



Development of selective partner preferences in captive male and female meadow voles, *Microtus pennsylvanicus*

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Intraspecific social systems vary considerably as a function of environmental parameters (Lott 1984, *Behaviour*, **88**, 266–325). For example, nonmonogamous species may engage in facultative partner preferences and parenting to offset the costs associated with harsher breeding conditions. Because no field or laboratory research has examined nonmonogamous meadow voles under suboptimal conditions (e.g. low-density summer populations or during colder months), it was not known whether meadow voles could form affiliative preferences for a specific partner. The aim of this experiment was to identify whether meadow voles develop selective partner preferences and if so, under what circumstances. We assessed partner preferences using a choice apparatus in which the test animal chose to spend time with a familiar mate or stranger. We paired and tested males and females (within photoperiod) under one of five different conditions: after 24 h, 10 days, or 23 days of cohabitation with mating or after 24 h or 10 days of cohabitation without mating. Male and female meadow voles rapidly formed selective partner preferences for a familiar mate when compared with controls in nearly every condition, regardless of photoperiod, cohabitation duration, or whether mating did or did not occur. Within 24 h, males directed significantly more aggression towards unfamiliar animals, and mating enhanced this effect. For females, 24 h of social cohabitation was sufficient to decrease aggression towards partners, but stranger-directed aggression appeared later, following delivery of the litter. These data suggest that meadow voles are capable of developing selective partner preferences and stranger-directed aggression and may have evolved these abilities to maximize reproductive success during the colder months of the year or under low population density during the summer breeding season.

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Small rodents with high reproductive potential often live in seasonally and socially unpredictable habitats and, consequently, have flexible social systems to best accommodate variable ecological circumstances (Eisenberg 1966). Although microtine species are often categorized as ‘monogamous’ (e.g. prairie voles, *Microtus ochrogaster*; pine voles, *M. pinetorum*) or ‘nonmonogamous’ (e.g. montane voles, *M. montanus*; meadow voles, *M. pennsylvanicus*), they frequently display intraspecific variation in social organization. For example, typically monogamous prairie voles have a polygynous mating system during winter breeding and under high population densities in east-central Illinois (Getz et al. 1987; McGuire et al. 1993), and habitually show polygyny in the more xeric habitat of eastern Kansas (Fitch 1957; M. Gaines, personal

communication). Similarly, typically monogamous pine voles display an array of mating systems in the field (Wolff 1985). In this species, cooperative polyandry is the most common alternative to monogamy, and is thought to occur as a result of intense predation (Fitzgerald & Madison 1983). In contrast, nonmonogamous and asocial montane voles can form extended maternal families (Jannett 1978) or polygynous mating systems (Jannett 1980) under high population density, and engage in facultative monogamy under low-density conditions (Jannett 1980; Berger et al. 1997). Similarly, promiscuous and asocial meadow voles may engage in monogamy under circumstances of acute predation (D. Madison, personal communication), and live socially in female, preweaning young and reproductive adult male nesting constellations during colder months (Madison et al. 1984).

Although facultative changes in affiliative and parenting behaviours can be posited for these free-living conditions (Emlen & Oring 1977; Clutton-Brock 1991), most of these field studies used radiotelemetry and repeat

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live-trapping methodology to determine whether males and females occupied coincident home ranges and nest sites (i.e. monogamy), or whether they maintained separate territories/ranges and nest sites (i.e. nonmonogamy). Thus, to our knowledge, no field studies have examined whether variations in spatial social organization (as indexed by radiotelemetry or live trapping) induced measurable changes in specific reproductive strategies (e.g. facultative presence or absence of partner preferences, mate guarding or paternal care). However, the possibility that microtines have the capacity to demonstrate facultative reproductive strategies merits further consideration.

In the laboratory, microtine paternal and affiliative behaviours have been examined extensively (e.g. Hartung & Dewsbury 1979; Williams et al. 1992; Insel et al. 1995). However, few laboratory studies have been conducted under specific circumstances in which captive animals would most likely engage in alternative mating and parenting strategies (see Roberts et al. 1998 for an exception). Admittedly, designing experiments to test whether changes in some ecological conditions (e.g. population density, predation intensity) induce variation in reproductive strategies are better executed in free-living, rather than captive populations. However, other environmental and social parameters (e.g. photoperiod, cohabitation) that may affect reproductive strategies can be simulated in the laboratory, and thus, testing of captive animals for the capacity to display affiliative and paternal behaviours can provide potentially valuable information for future field investigations.

Meadow voles provide an excellent model in which to examine how social and environmental circumstances potentially induce changes in reproductive strategies as their spatial organization varies seasonally. Under these reliably changing ecological circumstances, the capacity to optimally allocate mating and parenting effort to best accommodate breeding conditions would be advantageous (Lott 1984).

The majority of meadow vole breeding occurs during the summer (hereafter the primary breeding season) when free-living male meadow voles typically maintain overlapping and diffuse ranges that encompass the mutually exclusive territories of several adult females (Madison 1980). Under these conditions, a male's reproductive success is maximized by engaging in mating effort rather than parenting effort (Clutton-Brock 1991), and consequently, summer meadow vole males rarely live on male-female coincident home ranges or display paternal care (Madison 1980).

