

Female Meadow Voles Have a Preferred Mating Pattern Predicted by Photoperiod, Which Influences Fertility

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MEEK, L. R. AND T. M. LEE. *Female meadow voles have a preferred mating pattern predicted by photoperiod, which influences fertility.* *PHYSIOL BEHAV* 54(6) 1201–1210, 1993.—In a previous study, Meek and Lee (26) found that female meadow voles mated within three distinct time periods after pairing with a male, and fertility was influenced by the time of mating and photoperiod. In the present studies, we tested the hypothesis that different patterns of mating would correlate with high and low fertility. We found two very different patterns of mating were correlated with high fertility. Females housed in long daylengths (long day; 14 h light/day) and mating within 48 h of pairing with a male, received few mounts, intromissions, and intromissions per ejaculatory series, with very short interintromission intervals. All long-day females utilizing this mating pattern ovulated, and 86–100% produced litters. We suggest this pattern of mating is associated with spontaneous estrus. In contrast to long-day females mating within 48 h, females housed in short daylengths (short day; 10 h light/day) and mating between 14–48 h after pairing with a male, received significantly more short mounts and intromissions, with longer interintromission intervals. These short day females all ovulated and 86% produced litters. We suggest that this pattern of mating is associated with an induced estrus. Short-day females that did not exhibit this pattern of mating (66%) produced far fewer litters. We discuss two mechanisms by which fertility may be inhibited in most short-day females.

Microtus pennsylvanicus Mating behavior Mating latency Estrus Ovulation Pregnancy

MEADOW voles (*Microtus pennsylvanicus*) are small (30–60 g) arvicoline rodents believed to be induced into behavioral estrus through contact with a mature male or his urine (29), and copulatory behavior subsequently induces ovulation (6,20). Meadow voles are seasonal breeders, with most females producing young from February to August. However, field researchers have reported significant winter breeding, with up to 50% of females found pregnant or lactating from November to January (4,5). Winter breeding appears to be dependent upon the existence of a small number of males (5%) that maintain spermatogenesis when daylengths shorten (16,27).

Because some males remain reproductively active in the winter, and behavioral estrus and ovulation are induced by the male, it is surprising that no more than 50% of females in the field are reported to be pregnant in the winter. This might be due to the low number of fertile males in winter. However, birthrates of laboratory females housed in short daylengths (10 h light:14 h dark; short-day; SD), when paired with males housed in long daylengths (14 h light:10 h dark; long-day; LD), do not exceed 20–30% (21). These data suggest that female meadow voles possess an independent, photoperiod-sensitive mechanism enabling

them to avoid pregnancy when environmental conditions do not favor production and rearing of young.

Meek and Lee (26) reported that intact nulliparous females paired with sexually experienced males produce three nonoverlapping mating groups based on mating latency. We suggested that these three groups differ behaviorally and that these differences result in altered fertility. Rapidly mating females (rapid onset; 7 min–9.4 h), probably require no contact with male urine to enter estrus, while females mating within 14.4–48 h of pairing with a male (intermediate onset) may be induced into estrus through contact with the male or his urine (3). The third group of females mated after a delay of up to 10 days (late onset) and had the lowest fertility of the three groups. In that study, we reported that short mating latencies predicted high rates of fertility and photoperiod predicted mating latency. Most LD nulliparous animals mated in the rapid onset (67%) and intermediate onset groups (26%), whereas SD nulliparous females were evenly divided among the three mating latency groups. Females housed in short daylengths produced fewer litters than LD females with the same mating latency. Thus, short photoperiods delayed the onset of mating and reduced fertility. This report extends the

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findings of our previous report and identifies differences in mating behavior among these three groups that may be responsible for the differences in fertility between mating latency groups and between LD and SD females within a mating latency group.

The reduced birthrate of SD females in all mating latency groups suggests that there is some aspect of copulatory behavior or postcopulatory physiological response that differs between LD and SD females and influences subsequent pregnancy. In rodents, species-specific vaginal codes with specific temporal and frequency characteristics exist which are necessary to trigger the hormonal changes needed for the maintenance of pregnancy (9). This vaginal code is of particular importance for induced ovulators. One component of rodent mating behavior that has consistently been implicated in successful pregnancy is the number and rate of intromissions received by the female (1,2,7-9,14,15,17,18,24,28). In all cases, too few intromissions or intromissions occurring outside the limits of some preferred rate disrupted subsequent fertility.

Meadow vole mating behavior has been examined previously in a laboratory by pairing LD males and females brought into estrus with exogenous estrogen (13). Meadow vole mating behavior is similar to that of other rodent species, with the male mounting the female from behind and grasping her flanks with his forepaws. A series of mounts and intromissions occur prior to ejaculation and 5 or 6 ejaculatory series (one or more mounts and intromissions terminating with an ejaculation) occur before satiety is achieved (13). Meadow vole mating is characterized by intravaginal thrusting and single or multiple intromissions prior to ejaculation, with no vaginal lock (13).

In an attempt to produce behavior in the laboratory that can be interpreted as relevant to field animals, we manipulated the animals as little as possible. For example, estrus was not hormonally induced but occurred naturally after continuous exposure to a male. In addition, mating behavior was not disrupted or truncated but was allowed to proceed to satiety by housing males and females together in pairs for several days. Animals were videotaped to prevent disruption of behavior by human disturbances. Animals were housed with sawdust bedding in containers similar in size and opacity to the containers used to house our breeding colony; a procedure that produces fertility rates comparable to field studies.

GENERAL METHOD

Subjects and Housing Conditions

The laboratory *M. pennsylvanicus* population used in these experiments are derived from wild stock obtained from North-western Pennsylvania and maintained as an outbred population (no sibling or first cousin matings) at the University of Michigan. Animals were housed individually or in pairs with Purina Mouse Chow (#5015) and water available ad lib.

Procedure

Females were born into long daylengths (14 h light/day; LD) and at weaning (3 weeks) were either kept in long daylengths (mimicking summer when all field adult females typically become pregnant) or placed into short daylengths (mimicking winter when field adult females may or may not produce a litter; 10 h light/day; SD) for 8 weeks. At 11 weeks of age each mature female was paired with a LD sexually experienced male known to have sired litters with both LD and SD females. All females had mature body sizes (>30 g) and patent vaginae. Each pair was maintained in the photoperiod previously experienced by the female and was housed in a 50 by 25 by 29 cm aquarium

containing 3 cm sawdust bedding. Exteriors of the aquaria were masked with paper to minimize disturbance.

