Variable mode of estrus affects female decision for multiple mating

Ines Klemme,a Saana Kataja-aho,a Jana A. Eccard,a and Hannu Ylönena,b
aDepartment of Biological and Environmental Science, University of Jyväskylä, Survontie 9, FI 40014 Jyväskylä, Finland and bKonnevesi Research Station, University of Jyväskylä, Sirkkamäentie 220, FI 44300 Konnevesi, Finland

Investigating patterns of variation in mating strategies may shed light on their evolutionary importance as well as their impact on the strength and shape of sexual selection. Multimale mating (polyandry) and mate choice are intensely studied subjects in the area of sexual selection, but little is known about intraspecific patterns of variation that may respond to dynamic cost-benefit balances. In a laboratory study, we investigated the mating behavior of female bank voles (Myodes glareolus) with distinctly different time and energy budgets: They were either in cycling estrus (CE) or postpartum estrus (PPE) shortly after delivery. Postpartum mating is highly constrained by time and energy due to maternal commitments to the newborn litter. All females had a free and simultaneous choice among 4 males that differed in scent-marking frequency (indicator of dominance) and body mass. We show that females in CE engaged significantly more often in multimale mating than females in PPE. Additionally, PPE females mated with males that scent marked more, whereas CE females seemingly mated indiscriminately. We discuss our findings in light of time and energy budgets of females and also consider alternative explanations such as pup protection against infanticidal males or physiological constraints to estrus modes. Key words: Clethrionomys = Myodes glareolus, cycling estrus, female choice, male dominance, polyandry, postpartum estrus. [Behav Ecol 23:361–367 (2012)]

INTRODUCTION

A female mammal’s reproductive strategy involves complex trade-offs, arising from time and energy allocation, mating, and lactation. Variation in any of these elements may alter female fitness and thus cause variation in reproductive strategies. Due to its impact on sexual selection, such variation has important evolutionary consequences. Yet, the causes of individual variation in mating behavior, especially related to dynamic cost-benefit balances, remain poorly known.

Mating with multiple males within one reproductive event is a widespread female reproductive strategy (Birkhead and Møller 1998) with varying levels within species (e.g., Valimäki and Kaitala 2006; Firman and Simmons 2008; McNamara et al. 2008; Long et al. 2010). Variation in the degree of remating is ultimately shaped by a cost-benefit balance. Although there is increasing evidence from empirical studies that females gain a range of benefits from mating with multiple males (Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Hosken and Stockley 2003; Simmons 2005), costs associated with remating may counteract these benefits. Such costs include loss of time or energy, increased risk of injury or predation, and exposure to male-borne parasites and diseases (Daly 1978; Magnhagen 1991; Thrall et al. 1997).

The degree of remating frequency may be shaped by whether or not females benefit directly or indirectly (Bretman and Tregenza 2005) and/or the magnitude of such benefits (Brooker et al. 1990; Petrie and Lipschitz 1994). Indirect evidence from studies showing an effect of environmental factors has suggested that the degree of multimale mating may also vary in relation to time- and energy-based constraints. For example, in the moth Lobesia botrana, food quality affects female size, and large females are more polyandrous than small ones (Torres-Vila et al. 2004). In addition, multimale mating has been shown to increase with density (Richardson and Burke 2001) and male-biased operational sex ratios (Klemme, Ylönen, et al. 2007), suggesting that remating increases with mate encounter rate and thus a potentially reduced mate search cost.

In addition to female remating rate, there is ample intraspecific variation in female choice (reviewed in Jennions and Petrie 1997). Although the benefits are obvious, female preference can be constrained also by time, energy, and predation pressure (Janetos 1980; Pomiankowski 1987; Real 1990). Consequently, a female’s level of choosiness will again depend on a cost-benefit balance (Pomiankowski 1987) and has, for example, been shown to decrease with increasing costs of sampling (e.g., Alatalo et al. 1988; Wong and Jennions 2003) and an increased predation risk (Hedrick and Dill 1993; Bonachea and Ryan 2011).