However, during the colder months of the year, meadow voles, like many rodents, live communally to conserve heat (Howard 1950). During this seasonal period, social nesting and breeding activity overlap in time and reproductive adult males have been observed nesting and sleeping with females and preweanling young (Madison et al. 1984). In autumn and winter in some years, up to 50% of meadow vole females produce litters (Tamarin 1977; Christian 1980), and in unusually warm winters, 100% of females may continue breeding (Webster & Brooks 1981). During winter, meadow vole

population densities are lower than they are in the primary breeding season, and the probability of offspring mortality is greater. In the laboratory, biparental care increases pup growth rates even under summer photoperiods, and this effect should be more salient under free-living conditions (Storey & Snow 1987). Thus, under marginal winter field conditions, pair bonding and biparental care may confer a selective advantage by affording postpartum mating opportunities with a known breeding female and increased offspring survivorship. Furthermore, males may gain a selective advantage even during the summer breeding season by engaging in facultative monogamy when population densities are low, as they are on a regular cyclical basis for meadow voles (Christian 1980; Taitt & Krebs 1985). Under these circumstances, guarding a female mate and providing paternal care may confer higher fitness than travelling long distances searching for additional mating opportunities (Dewsbury 1985). Thus, long-term cohabitation, particularly of breeding pairs, may promote the development of strong partner preferences and, subsequently, biparental care.

Although no research has systematically examined partner preference formation in free-living or captive meadow voles under winter, short day lengths (SD) or summer, long day lengths (LD), some laboratory data are available for related social behaviours in LD animals. Whether meadow vole sires display paternal behaviour varies between laboratories and experimental conditions. Some researchers (Gruder-Adams & Getz 1985; Oliveras & Novak 1986; Wang & Novak 1992) have found little evidence of paternal behaviour, whereas other researchers (Hartung & Dewsbury 1979; Wilson 1982; Storey & Snow 1987; Storey & Walsh 1994; Storey & Joyce 1995; Parker & Lee, *in press*) report appreciable to extensive male care of young. In populations in which males are readily paternal, male meadow voles may share the nest of a female mate rather than establishing an independent nest site (Storey et al. 1994), show considerable paternal care (Hartung & Dewsbury 1979; Dewsbury 1983; Storey et al. 1994), drive off intruders (Storey 1986, 1994, 1996; DeCatanzaro & Storey 1989), and even mate with an unfamiliar female without diminishing paternal care (Storey & Snow 1987; Storey et al. 1994). Rearing and experimental conditions aside, another possibility suggested by this behavioural variation is that mating and parenting behaviours are facultative and population specific in this species. This combination of evidence suggests that it would be valuable to determine whether captive meadow voles form selective partner preferences in response to any of the seasonal changes (e.g. photoperiod, social cohabitation) that free-living voles routinely experience. Our laboratory manipulations were designed to pursue this issue.

The aim of this experiment was to determine whether meadow voles are capable of forming selective, opposite-sex partner preferences and displaying stranger-directed aggression, two defining features of pair bonding (Williams et al. 1992; Insel et al. 1995). Laboratory research on monogamous prairie voles has implicated several social variables in the onset of these behaviours.

Table 1. Selective partner preference testing

Subjects	Mating condition			Nonmating condition	
	Day 1/2	Day 10/11	Day 23/24	Day 1/2	Day 10/11
SD females	12	7	13	8	10
SD males	14	8	15	10	10
LD females	14	9	14	10	4
LD males	15	10	14	10	4

Values are sample sizes of captive adult male and female meadow voles tested for the development of partner preferences and stranger-directed aggression under various social and environmental circumstances. Independent variables were as follows: photoperiod (e.g. winter, short days, SD, or summer, long days, LD), sexual relationship (i.e. cohabitation with mating or without mating) and length of cohabitation.

In female prairie voles (Williams et al. 1992), 6 h of cohabitation with mating and 24 h of cohabitation without mating are both sufficient to create strong, selective partner preferences. Longer durations of cohabitation (e.g. 48 h) do not increase the strength of the preference. Female prairie voles do not differ in the frequency of aggressive bouts directed to the mate versus an unfamiliar male, as they engage in very little aggression. For male prairie voles (Insel et al. 1995), cohabitation without mating is insufficient to induce partner preferences or stranger-directed aggression, as males require 24 h of cohabitation with mating to initiate pair-bonding behaviours.

Because specific partner preferences and stranger-directed aggression do not consistently appear in typically nonmonogamous species, it seems likely that specific circumstances (i.e. the coincidence of multiple variables) are required to instate these behaviours. To test whether meadow voles develop similar selective partner preferences or display stranger-directed aggression, we chose to examine social and environmental variables that would be likely to produce changes in affiliative and aggressive behaviours under field conditions: sexual relationship, length of cohabitation and photoperiod.