Pairs were videotaped continuously with a time-lapse Panasonic Low Light Camera and extended-play VCR. A 25 W red light provided illumination for the camera during the dark portion of the photoperiod.

Pairs were videotaped for 12-24 h after copulation had ceased or for 2 weeks, whichever occurred first. No pair was observed to resume mating following a 4 h hiatus.

Statistical Analysis

Behavioral measures were analyzed with a 2-way ANOVA for mating latency, photoperiod, and mating latency by photoperiod interactions. Planned contrasts were made with Bonferroni adjusted probabilities using the adjusted error terms from the two-way ANOVA for each behavioral measure. Bartlett's chi-square was used to measure homogeneity of variance. Differences in interintromission intervals, litter production, interrupted mounts, and ovulation were analyzed with chi-square. Survival analysis (BMDP) was used to analyze the temporal pattern of interintromission intervals (10,24,25). Significant differences in temporal patterning of interintromission intervals were determined with chi-square. Probability values less than or equal to 0.05 were considered significant. Values are given as mean \pm SEM.

EXPERIMENT 1

Method

The mating behavior of 31 nulliparous animals is described in this paper. A previous paper described how nulliparous female meadow voles mate within three distinct mating latency groups: rapid onset (mating between 7 min-9.4 h), intermediate onset (mating between 14.4-48 h), and late onset (mating after 58 h) (26). The females in this study consist of seven long-day and six short-day females mating in the rapid onset group, four long-day and six short-day females mating in the intermediate onset group, and two long-day and six short-day animals mating in the late onset group.

Females remained in the same experimental photoperiod after copulation until litters were produced or for 25 days (which exceeds the length of gestation).

Three basic components of mating behavior were analyzed: mounts, intromissions, and ejaculations (defined below). Mating behavior that did not terminate in an ejaculation was not included in any analysis. The frequency and duration of mating behaviors and the intervals between them were quantified.

Group means were calculated by averaging total frequency or duration of a behavior for each group of animals (method 1) or by calculating a mean for each animal and then deriving a group average from the individual means (method 2). The method used is noted below for each behavior analyzed.

Frequencies

Ejaculation Frequency (EF). An ejaculation was recorded when a mounted male demonstrated a spasmodic muscle movement, followed by a subsequent period of immobility, dismount, and genital grooming (12) (method 1).

Interrupted Mounts (%) (MI). An interrupted mount was recorded when females continued moving while mounted, eventually pulled away from the male and were chased and remounted within 15 s. Chi-square analysis was performed on the percentage of mounts that were interrupted in each group.

Intromission Frequency (IF). An intromission was recorded if the male achieved vaginal penetration with intravaginal thrusting during a mount. Mounts with intromissions in voles can be distinguished from mounts without intromissions by deep pelvic thrusts that occur at the rate of about 1/s (13,28). Because mounts with intromissions (penetration) can be difficult to distinguish from mounts without intromissions (no penetration) on videotape, an additional time criteria was used if identification of mounts with intromissions was uncertain. In the field vole (*Microtus agrestis*) (28), mounts with intromissions tended to be longer than mounts without intromissions. The shortest mean intromission length recorded was 11 s (28). Our observations of mounts with and without intromissions in which identification of each was unequivocal determined that the mean duration of mounts with intromissions (18.3 s) was more than twice as long as that of mounts without intromissions (7.3 s) in meadow voles. Although only 12% of mounts without intromissions were greater than 12 s long, 70% of mounts with intromissions were longer than 12 s. Thus, when the designation of a behavior as a mount with an intromission was in doubt, any mount continuing 12 s or longer was designated as a mount with intromission. These will be referred to in the text simply as intromissions or intromission frequency (method 1).

Intromissions per Ejaculatory Series (IE). A ratio of the total number of intromissions divided by the total number of ejaculations was calculated for each animal (method 1).

Litter Production (LP). Successful litter production was recorded for each female that mated and subsequently produced a litter. Chi-square analysis was used to determine differences in the percentage of females giving birth in each group.

Mount Frequency (MF). A mount consisted of the male placing his forefeet on the back of the female and tightly grasping her flanks (13). A mount was recorded if the male mounted but did not achieve penetration of the vagina. If the designation of a behavior as a mount (no penetration) or intromission (penetration) was difficult to determine from the videotape, any mount terminating prior to 12 s was designated as a mount (see explanation under intromission frequency) (method 1).

Latencies

Mating Latency (ML). Mating latency was the time elapsed between initial pairing and the beginning of the first intromission (method 1).

Ejaculation Latency (EL). This was the time elapsed between the first intromission in an ejaculatory series and the ejaculation in that series (method 2).

Postejaculatory Interval (PEI). The postejaculatory interval was the interval between an ejaculation and the next intromission. A mean was calculated for each animal by summing all PEIs and dividing by the number of intervals. A group mean was then calculated for each group.

Durations

Interintromission Interval (III). The duration of each interval separating intromissions was recorded within each ejaculatory series. These values were analyzed using survival analysis (24,25).

Mating Duration (MD). The time elapsed from the beginning of the first intromission in the first ejaculatory series to the end of the final ejaculation in the final ejaculatory series (method 1).

Mean Intromission Length (MIL). For each animal the duration of intromissions in all ejaculatory series was divided by the number of intromissions (method 2).

RESULTS

There were no main effects of mating latency, photoperiod, or interaction effects of mating latency and photoperiod on mating duration, ejaculation latency, ejaculation frequency, or post-ejaculatory intervals. Therefore, a detailed analysis of these measures is not reported.

Mating latency had a significant effect on intromission frequency, mount frequency, mean intromission length, and intromissions per ejaculatory series. There was no main effect of photoperiod or an interaction effect of mating latency and photoperiod on any of these measures. Therefore, the differences in mating behavior will be described between the three mating latency groups (rapid onset, intermediate onset, and late onset). Unless specifically noted, each mating group includes both LD and SD females, because within a mating latency group, few behavioral differences were found between animals with different photoperiodic manipulations. Where photoperiod differences were found, they are reported.

Mating behavior of females differed dramatically between the three mating latency groups. Rapid onset females always produced the lowest frequencies of behaviors and longest mean intromission lengths, and late onset females always had the greatest frequencies of mounts and intromissions. The mating behavior pattern of a representative female from each mating latency group is shown in Fig. 1.

