



## Genetic relatedness of mates does not predict patterns of parentage in North American red squirrels

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(Received 4 October 2006; initial acceptance 9 November 2006;  
final acceptance 26 December 2006; published online 23 August 2007; MS. number: A10581R)

Previously limited to laboratory studies, the deleterious effects of inbreeding are now being revealed in a number of wild systems. Female North American red squirrels, *Tamiasciurus hudsonicus*, show high levels of multimale mating and little to no overt precopulatory mate selection. We hypothesized that the negative repercussions of inbreeding should select for a female's ability to select sperm from more distantly related males. Consequently, successful sires should be less genetically related to the female than are unsuccessful males. We tested this hypothesis using both an analysis of absolute success among all copulating males and also relative success of sires within multiply sired litters. Pairwise genetic relatedness and paternity were determined through molecular analysis of tissue samples collected from reproductive females, copulating males and resultant offspring. In contrast to other systems, we found no evidence that the genetic similarity of mates predicts patterns of parentage in red squirrels. Genetic relatedness did not predict whether a copulating male would sire any offspring, and relative success of sires within multiply sired litters was unrelated to their relatedness to the dam of the litter. Furthermore, selection for inbreeding avoidance mechanisms may be minimal, as there were no observable negative fitness repercussions to inbreeding. We detected no relationship between the genetic relatedness of an offspring's parents and its neonatal mass, growth rate or survival to reproductive age. In red squirrels, we found no evidence of parentage patterns based on genetic similarity of mates, and this phenomenon may be less universal than previously thought.

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**Keywords:** compatible genes; genetic relatedness; inbreeding; litter; microsatellite; multimale mating; North American red squirrel; paternity; sperm competition; *Tamiasciurus hudsonicus*

The evolution of multimale mating has remained a contentious issue despite widespread study in taxa ranging from insects to vertebrates (Jennions & Petrie 2000, and references therein). In males, the benefits of multi-female mating are well established (i.e. elevated reproductive success), but in females, maximum offspring production is limited, making multimale mating 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 multimale mating 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 intrinsic effects of paternal genes directly affect the fitness of resultant offspring, compatible genes hypotheses postulate that it is the interaction between paternal and maternal genomes that primarily influences offspring fitness (Mays & Hill 2004).

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

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The deleterious effects of inbreeding are well 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 multiply mated females to influence the paternity of their offspring through postcopulation 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 postcopula 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. Bishop 1996; Olsson et al. 1996; Stockley 1999; Kraaijeveld-Smit et al. 2002; Thuman & Griffith 2005). In these investigations, males that sired a higher proportion of offspring in individual litters/clutches were less genetically related to the female than were males that sired fewer offspring. Interestingly, the influence of genetic relatedness on parentage patterns has been largely seen in oviparous species. The sole study of a eutherian mammal (common shrew, *Sorex araneus*), however, found no evidence of sperm selection by females (Stockley 1997). This finding runs counter to the hypothesis of Zeh & Zeh (2001) that, because the arena for genomic conflicts created by embryonic development is within the female, viviparous species should be the species most likely to show patterns of parentage based on genetic compatibility. Clearly, further studies on viviparous species are required.

In this study we investigated whether genetic similarity of mates predicted observed patterns of parentage in North American red squirrels, *Tamiasciurus hudsonicus*. Levels of multimale mating in female red squirrels are among the highest recorded for all sciurid species (mean  $\pm$  SE mates/female =  $6.3 \pm 0.4$ ,  $N = 29$  oestrous bouts; Boellstorff et al. 1994; Murie 1995; J. E. Lane & S. Boutin, unpublished data), and females show little to no overt precopulatory mate choice. Females mate with the majority of males attending their mating chase and do not bias copulations in favour of 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 postcopulation inbreeding avoidance mechanisms. Moreover, paternity-biasing mechanisms, such as mating-order effects (Lacey et al. 1997) and copulatory plugs (Koprowski 1992), which could obscure a potential relationship between relatedness and siring success, are not known for red squirrels (J. E. Lane & S. Boutin, unpublished data). We thus predicted that females would bias paternity of their offspring in favour of more distantly related males.