METHODS

Study Animals and Housing Conditions

Study animals, derived from wild-caught voles indigenous to northwestern Pennsylvania and southwestern New York, U.S.A., were born to continuously cohabiting breeding pairs housed under long day lengths (LD; 14 h light/day) in an established colony at the University of Michigan. We removed weanling meadow vole pups from the dam and sire at 18 days of age, housed them in same-sex sibling pairs, and placed them in either LD or short day conditions (SD; 10 h light/day). A total of 142 males (69 LD and 73 SD males) and 135 females (67 LD and 68 SD females) were used in this experiment. We housed study animals in polypropylene cages (26.67 × 21.59 × 13.97 cm) on pine shaving bedding with food (Purina mouse chow No. 5015) and water available ad libitum. Animal rooms were maintained at 21 ± 2 °C with low ambient noise conditions. Study animals resided in sibling pairs from 20 to 50 days of age, when they were

separated into individual housing for 3 weeks. Study animals remained so housed until the beginning of the experimental procedure.

Experimental Design

At 71 days of age, animals either remained housed alone, or were paired with an (unrelated) animal of the opposite sex. Pairs were placed in 10-gallon (37.85-litre) aquaria for videotaping (with ad libitum access to food and water) to determine whether mating occurred. During the dark phase of the light cycle, pairs were continuously videotaped with a Panasonic camera and wide-angle lens on a time-lapse video-cassette recorder using low illumination red light. The first partner preference testing (described below) occurred either within 24–36 h after mating, or in the pairs that did not mate, 24–36 h after pairing. Only study animals that mated within the first day of pairing were used for the mating condition to minimize the confounding effects of increased cohabitation time, relative to the nonmating group. We also tested another group of study animals for partner preferences after 10–11 days of mated or unmated cohabitation. After pairing, we continuously videotaped nonmating pairs to ensure that mating did not occur prior to preference testing. We tested the preferences of mating pairs 48–72 h after the birth of the litter (after 23–24 days of cohabitation; see Table 1). Control animals were tested once, at comparable ages to study animals at the first postmating test. (Because unpublished data from our laboratory indicated that the behaviour of unpaired animals tested with two unfamiliar, opposite-sex, stimulus animals of comparable ages to the experimental groups did not differ, we tested only control animals once to simplify data analysis.) It should be noted that a cross-sectional design was used rather than a within-subjects design. This design was chosen so that pairs were only tested once, which eliminated the potential confound of multiple experiences in the testing device and duration of cohabitation.

Preference Testing

Behavioural testing was conducted in a Plexiglas three-arm affiliation device (39.37 × 24.13 × 27.94 cm). The

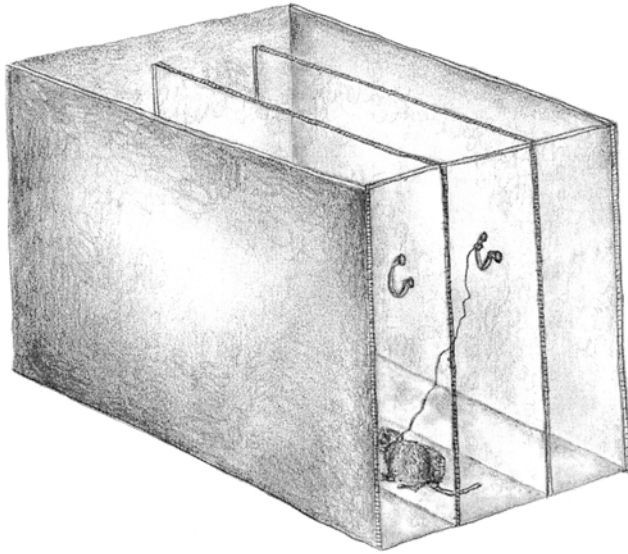


Figure 1. Drawing of the Plexiglas testing apparatus (39.37×24.13×27.94 cm) used to assess opposite-sex, selective partner preferences in male and female meadow voles.

device consisted of three equal compartments, connected by a runway on one side of the device (see Fig. 1). In one of the compartments, we loosely tethered an animal from the test pair with a 10.15-cm nylon tie (Radio Shack) and wire fishing clip secured to the back wall of the compartment. (We secured nylon ties while test animals were lightly anaesthetized with Halothane.) This familiar stimulus animal was able to move freely in its end of the compartment. We tethered a second unfamiliar stimulus animal of the same sex, photoperiod and age as the first stimulus animal in an identical fashion in a second arm of the device. The third arm of the device remained empty. We allowed tethered stimulus animals to become familiar with the new environment for 10 min before introducing the test animal into the device. During the 180-min preference test, the test animal could move freely throughout the entire testing device. We carried out each test during the lighted phase of the light cycle and videotaped each 3-h test with a Panasonic camera and wide-angle lens on a time-lapse video-cassette recorder. We later scored behavioural tests with a computer program for counts and durations of amicable and agonistic behaviour between the test and stimulus animals. We determined behaviour to be amicable when the test animal engaged in side-by-side contact with a stimulus animal (see Williams et al. 1992). We determined behaviour to be aggressive when the test animal engaged in charging, attacking, biting, or boxing with a stimulus animal (see Ferkin 1988). Because the scoring program only tabulated time in minutes (and, thus, seconds could only be approximated), we chose to analyse only counts of aggressive behaviours (as these lasted under 1 min in duration) and only duration of side-by-side contact (as duration of time spent together was a more accurate index of affiliative preference and lasted for long bouts and few counts). We also calculated the amount of time the test animal spent alone in the third arm of the device.