Females in the rapid onset mating latency group had significantly lower frequencies of intromissions and intromissions per ejaculatory series and longer mean intromission lengths than females in the intermediate and late onset mating latency groups ($p \leq 0.05$, Fig. 2A, C, D). Rapid onset females did not differ from intermediate onset females in mount frequency ($p > 0.05$, Fig. 2B), but received significantly fewer mounts than late onset females ($p \leq 0.01$). There was only one incidence out of a total of 66 mounts where a rapid onset female interrupted a mount/intromission (0.015%, Fig. 2E). Sixty-nine percent of females mating in the rapid onset group produced a litter (Fig. 2F). Mating behavior of LD and SD females in the rapid onset group did not differ significantly for any measure (Table 1).

Behavior of the intermediate onset mating latency group tended to be intermediate in frequency and duration to that of the other groups, but there were no significant differences between intermediate onset and late onset females in the frequency of intromissions, mounts, ejaculations, intromissions per ejaculation, or in mean intromission length ($p > 0.05$; Fig. 2A-D). However, intermediate onset females never interrupted mounts, and 91% produced a litter (Fig. 2E, F). Although mounts, intromissions, intromissions per ejaculatory series, and mean intromission length did not differ significantly between LD and SD females in the intermediate onset group, frequencies and durations of behaviors of LD-intermediate onset females were more similar to those of rapid onset females, while frequencies and durations of SD-intermediate onset females were more like those of late onset females (Table 1).

Females in the late onset mating group demonstrated the greatest frequencies of all behaviors and the shortest mean intromission lengths (Fig. 2A-D). Forty-four percent of mount/intromission attempts were interrupted by the late onset females, significantly more than rapid or intermediate onset females ($p \leq 0.01$). Late onset females produced litters only 33% of the time (Fig. 2F). The late onset group was composed of two LD and six SD females. Although there were no significant differences between LD and SD females in this group, the high frequencies of mounts and intromissions were attributable primarily to the SD females. Mounts, intromissions, mean intromission

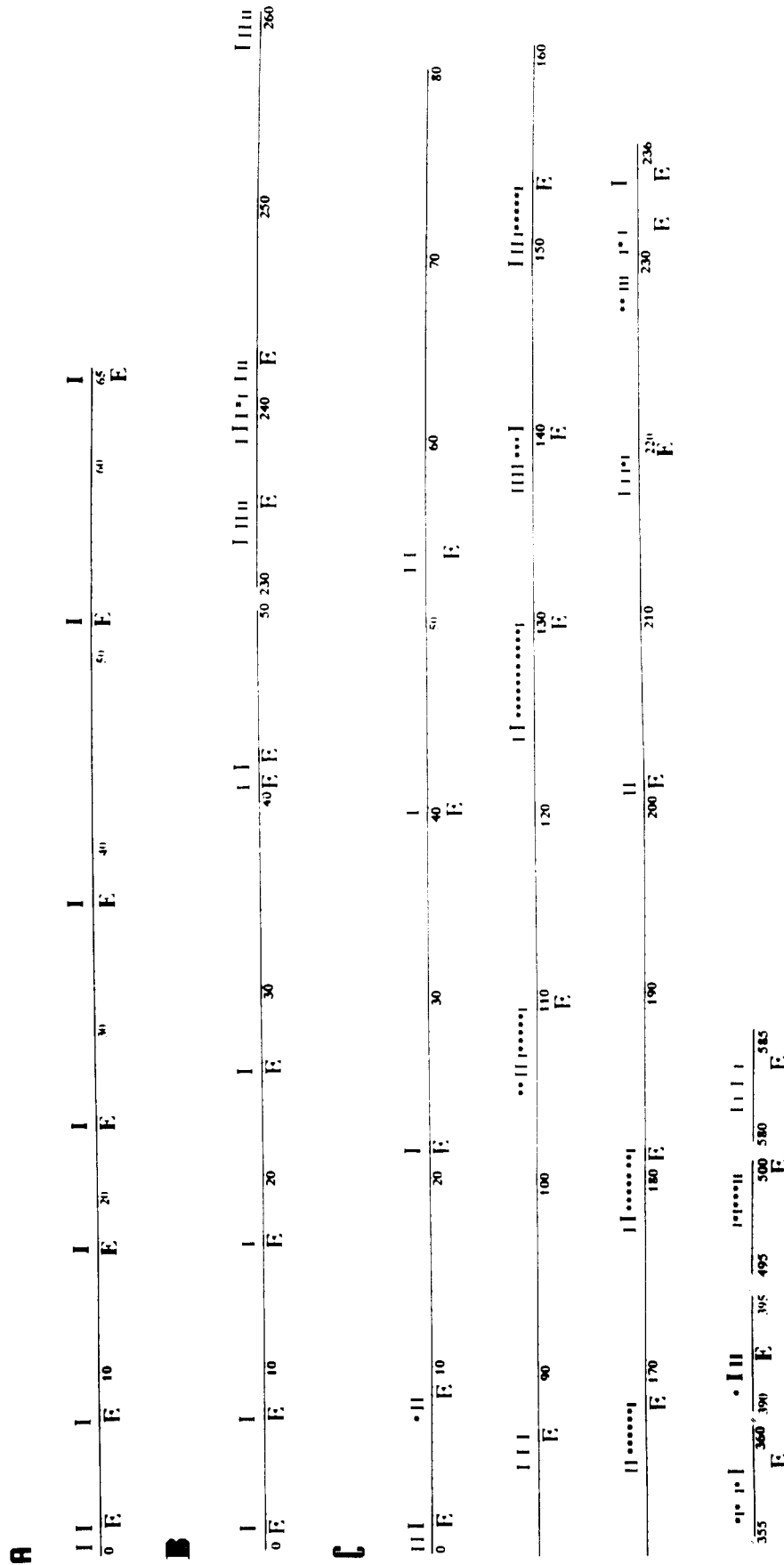


FIG. 1. Temporal mating patterns of three representative animals in the three mating latency groups: (A) rapid onset (RO), (B) intermediate onset (IO), and (C) late onset (LO). Numbers indicate time in minutes, with time 0 indicating the start of the first intramission and the end of the timeline indicating the natural termination of mating. The spacing between events represents real time. I designates an intramission, with the size of the I indicating the duration of the intramission. Small I = intramission lasted less than 10 s. Medium I = intramission lasted 10 - 19 s. Large I = intramission lasted 20 s or longer. E designates an ejaculation. *Designates a mount.

