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Multiple paternity in meadow voles (*Microtus pennsylvanicus*): investigating the role of the female

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Abstract Multiple paternity in single litters conceived in the wild was recently demonstrated in meadow voles (*Microtus pennsylvanicus*). In this study, we used an experimental approach (males tethered and females allowed to mate freely with one or several males) to investigate the role of female meadow voles in multiple paternity. We found that among 29 (of 39) females that copulated during our experiment, 79.3% chose to mate with more than one male. Female behavior in meadow voles thus clearly promotes multiple paternity and their role is an active one. Some of the hypotheses explaining promiscuity in meadow voles should be reconsidered in light of this result. We do not know the primary determinant of female mate choice, but male body mass played a secondary role in driving female preferences. The partial dependence between male body mass and female choice, coupled with the active role played by females, indicates that intersexual selection has the potential for reinforcing the effects of intrasexual selection (male-male dominance relationships) in this species. Finally, we demonstrate that the time period over which tests are conducted is an important part of the design of experiments aimed at understanding the role of females in multiple paternity.

Key words Sexual selection · Female choice · *Microtus pennsylvanicus* · Multiple paternity · Meadow vole

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Introduction

Multiple paternity of litters or broods results from the insemination of a female by more than one male. Multiple paternity has now been reported for a wide range of taxonomic groups (e.g., birds: Burke et al. 1989; Price et al. 1989; mammals: Stockley et al. 1993; Boellstorff et al. 1994; reptiles: Schwartz et al. 1989; fishes: Borowsky and Kallman 1976; Travis et al. 1990; invertebrates: Watson 1991; Reichardt and Wheeler 1996). A female can mate with several males as a result of either forced copulations, non-resistant acceptance of multiple males, or solicitations by the female to multiple males (reviews in Schwagmeyer 1984; Petrie et al. 1992; Reichardt and Wheeler 1996). Proposed explanations of multiple paternity thus fall into two categories: (1) females play a passive role and it is the unwillingness or inability of males to monopolize access to receptive females that leads to multiple paternity; (2) females play an active role and female benefits are the driving force behind multiple paternity. The first category emphasizes the role of intrasexual selection (male-male competition) whereas the second stresses the role of intersexual selection (female choice).

In mammals, multiple paternity has been observed in species as diverse as shrews (e.g., *Sorex araneus*, Stockley et al. 1993), ground squirrels (e.g., *Citellus tridecemlineatus*, Hanken and Sherman 1981), and bears (e.g., *Ursus americanus*, Schenk and Kovacs 1995). Yet the role of female mammals in multiple paternity often remains obscure, since it is difficult to disentangle the respective roles of intra- and intersexual selection (but see e.g. Foltz and Schwagmeyer 1989; Murrie 1995). At a proximate level, the extent of multiple paternity in a population will be influenced by the ability of males to protect their paternity, by the ability of males to gain access to already mated females, and by the extent to which females seek copulations with several males. Each of these three players has its own reproductive interests, which are likely to conflict with those of the others in complex ways. Therefore, understanding multiple paternity will depend critically on identifying the role of these three categories of individuals.

Arvicoline rodents offer interesting opportunities to study mammalian mating systems because their use of space can be relatively easily manipulated (see Davies 1991). A few investigators have manipulated the use of space by males to prevent them from interacting, and thus experimentally separated intra- from intersexual selection to understand some particular aspects of arvicoline mating systems. Males were either tethered (e.g., Shapiro and Dewsbury 1986; Gubernick and Nordby 1993; Horne and Ylönen 1996) or placed in separate cages (e.g., Solomon 1993; Gubernick and Addington 1994; Agrell 1997) and females then allowed to associate with preferred males. This powerful experimental approach, however, has not been used to explore the role played by females in multiple paternity.