We tested males and females of a pair on subsequent days and counterbalanced this design between pairs.

Ethical Note

As described above, we permitted aggressive behaviour between a free-roaming test animal and a tethered stimulus animal. In this experiment, agonistic interactions were infrequent, mild and brief. Of the 251 preference tests conducted in this study, no test was terminated early due to acute aggression. Furthermore, we inspected test and stimulus animals following each test, and no signs of physical injury were evident.

Data Analyses and Statistics

We conducted all design verification and experimental analyses separately for male and female subjects. Control data for males and females will be presented in the same section, whereas the experimental data for males and females will be presented separately. SYSTAT (version 7.0) was used for all analyses. Differences were considered significant when $P < 0.05$.

Design verification analyses and statistics

Several method verification analyses were needed to assess whether pre-existing behavioural preferences existed that might compromise the integrity of the experimental design. Thus, it was critical to determine whether meadow voles displayed location preferences in the testing apparatus in the absence of stimulus animals, whether the location of the stimulus animals in the apparatus influenced the behaviour of the test animals, and whether testing on consecutive days during the different testing times differentially affected the development of affiliative preferences.

We examined potential location preferences within the testing apparatus by placing the test animal ($N = 12$ males, 6 LD and 6 SD; $N = 14$ females, 6 LD and 8 SD) into the device alone for 3 h without stimulus animals. During behavioural scoring, we quantified the duration of time the test animal spent in the possible areas of the apparatus (e.g. the three compartments). We assessed location preferences using a repeated measures analysis of variance (ANOVA) across the areas, with photoperiod (LD, SD) as a factor. Second, we tested the hypothesis that test animals ($N = 20$ males, 10 LD and 10 SD; $N = 20$ females, 10 D and 10 SD) would randomly select a partner when both potential, opposite-sex, photoperiod-matched partners were unfamiliar, either spending little time with either, or equal time with both via a within-subjects repeated measure ANOVA across the three compartments, with photoperiod as a factor. For both location preference analyses, we tested significant differences with C-matrix post hoc tests and adjusted P values for protection against multiple comparisons. Third, because males and females from pairings were tested on 1 of 2 days at each test interval (either day 1/2, day 10/11, or day 23/24), it was necessary to determine whether an order effect of test day on preference behaviour existed. We performed this

analysis separately for each of the five test times (i.e. cohabitation with mating after 1/2 days, 10/11 days, 23/24 days; and cohabitation without mating after 1/2 days and 10/11 days). Day effects were assessed using a repeated measures ANOVA across time spent with partner, stranger and alone, with photoperiod (LD, SD) and the day of test as factors.

Experimental data analysis and statistics

Analyses within groups. We assessed affiliative preferences similar to Williams et al. (1992). We determined preferences by quantifying the number of minutes test subjects spent in side-by-side contact with each stimulus animal (e.g. partner and stranger). We tabulated the number of aggressive interactions with each stimulus animal. We compared each of these behaviours (e.g. contact time, aggressive bouts) between the partner and stranger, using paired *t* tests within each experimental condition. When an effect of photoperiod was found in the between-group analyses for affiliative or aggressive behaviours, we performed within-group analyses (i.e. paired *t* tests) separately for each photoperiod. It should be noted that statistical analyses did not include LD animals at 10–11 days of cohabitation without mating in either the within- or between-subject groups. Because nearly all LD animals mate within 48 h of pairing (Meek & Lee 1993), obtaining LD animals after 10 days of unmated cohabitation proved difficult. Furthermore, given that failure to mate after 10 days of cohabitation might be correlated with other atypical social behaviours, we excluded the data from the study animals we did test.

Analyses between groups. To examine whether LD and SD mated and unmated animals changed their affiliative or aggressive behaviour over time, differed from each other, or differed from control animals, we used a multivariate ANOVA where photoperiod, mating status (mated, unmated, or control) and test time were factors and duration in each compartment (e.g. time spent in side-by-side contact with the partner, stranger, or alone) or aggressive behaviour (e.g. number of aggressive bouts with each stimulus animal) was the primary effect tested. When a significant within-subjects interaction was observed between a single factor and time in each compartment, we ran Tukey's post hoc pairwise comparisons holding constant the C-matrix for main effects of time (or counts) in each compartment. When factor and interaction effects were significant, we generated and tested a C-matrix for main effects of time in compartments, and an A-matrix for testing differences between photoperiods and mating status. We performed a C-matrix holding constant the A-matrix for photoperiod or mating status testing for factor interactions. Because Insel et al. (1995) found that 24 h of cohabitation with mating (but not 24 h of cohabitation without mating) induced aggressive behaviour in male prairie voles, we also conducted a similar analysis that collapsed across duration of time spent together prior to the delivery of the litter (i.e. the factors were: cohabitation with mating, cohabitation without mating, and controls) to examine whether mating had a similar effect on male meadow voles.

RESULTS

Method Verification

Area preferences without stimulus animals

No significant differences appeared in time spent between the areas of the test apparatus, indicating that no side preference existed for either males or females. No effect of photoperiod was observed, and there was no interaction between photoperiod and area of apparatus preference.