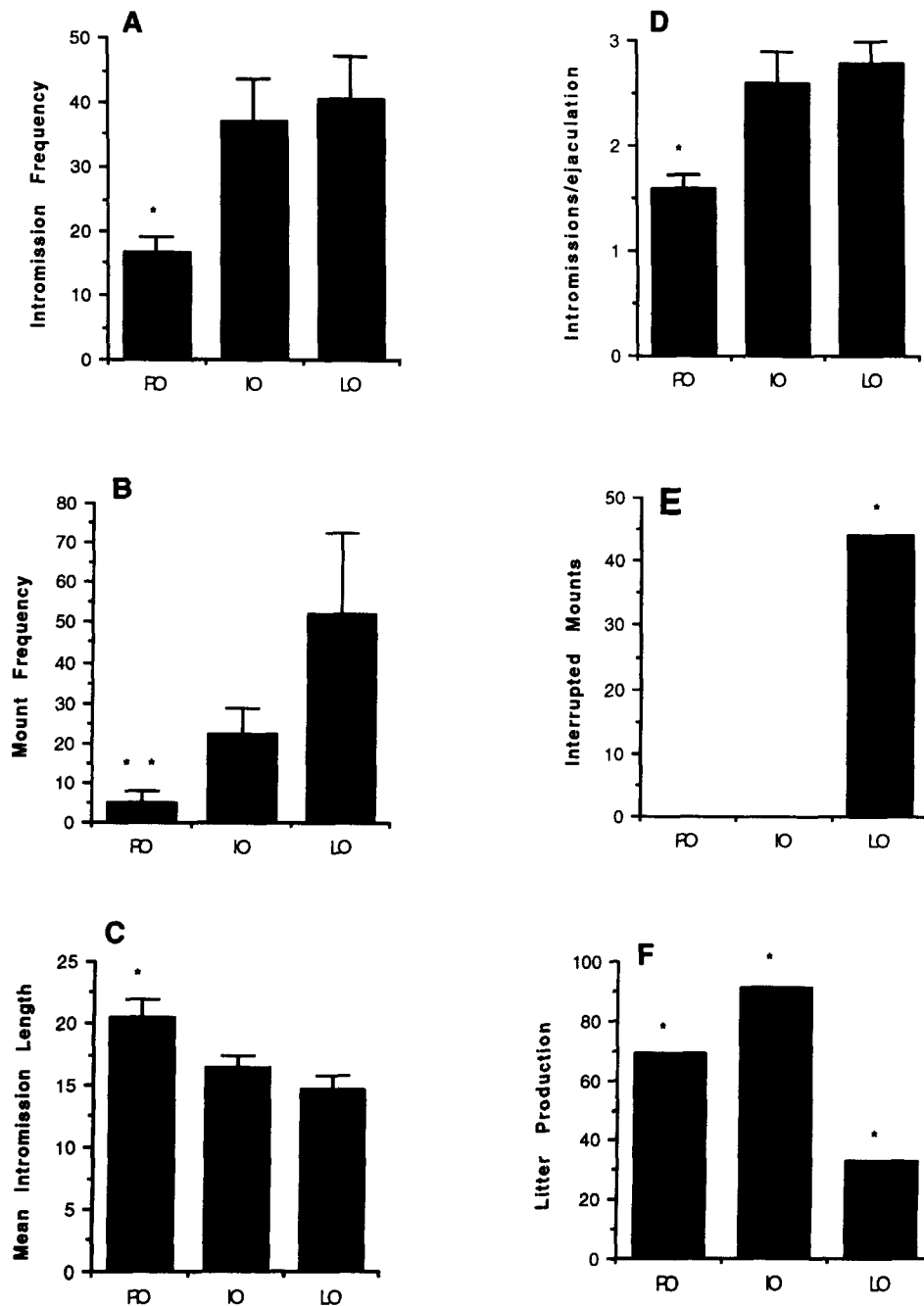


FIG. 2. Five mating behaviors and litter production for RO, IO, and LO females. Group abbreviations are the same as used in Fig. 1. *Indicates that group is significantly different from all other groups. **Indicates that RO is significantly different from LO.

length, and intromissions per ejaculatory series of LD-late onset females were more similar to those of LD-intermediate onset females than to SD-late onset females (Table 1).

Survival analysis was done to determine if significant differences existed among the three mating latency groups and between photoperiodic groups within mating latency groups in interintromission intervals (IIIs). Eighty-three percent of rapid onset interintromission intervals are shorter than 20 s in length (Fig. 3A). In comparison, only 70% of late onset IIIs and 54% of

intermediate onset IIIs are shorter than 20 s. The percentage of intervals that are shorter than 20 s differ significantly among all groups ($p \leq 0.05$).

Photoperiod significantly affected IIIs in the intermediate and late onset mating latency groups. Interintromission intervals for LD and SD females in the rapid onset group are almost identical (Fig. 3B), but IIIs of LD and SD females in the intermediate (Fig. 3C) and late onset (Fig. 3D) groups differ significantly. For example, 91% of IIIs in LD intermediate onset females are less

TABLE 1
MATING BEHAVIOR OF LD AND SD FEMALES IN THE RAPID, INTERMEDIATE, AND LATE ONSET MATING GROUPS

Group	Intromission Frequency	Mount Frequency	Mean Intromission Length (s)	Intromissions per Ejaculation	Percent IIs <20 s in Length	Litter Production
LD-RO	15.1 ± 3.4*	5.7 ± 4.6†	20.1 ± 1.5*	1.5 ± .2‡	84%	86%
SD-RO	18.3 ± 4.0*	9.8 ± 5.3†	21.1 ± 2.5*	1.5 ± .3‡	84%	50%
LD-IO	29.8 ± 5.1*	17.3 ± 6.8*	15.7 ± 2.6	2.8 ± .4	91%	100%
SD-IO	42.0 ± 10.2	25.8 ± 10.0	16.9 ± .7	2.1 ± .3	34%	86%
LD-LO	30.0 ± 4.0	22.0 ± 14.0	16.4 ± 3.8	2.7 ± .7	36%	50%
SD-LO	44.2 ± 8.3	62.3 ± 25.8	14.1 ± 1.2	2.8 ± .3	71%	33%

LD-RO = long day rapid onset.

SD-RO = short day rapid onset.

LD-IO = long day intermediate onset.

SD-IO = short day intermediate onset.

LD-LO = long day late onset.

SD-LO = short day late onset.

IIs = interintromission intervals.

* Significantly different from SD-late onset.

† Significantly different from SD-intermediate onset and SD-late onset.

