Differences in Affiliative Behavior, Pair Bonding, and Vaginal Cytology in Two Species of Vole (*Microtus ochrogaster* and *M. montanus*)

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Prairie voles (*Microtus ochrogaster*) and montane voles (*M. montanus*) display marked differences in social organization in the field. Trios of 1 male and 2 females were studied in a large enclosure for a 10-day period. Prairie voles spent 59% of the observation time in side-by-side contact, whereas montane voles spent only 7% of the time in contact. Vaginal smears indicated female-female suppression of estrus in prairie voles; female montane voles appeared to cycle in the presence of males. Male prairie voles preferentially paired and nested with 1 of the females, and vaginal estrus generally followed pair formation by 2 days. Male montane voles did not spend time preferentially with either female, even after mating. These results suggest that the contrasting mating systems of these species result from differences in the propensity for affiliative behavior and social bonding rather than from mate availability or female receptivity.

Social organization and mating systems can vary among, and even within, populations of the same species (e.g., Lott, 1984; Sachser, 1986). Nevertheless, species do display characteristic forms of social organization that are the result of stable differences in behavior (Dewsbury, 1988; Mason, 1974). When animals from closely related species that display different forms of social organization are examined in the laboratory, species-typical behavioral profiles emerge. Contrasts in these profiles represent those traits and propensities that were at a selective advantage in certain ecological and social contexts. They also represent those traits responsible for generating differences in social organization. The aim of our research is to delineate key behavioral differences between prairie voles (*Microtus ochrogaster*) and montane voles (*M. montanus*) in a large, indoor enclosure. Our overall objective is to use these profiles to understand better the processes and mechanisms by which differences in the traits of individual animals are translated into adaptive patterns of spacing, mating, and demography at the group or population level.

Although closely related phylogenetically and morphologically, prairie and montane voles display marked differences in social organization in the wild. Prairie voles are generally monogamous and live in extended family groups, whereas montane voles are polygamous and solitary, and the females may abandon their young at about 2 weeks after birth (Getz, 1985; Getz & Carter, 1980; Getz, Carter, & Gavish, 1981; Getz & Hofman, 1986; Jannett, 1980, 1982). The data indicating contrasting mating systems in natural populations of prairie and montane voles are also supported by a growing

number of laboratory studies in which these species have been examined either individually (e.g., Carter, Getz, & Cohen-Parsons, 1986; McGuire & Novak, 1986; Thomas & Birney, 1979), in comparison with other species (e.g., Dewsbury, 1985; Hartung & Dewsbury, 1979; McGuire & Novak, 1984; Oliveras & Novak, 1986; Wilson, 1982a, 1982b), or in direct comparison with each other (e.g., Shapiro, Austin, Ward, & Dewsbury, 1986; Shapiro, Meyer, & Dewsbury, 1989).

In addition to behavioral differences, prairie and montane voles individually housed under identical conditions in the laboratory display a clear species difference in the appearance of their vaginal cytology. Prairie vole smears are dominated almost entirely by leukocytes, which generally indicates diestrus (Richmond & Conaway, 1969a, 1969b), whereas montane voles smears are dominated by cornified cells, which generally indicates estrus (Sawrey & Dewsbury, 1985). These differences may have important implications because the dynamics of female receptivity within a population can be an important proximate determinant of mating system diversity in the field (e.g., Emlen & Oring, 1977).

This research directly compares, for the first time, the social behavior of these species in a large test enclosure. Trios of 1 male and 2 females were observed for a period of 7 days. Species differences in patterns of nesting, pair bonding, affiliative behavior, and aggression were recorded. In addition to these behavioral measures, vaginal smears were taken daily. We tested the hypothesis that species differences in vaginal histology would emerge in a social context and that these differences would have functional or adaptive significance.

