*, **67**, 975-984.

Tregenza, T. & Wedell, N. 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution*, **52**, 1726-1730.

Tregenza, T. & Wedell, N. 2000. Polyandrous females avoid costs of inbreeding. *Nature*, **415**, 71-73.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871-1971* (Ed. by B. Campbell), pp. 136-179. Chicago: Aldine Publishing Company.

Wang, J. 2002. An estimator for pairwise relatedness using molecular markers. *Genetics*, **160**, 1203-1215.

Waterman, J. M. 1998. Mating tactics of male Cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Animal Behaviour*, **56**, 459-466.

Wolff, J. O. & Dunlap, A. S. 2002. Multi-male mating, probability of conception, and litter size in the prairie vole (*Microtus ochrogaster*). *Behavioural Processes*, **58**, 105-110.

Wolff, J. O. & Macdonald, D. W. 2004. Promiscuous females protect their offspring. *Trends in Ecology and Evolution*, **19**, 127-134.

Wolff, J. O. & Sherman, P. W. 2007. *Rodent Societies: An Ecological and Evolutionary Perspective.* Chicago: University of Chicago Press.

Table 2.1. Hypothesized cryptic direct, and indirect, genetic, benefits of multi-male mating in North American red squirrels (*Tamiasciurus hudsonicus*)[a]

Hypothesis	Explanation	Predictions	References
Fertility assurance[b]	MMM as a bet-hedging strategy against either sperm-depleted or genetically incompatible males	• MMM positively correlates with pregnancy rate and/or litter size • MMM does not correlate with offspring quality • MMM does not correlate with litter allelic diversity or multiple paternity • MMM does not correlate with litter fate, after controlling for variation in litter sizes	Stockley et al. (1993), Olsson et al. (1996), Hoogland (1998), Keil et al. (1999), Preston et al. (2001), Stockley (2003)
Infanticide avoidance	MMM serves to confuse paternity of copulating males, thereby reducing the probability that a male will kill resultant offspring	• MMM correlates positively with litter fate • MMM does not correlate with pregnancy rate and/or litter size • MMM does not correlate with litter allelic diversity or multiple paternity • MMM does not correlate with offspring quality	Hrdy (1979), Wolff & Macdonald (2004)
Genetic benefits to offspring[c]	MMM results from females either 'trading up' copulatory partners or to enable post-copulatory mechanisms to select genetically superior (i.e. 'good genes' hypotheses) or more compatible sperm (i.e. 'compatible genes' hypotheses)	• MMM positively correlates with offspring quality • MMM may correlate positively with litter size (if mating with genetically incompatible males results in early reproductive failure) • MMM may correlate positively with litter fate (if higher quality offspring are more likely to emerge)	Jennions & Petrie (2000), Tregenza & Wedell (2000), Neff & Pitcher (2005)

Table 2.1. *cont.*

Hypothesis	Explanation	Predictions	References
Genetic benefits to offspring *cont.*[c]		• MMM does not correlate with litter allelic diversity or multiple paternity	
Increase genetic diversity of litter	MMM results in genetically diverse litters which females use as a bet-hedging strategy against unpredictable future environmental conditions.	• MMM positively correlated with litter allelic diversity and/or multiple paternity • MMM does not correlate with litter size • MMM does not correlate with offspring quality • MMM does not correlate with litter fate	Baer & Schmid-Hempel (1999), Jennions & Petrie (2000), Cohas et al. (2007)

[a] Adapted from Wolff & Macdonald (2004), however, only includes those hypotheses relevant to the mating system (scramble-competition promiscuity) and social structure (individual based territoriality; strictly maternal care of offspring) of red squirrels.

[b] While occasionally treated separately, the underlying mechanisms for both fertility assurance and increased litter size are likely the same (i.e. mating multiply in response to the risk of mating with a sperm-depleted or genetically incompatible male). I have, therefore combined both predictions under one hypothesis.

[c] Due to the difficulty of differentiating good gene benefits from compatible gene benefits, I have grouped both under one hypothesis (i.e. MMM to increase the genetic quality of young).

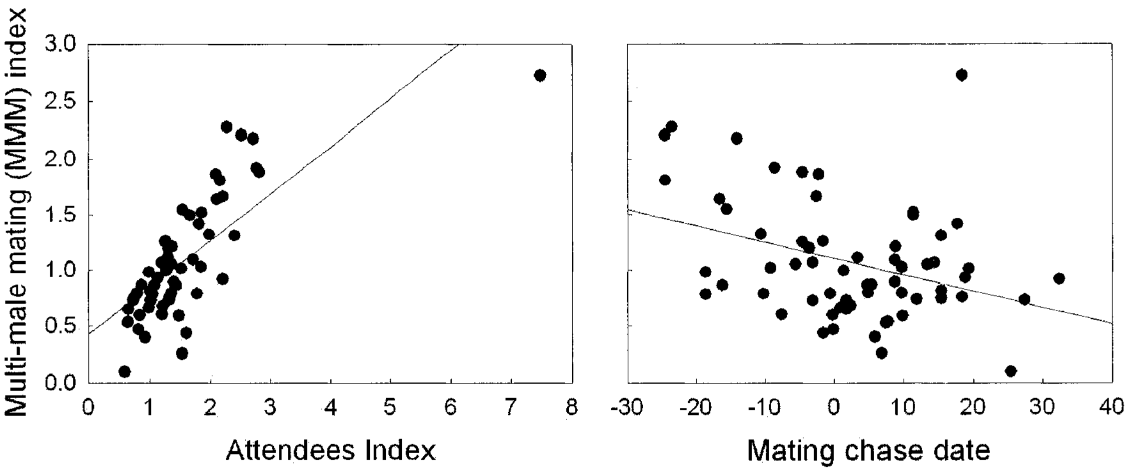

Figure 2.1. Multi-male mating index of female North American red squirrels as a function of the number of attending males index and Julian date (*N* = 62 mating chases). Multi-male mating and number of attending males indices were calculated as the number of observed males copulating with the female, and attending the female's mating chase, respectively, divided by the product of the total observation time and the proportion of 'in-sight' observations. Julian date is calculated as the linear deviation of the females mating chase date from the mean mating chase date for each year.

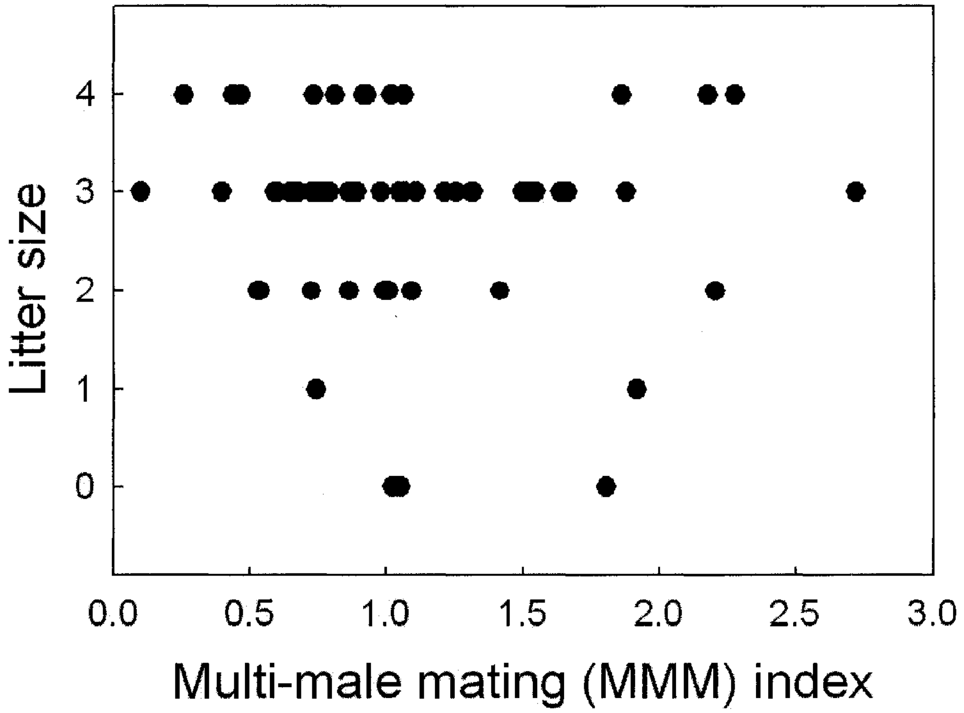

Figure 2.2. Litter size (N = 60 litters) of female North American red squirrels as a function of their multi-male mating index (number of mates·(observation time·proportion of in-sight observations)$^{-1}$).

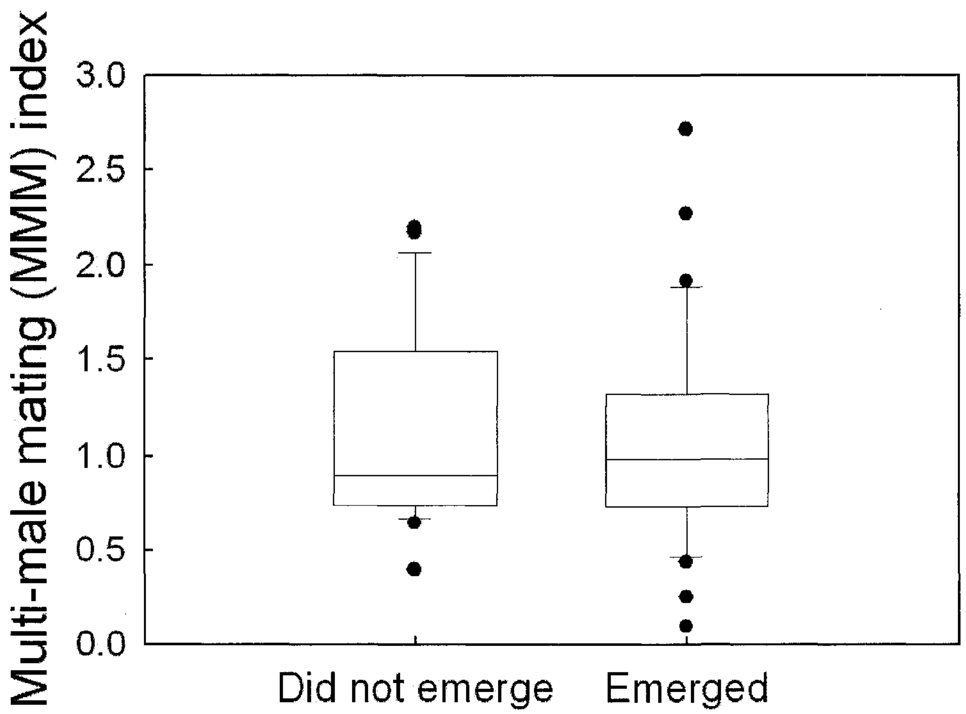

Figure 2.3. Multi-male mating index (number of mates·(observation time·proportion of in-sight observations)$^{-1}$) of female North American red squirrels that did (*N* = 34 females) and did not (*N* = 18 females) wean at least one offspring from their litter. Box plots show 25th and 75th percentiles (box), median (line within the box), 10th and 90th percentiles (whiskers) and data points outside the 10th and 90th percentiles.

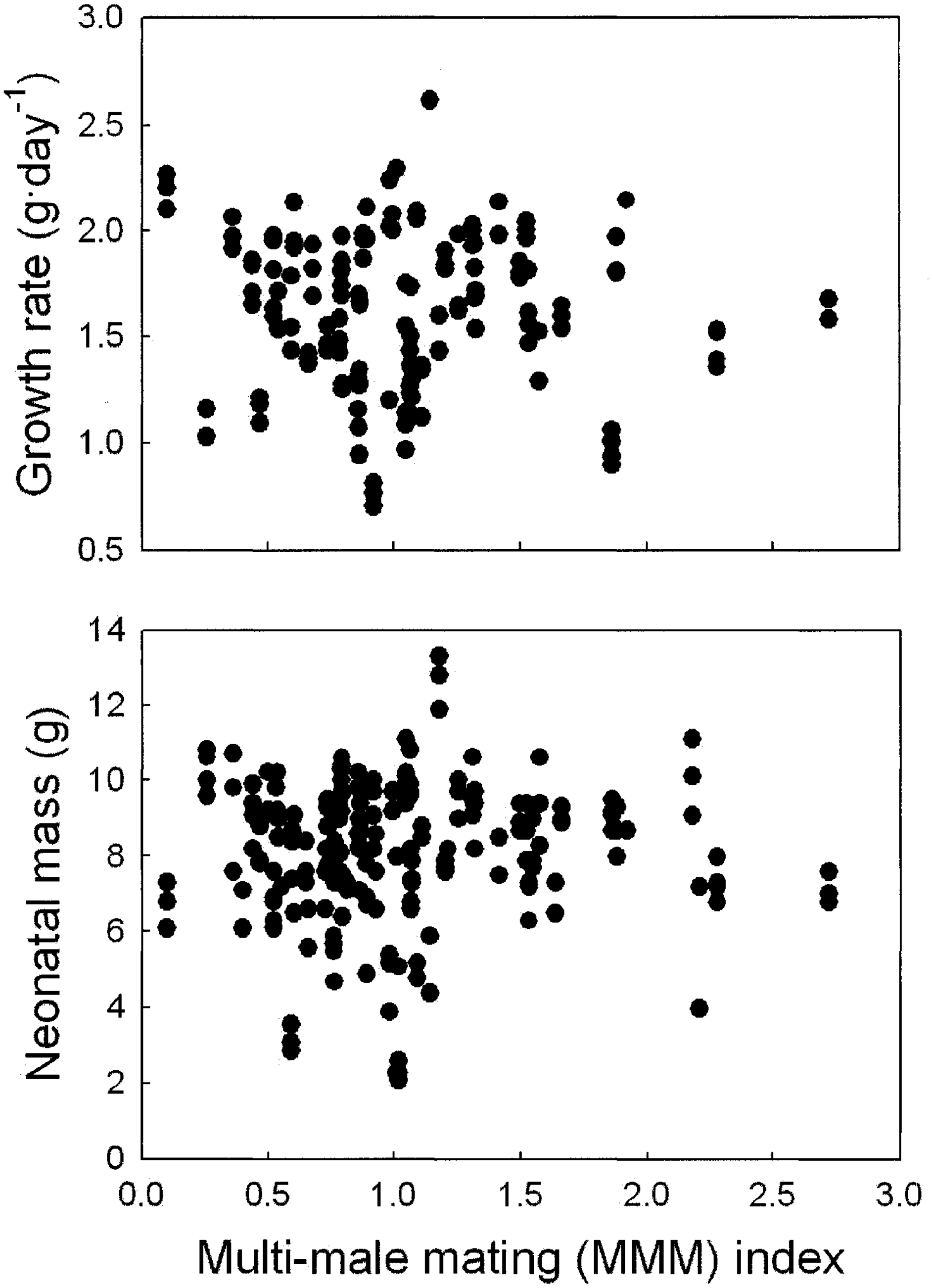

Figure 2.4. Neonatal mass (g; $N = 215$) and growth rate (g·day^{-1}; $N = 148$) of offspring in litters of female North American red squirrels as a function of their multi-male mating index (number of mates·(observation time·proportion of in-sight observations)$^{-1}$).

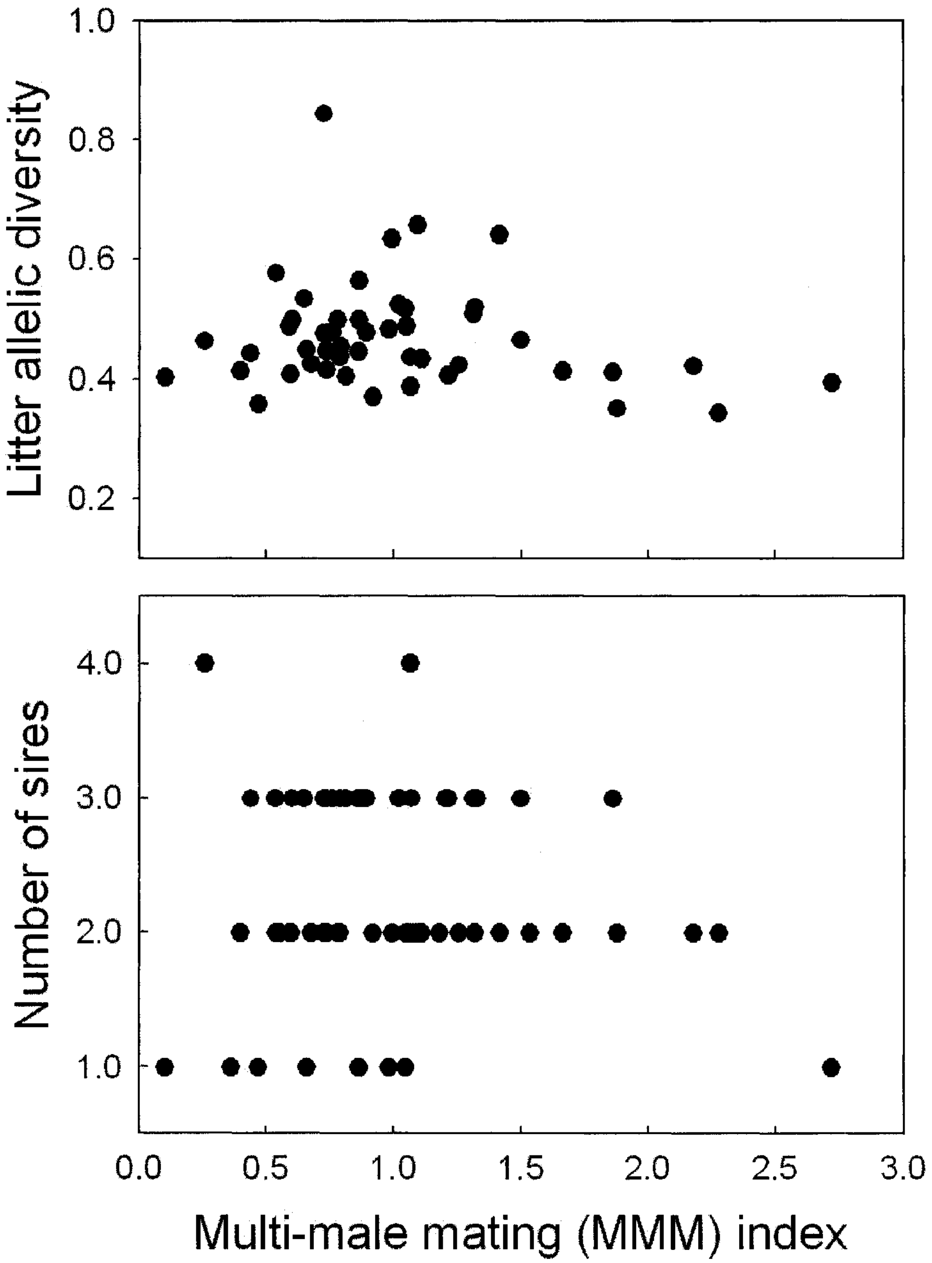

Figure 2.5. Litter allelic diversity (number of different alleles·(number of total alleles)$^{-1}$) and multiple paternity (number of sires represented) in litters of female North American red squirrels (*N* = 48 polytocous litters) as a function of the females' multi-male mating index (number of mates·(observation time·proportion of in-sight observations)$^{-1}$).

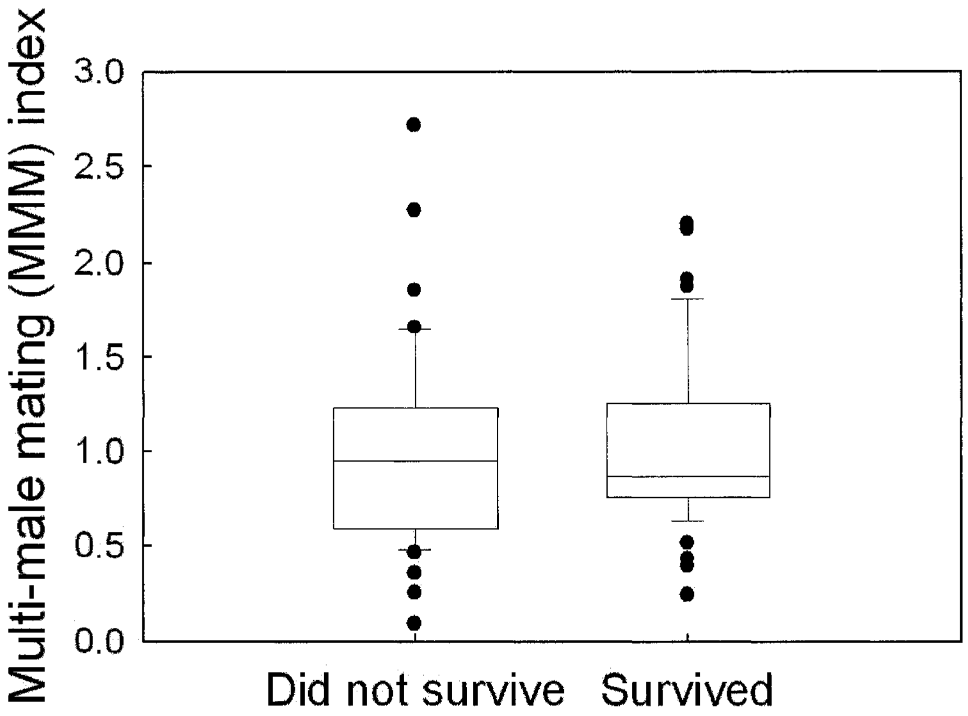

Figure 2.6. Multi-male mating index (number of mates·(observation time·proportion of in-sight observations)$^{-1}$) of female North American red squirrels that did (*N* = 28) and did not (*N* = 34) survive over the winter following observation of their mating chases. Box plots show 25th and 75th percentiles (box), median (line within the box), 10th and 90th percentiles (whiskers) and data points outside the 10th and 90th percentiles.

