

Microsatellite analysis of multiple paternity and male reproductive success in the promiscuous snowshoe hare

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Abstract: Few genetic studies have addressed patterns of paternity in promiscuous mammals. I used microsatellite DNA primers developed in the European rabbit to analyze paternity in the promiscuous snowshoe hare (*Lepus americanus*). Sixty-five offspring, their 12 mothers, and their 24 putative fathers were genotyped at seven polymorphic loci (3–22 alleles/locus). Paternal allele counts and likelihood-based paternity assignments confirmed that multiple paternity occurs in snowshoe hare litters. However, the estimated frequency of multiple paternity was lower than expected in an unstructured promiscuous mating system. A relatively low variance in male reproductive success indicated that no males dominated paternity. A few males did achieve significantly more paternities than average, largely by fathering one or two complete litters rather than a few offspring in many litters. The results suggest that successful multiple mating is limited among both male and female snowshoe hares. An important role for pre- and (or) post-copulatory competition is implied.

Résumé : Les patterns de paternité des mammifères qui pratiquent la promiscuité sexuelle ont rarement fait l'objet d'études génétiques. J'ai utilisé des sondes d'ADN obtenues chez des lapins d'Europe pour analyser la paternité de lièvres d'Amérique (*Lepus americanus*) qui s'accouplent avec n'importe quel partenaire. J'ai déterminé le génotype de 65 rejetons, de leurs 12 mères et de leurs 24 pères putatifs à sept locus polymorphes (3–22 allèles/locus). Le dénombrement des allèles paternels et l'assignation de la paternité basée sur la vraisemblance confirment que les portées de lièvres peuvent être issues de plusieurs pères. Cependant, la fréquence estimée des paternités multiples s'est avérée moins élevée que prévu dans un système où les accouplements se font sans discrimination des partenaires. La variance relativement faible du succès de la reproduction des mâles indique qu'aucun mâle ne contrôle la paternité. Quelques mâles réussissent plus de paternités que la moyenne, surtout en engendrant une ou plusieurs portées complètes plutôt que quelques rejetons dans plusieurs portées. Ces résultats semblent indiquer que le succès des accouplements multiples est mitigé chez les mâles et les femelles du lièvre d'Amérique. La compétition joue un rôle important avant et après l'accouplement.

[Traduit par la Rédaction]

Introduction

Multiple paternity occurs when offspring from a single litter or brood are fathered by more than one male. The frequency of multiple paternity has important implications for the intensity of sexual selection and sperm competition (Reynolds 1996; Birkhead and Moller 1998; FitzSimmons 1998; Kelly et al. 1999). Relative to single paternity, multiple paternity may also increase the genetic diversity of offspring (Williams 1975), increase effective population size (Sugg and Chesser 1994), reduce inbreeding (Stockley et al. 1993), increase intrapopulation gene flow (Kelly et al. 1999), influence interactions among offspring (Ridley 1993), and decrease estimated genetic correlations in heritability studies (Rhen and Lang 1995).

Genetic studies of paternity have challenged our understanding of mating systems (Reynolds 1996; Birkhead and Moller 1998; Hughes 1998). In mammals, genetic analysis has both revealed multiple paternity in seemingly monogamous species (Goossens et al. 1998) and confirmed strict monogamy (Ribble 1991; Brotherton et al. 1997). While many genetic studies have focused on monogamous species, only 3% of mammalian mating systems are classified as monogamous (Kleiman 1977) and over 90% are considered polygynous or promiscuous (Clutton-Brock 1989). Few genetic studies have investigated patterns of paternity in the latter class of species, but results to date suggest significant variation in the frequency of multiple paternity: 80% or greater has been found in several promiscuous species (Stockley et al. 1993; Boellstorff et al. 1994; Baker et al. 1999), whereas less than 20% has been reported in others (Ribble and Millar 1996; Lacey et al. 1997). Such differences in multiple paternity reflect considerable variation in mating behaviour and reproductive success, much of which had been previously undetected and which remains poorly understood.

