



## Female multiple mating and paternity in free-ranging North American red squirrels

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Multimale mating (MMM) is common in female mammals, but our understanding of its evolutionary significance in this taxon lags behind that in others (e.g. invertebrates and birds). To date, the majority of research on mammalian MMM has been conducted under laboratory conditions, and the extent to which these findings have relevance for natural populations is little known. We quantified MMM in a free-ranging population of North American red squirrels, *Tamiasciurus hudsonicus*, and investigated its hypothesized influences and consequences. We observed 85 mating chases, representing 62 individual females, over three mating seasons and calculated standardized indexes of MMM and number of copulations. Extrinsic elements of mating chases (i.e. an index of the number of attending males and date) explained variation in MMM, while traits of the females (age and pre-oestrous body mass) did not correlate with MMM. Hypotheses of cryptic direct benefit (fertility assurance and infanticide avoidance) were not supported because MMM did not influence pregnancy rate, litter size or nest fate. There was also no correlation between MMM and offspring quality, multiple paternity or litter allelic diversity, and therefore, no support for hypotheses of genetic benefit. In addition to not accruing observable benefits to MMM, females did not incur a detectable cost, thus rendering MMM selectively neutral. Rather, in line with traditional explanations, female mating behaviour in red squirrels appears to be a passive response to selection on multifemale mating in males.

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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 choosy and monogamous, with multimale mating (MMM) arising indirectly as a consequence of selection

for multifemale mating in males (reviews in Schwagmeyer 1990; Petrie et al. 1992; Wolff & Macdonald 2004). Behavioural and genetic studies, however, have revealed widespread MMM by 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 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

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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 potentially negating 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. Although social structures of 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.

Because of the difficulties inherent in monitoring the natural mating behaviour of free-ranging mammals, most studies of MMM have been 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 our knowledge, only two mammalian studies have directly investigated these benefits under ecologically relevant conditions. When Fisher et al. (2006a) experimentally mated female brown antechinuses, *Antechinus stuartii*, to single or multiple males and released their offspring into the wild, they found that MMM increased offspring survival, thereby providing support for genetic benefit hypotheses. Hoogland (1998), in contrast, found that the benefits to Gunnison's prairie dogs, *Cynomys gunnisoni*, arose through a cryptic direct benefit (i.e. fertility assurance): females that had at least three mates showed elevated pregnancy rates and litter sizes relative to females that had only one or two mates. 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).

We studied the behavioural and genetic mating system of North American red squirrels, *Tamiasciurus hudsonicus*, and measured the quality, quantity and fate of resultant offspring to test the relevant hypotheses of MMM for a free-ranging mammal (Table 1). Attributes of the natural history of red squirrels allowed us 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 us to directly observe individual oestrous females and enumerate their mates. In addition, the majority of young are born in easily accessible arboreal grass nests, allowing us immediate access after parturition. We were thus able to measure attributes of litters (e.g. offspring mass, litter size) at the earliest possible stage. We, 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, we estimated the strength of selection on MMM, using standardized selection gradients (Lande & Arnold 1983).

## METHODS

### Study Area and Population

We 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 our study site. Red squirrels clip the new spruce cones in the autumn (August–October) of each year and cache them in a larder hoard (middens; Smith 1968). These middens form the centre 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 in response to food availability. During the 3 years of this study, food conditions were low and squirrel densities remained below 2.2 squirrels/ha (LaMontagne 2007; S. Boutin, unpublished data).

We captured individuals in live traps (Tomahawk Live Trap, Tomahawk, WI, U.S.A.) 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 at that time received unique alphanumeric eartags. Ages of such focal females ( $N = 45$ ) were known with certainty. Any immigrating adults received eartags on first capture and, for these focal females ( $N = 18$ ), we treat age as a minimum estimate (i.e. age = 1 in year of first capture). Restricting our analyses to include only females of known age did not influence our main conclusions. Each individual was also given a unique combination of one or two coloured wires, threaded through their eartags, during their first capture each season to allow for identification from a distance. Females were outfitted with radiocollars (model PD-2C, 4g, Holohil Systems Limited, Carp, Ontario, Canada) 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-oestrous body mass.

