

Development of Partner Preferences in Female Prairie Voles (*Microtus ochrogaster*): The Role of Social and Sexual Experience

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Prairie voles (*Microtus ochrogaster*) exhibit a monogamous mating system characterized by long-term pair bonds between mates. The purpose of this study was to examine the effect of cohabitation time and sexual experience on the development of pair bond formation in female prairie voles. Females that were allowed to cohabit for 24 hr or more, with or without mating, exhibited a strong social preference for a familiar partner versus a strange male. Females that cohabited and mated for 6 hr showed strong preferences for a familiar partner, while cohabitation for less than 24 hr, without mating, did not result in preferences for the familiar male. These results indicate that mating was not essential for partner preference formation; however, preferences developed more rapidly when mating occurred. © 1992 Academic Press, Inc.

Field and laboratory data suggest that the social organization of prairie voles (*Microtus ochrogaster*) is predominantly characterized by monogamy (Getz, 1978; Thomas and Birney, 1979; Getz and Carter, 1980; Carter and Getz, 1985; Getz, Hofmann, and Carter, 1987; Dewsbury, 1987). In the laboratory, female prairie voles that are pregnant and living with a mate show aggression toward unfamiliar males. Females in postpartum estrus preferentially mate with their partner, and usually show aggression toward and rebuff mating attempts by strange males (Getz, Hofmann, and Carter, 1981, Williams, unpublished data). Pregnant female prairie voles also show preferences for the odor of their mate, versus that of a stranger (Newman and Halpin, 1988). These observations suggest the presence, in pregnant or postpartum female prairie voles, of pair bonds characterized by sexual and social preferences for familiar sexual partners. In general, sexual and social preferences have been more difficult to document in sexually naive females or in females given limited amounts of copulatory experience. For example, females allowed 18 hr of nonsexual cohabitation with a male engaged in equal amounts of sexual behavior with the familiar male and with a stranger. Similarly, females in natural estrus that had been allowed to copulate *ad libitum* with a given male

for 2 or 24 hr did not show significant preferences for the sexual partner. In this experiment, tests were conducted either as a dyadic encounter or by tethering the familiar and unfamiliar male. However, each test was only 10 min in duration and was conducted in a 10-gallon aquarium. In relatively brief tests, females tend to move back and forth between males (Carter and Getz, 1985). This test paradigm confined the female, and may have resulted in an overestimation of the willingness of the female to mate or spend time in the presence of a given partner. In addition, brief tests may assess investigatory behaviors rather than, or in addition to, preferences.

The general goal of the present study was to describe factors contributing to partner preference formation in female prairie voles. Females were tested for preferences using a relatively large, three-chamber test apparatus, designed to maximize female choice, and behavioral preferences were assessed over an 8-hr test period. Pilot data indicated that postpartum females tested in this apparatus show clear social and sexual preferences for their mate, and that social preferences for the mate were significant after 1 hr of testing and remained stable over a 24-hr testing period.

EXPERIMENT 1

As described above, in prairie voles preferences for a familiar partner are observed in pregnant females and during postpartum estrus, but have been difficult to document in sexually inexperienced females or females that have recently mated. In Experiment I, we compared the effects of differing amounts of sexual and nonsexual cohabitation time on subsequent partner preferences. Females were allowed to cohabit with a sexually experienced male for periods of either 24 or 48 hr. During this cohabitation, sexual behavior was either permitted, by testing gonadally intact females in natural estrus, or prevented by using ovariectomized females. Following the cohabitation period, females were given a partner preference test.

Methods

Subjects. Prairie voles used in these experiments were laboratory-bred animals derived from wild stock caught in Champaign, Illinois. Animals were maintained routinely under a 14 L:10 D photoperiod in polycarbonate cages (20 × 25 × 45 cm) on heat-treated beta chip bedding. Purina rabbit chow and water were provided *ad libitum*. Litters were weaned at 21 days of age, separated into unisexual sibling groups of two to four animals per cage in a room with same-sexed animals. Animals remained with their siblings until assigned randomly to an experimental group at approximately 60 days of age.

Groups differed in their experiences with a partner, including the duration of cohabitation and whether the pair mated during cohabitation.

Groups included females that cohabit with a male for (1) 24 hr without sexual interactions (24 HR-NS, $N = 11$), (2) 48 hr without sexual interactions (48HR-NS, $N = 10$), or (3) 48 hr with *ad libitum* sexual interactions (48HR-SEX, $N = 8$).