To evaluate the potential selective pressures associated with mate choice based on genetic similarity, we compared

the effect of parental genetic relatedness on two aspects of offspring performance: neonatal mass and growth rate. In this eutherian species, acquisition of a territory and food cache (middens) is thought to be necessary for overwinter survival, placing a strong selective pressure on developing and weaning early (McAdam & Boutin 2004). We 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 we have restricted our analyses to the genetic relatedness of mates.

## METHODS

### Study Population and Field Methods

We studied a population of red squirrels near Kluane National Park in the southwest Yukon during 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; Becker 1992) with females producing a single litter, each year, after a 35-day gestation period. 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 1 day. During the ensuing 'mating chase', males congregate on the oestrous female's territory, and chase and attempt to copulate with her, and the female copulates with many or all attending males (mean  $\pm$  SE =  $6.3 \pm 0.4$ ,  $N = 29$  oestrous bouts; J. E. Lane & S. Boutin, unpublished data). We followed individual females ( $N = 65$ ) for an average of  $9.6 \pm 0.2$  h during a total of 89 oestrous periods (2003:  $N = 46$ ; 2004:  $N = 23$ ; 2005:  $N = 20$ ). Six females contributed records in all 3 years, and 12 females contributed records in 2 years. 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). 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 ear tags, allowing for identification from a distance. All oestrous females and a mean  $\pm$  SE of  $91 \pm 1\%$  of males in each chase were individually identifiable. Because many copulations occur underground, we judged a copulation to have occurred when a female was followed underground and the pair remained there for a minimum of 60 s (Waterman 1998). Copulations of tree squirrels generally last less than 60 s

(Koprowski 2007), which is sufficient for fertilization in red squirrels (J. E. Lane & S. Boutin, unpublished data). We also deemed a copulation to have occurred if copulatory vocalizations could be heard, regardless of the time spent underground. During and following underground consortships, we 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 our assumption that these consortships represented underground copulations.

We checked nests soon after birth to sample offspring for genetic analyses and to measure neonatal mass ( $N = 159$ ). A small tissue biopsy from the ear was collected with sterile scissors and preserved in 70% ethanol. Red squirrel litters can be multiply sired (J. E. Lane, S. Boutin, M. R. Gunn, J. Slate & D. W. Coltman, unpublished data), so a unique sex–ear notch combination was used for each individual offspring, allowing for subsequent discrimination of individuals. We also checked nests at approximately 1 month postparturition to determine juvenile growth rates ( $N = 156$ ) and to 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 analysis when initial mass was greater than 50 g (ca. 25 days old), or final mass was greater than 100 g (ca. 57 days old), or when less than 5 days elapsed between measurements. For nest checks occurring after the date of parturition, we 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. Our ability to document overwinter survival with confidence was enhanced by 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), as well as the 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 has been shown not to bias measurements of fitness (M. M. Humphries, A. G. McAdam & S. Boutin, unpublished data). The Biosciences Animal Policy and Welfare Committee at the University of Alberta approved all protocols.

### Molecular Analyses and Paternity Assignment

Details of the molecular methods for microsatellite loci isolation and paternity assignment are provided elsewhere (Gunn et al. 2005; J. E. Lane, S. Boutin, M. R. Gunn, J. Slate & D. W. Coltman, unpublished data). 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 (Appendix, Table A1). Maternity was determined by behavioural observation at the nest and paternity was assigned at 95% confidence using CERVUS 2.0 (Marshall et al. 1998). Analyses were conducted for each year (2003, 2004 and 2005) separately.

### Estimation of Genetic Relatedness

We 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  $\pm$  SE genetic relatedness between mothers and their offspring (predicted value =  $0.5$ ; Wang 2002) was  $0.489 \pm 0.004$  (range  $0.24$ – $0.94$ ,  $N = 438$ ), and the mean  $\pm$  SE genetic relatedness between grandmothers and their grandoffspring (predicted value =  $0.25$ ; Wang 2002) was  $0.239 \pm 0.015$  (range  $0.11$ – $0.55$ ,  $N = 89$ ).