Area preferences with stimulus animals

Control experiments involving a male and two unfamiliar females or a female and two unfamiliar males further tested the validity of the method. With animals present in the test compartments, overall male area preferences significantly differed ($F_{2,36}=16.793$, $P<0.0001$) such that males preferred to spend more time alone (time with stimulus animal 1 versus alone: $F_{2,18}=5.319$; $P=0.015$; time spent with stimulus animal 2 versus alone: $F_{2,18}=17.369$, $P<0.0001$) but did not show a preference between the two unfamiliar animals. No main effects for photoperiod or interaction effects with area preference were observed. Like males, females showed strong area preferences ($F_{2,36}=41.552$, $P<0.0001$), did not differ by photoperiod, nor was an interaction between photoperiod and area preference observed. Females preferred to spend time alone in the empty compartment rather than spend time with stimulus animal 1 ($F_{2,18}=15.659$, $P<0.0001$) or stimulus animal 2 ($F_{2,18}=31.383$, $P<0.0001$), and also like males, females did not differ in time spent in contact with stimulus animals.

Order effects for test days

Statistical analysis ruled out order effects for test days. No significant differences appeared between the two consecutive test days for either mated or unmated female and male test animals after 1–2 days of cohabitation with mating or without mating, 10–11 days of cohabitation after mating or without mating, or 23–24 days of cohabitation after mating.

Experimental Analyses: Partner Preferences of Males

Within-group partner preferences

Male meadow voles established strong, specific affiliative preferences for a familiar female within 24 h of paired cohabitation. Paired *t* tests within each experimental group revealed that all experimental males engaged in 10 times the amount of side-by-side contact with the familiar female as with the unfamiliar female (see Fig. 2).

Between-group partner preferences

Across groups, male meadow voles formed specific partner preferences within 24 h of paired cohabitation, regardless of photoperiod, mating, or duration of time spent together. Multivariate ANOVA revealed a main

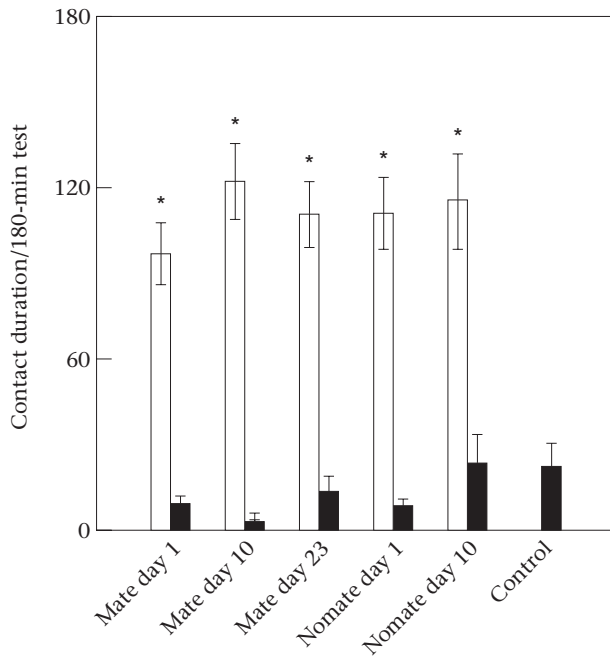


Figure 2. Mean (\pm SE) male duration of side-by-side contact with female partners (\square) and strangers (\blacksquare) during 180-min preference tests. *Indicates a minimum significant difference ($P < 0.05$) between time spent with partner and stranger within each group. Mate day 1 ($t_{28} = 7.644$, $P < 0.0001$), mate day 10 ($t_{18} = 9.330$, $P < 0.0001$), mate day 23 ($t_{28} = 7.004$, $P < 0.0001$), nomate day 1 ($t_{19} = 8.501$, $P < 0.0001$), nomate day 10 ($t_9 = 2.912$, $P = 0.017$), control ($t_{19} = -0.198$, $P = 0.845$).

effect for test condition ($F_{5,118} = 2.356$, $P = 0.045$) and an interaction effect for test condition and amount of time spent in each compartment ($F_{10,236} = 6.978$, $P < 0.0001$). For each compartment, Tukey post hoc pairwise comparisons were run. All experimental males differed significantly from control males (but not from each other) in the amount of time spent with a familiar partner and the amount of time spent alone. There were no observed differences among any of the conditions in time spent with an unfamiliar female ($F_{5,118} = 1.006$, $P = 0.417$). In all conditions, test males spent 61% of the 3-h affiliation test in side-by-side contact with the familiar partner, compared with 6% of time with an unfamiliar stimulus female and 33% of time alone. In contrast, control males spent 75% of the 3-h test alone, and only 25% of the time in contact with the unfamiliar stimulus females.

Experimental Analyses: Aggressive Behaviour by Males

Within-group aggressive behaviour

In all five experimental conditions, males displayed significantly more aggression towards the unfamiliar female than the familiar partner (paired t test: mate day 1: $t_{28} = -3.686$, $P = 0.001$; mate day 10: $t_{17} = -2.654$, $P = 0.017$; mate day 23: $t_{28} = -2.542$, $P = 0.017$; nomate day 1: $t_{19} = -2.856$, $P = 0.010$; nomate day 10: $t_9 = -2.475$, $P = 0.035$; control: $t_{19} = 1.154$, $P = 0.263$; see Fig. 3).