Method

Subjects

A total of 15 prairie vole (*Microtus ochrogaster*) trios and a total of 11 montane vole (*M. montanus*) trios were used in this experiment. Trios of 1 male and 2 females were used because we had found that

the use of more than 1 male per group resulted in the death of several animals due to male–male aggression. All animals were laboratory bred and had been in the colony for several years before this study. Animals had been weaned at 21 days of age and were housed in unisex, sibling groups. Animals between 60 and 90 days of age were arbitrarily chosen for testing and placed in individual cages (29 cm wide × 19 cm high × 13 cm deep) with wood shavings as bedding. They were kept in individual cages for 1 week before testing, and they had continuous access to Purina Rabbit Chow (Ralston-Purina, St. Louis, MO) and water. There was a 16:8-hr light/dark photoperiod of white fluorescent light, with lights out at 1200 hr; dim red lights were on at all times. None of the animals used in this study had any prior sexual experience.

Apparatus

Two test enclosures were used in this study, one for prairie voles and one for montane voles. The enclosures were 1.19 m wide × 0.59 m high × 0.32 m deep, with the front wall made of transparent Plexiglas. A mixed substrate of 50% peat and 50% wood shavings was used in each apparatus. Several rocks and branches were placed about each enclosure. In addition, each enclosure contained three individual polycarbonate cages (29 cm wide × 19 cm high × 13 cm deep); plastic cylinders 18.4 cm long and 5.1 cm in diameter served as entrances to each cage. These cages were present so that each animal had the opportunity to nest separately. Purina Rabbit Chow was available ad libitum, and there was a water bottle at all times at the rear of each compartment.

Procedure

Vaginal smears were taken from females for 3 days before they were introduced into the test enclosures. On the morning of the 1st day of behavioral testing, all animals were weighed and marked for identification. A small patch of fur was clipped from the neck of one female and the rump of the other; males remained unmarked. Pairs of females were placed in the test enclosure and observed for 3 days. The male was placed in the apparatus on Day 4, and the trios were observed for 7 days.

There were three 10-min observation periods daily. The first observation period was between 0900 and 1200 hr, the second between 1200 and 1600 hr, and the third between 1600 and 2100 hr. Observation periods were designed to be evenly spaced throughout the day with one sampling period at the end of the dark phase, one in the middle of the light phase, and one at the beginning of the next dark phase. Previous work has shown that these species display identical, acyclic activity patterns (Baumgardner, Ward, & Dewsbury, 1980; Dewsbury, 1980). Behavioral observations were recorded by hand on prepared data sheets.

Behavioral Measures

Scored behavioral patterns included the frequency of rough-and-tumble fighting (vigorous fighting in which both animals tumble end-over-end), chases (one animal pursues another), and boxing (sparring with rapid forepaw–forepaw contact in an upright position). In addition, the frequency of body-nosing (brief nose-to-body contact episodes lasting less than 5 s) was recorded for both males and females. As a measure of affiliative behavior, huddling duration was scored. Huddling was scored when at least two animals rested in side-by-side contact with each other for at least 5 s. Durations were recorded with a stop watch. Scores were recorded for interactions between females only, between males and females, and for huddling duration among all three animals as a group (trio).

Vaginal Cytology

Vaginal smears were taken once each day, immediately after the second observation period. Smears were taken for 3 days before the females were put in the enclosure while they were individually housed, for 3 days after they were put in the enclosure when the 2 females were together, and for the 7 days after the introduction of the male into the enclosure.

The technique for obtaining a smear consists of inserting a small wire loop into the vagina. The smears were then stained with toluidine blue and examined microscopically. They were assessed for percentages and types of cells present as for laboratory rats (e.g., McClintock, 1983, 1984) and were classified as diestrus if they consisted of at least 50% leukocytes or as estrus if they contained at least 50% cornified cells.

