Prediction of fertility by mating latency and photoperiod in nulliparous and primiparous meadow voles (*Microtus pennsylvanicus*)

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Mating behaviour and litter production of female meadow voles (*Microtus pennsylvanicus*) housed in long (14 h light:10 h dark; long day; LD) or short (10 h light:14 h dark; short day; SD) photoperiods were monitored to determine whether the reduced birthrate of SD females resulted from a lack of copulation. All females mated, but fewer SD females gave birth. LD and SD females fell into three distinct groups based on mating latency. The rapid onset group (RO) mated between 7 min and 9 h after pairing, the intermediate onset group (IO) mated between 16–44 h and the late onset group (LO) mated after 58–262 h of male contact. Sixty-seven per cent of LD females were assigned to group RO, 27% to IO, and 6% to LO. In contrast, 30% of SD females were assigned to group RO, 35% to IO and 35% to LO. Fertility was predicted by mating latency. Sixty-nine per cent of RO, 93% of IO and 33% of LO animals gave birth. In a further experiment, a small-mouthed cup was added to the environment to serve as an escape for females wishing to avoid mating. Although females did not use the cup to escape male approaches, mating occurred in only 66% of SD females, but was observed in all LD females. In a final experiment, mating latency and litter production were recorded in primiparous LD and SD females initially observed in the first experiment. Group LO was eliminated in parous females; all primiparous LD and SD females mated within 48 h. Birthrates of LD (82%) and SD (73%) parous females were increased compared with birthrates of nulliparous females (LD = 65%; SD = 55%). These observations suggest that long day-length and parity increase spontaneous oestrus in meadow voles (50% of nulliparous and 80% of primiparous RO animals mated in less than 1.5 h). Females in the IO and LO groups are probably induced into oestrus, as normally described for arvicoline rodents, by direct male contact. Rapid mating (<48 h) predicts greater fertility for both LD and SD females, while delayed mating (>58 h) predicts low fertility. Parity decreases mating latency and increases litter production. Short day females produce fewer litters than LD females in equivalent groups.

Introduction

Female voles (*Microtus*) are thought to be induced into behavioural oestrus by exposure to the urine of reproductively competent males (Sawrey and Dewsbury, 1985). Baddaloo and Clulow (1980) demonstrated that young female meadow voles (*Microtus pennsylvanicus*) develop heavier uteri and ovaries after exposure to a mature male, suggesting that behavioural oestrus in meadow voles also depends upon male chemical stimulation. As in many other species of vole, the induction of ovulation by copulatory stimulation is well documented in meadow voles (Lee and Horvath, 1969; Clulow and Mallory, 1970; Lee *et al.*, 1970). This mode of induction of oestrus and ovulation suggests that most mature healthy female meadow voles exposed to a reproductively competent male should become pregnant.

However, litter production in laboratory-housed females kept under short winter-like photoperiod conditions (10 h light:14 h dark; short day; SD) paired with reproductively competent males that have been housed in long summer-like photoperiods (14 h light:10 h dark; long day; LD) is low; only 20–30% of SD females give birth (Imel and Amann, 1979; Lee *et al.*, 1987). Field studies indicate that winter breeding occurs; 0–50% of females are found pregnant in the winter of any given year (Christian, 1971, 1980). In contrast, more than 95% of females in the field become pregnant during the summer (McShea and Madison, 1989) and 65–90% of laboratory females housed in LD give birth (Imel and Amann, 1979; Lee *et al.*, 1987). This suggests that adult female meadow voles do not rely solely on male photoperiodic responses (regression of testes in response to short days) to limit litter production, but possess an independent photoperiod-sensitive mechanism enabling them to avoid pregnancy under predictable, adverse conditions such as winter.

Short-day females may avoid pregnancy through a variety of behavioural or physiological methods. The most straight-

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forward mechanism would be insensitivity to oestrus-inducing male urine, resulting in fewer matings. Alternatively, females may be induced into oestrus by males and mate, but limit fertility through post-copulatory mechanisms.

The experiments reported here were designed to investigate whether SD females avoid pregnancies through a reduction or alteration in mating behaviour. Experiment 1 investigated the mating behaviour and subsequent fertility of mature, nulliparous female meadow voles housed under long or short day-length conditions. Experiment 2 investigated whether increased environmental complexity, which allowed females to avoid copulation, resulted in reduced fertility in SD females compared with LD females. It is well known that parity increases fertility in rodents (Bronson, 1989), and previous work in both voles and non-microtines indicates that photoperiod has no impact on *postpartum* oestrus (Tamarin, 1977; Charlton *et al.*, 1978; Beasley *et al.*, 1981). Meadow voles, however, may not have access to fertile males during the winter at the time of a *postpartum* oestrus. In Expt 3 we therefore examined the influence of photoperiod on mating behaviour and fertility in parous females, 8 weeks after their first litter was removed.