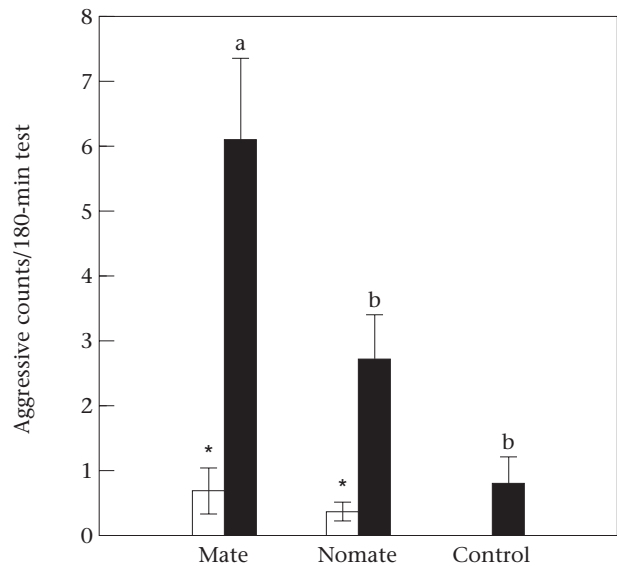


Figure 3. Mean (\pm SE) frequency of aggressive interactions by males towards female partners (\square) and strangers (\blacksquare) during 180-min preference tests. 'a' indicates a minimum significant difference ($P < 0.05$) between groups with 'b', whereas those with 'b' did not differ. *See Fig. 2.

Between-group aggressive behaviour

Experimental males that mated and then lived with the female for 10 days were significantly more aggressive to the unfamiliar female when compared with control males (ANOVA: $F_{5,118} = 2.495$, $P = 0.035$; Tukey post hoc HSD multiple comparison: $P = 0.041$), but did not differ from other experimental males. Experimental males did not differ in hostility to the familiar partner, and control males had low aggression towards both unfamiliar females. No additional effects or interactions were observed for photoperiod or test time.

Similar to prairie voles, mating and 24 h of cohabitation appeared to have a significant effect on increasing aggression directed towards strangers. Whereas mated and unmated males differed in aggression to stranger versus partner (paired t test: mated: $t_{75} = -5.07$, $P < 0.0001$; unmated: $t_{33} = -3.41$, $P = 0.002$) and controls did not (control: $t_{19} = 1.154$, $P = 0.263$), MANOVA revealed a significant effect for mating status on aggression towards the stranger ($F_{2,98} = 6.226$, $P = 0.003$). Post hoc multiple comparisons revealed that mated males were significantly more aggressive to strange males than either unmated ($P = 0.021$) or control males ($P = 0.001$). Unmated and control males did not differ from each other (see Fig. 3).

Experimental Analyses: Partner Preferences of Females

Within-group partner preferences

All females spent significantly more time in side-by-side contact with the familiar partner (55% of the time) than with the stranger (6% of the time). Results of the

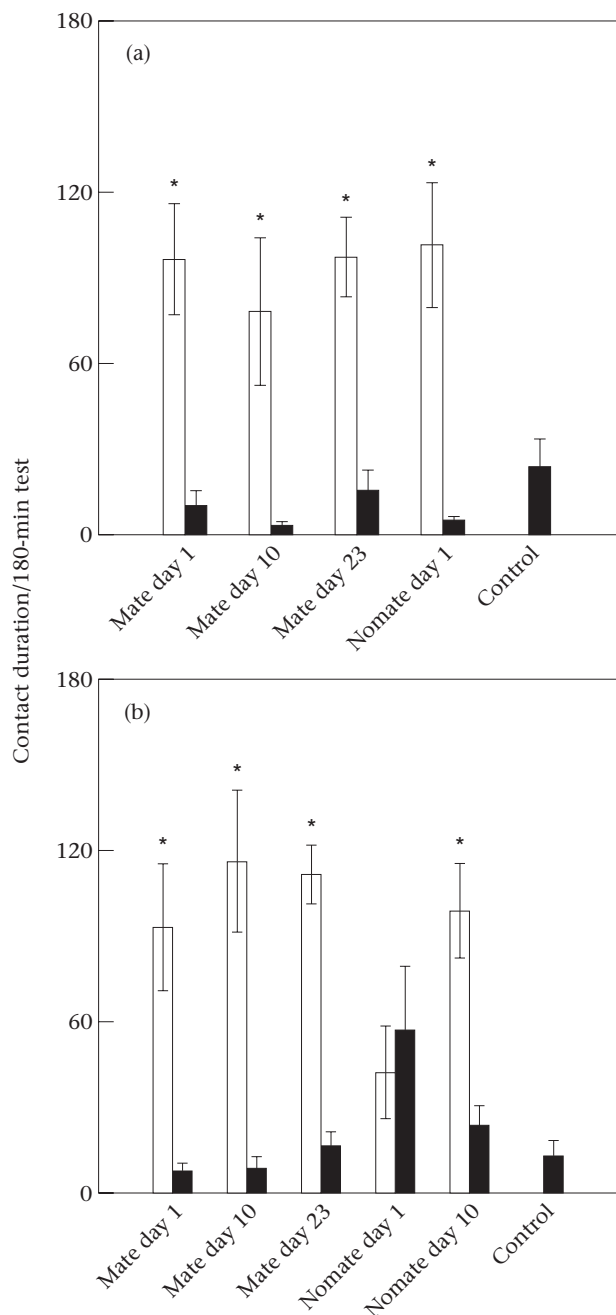


Figure 4. Mean (\pm SE) duration of side-by-side contact with male partners (\square) and strangers (\blacksquare) during 180-min preference tests. (a) LD females; (b) SD females. *See Fig. 2.