Results

Behavior

Species differences recorded in the apparatus are summarized in Table 1. There were no significant differences between species on any behavioral measure when just female–female interactions were considered. When interactions between males and females were considered, however, prairie voles displayed significantly lower frequencies of body-nosing, chases, and boxing than did montane voles. When composite frequencies of aggression (i.e., sum of Chases + Rough-and-Tumble Fighting + Boxing) are plotted across time (Figure 1), it is evident that aggression remained fairly low and constant for prairie voles throughout the 10-day observation period; for montane voles aggression was highest on Day 4, when the male was introduced, and decreased rapidly thereafter. As can be seen from Table 1, aggression in montane voles was primarily in male–female interactions.

Table 1

Mean (± SEM) Total Instances of Behavior and Huddling Duration (in Minutes) During 10-Day Observation Period for Prairie and Montane Vole Groups in the Seminatural Apparatus

Measure	Prairie voles M ± SEM	Montane voles M ± SEM	p
Body-nosing			
Female–female	2.6 ± 1.0	2.4 ± 0.8	ns
Male–female	7.8 ± 1.9	21.6 ± 4.9	<.006
Rough-and-tumble fighting			
Female–female	0.2 ± 0.1	0.8 ± 0.7	ns
Male–female	0.1 ± 0.1	0.8 ± 0.7	ns
Chases			
Female–female	1.4 ± 0.6	1.4 ± 0.8	ns
Male–female	0.4 ± 0.1	5.7 ± 2.3	<.008
Boxing			
Female–female	0.6 ± 0.4	0.2 ± 0.1	ns
Male–female	0.1 ± 0.1	3.6 ± 0.9	<.001
Huddling			
Female–female	23.2 ± 5.6	10.3 ± 3.0	ns
Male–female	59.7 ± 8.9	3.6 ± 1.5	<.001
Trio	29.4 ± 8.4	0.0	<.009

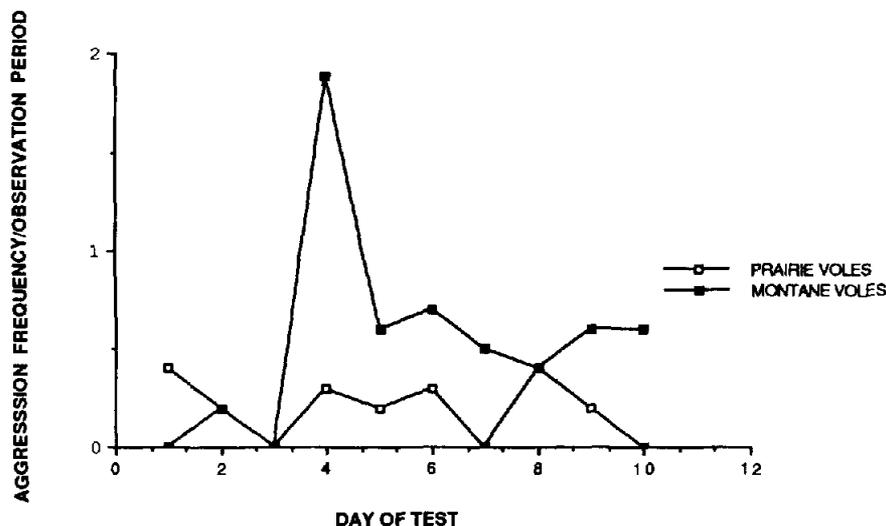*Note.* Statistical results are based on independent samples *t* tests.

Figure 1. Mean scores of aggression for prairie and montane vole trios during 10-min observation period in the seminatural enclosure. (Scores represent the composite frequencies of Chase + Rough-and-Tumble Fighting + Boxing.)

One of the key differences highlighted in these results is the degree of contact proneness displayed by these two species when the male was present (Figure 2). Prairie voles spent an appreciable amount of each observation period in direct physical contact with another animal. From the introduction of the male on Day 4, prairie voles spent a mean of 59% of the total observation time in side-to-side contact either as pairs or as a trio. Montane voles, on the other hand, spent only 7% of the total observation time huddling in male-female or female-female pairs and were never observed to huddle or sit in contact as a trio.

