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Mating patterns and reproductive success in the bushy-tailed woodrat (*Neotoma cinerea*), as revealed by DNA fingerprinting

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Abstract The mating patterns and reproductive success of the bushy-tailed woodrat (*Neotoma cinerea*) were investigated over a 3-year period (1992–1994) using DNA fingerprinting. Paternity was determined by genetic analysis of 58 juveniles of known maternity from 35 litters. Analysis of DNA fingerprints revealed that all offspring within a litter were fathered by a single male; the statistical probability of detecting multiple males mating with a female was high, indicating that multiple paternity would have been detected had it occurred. However, individual males did not father more than one litter from a given female either within or between years. At least 75% of females and 57% of males successfully produced offspring each year. The finding that all littermates are first-order relatives may contribute to the high level of female cooperation in this species.

Key words *Neotoma cinerea* · DNA fingerprinting · Reproductive success · Paternity

Introduction

Mating system theory has generally proposed that the mating system of a particular species can be assessed based on the degree of parental care, the ability to monopolize mates, variance in male and female reproductive success, and the spatial distribution of the sexes (Reynolds 1996). While early investigations were mostly restricted to assessing the mating system based on spatial distribution and/or copulatory behavior (Emlen and Oring 1977), more recent approaches have utilized genetic techniques such as DNA fingerprinting and electrophoresis to quantify mating success (see Queller et al. 1993; Avise 1994).

In many cases, quantification of reproductive success has confirmed the classification of the mating system based on behavioral data (Burke et al. 1989). However, some investigations into the mating systems of birds have revealed that the pattern of reproductive success within the population could not have been assessed from behavioral observations, leading to major revisions in the classification of the mating system of many avian species (Gowaty and Karlin 1984; Põldmaa et al. 1995).

While studies combining the results of behavioral observation and genetic analyses of paternity in birds are common (Westneat 1990; Pinxten et al. 1993), there are fewer investigations into mammalian mating patterns that combine field-based behavioral data with genetic analyses of paternity. Within this group of investigations, small mammals have been the focus of many studies (Ribble 1991; Ribble and Millar 1996; Travis et al. 1996). Such organisms are ideal for these studies because they are well studied in terms of other aspects of behavior and ecology. In addition, they have short gestation periods, frequently occur at high densities and usually have more than one offspring per litter, making investigations into multiple paternity possible.

The application of genetic techniques to the study of small-mammal populations has demonstrated that many species exhibit some level of multiple paternity within litters (Xia and Millar 1991; Boellstorff et al. 1994; Travis et al. 1996). In contrast, monogamous species of small mammals exhibit no multiple paternity (Ribble 1991). Therefore, given that many small mammals have access to multiple mates, and that often a considerable number of females mate with multiple males (Xia and Millar 1991; Boellstorff et al. 1994; Travis et al. 1996), there must be some inherent benefits for females that mate with more than one male (Boonstra et al. 1993). Many hypotheses explain the benefits of multiple mating by females (see Reynolds 1996 for review), including

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lowered probability of infanticide by mated males (Wolff 1985) and increased probability of successful fertilization (Dewsbury 1984).

The bushy-tailed woodrat (*Neotoma cinerea*) is a nocturnal cricetid rodent, distributed throughout the mountainous regions of western North America, from the Yukon to New Mexico (Burt and Grossenheider 1976). Throughout this range, its local distribution is constrained by the number of suitable den sites, which are located in rocky outcrops, talus slopes, and caves (Finley 1958, Escherich 1981). This reliance on suitable rocky habitat primarily for thermoregulatory reasons (Brown 1968) forces *N. cinerea* to establish nest sites that are often located at large distances from food (Topping and Millar 1996a). In addition, this species shows relatively high levels of cooperation among related females, usually resulting in daughters experiencing a greater probability of survival to reproductive maturity in the presence of her mother (Moses and Millar 1994). Previous research has suggested that the mating system is harem polygyny, due to the high degree of sexual dimorphism (Finley 1958; Escherich 1981), the asynchrony of estrus (Egoscue 1962), and the aggregated distribution of females (Escherich 1981; Hickling 1987). However, we have recently documented the spatial distribution of males and females (Topping and Millar 1996a), which revealed that both males and females overlap considerably with the other sex.

The purpose of this study was to measure the male reproductive success of *N. cinerea* using DNA fingerprinting to determine the paternity of all juveniles born during the period of study. By evaluating the variance in male reproductive success relative to that of females, and examining litters for evidence of multiple paternity, the pattern of male mating success and male mating strategies can be determined. The ecology of *N. cinerea* makes it an attractive species for an investigation of reproductive behavior. The potential for interaction with a large number of males (Topping and Millar 1996a), coupled with the high level of female cooperation and kin selection in the population (Moses and Millar 1992, 1994), provides a unique opportunity to investigate whether the benefits of multiple paternity are offset by the benefits of kin selection, which are more likely to occur in closely related litters. An understanding of the extent to which litters with high or low genetic relatedness occur in *N. cinerea* may provide important information on the role of mating behavior in determining social interactions within the population.