**Table 1.** Hypothesized cryptic direct and indirect genetic benefits of multimale mating (MMM) in North American red squirrels (*Tamiasciurus hudsonicus*)\*

Hypothesis	Explanation	Predictions	References
Fertility assurance†	MMM as a bet-hedging strategy against either sperm-depleted or genetically incompatible males	MMM is positively correlated with pregnancy rate and/or litter size MMM is not correlated with offspring quality MMM is not correlated with litter allelic diversity or multiple paternity MMM is not correlated 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 is positively correlated with litter fate MMM is not correlated with pregnancy rate and/or litter size MMM is not correlated with litter allelic diversity or multiple paternity MMM is not correlated with offspring quality	Hrdy 1979; Wolff & Macdonald 2004
Genetic benefits to offspring‡	MMM results from females either 'trading up' copulatory partners or to enable postcopulatory mechanisms to select genetically superior (i.e. 'good genes' hypotheses) or more compatible sperm (i.e. 'compatible genes' hypotheses)	MMM is positively correlated with offspring quality MMM may be positively correlated with litter size (if mating with genetically incompatible males results in early reproductive failure) MMM may be positively correlated with litter fate (if higher-quality offspring are more likely to emerge) MMM is not correlated with litter allelic diversity or multiple paternity	Jennions & Petrie 2000; Tregenza & Wedell 2002; Neff & Pitcher 2005
Increase genetic diversity of litter	MMM results in genetically diverse litters that females use as a bet-hedging strategy against unpredictable future environmental conditions	MMM is positively correlated with litter allelic diversity and/or multiple paternity MMM is not correlated with litter size MMM is not correlated with offspring quality MMM is not correlated with litter fate	Baer & Schmid-Hempel 1999; Jennions & Petrie 2000; Cohas et al. 2007

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

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

‡Owing to the difficulty of differentiating good gene benefits from compatible gene benefits, we have grouped both under one hypothesis (i.e. MMM to increase the genetic quality of young).

## 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 oestrus 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 (J. E. Lane & S. Boutin, unpublished data). Upon locating an oestrous 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 we monitored 46 mating

chases through to completion. In 2004 and 2005, owing to logistical constraints, we monitored a smaller sample ( $N = 23$ , 2004;  $N = 16$ , 2005) through to completion.

For mating chase observations, we used a combination of focal-animal sampling of the oestrous female, scan sampling for attending males and all-occurrence sampling of mating behaviour (Altmann 1974; Martin & Bateson 1986). This protocol allowed us to identify and enumerate both attending and copulating males and to 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 oestrous females and  $91 \pm 1\%$  of males in each mating chase were individually identifiable. Because many copulations occur underground, we also judged copulations as occurring when a female was followed underground by a male and the pair remained there for a minimum of 60 s or made copulatory vocalizations, regardless of the time spent underground. Copulations of tree squirrels generally last less than 60 s (Koprowski 2007), which is adequate for fertilization in red squirrels (J. E. Lane & S. Boutin, unpublished data). This criterion has been used previously in this (Lane

et al. 2007) and other (Waterman 1998) systems. We also noted whether the female was in or out of sight, every 10 min. We then calculated standardized indexes 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.

### Offspring Measurements

We 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. We checked nests a second time at approximately one month postparturition 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, we measured offspring mass at both nest checks with a portable electronic balance ( $\pm 0.1$  g) and, to ensure linearity, we excluded offspring from our analyses when their initial mass was greater than 50 g (ca. 25 days old), their final mass was greater than 100 g (ca. 57 days old), or when fewer than 5 days elapsed between measurements. 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 ( $N = 118$ ), we 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, we 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 postparturition (Berteaux & Boutin 2000). During this period we used 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 survival to reproductive age as well as overwinter survival of females. Our ability to document overwinter survival with confidence is enhanced by the territorial social structure of red squirrels, the size of our study area (80 ha) relative to the natal dispersal distance of red squirrels (mean  $\pm$  SE =  $96 \pm 94$  m; Berteaux & Boutin 2000) and the relatively poor quality of the surrounding habitat. The overwinter survival of offspring is an established measure of fitness in this study system (e.g. McAdam & Boutin 2003a) and dispersal does not bias fitness calculations (Kerr et al. 2007). Females were also monitored in the year following mating season observations to determine whether 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.