Multiple paternity in single litters conceived in the wild was recently demonstrated for meadow voles (*Microtus pennsylvanicus*) (Boonstra et al. 1993). The social organization of meadow voles is characterized by female territoriality, whereas male home ranges overlap those of several other males and females (Madison 1980; Webster and Brooks 1981; Ostfeld et al. 1988). Mating systems or mating behaviors of *Microtus* have been extensively studied in the field (FitzGerald and Madison 1983; Ostfeld 1986; Zwicker 1989; Lambin et al. 1992) and the laboratory (Fuentes and Dewsbury 1984; Shapiro and Dewsbury 1986; Storey 1994; Salo and Dewsbury 1995; Storey et al. 1995). To date, however, meadow voles and field voles (*M. agrestis*; Agrell 1997) are the only two species of *Microtus* for which multiple paternity has been observed. Boonstra et al. (1993) suggested that multiple paternity in meadow voles may result from females accepting multi-male insemination and males failing to monopolize mating access to estrous females, but no data exist to support or reject this hypothesis.

Here we use an experimental approach (males tethered and females allowed to mate with one or several males) to examine the role of female meadow voles in multiple paternity. We address three objectives. First, we test the null hypothesis that females choose to mate with only one male in the absence of any male harassment. Failure to reject this null hypothesis would suggest that females actively seek multiple mates and thus play an active role in multiple paternity. Second, since male dominance relationships have been shown to affect female mate choice in some arvicolines (Shapiro and Dewsbury 1986; Horne and Ylönen 1996), and dominance is size dependent in meadow voles (Turner and Iverson 1973), we test the hypothesis that larger males are preferred over smaller ones. Testing this hypothesis is pertinent because female choice (intersexual selection) could potentially reinforce or undermine the effects of male dominance relationships (intrasexual selection). Third, experiments dealing with female mate choice in small mammals have been performed over very different time periods, ranging from minutes (Salo and Dewsbury 1995; Horne and Ylönen 1996) to days (Gubernick and Nordby 1993; Agrell 1997). Here we quantify the effect of the length of the observation period on the results obtained, to identify potential biases dependent on duration of the experiment.

Methods

Animals

Meadow voles originated from a captive colony that was periodically outbred with wild voles. They were housed individually in plastic cages (15 × 22 × 45 cm) with wire tops, and kept at 18 °C on a 16:8 h light/dark photoperiod. Bedding consisted of wood shavings, cotton was provided for nesting material, and water was provided ad libitum. Voles were maintained on Purina rabbit chow after weaning.

We used adults ≥2 months old. Individuals for a given test were unrelated and unfamiliar with each other. Females were brought into behavioral estrus with intramuscular injections of 0.06 mg β-estradiol 3-benzoate (Sigma) 72 h before testing (Gray and Dewsbury 1975). Females had perforate vaginas when introduced into the choice arena. All individuals were weighed with an electronic balance (± 0.1 g) just before being introduced into the choice arena. We tried to select males so that there was at least a 10-g difference between the lightest and the heaviest male used in a given test. Trials were conducted during the dark phase of the daily cycle (for practical rather than biological reasons), from 26 January to 15 June 1994. All husbandry practices and experimental procedures were approved by the University Animal Care Committee and met the guidelines of the Canadian Council on Animal Care.

Apparatus

The choice tests were carried out in a 57 × 30 × 15 cm arena made of plywood painted gray with a Plexiglas top. The arena was divided into four compartments by plywood screens and its floor was covered with sawdust. Three adjacent 19 × 22 × 15 compartments (male chambers) were contiguous to a 57 × 8 × 15 female compartment. The screens allowed olfactory and auditory communication between males but prevented visual and physical contacts. Each male chamber had a 15-cm^2 doorway allowing the female free passage between her compartment and each of the three male chambers. No direct passage was possible between the male compartments. Each male compartment was provided with a 20-cm tether to confine the movement of males. The tethers consisted of a flexible steel leash attached at one end to a plastic collar (nylon-wire ties) placed loosely around each male's neck. The other end of the leash was attached to a clip running on a steel bar which was mounted across the top of the compartment. Each male was thus allowed free movement in his own chamber but prohibited access to other compartments. Females were free to move back and forth between males or could choose to stay in their own compartment that none of the males could reach. Bedding, food, and water were provided in each compartment. Three 100-W red incandescent bulbs placed 40 cm above the apparatus lighted the arena during the tests. The arena was cleaned thoroughly with ethanol after each trial.

Procedure

Animals were allowed to habituate to the choice arena during a 30-min period, during which the female was confined to her compartment by metal doors and the males were tethered in their respective chambers. The test began at the end of the habituation period, when the doors were lifted, giving the female access to the whole arena. We videotaped all activities occurring in the test apparatus for 10.5 h with a video camera (Hitachi model VK-C830) and a timelapse video cassette recorder (GYYR model TLC-1400).