CHAPTER 3. GENETIC RELATEDNESS OF MATES DOES NOT PREDICT PATTERNS OF PARENTAGE IN NORTH AMERICAN RED SQUIRRELS[2]

3.1 INTRODUCTION

The evolution of female promiscuity has remained a contentious issue despite widespread study in taxa ranging from insects to higher vertebrates (Jennions & Petrie 2000, and references therein). In males the benefits of promiscuity are well established (i.e. elevated reproductive success), but the limited maximum offspring production of females makes female promiscuity more enigmatic (Bateman 1948; Trivers 1972). No fewer than nine hypotheses have been put forward to explain this behaviour and most rely on material benefits to the female (e.g. nuptial gifts and paternal care of offspring; reviewed in Birkhead & Parker 1997; Wolff & Macdonald 2004). In most species of mammals, and in other groups lacking paternal care, explanations of female promiscuity largely rely on genetic benefits to the female (Jennions & Petrie 2000, but for examples of more cryptic direct benefits see Hoogland 1998; Wolff & Macdonald 2004). These genetic benefits can be broadly grouped into 'good genes' hypotheses (Møller & Alatalo 1999), 'compatible genes' hypotheses (Tregenza & Wedell 1998), or a combination of the two (Neff & Pitcher 2005). While good genes hypotheses postulate that the intrinsic effects of paternal genes have a direct impact on the fitness of resultant offspring, compatible genes hypotheses put forward that the interaction of the paternal and maternal genomes plays the primary role in influencing the offspring's fitness (Mays & Hill 2004).

Perhaps in no other context are the benefits of compatible genes more apparent than under inbreeding depression. The deleterious effects of inbreeding are well

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[2] A version of this chapter has been published. Lane et al. 2007. *Animal Behaviour*, **74**, 611-619.

established under laboratory conditions (Connor & Bellucci 1979; Lacy et al. 1996; Dudash & Carr 1998), and have recently been shown to have comparable, or enhanced effects in the wild (Crnokrak & Roff 1999; Meagher et al. 2000; Keller & Waller 2002; Kruuk et al. 2002). The reduced fitness of offspring resulting from a mating of two closely related individuals should strongly select for effective inbreeding avoidance mechanisms.

There is currently much interest in the potential for promiscuous females to influence the paternity of their offspring through post-copulation processes (Eberhard 1996; Zeh & Zeh 1996). Cryptic female choice can operate through biasing sperm competition, active selection of sperm, or through differential investment to developing offspring (including selective abortion; Jennions & Petrie 2000). Operating separately, or in combination, these mechanisms may allow females to avoid inbreeding post copula and ensure the highest genetic quality of their offspring. A growing number of studies are showing that patterns of parentage can be influenced by the genetic relatedness of mates (e.g. Olsson et al. 1996; Bishop 1996; Stockley 1999; Kraaijeveld-Smit et al. 2002; Thuman & Griffith 2005). In these investigations males siring a higher proportion of offspring in individual litters/clutches were less genetically related to the female than males siring fewer offspring. Interestingly, the significant effects of genetic relatedness on influencing parentage patterns have been largely seen in oviparous species. The sole study of a eutherian mammal (*Sorex araneus*), however, found no evidence of sperm selection by females (Stockley 1997). This runs counter to the hypothesis of Zeh & Zeh (2001) that, due to the arena for genomic conflicts created by embryonic development within the female, viviparous species should be the most likely to show patterns of parentage based on genetic compatibility. Clearly, further studies on viviparous species are required.

In this study I investigated whether genetic similarity of mates predicted observed patterns of parentage in North American red squirrels (*Tamiasciurus hudsonicus*; henceforth red squirrel). Female red squirrels engage in among the highest levels of promiscuity of all Sciurid species (mean ± S.E. mates/female = 6.9 ± 0.2, N = 85 estrous bouts; Chapter 2; Boellstorff et al. 1994; Murie 1995) and show little to no overt pre-copulatory mate choice. Females mate with the majority of males attending their mating chase and do not bias copulations to favour less genetically related males. The relatively indiscriminate mating behaviour and insemination by multiple males makes red squirrels an ideal system to test for the presence of post-copulation inbreeding avoidance mechanisms. Moreover, paternity biasing mechanisms, such as mating-order effects (Lacey et al. 1997) and copulatory plugs (Koprowski 1992), that could obscure a potential relationship between relatedness and siring success, are not known from red squirrels (J. E. Lane and S. Boutin unpublished data). I thus predicted that females would bias paternity of their offspring to favour more distantly related males.

To evaluate the potential selective pressures associated with mate choice based on genetic similarity, I compared the effect of parental genetic relatedness on two aspects of offspring performance: neonatal mass and growth rate. In this euthermic species, acquisition of a territory and food cache (midden) is thought to be necessary for over-winter survival, placing a strong selective pressure on developing and weaning early (McAdam & Boutin 2004). I also directly determined the effect of parental relatedness on one component of fitness (offspring survival to reproductive age, year 1). While previous studies have incorporated offspring heterozygosity in such analyses, recent theoretical (Balloux et al. 2004) and empirical work (Slate et al. 2004) has cast doubt on the utility of this metric. The correlation between individual heterozygosity and inbreeding coefficients is often weak, at best, and usually only observable with a far greater number of microsatellites than is typically used in field studies (Pemberton 2004). For this reason I have restricted my analyses to the genetic relatedness of mates.

3.2 METHODS

3.2.1 Study Population and Field Methods

I studied a population of red squirrels near Kluane National Park in the southwest Yukon from 2003-2005. Details of the study population and landscape have been reported previously (e.g. Humphries & Boutin 2000; McAdam & Boutin 2004). The mating season for this population usually commences in late winter (late-January to mid-February) with females producing a single litter, each year, after a 35-day gestation period. Behavioural estrus commences with the receptive female emerging from the nest in the morning and continues until the female retires to the nest in the evening, thereby encompassing the full activity period for one day. During the ensuing 'mating chase' males congregate on the estrous female's territory, chase and attempt to copulate with her and the females copulate with many or all attending males (Mean ± S.E. = 6.9 ± 0.2, N = 85 estrous bouts; Chapter 2). I followed individual females (N = 65) for an average of 9.6 ± 0.2 h during a total of 89 estrous periods (N = 46 (2003), 23 (2004), 20 (2005)). Six females contributed records in all three years and 12 females contributed records in two years. I employed a combination of focal-animal sampling of the estrous female, scan sampling for attending males and all-occurrence sampling of mating behavior (Altmann 1974; Martin & Bateson 1986). Copulating males were identified as mating with the female and attending males were identified as chasing and attempting to copulate with the female. Individuals in the population were marked with unique combinations of coloured wiring, threaded through permanent eartags, allowing for identification from a distance. All estrous females and 91 ± 1 % of males in each chase were individually identifiable. Because a large number of copulations occur underground, I opted to follow Waterman (1998) and judge underground copulations as occurring when a female was followed underground by a male and the pair remained there for a minimum of 60 s. Copulations of tree squirrels generally last < 60 s (Koprowski In press) and this duration is adequate for fertilization in red squirrels (J. E. Lane and S. Boutin unpublished data). I also deemed a copulation as occurring if copulatory vocalizations could be heard, regardless of the time spent underground. During and following underground consortships I observed similar behaviours to those during and following aboveground copulations (e.g., time spent in proximity, occurrence of copulatory vocalizations, and genital grooming) thereby providing confidence in my assumption that these consortships represented underground copulations.

I checked nests soon after birth to sample offspring for genetic analyses and measure neonatal mass ($N = 156$). A small tissue biopsy from the ear was collected with sterile scissors and preserved in 70 % ethanol. Red squirrel litters can be multiply sired (Chapter 2) so a unique sex-ear notch combination was used for each individual offspring, allowing for subsequent discrimination of individuals. I checked nests a second time at approximately one month post-parturition to determine juvenile growth rates ($N =$

159), and tag all offspring with unique alphanumeric eartags. Growth rates during this period follow a linear trajectory (Boutin & Larsen 1993; McAdam et al. 2002; McAdam and Boutin 2003a, b) and were calculated following McAdam & Boutin (2003b). Briefly, offspring mass at both nest checks was measured with a portable electronic balance (± 0.1 g) and any offspring with an initial mass greater than 50 g (ca. 25 days old), a final mass greater than 100 g (ca. 57 days old), or less than five days between measurements were excluded from analyses to ensure linearity. For nest checks occurring after the date of parturition, I used the individual's growth rate and known parturition date to estimate neonatal mass. Population-wide census trapping during the winter and spring following birth in 2004 and 2005 was used to determine offspring survival to reproductive age. The ability to document over-winter survival with confidence is enhanced by the size of the study area (80 ha) relative to the natal dispersal distance of red squirrels (Mean ± SE = 96 ± 94 m; Berteaux & Boutin 2000), as well as the poor quality of the surrounding habitat. The over-winter survival of offspring is an established measure of fitness in this study system (e.g. McAdam & Boutin 2003) and dispersal has been shown to not bias measurements of fitness (Kerr et al. 2007). The Biosciences Animal Policy and Welfare Committee at the University of Alberta approved all protocols.

3.2.2 Molecular Analyses and Paternity Assignment

Details of the molecular methods for microsatellite loci isolation and paternity assignment have been provided elsewhere (Gunn et al. 2005; Chapter 2). Briefly, DNA was extracted from preserved tissue using either an acetate-alcohol precipitation protocol (Bruford et al. 1998) or DNeasy Tissue extraction kits (Qiagen, Venlo, The Netherlands), and polymerase chain reaction (PCR) amplification was performed for a panel of 16 microsatellite loci (Table 3.1). Maternity was determined by behavioural observation at the nest and paternity was assigned at 95% confidence using CERVUS 2.0 (Marshall et al. 1998). Separate analyses were conducted for each of the years 2003, 2004 and 2005.

3.2.3 Estimation of Genetic Relatedness

I calculated the degree of genetic relatedness as the pairwise genetic relatedness value, *r* (Wang 2002), in the program SPAGeDi 1.2 (Hardy & Vekemans 2002). This relatedness coefficient estimates the degree of genetic similarity of two individuals by using the proportion of shared alleles between the individuals weighted by the allele frequencies in the whole population. Resulting values can range from –1.0 to 1.0. Values close to zero represent two relatively unrelated individuals and increasingly positive values between mates signify higher levels of inbreeding. Outbred matings would result in a pairwise genetic relatedness of $r < 0$. The mean ± S.E. genetic relatedness between mothers and their offspring (predicted value = 0.5; Wang 2002) was 0.489 ± 0.004 (Range = 0.24-0.94, $N = 438$), and the mean ± S.E. genetic relatedness between grandmothers and their grand-offspring (predicted value = 0.25; Wang 2002) was 0.239 ± 0.015 (Range = -0.11-0.55, $N = 89$).

3.2.4 Statistical Analysis

I used a generalized linear mixed-effects model (GLMM) to determine whether genetic similarity influenced patterns of paternity among observed copulating males. The dependent variable in this analysis was number of offspring sired (range = 0-4) in individual litters and was modeled with a Poisson error structure. Males observed copulating, but not siring offspring, were assigned an offspring sired value of 0. I fitted pairwise genetic relatedness of the copulating males and the estrous females as a fixed effect (N = 302). Data were nested within mating chases, and therefore mating chase was fitted as a random effect. I analyzed data from 68 of the 89 observed mating chases (14 of the observed females came into estrus, but did not produce a litter and I did not have a DNA sample, and was therefore unable to calculate genetic relatedness, for 8 of the females that did produce a litter). Year (2003, 2004 or 2005) was tested as a three-level categorical explanatory term in the model.

A least squares linear regression analysis was used to compare the relative success of sires within mixed paternity litters. This analysis facilitates comparison between my results and previous work, as this has been the method most commonly employed (e.g. Olsson et al. 1996; Stockley 1997; Kraaijeveld-Smit et al. 2002). Also, it controls for males that copulated but failed to ejaculate because only successful sires are incorporated. Similar to the previous studies, I standardized data to yield the relative proportions of offspring sired within multiply sired litters and the relative genetic similarity of sires to the female. The relative proportion of offspring sired by each male was calculated as the observed percentage of offspring in the litter, minus the proportion expected by chance (e.g. 50% if two males sired offspring in the litter, 33% if three). Relative genetic similarity was calculated as the male's pairwise genetic relatedness to the female minus the mean pairwise genetic relatedness of all sires. To avoid pseudoreplication, I randomly selected one litter for females contributing records in multiple years. Additionally, only litters in which all offspring were assigned a sire at

95% confidence were used, yielding a total of 36 multiply sired litters for the analysis. As this analysis does not include males that copulated but failed to sire any offspring, litters for which I did not observe the estrous bout of the female are included. Litters were used as independent data points in this analysis, with each represented by one male selected at random.

To determine whether any level of siring success, for which I had an adequate sample size (offspring sired = 0 (N = 210), 1 (N = 54), 2 (N = 8)), deviated significantly from zero, signifying non-random parentage patterns, I used one-sample t tests. A two-sample Kolmogorov-Smirnoff test was used to determine whether the distribution of pairwise relatedness of copulatory partners (N = 345) differed from that of successful parents of offspring (N = 300). Pseudoreplication was dealt with in these two analyses by randomly selecting only one record for each male-female pair.

Two separate linear mixed effects models (LMEs) were used to investigate the effect of parental genetic relatedness on offspring performance. In both models the pairwise relatedness of the parents was fitted as a fixed effect (N = 159 (neonatal mass); N = 156 (growth rate)) and year (2003, 2004, or 2005) was tested as a three-level categorical explanatory term. As birth date has been shown to have a significant effect on both of these traits (J. E. Lane, S. Boutin, M. R. Gunn, J. Slate, D. W. Coltman unpublished data) it was included in the model as a covariate. In these analyses multiple offspring from individual females do not represent independent data points, therefore female ID (N = 56) was fitted as a random effect. The dependent variables in the two models were offspring neonatal mass and growth rate. To determine whether parental relatedness influenced survival to year 1, I used a logistic GLMM with a binary dependent variable (1 or 0 for survived ($N = 41$) or not ($N = 126$), respectively). Year was tested as a two level (2003 or 2004) categorical explanatory term and female ID ($N = 56$) was fitted as a random effect to accommodate that offspring from the same dam do not represent independent samples.

The GLMMs and LMEs were implemented in S-PLUS (ver. 7.0; Insightful 2001). I initially included a quadratic term for pairwise genetic relatedness (r^2) in these analyses, but removed it, as non-significant, in all cases. The least squares linear regression, *t* tests and Kolmogorov-Smirnoff tests were conducted using Systat software (ver. 9.0; SPSS 1998). I determined what statistical power I had to detect a medium effect (Cohen 1988) for the least squares linear regression ($f^2 = 0.15$) and *t* tests ($f = 0.25$) using the software package G*POWER (ver 2.1; Erdfelder et al. 1996). As GLMMs and LMEs are fitted using likelihood procedures (penalized quasi-likelihood and maximum likelihood, respectively; Venables and Ripley 2002), power analyses can be most easily conducted using simulation. To test the ability of these models to detect varying effect sizes I simulated data sets based on the parameterized models and recorded the estimated coefficients when the models were fitted to the simulated data. For all simulations I ran 100 iterations and varied the slope of *r* by 0.1. I report the effect size resulting in an estimated power of 0.8, and the detectable response to a change in *r* from 0 to -0.25.

3.3 RESULTS

Over the course of this study I was able to assign paternity to 327 offspring from 139 litters (mean ± S.E. litter size = 3.0 ± 0.7, range = 1-5; incorporating littermates with unassigned paternity). I was able to assign paternity to all offspring in the litter in 77 cases, 68 of which were polytocous litters. Forty-five of these 68 litters were multiply sired (mean ± S.E. = 2.3 ± 0.1 sires, range = 2-3). Seventy-five of the total number of litters (162 offspring) were from females for which I observed their mating chase (14 females came into estrus but did not produce a litter).

3.3.1 Genetic Relatedness of Mates and Patterns of Parentage

I recorded a number of instances of assumed inbreeding during my mating-season observations. Nineteen percent of the copulatory pairs ($N = 66$) had an estimated pairwise relatedness of $r > 0.125$, and 5 % ($N = 18$) had an estimated pairwise relatedness of $r > 0.25$, including one pair at $r > 0.50$ (estimated as first order relatives; Fig. 3.1).

The absolute number of resultant offspring that a copulating male sired in a litter was not predicted by his pairwise genetic relatedness to the dam of the litter (GLMM: $\chi^2_1 = 0.31$, $P = 0.58$; Fig. 3.2). For this analysis, I should be able to detect a slope of -0.6 for r. In other words, if the actual r is -0.25, then I have sufficient power to detect that such males sire, on average, a factor of 1.16 (or more) offspring relative to males for which r is 0. Similarly, despite reasonable statistical power (0.62; Jennions and Møller 2003), the relative proportion of offspring sired by a male in multiply sired litter was not related to his relative pairwise relatedness to the dam of the litter (least squares linear regression: $F_{1,34} = 0.20$, $R^2 = 0.01$, $P = 0.66$; Fig. 3.3).

The relatedness at each level of siring success (number of offspring sired = 0-2, sample size inappropriate for 3 ($N = 4$) or 4 ($N = 1$) offspring sired) did not deviate significantly from zero (one-sample t test: $t_{7\text{-}209} = -0.02\text{--}0.52$, $P = 0.60\text{-}0.98$) suggesting that the average copulatory pair within each of these groups is not more or less related than would be expected by chance. Statistical power for these analyses ranged from 0.95 (offspring sired = 0) to 0.09 (offspring sired = 2). The apparent lack of post-copulatory mechanisms based on genetic relatedness yields a distribution of pairwise relatedness of parents of offspring that does not deviate significantly from that of copulatory pairs (two-sample Kolmogorov-Smirnoff test: $t_{644} = 0.08$, $P = 0.28$; Fig. 3.1).

3.3.2 Parental Genetic Relatedness and Offspring Performance and Fitness

Despite relatively high variation in parental pairwise genetic relatedness (Range = -0.32-0.51, $N = 159$), I found no suggestion that this variable influenced my measurements of offspring performance or fitness. There was no relationship between genetic relatedness and either neonatal mass or offspring growth rate (neonatal mass: LME: $F_{1,99} = 0.88$, $P = 0.35$; growth rate: LME: $F_{1,96} = 0.17$, $P = 0.68$; Fig. 3.4). For these analyses, I should be able to detect slopes of -0.8 (neonatal mass) and -0.2 (growth rate) for parental r (detectable response: offspring should be, on average, at least 0.2 g heavier at birth and should grow at least 0.05 g/day faster). Additionally, whether or not an individual survived to year 1 was not influenced by the pairwise genetic relatedness of its parents (GLMM: $\chi^2_1 = 0.02$, $p = 0.91$; Fig. 3.5). I should be able to detect a slope of –1.0 for this analysis (detectable response: offspring should have at least a 5.3 % greater probability of surviving).

3.4 DISCUSSION

I found no compelling evidence for the role of genetic similarity in influencing parentage patterns in red squirrels. Specifically, pairwise genetic relatedness of copulating males to the estrous female was unrelated to siring success, and relative success of sires within litters was not predicted by relative relatedness to the dam. Further, the relatedness at no level of siring success deviated significantly from zero and the frequency distribution of successful parents mirrored that of copulating males and estrous females. Red squirrels thus appear to deviate from the observed parentage patterns in all but one previous investigation (Bishop 1996; Olsson et al. 1996; Wilson et al. 1997; Stockley 1999; Kraaijeveld-Smit et al. 2002; Mack et al. 2002; Thuman & Griffith 2005). The sole exception being that by Stockley (1997) of common shrews (*Sorex araneus*) where patterns were similar to those found in my study. While copulations among genetically related individuals occur in *S. araneus* (Stockley et al. 1993), females were not shown to be capable of sperm selection for genetically dissimilar mates (Stockley 1997).

While definitive conclusions regarding inbreeding depression in red squirrels are premature without a detailed pedigree analysis (Pemberton 2004), current data suggest that these effects may be minimal. Neither the performance, nor fitness of individual offspring was influenced by the genetic relatedness of their parents. There may consequently be, at most, small selective benefit to a female's ability to bias paternity in favour of genetically dissimilar males.

One potential variable influencing the effects of genetic relatedness on parentage patterns is the historical degree of inbreeding in the population. Small, isolated populations or those exhibiting a mating system with relatively high male reproductive skew are potentially more vulnerable to inbreeding depression (Keller & Waller 2002). Whereas larger, more connected populations, with more equally distributed reproductive success, should be expected to show lower levels. Those populations that have historically experienced higher levels of inbreeding, where mating between genetically related individuals is currently more common, should show predictably higher selection for effective inbreeding avoidance mechanisms. Indeed, of those studies shown to exhibit post-copulatory selection for genetically dissimilar sires, many used individuals from populations with high observed (*Lacerta agilis*; Olsson et al. 1996), or inferred (lek breeding *Antechinus agilis*; Kraaijeveld-Smit et al. 2002 and *Philomachus pugnax*; Thuman & Griffith 2005) levels of inbreeding. My study, on the contrary, used individuals from a large population with high connectivity to others. Additionally, the mating system of red squirrels is a form of scramble-competition (Chapter 4) with less male reproductive skew than in traditional resource or female defense polygynous systems. Wild populations without pronounced inbreeding have been shown to possess post-copulatory paternity biasing mechanisms, however, (*Gryllodes supplicans*; Stockley 1999), and one inbred population was not shown to possess these mechanisms (*S. araeneus*; Stockley 1997). An explicit test of the hypothesis that the inbreeding history of a population influences currently observed mechanisms of post-copulatory mate selection should evaluate these paternity-biasing mechanisms in natural or lab-reared populations of the same species exhibiting relatively high and low degrees of inbreeding.

In conclusion I have shown that female red squirrels do not appear to have the ability to bias paternity of their litters based on genetic relatedness of potential sires. Furthermore, in this relatively outbred population, selection for this ability appears minimal. Previous analyses have tended to focus on more inbred populations and, as such, findings from these analyses may not be as general as was previously believed. I advocate that future analyses explore parentage patterns in populations ranging in levels of inbreeding, either naturally or through laboratory rearing. In addition, detailed investigations into the social and genetic context of female promiscuity in red squirrels and other mammalian species should help to provide insight into the evolution and maintenance of this behaviour.

3.5 REFERENCES

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behavior*, **9**, 227-267.

Balloux, F., Amos, W. & Coulson, T. 2004. Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology*, **13**, 3021-3031.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, **2**, 349-368.

Birkhead, T. R. & Parker, G. A. 1997. Sperm Competition and Mating Systems. In: *Behavioural Ecology: An Evolutionary Approach*. 4th edn. (Ed. by J. R. Krebs & N. B. Davies), pp. 121-148. Oxford: Blackwell Scientific.