In addition to spending more time huddling, prairie vole males usually displayed a preference for 1 of the 2 females of the trio; this was scored when 75% or more of the male-female huddling time was spent with a particular female. In 8 of the 10 trios, the males displayed this preference within 24 hr of introduction.

Vaginal Cytology

The two species displayed significant differences in their vaginal cytology when housed in isolation. During the 3 days that the females were still housed in their home cages, 18 of the 22 (82%) montane voles displayed at least 1 day of vaginal estrus. In contrast, of the 30 female prairie voles observed, only 6 (20%) showed at least one estrous smear during the first 3 days, $x^2(1, N = 52) = 19.53, p < .001$.

Figure 3 depicts the stage of vaginal estrus plotted for a total of 10 days for a representative pair of female prairie and montane voles. In 9 of 15 (60%) prairie vole groups, only 1 female of the pair ever displayed vaginal estrus. That is, the other female of the trio remained in diestrus for the entire 10-day period in the test enclosure. In 4 groups both females displayed estrous smears, and in 2, neither female did. As a result of this pattern, the cumulative percentage of female

prairie voles that displayed vaginal estrus only reached 56% (Figure 4).

Males paired with females in all 9 of the groups in which only 1 female displayed vaginal estrus. However, it was not always with the female that displayed the estrus smear. In fact, of the 10 females with which males paired, 3 never displayed an estrous smear, and of the 7 that did, the average time for appearance of an estrous smear was 2.01 ± 0.53 days after pair formation. Of interest is the fact that in 3 groups, the unchosen female was the one to display an estrus smear. Thus, it appears that a female's estrous stage was neither a cause nor a result of a male's preferentially associating with that particular female.

This pattern was supported by the incidence of copulatory behavior. During the observation period, copulation was observed in only 5 of the 15 prairie vole groups and always at least 4 days after the male had been introduced.

In contrast to this pattern, 100% of the female montane voles had experienced at least one vaginal estrus by the end of the testing period. Nevertheless, male montane voles did not spend time nesting or in side-to-side contact with either female of the pair even after mating. Copulation was observed in 6 of the 11 montane vole groups: One copulation was observed in montane voles on the day the male was introduced, and the remaining copulations occurred after Day 8.