paired t tests were as follows and are collapsed across photoperiod when relevant: mate day 1: $t_{25}=6.120$, $P<0.0001$; mate day 10: $t_{15}=5.253$, $P<0.0001$; mate day 23: $t_{26}=8.877$, $P<0.0001$; nomate day 10: $t_9=4.437$, $P=0.002$; control: $t_{19}=-0.720$, $P=0.480$. The only exception was that SD females after 24 h of unmated cohabitation did not show a significant preference for either stimulus animal ($t_7=-0.550$, $P=0.599$), whereas LD females did ($t_9=4.828$, $P=0.001$; see Fig. 4).

Between-group partner preferences

Within 24 h of mated cohabitation, LD and SD female meadow voles developed a strong preference for their familiar mate compared with unfamiliar controls. The only group that failed to develop a partner preference was SD females after 24 h of cohabitation without mating when compared with controls ($P=0.356$). In contrast, LD females that cohabited with a partner (for 24 h) with or without mating both developed strong preferences. After 10 days of cohabitation without mating, SD females developed a partner preference that was equivalent to that of SD females after 24 h of mated cohabitation (see Fig. 4).

Within photoperiod, all experimental SD female groups spent significantly more time with their familiar partner than those in the control group or 24 h of unmated cohabitation group. In contrast, the 24 h of unmated cohabitation group spent significantly more time with the unfamiliar stranger than every experimental and control group.

Females in every experimental LD group spent significantly more time with their familiar partner compared with the unfamiliar animal. No differences were observed in time spent in side-by-side contact with the stranger for any group, and control animals spent significantly more time alone when compared with all experimental groups.

Finally, when comparing LD and SD females within each group (e.g. SD and LD females after 24 h of mated cohabitation), the only significant differences observed were between SD and LD females after 24 h of unmated cohabitation. LD females spent significantly more time with the familiar partner, while SD females spent more time with the unfamiliar partner, but they did not differ on time spent alone.

Experimental Analyses: Aggressive Behaviour by Females

Within-group aggressive behaviour

Postpartum females (which had delivered a litter 48–72 h prior to behavioural testing) showed significantly more aggression towards the unfamiliar male than towards their partner ($t_{26}=-2.422$, $P=0.023$). No other aggression differences between the partner and the stranger were found for the other experimental groups or the control group (see Fig. 5).

Between-group aggressive behaviour

MANOVA revealed a main effect for test group (but not photoperiod) for aggression to partner ($F_{5,109}=3.468$, $P=0.006$) and to stranger ($F_{5,109}=3.248$, $P=0.009$). The first post hoc test indicated that every experimental group engaged in significantly less aggressive interactions with the familiar partner when compared with controls. The second post hoc test revealed that postpartum females displayed significantly or near significantly more aggression to the strange male when compared with all other groups (see Fig. 5).

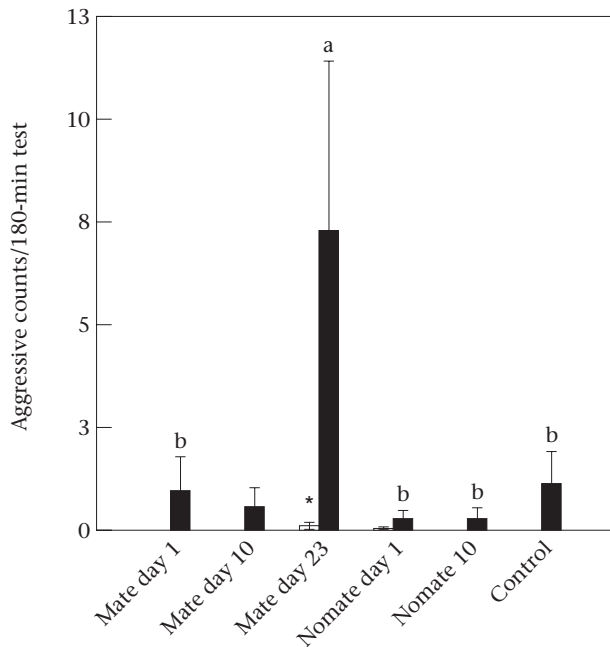


Figure 5. Mean (\pm SE) frequency of aggressive interactions by females towards male partners (\square) and strangers (\blacksquare) during 180-min preference tests. 'a' and 'b' see Fig. 3. *See Fig. 2.