Methods

Live trapping and tissue collection

The study was conducted in the foothills of the Canadian Rocky Mountains, southwestern Alberta (51°N, 115°W), from April to September, 1992–1994. These months overlap the breeding season of *N. cinerea* in this location (Hickling 1987; Moses 1992). *N. cinerea* from four adjacent rocky outcrops (170–300 m in length) were monitored by weekly live trapping and radio telemetry over a study area of approximately 30 ha (Topping and Millar 1996a). All animals were ear tagged (National Band and Tag model 1005-1), and weight (g), body length (mm), and reproductive status were recorded at each capture. Males were assessed as having scrotal (reproductive) or abdominal (non-reproductive) testes. Females were assessed as pregnant (distended abdomen and weight gain of >30 g from last capture), lactating (raised nipples and hair loss around nipples), pregnant and lactating, or postreproductive (no signs of pregnancy or lactation). Females undergo postpartum estrus, resulting in some females being simultaneously pregnant and lactating. Usually, females produce one to two litters, each of one to five young per litter in southwestern Alberta, but rarely give birth to more than four individuals within a litter (Moses 1992). Radio transmitters (Holohil Systems, model no. PD-2C) were fitted to all reproductively active, resident animals in early May. For the purpose of this study, residents were classed as previously captured and overwintered animals, or animals captured on at least two occasions during the first 3 weeks of study in each year. Den sites were located during the day, using radio telemetry. Female *N. cinerea* are known to occupy a single den for the duration of the breeding season (Hickling 1987; Moses 1992). Radio telemetry was also used to identify the location of male dens. If an individual was not fitted with a transmitter, we determined den site by live trap location in proximity to known den sites. Animals of both sexes were considered to be residents on the outcrop containing their den site.

To perform genetic analysis on the population, a sample of tissue was taken from both ears at the initial capture of each individual. Tissue samples were stored in 1.5-ml cryovials, and kept in a thermos flask containing cold packs until transfer to a −70 °C freezer within 2.5 h after collection.

Maternity of weaned juveniles captured on the study area was assessed by the estimated age of the juvenile, and location of capture relative to female den sites. Weight of the juvenile was used to estimate age and birth date, within ± 7 days (Moses 1992). Date of conception was calculated as parturition date minus 30 days gestation (Egoscue 1962). The estrus period (duration of female sexual receptivity) of bushy-tailed woodrats is 4–6 days (Egoscue 1962; Escherich 1981), so for the purpose of this investigation, estrus was defined as the 5-day period prior to conception. Estimated birth date, combined with the reproductive status of resident females on the outcrop where the juvenile was captured, was used to identify the maternity of juveniles. Asynchrony in the estrus periods of *N. cinerea* also aided in the identification of the juveniles' dam. If maternity could not be confidently determined from demographic data, DNA fingerprinting was used to identify the mother. The genetic relatedness (D) between juveniles and potential mothers was calculated as $D = 2 \times$ (bands shared)/number of female bands + number of juvenile bands (Wetton et al. 1987). Maternity was assessed by comparing the D value from known mother-juvenile relationships.

RFLP fingerprinting protocol

Tissue samples (20–55 μg) were digested following Ribble (1991). Genomic DNA was isolated by a series of phenol:chloroform extractions, then precipitated in 100% ethanol, pelleted out via centrifugation, and suspended in 50 μl of 1 × TBE. Samples were stored at 4 °C. Ten micrograms of genomic DNA was subsequently digested by adding *Alu*I to a restriction solution containing 10 mM spermidine (Maniatis et al. 1982). Differential-sized fragments within each digested sample were separated by electrophoresis (Thorne 1967) on a 15 cm × 20 cm 0.8% agarose gel for 18–20 h at 2 V/cm. Gels were washed twice in denaturing solution (1.5 M NaCl, 0.5 M NaOH) resulting in single-stranded DNA. This was followed by three washes in neutralizing solution (2.5 M ammonium acetate), after which the DNA was transferred to a nitrocellulose membrane (Schleicher and Schuell) by Southern

transfer (Southern 1975), using 1 M ammonium acetate, 0.02 M NaOH.

We used an RNA template ($[AGGGCUGGAGG]_{54}$) analogous to probe 33.6 (Jeffreys et al. 1985a) in the hybridization reactions (Ribble 1991). The probe was radiolabelled with ^{32}P during a transcription reaction (Ribble 1991). Phenol:chloroform and chloroform extractions were used to remove any non-specific transcription. Membranes were exposed to a hybridization solution ($5 \times$ SSPE, DI formamide, $1 \times$ Denhardts solution, 12,000 μg tRNA and dextran sulfate) containing the radiolabelled probe for 18–24 h. Membranes were then washed in $2 \times$ SSPE, 0.2% SDS at 55–65 °C until background counts reached 1–3000 cpm, after which they were exposed to X-ray film (Kodak X-OMAT AR) with intensifying screens for 5–25 days.