Discussion

The most striking species difference to emerge from our study is the overall difference in contact proneness between the species. This behavioral propensity, perhaps more than any other, may be responsible for generating the marked differences in social organization that these species display in the wild. Immediately after introduction of the male, prairie voles spent a large percentage of time in side-to-side contact
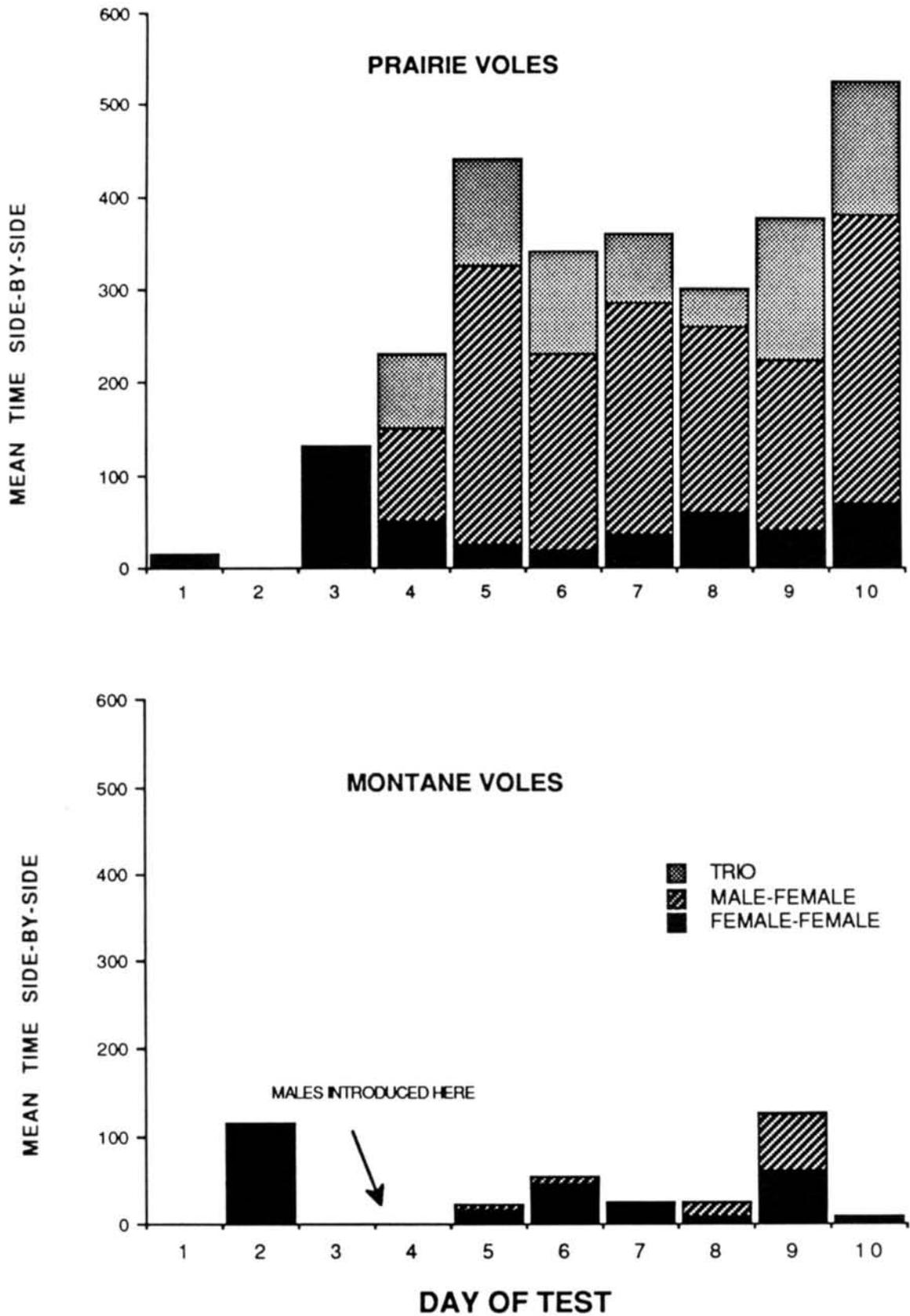
Figure 2.   Mean huddling duration in seconds for prairie and montane voles during 10-min observation periods.
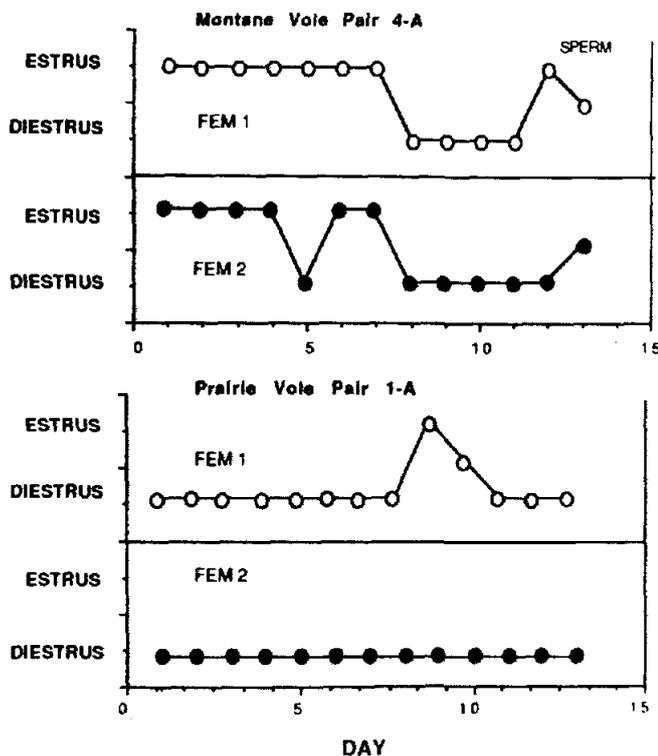
Figure 3. The stage of vaginal estrus for a representative pair of females of each species. (Females were in isolated cages during Days 1-3; in female-female pairs in the seminatural enclosure during Days 4-6; in trios of 1 male and 2 females in the seminatural enclosure during Days 7-13.