Bishop, J. D. D. 1996. Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. I. Autoradiographic investigation of sperm movements in the female reproductive tract. *Proceedings of the Royal Society of London, Series B*, **263**, 369-376.

Boellstorff, D. E., Owings, D. H., Penedo, M. C. T. & Heresk, M. J. 1994. Reproductive behavior and multiple paternity of California ground squirrels. *Animal Behaviour*, **47**, 1057-1064.

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

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1998. Multilocus and single-locus DNA fingerprinting. In: *Molecular Genetic Analysis of Populations: A Practical Approach.* (Ed. by A. R. Hoelzel), pp. 225-269. Oxford: IRL Press.

Cohen, J. 1988. *Statistical power analysis for the behavioral sciences.* 2nd edn. Hillsdale: Lawrence Erlbaum.

Connor, J. L. & Bellucci, M. J. 1979. Natural selection resisting inbreeding in captive wild house-mice (*Mus musculus*). *Evolution*, **33**, 929-940.

Crnokrak, P. & Roff, D. A. 1999. Inbreeding depression in the wild. *Heredity*, **83**, 260-270.

Dudash, M. R. & Carr, D. E. 1998. Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems. *Nature*, **393**, 682-684.

Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice.* Princeton: Princeton University Press.

Erdfelder, E., Faul, F. & Buchner, A. 1996. GPOWER: A general power analysis program. *Behavior Research Methods, Instruments, & Computers*, **28**, 1-11.

Gunn, M. R., Dawson, D. A., Leviston, A., Hartnup, K., Davis, C. S., Strobeck, C., Slate, J. & Coltman, D. W. 2005. Isolation of 18 polymorphic microsatellite loci from the North American red squirrel, *Tamiasciurus hudsonicus* (Sciuridae, Rodentia), and their corss-utility in other species. *Molecular Ecology Notes*, **5**, 650-653.

Hardy, O. J. & Vekemans, X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618-620.

Hoogland, J. L. 1998. Why do female Gunnison's prairie dogs copulate with more than one male? *Animal Behaviour*, **55,** 351-359.

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

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

Jennions, M. D. & Møller, A. P. 2003. A survey of the statistical power of research in behavioral ecology and animal behavior. *Behavioral Ecology*, **14**, 438-445.

Jennions, M. D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews of the Cambridge Philosophical Society*, **75**, 21-64.

Keller, L. K. & Waller, D. M. 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**, 230-241.

Kerr, T. D., Boutin, S., LaMontagne, J. L., McAdam, A. G. & Humphries, M. M. 2007. Persistent maternal effects on juvenile survival in North American red squirrels. *Biology Letters*, **3**, 289-291.

Koprowski, J. L. 1992. Removal of copulatory plugs by female tree squirrels. *Journal of Mammalogy*, **73**, 572-576.

Koprowski, J. L. In press. Alternative reproductive tactics and strategies of tree squirrels. In: *Rodent Societies: An Ecological and Evolutionary Perspective.* (Ed. by J. O. Wolff & P. W. Sherman). Chicago: University of Chicago Press.

Kraaijeveld-Smit, F. J. L., Ward, S. J., Temple-Smith, P. D. & Paetkau, D. 2002. Factors influencing paternity success in *Antechinus agilis*: last-male sperm precedence, timing of mating and genetic compatibility. *Journal of Evolutionary Biology*, **15**, 100-107.

Kruuk, L. E. B., Sheldon, B. C. & Merilä, J. 2002. Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). *Proceedings of the Royal Society of London, Series B*, **269**, 1581-1589.

Lacey, E. A., Wieczorke, J. R. & Tucker, P. K. 1997. Male mating behavior and patterns of sperm precedence in Arctic ground squirrels. *Animal Behaviour*, **53**, 767-779.

Lacy, R. C., Alaks, G. & Walsh, A. 1996. Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. *Evolution*, **50**, 2187-2200.

Mack, P. D., Hammock, B. A. & Promislow, D. E. L. 2002. Sperm competitive ability and genetic relatedness in *Drosophila melanogaster*: similarity breeds contempt. *Evolution*, **56**, 1789-1795.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural conditions. *Molecular Ecology*, **7**, 639-655.

Martin, P. & Bateson, P. 1986. *Measuring behavior: an introductory guide.* Cambridge: Cambridge University Press.

Mays, H. L. & Hill, G. E. 2004. Choosing mates: good genes versus genes that are a good fit, *Trends in Ecology and Evolution*, **19**, 554-559.

McAdam, A. G. & Boutin, S. 2003a. Variation in viability selection among cohorts of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Evolution*, **57**, 1689-1697.

McAdam, A. G. & Boutin, S. 2003b. Effects of food abundance on genetic and maternal variation in the growth rate of juvenile red squirrels. *Journal of Evolutionary Biology*, **16**, 1249-1256.

McAdam, A. G. & Boutin, S. 2004. Maternal effects and the response to selection in red squirrels. *Proceedings of the Royal Society of London, Series B*, **271**, 75-79.

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

Meagher, S. D., Penn, J. & Potts, W. K. 2000. Male-male competition magnifies inbreeding depression in wild house mice. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 3324-3329.

Møller, A. P. & Alatalo, R. V. 1999. Good-genes effects in sexual selection. *Proceedings of the Royal Society of London, Series B*, **266**, 85-91.

Murie, J. O. 1995. Mating behavior of Columbian ground squirrels. I. Multiple mating by females and multiple paternity. *Canadian Journal of Zoology*, **73**, 1819-1826.

Neff, B. D. & Pitcher, T. E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19-38.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelström, H. 1996. Sperm selection by females. *Nature*, **383**, 585.

Pemberton, J. 2004. Measuring inbreeding depression in the wild: the old ways are the best. *Trends in Ecology and Evolution*, **19**, 613-615.

Réale, D., Berteaux, D., McAdam, A. G. & Boutin, S. 2003. Lifetime selection on heritable life-history traits in a natural population of red squirrels. *Evolution*, **57**, 2416-2423.

Slate, J., David, P., Dodds, K. G., Veenvliet, B. A., Glass, B. C., Broad, T. E. & McEwan, J. C. 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity*, **93**, 255-265.

SPSS Inc. 1998. *Systat user's guide. Version 9. Standard Version*. Chicago: SPSS Inc.

Stockley, P. 1997. No evidence for sperm selection by female common shrews. *Proceedings of the Royal Society of London, Series B*, **264**, 1497-1500.

Stockley, P. 1999. Sperm selection and genetic incompatibility: does relatedness of mates affect male success in sperm competition? *Proceedings of the Royal Society of London, Series B*, **266**, 1663-1669.

Stockley, P., Searle, J. B., Macdonald, D. W. & Jones, C. S. 1993. Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. *Proceedings of the Royal Society of London, Series B*, **254**, 173-179.

Thuman, K. A. & Griffith, S. C. 2005. Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. *Animal Behaviour* **69**, 765-770.

Tregenza, T. & Wedell, N. 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution*, **52**, 1726-1730.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871-1971* (Ed. by B. Campbell), pp. 136-179. Chicago: Aldine Publishing Company.

Venables, W. N. & Ripley B. D. 2002. *Modern Applied Statistics with S. Fourth Edition.* New York: Springer.

Wang, J. 2002. An estimator for pairwise relatedness using molecular markers. *Genetics*, **160**, 1203-1215.

Waterman, J. M. 1998. Mating tactics of male Cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Animal Behaviour*, **56**, 459-466.

Wilson, N., Tubman, S. C., Eady, P. E. & Robertson, G. W. 1997. Female genotype affects male success in sperm competition. *Proceedings of the Royal Society of London, Series B*, **264**, 1491-1495.

Wolff, J. O. & Macdonald, D. W. 2004. Promiscuous females protect their offspring. *Trends in Ecology and Evolution*, **19**, 127-134.

Zeh, J. A. & Zeh, D. W. 1996. The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society of London, Series B*, **263**, 1711-1717.

Zeh, J. A. & Zeh, D. W. 2001. Reproductive mode and the genetic benefits of polyandry. *Animal Behaviour*, **61**, 1051-1063.

Table 3.1. Microsatellite loci used in the study of North American red squirrels, including the number, and size range, of alleles at each locus, as well as observed (H_O) and expected (H_E) heterozygosities. Values are based on the genotypes of 716 individuals (adults and juveniles).

Locus	Number of alleles	Size range (bp)	H_O	H_E
Thu03	10	221-239	0.77	0.80
Thu08	12	180-216	0.73	0.77
Thu14	11	268-288	0.83	0.80
Thu23	13	181-205	0.80	0.80
Thu25	10	182-206	0.78	0.75
Thu31	10	129-147	0.79	0.76
Thu32	11	267-289	0.8	0.81
Thu33	10	143-161	0.83	0.81
Thu37	10	155-182	0.36	0.37
Thu38	8	290-306	0.47	0.50
Thu40	13	378-402	0.89	0.89
Thu41	12	236-283	0.77	0.79
Thu42	10	232-260	0.76	0.78
Thu49	12	174-208	0.74	0.74
Thu50	10	273-293	0.64	0.69
Thu55	14	254-280	0.79	0.79
Mean ± SE	11.0 ± 0.4		0.73 ± 0.03	0.74 ± 0.03

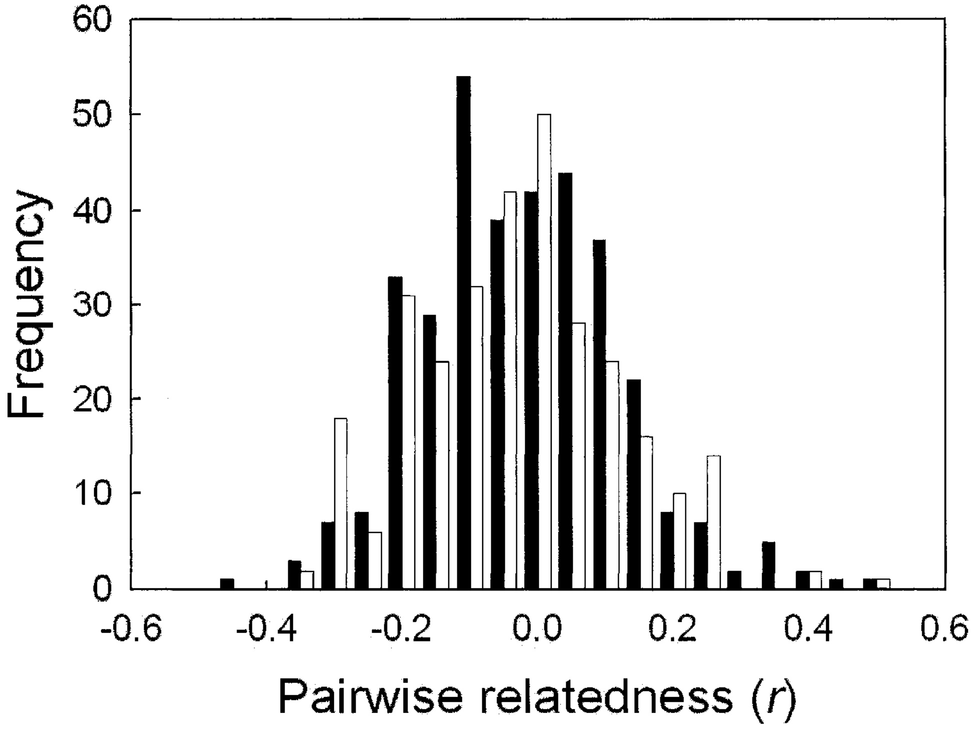

Figure 3.1 Frequency distribution of pairwise genetic relatedness of observed copulatory partners (black bars; *N* = 345) and parents of offspring born into the population of North American red squirrels (white bars; *N* = 300).

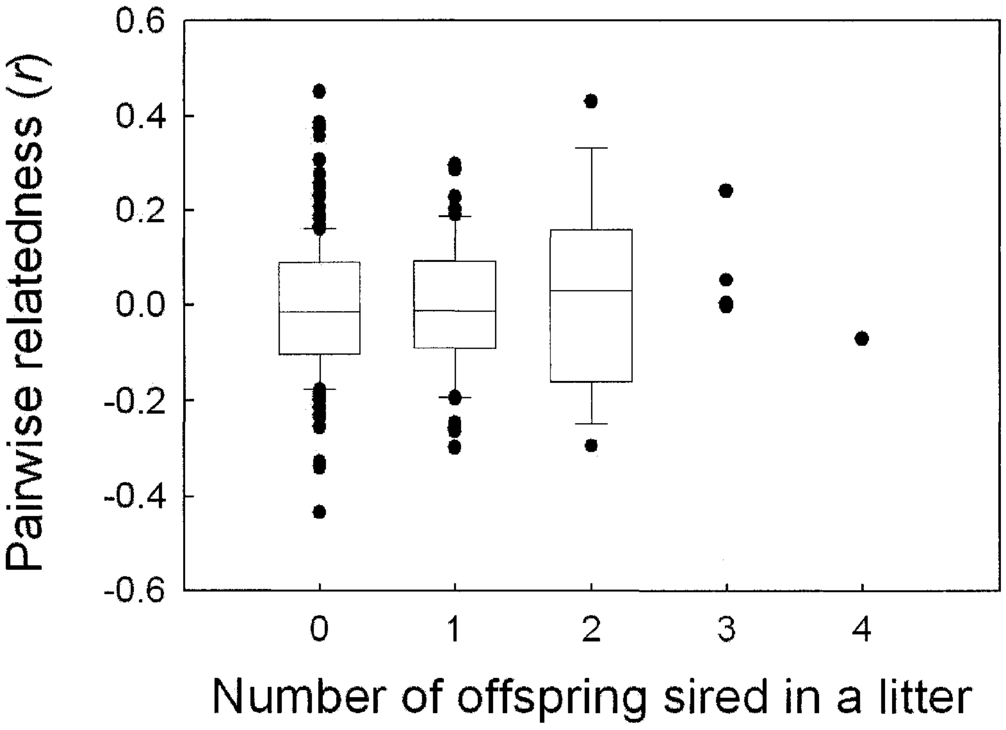

Figure 3.2. Pairwise genetic relatedness between copulating males and estrous females for males siring zero ($N = 232$), one ($N = 56$), two ($N = 9$), three ($N = 4$) and four ($N = 1$) offspring in a litter in North American red squirrels. Box plots for 0 - 2 offspring show 25th and 75th percentiles (box), median (line within the box), 10th and 90th percentiles (whiskers) and data points outside the 10th and 90th percentiles. Points for 3 - 4 offspring represent raw data.

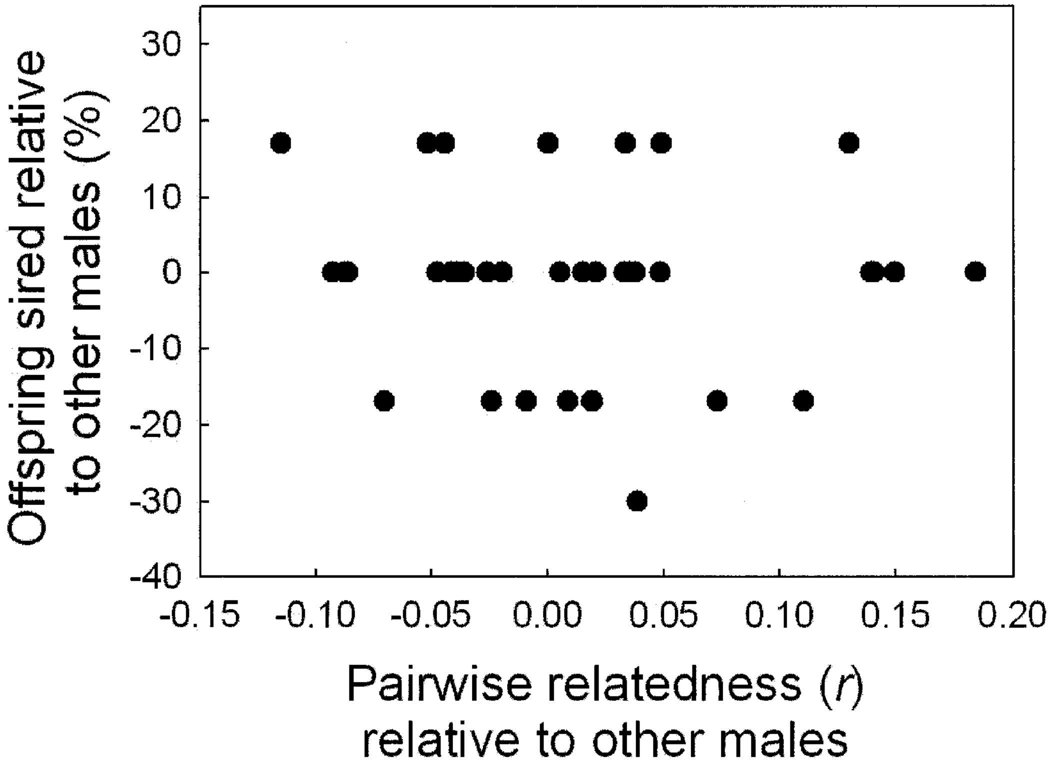

Figure 3.3. Relative number of offspring sired within multiply sired (N = 36) litters by males as a function of their relative pairwise genetic similarity to the estrous female in North American red squirrels.

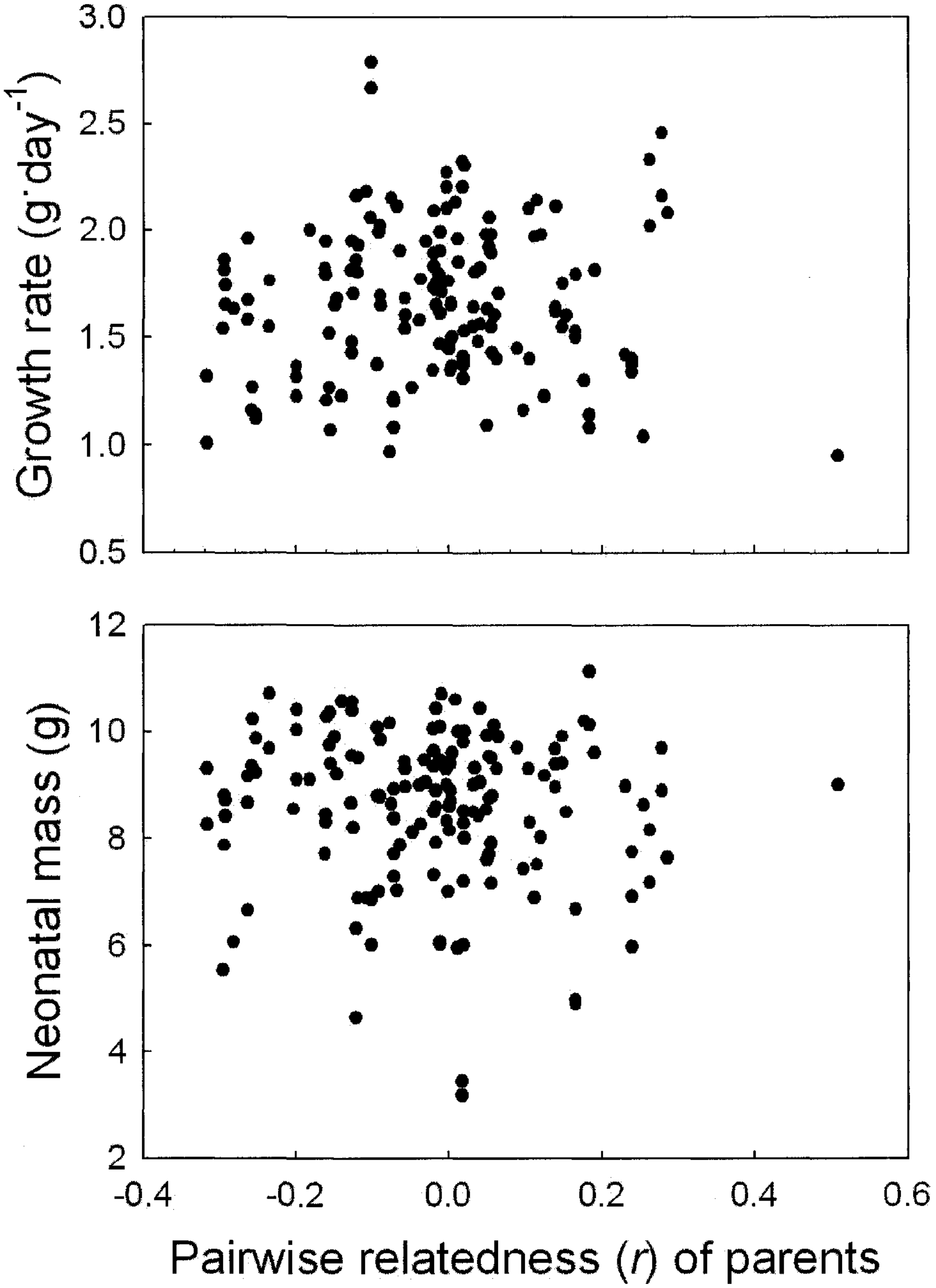

Figure 3.2. Neonatal mass (*N* = 159) and growth rate (*N* = 156) of North American red squirrel offspring as a function of the pairwise genetic relatedness of parents.