Blot scoring and identification of putative father

Since female home ranges overlapped with the home ranges of more than one male (Topping and Millar 1996a), assessment of a putative father based on behavioral data was not possible. In addition, such a restricted analysis could preclude the possibility that a non-resident male fathered all or part of a litter. Therefore, DNA samples from all males captured at least once in a given year were run on gels containing samples from the mother and offspring. These included all resident males from the study area in each year. Furthermore, the nearest suitable woodrat habitat (located 1–5 mean home range diameters from the study area; Topping and Millar 1996b) was trapped, and tissue samples taken from all animals captured. Males captured on the adjacent habitat were included on the gels, to ensure that they could not be the father of any offspring born on the study area. Due to the large number of males in 1992 and 1994, multiple gels were run for each maternal lineage in order to compare the fingerprints from mother and juveniles with all potential fathers. All radiographs were scanned on a Bio-Rad Gel Doc 1000 system, which allowed each lane of the gel to be scanned separately. The resultant output was an optical density graph, where the individual fingerprint consisted of a series of peaks, representing the bands (Fig. 1). The Bio-Rad Gel Doc software permitted two or more graphs to be overlaid, resulting in accurate comparison of banding patterns. As restriction using *Alu*I resulted in a large number of small (<2 kb) restriction fragments, bands were scored between 23 and 2 kb on all radiographs. Bands in the juveniles lanes were assessed as either maternally or paternally derived, based on position (± 1.0 mm; Burke and Bruford 1987) and intensity (height of the peak on the resulting graph). For the purpose of paternity identification, we considered only those bands that did not derive from the mother, i.e., all bands that were

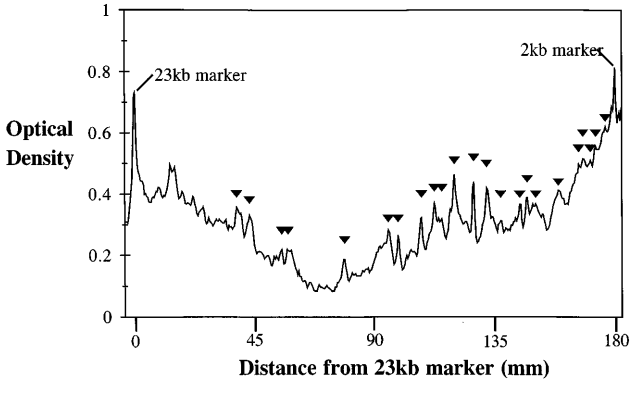

Fig. 1 Plot of output from optical scan of a DNA fingerprint. Optical density is graphed against distance from the 23-kb marker. Distinct peaks indicate the location of a band. Positions of bands ($n = 23$) on the autoradiograph are denoted by *solid triangles*. The two large peaks on this graph represent markers at 23 and 2 kb

either paternally inherited, or shared by the father and the mother. When a juvenile shared bands with both father and mother (i.e., those that could not be assigned to just one parent), they were always assigned as maternally inherited. This reduced the number of diagnostic bands that a father shared with a juvenile, thus reducing his coefficient of relatedness (Wetton et al. 1987), but ensured a higher probability of classifying only the true father as the sire. By considering only those bands that could have been transmitted from the father (diagnostic paternal bands), we identified a putative father. Males were considered putative fathers if they possessed all or all but one of the diagnostic bands, to allow for novel bands arising from mutation (Jeffreys et al. 1985a,b).

To determine whether the putative father could be reliably assigned as the genetic father, we calculated the probability that (1) an unrelated male possessed all the diagnostic bands, and (2) a related male possessed all the diagnostic bands. This value was calculated as $p = X^m$ (Jeffreys et al. 1985b), where X is the degree of band sharing among unrelated males, and m, the number of diagnostic bands employed in the identification of a putative father for each litter. In contrast, $p = [(1 + X)/2]^m$ for related males (Hill 1986). Following Ribble (1991), we assumed equal allele frequencies, unlinked loci, and an equal probability of detecting differences across fragment sizes (Jeffreys et al. 1985 a,b; Hill 1986; Cohen 1990).

Probability of detecting multiple paternity

Because mammalian ova are usually ovulated and fertilized simultaneously, multiple paternity will result only if viable sperm from more than one male are present in the oviduct at the time of ovulation (Gomendino and Roldan 1993). It has been suggested that sperm competition in mammals resembles a raffle, where the male with the largest amount of sperm is more likely to fertilize the ova (Parker 1984; Parker et al. 1990). The manner by which sperm competition may operate in *N. cinerea* is unknown, but studies on other rodents have demonstrated first male (Martan and Sheppard 1976; Huck et al. 1985; Foltz and Schwagmeyer 1989), last male (Dewsbury and Baumgardner 1981; Oglesby et al. 1981) or no order advantages (Dewsbury and Hartung 1980; Kenagy and Trombulak 1986). Overall, it appears that the probability of paternity is directly related to the proportion of viable sperm present at the appropriate time for fertilization to take place. We therefore developed a model to evaluate various sperm competition scenarios, which could result from any of these three situations.