The mating system of snowshoe hares is poorly defined. Most species of the genus *Lepus*, including the snowshoe hare, are thought to have promiscuous mating systems

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(Banfield 1974; Flux 1981). In previous studies snowshoe hares have displayed traits consistent with promiscuous mating (Clutton-Brock 1989) such as limited parental care (Graf and Sinclair 1987; O'Donoghue and Bergman 1992), overlapping home ranges (Boutin 1979), and multiple mating in captivity (Graf 1981). However, snowshoe hare behaviour is difficult to observe in the field (Graf 1981) and the mating system has not been well studied. The goal of this study was to examine mating behaviour in a wild population of snowshoe hares by using genetic markers to determine the frequency of multiple paternity and assess male reproductive success. Given that snowshoe hares mate promiscuously in captivity, and that female home ranges are overlapped by those of several males at peak hare densities (e.g., three to seven males; Boutin 1979; Burton 2001), I predicted that the majority of snowshoe hare litters would be fathered by more than one male and that reproductive success would be widespread among males.

Materials and methods

Sample collection

The study was conducted over a 1-km² area near Kluane Lake, Yukon Territory (61° N, 138° W). The local forest is dominated by white spruce (*Picea glauca*) with an understory of grey willow (*Salix glauca*), bog birch (*Betula glandulosa*), and soapberry (*Shepherdia canadensis*), as described by Douglas (1974). I livetrapped hares beginning in May 1999 using Tomahawk traps (Tomahawk Live Trap Co., Tomahawk, Wis., U.S.A.) distributed in areas of high hare activity (i.e., fresh pellets, heavy browsing, runways). Traps were baited with alfalfa cubes, apples, and rabbit chow and were set late in the evening and checked early in the morning. The reproductive stage of any females captured was monitored using body mass and lactational tissue colour and by gentle palpation of the abdomen. Females near parturition were kept in 60 × 60 × 120 cm chicken-wire cages until the young were born (following O'Donoghue and Krebs 1992; see also Stefan and Krebs 2001). A small amount of ear tissue was collected from the mother and each newborn using a 3-mm biopsy punch (Mader Instrument Corp., Stamford, Conn., U.S.A.). Tissue samples were placed in 95% ethanol at the time of collection and frozen within 1–2 h. Immediately after sampling I returned the mother and offspring to the site of her original capture in the field. All adult hares from which tissue was sampled were identified with a Monel No. 3 ear tag (National Band and Tag Co., Newport, Ky., U.S.A.).

Eleven females were taken into captivity during the time of the first litter (24 May – 2 June 1999). Eight of these produced litters between 31 May and 11 June and the other three were released without having given birth. Seven females were taken into captivity between 5 and 11 July 1999, the time of the second litter (five of the seven had produced first litters in captivity). All seven produced litters between 9 and 22 July 1999. I also obtained samples for two additional litters by collecting tissue from two pregnant females (and their fetuses) killed on the nearby Alaska Highway. The average number of offspring per litter was 3.8 (range 1–5). Tissue samples were also collected from 24 adult males trapped near the females to identify potential fathers (Table 1).

One of the second litters was excluded from the multiple-paternity analysis because it contained only one leveret. For the paternity assignment and reproductive-success analyses, the two roadkill females and their litters (10 leverets in total) were excluded, since they were most likely not resident in the same area as the males (Table 1).

Genetic analysis

I extracted DNA from the ear-tissue samples and amplified each sample at eight microsatellite loci originally developed in the European rabbit (Sol03, Rico et al. 1994; Sol33, Surridge et al. 1997; Sat2, Sat3, Sat5, Sat12, Sat13, and Sat16, Mougél et al. 1997). Amplification conditions were similar to those originally described and are detailed in Burton et al. (2002).

I calculated allele frequencies and heterozygosity for each locus over all of the sampled individuals (including offspring) using the program GENEPOP version 3.1 (Raymond and Rousset 1995; Table 2). Each locus was tested for adherence to Hardy–Weinberg equilibrium (HWE) and genotypic linkage equilibrium in GENEPOP using the genotypic data for the adults only (offspring were excluded from these tests to avoid violating the assumption of independent sampling). None of the loci were in linkage disequilibrium and all except Sat5 conformed to HWE. The Sat5 locus showed significant heterozygote deficiency ($P < 0.0001$; see Table 2), presumably owing to one or more high-frequency non-amplifying (null) alleles, and was therefore excluded from any further analysis.

Data analysis

Multiple paternity

The genotype of each offspring in a litter was compared with the mother's genotype to identify the maternal and paternal alleles for each locus. I identified paternal alleles as (i) an allele present in the offspring that is not present in the mother; (ii) an allele present in homozygous condition in the offspring; or (iii) one of the two alleles of a heterozygous offspring with a genotype identical to the mother's (which allele is paternal cannot be determined in this case). If the minimum number of paternal alleles required to explain the observed genetic variation in a litter is greater than two, multiple paternity can be assumed for that locus. The robustness of this assumption increases with the number of different loci that meet the criterion (FitzSimmons 1998).