Females of most species of rodents can have 2 forms of estrus: postpartum estrus (PPE), where females become receptive and mate shortly after giving birth, and cycling estrus (CE), which occurs in virgins, in nonvirgins that enter a new breeding season, and after no or an unsuccessful mating in PPE (reviewed in Dewsbury 1990). Postpartum mating is beneficial because it reduces interbirth intervals, but it is also energetically costly and imposes time constraints (Gilbert 1984). In comparison with females mating in CE, which only face energetic demands for self-maintenance, females mating in PPE are required to trade-off the time and energy spent on nursing and nest defense with those on acquiring copulations. Lactation is the most energetically expensive component of...
mammalian reproduction with costs reaching 66% to >200% of nonlactating requirements (for review, see Sikes and Ylönen 1998). Moreover, females in PPE need to protect their offspring from nest predators and infanticidal conspecifics at a time when they are most vulnerable. Taken these together, one might expect different mating strategies of females mating in each mode of estrus. Indeed, it has been shown that females in PPE devote less time to mating than females in CE (Gilbert et al. 1980; Hedricks and McClintock 1985; Witt et al. 1990; delBarco-Trillo and Ferkin 2007). However, whether females in PPE and CE differ in their propensity to remate and differ in mate assessment efforts has not been investigated.

The bank vole Myodes glareolus is a common rodent species exhibiting PPE up to 36 h after delivery (Gustafsson et al. 1980). Its spatial distribution, with female territories that are overlapped by several male home ranges (Bondrup-Nielsen and Karlsson 1985), suggests a polyandrous mating system. Indeed, studies in the wild and laboratory confirm that female bank voles actively mate with multiple males within one reproductive event (Klemme, Eccard, et al. 2007; Klemme, Ylönen, et al. 2007) and benefit from this mating strategy in the form of increased pregnancy rates via increased reproductive stimulation (Klemme, Eccard, et al. 2007), offspring with an increased reproductive potential (Klemme et al. 2008) and increased offspring survival most likely via infanticide avoidance (Klemme and Ylönen 2010). Males form clear dominance hierarchies (Rozenfeld et al. 1987 and references therein), and body size has been found to correlate positively with dominance status (Horne and Ylönen 1998). Female bank voles prefer a dominant over a subordinate male as an initial mate (Horne and Ylönen 1996) but will mate with both in quick succession when given the opportunity (Klemme, Eccard, Gerlach, et al. 2006; Klemme, Eccard, and Ylönen 2006). Paternity is biased toward dominant males when females mate with both a dominant and a subordinate male (Klemme, Eccard, and Ylönen 2006).

Here, we placed females in either CE or PPE in a mating arena with 4 confined males, for which we recorded scent marking frequency (indicator of dominance) and body mass. We tested the hypotheses that females in PPE, facing a trade-off between maternal duties and mating, 1) show a lower degree of remating frequency and 2) invest less time in mate sampling and as a consequence are less choosy than females in CE.

MATERIALS AND METHODS

Reproductive biology of bank voles

Bank vole populations breed seasonally in Fennoscandia, typically from May until September (Kaikusalo 1972; Koivula et al. 2005). Ovulation is induced by mating. Thus, females only mate when in estrus and only ovulate following copulation (Andersson and Gustafsson 1982). When forced out of the postpartum cycle, CE recurs regularly with a cycle length of 4–5 days (Bujalska and Petrušević 1983). Gestation lasts 18–20 days for females who have mated during CE and 20–24 days for females who have mated during PPE due to delayed implantation to avoid litter overlap (nursing period about 20 days; Gustafsson et al. 1980). A female can have up to 4 litters per breeding season. Litter size ranges usually from 4 to 7 pups (Bujalska and Petrušević 1983).

An ejaculatory series in bank voles consists of several intermissions (a single deep pelvic thrust) without sperm transfer and one ejaculation with sperm transfer (Milligan 1979). At ejaculation, the insertion is prolonged and the male falls to one side carrying the female over with him, making it easy to differentiate ejaculations from intermissions without sperm transfer (Milligan 1979).

The care and use of these animals were in accordance with the ethical guidelines in Finland, under permission 35/31.5.2004 (Board for Animal Experimentation of the University of Jyväskylä).

Experimental animals

The animals used in the experiment were the F2 progeny of wild bank voles trapped in Central Finland. They were housed singly in standard mouse cages (43 × 26 × 15 cm) on a 16:8 h light: dark photoperiod. Water and food were provided ad libitum, and sawdust and hay were used as bedding. The age and reproductive history of all females were controlled for, so that there were no differences between the treatments. Male and female voles were 41–53 weeks old with CE and PPE females being on average ± standard deviation (SD) 47.0 ± 3.4 and 48.1 ± 3.6 weeks, respectively. All males were known to be fertile; each had sired 1–2 litters prior to the experiment. All females had given birth to 1 litter; PPE females within 12 h prior to the mating trial and CE females at least 4 weeks before the experiment, that is, they were not lactating at the time of the experiment.