The small weaned litter sizes of *N. cinerea* may result in a low probability of detecting multiple paternity within a litter, even if more than one male mates with a female. Because such a distinction is vital to determining the reproductive strategies of both males and females, we quantitatively describe the probability of observing single paternity in a litter of two or more juveniles, even if more than one male had mated with the female. As a conservative estimate, we considered a scenario where, if multiple mating occurs, only two males mate with a female. The probability that male A or B fertilized a given ovum were set at P_A and P_B, respectively, for a number of sperm competition scenarios (i.e., for a 1:1 fertilization probability ratio, $P_A = P_B = 0.5$). The probability of male K siring all juveniles within a litter is P_K^N, where N is the weaned litter size. Therefore, within a litter of size N, the probability of observing single paternity ($P_{sp(N)}$) is $\sum(P_K^N)$. From there, we can calculate the probability that we detect multiple paternity within a single litter ($P_{mult(N)}$) as $[1 - (P_{sp(N)})] \times R$, where R is the proportion of litters that involve more than one male mating with a female. For this R value, the probability of not detecting at least one litter with multiple paternity (P_{nm}), can be calculated as $[(1 - P_{mult(N)})^{L2}] \times [(1 - P_{mult(N)})^{L3}]$, where LN = number of litters of size N. The corresponding probability of detecting multiple paternity is therefore $(1 - P_{nm})$, or P_m.

The mechanisms of sperm competition are unknown in *Neotoma*, so P_m was calculated for five different sperm competition scenarios involving different ratios of viable sperm from males A and B that are present in the female reproductive tract at the time

of ovulation. These ranged from a "fair raffle," where each male had an equal chance of fertilizing a given offspring, to a scenario where male A had a five times greater chance of fertilizing a given ovum (5:1 sperm ratio). A 5:1 ratio was chosen as the upper limit, as it represents a sperm competition scenario where one male has a much higher chance of fertilizing an ovum. On the basis of previous studies investigating multiple paternity, the ratio of offspring fathered by one male to the other has been reported as 3.2:1–1.27:1 for *Peromyscus maniculatus* (Dewsbury and Baumgardner 1981), 2:1–1.56:1 for *Spermophilus tridecemlineatus* (Schwagmeyer and Foltz 1990), and 1.63:1–1.12:1 for tree swallows (Barber et al. 1996). As a 5:1 sperm ratio greatly exceeds these reported values, we are confident that this represents an upper limit in our model. By calculating P_m for each sperm competition scenario over a range of R values (0–100%), we plotted a graph of P_m versus R. For each curve, we determined the proportion of litters resulting from two males mating with an estrus female that resulted in a significant probability of our being able to detect multiple paternity (i.e., $P_m \geq 0.95$).

Male reproductive success within a year was defined as the number of offspring sired by each male that survived until emergence following weaning. To confirm that the reproductive success of resident males was restricted to the study area, the outcrops on either side of the study area were also trapped, and tissue samples taken from reproductively active females and their juveniles. The DNA fingerprints of juveniles from the nearest suitable habitat to the study area were then analyzed to determine whether litters outside the study area were sired by males resident on the study area.

All values are reported as mean (±1SE), except where noted otherwise. Statistical significance was accepted at $P \leq 0.05$.

Results

Litter size and maternity

A total of 58 weaned juveniles from 35 litters was recorded on the study area. In 27 litters, the maternity of juveniles could be assigned, based on the capture location relative to the den of a female, and the stage of reproduction of the female. In the remaining 8 litters, maternity could not be determined from demographic data, but was restricted to one of two potential mothers, whose den sites and date of parturition were relatively close. In these cases, maternity was assessed using DNA fingerprinting. The mean (±SD) genetic relatedness (D) for all known mother-offspring pedigrees was 0.57 ± 0.08 (range = 0.40–0.71). This range of values was used to determine maternity in the 8 litters where maternity was uncertain. In these litters, one female showed a genetic relatedness value that was within the range of known values (mean = 0.53, range = 0.45–0.59), while the other female had a D value that was outside this range (mean = 0.29, range = 0.17–0.38). The female with the largest D value was identified as the mother of the offspring, provided that the other female had a D value that fell outside the range of 0.40–0.71. There was no difference in the size of successful litters among years ($F = 0.28$, $df = 2,32$, $P = 0.756$).

DNA fingerprinting and paternity identification

Pedigrees were analyzed by DNA fingerprinting for all 35 litters. The mean (±SE) number of bands scored for mothers, fathers, and offspring are shown in Table 1. Due to the protocol employed for paternity analysis, the proportion of bands shared between father and juvenile was often low (min. = 0.27). However, these values reflect the small number of diagnostic paternal bands in some litters, rather than an indication of the accuracy of determining paternity. A comparison of the proportion of juvenile bands shared by the putative father and the next most likely father demonstrates that the closest other male always shared a much lower proportion of bands than the putative father (Fig. 2). Having identified a putative father, the probability of having incorrectly identified him as the sire of the litter was calculated. The mean proportion of shared bands among unrelated males was 0.14 (range: 0.05–0.25). This was calculated by comparing the banding patterns of seven

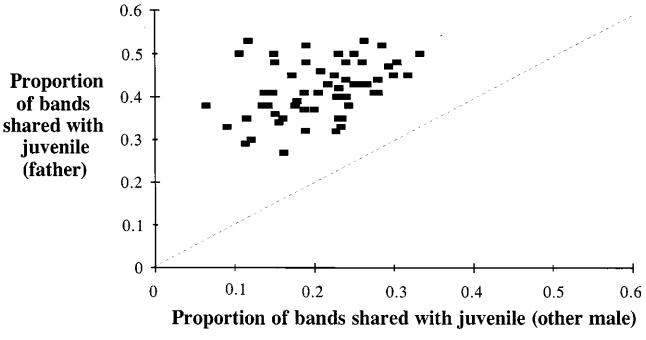

Fig. 2 Plot of the proportion of juvenile bands shared by the father against the highest proportion of bands shared by another male for each juvenile ($n = 57$). The *line* represents a situation where both proportions (father and other male) are the same for a single juvenile