The presence of only one or two paternal alleles in a litter does not necessarily preclude multiple paternity, as several different males could have contributed the same allele. For this reason I calculated a detection index (d), defined as the probability of detecting the presence of alleles from more than one putative father, given the population allele frequencies (FitzSimmons 1998). This was calculated per locus as

$$d = 1 - 2a_2 + a_3 + 3(a_2a_3 - a_5) - 2(a_2^2 - a_4)$$

where $a_x = \sum_{i=1}^n p_i^x$ and p_i is the frequency of the i th allele for n alleles (Westneat et al. 1987; FitzSimmons 1998). The probability of detecting multiple paternity across all loci (D) was calculated as

Table 1. Numbers of individuals sampled and included in the different analyses.

	No. of mothers	No. of litters	No. of offspring
Total	12	17	65
Multiple-paternity analysis*	12 (10)	16 (14)	64 (54)
Reproductive-success analysis	10	15	55

Note: One litter had to be excluded from multiple paternity analyses, as it contained only one leveret. The two roadkill litters (2 mothers, 10 offspring) were excluded from analyses involving the sampled males, since they were likely resident in a different area. Number of males sampled was 24.

*Value in parentheses are from the CERVUS analysis.

$$D = 1 - \prod_{i=1}^m (1 - d_i)$$

for m loci (Westneat et al. 1987; FitzSimmons 1998).

I also used two likelihood-based paternity-inference methods as alternatives to estimating multiple paternity from direct counts of paternal alleles. The computer program CERVUS, version 1.0 (Marshall et al. 1998) uses the observed multi-locus genotypes to determine the most likely father for each offspring from a pool of candidate males. It calculates statistical significance for these assignments based on simulations using population allele frequencies and estimates of genotyping error and sampling bias. Multiple paternity can be inferred from this program if offspring from the same litter are assigned to different fathers at a high confidence level. Since there is a trade-off between the number of paternity assignments and their accuracy (Marshall et al. 1998), I included results for strict (95%) and relaxed (80%) levels of confidence. Simulation parameters were as follows: 10 000 cycles, 100% of loci typed, an error rate of 0.001, and a pool of 50 candidate males of which 48% were sampled. The error rate was determined by testing the accuracy of maternity assignments for the offspring and known mothers. Candidate-male parameters were estimated based on previously reported densities, home-range overlaps, and capture probabilities for snowshoe hares in the Kluane region (Boutin 1979; Boulanger 1993), and confirmed by consistency between the observed and expected numbers of resolved paternities in CERVUS (for details see Burton 2001).

The program KINSHIP, version 1.3 (Goodnight and Queller 1999), also performs likelihood calculations and determines statistical significance for hypotheses about pedigree relationships between pairs of individuals. The significance of putative father-offspring relationships was assessed using 10 000 pairs of individuals generated from the overall allele frequencies (see Goodnight and Queller 1999). Since the paternity assignments in both KINSHIP and CERVUS depend on the pool of potential fathers sampled, I also used KINSHIP to assess the likelihood that offspring in a litter were full siblings against the null hypothesis that they were only maternal half-siblings. KINSHIP also generated pairwise relatedness (r) values between all offspring. Full siblings are theoretically expected to have $r = 0.5$, whereas half-siblings are expected to have $r = 0.25$, although in practice there is considerable variation around those values (Queller and Goodnight 1989; Blouin et al. 1996).

Male reproductive success

I first examined the distribution of male reproductive success by comparing the frequencies of paternal alleles in the offspring with the allele frequencies in the adult males sampled. If all males were equally successful, the observed paternal allele frequencies should follow the distribution found in the adult males. I performed a likelihood-ratio χ^2 goodness-of-fit test for each locus in program JMP IN (version 3.2.1, SAS Institute Inc.) and calculated Fisher's combined probability across all loci (Sokal and Rohlf 1995). Within a locus, alleles with very low expected frequencies were grouped together. When considering alleles for which the precise frequency in the offspring was uncertain (see *iii* above), I used a conservative estimate that maximized similarity with the adult male frequencies.