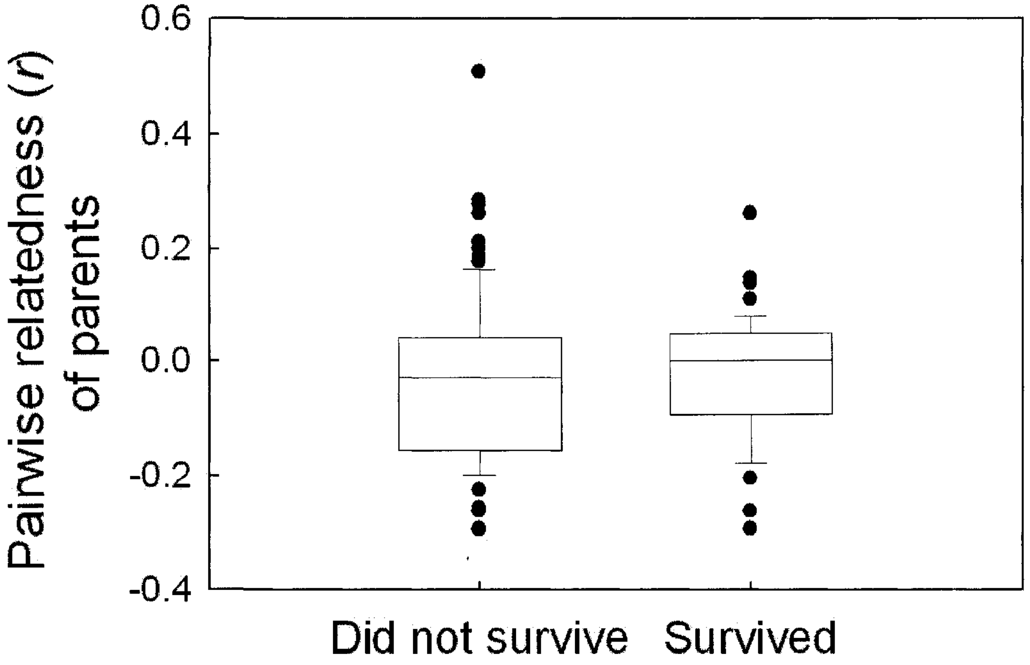

Figure 3.3. Pairwise genetic relatedness of North American red squirrel parents of offspring that did (*N* = 41) and did not (*N* = 126) survive to reproductive age (year 1). Box plots show 25th and 75th percentiles (box), median (line within the box), 10th and 90th percentiles (whiskers) and data points outside the 10th and 90th percentiles.

CHAPTER 4. SEXUALLY-SELECTED BEHAVIOUR: RED SQUIRREL MALES SEARCH FOR REPRODUCTIVE OPPORTUNITY

4.1 INTRODUCTION

The concept of sexual selection was originally formulated by Darwin (1871) to account for the maintenance of conspicuous sexually dimorphic traits not favoured by natural selection. It is not surprising then that sexual selection theory has primarily advanced through the study of model systems with prominent morphological dimorphism. In mammals, differences in reproductive success among males can be pronounced (e.g. Leboeuf 1974; Clutton-Brock et al. 1982), and traditional explanations for these asymmetries rely on variation in traits that enable males to defend sexually receptive females from rival males (Clutton-Brock et al. 1979; Clutton-Brock 1988; Andersson 1994). Although the overt nature of male-male combat, and its associated traits, have led to female defense being the most studied of all mammalian mating systems, it is important to realize that it represents only one point along a continuum. If females are clumped in space, and/or come into estrus asynchronously, dominant males should be able to defend access to receptive females, giving rise to a female defence mating system (Emlen & Oring 1977; Ims 1988). Conversely, if females are spatially dispersed, and/or breed synchronously, they become economically indefensible and a scramble competition mating system is more likely (Ims 1988).

Scramble competition mating systems are considered common in both anurans (Wells 1977; Sztatecsny et al. 2006) and insects (Thornhill & Alcock 1983; Rank et al. 2006; Moya-Larano et al. 2007), but their prevalence in mammals and most other taxa is unknown. In addition, the avenues through which males achieve reproductive success favour more subtle, and hence difficult to study, adaptations over the conspicuous morphological traits favoured in female defence systems (Schwagmeyer & Woontner 1986; Kruuk et al. 2002). Male mating success in scramble competition mating systems should correlate with variation in traits that enable males to efficiently locate and follow estrous females, such as search effort and ability (Schwagmeyer & Woontner 1986; Schwagmeyer & Parker 1987; Schwagmeyer et al. 1998; Spritzer et al. 2005a,b). Sexual selection acting on these traits has been proposed to explain why male home ranges of many species expand during the mating season, while those of females remain relatively consistent throughout the year (e.g. Erlinge & Sandell 1986; Stockley et al. 1994; Kappeler 1997; Edelman & Koprowski 2006). Similarly, evolutionary explanations for the consistent male-female spatial ability asymmetries observed under both laboratory (McNemar & Stone 1932; Joseph et al. 1978) and field (Schwagmeyer et al. 1998; Spritzer et al. 2005a) conditions typically invoke sexual selection (Gaulin & Fitzgerald 1986, 1989). Proper evaluation of these hypotheses, however, requires analyses that extend beyond correlations with mating success. While often called upon for such explanations, the actual strength and shape of sexual selection acting on these traits has not been quantified. In their review of published estimates of phenotypic selection, Kingsolver et al. (2001) concluded that sexual selection is, on average, stronger than viability selection. More than 80% of these estimates, however, were for morphological traits. By comparison, estimates of selection on behavioural attributes comprised less than 2% of those published and I am unaware of any studies quantifying selection on male traits in a scramble competition mating system.

I studied a free-ranging population of North American red squirrels (*Tamiasciurus hudsonicus*; hereafter red squirrel) to test the hypothesis that sexual selection operates on male searching behaviour. As a first step, through detailed behavioural observations of both males and females during the mating season, I characterized the mating system of this population. Red squirrels at my Yukon study site rely primarily on the seeds of white spruce (*Picea glauca*), which they hoard in a central food cache (midden; Smith 1968). These middens are thought to be necessary for over-winter survival (Rusch & Reeder 1978; McAdam & Boutin 2003) and form the center of individual territories that aredefended against members of both sexes year-round (Smith 1968; Price et al. 1990). As a result, males must temporarily vacate their territories to locate spatially dispersed receptive females, thereby setting the stage for a scramble competition mating system. Studies of other Sciurids, however, (e.g. Farentinos 1972; Thompson 1977; Koford 1982; Wauters et al. 1990; Koprowski 1993; Schulte-Hostedde & Millar 2004) have suggested elements of both female defence and sperm competition. In those species exhibiting a female defence component, males congregate in the vicinity of females approaching estrus and more dominant males attempt to maintain exclusive reproductive access. In addition, intraspecific variation in testis size has previously been shown to influence reproductive success (Schulte-Hostedde & Millar 2004). Therefore, I also examined to what extent, if any, female defence and sperm competition may play a role in the mating system of red squirrels. Finally, I quantified the strength and shape of selection acting on male behavioural traits with standardized selection gradients (Lande & Arnold 1983).

4.2 METHODS

I studied a population of red squirrels near Kluane National Park in the southwest Yukon from 2003-2005 (61° N, 138° W). Individuals were resident on two 40 ha study grids bisected by the Alaska Highway. Details of the study landscape (Krebs et al. 2001) and population (e.g. Humphries et al. 2005; Boutin et al. 2006; McAdam et al. 2007) have been reported previously. Briefly, I captured individuals in live traps (Tomahawk Live Trap, Tomahawk, Wisconsin) placed on, or in the immediate vicinity of, their middens. All individuals born into the study population were originally handled in their natal nest and had there received unique alphanumeric ear tags. Ages of such focal males ($N = 68$) were known with certainty. Any immigrating adults received eartags on first capture and, for these focal males ($N = 38$), I treat age as a minimum estimate (i.e. age = 1 in year of first capture). Restricting my analyses to include only males of known age did not influence any of my main conclusions. Each individual was also given a unique combination of one or two coloured wires, threaded through their eartags, on first capture of the season to allow for identification from a distance. Male body mass (g; using a 500 g Pesola spring scale) was recorded at each capture in 2003-2005 and in 2004 and 2005 I also measured testis size (mm; using a ruler placed along the long-axis of the left testis) of captured males. The means of all measurements (body mass: range = 1-16; testis size: range = 3-12) taken during the mating season, for these two traits, were used in analyses. The Biosciences Animal Policy and Welfare Committee at the University of Alberta approved all protocols.

4.2.1 Mating Season Observations

Details of the mating season for this population, and observations employed, are provided elsewhere (Lane et al. 2007; Lane et al. In Press). Briefly, mating usually commences in mid-late winter (Lane et al. 2007; S. Boutin, unpublished data) with selection favouring early breeding by females (Réale et al. 2003). Females typically produce a single litter, each year, after a 35-day gestation period, but will occasionally attempt a second litter after litter loss and, rarely, following a successful litter (Boutin et al. 2006). Behavioural estrus commences with the receptive female emerging from the nest in the morning and continues until the female retires to the nest in the evening, thereby encompassing the full activity period for one day. All resident females were monitored daily for reproductive activity in 2003-2005 and I monitored 85 mating chases (N = 46 (2003), 23 (2004), 16 (2005)) through to completion.

For focal mating chases, I employed a combination of focal-animal sampling of the estrous female, scan sampling for attending males and all-occurrence sampling of mating behavior (Altmann 1974, Martin & Bateson 1986). This protocol allowed me to identify both attending and copulating males. Because a large number of copulations occur underground, I judged these copulations as occurring when a female was followed underground by a male and the pair remained there for a minimum of 60 s or if copulatory vocalizations could be heard, regardless of the time spent underground. Copulations of tree squirrels generally last less than 60 s (Koprowski 2007) and this duration 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. 2007) and other (Waterman 1998) systems.

4.2.2 Searching Information

I quantified two searching behaviour metrics: search ability and search effort. The number of estrous females located by males (*N* = 69 (2003), 47 (2004), 48 (2005) males analyzed) over the mating season, each year, was taken to represent search ability (sensu Schwagmeyer & Woontner 1986). As the number of mating chases followed varied from year to year, I standardized each male's value by dividing by the annual population mean. The home range size of males (*N* = 63 (2003), 41 (2004), 42 (2005)) during the mating season was taken to represent search effort. For comparison, I also calculated home range sizes of males during the non- (post-) mating season (*N* = 57 (2003), 39 (2004), 51 (2005)) and females during both seasons (mating: *N* = 62 (2003), 49 (2004), 61 (2005); non-mating: *N* = 53 (2003), 53 (2004), 52 (2005)). Study grids were staked and flagged at 15 or 30 m intervals to provide spatial references for all location data and I compiled three types of spatial data for home-range analyses. In both seasons, and for both sexes, I recorded the trap locations of individuals and noted the location of any individuals seen opportunistically (for example, during regular trapping rounds). A sample of individuals (males: *N* = 43 (2003), 32 (2004), 27 (2005); females: *N* = 69 (2003), 49 (2004), 63 (2005)) was also outfitted with radio-collars (model PD-2C, 4 g, Holohil Systems Limited, Carp, Ontario) and I used radio-telemetry to augment my spatial data. During the mating season, I obtained point locations (2003) or conducted behavioural focals (2004 and 2005; recording spatial locations every 30 s for 10 min) for males and, for females, I obtained point locations in each year (2003-2005). Post-mating season spatial data for females was also provided, through coordination with other projects, in the form of behavioural focals (recording spatial locations every 30 s for 10 min) and nest site locations. Red squirrels become accustomed to human observers rapidly, following a brief period of acclimation (Smith 1968). This population has been the focus of research for 20 years, and behavioural focals have been employed since its inception (e.g. Stuart-Smith & Boutin 1995; Humphries & Boutin 2000; Lane et al. In Press). I am thus confident that my presence did not affect natural ranging behaviour of observed individuals.

Home range sizes were estimated with minimum convex polygons (MCP). While 95% MCPs are traditionally used for home range analyses (White & Garrott 1990) and are less vulnerable to being confounded by the number of data points used in the calculation, off-territory excursions, most relevant to my hypotheses, are largely excluded from these calculations. I thus retained all data and calculated 100% MCPs. To cope with its potential confounding influence, I include the number of data points used (range: 3-485) as a covariate in all analyses, following Kenward (2001).

4.2.3 Molecular Analyses and Paternity Assignment

Details of the molecular methods for microsatellite loci isolation and paternity assignment have been provided elsewhere (Gunn et al. 2005; Lane et al. In Press). Briefly, DNA was extracted from preserved tissue using either an acetate-alcohol precipitation protocol (Bruford et al. 1998) or DNeasy Tissue extraction kits (Qiagen, Venlo, The Netherlands). Polymerase chain reaction (PCR) amplification was performed for a panel of 16 microsatellite loci (for details of microsatellite loci used, including the number and size range of alleles at each locus, as well as observed (H_O) and expected

(H_E) heterozygosities see Lane et al. (2007)). Maternity was determined by behavioural observation at the nest and paternity was assigned at 95% confidence using CERVUS 2.0 (Marshall et al. 1998). Separate analyses were conducted for each of the years 2003, 2004 and 2005.

4.2.4 Male Success

I quantified both male relative mating (i.e. number of females mated with divided by the annual mean) and relative reproductive success (i.e. number of offspring sired divided by the annual mean) for each year. I also calculated the opportunity for sexual selection on both metrics (I) as the square of the coefficient of variation ((standard deviation/mean)*100) (Crow 1958; Arnold & Wade 1984a,b).

4.2.5 Statistical and Selection Analyses

I used a linear mixed-effects model (LME) to determine whether sex and/or season influenced home range size. Home range sizes varied over five orders of magnitude and were thus $\log_{10}$-transformed prior to analysis as suggested by Kenward (2001). I fitted sex and season as fixed effects and included the sex*season interaction. Individuals (male: $N = 129$; female: $N = 113$) are represented in multiple years and across both seasons. Individual ID is therefore fitted as a random effect. Year (2003, 2004 or 2005) and study grid were included as three and two-level, respectively, categorical explanatory terms in the model. The $\log_{10}$-transformed number of data points used in the MCP calculations was also included as a covariate.

To investigate whether my calculated searching metrics explained male success, I used generalized linear mixed-effects models (GLMMs) with Poisson error structures. In all cases, year and study grid were tested as categorical variables, and minimum age, $(\text{minimum age})^2$ and body mass were included as covariates. Testis size was also fitted as a covariate in analyses on male reproductive success. Treating testis size as the residual from a mass/testis size regression did not change my conclusions. The quadratic term for age was included to examine whether a senescent decline in female quality with age (McAdam et al. 2007) is also observable in males. In my first two analyses I tested whether either search ability (i.e., relative number of females located during the mating season) of males or their search effort (i.e., $\log_{10}$(mating season home range size)) influenced relative mating success. In the next two analyses I tested whether either of these two variables influenced relative reproductive success. The number of females that a male located divided by the annual population mean and the $\log_{10}$-transformed home range sizes, respectively, were fitted as the fixed effects. The number of females copulated with divided by the annual population average represented the dependent variable in the mating success analyses and the number of offspring sired divided by the annual population average represented the dependent variable in the reproductive success analyses.

I calculated selection on the two behavioural traits using standardized linear and non-linear selection gradients (Lande & Arnold 1983). I obtained the standardized linear selection gradients (β'_i) from the multiple regression of my success metrics (relative number of females copulated with and number of offspring produced) on either the number of estrous females located or the home range size as well as average body mass and minimum age. Testis size was also included in the models on reproductive success. Trait values were standardized to a mean of zero. Quadratic (γ'_{ii}) and correlational (γ'_{ij}) selection gradients were estimated in a separate linear model that included all linear, quadratic and pair-wise cross-products of traits. Standard errors were generated by jackknifing, and I corrected for multiple comparisons of selection gradients (2 (mating success) or 3 (reproductive success) traits x 3 gradients) with sequential Bonferonni correction (Rice 1989). All analyses were implemented in R (ver. 2.6.0; R Core Development Team) and values are presented as mean ± 1 S.E..

4.3 RESULTS

Mating seasons commenced in mid-late winter of each year (2003: 19-Feb; 2004: 5-Mar; 2005: 21-Mar). In 2004 and 2005 the mating seasons extended to the late spring (2003: 4-June; 2004: 19-May), while in 2005 it extended to mid-summer (17-July). The latter was characterized by five preceding years of low food, and was the last before a mast year of high food abundance. Many females (as described in Lane et al. In Press) lost their earlier litters and re-cycled and females also re-cycled following successful litters (as described in Boutin et al. 2006) this year. Females entered estrus asynchronously, resulting in temporally dispersed reproductive opportunities for males and a male-biased daily operational sex ratio (mean = 25 males/female; Lane et al. In Press).

The home ranges of the two sexes showed dissimilar patterns across the two seasons (sex*season: LME: $F_{1,409} = 112.87$, $P < 0.001$; Fig. 4.1). During the non- (post-) mating season, male home ranges averaged 6194.04 ± 887.13 m^2 ($N = 147$) and during the mating season they expanded by almost 10-fold (mean = 59827.06 ± 3416.45 m^2; $N =$

177). These values represent multiples of 1.51 and 14.59, respectively, of average male territory sizes (i.e., actively defended, core, areas LaMontagne 2007). Female home ranges, by contrast, varied more moderately throughout the year (Fig. 4.1). During the non-mating season, female home ranges averaged 8470.06 ± 781.05 m^2 ($N = 158$), while during the mating season they expanded to 16188.83 ± 1891.13 m^2 ($N = 172$). These values represent multiples of 2.07 and 3.86 of the average female territory sizes (LaMontagne 2007).

Male mating success was correlated with my measures of both search effort and ability. Relative mating success increased with the relative number of estrous females located (GLMM: $\chi^2_1 = 386.36$, $P < 0.001$; Fig. 4.2a) and with $\log_{10}$(home range size) (GLMM: $\chi^2_1 = 39.21$, $P < 0.001$; Fig. 4.2b). In contrast, mating season body mass, minimum age, and (minimum age)^2 showed weaker and inconsistent relationships with mating success. In the search ability analysis (i.e., when controlling for the relative number of females located) neither minimum age (GLMM: $\chi^2_1 = 2.90$, $P = 0.09$) nor (minimum age)^2 (GLMM: $\chi^2_1 = 1.97$, $P = 0.17$) were related to mating success while mating season body mass showed a weak negative relationship (GLMM: $\chi^2_1 = 9.55$, $P < 0.01$). In the search effort analysis (i.e., when controlling for home range size), however, minimum age (GLMM: $\chi^2_1 = 12.69$, $P < 0.001$) and (minimum age)^2 (GLMM: $\chi^2_1 = 12.91$, $P < 0.001$) were correlated with mating success, while mating season body mass was unrelated to mating success (GLMM: $\chi^2_1 = 3.39$, $P = 0.07$).

Male reproductive success was similarly correlated with my measures of both search effort and ability (Fig. 4.2). The relative number of offspring sired increased with the relative number of estrous females located (GLMM: $\chi^2_1 = 9.81$, $P = 0.002$; Fig. 4.2c) and with $\log_{10}$(home range size) (GLMM: $\chi^2_1 = 15.01, P < 0.001$; Fig. 4.2d). In the search ability analysis (i.e., when controlling for the relative number of females located) minimum age (GLMM: $\chi^2_1 = 5.66, P = 0.02$) and (minimum age)^2 (GLMM: $\chi^2_1 = 4.94, P = 0.03$) were correlated with reproductive success while mating season body mass was unrelated to reproductive success (GLMM: $\chi^2_1 = 0.09, P = 0.76$). In the search effort analysis (i.e., when controlling for home range size), however, none of minimum age (GLMM: $\chi^2_1 = 2.08, P = 0.15$), (minimum age)^2 (GLMM: $\chi^2_1 = 0.23, P = 0.63$) or mating season body mass were related to mating success (GLMM: $\chi^2_1 = 0.62, P = 0.43$). Testis size was unrelated to the relative number of offspring sired in the search ability analysis (GLMM: $\chi^2_1 = 0.79, P = 0.39$), but was significant in the search effort analysis (GLMM: $\chi^2_1 = 5.28, P = 0.02$).

The opportunity for sexual selection on males was 0.64 for mating success and 1.52 for reproductive success, and sexual selection favoured both male search effort and ability (Table 4.1). In order, the strongest selection gradients were for search ability (i.e. relative number of females located) on mating success ($\beta'_i = 0.67 \pm 0.03$; one-sample t test: $t_{157} = 20.33, P < 0.001$), search effort (i.e. home range size) on reproductive success ($\beta'_i = 0.58 \pm 0.15$; one-sample t test: $t_{73} = 3.91, P < 0.001$), search ability on reproductive success ($\beta'_i = 0.45 \pm 0.16$; one-sample t test: $t_{82} = 2.78, P = 0.006$) and search effort on mating success ($\beta'_i = 0.34 \pm 0.07$; one-sample t test: $t_{144} = 2.66, P < 0.001$). Selection did not show any non-linear (stabilizing or disruptive) elements, but there was evidence for correlational selection on search ability and mass for mating success ($\gamma'_{ij} = 0.210 \pm 0.036$; one-sample t test: $t_{154} = 5.833, P < 0.001$; Table 4.1).

4.4 DISCUSSION

Scramble competition mating systems are predicted to predominate over female defence systems when either temporal clumping of reproductive opportunities or spatial dispersion of females renders them indefensible (Ims 1988; Sandell & Liberg 1992; Murphy 1998). Unlike many Sciurids (e.g. ground squirrels and chipmunks), red squirrels remain active throughout the winter (Humphries et al. 2005). This results in an extended mating season and temporally dispersed mating opportunities (Lane et al. In Press). Elements of female defence could thus be predicted, and previous studies of Sciurid mating systems have revealed it to varying degrees (Farentinos 1972; Thompson 1977 Koford 1982; Wauters et al. 1990; Koprowski 1993; Koprowski 2007, but see Schwagmeyer & Woontner 1986). Despite this, red squirrels exhibited an almost exclusively scramble competition mating system. Females showed a modest increase in home ranges size across the two seasons, potentially because they were searching for vacant territories to subsequently relinquish to offspring (Boutin et al. 2000) or because they were pilfering food stores from vacant male neighbours (Gerhardt 2005). Male home ranges, by contrast, expanded almost 10-fold as they vacated their territories to search for, and mate with, females in, or approaching, estrus. Furthermore, both male mating and reproductive success were correlated with male searching behaviour and body mass was, at most, a minor influence.

A key ecological aspect preventing female defence in the mating system of red squirrels is the spatial dispersion of females and their territorial social structure. With the higher degree of sociality in previously studied Sciurids, males are able to congregate in the area surrounding females in the days preceding her estrus, and more dominant males attempt to maintain exclusive access to the female (e.g. Wauters et al. 1990). For male red squirrels, however, this would require abandoning defence of their territories, while incurring aggression from the territory owners neighbouring the female. Thus, although reproductive opportunities are temporally dispersed, the strict territoriality of red squirrels in my study population prevents males from attempting to defend females approaching estrus, resulting in a scramble competition mating system.