Materials and Methods

Animals

At weaning (3 weeks of age), 96 virgin female voles (*Microtus pennsylvanicus*) born and raised under long day conditions (14 h light:10 h dark; LD) from the laboratory colony at the University of Michigan either remained under long day conditions ($n = 53$) or were placed in short photoperiod conditions (10 h light:14 h dark; short day, SD; $n = 43$) for 8 weeks. Females were near to sexual maturity at 3 weeks of age, as determined by perforate vaginae. Animals were housed individually or in same sex pairs with Purina Mouse Chow (no. 5015; Purina Mills, Inc., St Louis, MO) and water was available *ad libitum*.

Videotaping procedure

After exposure to the experimental photoperiod for 8 weeks, each female was placed in a 10 gallon aquarium with an LD male known to have sired litters with both LD and SD females. All females had perforate vaginae at this time. Pairs were maintained in the experimental photoperiod of the female. Sawdust bedding was provided and was not changed while animals were paired. The outside of the aquarium was masked with paper to minimize disturbance.

The behaviour of each pair was videotaped continuously with a 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 until post-copulation satiety occurred or for 2 weeks, whichever occurred first. All pairs in Expt 1 were videotaped for 12–24 h after copulation had ceased to allow for the possibility that copulatory activity would resume after a lengthy hiatus. No pairs were observed to resume mating after a 4 h hiatus; thus for Expts 2 and 3, satiety was defined as 4 h

without mating. Within 24 h of copulation, females were separated from the male and remained in the same photoperiod for 3 weeks or until litters were produced.

Mating behaviour

For each pair, mating latency, frequency of mounts, intromissions and ejaculations, the total length of all ejaculatory series (including and excluding the post-ejaculatory interval), mean inter-intromission interval and litter production were recorded. Mating latency and litter production only are described in this paper. Mating latency was defined as the time from initial pairing until the first intromission. Litter production was defined as production of pups 3 weeks after copulation.

Statistical analysis

Mating latency was analysed using a two-tailed Student's *t* test. Bartlett's chi square was used to measure homogeneity of variance. Differences in litter production were analysed by chi-square analyses. Probability values less than or equal to 0.05 were considered significant. Values are given as means $\pm$ SEM.

Experiment 1

LD ($n = 30$) and SD ($n = 20$) animals were paired with LD males and videotaped as stated in the videotaping procedure.

Experiment 2

LD ($n = 12$) and SD ($n = 12$) females were paired with LD males and videotaped as in Expt 1. A small-mouthed container (4.4 cm diameter at the mouth, 10 cm in length) open at one end and large enough for only one vole to enter was added to the environment. Mating latency and litter production was monitored as in Expt 1.

Experiment 3

After pups were weaned, 22 LD primiparous females from Expt 1 were kept under LD ($n = 11$) or SD ($n = 11$) conditions for 8 weeks. They were then paired with a male, videotaped and maintained in the photoperiod of mating until pups were weaned as previously described.

Results

Experiment 1

All animals mated within 2 weeks. However, mating latency varied greatly between LD and SD animals. SD females began mating significantly later than LD animals (LD = 805.1 ± 289.5 min; SD = 3886.4 ± 1131.1 min, $P < 0.05$). This difference was significant despite the lack of homogeneity of variance ($P < 0.05$). All LD females mated within 3.2 days; 50% mated in less than 1.5 h (range = 7–4648 min). In contrast, only five

Fig. 1. Cumulative increase in the percentage of (□) LD (long day; 14 h light:10 h dark) and (◆) SD (short day; 10 h light:14 h dark) female meadow voles mating in Expt 1 with time. Columns denoting periods of no mating by LD and SD females separate groups RO, IO and LO. The first column corresponds to 7 h, the second to 14.4 h. RO: rapid onset group; IO: intermediate onset group; LO: late onset group.

of 20 SD females mated within 1.5 h (range = 8–15 720 min). Ranking animals by mating latency produced three distinct mating patterns (Fig. 1).

The rapid onset group (RO) comprised 26 animals (77% LD; 23% SD) mating within 9 h after pairing with a male. The intermediate onset group (IO) contained 15 animals (53% LD; 47% SD) mating between 16 and 44 h after pairing, whereas the late onset group (LO) consisted of nine animals (22% LD; 78% SD) mating 58–262 h after pairing with the male. The divisions between these groups were very clear, with no animals mating between 9 and 16 h, or between 44 and 58 h after exposure to the male (Fig. 1). No other interval of 7 h without mating occurred before 64 h after pairing.