We conducted 30 mating trials with 30 females and 44 males. Fifteen females were in CE at the time of each mating trial. The remaining experimental females (15) were in PPE and were placed in the mating arena together with their litter. This was done to simulate as natural conditions as possible (threat of infanticide, absence of litter may cause active search behavior) and to be able to observe the time devoted to maternal care during the trial.

Fifteen male groups were established, consisting of 4 males each. Due to low numbers of available fertile males, 16 males were used in 2 groups. However, each group always consisted of different male combinations. Each male group was used once in the PPE and once in the CE treatment. The presentation of PPE and CE females was randomized for each male group, with 7 groups being used first with a female in PPE. The experimental animals in each mating trial were unrecorded. A minimum of 5 days passed between trials involving the same males to allow for sperm replenishment.

Mating arena

The mating arena consisted of 3 sides of plywood and 1 side of Perspex to permit viewing (1.5 × 1.5 × 0.6 m; Figure 1). In each corner, there was a triangular 0.08 m² male compartment separated by a wire mesh from the central female compartment. Each wire mesh wall contained 2 holes (1 × 1 cm) to allow females to enter/exit. Holes were located in the corners of the male compartments so that males could not pursue or corner the female entering their compartment, but the female could escape. All males were prevented from leaving their compartment by a spike collar, made of 3 cable binders with a spike length of 0.5 cm and weighing about 0.4 g (i.e., 1.8% of the mean male body mass in this study: 22.6 ± 2.8 g). The spike collars did not prevent the males from mating or other normal behavior, such as grooming. Females were free to move within the entire arena. A nest-box (25 × 25 × 10 cm) made of plywood was placed in the middle of the female compartment. For each trial, the nest-box was lined with soiled bedding of each female’s home cage. Water and food pellets were provided ad libitum. Brown packing paper was placed under the arena to collect the urine markings of each male.

Experimental procedure

Before each trial females were tested to verify estrus. Each female was placed in a standard mouse cage together with a randomly chosen male that was not used in the experiment and
observed for the typical lordosis mating behavior. When observed, the pair was separated and the female was placed in the arena.

The males were weighed and placed to the arena and given a 10-min habituation period before the female was placed into the nest-box. The trial started immediately after the female was released and was monitored from an adjacent room through a one-way-window. We recorded the time females spent in the nest-box, outside of the nest-box in the central compartment, and the time interacting with each particular male. In addition, all copulatory behavior (i.e., the number of intromissions and ejaculations) was documented.

Trials were continued until 1 h elapsed without the occurrence of any mating interest of the female. To verify that this time period can be used to characterize sexual activity, we videotaped 1 PPE trial and 1 CE trial for 12 h following the 1 h of sexual inactivity. In both cases, the females did not show any sexual activity after we had ended the behavioral observation.

Male scent marking and body weight

Scent marks are a common form of signaling in mammals (Gosling and Roberts 2001) and are used by bank voles in male hierarchical interactions where the degree of marking is positively related to social status (Rozenfeld et al. 1987). Males had no direct contact with each other in the mating arena. However, they were able to perceive each other through the mesh walls. Thus, following each trial, we assessed the fine-trace urination marks on the brown paper lining the male compartments. The markings were analyzed visually by comparing them with the results of the previous trial. The marking frequency was assessed as a measure of dominance (Horne and Ylönen 1996; Klemme, Eccard, and Ylönen 2006), females were present during scent marking in this study. It is likely that females scent marked in the males’ compartments, but female marking intensity and UV reflectance are significantly reduced in comparison with males (Koivula, Koskela, et al. 1999; Koivula, Viitala, et al. 1999). Because bank vole females perceive male status via chemical cues (Hoffmeyer 1982; Kruczek 1997), their presence may promote scent marking, and therefore, marking intensity may also be related to the amount and duration of female visits rather than social status alone. However, it is important to note that within-group rank was repeatable over the 2 tests involving 2 different females (Kohen’s weighted kappa $k_w = 0.778$; Fleiss and Cohen 1973). Also, all males were able to perceive the female in the arena indicated by a noticeable agitated behavior.