In females that were ovariectomized, surgery was conducted under sodium pentobarbital (50 mg/kg) anesthesia. Females were allowed 2 weeks of recovery prior to group assignment. The groups requiring cohabitation without sexual interactions comprised naive, ovariectomized females. The group experiencing sexual interactions comprised naive, intact females in natural estrus. To induce estrus, females were placed with a strange male for 15 min and then remained alone in a cage containing male-soiled bedding for 48 hr (Carter, Witt, Schneider, Harris and Wolkening, 1987). Experimental pairs were videotaped during the cohabitation period to confirm behavioral categories.

Preference test. Partner preferences were assessed using a Y-shaped apparatus that consisted of three identical plastic cages ($20 \times 25 \times 45$ cm). An uninhabited cage was connected by hollow tubes (7.5×16 cm) to two parallel cages housing stimulus animals. During the preference test, females were first placed in the uninhabited cage and were free to move throughout the apparatus, but stimulus males were tethered within their respective chambers. Stimulus males consisted of a familiar male designated as the "partner" and an unfamiliar male designated as the "stranger." Strangers were sexually experienced males that had been previously screened with a sexually receptive female, but that were housed alone prior to testing. Preference tests for all subjects lasted 8 hr, although some subjects were tested for periods of up to 24 hr and continued to show consistent behavioral responses throughout the longer test periods. Tests were monitored on a Panasonic time-lapse video recording system. Tapes made on this system compressed 12 hr of real time on tapes that were replayed in 1 hr. Each tape was replayed and the frequencies and/or durations (in seconds) of the behaviors below were recorded (by an experimentally blind observer) using the S & K Computerized Event Recorder System (Buffalo, NY). Data were subsequently converted into real time measures and segmented into 1-hr intervals for analysis. The behaviors recorded included time in each cage, time in physical (side-by-side) contact with each male, aggression, and sexual behavior. An aggressive bout was recorded when fighting, biting, or chasing was observed. A bout of sexual behavior was recorded when the female assumed an immobile posture with her back flat or concavely arched (lordosis) in response to mounting by the male.

Statistical Analyses

Nonnormally distributed data, such as frequency of aggressive bouts, were compared across groups using a Kruskal-Wallis H test. Social preference was defined on the basis of the duration of time (cumulative

seconds) the female spent in side-by-side contact with a male. Preferences within each group were assessed by comparing contact time with the partner to contact time spent with the stranger using paired *t*-tests. Groups were compared to each other using a preference ratio score. Ratios were obtained by dividing contact time with the partner by contact time with the stranger. The natural log of the ratio was used in all statistical tests. The natural log transform corrected both heteroscedasticity and skew. Data across 8 hr of testing were segmented into 1-hr blocks to evaluate the consistency of the preference ratio measure across time. Log ratios for each hour (noncumulative) were compared across groups using a two-factor repeated measure ANOVA. Post hoc comparisons were made using the Sheffe *F* tests. An α of 0.05 was required throughout.

Results

Aggression. Groups did not differ in the frequency of aggression bouts directed by the experimental female toward the familiar partner ($H(2) = 3.39, P = 0.184$) or toward the stranger ($H(2) = 1.46, P = 0.308$) or the frequency of aggressive bouts directed toward the experimental female by the strange male ($H(2) = 0.65, P = 0.184$). There was no instance of aggression directed toward the test female by the familiar male partner. Aggression on the part of the females was infrequent, with only two females showing brief aggression toward the familiar partner and eight females showing aggression toward the stranger.

Sexual behavior. Because of the virtual lack of sexual behavior across groups, sexual preferences could not be assessed. Mating was seen in only one of 29 preference tests in the experiment. One female in the 24HR-NS group showed lordosis toward both males during the 24-hr preference trial.

Social preference. All three experimental groups showed a highly consistent preference for the partner throughout the preference test (Fig. 1). Preference ratios did not differ significantly across groups or across the 8 hr of testing.

When comparing the amount of time spent with the partner versus the stranger during the first hour of the test (Fig. 1), the preference for the partner was significant in the 24HR-NS and the 48 HR-NS groups ($t(10) = 3.91, P = 0.003; t(9) = 2.26, P = 0.05$, respectively), and approached significance in the 48HR-SEX group ($t(7) = 2.24, P = 0.06$). By the end of the second hour (cumulative score over 2 hr) of testing, all three groups spent significantly more time with the mate versus the stranger (24HR-NS, $t(10) = 3.48, P = 0.006$; 48HR-NS, $t(9) = 4.41, P = 0.002$; 48HR-SEX, $t(7) = 3.07, P = 0.018$, respectively) and a similar pattern was seen when data for the first 3 hr were compared (Fig. 2).