### Statistical Analysis

We 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 modelled with a Poisson error structure. Males observed copulating, but not siring offspring, were assigned an offspring sired value of  $0$ . We fitted pairwise genetic relatedness of the copulating males and the oestrous females as a fixed effect ( $N = 302$ ). Data were nested within mating chases, and therefore mating chase was fitted as a random effect. We analysed data from 67 of the 89 observed mating chases (9 of the observed females came into oestrus but did not produce a litter, and in 13 cases, we did not have a DNA sample from either the female ( $N = 8$ ) or offspring from the resultant litter ( $N = 5$ ). 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 our 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, we 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 males sired offspring). 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, we randomly selected one litter for females contributing records in multiple years. In addition, 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 (which also included some litters for which we did not observe the oestrous bout of the female). 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 we had an adequate sample size (offspring sired = 0 ( $N = 210$ ), 1 ( $N = 54$ ), 2 ( $N = 8$ )) deviated significantly from zero, signifying nonrandom parentage patterns, we used one-sample  $t$  tests. A two-sample Kolmogorov–Smirnov 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 (neonatal mass:  $N = 159$ ; growth rate:  $N = 156$ ) and year (2003, 2004, 2005) was tested as a three-level categorical explanatory term. Because birth date significantly affects both of these traits (J. E. Lane, S. Boutin, M. R. Gunn, J. Slate & D. W. Coltman, unpublished data), we included it 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, we used a logistic GLMM with a binary dependent variable (1 = survived,  $N = 41$ ; 0 = did not survive,  $N = 126$ ). Year was tested as a two-level (2003 or 2004) categorical explanatory term, and female ID ( $N = 56$ ) was again fitted as a random effect, because offspring from the same dam did not represent independent samples.

The GLMMs and LMEs were implemented in S-PLUS (ver. 7.0; [Insightful 2001](#)). We initially included a quadratic term for pairwise genetic relatedness ( $r^2$ ) in these analyses, but removed it, as nonsignificant, in all cases. The least-squares linear regression,  $t$  tests and Kolmogorov–Smirnov tests were conducted using SYSTAT software (ver. 9.0; [SPSS 1998](#)). We determined what statistical power we 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; [Erdfeider et al. 1996](#)). As GLMMs and LMEs are fitted using likelihood procedures (penalized quasi-likelihood and maximum likelihood, respectively;

[Venables & Ripley 2002](#)), power analyses can be most easily conducted using simulation. To test the ability of these models to detect varying effect sizes, we 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, we ran 100 iterations and varied the slope of  $r$  by 0.1. We report the effect size resulting in an estimated power of 0.8 and the detectable response to a change in  $r$  from 0 to  $-0.25$ .

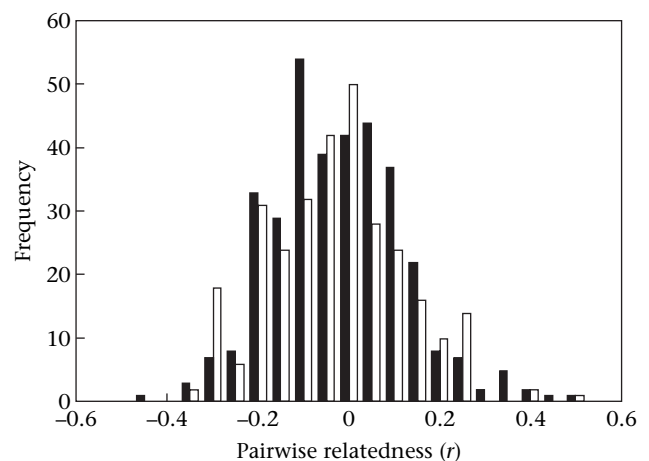
## RESULTS

Over the course of this study we were able to assign paternity to 327 offspring from 139 litters (mean  $\pm$  SE litter size =  $3.0 \pm 0.7$ , range 1–5; incorporating littermates with unassigned paternity). We were 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  $\pm$  SE =  $2.3 \pm 0.1$  sires, range 2–3). Seventy-five of the total number of litters (162 offspring) were from females for which we observed their mating chase (9 females came into oestrus but did not produce a litter).