‡ Significantly different from LD-intermediate onset, LD-late onset, and SD-late onset.

than 20 s in length. In contrast, only 34% of the IIs of SD intermediate onset females were less than 20 s in length ($p \leq 0.05$). Interintromission intervals of LD late onset females were longer than those of SD late onset females. Only 36% of IIs for LD late onset females were less than 20 s in length, while 71% of IIs in SD late onset females were less than 20 s in length ($p \leq 0.05$).

Comparing the duration of IIs revealed similarities among Table 1 groups. IIs of LD intermediate onset females (91% < 20 s) are similar to those of LD rapid onset (84% < 20 s) and SD rapid onset females (84% < 20 s). The pattern of IIs in SD intermediate onset females is, however, significantly different from the above three groups (34% < 20 s; $p \leq 0.05$), but indistinguishable from IIs in LD late onset females (36% < 20 s). IIs of SD late onset females were intermediate to all other groups (71% < 20 s in length).

Although fertility of LD and SD females did not differ significantly within each mating latency group (due to small n s), SD females consistently gave birth at a lower rate than LD females. Because there is no reason to assume that the cause of infertility is the same for all infertile animals, mating behavior for infertile females in the rapid onset ($n = 3$ SD; $n = 1$ LD) and late onset groups ($n = 4$ SD; $n = 1$ LD) was compared. Infertile females in the late onset group received significantly more intromissions and intromissions per ejaculatory series with shorter mean intromission lengths than infertile females in the rapid onset group (Fig. 4A-C; $p \leq 0.05$). No infertile female in the rapid onset group interrupted mounts, but 37% of mounts in the late onset infertile group were interrupted by the female (Fig. 4D; $p \leq 0.01$).

Mating behaviors of infertile and fertile females within each mating latency group were equivalent (RO infertile vs. RO fertile; IO infertile vs. IO fertile; LO infertile vs. LO fertile; $p > 0.05$). However, mating behavior differed between RO and LO fertile females; fertile RO females had significantly fewer intromissions, mounts, ejaculations, and intromissions/ejaculation than did fertile LO animals (Table 2, $p \leq 0.05$). Only 1 of 66 total mounts were interrupted by fertile RO animals, while 50% (112/222) of mounts were interrupted by fertile LO females ($p \leq 0.05$).

EXPERIMENT 2

Method

Females were housed and videotaped as in Experiment 1. Three days after mating, 44 virgin females (LD $n = 22$; SD $n = 22$) were euthanized with an overdose of pentobarbital sodium and ovaries removed and examined for corpora lutea. The presence of corpora lutea, which are readily visible macroscopically, indicate that ovulation has occurred.

Results

All long- and short-day females mating in less than 48 h ovulated (rapid and intermediate onset; $n = 22$) but 42% of LD and 40% of SD females mating 58 h or more after pairing with a male did not ovulate.

Discussion

This study confirms the relationship reported in the preceding study (26) between mating latency and fertility and, in addition, identifies the type of mating behavior that results in high rates of fertility in each mating latency group. LD female meadow voles have two successful patterns of mating. Females had high subsequent fertility (rapid onset = 86%; intermediate onset = 100%) if they mated quickly (within 48 h), with few mounts and intromissions and short interintromission intervals. SD females were more likely to produce a litter (intermediate onset group = 86%) if they delayed mating until 14–48 h after pairing with a male and received many short mounts and intromissions with much longer interintromission intervals than LD females.

Females in long and short days in all mating groups that did not use one of these patterns were likely to have reduced fertility. For example, all LD and SD animals mating in the rapid onset group received low frequencies of long-duration intromissions and mounts, with very short IIs. All females in this group demonstrated lordosis and proceptive behaviors, and only once did a female interrupt a mount or intromission. There were no differences in mating behaviors, including IIs, between LD and SD females. All rapid onset females, regardless of photoperiod, ovulated after mating. Fertility rate, however, was much higher