Patterns of reproductive success in red squirrels follow theoretical predictions (Bateman 1948; Trivers 1972; Emlen & Oring 1977). Although the opportunity for sexual selection on males was moderate, relative to previously studied female defence mating systems (e.g. Coltman et al. 2002), it was approximately 3-fold larger than on females for mating success (0.23) and 10-fold larger than on females for reproductive success (0.16). In contrast to female defence mating systems (e.g. Kruuk et al. 2002), however, sexual selection favoured behavioural, rather than morphological, traits.

The strength of selection on both male searching behaviour metrics is similar to previous estimates for secondary sexual characters in female defence mating systems. For example, using lifetime reproductive success, the standardized selection gradient on antler size in male red deer (*Cervus elaphus*) was found to be 0.44 (± 0.18 S.E.) (Kruuk et al. 2002). Whether selection on searching behaviour will be weaker when considering lifetime, rather than annual, reproductive success in red squirrels is currently unknown. On the other hand, the strength of selection I observed may represent an underestimate for scramble competition mating systems in general. In thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*), males who expend more search effort are more likely to arrive early at a females' mating chases (Schwagmeyer et al. 1998) and a queuing convention promotes early mating by these males relative to later-arriving competitors (Schwagmeyer & Parker 1987). Unlike red squirrels, which do not exhibit patterns of sperm precedence (sensu Lacey et al. 1997, J. E. Lane & S. Boutin, unpublished data), a first-male precedence is apparent in thirteen-lined ground squirrels and this further strengthens the competitive advantage to early arriving males (Foltz & Schwagmeyer 1989). Thus, the tight linkages between searching behaviour, mating success and, ultimately, reproductive success provide consistent directional benefits to early arriving males. I would predict, therefore, that the strength of selection on searching behaviour should be even stronger for thirteen-lined ground squirrels than red squirrels.

Sperm competition has been implicated as an important component of genetic mating systems (e.g. Preston et al. 2001; Schulte-Hostedde & Millar 2004) and may be an important determinant of reproductive success in red squirrels. Although searching behaviour of males was the primary influence on both measures of male success, its level of significance decreased for reproductive, relative to mating, success. In addition, levels of female multi-male mating (MMM) in red squirrels are among the highest recorded for any Sciurid (Lane et al., In Press), and patterns of sperm precedence are not evident. Testis size, however, was at most a minor influence on reproductive success. This discrepancy could be either due to sperm quality (or compatibility) being a more important determinant than quantity, or to testis size being an inaccurate reflection of ejaculate investment. Females in other species show selection for both good and compatible genes (Olsson et al. 1996; Neff & Pitcher 2005), and although female red squirrels do not bias paternity based on genetic relatedness, they may use more sophisticated cues (e.g. selection based on MHC genotypes; Neff & Pitcher 2005).

Previous work identifying intraspecific variation in testis size as an influence on reproductive success in a Sciurid used a hibernating species (yellow pine chipmunks (*Tamias amoenus*); Schulte-Hostedde & Millar 2004), with short intense bouts of mating. The temporal dispersion of reproductive opportunities in red squirrels, by contrast, likely places little draw on male's spermatogenesis abilities, thereby requiring comparatively smaller testes. In support of this contention is that, although female red squirrels are highly promiscuous, mean testis size falls below the interspecific regression of testis size on body mass (Kenagy & Trombulak 1986).

Female red squirrels mate with the majority of males that attend their mating chase (Lane et al. In Press). As a result, the most direct influence on a male's reproductive success that I have observed is the number of estrous females that he located during the mating season. The question remains then, why males don't simply attend more mating chases? At least three explanations could exist for variation in chase attendance by males. First, males may vary in temperament. Recent work in this population has identified a shy-bold continuum in females that is repeatable across time (Boon et al. 2007). Should males also exhibit this continuum, 'bold' males may be more predisposed to venture off-territory in search of reproductive opportunity, while 'shy' males limit mating to neighbouring females. Second, males may vary in spatial ability and/or memory and this affects their ability to find and/or remember the location of females approaching estrus (sensu Schwagmeyer et al. 1998). Finally, males may vary in condition. Red squirrels are relatively long-lived for a small mammal, with a maximum lifespan of 9 years (McAdam et al. 2007). Consequently, males currently in poor condition, or with few energetic resources, may invest less in current, so as to not sacrifice future, reproduction.

Due to the complexities and subtleties inherent in how males achieve success under scramble competition mating systems, our understanding of the evolutionary significance of these male traits lags considerably behind relative to other systems (e.g. Maynard Smith 1985; Kruuk et al. 2002). This discrepancy not only influences our understanding of mammalian sexual selection but also has important implications for the evolution, genetic structure and ecology of populations. For example, differential patterns of male reproductive success between female defence and scramble competition mating systems should have direct consequences for the fine-scale genetic structure of populations. In female defence systems, reproductive skew accentuates the genetic structure because successful males create clusters of relatives (Nussey et al. 2005), whereas in scramble competition systems, the most successful males are those that range the furthest and therefore disrupt the genetic structure. In addition, the more moderate skew among male reproductive success in scramble competition, relative to other mating systems, lessens the influence of male reproductive success on effective population sizes (Sugg & Chesser 1994). These predictions are in accordance with previous analyses showing that, despite strong philopatry, inbreeding depression is low in this population, and, consequently, females do not exhibit patterns of mate (or gamete) selection based on genetic relatedness (Lane et al. 2007). I suggest that future studies investigate the ways in which males achieve reproductive success in less represented mating systems. A better appreciation of the strength and shape of sexual selection on a more diverse suite of behaviours should aid our understanding of mammalian reproductive ecology and evolution.

4.5 REFERENCES

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behaviour*, **49**, 227-267.

Andersson, M. B. 1994. *Sexual Selection*. Princeton, NJ: Princeton University Press.

Arnold, S. J. & Wade, M. J. 1984a. On the measurement of natural and sexual selection: theory. *Evolution*, **38**, 709-719.

Arnold, S. J. & Wade, M. J. 1984b. On the measurement of natural and sexual selection: applications. *Evolution*, **38**, 720-734.

Bateman, A. J. 1948. Intra-sexual selection in drosophila. *Heredity*, **2**, 349-368.

Boon, A. K., Reale, D. & Boutin, S. 2007. The interaction between personality, offspring fitness and food abundance in North American red squirrels. *Ecology Letters*, **10**, 1094-1104.

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

Boutin, S., Wauters, L. A., McAdam, A. G., Humphries, M. M., Tosi, G. & Dhondt, A. A. 2006. Anticipatory reproduction and population growth in seed predators. *Science*, **314**, 1928-1930.

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1998. Multilocus and single-locus DNA fingerprinting. In: *Molecular Genetic Analysis of Populations: a Practical Approach* (Ed. by Hoelzel, A. R.), pp. 225-269. Oxford: IRL Press.

Clutton-Brock, T. H., Albon, S. D., Gibson, R. M. & Guinness, F. E. 1979. The logical stag: adaptive aspects of fighting in red deer (*Cervus elaphus L.*). *Animal Behaviour*, **27**, 211-225.

Clutton-Brock, T. H., Guinness, F. E. & Albon, S. D. 1982. *Red Deer: Behaviour and Ecology of Two Sexes*. Chicago: University of Chicago Press.

Clutton-Brock, T. H. 1988. *Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems*. Chicago: University of Chicago Press.

Coltman, D. W., Festa-Bianchet, M., Jorgenson, J. T. & Strobeck, C. 2002. Age-dependent sexual selection in bighorn rams. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **269**, 165-172.

Crow, J. F. 1958. Some possibilities for measuring selection intensities in man. *American Anthropologist*, **60**, 1-13.

Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex.* Murray: London.

Edelman, A. J. & Koprowski, J. L. 2006. Seasonal changes in home ranges of Abert's squirrels: Impact of mating season. *Canadian Journal of Zoology*, **84**, 404-411.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and evolution of mating systems. *Science*, **197**, 215-223.

Erlinge, S. & Sandell, M. 1986. Seasonal changes in the social organization of male stoats, *Mustela erminea*: an effect of shifts between 2 decisive resources. *Oikos*, **47**, 57-62.

Farentinos, R. C. 1972. Social dominance and mating activity in tassel-eared squirrel (*Sciurus aberti ferreus*). *Animal Behaviour*, **20**, 316-326.

Foltz, D. W. & Schwagmeyer, P. L. 1989. Sperm competition in the 13-lined ground-squirrel: differential fertilization success under field conditions. *American Naturalist*, **133**, 257-265.

Gaulin, S. J. C. & Fitzgerald, R. W. 1986. Sex differences in spatial ability: an evolutionary hypothesis and test. *American Naturalist*, **127**, 74-88.

Gaulin, S. J. C. & Fitzgerald, R. W. 1989. Sexual selection for spatial-learning ability. *Animal Behaviour*, **37**, 322-331.

Gerhardt, F. 2005. Food pilfering in larder-hoarding red squirrels (*Tamiasciurus hudsonicus*). *Journal of Mammalogy*, **86**, 108-114.

Gunn, M. R., Dawson, D. A., Leviston, A., Hartnup, K., Davis, C. S., Strobeck, C., Slate, J. & Coltman, D. W. 2005. Isolation of 18 polymorphic microsatellite loci from the North American red squirrel, *Tamiasciurus hudsonicus* (Sciuridae, Rodentia), and their cross-utility in other species. *Molecular Ecology Notes*, **5**, 650-653.

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

Humphries, M. M., Boutin, S., Thomas, D. W., Ryan, J. D., Selman, C., McAdam, A. G., Berteaux, D. & Speakman, J. R. 2005. Expenditure freeze: the metabolic response of small mammals to cold environments. *Ecology Letters*, **8**, 1326-1333.

Ims, R. A. 1988. Spatial clumping of sexually receptive females induces space sharing among male voles. *Nature*, **335**, 541-543.

Joseph, R., Hess, S. & Birecree, E. 1978. Effects of hormone manipulations and exploration on sex differences in maze learning. *Behavioral Biology*, **24**, 364-377.

Kappeler, P. M. 1997. Intrasexual selection in *Mirza coquereli*: evidence for scramble competition polygyny in a solitary primate. *Behavioral Ecology and Sociobiology*, **41**, 115-127.

Kenagy, G. J. & Trombulak, S. C. 1986. Size and function of mammalian testes in relation to body size. *Journal of Mammalogy*, **67**, 1-22.

Kenward, R. 2001. *A Manual for Wildlife Radio Tagging*. London: Academic Press.

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

Koford, R. R. 1982. Mating system of a territorial tree squirrel (*Tamiasciurus douglasii*) in California. *Journal of Mammalogy*, **63**, 274-283.

Koprowski, J. L. 1993. Alternative reproductive tactics in male eastern gray squirrels - making the best of a bad job. *Behavioral Ecology*, **4**, 165-171.

Koprowski, J. L. 2007. Alternative reproductive tactics and strategies of tree squirrels. In: *Rodent Societies: an Ecological & Evolutionary Perspective* (Ed. by Wolff, J. & Sherman, P. W.), pp. 86-95. Chicago: University of Chicago Press.

Krebs, C. J., Boutin, S. A. & Boonstra, R. 2001. *Ecosystem Dynamics of the Boreal Forest: the Kluane Project.* Oxford: Oxford University Press.

Kruuk, L. E. B., Slate, J., Pemberton, J. M., Brotherstone, S., Guinness, F. & Clutton-Brock, T. 2002. Antler size in red deer: heritability and selection but no evolution. *Evolution*, **56**, 1683-1695.

Lacey, E. A., Wieczorek, J. R. & Tucker, P. K. 1997. Male mating behaviour and patterns of sperm precedence in arctic ground squirrels. *Animal Behaviour*, **53**, 767-779.

LaMontagne, J. M. 2007. Spatial and temporal variability in white spruce (*Picea glauca*) cone production: individual and population responses of North American red squirrels (*Tamiasciurus hudsonicus*). Ph.D. Thesis. University of Alberta.

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

Lane, J. E., Boutin, S., Gunn, M. R., Slate, J. & Coltman, D. W. 2007. Genetic relatedness of mates does not predict patterns of parentage in North American red squirrels. *Animal Behaviour*, **74**, 611-619.

Lane, J. E., Boutin, S., Gunn, M. R., Slate, J. & Coltman, D. W. In Press. Female multiple mating and paternity in free-ranging North American red squirrels. *Animal Behaviour*.

Leboeuf, B. J. 1974. Male-male competition and reproductive success in elephant seals. *American Zoologist,* **14**, 163-176.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7, 639-655.

Martin, P. R. & Bateson, P. P. G. 1986. *Measuring Behaviour: an Introductory Guide.* Cambridge: Cambridge University Press.

Maynard Smith, J. 1985. Sexual selection, handicaps and true fitness. *Journal of Theoretical Biology*, **115**, 1-8.

McAdam, A. G. & Boutin, S. 2003. Variation in viability selection among cohorts of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Evolution*, **57**, 1689-1697.

McAdam, A. G., Boutin, S., Sykes, A. K. & Humphries, M. M. 2007. Life histories of female red squirrels and their contributions to population growth and lifetime fitness. *Ecoscience*, **14**, 362-369.

McNemar, Q. & Stone, C. P. 1932. Sex difference in rats on three learning tasks. *Journal of Comparative Psychology*, **14**, 171-180.

Moya-Larano, J., El-Sayyid, M. E. T. & Fox, C. W. 2007. Smaller beetles are better scramble competitors at cooler temperatures. *Biology Letters*, **3**, 475-478.

Murphy, C. G. 1998. Interaction-independent sexual selection and the mechanisms of sexual selection. *Evolution*, **52**, 8-18.

Neff, B. D. & Pitcher, T. E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19-38.

Nussey, D. H., Coltman, D. W., Coulson, T., Kruuk, L. E. B., Donald, A., Morris, S. J., Clutton-Brocks, T. H. & Pemberton, J. 2005. Rapidly declining fine-scale spatial genetic structure in female red deer. *Molecular Ecology*, **14**, 3395-3405.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelstrom, H. 1996. Sperm selection by females. *Nature*, **383**, 585-585.

Preston, B. T., Stevenson, I. R., Pemberton, J. M. & Wilson, K. 2001. Dominant rams lose out by sperm depletion *Nature*, **409**, 681-682.

Price, K., Boutin, S. & Ydenberg, R. 1990. Intensity of territorial defense in red squirrels: an experimental test of the asymmetric war of attrition. *Behavioral Ecology and Sociobiology*, **27**, 217-222.

Rank, N. E., Yturralde, K. & Dahlhoff, E. P. 2006. Role of contests in the scramble competition mating system of a leaf beetle. *Journal of Insect Behavior*, **19**, 699-716.

Réale, D., Berteaux, D., McAdam, A. G. & Boutin, S. 2003. Lifetime selection on heritable life-history traits in a natural population of red squirrels. *Evolution*, **57**, 2416-2423.

Rusch, D. A. & Reeder, W. G. 1978. Population ecology of Alberta red squirrels. *Ecology*, **59**, 400-420.

Sandell, M. & Liberg, O. 1992. Roamers and stayers: a model on male mating tactics and mating systems. *American Naturalist*, **139**, 177-189.

Schulte-Hostedde, A. I. & Millar, J. S. 2004. Intraspecific variation of testis size and sperm length in the yellow-pine chipmunk (*Tamias amoenus*): implications for sperm competition and reproductive success. *Behavioral Ecology and Sociobiology*, **55**, 272-277.

Schwagmeyer, P. L. & Woontner, S. J. 1986. Scramble competition polygyny in 13-lined ground-squirrels: the relative contributions of overt conflict and competitive mate searching. *Behavioral Ecology and Sociobiology*, **19**, 359-364.

Schwagmeyer, P. L. & Parker, G. A. 1987. Queuing for mates in 13-lined ground-squirrels. *Animal Behaviour*, **35**, 1015-1025.

Schwagmeyer, P. L., Parker, G. A. & Mock, D. W. 1998. Information asymmetries among males: implications for fertilization success in the thirteen-lined ground squirrel. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 1861-1865.

Smith, C. C. 1968. Adaptive nature of social organization in genus of tree squirrels *Tamiasciurus*. *Ecological Monographs*, **38**, 31-&.

Spritzer, M. D., Meikle, D. B. & Solomon, N. G. 2005a. Female choice based on male spatial ability and aggressiveness among meadow voles. *Animal Behaviour*, **69**, 1121-1130.

Spritzer, M. D., Solomon, N. G. & Meikle, D. B. 2005b. Influence of scramble competition for mates upon the spatial ability of male meadow voles. *Animal Behaviour*, **69**, 375-386.

Stockley, P., Searle, J. B., Macdonald, D. W. & Jones, C. S. 1994. Alternative reproductive tactics in male common shrews - relationships between mate-searching behavior, sperm production, and reproductive success as revealed by DNA-fingerprinting. *Behavioral Ecology and Sociobiology*, **34**, 71-78.

Stuart-Smith, A. K. & Boutin, S. 1995. Behavioral differences between surviving and depredated juvenile red squirrels. *Ecoscience*, **2**, 34-40.

Sugg, D. W. & Chesser, R. K. 1994. Effective population sizes with multiple paternity. *Genetics*, **137**, 1147-1155.

Sztatecsny, M., Jehle, R., Burke, T. & Hoedl, W. 2006. Female polyandry under male harassment: the case of the common toad (*Bufo bufo*). *Journal of Zoology*, **270**, 517-522.

Thompson, D. C. 1977. Diurnal and seasonal activity of grey squirrel (*Sciurus carolinensis*). *Canadian Journal of Zoology*, **55**, 1185-1189.

Thornhill, R. & Alcock, J. 1983. *The Evolution of Insect Mating Systems*. Cambridge: Harvard University Press.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man, 1871-1971* (Ed. by Campbell, B. G.), pp. 136-179. Chicago: Aldine

Waterman, J. M. 1998. Mating tactics of male cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Animal Behaviour*, **56**, 459-466.

Wauters, L., Dhondt, A. A. & Devos, R. 1990. Factors affecting male mating success in red squirrels (*Sciurus vulgaris*). *Ethology Ecology & Evolution*, **2**, 195-204.

Wells, K. D. 1977. Social behavior of anuran amphibians. *Animal Behaviour*, **25**, 666-693.

White, G. C. & Garrott, R. A. 1990. *Analysis of Wildlife Radio-tracking Data*. San Diego: Academic Press.

Table 4.1. Standardized linear, quadratic and correlational selection gradients (± S.E.) for male searching effort ($\log_{10}$(home range size) and ability (number of estrous females located) based on both male mating success (number of estrous females mated with) and reproductive success (number of offspring sired). Boldface gradients were significantly different from zero based on S.E. generated by jackknifing after sequential Bonferroni correction. Sample sizes are included in parentheses.

Success measurement	Linear selection gradient (β'_i)		Quadratic selection gradient (γ'_{ii})		Correlation selection gradient (γ'_{ij})			
	Effort	Ability	Effort	Ability	Effort x Mass	Effort X Testis	Ability X Mass	Ability X Testis
Mating success	**0.336 ± 0.072** (145)	**0.671 ± 0.033** (162)	0.095 ± 0.037 (145)	0.066 ± 0.029 (162)	0.288 ± 0.168 (145)	N.A.	**0.210 ± 0.036** (162)	N.A.
Reproductive success	**0.582 ± 0.149** (80)	**0.450 ± 0.161** (88)	0.168 ± 0.158 (80)	0.111 ± 0.251 (88)	0.801 ± 0.406 (80)	0.958 ± 0.519 (80)	0.772 ± 0.392 (88)	0.787 ± 0.539 (88)

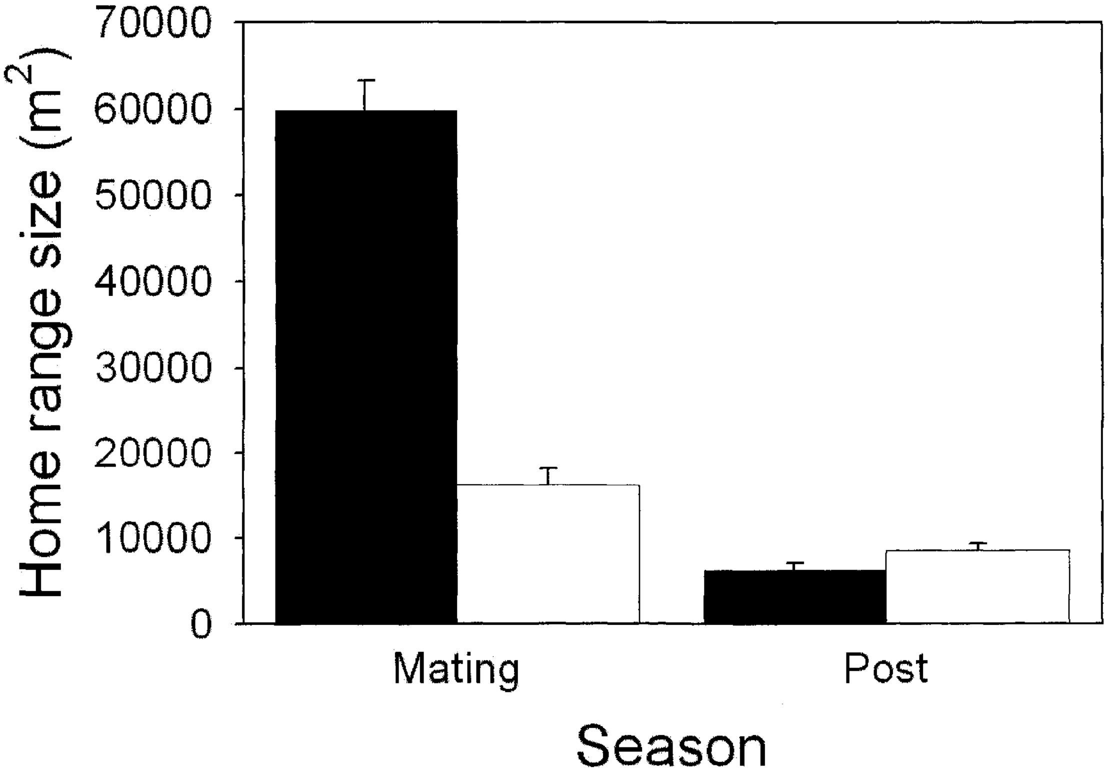

Figure 4.1. Home range sizes, calculated as 100 % minimum convex polygons, of male (black bars) and female (white bars) North American red squirrels measured during the mating (male: *N* = 177; female: *N* = 172) and non- (post-) mating (male: *N* = 147; female: *N* = 158) seasons.