The videotapes were later scored by four observers during rapid playback with a 6:1 record/playback ratio. Data were recorded using the software The Observer (Noldus 1991). For the analyses, each 10.5-h test was split into seven 1.5-h periods. We recorded for each period the number of female visits to each compartment, the length of visits, and the number of times females copulated with each male. Copulatory behavior in voles consists of three primary events (Gray and Dewsbury 1975): mounts (without vaginal penetration), intromissions (mounts with insertion and repeated pelvic thrusting) and ejaculations (mounts with insertion and sperm transfer). In this experiment, we defined as "copulation" any mount associated with pelvic thrusting, but did not attempt to separate intromissions from ejaculations. We checked at the end of 28 of the 39 trials for spermatozoa in the vaginal tract of the female. We found spermatozoa in 20 (71.4%) of the 28 checks. Copulations had occurred in all of these trials, and we never found spermatozoa when no copulation had occurred. This indicates that our experimental restriction of space use by males did not prevent them from performing the complete sequence of behaviors associated with insemination. The between-observer reliability was ≥ 95% for each category of behavior.

Data analyses

Thirty-nine trials were completed successfully. A different female was used in each trial. Each of 48 males was used in 2.44 (± 0.21 SE) trials, on average (range: 1–5). To increase uniformity in the readiness of males to mate, a minimum of 1 week elapsed between two trials for a given male.

There were three potential sources for lack of independence in our data set. First, some males were used in more than one trial, and one may argue that for this reason trials were not completely independent. Each test represented a choice by a unique female among a unique combination of males, however, and it is females, not males, that were the sampling units. We thus decided to consider the different scores for each trial as independent. Second, the activities of a female in one compartment influenced the activities of that female in other compartments. For each category of behavior investigated (e.g., time spent in each compartment), we thus compared compartments using ranks within trials rather than raw data. Third, in a given trial, the behavior of a female during a 1.5-h period was not independent of the behavior of that female in other 1.5-h periods. Again, we analyzed the effect of time on the activity of females using ranks instead of raw data.

We used non-parametric tests (Kruskal-Wallis one-way ANOVA, Wilcoxon signed-rank test, Friedman two-way analysis of variance by ranks; Siegel 1956), when data were not distributed normally (Shapiro-Wilk *W*-tests, SAS 1994a) or variances were not homogeneous (O'Brien's tests, SAS 1994a). We used *G*-tests for differences in the number of individuals that exhibited selected behaviors (Sokal and Rohlf 1981). Analyses were carried out with JMP version 3.0 (SAS 1994b). All statistical tests were two-tailed and significance levels were set at 0.05. Values provided are the mean ± SE.

Results

General activity of the females

Females entered their own compartment more often than any of the male chambers (Friedman test: $\chi^2_r = 50.50$, $df = 3$, $P < 0.001$; Table 1), and they spent more time in their compartment than in any of the male compartments (Friedman test: $\chi^2_r = 65.41$, $df = 3$, $P < 0.001$; Table 1). There was no significant difference in the number of visits to each of the male compartments (Friedman test: $\chi^2_r = 3.96$, $df = 2$, $P = 0.138$; Table 1) but females spent more time in one of the male compartments (Friedman test: $\chi^2_r = 9.70$, $df = 2$, $P = 0.008$; Table 1). This did not translate into a higher number of copulations observed in this preferred compartment (Friedman test: $\chi^2_r = 0.63$, $df = 2$, $P = 0.730$; Table 1), nor did we observe that more males copulated in this preferred compartment (number of tests where the male placed in compartment 1 copulated = 21; compartment 2 = 20; compartment 3 = 18; *G*-test: $G = 0.1$, $P > 0.5$). We thus consider in subsequent analyses that the location of a male in the arena did not affect its mating probability.

Did females choose to mate with one or several males?