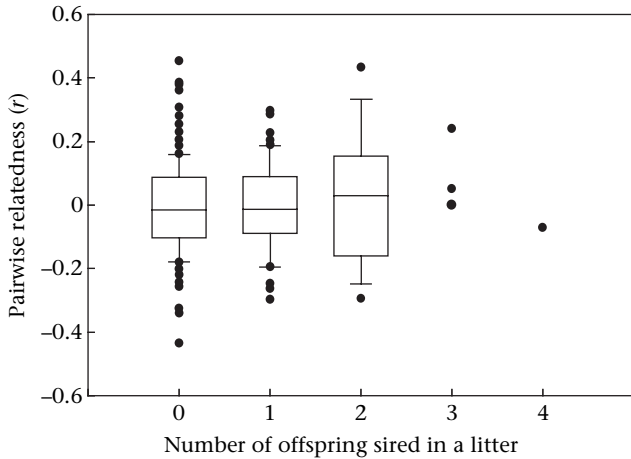
### Genetic Relatedness of Mates and Patterns of Parentage

We recorded a number of instances of assumed inbreeding during our mating-season observations. Nineteen per cent 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. 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:  $\chi_1^2 = 0.31$ ,  $P = 0.58$ ; [Fig. 2](#)). For this analysis, we



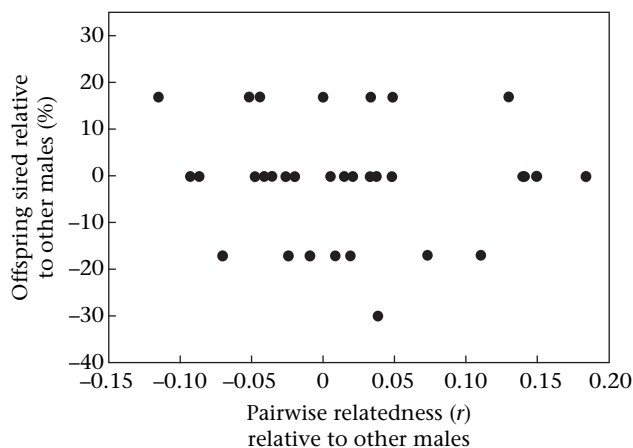
**Figure 1.** Frequency distribution of pairwise genetic relatedness of observed copulatory partners (■;  $N = 345$ ) and parents of offspring born into the population of North American red squirrels (□;  $N = 300$ ).



**Figure 2.** Pairwise genetic relatedness between copulating males and oestrous 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.

should be able to detect a slope of  $-0.6$  for  $r$ . In other words, if the actual  $r$  is  $-0.25$ , then we 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 & 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).

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: 0 offspring sired:  $t_{209} = -0.46$ ,  $P = 0.65$ ; 1 offspring sired:  $t_{53} = -0.53$ ,

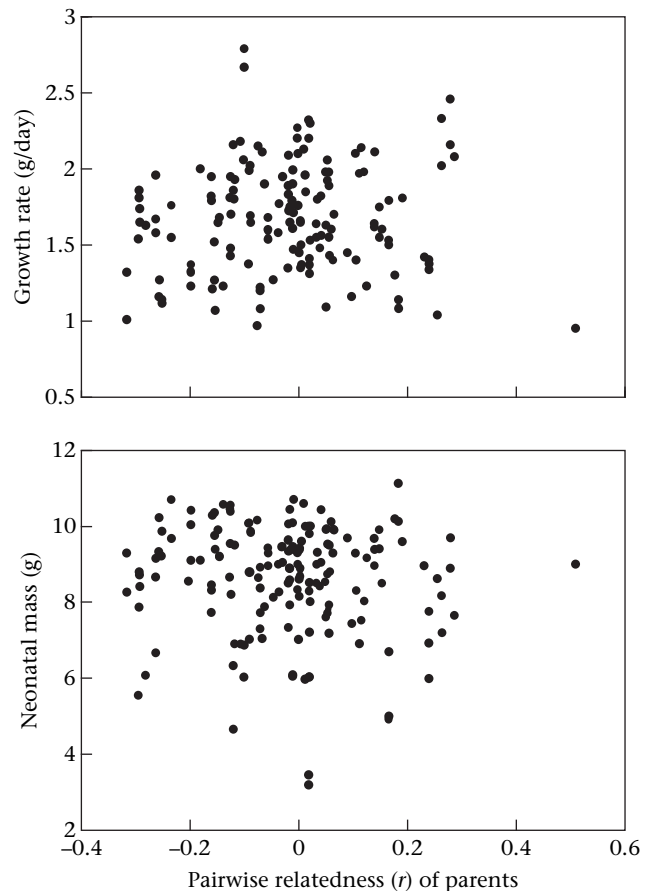


**Figure 3.** Relative number of offspring sired within multiply sired ( $N = 36$ ) litters by males as a function of their relative pairwise genetic similarity to the oestrous female in North American red squirrels.