Paternity assignments from programs CERVUS and KINSHIP were used to determine how many offspring were assigned to each male. I included CERVUS results significant at a relaxed confidence level of 50% to provide a "maximum" estimate of reproductive success (Coltman et al. 1998). I also used KINSHIP to test the relatedness of offspring independently of the candidate fathers: the number of offspring in a litter that had paternal half-siblings in other litters was estimated and the total proportion of half-sibling relationships determined. If a small number of males were responsible for most of the paternity, it would be expected that a large proportion of offspring from different litters would be classified as half-siblings.

Results

Multiple paternity

Four of 16 litters (25%) showed evidence of multiple paternity from the minimum number of paternal alleles (Table 3). One litter had three different paternal alleles at both the Sat3 and the Sat2 loci, two other litters had three paternal alleles at Sat2, and another litter had a third paternal allele at Sat12. The genotypes at the other loci in these litters and at all loci in the remaining 12 litters could be explained with only one or two different paternal alleles. Direct evidence for multiple paternity was thus seen in 5 of 112 possible cases (7 loci \times 16 litters) and mainly at the most variable locus (3 of 5 cases at Sat2). There were 20 other cases where a third paternal allele was possible, but because the maternal and paternal alleles could not be distinguished (the offspring and mother had the same genotype), the minimum possible number of paternal alleles was two. The probability of detecting an allele from more than one putative father across all seven loci was high ($D = 0.994$). Three of the loci, Sol03, Sat3, and Sat13, had one or two common alleles in the population and thus had low power to detect different paternal alleles (Table 2). Nevertheless, Sat3 did show a third allele for one litter (Table 3). There were three cases at Sat13 where a mismatch occurred between mother and offspring. In all three cases the mother and offspring appeared homozygous for different alleles, indicating the likely presence of a low-frequency null allele.

There was low power to detect multiple paternity in the multi-locus paternity assignment analyses, as many offspring were not assigned fathers with high confidence (14–37 unassigned offspring; Table 4). Estimates of multiple paternity

Table 2. Numbers of alleles, allele size range, observed (H_o) and expected (H_e) heterozygosities, and the probability of detection (d) for each locus; the power to detect multiple paternity over all loci combined (D) is also given.

Locus	No. of alleles	Allele size range (base pairs)	H_o	H_e	d
Sol03	5	268–279	0.396	0.380	0.239
Sol33	8	212–221	0.733	0.774	0.527
Sat2	22	218–255	0.911	0.918	0.839
Sat3	5	138–160	0.485	0.488	0.221
Sat5 ^a	10	197–233	0.426 ^a	0.710 ^a	—
Sat12	7	112–136	0.693	0.706	0.505
Sat13	3	119–123	0.297	0.299	0.163
Sat16	7	95–115	0.733	0.835	0.659
Average ^b	8.38 (8.14)		0.584 (0.607)	0.639 (0.629)	$D = 0.994$

Note: The total number of individuals sampled was 101.

^aSat5 had a highly significant heterozygote deficiency ($P < 0.0001$) and was therefore excluded from further analysis.

^bNumbers in parentheses show the average value excluding Sat5.

Table 3. Results of the multiple-paternity analyses, showing the minimum number of paternal alleles detected for each litter at each locus and the corresponding minimum number of fathers, and the number of fathers assigned to each litter in programs CERVUS^b and KINSHIP.

Litter ^a code no.	Minimum number of paternal alleles at locus:							Minimum no. of fathers	CERVUS ^b		
	Sol03	Sol33	Sat2	Sat3	Sat12	Sat13	Sat16		95%	80%	KINSHIP
7950L1 (3)	1	2	3	1	2	2	2	2	1 ^c	1 ^c	1
7950L2 (4)	2	2	2	2	2	2	2	1	1	2	2
5925L1 (4)	1	2	2	2	1	2	1	1	1	1	1
5925L2 (4)	1	1	2	1	1	2	1	1	1	1	1
418L1 (4)	2	1	2	2	2	1	2	1	0	0	0
418L2 (4)	1	2	2	1	1	2	2	1	1	1	1
8220L1 (3)	2	2	1	2	1	1	1	1	1	1	1 ^c
474L1 (5)	1	2	3	2	2	2	2	2	0	0	1 ^c
8010L1 (3)	1	2	2	2	2	1	2	1	0	0	0
7973L2 (4)	1	2	2	1	2	1	2	1	0	0	0
8270L2 (5)	2	2	3	3	2	1	2	2	0	1 ^c	0
9412L1 (3)	2	2	1	1	2	1	2	1	1 ^c	1 ^c	0
9412L2 (5)	1	2	2	1	2	1	2	1	0	2	2
7901L1 (3)	2	2	2	2	3	1	2	2	1	1	1
RK1 (5)	2	2	2	2	2	1	2	1	—	—	—
RK2 (5)	2	1	2	1	2	1	2	1	—	—	—

Note: Instances of multiple paternity are in boldface type.