Table 1 Summary of gel scoring data. The mean (±SE) number of bands scored for mother, fathers and juveniles across all gels. The mean (±SE) number and proportion of juvenile bands shared for both mothers and fathers are also shown. Ranges are given in parentheses

	Mother (n = 35)	Father (n = 35)	Juvenile (n = 58)
Mean (±SE) number of scoreable bands	28.3 ± 0.7 (16–37)	28.3 ± 1.2 (18–38)	278 ± 0.8 (17–39)
Mean (±SE) number of juvenile bands shared	16.3 ± 0.6 (8–27)	11.3 ± 0.3[a] (7–16)	
Mean (±SE) proportion of juvenile bands shared	0.58 ± 0.01 (0.47–0.73)	0.41 ± 0.01[a] (0.27–0.53)	

[a] Values for males refer to paternally inherited bands only. Bands shared by mother, father and juvenile are not considered as paternally inherited

males, each from a geographically distinct location outside the study area. The distance between trapping areas used to capture these males was ≥10 km; therefore, the males were likely to be unrelated. The chance of an unrelated male possessing the same diagnostic paternal bands ranged from 1.1×10^{-6} (7 paternal bands) to 2.2×10^{-14} (16 paternal bands). The corresponding calculation for related males showed that the probability of a related male having the same pattern of diagnostic bands ranged from 1.9×10^{-2} (diagnostic bands = 7) to 1.2×10^{-4} (diagnostic bands = 16). These values are in the same range as values reported by Ribble (1991). Given these probabilities, and the fact that all males were included in the paternity analysis, it was concluded that the putative father could be assigned as the genetic father, even when the proportion of bands shared with the juvenile was low (Fig. 2). Assuming correct maternity and paternity for all pedigrees, the mutation rate (i.e., the number of juvenile bands that were not maternally or paternally transmitted) was 8.7×10^{-3} (14/1611). No more than one mutation was observed in any juvenile. In addition, all males that were classed as resident on the study area could not be assigned as the father of offspring born on the next closest habitat suitable for woodrats. Therefore, the reproductive success of all resident males and females was restricted to litters born on the study area.

Characteristics of mating patterns

Complete pedigrees for all individuals born on the study area are shown in Fig. 3. The mean, variance and range of reproductive success for males and females on the study area in all years are shown in Table 2. Comparison of the variance in male and female reproductive success (variance ratio test, Zar 1984) revealed no significant differences in 1992 ($F = 2.46$, $df = 13,15$, $P = 0.1$), 1993 ($F = 1.54$, $df = 7,10$, $P = 0.5$), or 1994 ($F = 1.04$, $df = 13,8$, $P > 0.5$), or when data from all years were combined ($F = 1.61$, $df = 35,35$, $P > 0.1$).There was no bias in the age of those animals that failed to produce any offspring in either males or females (yearlings vs adults). Reproductively active female woodrats produced zero to two successful litters per year (Fig. 3). Male woodrats fathered zero to three litters during the breeding season, although a male never successfully produced offspring with the same female twice (either within or between years). No incestuous matings were observed during the 3 years of study (Fig. 3).

Of the 35 litters born over the period of study, 17 consisted of more than one juvenile (Fig. 3). Within these 17 litters, there was no evidence for multiple paternity, which suggests that the male that sired the litter was the only male with which a female mated. To test this suggestion, the probability of detecting single paternity, when more than one male mated with a female was calculated. Fig. 4 shows the probability of detecting multiple paternity (P_m) when two males mate with an estrus female in 0–100% of litters (% multiple mates) for five different sperm ratios. Arrows indicate the value for % multiply mating females above which there is a statistically significant probability of detecting multiple paternity (i.e., $P_m \geq 0.95$) for each sperm ratio curve. This value ranges from 27.5% of females (sperm ratio = 1:1) to 48.5% of females (sperm ratio = 5:1).

Over all years, six females produced a second litter within a season, all of which were sired by a male other than the one that sired the first litter. In five of the six cases, the sire of the first litter was still present and reproductively active on the study area. In addition, seven females produced litters in more than one year. For four females, the sire of the litter from the previous year was no longer present on the study area. However, in the other three cases, the previous mate was present, yet the litter was fathered by another male.

The majority of litters (23 of 35) resulted from matings between males and females from the same outcrop. The remaining 12 litters (34%) resulted from a pairing of animals from adjacent outcrops. Home range data were available for 22 male-female pairs that successfully produced offspring (Topping and Millar 1996a). All animals that produced offspring together had overlapping home ranges during the breeding season. However, each females' home range was overlapped by an average of 5.6 males for all years, and the male that overlapped the most with the female (over the duration of the breeding season) fathered a total of only 4/22 litters (18%). Because the radio telemetry protocol involved recording information on as many individuals as possible in a session, we did not obtain any detailed information on the movements of estrus females and their location relative to males. If an outcrop had more than one resident female, no male was able to monopolize all the females on the outcrop, even if he was the only resident. When females on the same outcrop were in estrus at the same time ($n = 6$), their litters were fathered by different males. The duration between the start of estrus periods of different females that produced litters with the same male is shown in Fig. 5. The mean duration was 18.5 days (range: 5–40), which is considerably longer than the duration of estrus.