$P = 0.60$ ; 2 offspring sired:  $t_7 = -0.02$ ,  $P = 0.98$ ), suggesting that the average copulatory pair within each of these groups was 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 postcopulatory mechanisms based on genetic relatedness yielded a distribution of pairwise relatedness of parents of offspring that did not deviate significantly from that of copulatory pairs (two-sample Kolmogorov–Smirnov test:  $t_{644} = 0.08$ ,  $P = 0.28$ ; Fig. 1).

### Parental Genetic Relatedness and Offspring Performance and Fitness

Despite relatively high variation in parental pairwise genetic relatedness (range  $-0.32$ – $0.51$ ,  $N = 159$ ), we found no suggestion that this variable influenced our 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. 4). For these analyses, we should be able to detect slopes of  $-0.8$  (neonatal mass) and  $-0.2$  (growth rate) for parental  $r$  (detectable response: offspring



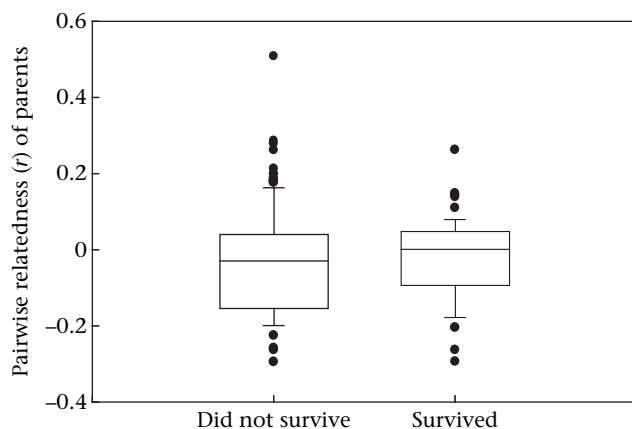
**Figure 4.** Neonatal mass ( $N = 159$ ) and growth rate ( $N = 156$ ) of North American red squirrel offspring as a function of the pairwise genetic relatedness of parents.

should be, on average, at least 0.2 g heavier at birth and should grow at least 0.05 g/day faster). In addition, 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. 5). We 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).

## DISCUSSION

We 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 oestrous female was unrelated to siring success, and relative success of sires within litters was not predicted by relative relatedness to the dam. Furthermore, relatedness did not deviate significantly from zero at any level of siring success and the frequency distribution of successful parents mirrored that of copulating males and oestrous 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 the common shrew, *S. araneus*, where patterns were similar to those found in our study. While copulations between 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), our evidence suggests that these effects may be minimal. Neither the performance, nor the fitness of individual offspring was influenced by the genetic relatedness of their parents. There may consequently be, at most, small selective benefit to



**Figure 5.** 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.

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 having 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 documenting postcopulatory selection for genetically dissimilar sires, many used individuals from populations with high observed (sand lizard, *Lacerta agilis*: Olsson et al. 1996), or inferred (lek-breeding species: agile antechinus, *Antechinus agilis*: Kraaijeveld-Smit et al. 2002; ruff, *Philomachus pugnax*: Thuman & Griffith 2005) levels of inbreeding. In contrast, we used individuals from a large population with high connectivity to other populations. In addition, the mating system of red squirrels is a form of scramble-competition polygyny (J. E. Lane & S. Boutin, unpublished data), with less male reproductive skew than that in traditional resource or female defence polygynous systems. Postcopulatory paternity-biasing mechanisms, however, may also operate in wild populations without pronounced inbreeding (decorated cricket, *Grylodes supplicans*: Stockley 1999), or be lacking in inbred populations (*S. araneus*: Stockley 1997). An explicit test of the hypothesis that the inbreeding history of a population influences currently observed mechanisms of postcopulatory mate selection should evaluate these paternity-biasing mechanisms in natural or laboratory-reared populations of the same species showing relatively high and low degrees of inbreeding.