^aThe number in parentheses is the number of offspring in the litter.

^bThe results include assignments under both strict (95%) and relaxed (80%) levels of confidence.

^cPaternity was assigned for only one offspring.

for litters in which fathers were assigned to more than one offspring were 0% (CERVUS 95% confidence level (CL)) and 28.6% (CERVUS 80% CL and KINSHIP; see Table 3). None of the four litters in which an extra paternal allele was detected gave a significant multiple paternity result in these analyses. Only 4 of the 16 litters had statistically significant full-sib relationships for all offspring in KINSHIP ($P < 0.05$), but the probability of misclassifying full sibs as maternal half-sibs was relatively high (0.503). A less conservative estimate of sibling relationships was made on the basis of the pairwise relatedness (r) values calculated in KINSHIP. Five litters (31%) had low mean r values (<0.4) with high standard deviations, indicating that some of the litter-

mates were likely half-sibs, and thus suggesting multiple paternity (Fig. 1). Two additional litters had intermediate r values (0.35–0.4), making paternity unclear. Although r values can be influenced by other factors, such as the relatedness of different parents, it seems likely that the large discrepancies between observed and expected values were due to multiple paternity.

Male reproductive success

There was a significant difference between the paternal allele frequencies and those in the adult males when all loci were combined ($P < 0.001$, not shown). This was largely due to discrepancies at Sol33 and Sat2 ($P < 0.01$). While some

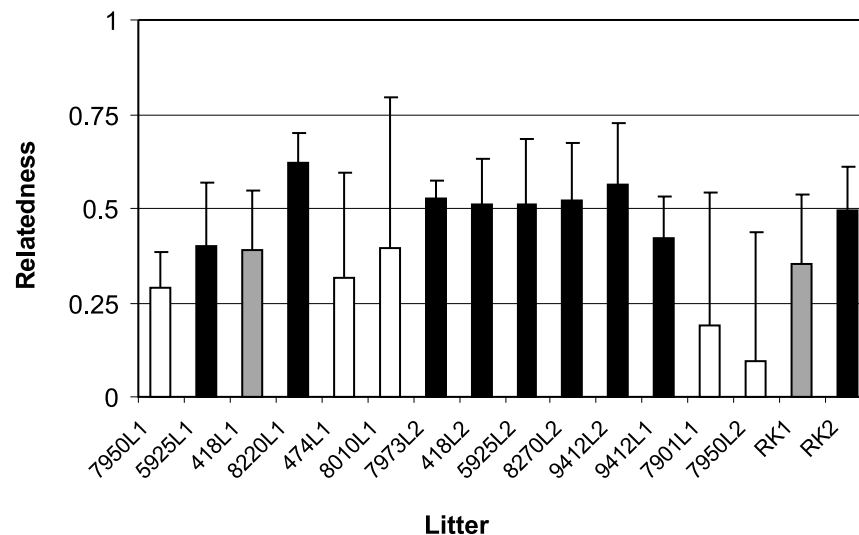
Table 4. Summary statistics for estimates of male reproductive success based on paternity assignments in CERVUS at 95, 80, and 50% confidence levels and in KINSHIP.

Analysis	No. of paternities assigned	Maximum per male	Mean per male	Variance	Standardized variance ^a
CERVUS					
95%	18	8	0.90	4.09	4.55
80%	27	8	1.35	5.71	4.23
50%	41	8	2.05	6.47	3.16
KINSHIP	23	8	1.15	6.34	5.52

Note: Four males that were not assigned paternities and were known to have died before the second oestrus period were excluded from these calculations.

^aThe variance divided by the mean (Boness et al. 1993).

Fig. 1. Mean pairwise relatedness (and standard deviation) among offspring within litters. Relatedness values, calculated in KINSHIP, have a theoretical value of 0.5 between full sibs and 0.25 between half-sibs. Open bars denote litters with values strongly suggestive of multiple paternity and light-shaded bars denote those with marginal results.



alleles were significantly over- or under-represented among the offspring, the majority of the paternal allele frequencies were similar to those in the candidate males (deviation = 0.065 ± 0.041 (mean \pm SD)). Most of the alleles at each locus that were detected in the entire population were accounted for in the paternal alleles (between 66.7 and 100% per locus, mean of 85%). There were 16 different paternal alleles at the most variable locus, Sat2, suggesting that a minimum of eight different males fathered the offspring in the 15 litters. When the number of different paternal alleles was compared with the number of different maternal alleles, there were more paternal alleles at every locus except Sat16, with an average of 1.3 times more alleles across all loci (range = 0.9–2.5).