Twenty-nine of 39 females (74.4%) copulated during the experiment. Of these, only 6 (20.7%) copulated with a single male, whereas 16 (55.2%) and 7 (24.1%) copulated with two and three males, respectively. The total number of copulations by females was significantly higher when more males were mated (Kruskal-Wallis test: $\chi^2_r = 9.33$, $df = 2$, $P = 0.009$; Table 2). The mean number of copulations per male, however, did not differ significantly according to the number of males involved (Kruskal-Wallis test: $\chi^2_r = 0.02$, $df = 2$, $P = 0.989$; Table 2). When females mated with two or three males, copulations were not evenly distributed among them (two males involved: 75.3% of copulations directed to the most mated male, Wilcoxon test: $Z = 3.85$, $P < 0.001$; three males involved: 50.0% of copulations directed to the most mated male, Kruskal-Wallis test: $\chi^2_r = 8.03$, $df = 2$, $P = 0.018$; Table 2). The males with which females mated first tended to

Table 1 Number of visits by female meadow voles to each of the four compartments of the choice arena, percentage of time spent by females in each of these compartments, and number of copulations performed by females in each male compartment ($n = 39$ trials for all variables)

Compartment	Number of visits to each compartment		Time spent in each compartment (%)		Number of copulations per compartment	
	Mean	SE	Mean	SE	Mean	SE
Male 1	66.7	11.3	8.3	1.5	8.5	3.2
Male 2	43.9	6.7	4.9	1.1	4.5	1.0
Male 3	78.3	13.8	12.1	2.2	5.1	1.2
Female	163.6	16.3	74.7	3.3	–	–

Table 2 Number of copulations by female meadow voles that mated with one, two or three males

Number of copulations	Females that mated with one male			Females that mated with two males			Females that mated with three males		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Total	10.7	3.0	6	25.6	7.3	16	33.4	7.5	7
Per male	10.7	3.0	6	12.8	3.6	16	11.1	1.9	7
With the most mated male				19.3	7.0	16	16.7	3.8	7
With the least mated male				6.3	0.5	16	5.4	1.4	7

Table 3 Body mass (g) of female meadow voles that mated with none, one, two, or three males

Female behavior	Body mass (g)		Range	
	Mean	SE	Min.	Max.
Did not mate ($n = 10$)	41.1	1.9	27.8	63.7
Mated with one male ($n = 6$)	37.1	2.8	33.2	64.7
Mated with two males ($n = 16$)	44.7	2.4	35.2	61.0
Mated with three males ($n = 7$)	40.4	3.4	31.0	73.2

perform more copulations than others, but differences were not significant (two males involved: 59.1% of copulations directed to the first mated male, Wilcoxon test: $Z = -1.93$, $P < 0.053$; three males involved: 42.4% and 34.0% of copulations directed to the first and second mated males, respectively, Kruskal-Wallis test: $\chi^2_r = 2.55$, $df = 3$, $P = 0.280$). We did not find any significant difference in body mass between the females that copulated with none, one, two, or three males (Kruskal-Wallis test: $\chi^2_r = 4.74$, $df = 3$, $P = 0.192$; Table 3).

Did females preferentially mate with heavier males?

Males were heavier than females (Kruskal-Wallis test: $\chi^2_r = 15.76$, $df = 3$, $P = 0.001$; Table 4) and their body mass did not differ significantly according to the compartment in which they were placed (Kruskal-Wallis test: $\chi^2_r = 2.07$, $df = 2$, $P = 0.356$; Table 4). The mass difference between the heaviest and the lightest male in a given test was high and significant [body mass of heaviest male, mean ± SE = 54.0 ± 1.0 g ($n = 39$); lightest male, mean ± SE = 40.1 ± 0.9 g ($n = 39$); Wilcoxon test: $Z = -6.89$, $P < 0.001$]. Males of intermediate body mass weighed on average 7.1 ± 0.9 g less than the heaviest male and 6.8 ± 0.8 g more than the lightest one.