We also ranked males based on their weight within the group with the lightest given a rank of 1 and the heaviest a rank of 4 (mean ± SD rank 1–4: 19.3 ± 1.4, 21.6 ± 1.7, 23.5 ± 2.0, and 26.0 ± 2.0 g). Urine marking rank and body mass rank were not related to each other (Friedman Test, $X^2 = 1.160, df = 3, P = 0.763$). This may be explained by single caging and ad libitum food. Body masses attained under such ideal living conditions may not reflect those under competitive situations in the wild (Klemme, Eccard, Gerlach, et al. 2006).

Paternity analysis

The procedure of paternity analysis has been described earlier (Klemme et al. 2008). Briefly, ear tissue was collected from those CE females that mated polyandrously ($N = 12$), their mates, and the resulting offspring. DNA was extracted using a standard protocol and then these individuals were genotyped at 6 microsatellite loci (Klemme et al. 2008). All offspring could be unambiguously assigned to one of the potential fathers in at least 5 of 6 loci used. All other potential fathers could be excluded in at least 2 loci.

Data analysis

Statistical analyses were performed with SPSS 16.0 and SAS v. 9.1. Comparisons of PPE and CE females were conducted using pairwise nonparametric tests. Means are given ± SD. Female preference and male reproductive success were analyzed with a general linear mixed model (GLMM) with either a “Poisson” or binomial error distribution as appropriate. As measures within a male group were repeated, we included group as a random factor using the RESIDUAL option. For GLMM’s, we present means ± standard error from the final model. $P$ values in post hoc least-squares (LS) means significance tests were adjusted using the Bonferroni correction.

RESULTS

Female mating behavior

All 30 experimental females mated during the trial, and all of them visited all 4 males in the arena. Only a single PPE female (1/15), compared with 13/15 of the CE females, mated with more than 1 male. Thus, the level of multimale mating was significantly different between the groups (McNemar test, $N = 15, P < 0.001$). The mean number of mates and ejaculations (including both same male partners and different males) were significantly greater for CE females than for PPE females (Table 1). The mean number of intromissions per ejaculatory series did not differ between the groups, but the total number of intromissions was significantly greater for CE females than for PPE females (Table 1).

The time of sexual activity (i.e., the time from the first intromission of the first ejaculatory series to the ejaculation of the last series) was significantly shorter for PPE than for CE females (Table 1). The proportion of time spent in the nest was significantly greater for PPE females than for CE females.
Table 1
Means ± SD for PPE and CE female mating behavior variables and statistical values from the Wilcoxon signed-rank test