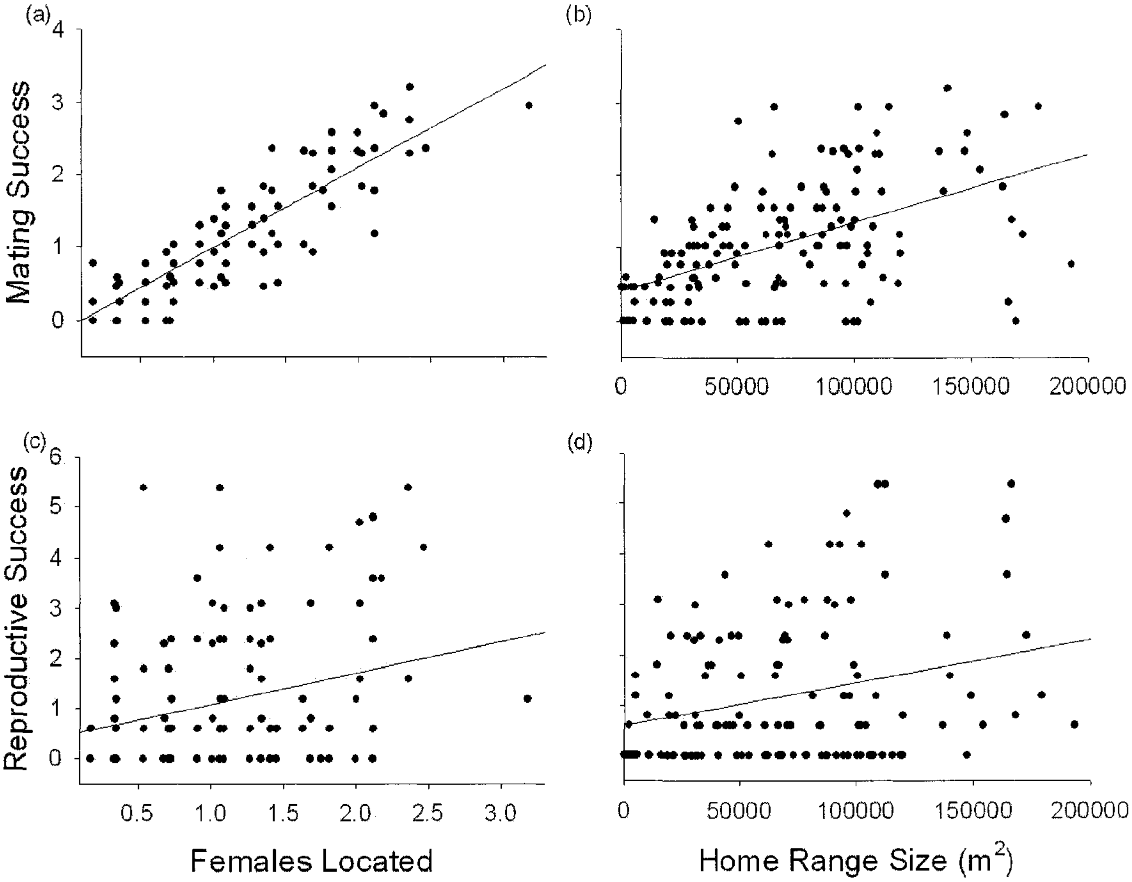

Figure 4.2. Relative mating success (panels (a), N = 106; (b) N = 98) and reproductive success (panels (c), N = 91; (d) N = 88) of male North American red squirrels as a function of the search ability (relative numbers of females located over the mating season; panels (a), (c)) and search effort ($\log_{10}$-transformed home range size); panels (b), (d)). Relative mating success, reproductive success and searching success were calculated as the number of estrous females copulated with, offspring sired, and estrous females located, respectively, divided by the annual population average. Home range sizes were calculated as 100% minimum convex polygons.

CHAPTER 5. BREEDING SEASON ENERGETICS OF MALE NORTH AMERICAN RED SQUIRRELS.

5.1 INTRODUCTION

Life history theory predicts that energy invested in current reproduction comes at the expense of survivorship and reproduction in subsequent years (Lack 1966; Williams 1966; Williams et al. 2006). Quantifying this energetic investment then allows for an understanding of the physiological mechanisms underlying observed life history patterns. Energetic costs to reproduction stem from two major components: energy invested in mating and energy invested in raising offspring (Trivers 1972; Michener & Locklear 1990). In most mammalian species, females provide the majority of parental care while males compete for access to reproductive females (Clutton-Brock 1988; Clutton-Brock 1991). Energy investment is thus unequally distributed between the two components, and the weighting of each component is opposite between males and females. Females allocate large amounts of energy to parental care and the energetic costs of gestation and lactation are well documented for a range of mammals (e.g. Loudon & Racey 1987; Sikes 1995; Huber et al. 1999). However, the corresponding costs to males of attracting and competing for females have remained relatively understudied (but see Salsbury & Armitage 1995; Coltman et al. 1998; Galimberti et al. 2007). Further, many of the energetic assessments (on both males and females) have inferred the energetic demands based on measures such as mass changes (e.g. Michener & Locklear 1990; Millesi et al. 1998) or food consumption (Sikes 1995), rather than directly measuring the energetic throughput of individuals (but see Humphries & Boutin 2000).

While estimates of mass loss during the breeding season can reasonably approximate energetic investment to reproduction in capital breeders (e.g. Deutsch et al. 1990; Galimberti et al. 2007), this technique is more limited for income breeders. Field metabolic rate (FMR), by contrast, is a direct measure of the energy expenditure of animals under natural conditions (Speakman 2000) and reflects both the amount of energy that animals need to acquire from the environment and that which is allocated to maintenance, growth and reproduction. The doubly labeled water technique (DLW; Nagy 1987; Speakman 1997), which estimates the carbon dioxide (CO_2) production of animals based on the differential washout of injected hydrogen (^2H) and oxygen (^{18}O) isotopes, has allowed for the measurement of FMR for a range of species and environments (reviewed in Nagy et al. 1999; Speakman 2000; Anderson & Jetz 2005; Nagy 2005) and provides the opportunity to measure energetic investment to reproduction across varying breeding strategies (including capital and income) and mating systems.

I employed the DLW technique to measure the breeding season energetics of free-ranging male North American red squirrels (*Tamiasciurus hudsonicus*; hereafter red squirrel), encountering two different potential fitness payoffs and across two different food levels. Specifically, I conducted three parallel studies to investigate the energy expenditure of males at both the population and individual level. First, I measured the daily energy expenditure, as FMR, of males across two contrasted years. One breeding season (2004) preceded a food shortage while the other (2005) preceded a year of superabundant food resources. Second, I determined whether males are limited in energy expenditure by current food stores by providing supplemental food to all individuals on one study grid. Finally, I investigated the interrelationship among behavioural reproductive effort, energy expenditure and reproductive success for individual males.

Red squirrels are conifer cone specialists, and the presence of a single tree species at my study site (white spruce, *Picea glauca*) results in extreme annual variability in food production (McAdam & Boutin 2003; Boutin et al. 2006). Years of superabundant food (mast crops) are interspersed with several (3-7) years of reduced food production (LaMontagne & Boutin 2007) and the density of squirrels closely tracks food abundance (S. Boutin, unpublished data). Individuals reproducing in breeding seasons preceding mast crops, consequently, have few competitors and high levels of food available to offspring, resulting in high over-winter survivorship of juveniles (McAdam & Boutin 2003). Female red squirrels have recently been shown capable of anticipating the upcoming food crop and observed life history patterns have seemingly evolved to optimize fitness in the context of fluctuating food resources. In breeding seasons preceding mast crops, litter sizes increase, females attempt second litters following successful first attempts and greater proportions of yearling females reproduce (Boutin et al. 2006). I predict that males will similarly attempt to optimize fitness by increasing energetic investment to reproduction in a year preceding a mast crop.

Although the potential fitness payoffs to males in breeding seasons preceding mast crops are high, the energy available for reproductive allocation is highest in the year following the mast crop (Smith 1968; McAdam & Boutin 2003). Mast crops typically follow multiple years of food shortage and males are likely to have expended most, or all, of their food caches (Rusch & Reeder 1978). To what extent males can take advantage of the fitness opportunities in breeding seasons preceding mast crops is, consequently, not known. I tested to what extent males are limited by current food availability by providing mast crop level food conditions on one study grid. Specifically, *ad libitum* food was provided throughout the winter and breeding season to all individuals and energy expenditure of these individuals was compared to those measured on control grids.

Investigations of energetic expenditure during reproduction in males have rarely incorporated individual comparisons, and of those that have, almost all have studied pinnipeds (e.g. Anderson & Fedak 1985; Deutsch et al. 1990; Galimberti et al. 2007). For example, southern elephant seals (*Mirounga leonina*) exhibit a harem defence polygynous mating system and males fast for the entire duration of the breeding season (Galimberti et al. 2002; Galimberti et al. 2007) Over this period males lose, on average, 25% of their initial body masses. Further, enhanced reproductive effort results in gains in both mating and reproductive success but comes at an energetic cost as mass loss increases linearly with effort (Galimberti et al. 2007). It is currently unknown for most other mammalian taxa what the energetic costs to elevated reproductive effort are. Similarly, whether males expending more energy experience elevated mating and/or reproductive success across different mating systems is unknown. I thus sought to determine individual energetic costs to reproductive effort and the consequences, in terms of reproductive success, to increased energy expenditure in male red squirrels.

Red squirrels exhibit a scramble-competition mating system in which both mating and reproductive success are limited by search effort (Chapter 4). What variables limit searching effort, however, are currently unknown. The relatively long duration of the breeding season, competition for mates, and the high thermoregulatory costs incurred by breeding during the winter (Pauls 1981) should cause this period to be energetically demanding on males. I thus hypothesized that searching effort is energetically costly, and male reproductive success is limited by energy expenditure. Specifically, I predict that search effort will correlate positively with daily energy expenditure and individuals expending greater amounts of energy during the breeding season will experience elevated reproductive success.

5.2 METHODS

5.2.1 Study Area and Population

I studied a free-ranging population of red squirrels resident on three study grids (two 40 ha (KL and SU) and one 60 ha (AG)) near Kluane National Park in the southwest Yukon (61° N, 138° W) in 2004 and 2005. Details of the study landscape (Krebs et al. 1995) and population (e.g. Humphries & Boutin 2000; Boutin et al. 2006; McAdam et al. 2007) have been reported previously. Briefly, both males and females defend individual food-based territories, centered on a food cache (midden). I captured individuals in live traps (Tomahawk Live Trap, Tomahawk, Wisconsin) placed on, or in the immediate vicinity of their middens. All individuals born into the study population were originally handled in their natal nest, and had there received unique alphanumeric ear tags. Ages of such focal males ($N = 32$) were known with certainty. Any immigrating adults received eartags on first capture, and for these focal males ($N = 10$), I treat age as a minimum estimate (i.e. age = 1 in year of first capture). Each individual was also given a unique combination of one or two coloured wires, threaded through their eartags, on first capture of the season to allow for identification from a distance. The Biosciences Animal Policy and Welfare

Committee at the University of Alberta approved all protocols for the capture and handling of red squirrels.

5.2.2 Food Abundance

Natural food abundance was estimated each year by recording an index of the spruce cones produced, using previously established protocols (e.g. Humphries & Boutin 2000; McAdam & Boutin 2003; Boon et al. 2007). The average number of visible cones on one side of the top 3 m of each of 298 designated trees (N = 85 (KL), N = 87 (SU), N = 126 (AG)) among the study areas was recorded in July of 2003, 2004 and 2005 for the control grids (KL and SU) and 2005 for the food-supplemented grid (AG). Squirrels harvest the available cones in August of each year (Q. E. Fletcher, S. Boutin, J. E. Lane, J. M. LaMontagne, A. G. McAdam & M. M. Humphries, unpublished data) and store them for subsequent consumption in underground tunnels on their middens. Because this index of cone abundance can vary over three orders of magnitude (Humphries & Boutin 2000), I ln-transformed these values prior to analysis.

Supplemental food was provided, in the form of *ad libitum* natural peanut butter (no salt or sugar added), for all individuals on one study grid (AG), beginning in the winter of 2004. Peanut butter is nutritionally similar to spruce cones and 1 kg of peanut butter provides the approximate caloric equivalent of 5000 spruce cones (calculated from Brink & Dean 1990; Nienstaedt & Zasada 1990). Individuals received an elevated feeder, centered over their midden and containing 1 kg of peanut butter. Peanut butter was replaced, prior to depletion, throughout the winter (October-May), ensuring constant food availability.

5.2.3 Reproductive Effort

I quantified behavioural reproductive (searching) effort of males on the two control study grids (KL and SU) in both 2004 and 2005, as their breeding season home range size (N = 22 (2004), 13 (2005)). Study grids were staked and flagged at 15 or 30 m intervals to provide spatial references for all location data and I compiled three types of spatial data for home-range analyses. I recorded the trap locations of individuals and noted the location of any individuals seen opportunistically (for example, during regular trapping rounds). Individuals were also outfitted with radio-collars (model PD-2C, 4 g, Holohil Systems Limited, Carp, Ontario) and I used radio-telemetry to augment my spatial data. Specifically, I conducted behavioural focals (recording spatial locations every 30 s for 10 min). Red squirrels become accustomed to human observers rapidly, following a brief period of acclimation (Smith 1968). This population has been the focus of research for 20 years, and behavioural focals have been employed since its inception (e.g. Stuart-Smith & Boutin 1995; Humphries & Boutin 2000; Lane et al. In Press). I am thus confident that my presence did not affect natural ranging behaviour of observed individuals.

Home range sizes were estimated with minimum convex polygons (MCP). While 95% MCPs are traditionally used for home range analyses (White & Garrott 1990) and are less vulnerable to being confounded by the number of data points used in the calculation, off-territory excursions, most relevant for my analyses, are largely excluded from these calculations. I thus retained all data and calculated 100% MCPs. To cope with its potential confounding influence, I included the number of data points (range: 7-485) used as a covariate in all analyses, following Kenward (2001).

5.2.4 Reproductive Success

I quantified both mating and reproductive success of sampled males on control study grids in both 2004 and 2005. Details of the mating season for this population, and observations employed, are provided elsewhere (Lane et al. 2007, Lane et al. In Press). Briefly, mating usually commences in mid-late winter (Lane et al. 2007; S. Boutin, unpublished data) with selection favouring early breeding by females (Réale et al. 2003). Females typically produce a single litter, each year, after a 35-day gestation period, but will occasionally attempt a second litter after litter loss and, rarely, following a successful litter (Boutin et al. 2006). Behavioural estrus commences with the receptive female emerging from the nest in the morning and continues until the female retires to the nest in the evening, thereby encompassing the full activity period for one day. All resident females were monitored daily for reproductive activity in 2004 and 2005 and I monitored 39 mating chases (N = 23 (2004), 16 (2005)) through to completion.

For focal mating chases, I employed a combination of focal-animal sampling of the estrous female and all-occurrence sampling of mating behavior (Altmann 1974; Martin & Bateson 1986), which allowed me to identify copulating males. Because a large number of copulations occur underground, I judged these copulations as occurring when a female was followed underground by a male and the pair remained there for a minimum of 60 s or if copulatory vocalizations could be heard, regardless of the time spent underground. Copulations of tree squirrels generally last less than 60 s (Koprowski 2007) and this duration 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. 2007) and other (Waterman 1998) systems. I quantified the mating success of males as the number of females copulated with during each breeding season. Because the number of mating chases followed varied over the two years, I standardized each male's value by dividing by the population mean (described in Chapter 4).

I quantified reproductive success of males as the number of offspring they sired divided by the annual population mean, as described in Chapter 4. Details of the molecular methods for microsatellite loci isolation and paternity assignment have been provided elsewhere (Gunn et al. 2005; Lane et al. In Press). Briefly, DNA was extracted from preserved tissue using either an acetate-alcohol precipitation protocol (Bruford et al. 1998) or DNeasy Tissue extraction kits (Qiagen, Venlo, The Netherlands), and polymerase chain reaction (PCR) amplification was performed for a panel of 16 microsatellite loci (for details of microsatellite loci used, including the number, and size range of alleles at each locus, as well as observed (H_O) and expected (H_E) heterozygosities see Lane et al. (2007)). Maternity was determined by behavioural observation at the nest and paternity was assigned at 95% confidence using CERVUS 2.0 (Marshall et al. 1998). Separate analyses were conducted for each of the two years.

5.2.5 Energy Expenditure

I measured the breeding season daily energy expenditure (DEE), as FMR, of 22 males in 2004 and 27 males in 2005 ($N = 15$ (control), $N = 12$ (food-add)) using the DLW technique (Speakman 1997). Seven of these males were measured in both years. Squirrels were captured, weighed and injected intraperitoneally with 0.5 mL of DLW [10% APE enriched ^{18}O water (Enritech, Rehovot, Israel) and 99% and ^{2}H (MSD Isotopes, Pointe-Claire, Québec, Canada) mixed in a ratio of 20:1]. Squirrels were held in the trap for a 60 min equilibration period to allow the isotopes to equilibrate in the body and then bled via a clipped toenail to obtain initial blood samples for isotope analysis. Squirrels were then released at the site of capture and recaptured, weighed and bled 3-5 days later, within a range of 0.03-8.28 h of the 24 h interval (25th percentile = 0.53 h, median = 1.32 h, 75th percentile = 2.63 h). Analysis of the isotope concentrations in the blood was conducted according the methods described in Ergon et al. (2004). I estimated CO_2 production using the single pool equation of Speakman (1997; eqn 7.17) and converted it to FMR ($kJ \cdot day^{-1}$) assuming a respiratory quotient (RQ) of 0.8. Both FMR and body mass values were subsequently $\log_{10}$-transformed prior to analysis. This procedure has been successfully used previously on this study population (Humphries and Boutin 2000; Humphries et al. 2005). The animal use committee at McGill University approved all protocols for the measurement of FMR in red squirrels.

An index of ambient temperature (T_a) during all FMR sampling intervals was obtained by calculating the mean daily temperature recordings between each individual's initial and final blood sample, as recorded by a nearby weather station in Haines Junction, Yukon (Haines Junction Environment Canada Station, http://climate.weatheroffice.ec.gc.ca; 60° N, 137° W; ca. 25 km from the study site).

5.2.6 Statistical Analyses

I used a general linear model (LM) with a three-way interaction term (year*age*searching effort) to investigate the combined and separate effects of these variables on daily expenditure of males during the breeding season. Year was incorporated as a two-level (2004 and 2005) categorical variable and age class was incorporated as a three-level categorical variable (yearling, mature (2-4 years old), and old ($\geq$ 5 years old)). Searching effort, represented as the $\log_{10}$-transformed home range size, was incorporated as a continuous variable. I also included T_a, and $\log_{10}$-transformed body mass as covariates. To analyze the within individual variation in DEE across the two years I used a paired-sample ttest. This analysis was restricted to control males sampled in both years.

I used a LM to determine whether the DEEs of males on the food-supplemented grid differed from those of males on the control grids in 2005. A complicating factor in this analysis is that food-supplemented females breed earlier than control females (Kerr et al. 2007). I thus attempted to pair DEE measurements by reproductive chronology rather than Julian date, and measured males on AG (median date = 30-Mar) earlier than KL and SU (median date = 15-Apr). Ambient temperature was included as covariate in this analysis to control for temperatures being warmer later in the season. Log_{10}-transformed body mass was also included as a covariate in the analysis and the food treatment*age class interaction was included to investigate whether the effects of food vary across age classes.

I used two LMs to determine whether DEE explained variation in either mating or reproductive success of males. In addition, to determine whether its potential effects are dependent on age class and/or year, I included the three-way interaction term (year*age class*DEE). Year and age class were incorporated as two- and three-level categorical variables, respectively. Relative mating success and reproductive success represented the independent variables in the two models. I also included T_a, and $\log_{10}$-transformed body mass as covariates.

5.3 RESULTS

The mean DEE of males, across the two years, was 386.10 ± 49.63 kJ•day^{-1}. The breeding season in 2005 preceded a mast crop on control grids (Fig. 5.1), with high over-winter survivorship of juveniles (S. Boutin, unpublished data) and, consequently, potential fitness to reproductive males. By contrast, the breeding season in 2004 preceded a cone crop failure on control grids, with low juvenile over-winter survivorship and potential fitness to males. Males appeared to anticipate the upcoming cone crop and elevated DEE accordingly (Fig. 5.2). While energy expenditure was relatively low (277.30 ± 6.78 kJ•day^{-1}) in 2004, mean values almost doubled in 2005 to 545.67 ± 69.32 kJ•day^{-1}. In 2004 males expended energy at approximately 2.2 times resting metabolic rate (RMR; i.e., of quiescent individuals recorded within their thermo-neutral zone; Pauls 1981). By contrast, in 2005 male FMR was 4.3 times RMR. The duration of the mating season was also longer in 2005, further exaggerating the variation in total breeding season energy expenditure. In 2004 the breeding season extended from 5-Mar to 4-Jun (duration = 91 d) and in 2005 it extended from 21-Mar to 17-Jul (duration = 118 d). Multiplying the breeding season DEE of individuals by the duration of the breeding season reveals that in 2005 male total breeding season energy expenditure was 2.6 times that of 2004.

Daily energy expenditure across the two years also varied according to searching effort and age class (LM: year*age class*searching effort: $F_{1,18} = 11.01$, $P = 0.004$). The significant interaction between year and search effort (and age class) resulted partially from the slope of the search effort to DEE relationship being much greater in 2005 than

2004. In 2004, the slope of search effort and DEE was $\beta = -0.01$, while in 2005 the slope was $\beta = 0.67$, indicating greater energy expenditure in 2005 than was explained by my measure of search effort (Fig. 5.3). The influence of age was non-linear on DEE, with yearling (541.04 ± 95.31 $kJ \bullet day^{-1}$, $N = 10$) and old (>5 years old) males (382.47 ± 60.66 $kJ \bullet day^{-1}$, $N = 11$) experiencing elevated DEE, as compared to mature (2-4 year old) males (291.76 ± 16.8 $kJ \bullet day^{-1}$, $N = 16$). Neither mating success (LM: $F_{1,25} = 1.11$, $P = 0.30$), nor reproductive success (LM: $F_{1,23} = 1.54$, $P = 0.22$), however, was correlated with DEE, and none of the interaction terms were significant in either of these two models (statistics not shown).