Table 4 Body mass (g) of meadow voles used in the choice test experiment ($n = 39$ for each class). Note that a given male may have contributed several times to the data set if he was used in more than one test (see Methods)

Compartment	Body mass (g)		Range	
	Mean	SE	Min.	Max.
Male 1	45.6	1.3	27.8	63.7
Male 2	47.7	1.4	33.2	64.7
Male 3	47.8	1.1	35.2	61.0
Female	41.8	1.3	31.0	73.2

We observed no significant relation between the number of copulations performed by males and their within-trial body mass rank [number of copulations performed by heaviest male, mean ± SE = 10.2 ± 22.5 ($n = 29$); intermediate male, mean ± SE = 6.3 ± 6.8 ($n = 29$); lightest male, mean ± SE = 7.9 ± 7.0 ($n = 29$); Friedman test: $\chi^2_r = 1.05$, $df = 2$, $P > 0.5$]. Similarly, the heavier males did not consistently copulate before lighter ones [mating rank of heaviest male, mean ± SE = 2.1 ± 0.8 ($n = 29$); intermediate male, mean ± SE = 1.8 ± 0.8 ($n = 29$); lightest male, mean ± SE = 2.1 ± 0.7 ($n = 29$); Friedman test: $\chi^2_r = 1.05$, $df = 2$, $P > 0.5$]. Although females did not mate preferentially with heavier males, body mass of males influenced female choices in a more subtle way; data from females that mated with multiple males ($n = 23$) showed that the degree of female preferences was related to the magnitude of body mass differences between available males (Fig. 1). The difference between the number of copulations performed by the males ranked first and second (in terms of number of copulations performed per trial) increased with the absolute difference in body mass between these two males ($P = 0.022$, Fig. 1a). The same relationship existed between the males ranked first and third ($P = 0.014$, Fig. 1b) but not between the males ranked second and third ($P = 0.177$, Fig. 1c). These results indicate that male body mass had a significant influence on the number of copulations performed by males, but was not the major force driving female preferences.

The effect of time on the activity and copulatory behavior of females

The behavior of the 29 females that copulated in this experiment changed during the course of the trials; the total number of copulations observed per 1.5-h period was much higher at the beginning of the tests than at the end (Friedman test: $\chi^2_r = 35.83$, $df = 6$, $P < 0.001$;

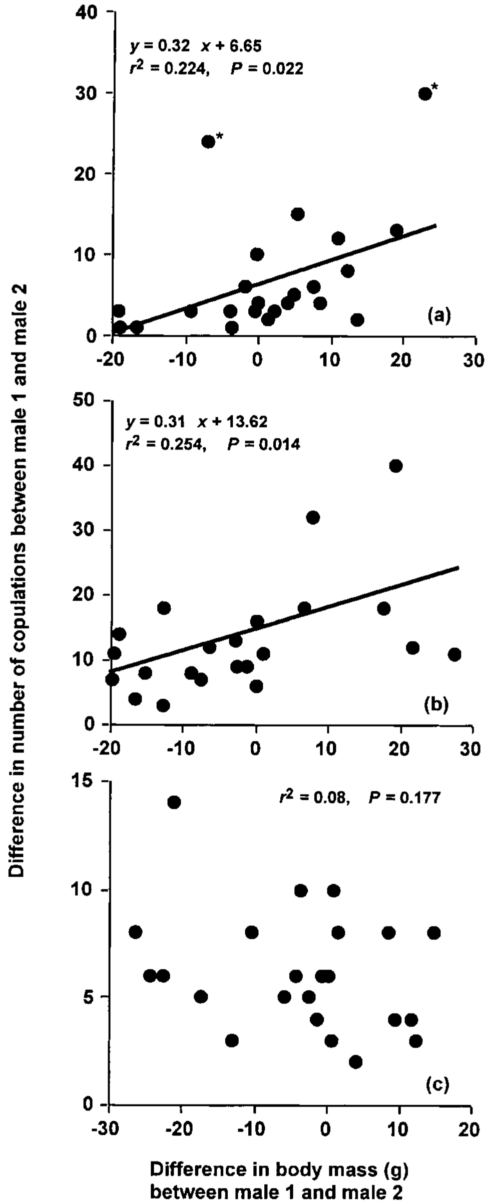

Fig. 1 Relationship between the difference in the number of copulations performed by males and the absolute difference in body mass between males. Data from trials where females mated with more than one male ($n = 23$). **a** Male 1 = male that performed the highest number of copulations, male 2 = male ranked second in terms of number of copulations performed. When the two data points identified by the *asterisks* are removed, $y = 0.23x + 5.03$, $r^2 = 0.323$, and $P = 0.007$. **b** Male 1 = male that performed the highest number of copulations, male 2 = male that performed the lowest number of copulations. **c** Male 1 = male ranked second in terms of number of copulations performed, male 2 = male that performed the lowest number of copulations