### 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, U.K.) and was normalized to a standard concentration of 10 ng/ $\mu$ l. 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, MA, U.S.A.) using 10  $\mu$ l reactions. Each 10  $\mu$ l reaction contained 1  $\mu$ l of genomic DNA, 1  $\mu$ M of each forward and reverse primer, 0.05  $\mu$ l of BioTaq DNA polymerase (Biolone, London, U.K.), 1  $\mu$ l of 10 $\times$  reaction buffer (20 mM  $(\text{NH}_4)_2\text{SO}_4$ , 75 mM Tris-HCl pH 9.0, 0.01% (w/v) Tween), 1.5 mM of  $\text{MgCl}_2$ , 4.65  $\mu$ l of  $\text{H}_2\text{O}$  and 1  $\mu$ l of dNTPs (0.2 mM of each dNTP). Details of PCR conditions and dye labelling 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, CA, U.S.A.), and genotypes were scored using GENEMAPPER 3.5 software (Applied Biosystems). 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 before 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 three 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 accepted only if there were two or fewer mismatches between offspring and both parents. The genotyping error rate based on known mother-offspring pair mismatches was approximately 2%.

We 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 we were unable to assign a sire were assumed to have been sired by an unsampled male ( $N = 25$  litters). If we were unable to assign a sire to more than one offspring within a litter ( $N = 7$  litters), we 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 unsampled male ( $N = 4$  litters). If values fell below this level, they were treated as half-siblings with different sires ( $N = 3$  litters).

### Statistical Analysis

We used a general linear model (LM) to determine whether traits of the female (pre-oestrous mass, minimum age and (minimum age)<sup>2</sup>) and/or traits of the mating chase (date and number of attending males index) influenced MMM. The quadratic term for female age was included owing 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, we 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 we 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) we fitted both the MMM and the number of copulations indexes 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 indexes 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), we 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)<sup>2</sup>, pre-oestrous 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 a 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, MMM index was fitted as a fixed effect and the potential covariates included in the models were female minimum age, (minimum age)<sup>2</sup>, pre-oestrous 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. Overwinter survivorship of the female, coded as a binary variable (1 = survived; 0 = did not survive) represented the dependent variable. We did not analyse whether MMM influenced the subsequent year's mating success, because all females that survived over the winter were observed to mate. We fitted the MMM and number of copulations indexes as fixed effects and included female minimum age, (minimum age)<sup>2</sup>, pre-oestrous body mass, mating chase date and litter size as potential covariates in the model.

We obtained the standardized linear selection gradient ( $\beta$ ) from the multiple regression of our fitness metric (number of offspring surviving over the winter) on the MMM index, female minimum age and pre-oestrous body mass (Lande & Arnold 1983). Trait values were standardized to a mean of zero, and relative fitness was calculated within each year to avoid confounding the selection gradient with covariances between environmental conditions, trait values and fitness. The standard errors were generated by jackknifing. Because of our limited sample size, we 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, The R Foundation for Statistical Computing, Vienna, Austria), and, unless otherwise noted, values are presented as means  $\pm$  1 SE. Sample sizes vary for analyses because of the suitability of records and therefore are included in the statistical output for analyses. For example, if we observed a female's mating chase, but she died before her estimated parturition date, she would be included in all analyses of MMM influences, but not in analyses relevant to hypothesized consequences.

### RESULTS

We followed 62 individual females for an average of  $9.5 \pm 0.3$  h during a total of 85 mating chases ( $N = 46$ , 2003;  $N = 23$ , 2004;  $N = 16$ , 2005). Five females contributed records in all 3 years, 12 females contributed records in 2 years and one female, who lost her litter and recycled in 2003, contributed two records for that year. During the 85 mating chases, females copulated with an average of  $5.8 \pm 0.3$  different males and the mean number of total copulations per female was  $22.8 \pm 1.9$ . This resulted in

a calculated mean MMM index and number of copulations index of  $1.0 \pm 0.1$  and  $3.6 \pm 0.2$ , respectively. To evaluate the validity of our MMM index, we selected 29 'high-quality' records and compared the actual number of observed mates with our calculated MMM index. For these mating chases, we observed the female for the majority of the day ( $10.4 \pm 0.3$  h), had a high percentage of recordings as 'in sight' ( $74.6 \pm 2.8\%$ ) 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 =  $3.21 + 3.68 \times$  (MMM index)), we then estimated the mean number of mates per oestrous female as  $6.9 \pm 0.2$ .