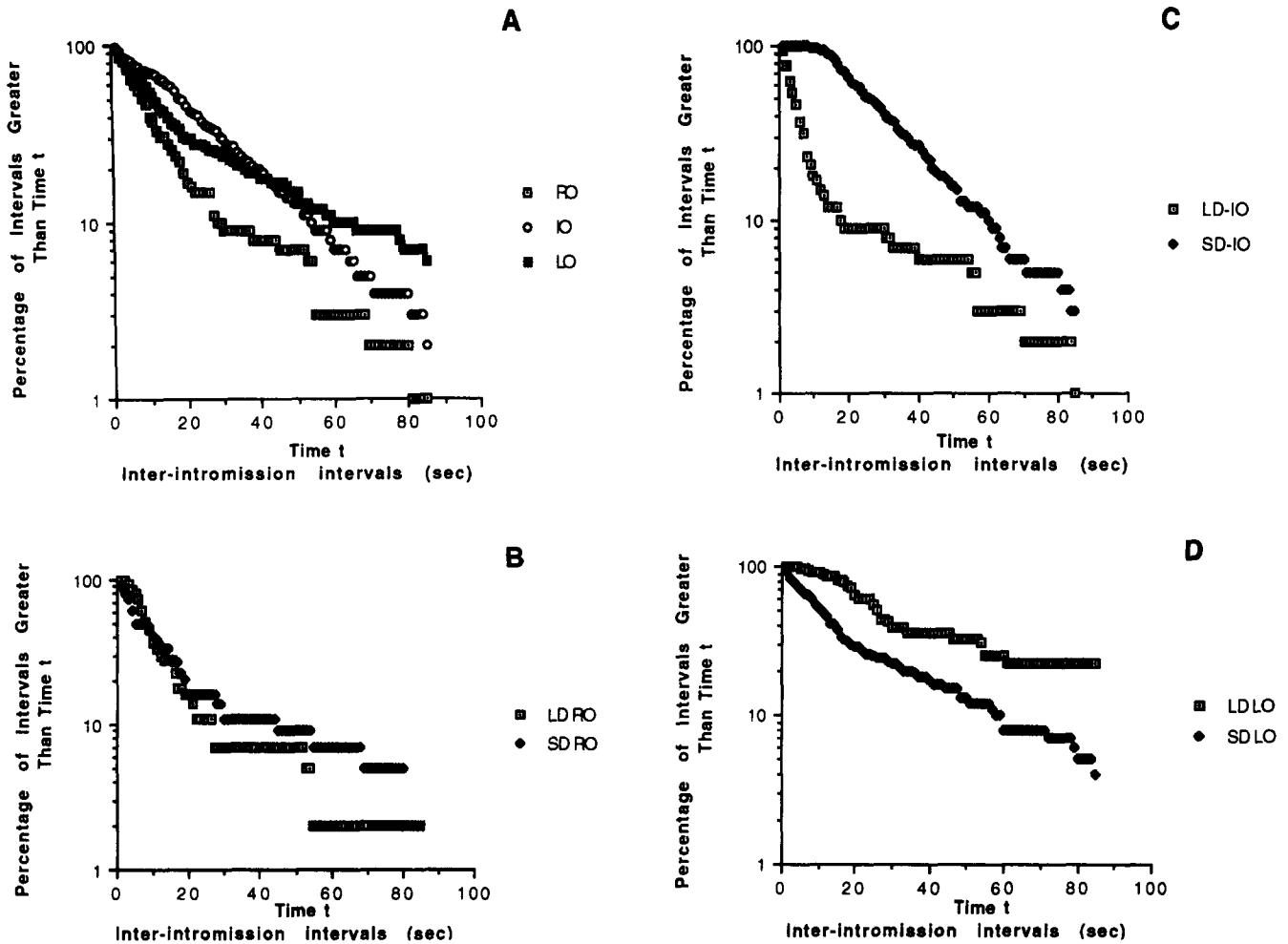


FIG. 3. Log survival plots of the temporal pattern of interintromission intervals (IIIs) in RO, IO, and LO females (see Fig. 1 for group abbreviations). In survival analysis, the steepness of the slope is proportional to the probability of a behavioral event occurring at time *t*. Note: graph redrawn from BMDP log survival plot. A = comparison of IIIs for RO, IO, and LO groups, with LD and SD females included within each group. B = comparison of IIIs for LD and SD females in the RO group. C = comparison of IIIs for LD and SD females in the IO group. D = comparison of LD and SD females in the LO group.

for LD females (86%), for which this appeared to be the preferred pattern of mating, than for SD females (50%). It appears that when LD females mate in less than 48 h they need less copulatory stimulation to ensure ovulation and the subsequent hormonal changes necessary to support pregnancy than do SD females.

In the intermediate onset group, there were no significant differences between LD and SD females in the frequency of intromissions or mounts, mean intromission length, intromission per ejaculatory series, or mount interruption, but the mating behavior of LD females in this group was, in many ways, more similar to that of rapid onset females (Table 1). Additionally, IIIs of LD females in the intermediate onset group were like those of rapid onset animals, while IIIs of SD intermediate onset females were significantly longer. Thus, the mating behavior of SD females in the intermediate onset group was quite different than that of SD rapid onset females, resulting in greater litter production (86%). In contrast, mating behavior of LD intermediate onset females resembled that of LD rapid onset females, also with high litter production (100%). All females in the intermediate onset group ovulated following copulation.

Meek and Lee (26) suggested that females mating in the rapid onset group entered estrus spontaneously, because 50% mated within 1.5 h after pairing with a male. It was not possible in the earlier analysis to determine whether intermediate onset females might also be classified as spontaneous if paired a day later. The analysis of IIIs, intromission frequency, mount frequency, mean intromission length, interrupted mounts, and fertility, suggests that LD intermediate onset females differ less from LD rapid onset females than they do from SD intermediate onset females (Table 1). Therefore, many LD intermediate onset females might have mated rapidly if pairing had occurred a day later, and thereby be classified as spontaneously entering estrus. In contrast, we conclude that while SD rapid onset females require no prior male contact for estrus, SD intermediate onset females probably were induced into estrus.

Although few behaviors were significantly different between females mating in the intermediate and late onset groups, late-mating SD animals consistently received higher frequencies of mounts and intromissions, with shorter durations than intermediate onset females. These SD late onset females were very