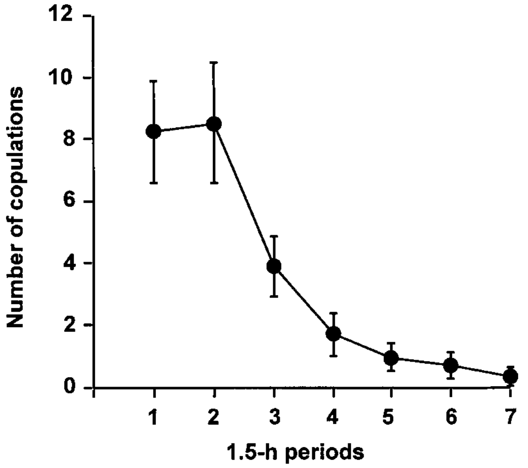

Fig. 2 Number of copulations performed by females ($n = 29$) in each of the seven successive 1.5-h periods of the choice trials (total duration of trials: 10.5 h). Values are means ± SE

Fig. 2). This was reflected in the higher activity of females at the beginning of the trials; the number of visits per 1.5-h period decreased through time (Friedman test: $\chi^2_r = 50.46$, $df = 6$, $P < 0.001$; Fig. 3), and females spent more time in their own compartment at the end of the tests than at the beginning (Friedman test: $\chi^2_r = 23.43$, $df = 6$, $P = 0.012$; Fig. 3). A time effect was also observed in the number of females that mated with none, one, two, or three males (Fig. 4); After 10.5 h of observation, 79.3% of the females that had copulated had mated with >1 male (Fig. 4), while only 34.8% of them had done so after 1.5 h. The first copulation was observed, on average, 916.0 ± 235.5 s ($n = 29$) after the beginning of the trial, while females copulated for the first time with a second male after 1422.4 ± 215.9 s ($n = 23$) and for the first time with a third male after 1901.1 ± 544.8 s ($n = 7$).

Discussion

Three main patterns emerge from this experiment: (1) when given the choice, female meadow voles were more

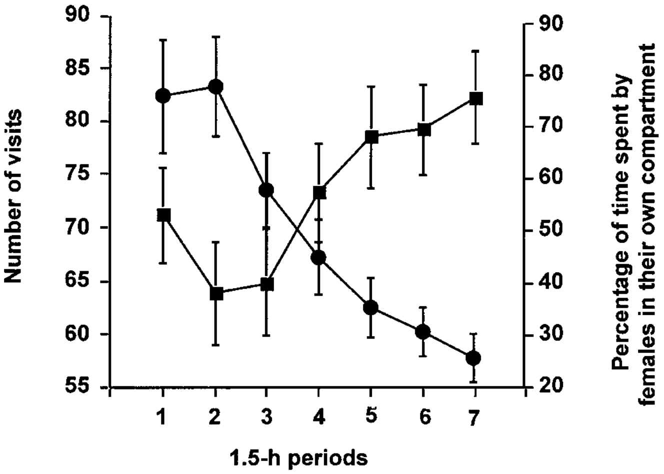

Fig. 3 Number of times females switched from one compartment of the choice arena to another (*circles*) ($n = 29$), and percentage of time females spent in their own compartment (*squares*), for each of the seven successive 1.5-h periods of the choice trials (total duration of trials: 10.5 h). Values are means ± SE

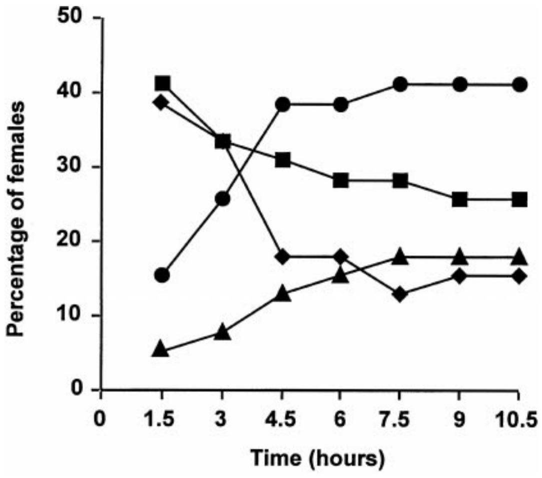

Fig. 4 Percentage of females ($n = 39$) that had copulated with no (*squares*), one (*diamonds*), two (*circles*), or three (*triangles*) males as a function of time elapsed since the beginning of the experiment

likely to mate with two or three males rather than with a single one; (2) male body mass influenced female preferences but this was not the major force driving female choices, and (3) some of our major results would have been very different if the duration of the choice tests had been shorter.