<table>
<thead>
<tr>
<th></th>
<th>PPE females</th>
<th></th>
<th>CE females</th>
<th></th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td></td>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of males copulated with</td>
<td>1.1±0.3</td>
<td></td>
<td>2.3±1.0</td>
<td></td>
<td>-3.169</td>
<td>0.002</td>
</tr>
<tr>
<td>Number of ejaculations</td>
<td>1.1±0.4</td>
<td></td>
<td>3.5±1.1</td>
<td></td>
<td>-3.198</td>
<td>0.001</td>
</tr>
<tr>
<td>Total number of intromissions</td>
<td>50.3±5.2</td>
<td></td>
<td>128.4±28.4</td>
<td></td>
<td>-5.486</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of intromissions per ejaculatory series</td>
<td>40.8±26.9</td>
<td></td>
<td>60.4±37.4</td>
<td></td>
<td>-1.647</td>
<td>0.100</td>
</tr>
<tr>
<td>Number of males with whom</td>
<td>2.1±1.2</td>
<td></td>
<td>3.0±0.9</td>
<td></td>
<td>-2.389</td>
<td>0.017</td>
</tr>
<tr>
<td>Number of male interactions with before first intromission</td>
<td>3.8±0.8</td>
<td></td>
<td>2.9±1.1</td>
<td></td>
<td>-1.845</td>
<td>0.065</td>
</tr>
<tr>
<td>Time of sexual activity</td>
<td>00:13:22</td>
<td></td>
<td>00:08:55</td>
<td></td>
<td>00:21:31</td>
<td>01:14:08</td>
</tr>
<tr>
<td>Time from first interaction to first intromission</td>
<td>00:13:51</td>
<td></td>
<td>00:12:31</td>
<td></td>
<td>00:45:25</td>
<td>01:14:08</td>
</tr>
<tr>
<td>Time from start of trial to the 1st interaction</td>
<td>00:13:22</td>
<td></td>
<td>00:08:55</td>
<td></td>
<td>00:21:31</td>
<td>00:29:36</td>
</tr>
<tr>
<td>Length of first ejaculatory series</td>
<td>00:37:41</td>
<td></td>
<td>00:36:51</td>
<td></td>
<td>00:24:19</td>
<td>00:14:12</td>
</tr>
<tr>
<td>Litter size</td>
<td>22.7±3.3</td>
<td></td>
<td>18.6±0.6</td>
<td></td>
<td>-2.901</td>
<td>0.028</td>
</tr>
<tr>
<td>Number of ejaculations</td>
<td>1.1±0.4</td>
<td></td>
<td>3.5±1.8</td>
<td></td>
<td>1.845</td>
<td>0.065</td>
</tr>
<tr>
<td>Number of males interacted with before first ejaculation</td>
<td>3.8±0.9</td>
<td></td>
<td>2.9±1.1</td>
<td></td>
<td>-1.845</td>
<td>0.065</td>
</tr>
<tr>
<td>Time of sexual activity</td>
<td>00:13:51</td>
<td></td>
<td>00:12:31</td>
<td></td>
<td>00:45:25</td>
<td>01:14:08</td>
</tr>
<tr>
<td>Time from first interaction to first intromission</td>
<td>00:13:22</td>
<td></td>
<td>00:08:55</td>
<td></td>
<td>00:21:31</td>
<td>00:29:36</td>
</tr>
<tr>
<td>Length of first ejaculatory series</td>
<td>00:37:41</td>
<td></td>
<td>00:36:51</td>
<td></td>
<td>00:24:19</td>
<td>00:14:12</td>
</tr>
<tr>
<td>Litter size</td>
<td>5.5±2.0</td>
<td></td>
<td>5.8±1.6</td>
<td></td>
<td>-0.967</td>
<td>0.334</td>
</tr>
</tbody>
</table>

Times are given as hh:mm:ss unless stated otherwise.

(Table 1). However, there was no difference between females in different estrus modes in the time from the start of the experiment to the first interaction between females and males and in the length of the first ejaculatory series (Table 1).

Female choice behavior

There was no difference in the time from the first interaction with a male to the first intromission for females in different estrus modes (Table 1). Both PPE and CE females had intromissions with significantly more males than they had ejaculations with (PPE: Z = -2.549, N = 15, P = 0.011; CE: Z = -2.640, N = 15, P = 0.008), suggesting that choice decisions extended beyond the first sexual activity. PPE females visited more males before the first ejaculation (in 13/15 cases all 4 males) than CE females (6/15), but this difference failed to reach significance (Table 1).

Although the male body mass had no effect on the proportion of time a PPE female would spend with them (GLMM F5,35 = 0.50, P = 0.684), there was a significant effect to marking frequency (F5,35 = 5.17, P = 0.003). A post hoc LS means significance test revealed that PPE females spent more time with males ranked 4 (0.47 ± 0.09) than with males ranked 2 (0.13 ± 0.04, F5,35 = -3.39, P = 0.008) and tended to spend more time with rank 4 males than with males ranked 1 (0.19 ± 0.05, b3 = -2.70, P = 0.056). A similar result was found for CE females (body mass; F5,35 = 0.11, P = 0.955; marking; F5,35 = 3.80, P = 0.015). Here, females spent significantly more time with males ranked 4 (0.40 ± 0.07) than with males ranked 1 (0.15 ± 0.04, b5 = -2.95, P = 0.028).

Because intromissions are believed to affect the number of sperm ejaculated (Dewsbury 1988), we also considered intromission number as a component of female choice. The number of intromissions a PPE female had with a male was neither affected by body mass nor by marking frequency (F5,35 = 0.82, P = 0.488 and F5,35 = 2.07, P = 0.115). The same was true for body mass in CE females (F5,35 = 0.12, P = 0.950). However, marking frequency did affect the number of intromissions CE females received (F5,35 = 2.90, P = 0.044). All adjusted P values of the post hoc LS means significance tests were non-significant, but the largest difference in the number of intromissions was found between males ranked 4 (70.2 ± 15.5) and 2 (26.9 ± 9.5, b3 = -2.37, P = 0.128) and males ranked 4 and 1 (27.8 ± 9.8, b3 = -2.18, P = 0.200).