A large proportion of the sampled males were not assigned paternities in the CERVUS and KINSHIP analyses (Fig. 2). The mean number of offspring assigned per male was thus low (0.90–2.05), and estimates of the standardized variance in reproductive success ranged from 3.16 to 5.52 (Table 4). The paternity assignments indicated that two of the five females whose first and second litters were sampled mated successfully with the same male for both litters, whereas the successive litters of the other three females were probably sired by different males. The mean proportion of relationships between offspring from different litters that

KINSHIP classified as paternal half-sibs was 5.14% ($\pm 2.3\%$ SD). Offspring from a single litter had paternal half-sibs in 25.7% ($\pm 15.0\%$ SD) of the other litters.

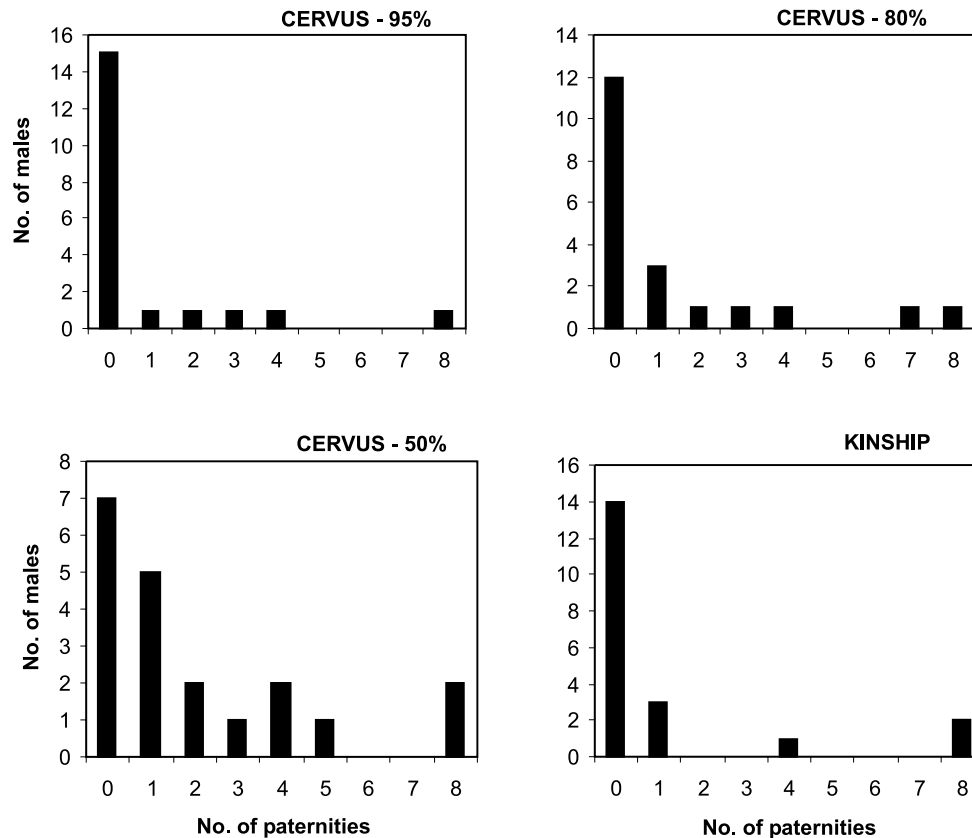
Discussion

Limitations of the analyses

In general, genetic studies of mating systems are affected by the number and variability of markers used (e.g., Blouin et al. 1996; Bernatchez and Duchesne 2000). Failure to detect multiple paternity or assign paternities with high confidence may simply be due to a lack of informative loci. The seven loci that I used showed a relatively high level of polymorphism and thus had a high combined probability of detecting multiple paternity. Using comparable or fewer polymorphic markers, higher levels of multiple paternity and variance in reproductive success have been reported for other species (e.g., Boellstorf et al. 1994; Goossens et al. 1998; Valenzuela 2000). Nevertheless, the power to detect different fathers increases with the number of alleles observed (see Table 2), and the use of more polymorphic loci would likely allow more precise estimates of paternity for snowshoe hares.