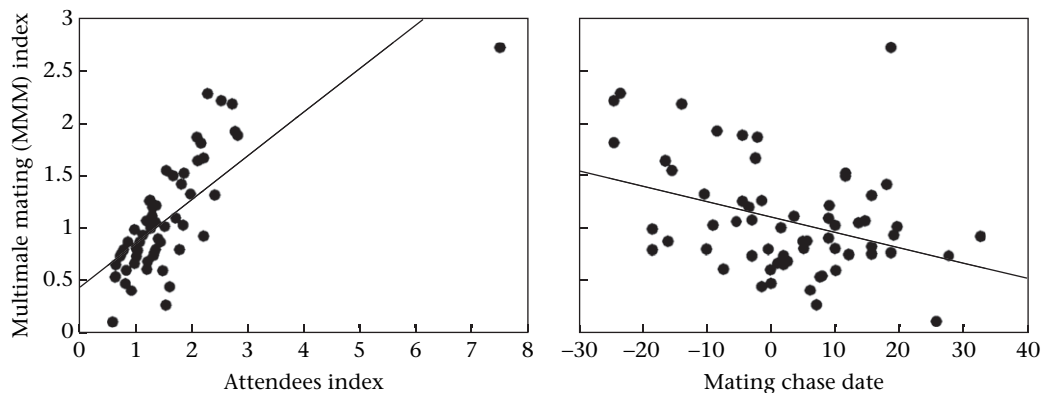
Individual levels of MMM were correlated with extrinsic aspects of the mating chase, and were unrelated to either measure of female quality (final LM:  $F_{3,58} = 50.49$ ,  $R^2 = 0.72$ ,  $N = 62$  independent mating chases  $P < 0.001$ ). Levels of MMM increased with the number of attending males index (LM:  $F_{1,58} = 121.68$ ,  $P < 0.001$ ; Fig. 1) and decreased with advancing date (LM:  $F_{1,58} = 23.82$ ,  $P < 0.001$ ; Fig. 1). In contrast, female minimum age (LM:  $F_{1,53} = 0.21$ ,  $P = 0.65$ ), (minimum age)<sup>2</sup> (LM:  $F_{1,54} = 0.35$ ,  $P = 0.56$ ), and pre-oestrous 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  $3.0 \pm 0.1$  offspring. Two females died during gestation (one female was killed by an avian predator 30 days after her mating chase and one female died as a result of a radiocollar 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) 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 oestrus (42.9%). In addition, 12 of the

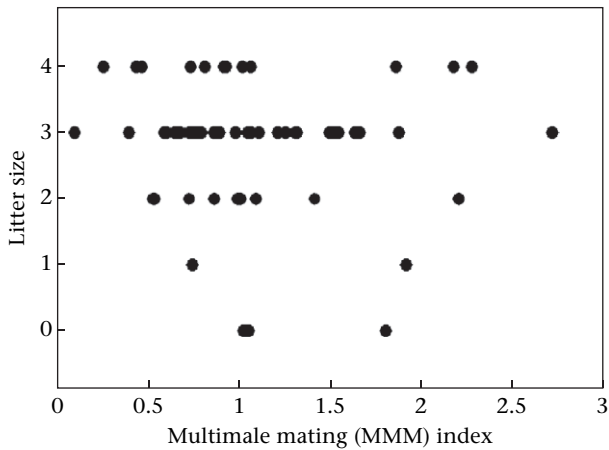
22 (54.5%) females that lost their litter postparturition but survived to the estimated litter emergence date, recycled. The probability that a female successfully had one offspring emerge from the nest ( $N = 52$  independent litters) was unrelated to her MMM index (GLM:  $\chi^2_1 = 0.21$ ,  $P = 0.65$ ; Fig. 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-fourths 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. 4).

Over the course of this study we were able to assign paternity to at least one offspring in each litter that resulted from 68 observed mating chases. The mean number of different alleles represented in polytocous litters ( $N = 63$ ) and the mean litter allelic diversity was  $41.8 \pm 0.7$  and  $0.47 \pm 0.1$ , respectively. Litter allelic diversity was unrelated to the MMM index (LM:  $F_{1,46} = 1.60$ ,  $N = 48$  independent mating chases,  $P = 0.21$ ; Fig. 5). The majority of the polytocous litters were multiply sired ( $52/63 = 82.5\%$ ), with a mean number of sires represented in litters as  $2.3 \pm 0.1$  (Fig. 5). For 42 litters, 38 of which were polytocous, we were able to assign paternity to all offspring. The majority of these polytocous litters were multiply sired ( $27/38 = 71.0\%$ ), with a mean of  $2.0 \pm 0.1$  sires represented. Levels of multiple paternity were, however, unrelated to females' MMM index, regardless of whether we restricted our analysis to litters for which we were able to assign all sires (GLM:  $\chi^2_1 < 0.01$ ,  $N = 31$  independent litters,  $P = 0.96$ ), or we included the entire data set (GLM:  $\chi^2_1 = 0.15$ ,  $N = 48$  independent litters,  $P = 0.70$ ).