That female red squirrels do not benefit from multimale mating through the selection of genetically dissimilar sires raises an interesting question about the mating system of this species. Namely, why do females show such high levels of multiple mating? In all sciurid species studied to date, females engage in some level of multimale mating (e.g. Boellstorff et al. 1994; Murie 1995), and explanations for this behaviour in ground squirrels, and most other mammals, largely rely on indirect (genetic) benefits (Jennions & Petrie 2000) or cryptic direct benefits (Hoogland 1998; Wolff & Macdonald 2004). In red squirrels, while there is no evidence of compatible genes hypotheses promoting multimale mating, these represent only one family of genetic postulates. It is plausible that cryptic female choice and sperm competition effects do occur in red squirrels, but these agents are selecting for good genes. Selection has been shown to operate on juvenile growth rate (McAdam & Boutin 2003b) and parturition date (Réale et al. 2003) in our study population, and both of these traits have heritable variation. If genetic variation for these traits is linked to traits of the sperm (a central tenet to good genes hypotheses for sperm competition; Mays & Hill 2004), then good genes mechanisms could

explain patterns of parentage in red squirrels and provide a selective benefit to multimale mating.

Cryptic direct benefits to multimale mating involve either fertility assurance or paternity confusion. In Gunnison's prairie dogs, *Cynomys gunnisoni*, females benefit from multiple mating by ensuring fertilization (Hoogland 1998), whereas in red squirrels, litter size does not vary as a function of number of copulatory partners (statistics not shown), suggesting that fertility assurance is a less likely explanation for red squirrels. Red squirrels do, however, have many characteristics of a mammalian species that is likely to have evolved multimale mating in order to confuse paternity and deter male infanticide (Wolff & Macdonald 2004). Namely, young are born altricial and females come into oestrus following loss of a litter. The extent to which infanticide influences the social and mating systems of tree squirrels is currently unknown. While infanticide has, to date, only been recorded for two species (striped bush squirrel, *Paraxerus cepapi*: de Villiers 1986; eastern grey squirrel, *Sciurus carolinensis*: Holm 1976), the difficulty of observing tree squirrel nests, which are typically high in the forest canopy, precludes many direct observations.

Infanticide avoidance may prove to be an important cryptic benefit, but it does not prohibit genetic benefits from operating, either with respect to compatible genes mechanisms as we have addressed or good genes mechanisms mentioned above. Regardless of whether females realize a direct selective benefit by mating with multiple males, the insemination by multiple males creates a competitive environment through which adaptive traits of the female or male (through sperm traits) could evolve. Female traits to select the sperm from males that will enhance offspring fitness and sperm traits to enhance male fitness should thus be selected for. We propose that future studies investigate the potential separate and combined effects of infanticide avoidance and good genes in the mating system of mammalian species.

In conclusion we 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. We 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 multimale mating in red squirrels and other mammalian species should help to provide insight into the evolution and maintenance of this behaviour.

### Acknowledgments

We are thankful to the 2003–2005 members of Squirrel Camp for help with fieldwork and to Elizabeth Anderson and Ainsley Sykes for coordinating research efforts. Katie Hartnup assisted with genotyping for the molecular paternity analyses, and Craig Aumann provided assistance with the GLMM and LME simulations. Helpful suggestions from

two anonymous referees improved an earlier version of this paper. This study was supported by funds from a National Sciences and Engineering Council of Canada (NSERC) Discovery Grant (S.B.), National Environment Research Council Standard Research Grant (D.W.C, J.S.), and a Department of Indian and Northern Affairs, Canada Northern Scientific Training Program Grant (J.E.L.). An NSERC Postgraduate Scholarship-B and Alberta Ingenuity Fund studentship supported J.E.L. This is paper no. 32 from the Kluane Red Squirrel Project.

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## Appendix

**Table A1.** 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

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

Values are based on the genotypes of 716 individuals (adults and juveniles).