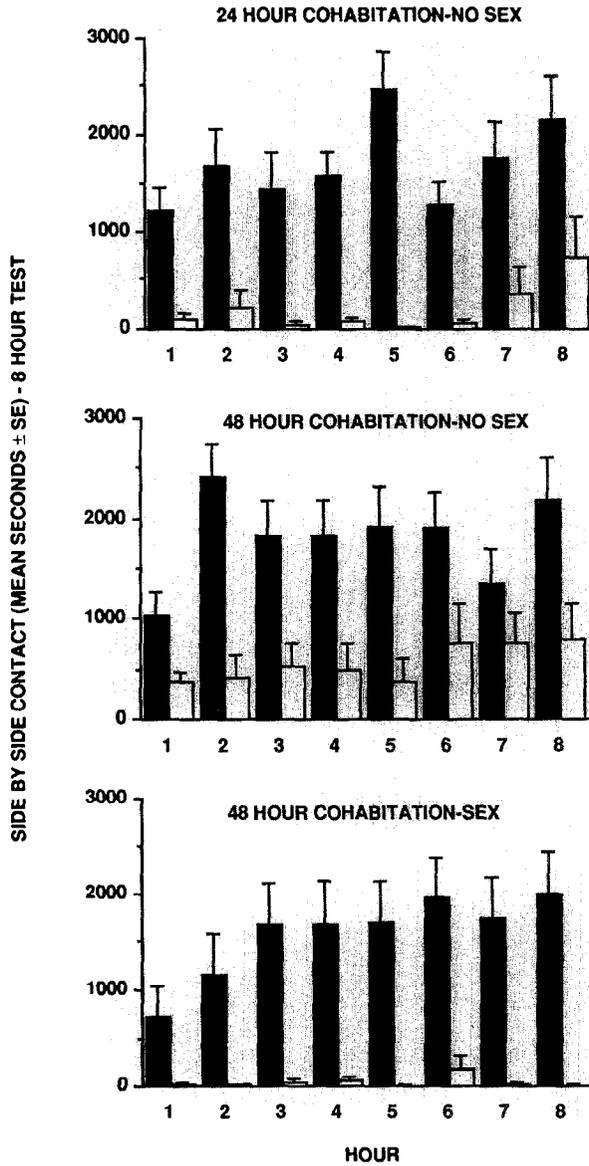


FIG. 1. Experiment I: Mean number of seconds (noncumulative) that each group spent in side-by-side contact with the partner (■) and the stranger (□) over 8 hr of testing.

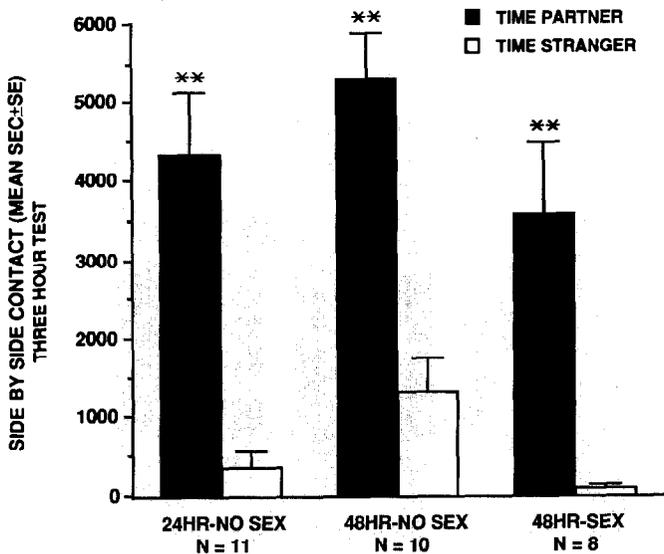


FIG. 2. Experiment I: Mean seconds (\pm SE) that each group spent in side-by-side contact with the partner and the stranger during a 3-hr preference test. Within each group, times were compared using paired t tests (** $P < 0.01$).

EXPERIMENT II

In Experiment I, females in all groups showed a significant preference for their familiar partner and there was no difference between groups in the magnitude of their preference. The results of that experiment indicated that 24 hr of cohabitation was sufficient for the development of a partner preference and sexual interactions did not further facilitate this preference.