In addition to realizing no observable benefit to MMM, females did not incur any realized cost. The mean proportion of females that survived over the winter was  $49.2 \pm 9.8\%$  and whether or not a female ( $N = 62$  females)



**Figure 1.** Multimale 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). Multimale mating and number of attending males indexes 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.

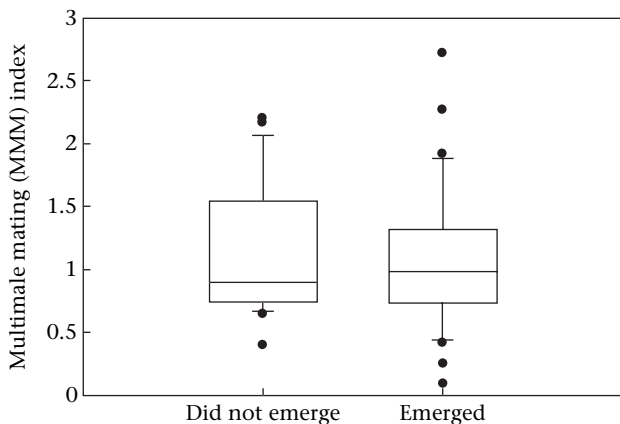


**Figure 2.** Litter size ( $N = 60$  litters) of female North American red squirrels as a function of their multimale mating index: number of mates/(observation time  $\times$  proportion of in-sight observations).

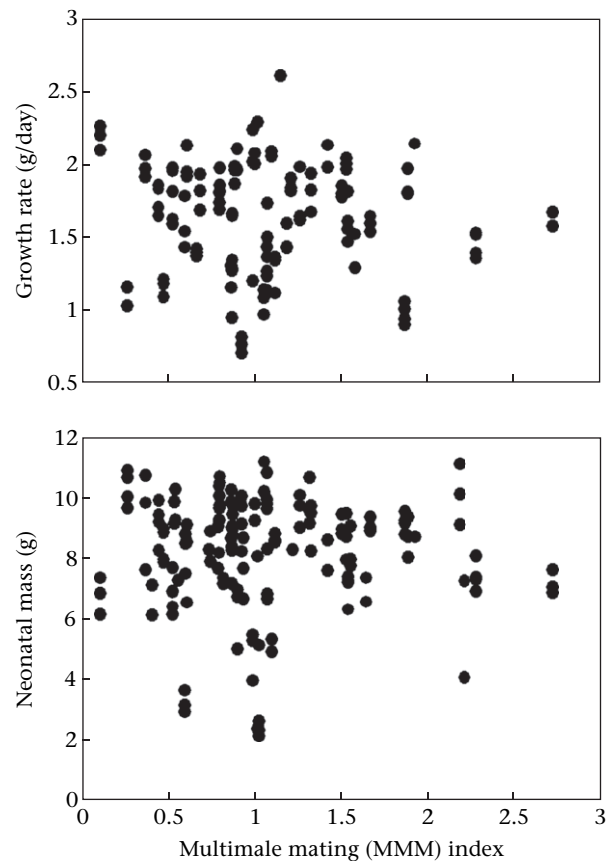
survived was unrelated to her MMM index (GLM:  $\chi^2_1 = 0.09$ ,  $P = 0.77$ ; Fig. 6). In addition, all females that survived over the winter mated the following year. The combination of both a lack of observed benefits and costs yielded MMM selectively neutral in free-ranging red squirrels. The standardized linear selection gradient was  $-0.37 \pm 2.87$ , and was not significantly different from 0 (one-sample  $t$  test:  $t_{62} = 0.13$ ,  $P = 0.90$ ).