The influence of age class is perhaps most clear in the within individual analysis on seven males across the two years (Fig. 5.4). Three of the seven males experienced elevated FMRs in 2005, as compared to 2004. The other four males showed similar or moderate decreases across the two years. The four males that showed similar or moderate decreases were either 3 ($N = 3$) or 4 years old ($N = 1$), while all the males that showed increases were 5 years old or greater ($N = 2$ (5 years old) or $N = 1$ (7 years old)). The mean difference across the two years was not statistically significant (paired sample ttest: $t_6 = 1.34$, $P = 0.23$)

Although a mast-crop occurred on both control grids in 2005, the food-supplemented grid experienced a cone-crop failure (Fig. 5.1). Despite this, food-supplemented males showed significantly higher DEE (840.51 ± 49.63 $kJ \bullet day^{-1}$) than control males (545.67 ± 69.32 $kJ \bullet day^{-1}$) this year (LM: $F_{1,25} = 7.63$, $P = 0.01$; Fig. 5.2)). These values represent 4.3 times (control) and 6.9 times (food supplemented) resting metabolic rates (Pauls 1981; Humphries & Boutin 2000).

5.4 DISCUSSION

The few studies that have investigated breeding season energetics of males have tended to focus on harem defence polygynous mating systems (e.g. Clutton-Brock et al. 1982; Deutsch et al. 1990; Galimberti et al. 2007, but see Michener & Locklear 1990; Salsbury & Armitage 1995). In these mating systems sexual size dimorphism is often exaggerated and the energetic costs to males are often large. The ability of larger males to withstand these energetic costs has been proposed as one explanation for why males can be several times greater in mass than females (Andersson 1994). Here I report a similarly large energy expenditure to reproductive effort in a small mammal. Mating season FMRs of male red squirrels averaged three times RMR, but single year estimates (2005) were over four times RMR, and under *ad libitum* food conditions were almost seven times RMR. By comparison, the FMR of lactating females averages between three and four times RMR (Humphries & Boutin 2000). Red squirrels, however, exhibit minimum size dimorphism, with males being only 5-10% heavier than females (Boutin & Larsen 1993).

Although red squirrels are not size dimorphic, they may exhibit analogous dimorphism in energy availability. Male red squirrels do not fast during the breeding season, but rather rely ingested energy to meet the demands of reproduction. They have thus traditionally been classified income breeders (sensu Drent & Daan 1980). Males do, however, acquire the energy required for reproduction in the autumn preceding the breeding season but, rather than store it as fat, hoard it in their midden (Smith 1968). Further, preliminary analyses on this study population have shown that males begin autumn hoarding earlier, and accumulate larger hoards, than females (Q. E. Fletcher, S.

Boutin, J. E. Lane, J. M. LaMontagne, A. G. McAdam & M. M. Humphries, unpublished data). Males could thus be considered capital breeders with their 'capital' being stored in an external food cache. Consequently, patterns of energy storage and reproductive investment in red squirrels follow theoretical predictions that sexual dimorphism is due to the ability of males to withstand high levels of energy expenditure during the breeding season (sensu Andersson 1994).

Although reproductive energy expenditure can be high in male red squirrels, it is not consistent across years. Under similar current food conditions, DEE almost doubled in the breeding season preceding a mast crop, as compared to the breeding season preceding a food shortage. Energy expenditure differences across 2004 and 2005 are further exaggerated because remating by females resulted in an extended mating season in 2005 (Chapter 4). Males thus appear to facultatively adjust reproductive effort according to potential fitness payoffs. Offspring survival is highest in mast crop years (McAdam & Boutin 2003) and elevated reproduction by females has been shown to incur a cost in the form of a reduced lifespan (Descamps et al. 2006). If males experience similar life history costs, they may allocate relatively little energy to reproduction when the fitness payoffs are low and expend greatly when the payoffs are high.

Males exhibited contrasting patterns of breeding season DEE among age classes. In general, yearlings and males in the oldest age class ($\geq$ 5 years old) expended greater energy than mature males (2-4 years old). In addition, at the individual level, while mature males exhibited similar, or diminished, DEE in 2005, the DEE of older males more than doubled. Thus, the males that are least likely to survive overwinter (old age class), showed an increase in reproductive energy investment, while those that are likely to survive to future breeding seasons (mature aged class) showed no increase, as would be predicted if current investment incurs a cost to future survival and reproduction (Descamps et al. 2007). Yearling males, however, showed similar levels of expenditure as the oldest males. This pattern is less easy to explain, but parallels that seen in females in this population (S. Descamps, S. Boutin, D. Berteaux & J.-M. Gaillard, unpublished data). Both yearling females and those in the oldest age class pay higher costs to reproduction (measured as future survival) than those in the mature age class and the differential costs were attributed to yearling females still growing during their first year of reproduction. If males are similarly still growing the metabolic investment to growth could compound the investment to reproduction.

In the scramble competition mating system of red squirrels, male mating success is primarily limited by search effort and ability as reflected by mating season home range size and the number of estrous females located, respectively (Chapter 4; Schwagmeyer & Woontner 1986). I hypothesized that elevated search effort entails an energetic cost and this, in turn, limits male mating success. Although I was unable to detect an influence of DEE on either mating or reproductive success, my sample size was low for these analysis, and I consequently had little power to detect potential effects. Daily energy expenditure did increase with elevated search effort but this relationship was only observable in the breeding season preceding the mast year. Further, the slopes of the relationship varied between the two years. I suggest, therefore, that reproductive effort does entail an energetic cost, but my search effort index provides only a minimum estimate. Mating season home range size provides a general picture of the area over which males searched for reproductive opportunity. It does not, however, provide an estimate of the intensity of the search effort. For example, males could have exhibited increased effort by making multiple forays to assess the status of neighbouring females that my data does not have the resolution to detect. Future studies quantifying the detailed activity budgets of males, in concert with mating season home range size, should help to clarify the behavioural correlates of increased DEE.

Small-scale spatial variation resulted in a mast crop occurring on the two control grids but not the experimental grid. Despite this, males still elevated DEE to the highest levels I recorded, suggesting that they allocate large amounts of energy to reproduction when not limited by current food availability. My results approximate what would be seen under natural conditions following a mast year. The food supplementation on AG, resulted in both high over-winter survivorship of individuals and energy available for reproduction (S. Boutin, unpublished data). As a result, while both reproductive opportunities (due to a high female density) and energy available for reproduction for males should have been high, competition for these opportunities was potentially intense (due to a high male density). To capitalize on these opportunities males consequently invested greater amounts of energy.

The reproductive success of male mammals has traditionally been assumed limited by reproductive opportunity, while that of females is limited by the energy that they can allocate to reproduction (Bateman 1948; Trivers 1972). As a result, the energetic costs of reproduction in females have been estimated for a variety of species (e.g. Loudon & Racey 1987; Sikes 1995; Huber et al. 1999; Byers et al. 2005), while analyses of the correlates of male reproductive success have tended to focus on morphological traits that attract females or allow males to defend them from competitors (Andersson 1994).

Where investigated, however, the costs of reproductive effort to males are often shown to be similar or more than females (e.g. Michener & Locklear 1990; Galimberti et al. 2007). I have similarly shown that the energetic costs to mating can be high for male red squirrels, surpassing those of lactation for females. Further, males showed evidence of both facultative suppression and external constraint on energy expenditure during the breeding season. Specifically, males adjusted energy expenditure to coincide with seasons of high fitness returns and also exhibited high expenditure when not limited by current food availability. My results, in combination with previous work, call into question the long-held assumption that energetic limitations to males are minimal as compared to females. I emphasize that, to develop a more general picture of the ecological limits to reproduction, future studies address the energetic costs to reproduction for both males and females.

5.5 REFERENCES

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behavior*, **9**, 227-267.

Anderson, S. S. & Fedak, M. A. 1985. Grey seal males: energetic and behavioural links between size and sexual success. *Animal Behaviour*, **33**, 829-838.

Anderson, K. J. & Jetz, W. 2005. The broad-scale ecology of energy expenditure of endotherms. *Ecology Letters*, **8**, 310-318.

Andersson, M. 1994. *Sexual Selection*. Princeton: Princeton University Press.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, **2**, 349-368.

Boon, A. K., Réale, D. & Boutin, S. 2007. The interaction between personality, offspring fitness and food abundance in North American red squirrels. *Ecology Letters*, **10**, 1094-1104.

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

Boutin, S., Wauters, L. A., McAdam, A. G., Humphries, M. M., Tosi, G & Dhondt, A. A. 2006. Anticipatory reproduction and population growth in seed predators. *Science*, **314**, 1928-1930.

Brink, C. H. & and Dean, F. C. 1990. Spruce seed as a food of red squirrels and flying squirrels in interior Alaska. *Journal of Wildlife Management*, **33**, 503-512.

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1998. Multilocus and single-locus DNA fingerprinting. In: *Molecular Genetic Analysis of Populations: A Practical Approach.* (Ed. by A. R. Hoelzel), pp. 225-269. Oxford: IRL Press.

Clutton-Brock, T. H. 1988. *Reproductive success*. Chicago: University of Chicago Press

Clutton-Brock, T.H. 1991. *The Evolution of Parental Care*. Princeton: Princeton University Press.

Clutton-Brock, T. H., Guinness, F. E & Albon S. D. 1982. *Red deer: The behaviour and ecology of two sexes*. Chicago: Chicago University Press.

Coltman, D. W., Bowen, W. D., Iverson, S. J. & Boness, D. J. 1998. The energetics of male reproduction in an aquatically mating pinniped, the harbour seal. *Physiological Zoology*, **71**, 387-399.

Descamps, S., Boutin, S., Berteaux, D. & Gaillard, J.-M. 2006. Best squirrels trade a long life for an early reproduction. *Proceedings of the Royal Society of London, Series B*, **273**, 2369-2374.

Descamps, S., Boutin, S., Berteaux, D. & Gaillard, J.-M. 2007. Female red squirrels fit Williams' hypothesis of increasing reproductive effort with increasing age. *Journal of Animal Ecology*, **76**, 1192-1201.

Deutsch, C. J., Haley, M. P. & Le Boeuf, B. J. 1990. Reproductive effort of male northern elephant seals: estimates from mass loss. *Canadian Journal of Zoology*, 68, 2580-2593.

Drent, R. H. & Daan, S. 1980. The prudent parent: energetic adjustments in avian breeding. *Ardea*, **68**, 225-252.

Ergon, T., Speakman, J. R., Scantlebury, M., Cavanagh, R. & Lambin, X. 2004. Optimal body size and energy expenditure during winter: why are voles smaller in declining populations? *American Naturalist*, **163**, 442-457.

Galimberti, F., Fabiani, A. & Sanvito, S. 2002. Measures of breeding inequality: a case study in southern elephant seals. *Canadian Journal of Zoology*, **80**, 1240-1249.

Galimberti, F., Sanvito, S., Braschi, C. & Boitani, L. 2007. The cost of success: reproductive effort in male southern elephant seals (*Mirounga leonina*). *Behavioral Ecology and Sociobiology*, **62**, 159-171.

Gunn, M. R., Dawson, D. A., Leviston, A., Hartnup, K., Davis, C. S., Strobeck, C., Slate, J. & Coltman, D. W. 2005. Isolation of 18 polymorphic microsatellite loci from the North American red squirrel, *Tamiasciurus hudsonicus* (Sciuridae, Rodentia), and their cross-utility in other species. *Molecular Ecology Notes*, **5**, 650-653.

Huber, S, Millesi, E., Walzl, M., Dittami, J. & Arnold. R. 1999. Reproductive effort and costs of reproduction in female European ground squirrels. *Oecologia*, **121**, 19-24.

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

Humphries, M.M., Boutin, S., Thomas, D. W., Ryan, J. D., Selman, C., McAdam, A. G., Berteaux, D. & Speakman, J. R. 2005. Expenditure freeze: the metabolic response of small mammals to cold environments. *Ecology Letters*, **8**, 1326-1333.

Kenward, R. E. 2001. *A manual for wildlife radio-tagging*. London: Academic Press.

Kerr, T. D., Boutin, S., LaMontagne, J. M., McAdam, A. G. & Humphries, M. M. 2007. Persistent maternal effects on juvenile survival in North American red squirrels. *Biology Letters*, **3**, 289-291.

Koprowski, J. L. 2007. Alternative reproductive tactics and strategies of tree squirrels. In: Rodent Societies: An Ecological and Evolutionary Perspective. (Ed. by J. O. Wolff & P. W. Sherman), pp. 86-95. Chicago: University of Chicago Press.

Krebs, C. J., S. Boutin and R. Boonstra. 2001. *Ecosystem dynamics of the boreal forest: the Kluane project.* New York: Oxford University Press.

Lack, D. 1966. *Population studies of birds*. London: Oxford University Press.

Lane, J. E., Boutin, S., Gunn, M. R., Slate, J. & Coltman, D. W. 2007. Genetic relatedness of mates does not predict patterns of parentage in North American red squirrels. *Animal Behaviour*, **74**, 611-619.

Lane, J. E., Boutin, S., Gunn, M. R., Slate, J. & Coltman, D. W. In Press. Female multiple mating and paternity in free-ranging North American red squirrels. *Animal Behaviour*.

LaMontagne, J. M. & Boutin, S. 2007. Local-scale variability and synchrony in mast seeding of *Picea glauca*. *Journal of Ecology*, **95**, 991-1000.

Loudon, A. S. I. & Racey, P. A. 1987. *Reproductive Energetics in Mammals. Symposia of the Zoological Society of London Number 57.* Oxford: Clarendon Press.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural conditions. *Molecular Ecology*, **7**, 639-655.

Martin, P. & Bateson, P. 1986. *Measuring behavior: an introductory guide.* Cambridge: Cambridge University Press.

McAdam, A. G. & Boutin, S. 2003a. Variation in viability selection among cohorts of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Evolution*, **57**, 1689-1697.

McAdam, A. G., Boutin, S., Sykes, A. & Humphries, M.M. 2007. Life histories of female red squirrels and their contributions to population growth and lifetime fitness. *Écoscience*, **14**, 362-369.

Michener, G. R. & Locklear, L. 1990. Differential costs of reproductive effort for male and female Richardson's ground squirrels. *Ecology*, **71**, 855-868.

Millesi, E., Huber, S., Dittami, J., Hoffmann, I. & Daan, S. 1998. Parameters of mating effort and success in male European ground squirrels, *Spermophilus citellus*. *Ethology*, **104**, 298-313.

Nagy, K. A. 1987. Field metabolic rate and food requirement scaling in mammals and birds. *Ecological Monographs*, **57**, 111-128.

Nagy, K. A. 2005. Field metabolic rate and body size. *Journal of Experimental Biology*, **208**, 1621-1625.

Nagy, K. A., Girard, I. A. & Brown, T. K. 1999. Energetics of free-ranging mammals, reptiles, and birds. *Annual Review of Nutrition*, **19**, 247-277.

Nienstaedt, H. & Zasada, J. C. 1990. *Picea glauca* (Moench) Voss. White spruce. Silvics of North America, vol. 1: Conifers. In: *Agriculture Handbook* 64. (Ed. by R. M. Burns & B. H. Honkala), pp. 204-226. Washington DC: U.S. Department of Agriculture Forest Service.

Pauls, R.W. 1981. Energetics of the red squirrel: a laboratory study of the effects of temperature, seasonal acclimatization, use of the nest and exercise. *Journal of Thermal Biology*, **6**, 79-86.

Réale, D., Berteaux, D., McAdam, A. G. & Boutin, S. 2003. Lifetime selection on heritable life-history traits in a natural population of red squirrels. *Evolution*, **57**, 2416-2423.

Rusch, D. A. & Reeder, W. G. 1978. Population ecology of Alberta red squirrels. *Ecology*, **59**, 400-420.

Salsbury, C. M. & Armitage, K. M. 1995. Reproductive energetics of adult male yellow-bellied marmots (*Marmota flaviventris*). *Canadian Journal of Zoology*, **73**, 1791-1797.

Schwagmeyer, P. L. & Woontner, S. J. 1986. Scramble competition polygyny in thirteen-lined ground squirrels: the relative contributions of overt conflict and competitive mate searching. *Behavioral Ecology Sociobiology*, **19**, 359-364.

Sikes, R.S. 1995. Costs of lactation and optimal litter size in northern grasshopper mice (*Onychomys leucogaster*). *Journal of Mammalogy*, **76**, 348-357.

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

Speakman, J. R. 1997. Doubly labeled water: theory and practice. London: Chapman and Hall.

Speakman, J. R. 2000. The cost of living: field metabolic rates of small mammals. *Advances in Ecological Research*, **30**, 177-297.

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

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871-1971* (Ed. by B. Campbell), pp. 136-179. Chicago: Aldine.

Waterman, J. M. 1998. Mating tactics of male Cape ground squirrels, *Xerus inauris*: consequences of year-round breeding. *Animal Behaviour*, **56**, 459-466.

White, G. C. & Garrott, R. A. 1990. *Analysis of wildlife radio-tracking data.* San Diego: Academic Press.

Williams, G. C. 1966. Natural selection, the costs of reproduction and a refinement of Lack's principle. *American Naturalist*, **100**, 687-690.

Williams, P. D., Day, T., Fletcher, Q. E. & Rowe, L. 2006. The shaping of senescence in the wild. *Trends in Ecology and Evolution*, **8**, 458-463.

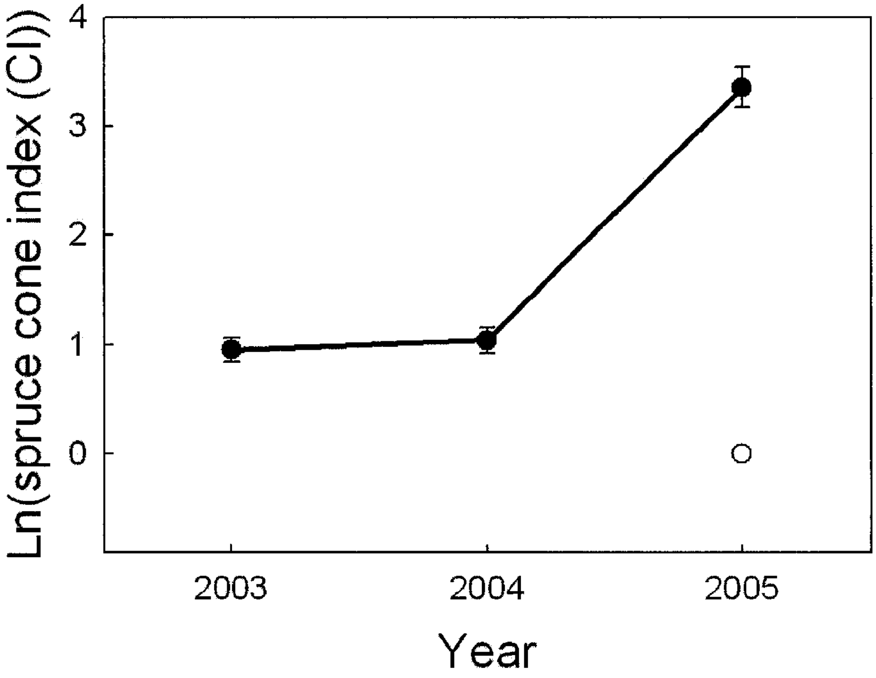

Figure 5.1. Food abundance on control and study grids. Energy available for current reproduction is produced in the previous year. Values represent the ln-transformed index of average spruce cone production. The solid line (closed circles) is the average production for two control grids (KL and SU; *N* = 172 trees), and the open circle is the production on one experimental grid (AG; *N* = 126 trees).

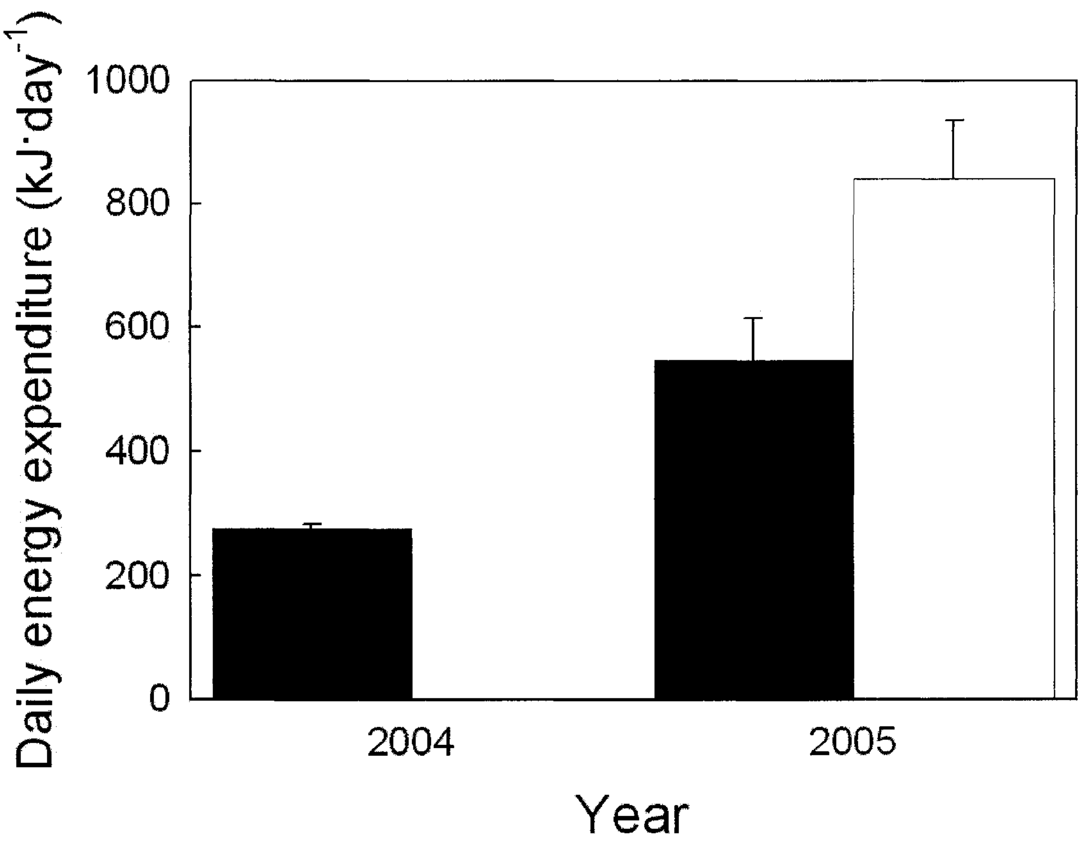

Figure 5.2. Mating season daily energy expenditure, measured as field metabolic rate (FMR), of male North American red squirrels across two years and two food conditions. The mating season of 2004 preceded a food shortage, while the 2005 season preceded a mast crop of superabundant food. Food supplemented squirrels (white bar; *N* = 12) received *ad libitum* natural peanut butter throughout the winter and mating season and control squirrels (black bars; *N* = 22 (2004), *N* = 15 (2005)) experienced natural food conditions.