PPE female choice for ejaculation was not affected by body mass (F5,35 = 0.95, P = 0.424) but by marking frequency (F5,35 = 3.33, P = 0.026; Figure 2A). Males ranked 4 were significantly more likely to ejaculate than males ranked 1 (b5 = -2.87, P = 0.035).

Eight CE females had ejaculations with 2 males, 1 with 3 males and 3 with all 4 males. The total number of ejaculations ranged from 2 to 7. There was no clear pattern of marking frequency or body mass on mating order. The number of times a CE female mated with a male was not affected by body mass or marking frequency (F5,35 = 0.04, P = 0.990 and F5,35 = 1.15, P = 0.338; Figure 2B).

To account for a potential cryptic female choice after multiple mating, we conducted paternity analyses for the offspring of those CE females that mated with multiple males and produced young (N = 12). Eight of these litters were sired by 2 males (66.6%) and about one-third of the males that mated failed to sire young (0.32 ± 0.26). Body mass and marking frequency were not related to the proportion of offspring sired (F5,37 = 0.55, P = 0.653 and F5,37 = 1.10, P = 0.363; Figure 2C). Within multiple sired litters, the proportion of offspring sired by the male with higher marking frequency did not deviate significantly from 0.50 (0.54 ± 0.23; one sample t-test, t7 = 0.676, P = 0.524). Because there was a lot of variation in the number of ejaculations and mates a female had as well as in the time delay between ejaculations, it was not possible to evaluate a pattern of mating order on paternity.

Female reproductive success

We investigated whether the differences in mating behavior of PPE and CE females affected their reproductive output. The probability of pregnancy did not differ between PPE females (12/15) and CE females (13/15; McNemar test, N = 15, P = 0.999). Successful pregnancy induction did not depend on the number of males (Binary Logistic Regression, PPE: W1 > 0.001, N = 15, P = 0.999; CE: W1 > 0.277, N = 15, P = 0.599) nor the number of ejaculations (PPE: W1 > 0.001, N = 15, P = 0.999; CE: W1 > 0.161, N = 15, P = 0.688). Pregnancy was significantly longer for PPE females than for CE females (Table 1). For PPE females, the length of pregnancy with the litter conceived during the experiment correlated positively with the size of the litter conceived and delivered before the experiment (R2 = 0.757, P = 0.001).

One PPE female died shortly before the delivery of the second litter, thus litter size data are only available for 11 pregnant PPE females. The litter size of PPE females and CE females did not differ significantly. Litter size did not depend
on the number of mates (PPE: $t$ test, $t_{10} = -0.251$, $P = 0.807$; CE: linear regression, $R^2 = 0.025$, $P = 0.605$). In CE females, there was no relationship between the number of ejaculations and litter size ($R^2 = 0.006$, $P = 0.798$).

**DISCUSSION**

In this study, we found significant variation in the mating behavior of female bank voles in different estrus modes. This variation is likely to be evident within individuals in the wild, as each female would mate at least once in CE and then in PPE after the birth of each litter. Females in PPE devoted less time on mating activities than females in CE and were less likely to mate with multiple males, with 7% compared with 80% mating with more than one male. Furthermore, our results may indicate that females in different estrus modes differ in their level of mate search effort and mate choice, though in the other direction to what we expected. PPE and CE females spend a comparable amount of time on mate sampling until the first sexual behavior occurred. However, there was a tendency for PPE females to visit more males before ejaculation than CE females. Furthermore, PPE females copulated most often with males that displayed high marking frequencies, whereas for CE females, the number of ejaculations and paternities were independent of scent marking and body mass.

There are at least 3 explanations for the observed plasticity in female remating frequency. First, females in PPE are constrained by time and energy due to maternal commitments to the newborn litter, including lactation and protection. Lactation is considered to be the most energetically demanding phase in reproduction, and in bank voles, energy needs are highest during lactation (Kaczmarz 1966; Trebaticka et al. 2006). Additionally, nest mortality is high in small mammals (Millar 2007). Infanticide by male and female conspecifics and nest predation (e.g., by shrews) may be counteracted by nest protection in bank voles (Ylönen and Horne 2002). Indeed, PPE females not only devoted less time than CE females on sexual activity but also spent more time in their nest than CE females. Finally, bank voles are a short-lived prey species with high rates of mortality. Thus, an existing litter would be expected to have the highest reproductive value compared with potential future litters. Support is evident in the delayed implantation after PPE mating; the delay of which is longer when the size of litter being nursed is larger (Gustafsson et al. 1980 and this study). Accordingly, PPE females may only mate as often as necessary for fertilization and then return to nurse and protect their litter.