## DISCUSSION

Our investigations of a free-ranging population of red squirrels support the original explanations of MMM occurring as a result of indirect selection for multifemale mating on males (Bateman 1948; Trivers 1972). During their single annual day of oestrus, all females except one were observed to mate multiple times and with multiple males, and the mean number of mates per female was high. Females were directly observed to copulate with  $5.8 \pm 0.3$  males, and we estimated that they may have



**Figure 3.** Multimale mating index (number of mates/(observation time  $\times$  proportion of in-sight observations)) of female North American red squirrels that did ( $N = 34$  females) and did not ( $N = 18$  females) have at least one offspring emerge from the nest.

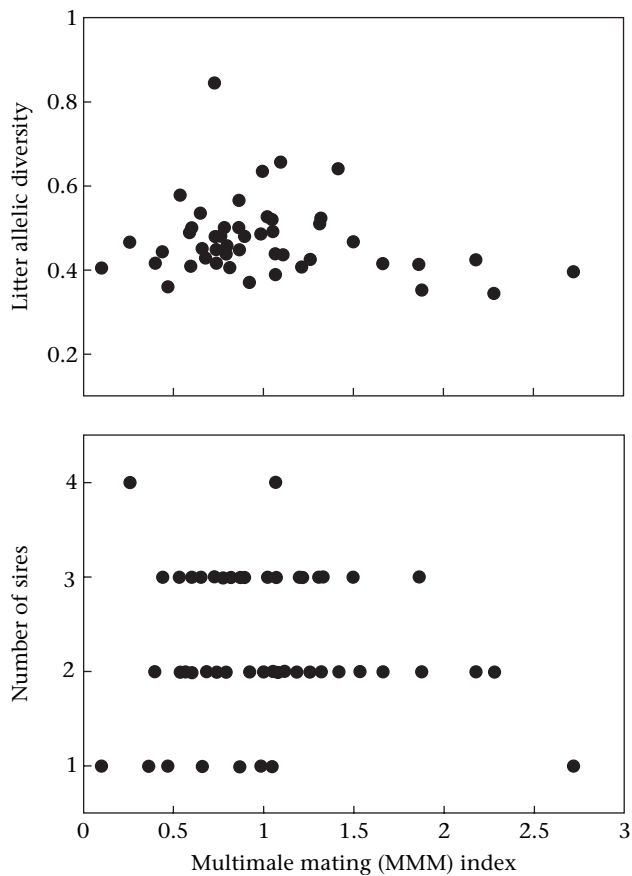


**Figure 4.** Growth rate (g/day;  $N = 123$ ) and neonatal mass (g;  $N = 169$ ) of offspring in litters of female North American red squirrels as a function of their multimale mating index: number of mates/(observation time  $\times$  proportion of in-sight observations).

copulated with  $6.9 \pm 0.2$ . In contrast to many laboratory studies, however, these high levels of MMM did not result in detectable direct benefits (fertilization assurance or infanticide avoidance) or indirect benefits (genetic quality or diversity).

## 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) found 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, we did not detect an increase in either pregnancy rate or litter size with MMM in red squirrels. Multimale

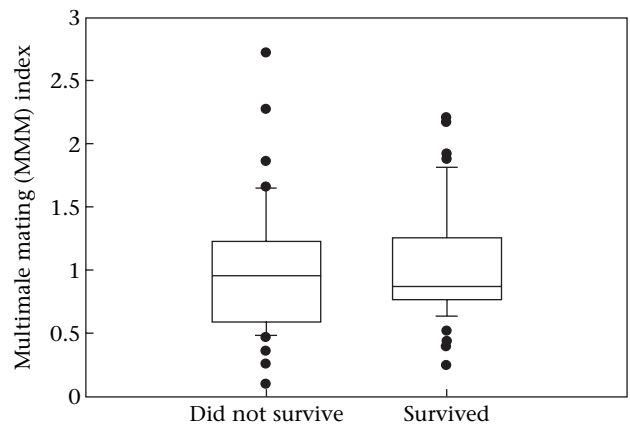


**Figure 5.** Litter allelic diversity (number of different alleles/number of total alleles) and multiple paternity (number of sires) in litters of female North American red squirrels ( $N = 48$  polytocous litters) as a function of the females' multimale mating index: number of mates/(observation time  $\times$  proportion of in-sight observations).

mating as a bet-hedging strategy against sperm-depleted males may be unnecessary in this species as sperm depletion is likely to be a relatively rare event. Unlike many ground squirrel species, which restrict mating to as little as 1–2 weeks following emergence from hibernation (Murie & Michener 1984), the mean duration of the mating season over the 3 years of this study was  $12.3 \pm 1.7$  weeks. This temporally dispersed mating season results in relatively fewer oestrous females per day, and should therefore place less of a drain on males' spermatogenesis abilities.