either as pairs or as a trio. Montane voles, on the other hand, spent almost no time sitting in contact, and they were never observed to nest together as male-female pairs or to sit in contact as a trio. This was true in spite of the fact that there were actually slightly higher numbers of observed copulations

and of smears with sperm cells in montane than in prairie voles. The lack of conesting and social contact in montane voles cannot, therefore, be attributed to a general absence of sexual receptivity on the part of females. Rather, social contact appears to be considerably less reinforcing for montane voles. Whether this is true of montane males, females, or both is an important question and remains to be determined.

In addition to prairie voles' overall levels of contact proneness, the males preferentially associated, or paired, with 1 of the 2 females. This is an important distinction because pair-bond formation, and not contact proneness, is correlated with monogamous mating systems. Indeed, levels of male-female affiliative behavior may be quite similar in monogamous and polygamous species for which the social organization of the polygamous species takes the form of large social groups (e.g., Mason, 1974).

Pair-bond formation between male and female prairie voles has been studied extensively by Carter and Getz and their colleagues (e.g., Carter et al., 1986). This process appears to involve a postcopulatory reduction in contact proneness of the mated pair toward conspecifics (Carter et al., 1986). Postmating aggression toward conspecifics has also been observed in monogamous pairs of gibbons in the field (Mitani, 1984) and titi monkeys in the laboratory (Anzenberger, Mendoza, & Mason, 1986) and may be an important proximate factor for maintaining monogamy in a wide variety of species.

In this study we observed no increase in levels of agonistic behavior after mating in prairie vole trios. However, agonistic behavior may have been mitigated by the fact that females had been housed together for 3 days before the introduction of the male and were thus familiar to each other in advance of male-female mating and pairing.

Another striking species difference in this study was revealed in the contrasting patterns in vaginal cytology, both in isolation and in the social context of the test enclosure. The vaginal smears of prairie vole females housed in isolation were dominated by leukocytes (diestrous); the smears of montane
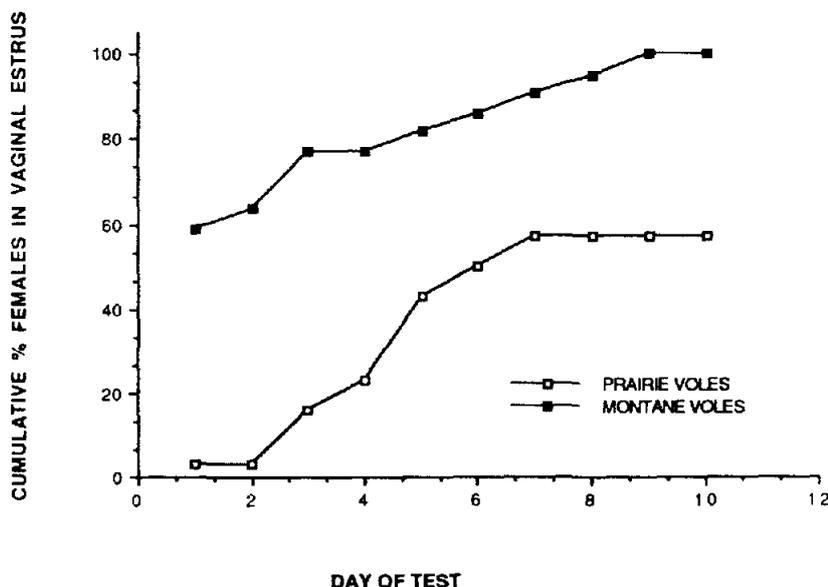

Figure 4. Cumulative percentage of females that displayed at least 1 day of vaginal estrus.