Experiment II was designed to test the hypothesis that sexual interactions might facilitate partner preference under conditions of shorter cohabitation. Pilot data indicated that 6 hr of cohabitation (without mating) was not sufficient for the development of a partner preference. Using a 6-hr cohabitation period, females that engaged in sexual interactions during cohabitation were compared to females that did not mate using the preference test described in Experiment I.

Methods

Subjects. All females were given 6 hr of cohabitation with a male and group differences were based on the estrus condition of the test female. Females were ovariectomized and allowed a 2-week recovery period before testing. Prior to cohabitation, females either were primed with estrogen to induce estrus or were left in anestrus. Estrogen-primed females received subcutaneous injections ($10 \mu\text{g}$ of estradiol benzoate (EB) suspended in 0.05 ml of sesame oil) for 2 consecutive days before the testing

on Day 3. This procedure induces behavioral estrus in this species, although (for unknown reasons) a significant percentage of females typically fail to respond to these procedures (Dluzen and Carter, 1979; Carter *et al.*, 1987). Therefore, EB-injected females could be further classified into females that mated or that did not mate with the partner. The three groups tested, then, included (1) EB-primed females that engaged in sexual interactions during cohabitation (6-EB-SEX, $N = 10$), (2) EB-primed females that did not mate (6-EB-NS, $N = 6$), and (3) nonprimed females (6HR-NS, $N = 13$). Interactions during the cohabitation test were monitored by time-lapse video to verify the behavioral classification of the females.

Test females were first placed in the cage of a stimulus male, allowed to cohabit, and monitored for a period of 6 hr. Immediately following the 6 hr cohabitation period, females were given the preference test described in Experiment I.

Since data from Experiment I indicated that significant partner preferences were apparent by the end of the second and third hour of testing, preference tests were shortened and statistical analyses were completed on the cumulative score at the end of the third hour of testing. All other procedures were identical to those described in Experiment I.

Results

Social preference. Only the females in the group that mated (6-EB-SEX) showed a preference for the partner (Fig. 3). The preference ratio differed significantly among the three groups ($F(2,26) = 4.15, P = 0.027$). Post hoc analysis revealed a significant difference between the 6HR-NS and the 6HR-EB-SEX group ($P < 0.05$, Sheffe F test). The 6HR-EB-SEX group spent significantly more time in contact with the partner versus the stranger ($t(9) = 3.45, P = 0.007$). Neither the 6HR-NS nor the 6HR-EB-NS group exhibited a preference for the partner over the stranger.

Sexual preference. Within the 6HR-EB-SEX group, 4 of the 10 females continued to show sexual behavior during the preference trial. These four females allowed the partner to mount and intromit, but did not mate with the stranger during the preference trial.

EXPERIMENT III

Experiments I and II indicate that females given at least 24 hr of cohabitation with a male or 6 hr of mating experience with a male show strong social preferences for a partner versus a stranger. These results are potentially confounded by the fact that the partner and the stranger may differ in their behavior toward the female. To eliminate the behavior of the stimulus male as a factor affecting female preferences, experimental females in Experiment III were tested with anesthetized males.

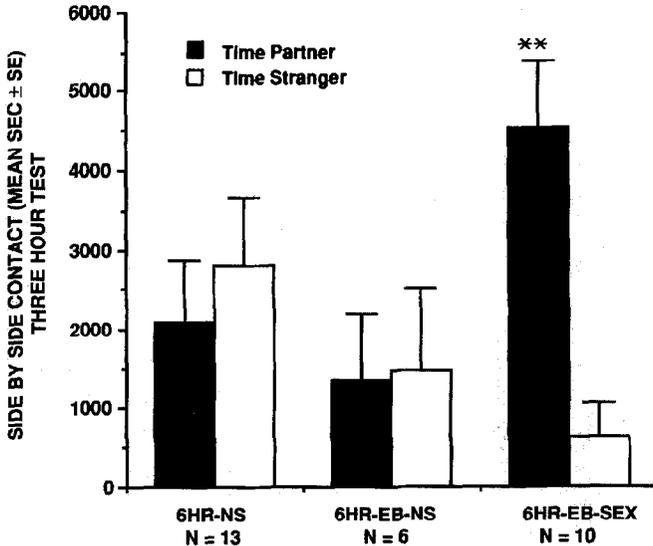


FIG. 3. Experiment II: Mean seconds (\pm SE) that each group spent in side-by-side contact with the partner and the stranger during a 3-hr preference test. Within each group, time in contact with the partner and the stranger was compared using paired t tests (** $P < 0.01$).