### Infanticide Avoidance

Red squirrels fit two of Wolff & Macdonald's (2004) key criteria for mammal species most likely to use MMM as a guard against infanticide: oestrous cycling is induced following litter loss, and females give birth to altricial young. We were, however, unable to detect an effect of MMM on rates of litter loss. Admittedly, absolute litter loss represents an indirect metric, and the 3 years of this study were conducted during relatively low food conditions when litter loss due to energetic limitations may have obscured that due to infanticide. To control



**Figure 6.** Multimale mating index (number of mates/(observation time  $\times$  proportion of in-sight observations)) of female North American red squirrels that survived ( $N = 28$ ) and did not survive ( $N = 34$ ) over the winter following observation of their mating chases.

for interindividual and interannual differences in energetic limitations, we included female minimum age, (minimum age)<sup>2</sup> and pre-oestrous body mass and year, respectively, as covariates in our analyses. The role of energy availability is potentially revealed by 2005 having the highest rates of litter loss of all 3 years. This year followed 6 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.

### 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, our evidence suggests that MMM is unlikely to be used to enhance offspring quality. The acquisition of a territory, and associated midden, is necessary for overwinter 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, we found no effect of MMM on either the neonatal mass or the 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 prevent females from limiting mating to these males, at the expense of their offspring. While evidence from laboratory studies of invertebrates (e.g. Tregenza & Wedell 1998) and one mammal (Fisher et al. 2006b) have corroborated genetic benefit hypotheses for MMM, field studies, primarily on extrapair 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.

We also found 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; Cohan et al. 2007). Although the majority of red squirrel litters were multiply sired, MMM was not correlated with the level of litter allelic diversity or the level of multiple paternity. Females are thus limited in their ability to manipulate the genetic diversity of their litters. One mechanism that could explain this constraint, sperm precedence, is not apparent in red squirrels (J. E. Lane & S. Boutin, unpublished data), so other factors influencing siring success may play a role. Both testis size (Schulte-Hostedde & Millar 2004) and copulation duration (Schwagmeyer & Foltz 1990) influence paternity patterns in other sciurid species and could overwhelm a female's ability to manipulate paternity patterns within her litter.

### Why are Female Red Squirrels Promiscuous?

We found that female red squirrels do not accrue a detectable benefit to MMM. However, we also found no obvious cost to MMM, and our selection analysis revealed that it is selectively neutral. Despite this, female red squirrels are among the most promiscuous sciurids studied to date (Boellstorff et al. 1994; Murie 1995). When we restricted our comparison to the number of copulating males observed in our 'high-quality' mating chases, red squirrels engaged in higher levels of MMM than all but one squirrel species (Boellstorff et al. 1994), and when we broadened our comparison to include the number of males estimated from our MMM index, red squirrels engaged in the highest level of MMM of any squirrel species. What causes such high levels of MMM in red squirrels? We suggest that, rather than actively mating for selective benefit, female red squirrels mate passively, as evidenced by the linear relationship between MMM and the number of attending males. Passive mating could arise either because females do not incur a cost from MMM, or because the costs of MMM do not exceed those of evading attending males. Indeed, one cost to females of evading attending males, vulnerability to predation above ground, could be greater than the cost of mating with multiple males below ground.

The majority of research on sciurid mating systems has been on ground squirrels, and given equal population densities, the higher levels of MMM in red squirrels may be a direct consequence of their relatively longer mating season, because there are fewer females in oestrus per day and the daily operational sex ratio (OSR) is more heavily male biased (Emlen & Oring 1977). For example, both red squirrels and thirteen-lined ground squirrels, *Spermophilus tridecemlineatus*, have a scramble-competition mating system (Schwagmeyer & Woontner 1986), yet the average daily OSR of red squirrels (25 males/female) is an order of

magnitude higher than that of thirteen-lined ground squirrels (1.9–5.9 males/female; Schwagmeyer & Brown 1983; Foltz & 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, we 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 males (but of lower quality) (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.