Birthrates of IO females (93%) were significantly higher than those of LO females (33%; $P < 0.05$) but did not differ from RO females (69%). RO and LO birthrates were not significantly different.

When the interaction of photoperiod and mating latency on birthrate was considered, SD birthrates accounted for the low overall birthrate in RO females; 75% of long day RO females gave birth but only 50% of short day females produced a litter. Birthrates for IO females in both photoperiods (LD = 100%; SD = 85%) were significantly higher than for LO females (LD = 50%; SD = 29%; $P < 0.05$). LD and SD birthrates did not differ significantly within any mating latency group, although SD birthrates were always lower than LD (Fig. 2).

Experiment 2

Mating patterns were altered in SD animals when a small container was added to the environment. All LD animals mated but four of 12 SD animals did not mate within 2 weeks. Mating latency was significantly shorter in LD than in SD animals (LD = 530.33 ± 289.61 min; SD = 2454.13 ± 968.38 min, $P < 0.05$, excluding the four SD animals that did not mate). When animals

Fig. 2. Comparison of birthrates of (□) LD and (◆) SD meadow voles in groups RO, IO and LO in Expt 1. RO: rapid onset group; IO: intermediate onset group; LO: late onset group.

were ranked by mating latency, they fell into the same three mating groups as in Expt 1. The percentage of LD females in each group was similar to Expt 1; RO = 67%; IO = 25%; LO = 8%. However, the percentage of SD females in each mating latency group in Expt 2 differed from that found in Expt 1. Only one of 12 SD females (8%) was assigned to group RO. In contrast, six of 12 (50%) were in IO and five of 12 (42%) in LO. Owing to the small number of animals involved, the shift in mating latency did not reach statistical significance. Birthrates for SD females in RO, IO and LO were 100%, 67% and 20%, respectively. Short day females that mated (eight of 12) achieved an overall birthrate of 75%. Long day birthrates did not differ from Expt 1.

Experiment 3

Mating latency in LD primiparous animals (192.9 ± 80.1 min) was shorter than in LD nulliparous females (805.1 ± 289.5 ,

Fig. 3. Comparison of mating latency and percentage of meadow vole females giving birth in Expts 1 and 3. SDN: short day nulliparous females; LDN: long day nulliparous females; SDP: short day primiparous females; LDP: long day primiparous females. Increases in birthrates with parity did not reach significance. Mating latency in SDN is significantly longer than all other groups ($P < 0.05$). (□) Mating latency; (◆) birthrate.

although this difference was not significant ($t = 1.399$, $P = 0.196$; two-tailed test; Fig. 3). Mean mating latency in SD primiparous females was similar to LD nulliparous animals and significantly shorter than SD nulliparous females ($P < 0.05$; Fig. 3). Birthrates in LD and SD primiparous groups were increased compared with nulliparous animals but with only 11 animals per group the differences were not significant (Fig. 3).

When primiparous animals were ranked by mating latency, all animals fell into groups RO and IO regardless of photoperiod. All LD and SD primiparous animals mated within 48 h; only five animals mated in group IO. Birthrates for primiparous RO (82%) and IO (60%) animals did not differ significantly from each other, or from RO and IO birthrates for nulliparous females.

When all animals from the three experiments ($n = 96$) were ranked by mating latency, the divisions into RO, IO and LO observed in Expt 1 were preserved, although non-mating intervals were slightly shorter. RO animals mated between 7 min and 9.4 h, IO mated between 14.4 and 48 h, and LO mated between 58 h and 2 weeks.

Discussion

These results indicate that female meadow voles paired with reproductively competent males mate within three, distinct, non-overlapping periods as defined by mating latency. Furthermore, fertility differs between these three mating latency groups, suggesting that mating latency indicates differences in the hormonal (oestrous) state among the three groups at the time of pairing with the male and predicts subsequent fertility. Short day lengths lengthen mating latency and inhibit fertility; SD females exhibited longer mating latencies and decreased fertility within each mating latency group than LD females in all

three experiments. When mating occurred in a slightly more complex environment, mating latency increased for SD females (four animals never mated), but was unchanged in LD females. For those SD females mating in the complex environment, fertility was increased, although overall fertility was unchanged. In contrast to nulliparous animals, all parous females mated within 48 h and fertility was greater than that of nulliparous females, suggesting that parity may permanently decrease the responsiveness of female meadow voles to inhibitory short day-lengths. Finally, the rapid onset to mating seen in RO animals (7 min–9.4 h) in LD and SD nulliparous females suggests that a large proportion of meadow voles (75% of LD and 30% of SD) may not need exposure to a male to achieve behavioural oestrus, but exhibit spontaneous oestrus. Parity increased the proportion of females exhibiting spontaneous oestrus to 91% for LD and 64% for SD.