Discussion

Mating patterns of male and female *N. cinerea*

Because all females were physiologically capable of reproduction (i.e., all females present within a year attempted at least one litter, as indicated by a significant weight gain or other sign of pregnancy), it is not surprising that at least 75% (Table 2) of females in each year produced a successful litter. Any variation in the reproductive success of females appears to be due to the differential survival of litters, the number of litters

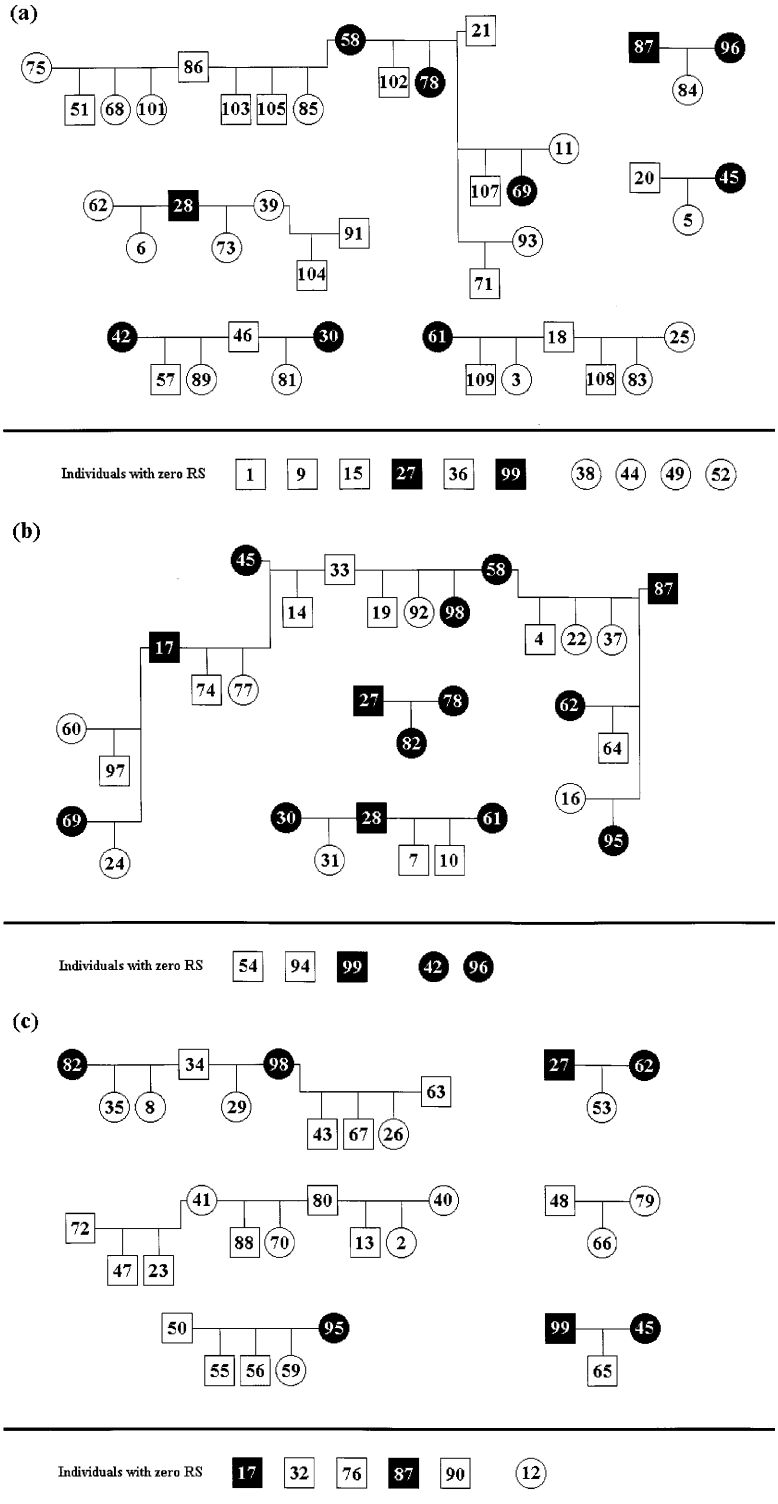

Fig. 3 Pedigrees for all litters born on the study area in 1992 (**a**), 1993 (**b**), and 1994 (**c**). Males and females represented by *dark squares* and *circles* are individuals that were present on the study area in more than one year. Multiple litters produced by females are indicated by being offset on different horizontal levels (*RS* reproductive success)

produced, and the litter size at birth, rather than the opportunity to breed (Moses 1992). Although the proportion of males achieving successful matings (57–63%) was lower than for females (75–89%), the variation in reproductive success was similar between the sexes in all years, indicating that among males, the distribution of reproductive success and mating opportunities was relatively broad (Fig. 3).