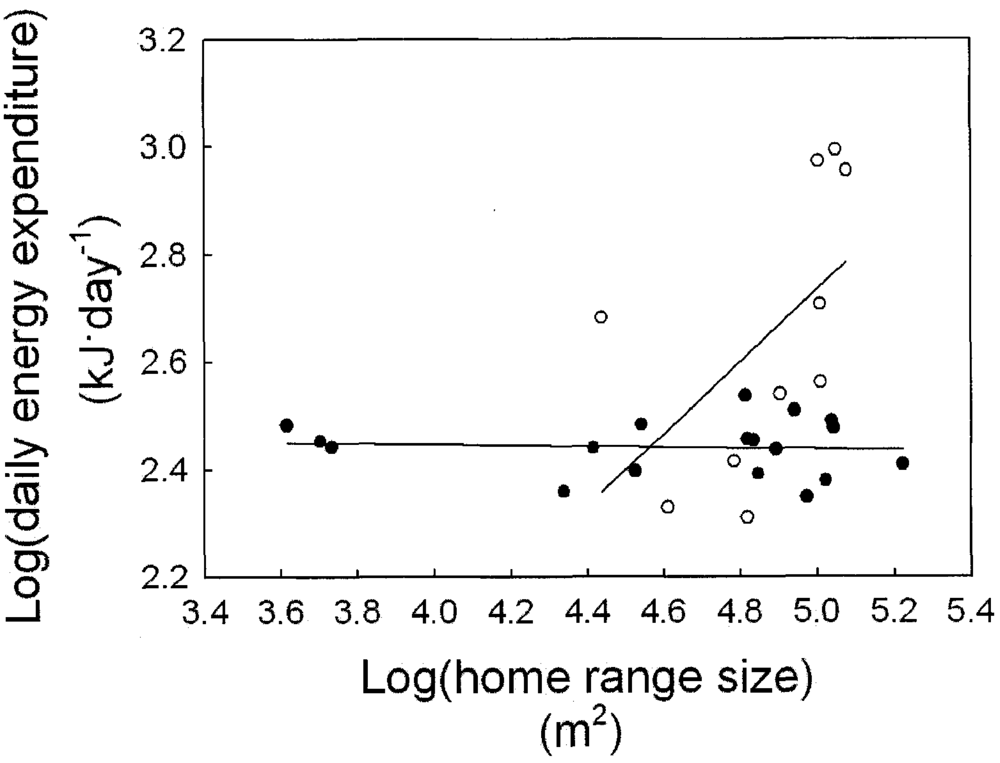

Figure 5.3. The influence of search effort on daily energy expenditure, measured as field metabolic rate (FMR) of male North American red squirrels in both 2004 (closed circles; *N* = 19) and 2005 (open circles; *N* = 11). Search effort was measured as $\log_{10}$-transformed home range size during the mating season.

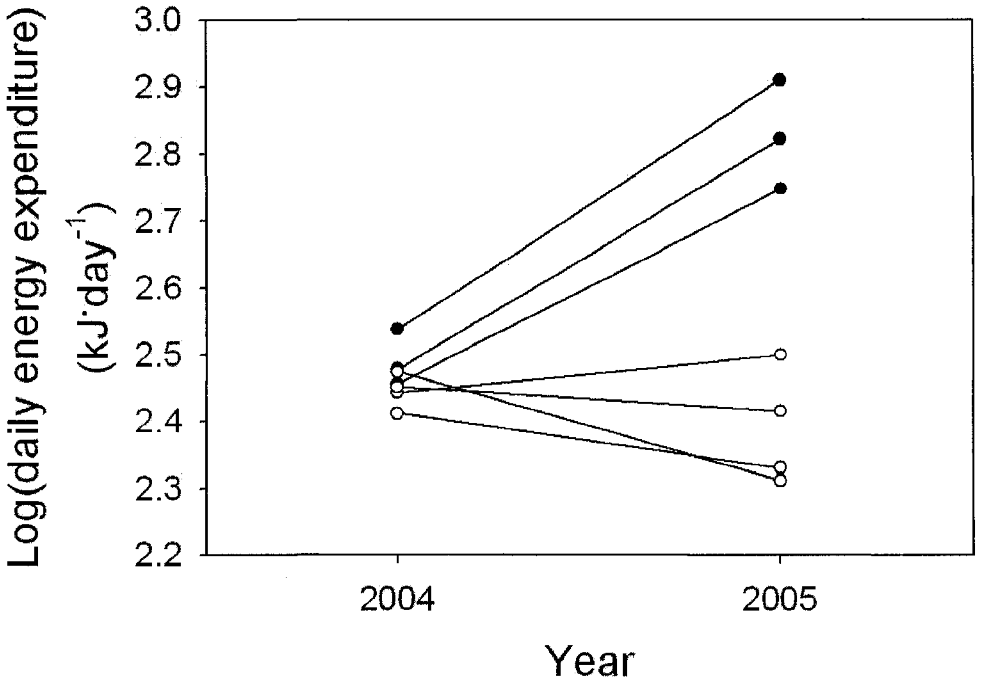

Figure 5.4. Mating season daily energy expenditure, measured as field metabolic rate (FMR), of seven male North American red squirrels that were measured in both 2004 and 2005. The top three lines (closed circles) are males in the oldest age class (> 5 year old), and the bottom four lines (open circles) are males in the mature age class (2-4 years old).

CHAPTER 6. GENERAL DISCUSSION AND CONCLUSIONS

Sexual selection theory predicts that the sex with the greatest potential rate of reproduction should compete for access to the limiting sex (Trivers 1972; Clutton-Brock & Vincent 1991; Andersson 1994). In the majority of mammalian species, females exclusively provide parental care, thereby limiting their potential rate of reproduction (Clutton-Brock 1991). The investment of males, by contrast, typically does not extend beyond their genetic contribution. The reproductive success of males should thus be limited by reproductive opportunity, while that of females should be limited by the resources available for allocation to offspring (Bateman 1948; Trivers 1972). This discrepancy is thought to be responsible for the development of exaggerated morphological dimorphism seen in many mammalian species, which allows males both to compete for access to females, and also to withstand the energetic costs of intense competition (Clutton-Brock et al., 1982; Andersson 1994; Kruuk et al., 2002).

North American red squirrels follow theoretical predictions for mammalian species. Males do not provide paternal care and the maternal care (including gestation and lactation) of females extends for over 100 d (Berteaux & Boutin 2000; Humphries & Boutin 2000). Females are hence limited in their potential rate of reproduction and males compete intensely for limited reproductive opportunities. The opportunity for sexual selection on males was consequently 3-fold larger than on females for mating success and 10-fold larger than on females for reproductive success. Red squirrels, however, exhibit minimal sexual dimorphism, with males being only 5-10% larger than females (Boutin & Larsen 1993).

6.1 SCRAMBLE COMPETITION

If females are clumped in space and/or come into estrus asynchronously, dominant males should be able to defend reproductive access to groups of females, resulting in a female defence mating system (Ims 1988; Murphy 1998). Under these conditions, male traits that allow them to outcompete rivals (e.g. sexual weaponry, large body size) should be selected for (Andersson 1994; Kruuk et al. 2002). Alternatively, if clumping of estrous bouts or spatial dispersion of females renders them indefensible, a scramble competition mating systems is predicted to predominate (Ims 1988; Sandell & Liberg 1992; Murphy 1998). As female defence is uneconomical under these conditions, sexual selection should favour male traits that allow them to locate and follow receptive females over traits promoting dominance over competitors.

In this study, I provided the first estimates of the strength of sexual selection for male traits in a scramble competition mating system. Although sexual selection favoured behavioural rather than morphological traits, the standardized selection gradients on these behavioural metrics were of equivalent magnitude to previous estimates for secondary sexual characters in female defence mating systems (e.g. Kruuk et al. 2002). Thus, although the traits selected for under scramble competition mating systems are more subtle, the strength of selection on them can be equally strong. Moreover, with slightly different parameters (e.g. first-male sperm precedence; Sherman 1989) the strength of selection on male behavioural traits could even be stronger than I recorded for male red squirrels.

6.2 ECOLOGICAL INFLUENCES

Their diurnal activity cycle and ease of observation have made members of the family Sciuridae amenable for the study of small mammal mating systems (summarized in Murie & Michener 1984; Schwagmeyer 1990; Koprowski 2007). The majority of research, however, has been based on hibernating species (e.g. Sherman 1989; Murie 1995; Michener & McLean 1996). By contrast, red, and other nearctic tree squirrels, rely on hoarded food resources to remain active throughout the winter (Vander Wall 1990; Humphries et al. 2005). Further, in northern coniferous forests red squirrels hoard food in a central food cache (midden), leading to strict individual territoriality (Smith 1968). The influence of these ecological variables is revealed through comparison of my results on red squirrels with those from previous work on ground squirrels.

6.2.1 Mating Season Duration

A fundamental biological difference (hibernation versus euthermia) results in the mating seasons of ground squirrels being compressed into as little as one or two weeks following hibernation (Dobson 1984; Schwagmeyer 1990), whereas the mating seasons of tree squirrels may extend over a period of months (e.g. Koprowski 1993). This temporal difference leads to few estrous females per day and a resultant heavily male biased daily OSR for tree squirrels (Emlen and Oring 1977). Relatively more males thus focus attention on receptive females. Whereas laboratory studies have provided mixed support for the hypothesized benefits to female multiple mating (e.g. Tregenza & Wedell 1998; Wolff & Dunlap 2002), this extrinsic ecological influence of the duration of the mating season was shown to have stronger predictive ability than any hypothesized selective benefit for explaining natural levels of female multiple mating in free-ranging red squirrels.

6.2.2 Territoriality

The prolonged mating season of red squirrels, and resulting temporally dispersed mating opportunities, could lead to the prediction of a female defence mating system, and elements of female defence have been reported, to varying degrees, in other eurthermic tree squirrels (e.g. Farentinos 1972; Thompson 1977; Wauters et al. 1990; Koprowski 1993). Red squirrels, however, exhibited a scramble competition mating system. In tree squirrels expressing higher degrees of sociality, males are able to congregate in the area surrounding receptive females, and more dominant males attempt to exclude rival males (e.g. Wauters et al. 1990). In an asocial, territorial species such as red squirrels however, this would require abandonment of male territories and concurrent aggression from the territory owners neighbouring the female. Thus, although reproductive opportunities are temporally dispersed, the spatial dispersion of females and strict territoriality of red squirrels prevents males from attempting to defend receptive females, promoting the scramble competition mating system.

6.2.3 Fluctuating Food Resources

The primary food resource for red squirrels at my study site is the seed of white spruce (*Picea glauca*). This species exhibits patterns of seed production whereby years of superabundant production (mast crops) are interspersed with several (3-7) years of reduced production (LaMontagne & Boutin 2007). This masting pattern, combined with relatively few competitors reproducing, results in high over-winter survivorship of juveniles, and potential fitness to males, in breeding seasons preceding mast crops years (McAdam & Boutin 2003). Males were shown to respond both to future and current food conditions, by expending significantly greater reproductive effort in breeding seasons preceding mast crops and under *ad libitum* food conditions. In addition, although males are not substantially larger than females, they do accumulate larger hoards in the autumn caching season preceding breeding. These results highlight the influence of resources on the reproductive behaviour of males, and lend support to the hypothesis that sexual size dimorphism has evolved, at least in part, to enable males to withstand extended periods of energetic investment during breeding.

6.3 FUTURE DIRECTIONS

6.3.1 Sperm Competition

Red squirrels represent a potentially powerful system to test hypotheses on sperm selection and competition. I recorded levels of female multiple mating in red squirrels that are among the highest reported for any Sciurid (Murie 1995), and patterns of sperm precedence are not evident. Although females appear to mate passively in response to selection for multiple mating patterns in males, the insemination by multiple males creates a competitive environment through which adaptive traits of the female (to select sperm) or male (through sperm traits) could evolve. Testis size, however, was at most a minor influence on reproductive success of males and genetic relatedness (based on neutral alleles) of mates did not predict patterns of parentage. Females may thus bias paternity for either good or compatible genes (Jennions & Petrie 2000; Olsson et al.

1996), using more sophisticated cues (e.g. selection based on MHC genotypes; Neff and Pitcher 2005). Further, to what extent, if any, males may strategically alter ejaculate investment according to varying risks of sperm competition (sensu Ramm & Stockley 2007) is currently unknown.

6.3.2 Behavioral Syndromes in Male Red Squirrels

Recent work in this population has identified a shy-bold continuum in female personality that is both repeatable across time and has fitness implications (Boon et al. 2007). The growth rate of offspring was shown to correlate with maternal activity levels and over-winter survival of offspring to correlate with maternal aggressiveness. Should males also exhibit this continuum, personality variation could help to explain variation in male search effort. Specifically, 'bold' males may be more predisposed to venture off-territory in search of reproductive opportunity, while 'shy' males focus attention on neighbouring females. Further, animal personality often exhibits moderate heritability (e.g. Dingemanse et al. 2002; Drent et al. 2003). Whether the influences of male and female personality are complimentary, or antagonistic, on offspring fitness could then help to explain the maintenance of personality variation in this population.

6.3.3 Spatial Genetic Structure

In red squirrels, the majority of juvenile dispersal distances are short (Mean ± S.E. = 96 ± 94 m; Berteaux & Boutin 2000) which, combined with long-term territory occupancy of individuals, could lead to fine-scale genetic structure (sensu Coltman et al. 1999). Preliminary analyses on this population (M. C. Andruskiw, J. E. Lane, D. W. Coltman and S. Boutin), however, have revealed minimal structuring. The movement of complete genomes, as juveniles, represents only one route through which genes disperse across the landscape, with male (or more accurately, male gametic) movements representing another significant contribution to the genetic neighbourhood (Crawford 1984). Although reproductive skew among males in female defence mating systems can accentuate the genetic structure (because successful males create clusters of relatives; Nussey et al. 2005), scramble competition mating systems create the opposite scenario. Specifically, the most successful males are those that range the furthest and therefore disrupt the genetic structure (Winters & Waser 2003). An iterative analysis examining the reciprocal interaction between spatial genetic structure and animal movement (juvenile and gametic) would provide insight into potential behavioural inbreeding avoidance mechanisms, and also how individual behaviour influences population-level genetic structure.

6.4 CONCLUSIONS

A combination of detailed behavioural observations, genetic profiling of, and phenotypic measurements on, offspring, as well as physiological estimates of reproductive effort allowed me to form a comprehensive description of the behavioural and genetic mating for a free-ranging population. In this scramble competition mating system, selection favoured behavioural traits that enabled males to encounter and mate with multiple females. Females, as well, showed high levels of multiple mating and multiple paternity was common. In contrast to some other systems (e.g. socially monogamous birds) and many laboratory studies, however, females did not experience a selective benefit from doing so. Rather, an extended mating season resulted in a highly male biased daily OSR, and females appeared to mate passively in response to higher levels of attending males. The ecological context of the mating system was revealed to have strong influences on both natural patterns of male and female reproductive behaviour.

6.5 REFERENCES

Andersson, M. 1994. *Sexual Selection*. Princeton: Princeton University Press.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, **2**, 349-368.

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

Boon, A. K., Réale, D. & Boutin. S. 2007. The interaction between personality, offspring fitness and food abundance in North American red squirrels. *Ecology Letters*, **10**, 1094-1104.

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

Clutton-Brock, T. H. 1991. *The Evolution of Parental Care*. Princeton: Princeton University Press.

Clutton-Brock, T. H., Guinness, F. E. & Albon, S. D. 1982. *Red deer: The behaviour and ecology of two sexes*. Chicago: Chicago University Press.

Clutton-Brock, T. H. & Vincent, A. C. J. 1991. Sexual selection and potential reproductive rates of males and females. *Nature*, **351**, 58-60

Coltman, D. W., Pilkington, J. G. & Pemberton, J. M. 1999. Fine-scale genetic structure in free-living ungulate population. *Molecular Ecology*, **12**, 733-742.

Crawford, T. J. 1984. The estimation of neighborhood parameters for plant populations. *Heredity*, **52**, 273-283.

Dingemanse, N. J., Both, C., Drent, P. J., Van Oers, K. & Van Noordwijk, A. J. 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour*, **64**, 929-938.

Dobson F. S. 1984. Environmental influences on Sciurid mating systems. In: *The Biology of Ground-dwelling Squirrels.* (Ed. by Murie, J. O. & Michener, G. R.), pp. 229-49. Nebraska: University of Nebraska Press.

Drent, P. J., Van Oers, K. & Van Noordwijk, A. J. 2003. Realized heritability of personalities in the great tit. *Proceedings of the Royal Society of London, Series B*, **270**, 45-51.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection and the evolution of mating systems. *Science*, **197**, 215-223.

Farentinos, R. C. 1972. Social dominance and mating activity in the tassel-eared squirrel (*Sciurus aberti ferreus*). *Animal Behaviour*, **20**, 316-326.

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

Humphries, M. M., Boutin, S., Thomas, D. W., Ryan, J. D., Selman, C., McAdam, A. G., Berteaux, D. and Speakman, J. R. 2005. Expenditure freeze: the metabolic responses of small mammals to cold environments. Ecology Letters, **8**, 1326-1333.

Ims, R. A. 1988. Spatial clumping of sexually receptive females induces space sharing among male voles. *Nature*, **335**, 541-543.

Jennions, M. D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biological Reviews of the Cambridge Philosophical Society*, **75**, 21-64.

Koprowski, J. L. 1993. Alternative reproductive tactics in male eastern gray squirrels-making the best of a bad job. *Behavioral Ecology*, **4**, 165-171.

Koprowski, J. L. 2007. Alternative reproductive tactics and strategies of tree squirrels. In: *Rodent Societies: An Ecological and Evolutionary Perspective.* (Ed. by J. O. Wolff & P. W. Sherman), pp. 86-95. Chicago: University of Chicago Press.

Kruuk, L. E. B., Slate, J., Pemberton, J. M., Brotherstone, S., Guinness, F. E. & Clutton-Brock, T. H. 2002. Antler size in red deer: heritability and selection but no evolution. *Evolution*, **56**, 1683-1695.

LaMontagne, J. M. & Boutin, S. 2007. Local-scale variability and synchrony in mast seeding of *Picea glauca*. *Journal of Ecology*, **95**, 991-1000.

McAdam, A. G. & Boutin, S. 2003. Variation in viability selection among cohorts of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Evolution*, **57**, 1689-1697.

Michener, G. R. & McLean, I. G. 1996. Reproductive behaviour and operational sex ratio in Richardson's ground squirrels. *Animal Behaviour*, **52**, 743-758.

Murphy, C. G. 1998. Interaction-independent sexual selection and the mechanisms of sexual selection. *Evolution*, **52**, 8-18.

Murie, J. O. 1995. Mating behavior of Columbian ground squirrels. I. Multiple mating by females and multiple paternity. *Canadian Journal of Zoology*, **73**, 1819-1826.

Murie, J. O. & Michener G. R. 1984. *The Biology of Ground-dwelling Squirrels.* Nebraska: University of Nebraska Press.

Neff, B. D. & Pitcher, T. E. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19-38.

Nussey, D. H., Coltman, D. W., Coulson, T., Kruuk, L. E. B., Donald, A. Morris, S. J., Clutton-Brock, T. H. & Pemberton, J. 2005. Rapidly declining fine-scale spatial genetic structure in female red deer. *Molecular Ecology*, **14**, 3395-3405.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelström, H. 1996. Sperm selection by females. *Nature*, **383**, 585.

Ramm, S. A. & Stockley, P. 2007. Ejaculate allocation under varying sperm competition risk in the house mouse. *Behavioral Ecology*, **18**, 491-495.

Sandell, M. & Liberg, O. 1992. Roamers and stayers: a model on male mating tactics and mating systems. *American Naturalist*, **139**, 177-189.

Schwagmeyer, P. L. 1990. Ground squirrel reproductive behavior and mating competition: a comparative perspective. In: *Contemporary Issues in Comparative Psychology* (Ed. By D. Dewsbury), pp. 175-196. Massachusetts: Sinauer.

Sherman, P. W. 1989. Mate guarding as paternity insurance in Idaho ground squirrels. *Nature*, **338**, 418-420.

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

Thompson, D. C. 1977. Reproductive behavior of the grey squirrel. *Canadian Journal of Zoology*, **55**, 1176-1184.

Tregenza, T. & Wedell, N. 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution*, **52**, 1726-1730.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871-1971* (Ed. by B. Campbell), pp. 136-179. Chicago: Aldine Publishing Company.

Vander Wall, S. B. 1990. *Food hoarding in animals*. Chicago: University of Chicago Press.

Wauters, L., Dhondt, A. A. & Devos, R. 1990. Factors affecting male mating success in red squirrels (*Sciurus vulgaris*). *Ethology Ecology & Evolution*, **2**, 195-204.

Winters, J. B. & Waser, P. M. 2003. Gene dispersal and outbreeding in a philopatric mammal. Molecular Ecology, 12, 2251-2259.

Wolff, J. O. & Dunlap, A. S. 2002. Multi-male mating, probability of conception, and litter size in the prairie vole (*Microtus ochrogaster*). *Behavioural Processes*, **58**, 105-110.