Females chose to mate with several males

We found that, when given the choice, most receptive female meadow voles mated with multiple males (Fig. 4). Storey et al. (unpublished data, in Storey 1994) noted that female meadow voles increased their approaches to subordinate males while engaged in mating bouts with dominants, and Salo and Dewsbury (1995) found that when female voles were presented with familiar and unfamiliar males, they tended to mate with both. Although the present study represents the first experiment aimed at analyzing female behavior in relation to multiple paternity in meadow voles, our results confirm previous reports suggesting that females play an active role in multiple paternity.

Boonstra et al. (1993) argued that the presence of promiscuity rather than polygyny in meadow voles was a result of the inability of some males to guard several females while others defend none. They attributed this inability to two factors. First, overwintered breeding males are similar in age and size, which decreases the variance in competitive ability between males. Such a low variance would lead to a more even distribution of females among males. Second, the habitat structure of meadow voles reduces the possibility that some males exclude others from mating a receptive female, because nearby males are difficult to detect and drive away. Our results indicate that female behavior is another, probably more powerful driving force behind promiscuity. By actively seeking fertilization by several males during her estrous period, a female may indeed be very efficient at preventing any male from successfully guarding her. We thus predict that promiscuity would occur in meadow voles even if their habitat was very different, or if variance in competitive ability of males was much higher. Such a prediction is easily amenable to testing in field or laboratory experiments.

Seeking to mate with several males is likely to entail costs (time and energy costs, increased risk of predation, risk of disease or parasite transmission; Lewis 1987) which should be compensated for by functional benefits. Many hypotheses have been proposed to explain multiple mating by females (hereafter numbered from 1 to 10). In the case of meadow voles, immediate material benefits to the female (1; Thornill and Alcock 1983) or increased parental care (2; Davies and Houston 1986) can be excluded as potential explanations because males do not provide food or material benefits to females, nor do they give paternal care to juveniles (but see Hartung and Dewsbury 1979, for paternal care in captivity). Alternatively, multiple mating could be a strategy against infanticide by males (3) since a mated male is less infanticidal than an unmated male (Wolff 1985). It could also represent a form of insurance against male infertility (4), whereby females guard against the possibility of some males being sterile or genetically defective (Gibson and Jewell 1982), or a means to increase the genetical diversity of offspring (5; Thornill and Alcock 1983) or to reduce the risk of inbreeding (6; Stockley et al. 1993). On the other hand, by mating with multiple males, females may ensure conceiving with genetically superior males by promoting sperm competition (7; Parker 1970). They may solicit at a high rate in order to assess some aspects of the "quality" of the males (8), since copulation is likely to be costly to males (Dewsbury 1982). All these potential benefits are not mutually exclusive and may well reinforce each other. They all emphasize the active role of females in multiple paternity.

One non-adaptative explanation of multiple paternity, namely the lower cost of remating with new males versus fighting off males (9; Alcock et al. 1977), can be rejected because females did mate with several males in our experimental conditions in which females could avoid harassment by males. One other non-adaptive hypothesis is that multiple mating may result from a genetic correlation between the sexes (10; Halliday and Arnold 1987), where multiple mating by males has been favored by selection. However, no evidence seems to currently support the idea of genetic correlations between the sexes in patterns of behavior (see references in Hunter et al. 1993).

We observed that 79.3% of the females that mated during our experiment did so with multiple males, whereas Boonstra et al. (1993) estimated that the proportion of litters sired by multiple males was 33.1% in their field conditions. Although these figures are difficult to compare because they come from artificial versus natural settings, the density of breeding adults may be a strong factor influencing the extent of multiple paternity (our experimental situation representing an extremely high density of voles). Support for this hypothesis comes from a recent experiment by Agrell (1997), who found in the laboratory that females of the closely related *M. agrestis* choose to mate predominantly with a single male when between-male distances are large, whereas they mate promiscuously if males are close to each other. Alternatively, one could suggest that males do manage to guard some of the females in the field, or simply that only a fraction of multiple matings result in multiple paternity.