DISCUSSION

In this experiment, we sought to determine whether captive male and female meadow voles developed selective, opposite-sex, partner preferences and stranger-directed aggression, and if so, under what circumstances. In particular, we wanted to ascertain whether mating, duration of cohabitation with a partner and photoperiod (e.g. long summer days, LD, or short winter days, SD) affected partner preference formation and the onset of stranger-directed aggression. Our results indicate that male meadow voles form rapid and selective partner preferences for a familiar mate within 24 h of pairing in every condition (Fig. 2). Male partner preferences established after 24 h of social cohabitation were not enhanced by mating, longer periods of cohabitation, or seasonal changes in day length. Similarly, 24 h of social cohabitation was sufficient to induce opposite-sex, stranger-directed aggression in all test males regardless of cohabitation duration or photoperiod (Fig. 3). However, cohabitation with mating enhanced stranger-directed aggression in males (when compared with cohabitation without mating or no cohabitation). Similarly, captive female meadow voles also established strong, selective partner preferences within 24 h of social cohabitation (Fig. 4). In nearly all groups, neither mating, longer cohabitation periods, nor day length enhanced the establishment of partner preferences. The only exception was that females housed under short day lengths failed to form partner preferences after 24 h of cohabitation in the absence of mating. However, following 10 days of social cohabitation without mating, SD female partner preferences were identical to those established after 24 h of cohabitation with mating. For females, social

cohabitation and the delivery of the litter influenced aggression directed towards strangers. Following the birth of the litter, females displayed significantly more aggression to strange males than to partners. In all other groups, although aggression to strangers did not generally increase when compared within groups (to the partner) or between groups (to controls), cohabitation did decrease aggression to a familiar partner within 24 h of pairing (Fig. 5).

In the laboratory, partner preferences have been defined as spending twice as much time in contact with a familiar partner relative to an unfamiliar conspecific (prairie voles, Insel et al. 1995). In our study, meadow vole males spent 61% of a 3-h preference test in contact with a familiar partner, compared with 6% of time with the unfamiliar female. Similarly, meadow vole females (unmated SD females after 24 h of cohabitation with-standing) spent 55% of the test in contact with a familiar partner, compared with 6% of the time with the unfamiliar male. These data represent much greater than a two-fold difference between time spent with the partner and time spent with the stranger. Furthermore, this is the first experimental evidence to show that a nonmonogamous microtine can establish partner preferences and stranger-directed aggression using the same social cues as a consistently pair-bonding species (i.e. the prairie vole).

Emlen & Oring (1977) have argued that monogamy is the default result of the inability of either sex to monopolize additional members of the opposite sex either directly or indirectly through resource control. However, most 'monogamous' species opportunistically exploit the 'polygamy potential' of changing environments that afford increased access to multiple mates (through a greater ability to control resources or via higher population densities). Under such circumstances, the costs and benefits of mating effort (e.g. energy/risk expenditure to secure matings) and parenting effort (energy/risk expenditure to produce/raise offspring) change, and males would be expected to allocate more effort to mating, rather than parenting, effort (Trivers 1972; Emlen & Oring 1977; Low 1978). This facultative polygyny has been documented in several monogamous species (Lott 1984). Conversely, facultative monogamy occurs under circumstances in which one sex no longer retains the capacity to monopolize multiple mates or control access to resources, or environmental circumstances are such that paternal effort is required to offset offspring mortality associated with uniparental care. Under such breeding circumstances, the failure to engage in facultative strategies that best accommodate 'suboptimal' field conditions carries negative fitness consequences for males and females. Consequently, selection may have favoured the capacity to facultatively display 'monogamous' or 'nonmonogamous' reproductive strategies under the appropriate circumstances (Lott 1984). Thus, rather than the exclusive property of 'monogamous' species, the establishment of partner preferences, stranger-directed aggression and paternal care may be best conceptualized as probabilistic clusters of behaviours that occur under specific environmental conditions that favour greater parental, rather than mating, strategies. However,

whether or not meadow vole partner preferences are facultative remains to be tested explicitly.

Although we have made an adaptive argument for the expression of partner preference formation and stranger-directed aggression in LD and SD pairs of meadow voles that cohabit after mating under low-density summer populations or during the colder months of winter respectively, exactly why unmated LD females retain the ability to form partner preferences and unmated, socially living SD females require many days to instate a social preference remains unclear. However, one proximate possibility is that because meadow vole reproductive activity decreases during winter months, reproductive physiology is frequently suppressed by winter conditions (Meek & Lee 1993). As this translates into a reduction in gonadal steroids that facilitate partner preference formation in prairie voles (Williams et al. 1992; Witt 1997), SD female meadow voles may experience a greater latency to activation of partner preferences. Therefore, LD females, having heightened reproductive physiology, may be primed to form rapid preferences if given the proper stimulus.

Future laboratory investigation of the establishment of opposite-sex partner preferences in captive meadow vole populations should consider other socioecological variables, such as restricted access to food, decreased ambient temperatures, and large seminatural enclosures, which might produce differences in the establishment of LD and SD partner preferences. Although laboratory research does not conclusively prove that captive behaviour occurs under free-living conditions, our laboratory evidence suggests that it would be valuable to examine in the field whether meadow voles engage in selective partner preferences under 'suboptimal' breeding conditions (e.g. low-density populations or the colder months of the year) that free-living voles routinely experience.

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