Detection of multiple paternity is also limited by the size of the litters sampled in that a minimum of three littermates

Fig. 2. Distribution of paternities among adult males according to CERVUS at confidence levels of 95, 80, and 50% and KINSHIP. Four males not assigned paternities and known to have died before the second oestrus period were excluded. Note that many paternities were unresolved in these analyses (see Table 4).



is required and the power increases with litter size. The litter sizes in this study allowed for the detection of multiple paternity in all but one litter; however, the average size, 3.8 (± 1.1 SD), was small. The addition of more litters might therefore also improve the precision of my multiple-paternity estimates. Similarly, a more complete census of the local snowshoe hare population would strengthen my estimates of the proportions of males and pregnant females sampled from the study area, thus allowing stronger conclusions to be drawn regarding the certainty of paternity assignments and estimates of variance in reproductive success.

Two other factors that can confound paternity analysis are null alleles and mutation (Pemberton et al. 1995; FitzSimmons 1998; Marshall et al. 1998). I found three mismatches between mother and offspring at the Sat13 locus that were most likely due to a null allele. The occurrence of such nonamplifying alleles could have “hidden” true paternal alleles and resulted in false paternity exclusions. However, I reran some of the paternity analyses excluding this locus and the results did not significantly change (data not shown). Some authors consider that an extra paternal allele at only one locus is likely due to mutation and that extra paternal alleles at several loci are the result of multiple paternity (FitzSimmons 1998; Valenzuela 2000). In my study, an extra paternal allele was detected at only one locus for three of four litters and at two loci for the fourth. Furthermore, three of the five extra alleles were found at Sat2, the most variable locus and one that is likely subject to high mutation rates. A mutation rate of 5.6×10^{-3} would be required to

explain the extra alleles by mutation alone, which is within the range reported in the literature (10^{-2} to 10^{-5} ; Jarne and Lagoda 1996). This raises the possibility that I have overestimated the frequency of multiple paternity. Nevertheless, I did not find mother-offspring mismatches for any loci except Sat13 (see above), which suggests that mutations were uncommon and unlikely to explain the observed extra paternal alleles.

Multiple paternity

I conclude that multiple paternity does occur in snowshoe hares. The frequency of its occurrence, however, is more difficult to determine. A conservative estimate, assuming extra alleles at only one locus in a litter are due to mutation (see above), would consider only one litter (6.25%) to have shown multiple paternity. On the other hand, up to 56% of the litters (9 of 16) showed some indication of having multiple fathers. Most of the analyses (i.e., minimum paternal allele count, CERVUS 80%, KINSHIP) suggested an intermediate frequency in the range of 25–31%. This level of multiple paternity confirms that at least some wild female snowshoe hares mate with multiple males during one oestrus period. The potential fitness benefits to females from multiple mating include fertility assurance, procurement of good genes, increased offspring viability, increased genetic diversity of offspring, and less harassment from courting males (Reynolds 1996; FitzSimmons 1998). However, the observed frequency of successful multiple mating in snowshoe hares is lower than expected. During the peak phase of the density

cycle, female hares are likely to encounter several different males during one oestrus period (e.g., three to seven; Boutin 1979; Burton 2001). If a female mated with three males, for example, and each male had an equal chance of fathering an offspring, the probability of multiple paternity in a litter of four would be $1 - (1/3)^4$, or 98.8%. The observed frequency is also lower than the 80–90% reported for several other promiscuous small mammals (e.g., Hanken and Sherman 1981; Stockley et al. 1993; Boellstorf et al. 1994; Baker et al. 1999). In fact, it is close to the range reported for some socially monogamous species (e.g., 34% in alpine marmots; Goossens et al. 1998).

There are several possible explanations for the low level of multiple paternity in snowshoe hares. Firstly, female hares may not frequently engage in multiple mating. Graf (1981) observed multiple mating only in captive hares. The male dominance hierarchies and female breeding dominance that Graf observed may restrict multiple mating in wild hares. Boutin (1979, 1980) suggested that females use their home ranges so as to avoid interactions with neighbouring females, and they may do the same to reduce encounter rates with males. Furthermore, Boutin's observation that both males and females have stable home ranges raises the possibility of stable mating associations. Postcopulatory sperm competition could also limit the number of males that fertilize one female. Sperm competition may influence fertilization in many promiscuous mammal species (Moller and Birkhead 1989; Gomendio et al. 1998). The size of the testes correlates with sperm competition in mammals (Kenagy and Trombulak 1986) and snowshoe hares have relatively large testes (~0.92% of body mass; R. Boonstra, personal communication). According to Kenagy and Trombulak's (1986) allometric relationship between mammalian testis mass and body mass, this corresponds to a relative testis size (observed/predicted) of 1.96, which is consistent with a high degree of sperm competition. The high synchrony of oestrus in female snowshoe hares (Cary and Keith 1979) also suggests an important role for sperm competition in male mating success.