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## References

- Altmann, J. 1974. Observational study of behavior: sampling methods. *Behaviour*, **9**, 227–267.
- Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: 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. doi:10.1038/16451.
- 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. doi:10.1890/0012-9658(2000)081[1311:BDIFNA]2.0.CO;2.
- 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. doi:10.1007/s002650050563.
- 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: *Behavioral 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. doi:10.1006/anbe.1994.1144.
- 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. doi:10.1126/science.1135520.
- 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. doi:10.1007/s00265-006-0341-7.
- 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. doi:10.1038/nature05206.
- 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. doi:10.1016/j.anbehav.2005.03.041.
- 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. doi:10.1038/nature01969.
- 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. doi:10.1111/j.1471-8286.2005.01022.x.
- 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. doi:10.1046/j.1471-8286.2002.00305.x.
- Hoogland, J. L. 1998. Why do female Gunnison's prairie dogs copulate with more than one male? *Animal Behaviour*, **55**, 351–359. doi:10.1006/anbe.1997.0575.
- 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. doi:10.1890/0012-9658(2000)081[2867:TDOOLS]2.0.CO;2.
- 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. doi:10.1017/S0006323199005423.
- 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. doi:10.1098/rsbl.2006.0615.
- Klemme, I., Eccard, J. A. & Ylönen, H. 2007. Why do female bank voles, *Clethrionomys glareolus*, mate multiply? *Animal Behaviour*, **73**, 623–628. doi:10.1016/j.anbehav.2006.07.010.
- 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.
- McAdam, A. G. & Boutin, S. 2003a. Variation in viability selection among cohorts of juvenile red squirrels (*Tamiasciurus hudsonicus*). *Evolution*, **57**, 1689–1697. doi:10.1554/02-393.
- 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. doi:10.1046/j.1420-9101.2003.00630.x.
- 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. doi:10.1554/0014-3820(2002)056[0846:MEATPF]2.0.CO;2.
- 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. *Ecoscience*, **14**, 362–369.

- Magnhagen, C. 1991. Predation risk as a cost of reproduction. *Trends in Ecology & 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. doi:10.1046/j.1365-294x.1998.00374.x.
- Martin, P. & Bateson, P. 1986. *Measuring Behavior: an Introductory Guide*. Cambridge: Cambridge University Press.
- 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*. Lincoln, 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. doi:10.1111/j.1365-294X.2004.02395.x.
- Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelström, H. 1996. Sperm selection by females. *Nature*, **383**, 585. doi:10.1038/383585a0.
- 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. doi:10.1007/BF00177775.
- Preston, B. T., Stevenson, I. R., Pemberton, J. M. & Wilson, K. 2001. Dominant rams lose out by sperm depletion. *Nature*, **409**, 681–682. doi:10.1038/35055617.
- 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. doi:10.1098/rspb.2004.2977.
- 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. doi:10.1554/02-346.
- Reynolds, J. D. 1996. Animal breeding systems. *Trends in Ecology & Evolution*, **11**, 68–72. doi:10.1016/0169-5347(96)81045-7.
- 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. doi:10.1007/s00265-003-0707-z.
- 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. Sunderland, 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. doi:10.1007/BF00295069.
- Schwagmeyer, P. L. & Foltz, D. W. 1990. Factors affecting the outcome of sperm competition in thirteen-lined ground squirrels. *Animal Behaviour*, **39**, 156–162. doi:10.1016/S0003-3472(05)80735-3.
- 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 and Sociobiology*, **19**, 359–364. doi:10.1007/BF00295709.
- 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. doi:10.1098/rstb.1993.0044.
- 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. doi:10.1146/annurev.ecolsys.36.102403.112501.
- 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. doi:10.1098/rspb.2002.2228.
- 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. doi:10.1098/rspb.1993.0143.
- 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. doi:10.1016/j.anbehav.2003.09.008.
- Tregenza, T. & Wedell, N. 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution*, **52**, 1726–1730.
- Tregenza, T. & Wedell, N. 2002. Polyandrous females avoid costs of inbreeding. *Nature*, **415**, 71–73. doi:10.1038/415071a.
- 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.
- 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. doi:10.1006/anbe.1998.0780.
- 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. doi:10.1016/S0376-6357(02)00022-0.
- Wolff, J. O. & Macdonald, D. W. 2004. Promiscuous females protect their offspring. *Trends in Ecology & Evolution*, **19**, 127–134. doi:10.1016/j.tree.2003.12.009.
- Wolff, J. O. & Sherman, P. W. 2007. *Rodent Societies: an Ecological and Evolutionary Perspective*. Chicago: University of Chicago Press.