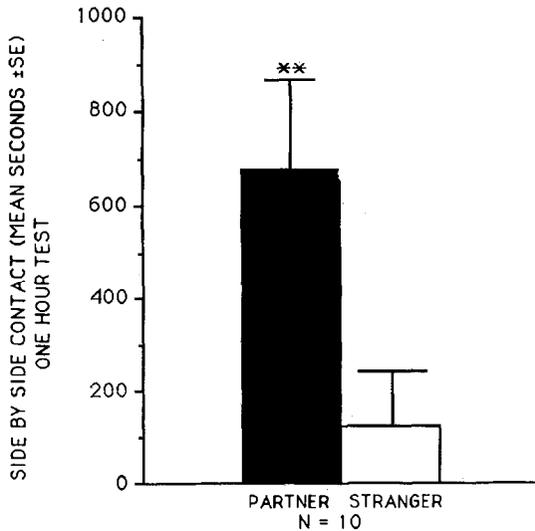


FIG. 4. Experiment III: Mean number of seconds (\pm SE) that females spent in side-by-side contact with the partner and the stranger in a 1-hr choice test in which both stimulus males were anesthetized (** $P < 0.01$, paired t test).

Methods and Subjects

Procedures were similar to those described previously, except that stimulus males were anesthetized with pentobarbital (50 mg/kg) prior to being placed in the preference apparatus and males were not tethered. Males typically regained consciousness after 1 hr and, therefore, preference testing occurred over a 1-hr period.

Experiment I indicated that females given at least 24 hr of cohabitation with a male show a social preference for that partner versus a stranger. Therefore, all experimental females had at least 24 hr of cohabitation time (cohabitation time ranged from 48 hr to 2 weeks) with a male before preference testing.

Results

Females showed a strong preference for the partner over the stranger (Fig. 4). Females spent significantly more time in side-by-side contact with the partner versus the stranger ($t(9) = 3.35$, $P = 0.009$).

DISCUSSION

In this study we have examined social and endocrine variables contributing to the formation of partner preferences in female prairie voles. Cohabitation for periods of at least 24 hr resulted in preferences for a familiar male partner. Social preferences established as a result of 24 hr of cohabitation were not further enhanced by mating or longer cohabitation periods. Six hours of cohabitation, in which mating occurred, resulted in a significant preference for the sexual partner; in contrast, 6 hr of cohabitation without mating did not result in partner preferences. Female voles will not mate without estrogen. Therefore, we cannot eliminate the possibility that estrogen-priming (leading to behavioral estrus) played a role in the facilitation of partner preferences. In this experiment, however, estrogen-injected females that did not mate did not exhibit preferences for a male after 6 hr of cohabitation. Results from this group suggest that exposure to estrogen, without mating, is not sufficient to induce a partner preference. Since females could not be randomly assigned to the 6-EB-NS versus 6-EB-SEX groups, we cannot discount the possibility that other unidentified factors that prevented mating also might have prevented the formation of partner preferences. Despite this unavoidable limitation, the results of this experiment suggest that events associated with estrus and/or mating facilitate partner preference formation.

Female preferences were not due to differential treatment by the partner versus the stranger since females tested with anesthetized males also demonstrated partner preferences. Cohabitation leads to partner preferences, and events associated with mating or estrus induction facilitate the onset

of partner preferences. These results agree with earlier observations of social preferences for the mate in pregnant and postpartum females (Getz *et al.*, 1981), and are in apparent contrast to reports that sexually naive females do not show social preferences for their mate (Carter and Getz, 1985). This discrepancy is probably due to differences in methodologies, since informal observations indicated that the first 30 min of the preference tests were characterized by high levels of exploratory behavior directed toward the stranger and the apparatus. Preference measures taken in brief tests may reflect investigatory patterns rather than preference patterns. Time spent in a chamber or in proximity to a male, particularly in brief encounters, potentially indexes both positive and negative social behaviors. Physical contact used in the present study is probably a more sensitive index of positive social interactions (Carter, Williams, and Witt, 1990). In the present experiment, partner preferences, even in sexually naive, nonaggressive females, were apparent when the testing trial exceeded the exploration period and when test procedures allowed the female to escape male contact.