voles were dominated by cornified cells (estrous). Whether isolated montane voles were in a state of behavioral as well as vaginal estrus is not clear. However, the appearance of sperm in the smears was always correlated with vaginal cornification. Male–female aggression peaked for montane voles after the introduction of the male. At this time the males pursued the females in an apparent attempt to initiate copulation. This may have been a reflection of behavioral estrus because montane voles generally engage in aggressive boxing and chasing before mating. It is worth noting that there was no comparable aggression between male and female prairie voles at this time. Even though prairie vole females were showing diestrus smears, the males and females began sitting in contact almost immediately.

In the social context of test enclosure, female–female suppression of estrus was evident in prairie but not montane voles. In both prairie and montane voles, ovulation is contingent on stimulus input, that is, either copulation or some form of physical interaction with a male (see Sawrey & Dewsbury, 1985, for review). In prairie voles, extended physical contact with a male or exposure to male urine will induce and maintain behavioral receptivity (Carter, Witt, Schneider, Harris, & Volkening, 1987). Moreover, when female prairie voles were housed across a wire-mesh barrier from a male, 71% showed an estrous smear. These findings are of particular relevance to the present work because females were exposed directly to a male as well as to male-soiled bedding for a total of 7 days. In spite of this, the pattern revealed was one in which 1 female of the pair displayed a diestrous smear the entire time. That contact with a male or its urine was not a sufficient stimulus in the present context is further supported by the fact that of 6 pairs in which only 1 female showed an estrous smear, the male paired either with the diestrous female or huddled with both equally.

Carter et al. (1986) also observed female–female suppression in 1-male–2-female prairie vole trios. Although they found some mating activity in 4 of 6 trios, they noted that the presence of 2 females "disrupts the behavior of one and perhaps both females in the trio" (p. 135). Getz, Dluzen, and McDermott (1983) also found that prairie vole females that have been exposed to a strange male do not display estrus if exposed to an inhibitory chemosignal in the urine of other females.

The evident suppression of estrus in prairie voles is in sharp contrast to the situation in montane voles, 100% of which experienced at least one and sometimes two or three estrus smears during the 7-day test period. Induced ovulators do not generally cycle even in the presence of males (Richmond & Conaway, 1969a). Our results, however, suggest this may not be the case for montane voles.

A guiding hypothesis of this research was that differences in vaginal cytology would have functional significance in generating contrasting mating system types. That is, females of monogamous species ought to display a pattern of suppression or synchrony, whereas females of polygamous species ought to cycle asynchronously (e.g., Emlen & Oring, 1977). The species differences that have emerged in our study support this prediction. Taken together, however, our results indicate that the estrous stage of females is most likely a complementary aspect rather than a direct cause of the contrasting mating

systems displayed by these two species. In other words, prairie voles appear predisposed to share the same nest and form long-term male–female associations in the field regardless of whether or not the female is in estrus and regardless of whether other potential mates are available. Conversely, montane voles are predisposed not to conest regardless of the estrous stage of the female; male and female montane voles did not conest or sit in contact even after mating within the confines of the enclosure. These observations strongly support the notion that the mating systems of these two species in the wild are not facultative. That is, the contrasting mating systems exhibited by these two species may be less a function of mate availability than of pronounced, species-typical differences in contact proneness and male–female bonding. Field data, at least for prairie voles, support this conclusion. Getz, Hofman, and Carter (1987) reported that although there is some variability, monogamy is the predominant type of mating system in the field regardless of season, population density, or reproductive rates.

Species differences in affiliative behavior and pair bonding, therefore, emerge as key proximate determinants of the contrasting mating systems which prairie and montane voles display in the wild. These behavioral traits are, in turn, mediated by species-typical differences in neural or hormonal mechanisms (Shapiro et al., 1989). Comparative studies are currently underway to elucidate the neural mechanisms that have been shaped by natural and sexual selection to mediate social bonding and other behavioral differences between these two species.

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