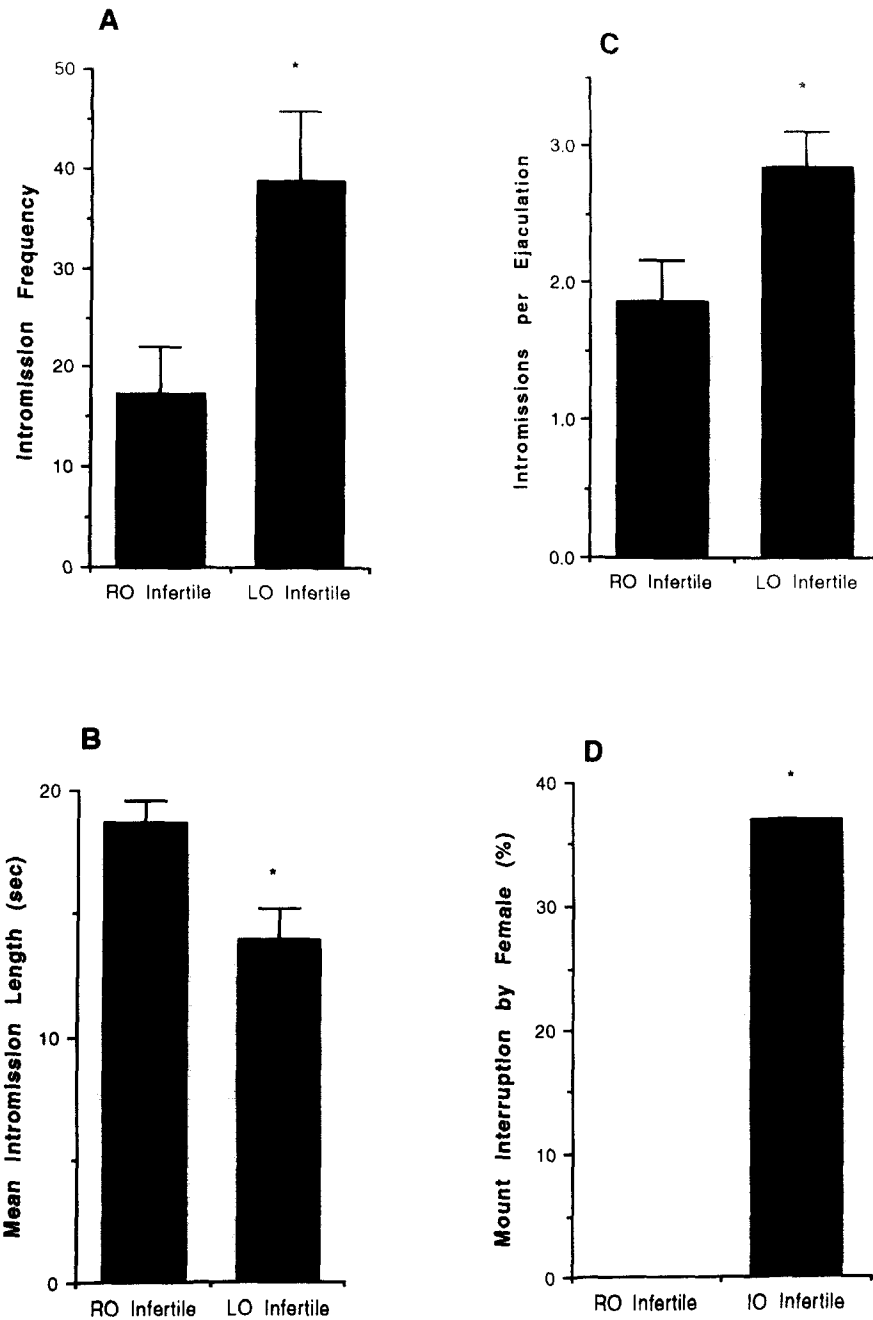


FIG. 4. Mating behaviors of infertile RO ($n = 4$) and LO ($n = 5$) animals. Group abbreviations are the same as used in Fig. 1. *Indicates significant differences.

uncooperative with the male, did not readily display lordosis, and interrupted over 40% of mounts and intromissions. Most IIIs were in the 11–33 s range, which was intermediate between those of SD rapid- and intermediate-mating females. This pattern of mating resulted in ovulation in only 60% of SD females mating in the late onset group, with 33% of those animals producing a litter. Because male meadow voles do not attempt copulation with nonestrous females, it appears that SD late onset females were attractive to males. Perhaps attractiveness (detected by the male from vaginal secretions), and behavioral receptivity (necessary for the female to exhibit receptive and proceptive behav-

iors), were dissociated in these females. It seems probable that the dissociation between vaginal and behavioral estrus is due either to low estrogen production or to SD-induced insensitivity to estrogen.

Because only two LD females delayed mating more than 58 h after pairing (Experiment 1), few conclusions can be drawn about the mating behavior of these animals. Their behavior did not differ significantly from that of SD late onset females, however, their mating behaviors and IIIs were most like those of SD intermediate onset females. Similarly, they did not interrupt mounts and intromissions. One of the two LD animals produced

TABLE 2
MATING BEHAVIOR OF FERTILE FEMALES IN RAPID AND LATE ONSET MATING LATENCY GROUPS

Mating Group	Intromission Frequency	Mount Frequency	Mean Intromission Length	Intromissions per Ejaculation	Ejaculation Frequency
Rapid Onset	16.3 ± 3.2*	4.2 ± 3.6*	21.3 ± 1.9	1.5 ± .1*	10.6 ± 1.6*
Late Onset	43.7 ± 12.2	30.3 ± 20.4	15.7 ± 2.2	2.5 ± .3	16.7 ± 2.7

* Indicates significantly less than late onset.

a litter following mating. However, like SD late onset females, only 58% of LD late onset females ovulated (Experiment 2). These animals may be induced into estrus, as they display a mating pattern typical of SD intermediate onset females, and are later than most LD females in becoming receptive.

The importance of the mating pattern, particularly IIIs, in determining subsequent pregnancy has been reported for several other species (1,8,9,25). It seems that the duration of IIIs is an important correlate of fertility in meadow voles. Females receiving the shortest IIIs (LD rapid and intermediate onset) demonstrated the highest birthrate (86% and 100%, respectively). Females receiving the longest IIIs (SD intermediate onset) also demonstrated a high birthrate (86%). In contrast, animals receiving intermediate IIIs (SD late onset) had the lowest birthrate (33%). Thus, there are two successful mating patterns: a) LD females mating within 48 h after pairing with a male, with short IIIs, and b) SD females mating between 14–48 h with very long IIIs.

Short-day females that mated in less than 9 h or after 58 h with intermediate IIIs had significantly fewer births than other groups of females ($p \leq 0.05$). It seems likely, however, that the primary cause of infertility is different in these two groups of SD females. Only 60% of short-day females mating in the late onset group, with intermediate length IIIs, ovulated. Thus, the primary cause of infertility is likely an inappropriate pattern of copulation necessary to stimulate LH release for ovulation. It appears that short photoperiods can regulate pregnancy by influencing the neuroendocrine axis such that sensitivity to males and the copulatory pattern is altered.

Short photoperiods apparently suppress the neuroendocrine axis in most females so that the incidence of spontaneous estrus (rapid mating) is greatly reduced. However, the reproductive axes of some females are insensitive to short days, and these females continue to mate rapidly after pairing with a male. SD females mating in the rapid onset group displayed mating behavior typical of spontaneously estrus LD females, and all ovulated. Litter production was probably reduced in the rapidly mating females because short daylengths suppress prolactin (30),

which is necessary for maintaining pregnancy. We hypothesize that the rapid onset mating pattern was probably insufficient to overcome the lower premating SD prolactin levels and support pregnancy, while the SD-intermediate onset pattern succeeded in stimulating both ovulation and pregnancy maintenance.

These are the first data to suggest that decreasing daylengths cause a change in mating strategy from spontaneous to induced estrus in meadow voles or any arvicoline rodent. Much of the work indicating that female voles must be induced into behavioral estrus has been done with prairie voles (*Microtus ochrogaster*), a monogamous species that often breeds throughout the winter [eg., (3)]. In contrast, meadow voles are primarily seasonal breeders, with a promiscuous breeding strategy. Animals are not colonial, but defend separate territories during the breeding season, with the territories of several females contained within larger male territories (22). Thus, spontaneous estrus in summer (LD) females would allow mating opportunities to be maximized for both males and females during times of optimal environmental conditions for producing young.

In the winter (SD), female meadow voles appear to maximize lifetime reproductive success using two very different strategies. One group of females, which are probably induced into estrus, mate and produce young at a high rate (4,5) despite short photoperiods (intermediate onset group). This strategy of winter breeding can be successful, as in *Peromyscus* (11), and has been shown to increase a female's contribution to the gene pool. However, most female meadow voles respond to short daylengths with physiological (decrease in prolactin) and behavioral changes (length of IIIs and aggression) that reduce the possibility of impregnation or successful pregnancy. Presumably, most females in fall and winter delay producing young until the hours of daylight increase and environmental conditions are more favorable.