The lack of multiple paternity in this study may be attributed to one of three factors. First, it may be that females are mating with more than one male during estrus, but are doing so at extremely low frequencies, leading to a low probability of detecting multiple paternity. However, this is an unlikely explanation, since all litters over a 3-year period were monitored, and no case of multiple paternity within a litter was observed. In

Table 2 Mean, variance and range of reproductive success for males and females (1992–1994). The proportion of resident animals of each sex that successfully produced offspring in each year of the study is also given

Year	Sex	*n*	Mean	Variance	Range	Proportion producing offspring
1992	Male	14	1.64	4.25	0–6	57%
	Female	16	1.44	1.73	0–5	75%
1993	Male	8	2.13	4.41	0–5	63%
	Female	11	1.55	2.87	0–6	82%
1994	Male	14	1.29	2.07	0–4	57%
	Female	9	2.00	2.00	0–4	89%

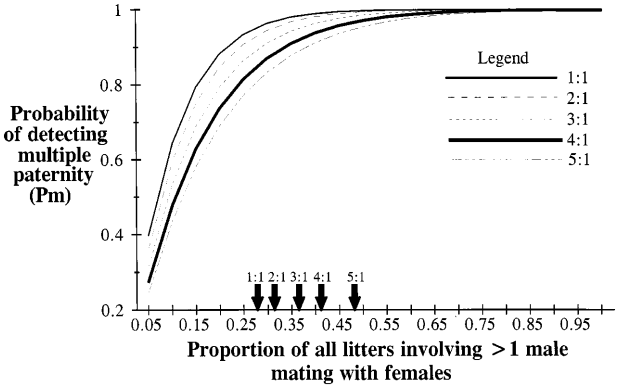

Fig. 4 Plot of the probability of detecting multiple paternity (P_m) against the proportion of litters where two males mate with the female during estrus. The *lines* represent different fertilization probability ratios. *Arrows* denote the percentage of matings above which there is a statistically significant probability of detecting multiple paternity for each fertilization probability ratio

addition, we would have a statistically significant probability of detecting multiple paternity if 27.5–36.5% of females mate with more than one male (Fig. 4). Because these values are relatively low compared to the majority of species already studied (Table 3), and given that all females exhibit significant home range overlap with more than one male (Topping and Millar 1996a), we suggest that there is strong evidence to reject the idea that females are mating with multiple males, and we are simply failing to detect it.

Alternatively, we may have been unable to detect multiple paternity using DNA fingerprinting as our method of paternity assessment. We also reject this option, because we were able to unambiguously assign paternity to each juvenile, preventing any misidentification of a juvenile's father.

The third explanation for the mating patterns observed is that a single male is fathering all offspring within a litter, either by being the only male that the female mates with during estrus (exclusive access), or by being the only male to mate with the female at the appropriate time in her estrus cycle to ensure paternity (temporal access). In either situation, the male ensures that only his sperm is capable of fertilizing the eggs released by the female. Female bushy-tailed woodrats undergo a postpartum estrus of 4–6 days (Egoscue 1962; Escherich 1981), and laboratory studies have indicated that many females became pregnant soon (24–48 h) after birth of the previous litter (Escherich 1981). Therefore, in order for a male to monopolize access to an estrus female and achieve paternity of the entire litter, he would only have to prevent other males from mating with her for a short period of time (Sherman 1989).

Given the potential for multiple paternity, as a result of the male-biased operational sex ratio (Egoscue 1962), and the large spatial overlap of males and females (Topping and Millar 1996a), the lack of multiple paternity is surprising. Under such conditions, exclusive mating access (either during the entire estrus period, or during the critical period of estrus where fertilization occurs) can be gained by males defending females and/or female choice of males. Because no observations of courtship behavior were possible, we cannot discriminate between these two alternatives, although support for both scenarios exists. Despite the lack of wounding observed in the male population (M. Topping, personal observation), males may still actively defend access to females through agonistic displays or other behavior. Males may also exhibit passive defense of females, through the production of copulatory plugs (Voss 1979), although the effectiveness of such plugs at preventing further copulations is questionable (Voss 1979; Eberhard 1985; Koprowski 1992). It is also possible that females are exhibiting choice for particular males, which then gain exclusive access to the females during estrus. In this situation, males do not need to actively defend females, but would devote more time to courting an estrus female that has not made a choice. Escherich (1981) documented one case of a captive female rejecting the advances of a male for up to 3 days, providing some support for the hypothesis that females exercise some degree of choice.