The social and dispersal behaviour of snowshoe hares may also not favour multiple paternity. For example, multiple mating may be advantageous for some female mammals in that it confuses paternity and prevents infanticide by adult males (Agrell et al. 1998), but infanticide has never been reported in snowshoe hares. Multiple paternity can also reduce inbreeding (Stockley et al. 1993), yet there may be little risk of inbreeding in hares, owing to frequent dispersal and low local relatedness (Burton and Krebs 2003). Similarly, multiple paternity may not be needed to maintain genetic diversity if hares have other effective mechanisms, such as high levels of gene flow between subpopulations (Burton et al. 2002).

The phase of the snowshoe hare population cycle (Keith 1990; Krebs et al. 2001) could affect the level of multiple paternity. Other studies have shown considerable variation in multiple paternity associated with changes in population density, habitat structure (Say et al. 1999), and predation pressure (Kelly et al. 1999), all of which change markedly during the snowshoe hare cycle (Hodges et al. 2001; Krebs et al. 2001). I expect that the frequency of multiple paternity would be greatest during the high-density phase (the time of

this study), since increased competition, greater home-range overlap, and elevated predation risk may all promote multiple mating in females (Kelly et al. 1999; Say et al. 1999).

Male reproductive success

The difference between the frequencies of paternal alleles in the offspring and those in the adult males suggests that reproductive success was not evenly distributed among these males. However, given that 15 litters and 24 males were sampled and that multiple paternity was infrequent, it is not surprising that some of the males did not achieve paternity. It is also likely that unsampled males contributed to the observed paternal alleles. Since most paternal allele frequencies were similar to those in the adult males and most of the alleles detected in the population were present among the paternal alleles, reproductive success was not limited to a few dominant males. The observed variation in paternal alleles suggests that a minimum of eight males fathered the 55 offspring. Given that 10 different females contributed the observed maternal alleles, the greater number of observed paternal alleles (by an average of 1.3 times across loci) suggests that more than 10 males achieved paternity.

The generality of the CERVUS and KINSHIP results is limited by the high number of unresolved paternities and the incomplete sampling of snowshoe hares in the study area. The results may thus overestimate the number of males who did not achieve paternity while underestimating those who fathered several offspring, potentially biasing the estimated variance in reproductive success. Nevertheless, the indication is that a few males do obtain considerably more paternities than average, generally as a result of fathering many offspring from one or two litters (rather than achieving paternity in many different litters). The low proportion of paternal half-sibs estimated in KINSHIP also suggests that individual males did not mate successfully with many different females. This implies that males are not able to fertilize all the females that they encounter. Estimates of the standardized variance in reproductive success (3.16–5.52) are consistent with those expected from low to moderate levels of polygyny (~1–5; Boness et al. 1993; Coltman et al. 1998, 1999) and are comparable to levels found in the socially monogamous white-toothed shrew, *Crocidura russula* (~3–5; Bouteiller and Perrin 2000). The indication is that male reproductive success is limited by competition in snowshoe hares, but that sexual selection is not likely to be very intense.

Relative trapping locations of the males and females provide supporting evidence of competition for mates with unequal reproductive success. For instance, eight different males were trapped at the same trap locations as four females; however, only three of these males were assigned paternity for their offspring. None of the other five males were assigned any paternities with high confidence, implying that they were outcompeted by the successful males. In two instances, males fathered offspring from the same female in successive litters despite the presence of several other males in the same area. It is unclear whether the females were demonstrating mate fidelity by choosing the same mate over time, or if the fathers were simply stronger competitors. Such questions warrant further investigation, since this species is not known to form any pair bonds.

In summary, expectations of unstructured promiscuous mating in snowshoe hares were not supported by my results. While the genetic evidence confirms that female hares have multiple mates, and that male reproductive success is not limited to a few dominant individuals, the finer complexities of the snowshoe hare mating system remain to be elucidated by combining these genetic techniques with detailed behavioural and experimental analysis.

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