In earlier studies (Carter and Getz, 1985; Carter *et al.*, 1990) we have attempted unsuccessfully to use sexual preferences as an index of pair bonds after 2 hr of mating. In the present study, very few females showed mating behavior during the preference test. However, our observation in Group 6-EB-SEX, that females that continued to mate did so only with their familiar partner, suggests that sexual preferences coincide with social preferences. As stated, the present study differed from earlier studies not only in the use of longer sexual exposures and preferences tests, but also in the use of a compartmentalized test apparatus designed to maximize female choice. Smaller, noncompartmentalized test chambers may complicate results by leading to the reflexive release of lordosis in experimental females because of forced proximity to stimulus males.

Despite repeated *ad libitum* mating over a 24-hr period during the cohabitation period in the 48HR-SEX group, only three of the eight females delivered live offspring. This finding was not expected and suggests that reproduction may have been inhibited by some aspect of the test paradigm.

During the course of this study aggression was rare. This finding is in contrast to observations of relatively high levels of aggression in postpartum females and females in mid-pregnancy (Getz and Carter, 1980; Getz *et al.*, 1981; Witt, Carter, Chayer, and Adams, 1990). This discrepancy is not due to differences in testing paradigms since we also have observed high levels of stranger-directed aggression in postpartum estrous females using the test apparatus described here (Williams and Carter, unpublished). We hypothesize that the stranger-directed aggression previously observed in pregnant and lactating female prairie voles is the result of hormonal factors associated with later stages of pregnancy and/or

lactation. Maternal aggression is viewed as a mechanism for the protection of offspring and is probably not a direct result of pair bond formation with a mate. However, maternal aggression is never directed toward the mate and, therefore, does not reflect the existence of a pair bond. Interestingly, the only females ($N = 2$) that showed high levels of aggression during the preference trial directed their aggression toward the stranger, and were later determined to have been approximately 2 days pregnant during the preference trial.

Sexual interactions have a variety of physiological and behavioral consequences, including the induction of pregnancy in mammals. In monogamous mammals, such as prairie voles, sexual interactions, and concomitant physiological changes (Carter *et al.*, 1990), also may influence the formation of partner preferences and subsequent social bonds.

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REFERENCES

- Carter, C. S., and Getz, L. L. (1985). Social and hormonal determinants of reproductive patterns in the prairie vole. In R. Gilles and J. Balthazart (Eds.), *Neurobiology*, pp. 18–36. Springer-Verlag, Berlin.
- Carter, C. S., Williams, J. R., and Witt, D. M. (1990). The biology of social bonding in a monogamous mammal. In J. Balthazart (Ed.), *Hormones, brain and behaviour in vertebrates. 2. Behavioural activation in males and females—Social interactions and reproductive endocrinology*, pp. 154–164. Karger, Basel.
- Carter, C. S., Witt, D. M., Schneider, J., Harris, Z. L. and Wolkening, D. (1987). Male stimuli are necessary for female sexual behavior and uterine growth in prairie voles (*Microtus ochrogaster*). *Horm. Behav.* **21**, 74–82.
- Dewsbury, D. A. (1987). The comparative psychology of monogamy. In D. W. Leger (Ed.), *Nebraska symposium on motivation*, pp. 1–50. Univ. of Nebraska Press, Lincoln.
- Dluzen, D. E., and Carter, C. S. (1979). Ovarian hormones regulating sexual and social behaviors in female prairie voles, *Microtus ochrogaster*. *Physiol. Behav.* **23**, 597–600.
- Getz, L. L. (1978). Speculation on social structure and population cycles of microtine rodents. *Biologist* **60**, 134–147.
- Getz, L. L., and Carter, C. S. (1980). Social organization in *Microtus ochrogaster* populations. *Biologist*, **62**, 56–69.
- Getz, L. L., Carter, C. S., and Gavish, L. (1981). Field and laboratory evidence for pair-bonding. *Behav. Ecol. Sociobiol.* **8**, 189–194.
- Getz, L. L., Hofmann, J. E., and Carter, C. S. (1987). Mating system and population fluctuations of the prairie vole, *Microtus ochrogaster*. *Amer. Zool.* **27**, 909–920.
- Newman, K. S., and Halpin, Z. T. (1988). Individual odours and mate recognition in the prairie vole, *Microtus ochrogaster*. *Anim. Behav.* **36**, 1779–1787.
- Thomas, J. A., Birney, E. C. (1979). Parental care and mating system of the prairie vole, (*Microtus ochrogaster*). *Behav. Ecol. Sociobiol.*, **5**, 171–186.
- Witt, D. M., Carter, S., Chayer, R., and Adams, K. (1990). Patterns of behaviour during postpartum oestrus in prairie voles, *Microtus ochrogaster*. *Anim. Behav.* **39**, 528–534.