Second, in many rodents, including bank voles, ovulation and/or the luteal function depend on the amount or pattern of vaginal stimulation (Clarke et al. 1970; Andersson and Gustafsson 1982; Dewsbury 1990). PPE and CE females may differ in hormone levels due to hormonal events associated with delivery and lactation (Carter et al. 1989) and may therefore require different levels of copulatory stimulation for a successful pregnancy induction. Indeed, PPE females of some species need less stimuli than CE for pregnancy initiation (reviewed in Dewsbury 1990). The same seems to be true for bank voles. Although females in CE increase the likelihood of pregnancy by mating multiply, either with the same or different males (Klemme, Eccard, et al. 2007), most PPE females in the present study successfully induced pregnancy, even though they mated only once and received a significantly reduced amount of vaginal stimulation. However, other potential benefits of multimale mating demonstrated for bank voles (Klemme et al. 2008; Klemme and Ylönen 2010; see INTRODUCTION), suggest that PPE females should remate with additional males, even if they do not require the stimulus of multiple copulations. A failure to do so may hint at the above discussed time and energy constraints.

Finally, artificial lab conditions may have caused the reduced mating behavior in PPE females. The mating arena was constructed so that all 4 males were relatively close to the females’ nest and may thus have represented a greater threat of infanticide than in the wild. However, in natural populations, a females’ home range is overlapped by home ranges of several males. Additionally, like most small rodent populations, bank vole numbers fluctuate considerably (Krebs and Myers 1974), and under high densities, it is not uncommon to find a large number of males within a close proximity to nests (Bondrup-Nielsen and Karlsson 1985).
Assuming that scent marking in this study is related to dominance status (though marking may have been incited by female visit; see MATERIALS AND METHODS), females in PPE appear to be more choosy than females in CE. This is in contradiction to our prediction, however, in line with earlier findings that demonstrated a preference of PPE bank vole females for dominant over subordinate males (Horne and Ylönen 1996). It is possible that the costs of choice making are small and outweighed by the benefits, at least in this experimental setup with all potential mates present in close proximity. In bank voles, male traits reflecting dominance are highly heritable (Horne and Ylönen 1998), making it beneficial for females to have their sons sired by dominant males (Pomiankowski 1988).

CE females showed some precopulatory preference for males with high marking frequency, indicated by a larger proportion of time spent with them and more intromissions. However, paternity success did not differ among males with different scent marking intensities. Again, this is consistent with an earlier field study using females in CE (Klemme and Ylönen 2010). The benefits of multimale mating (see INTRODUCTION) may perhaps outweigh the benefits of having most or all offspring sired by a dominant male. For example, male infanticide can potentially be avoided by multimale mating in bank voles (Klemme and Ylönen 2010). CE females may be thus making the best of a bad job by accepting matings with subordinate males (Woelfl and Macdonald 2004).

In conclusion, females in PPE and CE show a distinct mating behavior. The difference in time and energy budgets in each mode of estrus seems the most plausible explanation for the varying frequency of remating. When mating with only one male, it might pay to invest in mate choice even if it is costly. However, it must be taken into account that these results were obtained in artificial laboratory conditions, were males were available simultaneously and in close proximity. Moreover, the males were confined. Under natural conditions, males may compete with each other for matings and also harass females. Therefore, the mating behavior and mating rates observed here should be verified under natural conditions.

Intraspecific plasticity in mating rates and female choice may ultimately affect the strength and outcome of sexual selection (Janetos et al. 2006) and thus explain the maintenance of variation in male sexual traits. Finally, as shown, the mode of estrus can be an important determinant of experimental results. As suggested earlier by Dewsbury (1990), the reproductive state of females should be carefully chosen when conducting experiments on mating behavior.

FUNDING

This research was funded by the Centre of Excellence in Evolutionary Biology (Academy of Finland).

We are grateful for valuable comments by Renée Firman, Paco García-Gonzalez, Ben Hatchwell, and 2 anonymous referees. We also thank Aira Muhija and Marko Haapakoski for help with animal maintenance.

REFERENCES