The boundaries of the three mating latency groups, RO, IO and LO, were preserved throughout three different paradigms ($n = 96$), suggesting that these differences depend upon hormonal differences among these groups that result in different mating latencies (oestrus) and a different birthrate. Mating latency and birthrate differed markedly in LO animals (primarily SD females), compared with RO or IO animals. LO females are apparently induced into behavioural oestrus (as defined by male approaches and subsequent copulation). However, their long mating latencies and aberrant mating behaviours (L. Meek and T. Lee, unpublished) indicate an abnormal oestrous state with either lower than normal blood concentrations of oestrogen or an insensitivity to the oestrogen. Experiment 2 suggests that, in the field, many of these females would avoid mating. We suggest that the low birthrate of LO females is a direct result of their aberrant mating behaviour (L. Meek and T. Lee, unpublished); that is, the aberrant mating pattern fails to induce the LH surge that is necessary for ovulation.

RO and IO females successfully produce young in SD or LD photoperiods. These animals may represent two distinct subpopulations, one which needs contact with male chemosignals to achieve oestrus (IO) and one which does not (RO); or they may represent a single group of animals that show a continuum of follicular development. This experiment did not distinguish between these possibilities.

However, the probability of nulliparous females falling into RO or IO groups was significantly influenced by photoperiod. Most LD females (67%) mated in RO with high success rate of litter production (75%). Those LD females mating in IO (27%) had a statistically equivalent birthrate (100%). In contrast, only 33% of SD females in Expt 1 (birthrate = 50%) and 8% in Expt 2 (one animal; a litter was produced) mated in group RO. Those SD females mating in IO had birthrates of 85% and 67% (Expts 1 and 2), respectively. These data indicate that SD females are more successful at producing litters if mating is delayed until 14–48 h after pairing with a male.

If RO animals have spontaneous oestrus, then long days (Expts 1 and 2) and parity (Expt 3) increase the probability that a female will have spontaneous oestrus and successfully produce a litter. Thus short daylengths probably inhibit spontaneous oestrus in nulliparous females and dramatically increase the number of females that do not mate until after 58 h of male contact or do not mate at all. Short day females mating in IO have a fertility equivalent to LD animals, whereas fertility is

very low for those that have not copulated by 48 h after pairing with a male (LO) or those that copulate before 9 h of male contact (RO).

Since male approaches to the female and subsequent copulation occur, all SD females are assumed to produce chemosensory or other oestrogen-dependent cues indicating oestrus to the male. The hormonal milieu of RO and IO animals from each photoperiod is probably very similar before and during mating, as their mating sequences are indistinguishable (L. Meek and T. Lee, unpublished). However, the fertility of SD females was subsequently reduced, compared with LD females, indicating that a post-copulatory mechanism may be involved in preventing winter pregnancies. For example, there may be failure of the post-copulatory prolactin rise necessary to maintain corpora lutea (Milligan, 1975; Milligan and MacKinnon, 1976).

The behavioural data indicate that LD and SD nulliparous and primiparous females are uniquely adapted hormonally to winter or summer conditions. Long day female meadow voles mate quickly (50% within 1.5 h) and have a high percentage of successful pregnancies in the laboratory. All primiparous females also mate quickly (within 48 h) and show increased litter success compared with nulliparous females, suggesting that primiparous female meadow voles do not respond to the inhibitory influence of short days. This lack of response to photoperiod by primiparous females has been noted previously in field voles (Charlton *et al.*, 1978), beach voles (Tamarin, 1977) and white-footed mice (Beasley *et al.*, 1981). Species with short natural lifespans may continue to reproduce despite decreasing daylengths to maximize lifetime litter production.

Under laboratory conditions mimicking the short daylengths of winter, a season when field pregnancies decline significantly, SD nulliparous females show decreased litter production compared with nulliparous LD females in all three mating latency groups. Despite the availability of high-quality food and warm ambient temperatures, short photoperiods delay mating and reduce litter production in nulliparous meadow voles in the laboratory. It appears that most SD females mate if a reproductively competent male is available. However, both in the field and the laboratory, an assessment of parity, photoperiod, temperature, general health, body weight and fat, and available nutrients probably ultimately determines the success of a pregnancy in winter photoperiod.

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