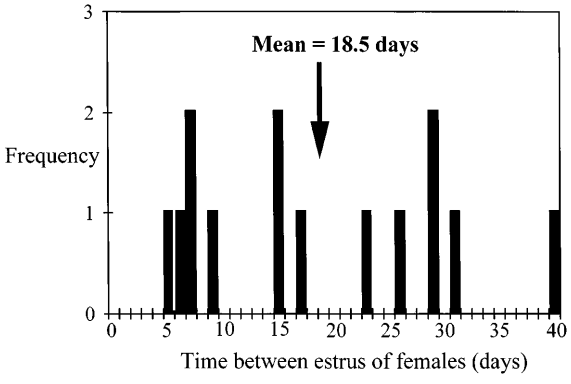

Fig. 5 Frequency distribution of the time between the start of estrus periods of females that mated with the same male (mean = 18.5 days)

Implications of mating patterns

The lack of multiple paternity in litters, coupled with the high availability of males for each female, suggests that multiple paternity within a litter provides no obvious benefit to either the female or her litter. Because all individuals within a litter are fathered by the same male, littermates are full, rather than half siblings. This may contribute to the strong degree of female sociality observed in *N. cinerea* (Moses and Millar 1992, 1994). The fact that mother-daughter and sister-sister relationships all involve first-order relatives may explain why cooperative sociality between female relatives is demonstrated so strongly in this species (Moses and Millar 1992, 1994). *N. cinerea* can differentiate between littermates and non-littermates (Moses and Millar 1992), leading to cooperative behavior that increases the reproductive success of close female relatives (Moses and Millar 1994). As such, cooperation may be greater if individuals within a litter are full, rather than half siblings.

Although females may benefit as a result of their entire litter being first-order relatives, it is noteworthy that when females produce two successful litters within a year (e.g., females 39 and 58 in 1992, females 45 and 58 in 1993, and females 41 and 98 in 1994; Fig. 3), they do not mate with the same male twice. This strategy may be explained by the social behavior of female woodrats. It appears that there are many benefits to producing first-order relatives within a litter, as evidenced by the cooperative behavior between relatives (Moses and Millar 1994). However, given the high mortality rates of juvenile woodrats (M. Topping, unpublished data), females restricting mating to a single male might be at a long-term disadvantage by reducing the genetic variation within each litter. Therefore, females may compensate for this by mating with other males in their subsequent reproductive attempts (either within or between years). This strategy leads to two benefits. First, female littermates are all full siblings, which appears to contribute to the cooperative sociality of *N. cinerea*. Second, females are ensuring genetic diversity between litters.

The pattern of reproductive success from DNA fingerprinting (Table 2) shows that variation in male and female reproductive success does not differ significantly, and that given the opportunity, both males and females will mate with more than one member of the opposite sex. Under these circumstances, we suggest that the most likely mating system would also be classed as some form of promiscuity, perhaps roving-male promiscuity (Clutton-Brock 1989), where males move from female to female, attempting to mate with as many receptive females as possible. However, such a classification would depend on a greater understanding of the dynamics of mate selection by both sexes, as well as the presence of any pair bonding, data for which are at present unavailable for this species. Therefore, until such information is available, we will restrict our comments on the mating system of *N. cinerea* to two points. First, given the pattern of mating behavior, coupled with the spatial distribution of both sexes (Topping and Millar 1996a), there is sufficient evidence to reject polygyny as a mating system. Second, we suggest that because our results show many similarities to what has previously been referred to as a promiscuous mating system, a working hypothesis for any future investigation into mating behavior can assume that the mating system is primarily promiscuous.

In conclusion, the lack of multiple paternity in this species is surprising, given the potential for it. The only other species of small mammals that have been documented as exhibiting single paternity within all litters (as revealed by DNA fingerprinting) have been those that are classed as traditionally monogamous (i.e., *P. californicus*; Ribble 1991). While we do not suggest that *N. cinerea* should be classed as monogamous, because there is no evidence to support behavioral monogamy, it is interesting to speculate why we observed this pattern of mating behavior. Perhaps females produce siblings that exhibit high levels of relatedness in order to facilitate the female cooperative social system. This investigation further reinforces the notion that we must investigate all aspects of mating behavior simultaneously. Interpreta-

Table 3 The minimum percentage of litters resulting from multiple males mating with an estrus female in natural populations. The method used to determine paternity is presented in parentheses: *PE* protein electrophoresis, *KA* karyotype analysis, *DF* DNA fingerprinting

Species	Percentage of litters with multiple paternity	Number of litters	Source
Cynomys gunnisoni	33% (DF)	21	Travis et al. 1996
Cynomys ludovicanus	3% (PE)	102	Hoogland and Foltz 1982
Clethrionomys rufocanus	21% (PE)	29	Kawata 1988
Microtus pennsylvanicus	33% (PE)	56	Boonstra et al. 1993
Peromyscus californicus	0% (DF)	28	Ribble 1991
Peromyscus leucopus	25% (PE)	61	Xia and Millar 1991
Peromyscus maniculatus	22% (DF)	18	Ribble and Millar 1996
Sorex araneus	25% (KA)	32	Searle 1990
Sorex araneus	89% (DF)	9	Stockley et al. 1993
Sorex araneus	40% (DF)	5	Tegelstrom et al. 1991
Spermophilus beecheyi	89% (PE)	9	Boellstorff et al. 1994
Spermophilus beldingi	78% (PE)	27	Hanken and Sherman 1981
Spermophilus brunneus	19% (PE)	26	Sherman 1989
Spermophilus parryi plesius	6% (PE & DF)	16	Lacey et al. 1997

tions drawn independently from the spatial distribution or the pattern of paternity for *N. cinerea* could lead to an inappropriate conclusion.

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