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REFERENCES

- Adler, N. T.; Zoloth, S. R. Copulatory behavior can inhibit pregnancy in female rats. *Science* 168:1480–1482; 1970.
- Andersson, C. B.; Gustafsson, T. O. Effect of limited and complete mating on ovaries and adrenals in bank voles, *Clethrionomys glareolus*. *J. Reprod. Fertil.* 64:431–435; 1982.
- Carter, C. S.; Witt, D. M.; Schneider, J.; Harris, Z. L.; Volkening, D. Male stimuli are necessary for female sexual behavior and uterine growth in prairie voles (*Microtus ochrogaster*). *Horm. Behav.* 21: 74–82; 1987.
- Christian, J. J. Fighting, maturity and population density in *Microtus pennsylvanicus*. *J. Mammal.* 52:556–567; 1971.
- Christian, J. J. Regulation of annual rhythms of reproduction in temperate small rodents. In: Steinberger, A.; Steinberger, F., eds. *Testicular development, structure and function*. New York: Raven Press; 1980:367–380.
- Clulow, F. V.; Mallory, F. F. Oestrus and induced ovulation in the meadow vole, *Microtus pennsylvanicus*. *J. Reprod. Fertil.* 23:341–343; 1970.
- Davis, H. N.; Gray, G. D.; Zerylneck, M.; Dewsbury, D. A. Ovulation and implantation in Montane voles (*Microtus montanus*) as a function of varying amounts of copulatory stimulation. *Horm. Behav.* 5:383–388; 1974.

8. Diamond, M.; Yanagimachi, R. Induction of pseudopregnancy in the golden hamster. *J. Reprod. Fertil.* 17:165; 1968.
9. Diamond, M. Intromission pattern and species vaginal code in relation to induction of pseudopregnancy. *Science* 169:995-997; 1970.
10. Dixon, W. J. BMDP statistical software manual. Berkeley: University of California Press; 1988.
11. Fairbairn, D. J. Why breed early? A study of reproductive tactics in *Peromyscus*. *Can. J. Zool.* 55:862-871; 1977.
12. Gray, G. D.; Dewsbury, D. A. A Quantitative description of copulatory behavior in prairie voles (*Microtus ochrogaster*). *Brain Behav. Evol.* 8:437-452; 1973.
13. Gray, G. D.; Dewsbury, D. A. A quantitative description of the copulatory behavior of meadow voles (*Microtus pennsylvanicus*). *Anim. Behav.* 23:261-267; 1975.
14. Gray, G. D.; Kenney, A. M.; Dewsbury, D. A. Adaptive significance of the copulatory behavior pattern of male meadow voles (*Microtus pennsylvanicus*) in relation to induction of ovulation and implantation in females. *J. Comp. Physiol. Psychol.* 91:1308-1319; 1977.
15. Gray, G. D.; Zerylnick, M.; Davis, H. N.; Dewsbury, D. A. Effects of variations in male copulatory behavior on ovulation and implantation in prairie voles, *Microtus ochrogaster*. *Horm. Behav.* 5: 389-396; 1974.
16. Jannett, F. J., Jr. Reproduction of the Montane vole, *Microtus montanus*, in the subnivean populations. In: Merritt, J. F., ed. Winter ecology of small mammals (special publication Carnegie Museum of Natural History) vol. 10; 1984:215-224.
17. Kenney, A. M.; Dewsbury, D. A. Effect of limited mating on the corpora lutea in montane voles, *Microtus montanus*. *J. Reprod. Fertil.* 49:363-364; 1977.
18. Kenney, A. M.; Hartung, T. G.; Davis, H. N.; Gray, G.; Zerylnick, M.; Dewsbury, D. A. Male copulatory behavior and the induction of ovulation in female voles: A quest for species specificity. *Horm. Behav.* 11:123-130; 1978.
19. Kenney, A. M.; Hartung, T. G.; Dewsbury, D. A. Copulatory behavior and the initiation of pregnancy in California voles, *Microtus californicus*. *Brain Behav. Evol.* 16:176-191; 1979.
20. Lee, C.; Horvath, D. J. Management of the meadow vole (*Microtus pennsylvanicus*). *Lab. Anim. Sci.* 19:88-91; 1969.
21. Lee, T. M.; Smale, L.; Zucker, I.; Dark, J. Role of photoperiod during pregnancy and lactation in the meadow vole (*Microtus pennsylvanicus*). *J. Reprod. Fertil.* 81:343-350; 1987.
22. Madison, D. M. Space use and social structure in meadow voles, (*Microtus pennsylvanicus*). *Behav. Ecol. Sociobiol.* 7:65-71; 1980.
23. McClintock, M. K. Simplicity from complexity: A naturalistic approach to behavior and neuroendocrine function. In: Silverman, I., ed. Laboratory and life: New directions for methodology of social and behavioral research, No. 8. San Francisco: Jossey-Bass; 1981: 1-19.
24. McClintock, M. K.; Anisko, J. J. Group mating among Norway rats I: Sex differences in the pattern and neuroendocrine consequences of copulation. *Anim. Behav.* 30:398-409; 1982.
25. McClintock, M. K.; Anisko, J. J.; Adler, N. T. Group mating among Norway rats II: The social dynamics of copulation: Competition, cooperation, and mate choice. *Anim. Behav.* 30:410-425; 1982.
26. Meek, L. R.; Lee, T. M. Prediction of fertility by mating latency and photoperiod in nulliparous and primiparous meadow voles (*Microtus pennsylvanicus*). *J. Reprod. Fertil.* 97:353-357; 1993.
27. Mihok, S. Life history profiles of boreal meadow voles (*Microtus pennsylvanicus*). In: Merritt, J. F., ed. Winter ecology of small mammals (special publication of Carnegie Museum of Natural History) vol. 10. 1984:91-101.
28. Milligan, S. R. Mating, ovulation and corpus luteum function in the vole, *Microtus agrestis*. *J. Reprod. Fertil.* 42:35-44; 1975.
29. Sawrey, D. K.; Dewsbury, D. A. Control of ovulation, vaginal oestrus and behavioural receptivity in voles (*Microtus*). *Neurosci. Biobehav. Rev.* 9:563-571; 1985.
30. Smale, L.; Nelson, R. J.; Zucker, I. Daylength influences pelage and plasma prolactin concentrations but not reproduction in the prairie vole, *Microtus ochrogaster*. *J. Reprod. Fertil.* 83:99-106; 1988.