Females did not prefer to mate with heavier males

Female meadow voles did not choose to mate with heavier males, despite the large variation in body mass between males and the fact that dominance is strongly related to body mass in meadow voles (Turner and Iverson 1973). In the monogamous *M. ochrogaster*, females do associate (Solomon 1993) and mate (Shapiro and Dewsbury 1986) preferentially with dominant males. Females of the promiscuous *Clethrionomys glareolus* also prefer to mate with dominant males (Horne and Ylönen 1996). Females of the promiscuous *Microtus montanus*, however, show no preference with regard to male dominance (Shapiro and Dewsbury 1986). This apparent diversity of female strategies led Shapiro and Dewsbury (1986) to propose that, to be able to conduct comparative analyses, it is as important to know species in which females do not display a preference as it is to know species in which they do. In spite of our results, we are reluctant to add meadow voles to the list of species where females show no mate preference with regard to male dominance. Indeed, Agrell (1997) found that female *M. agrestis* preferred dominant males when male density was low but showed no preference when male density was high (see previous section), suggesting that female preference may be context rather than species dependent. If such intraspecific variation is confirmed, then experimental manipulation of the social or ecological context of mate choice should prove to be a powerful tool to investigate the benefits of female choices. Moreover, although females did not prefer heavier males in our experiment, they did not distribute copulations evenly among males (Table 2), and the degree of female preferences was related to the difference in body mass between males (Fig. 1). Thus factors other than dominance did constitute the primary force affecting female preferences, but male dominance may have been a secondary player in driving female choices. The partial dependence between male body mass and female choice, coupled with the active role played by females, indicates that intersexual selection has the potential for reinforcing the effects of intrasexual selection (male-male dominance relationships) in meadow voles.

We have not identified the factors driving female preferences. Familiarity with males has been shown to positively affect female mate choice in meadow voles (Salo and Dewsbury 1995), but individuals were unfamiliar to each other in our study conditions. Alternatively, differences in solicitations by the males could potentially play a role in generating female choices (Webster et al. 1982). We did not attempt to quantify male approach patterns, however, and no obvious differences were noted between males. Many other differences between males can potentially influence female choice. For example, attractiveness of male odors to females varies with plasma testosterone concentration in meadow voles (Ferkin et al. 1994), and this might translate to mating preferences. Small mammals live in a sensory universe very different from that of humans, and we did not detect all cues used by females to choose their mates. More generally, the question of how animals choose their mates is a cloudy one (Gibson and Langen 1996).

Female behavior over time during the experiment

The females that mated more than one male during our trials had already done so on average 23 min after the beginning of the tests. There was a large variance around this mean value, however, so that it is only after 6 h that the proportions of females that had mated none, one, two, or three males began to reach asymptotic values (Fig. 4). Female copulatory behavior was still intense after 4.5 h (Fig. 2). It is thus crucial that future studies of multiple mating in small mammals include trials over extended periods of time. This contrasts with the shorter tests that have sometimes been conducted to measure female mate preferences in *M. pennsylvanicus* (e.g. ≤ 1 h, Salo and Dewsbury 1995). The optimal length of trials is likely to vary with species, however, so one should ideally stratify the results into discrete time periods, as we did, to verify that asymptotic values are obtained for the behavior considered. One major reason for this recommendation is that there does not seem to be any mating order effect in meadow voles (Dewsbury 1993; Storey et al. 1995) and other arvicolines (e.g. *M. ochrogaster*, Dewsbury and Baumgardner 1981). If this is the case, the last matings of a sequence are as likely to contribute to the progeny as are the first ones, and are thus equally important to consider.

In summary, we have demonstrated that female meadow voles play an active role in multiple paternity, and we believe that such a result should lead us to reconsider some of the hypotheses explaining promiscuity in the species. We also found that some variables other than body mass of males are the main factors driving preferences of females, although male body mass also had a significant influence on female choices. Finally, we show that the time period over which tests are conducted to examine multiple mating of female small rodents is an important part of the experimental design.

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