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Does risk of small mustelid predation affect the oestrous cycle in the bank vole, *Clethrionomys glareolus*?

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Abstract. Female bank voles suppress their reproduction when the risk of small mustelid predation is high. The mechanism for this reproductive suppression is unknown. Because rodents are known to alter their oestrous cycle in response to changing environmental conditions, the effect of predation risk on the oestrous cycle of bank vole females was studied. The oestrous cycles of 24 females divided into two treatments (predation risk and control) were observed for 20 days using female receptivity as an indication of oestrus. Voles exposed for 2–3 h a day for 20 days to the close presence of a least weasel, *Mustela nivalis nivalis*, had fewer oestrous cycles than control females exposed to a domestic rabbit, *Oryctolagus cuniculus*. Females under predation risk had more abnormally long cycles than did control females. The number of days females were in oestrus tended to be lower in the predator-exposure group than in the control group. For those females that performed lordosis, the latency to lordosis did not differ between treatments. The amount of food consumed or weight change in females did not differ between treatments. The results indicate that female bank voles may respond to predation risk by suppressing their oestrous cycle. Suppressed oestrus may be a mechanism for the breeding suppression observed under the risk of small mustelid predation in female voles. Whether females suppressing oestrus have selective advantage in terms of future survival requires further study.

The least weasel, *Mustela nivalis nivalis*, and the stoat, *M. erminea*, both small mustelids, are the main predators of the bank vole and the field vole, *Microtus agrestis* (Erlinge 1975; Tapper 1979; Korpimäki et al. 1991). Both species suppress or delay their reproduction when there is a risk of small mustelid predation (Ylönen 1989; Korpimäki et al. 1994; Ylönen & Ronkainen 1994; Koskela & Ylönen 1995; but see Korpimäki et al. 1994 for the field vole). However, the underlying mechanism for this suppression is unknown.

Small mustelids use olfactory cues to find voles. According to laboratory studies (Cushing 1984, 1985), least weasels prefer oestrous females over anoestrous ones when hunting for prey. When predation risk is high, suppressing oestrus or increasing the length of the oestrous cycle could therefore be advantageous for female voles if it decreased their vulnerability to predators. Suppressed oestrus would in turn lead to breeding suppression.

Olfactory cues are crucial in inducing and suppressing oestrus in microtines (Richmond & Stehn 1976; Brown 1985). Some rodents, such as the house mouse, *Mus musculus*, the prairie deermouse, *Peromyscus maniculatus bairdii*, and the field vole, are known to respond to changing environmental conditions, such as crowding, by changes in their oestrous cycle (Chitty & Austin 1957; Whitten 1959; Champlin 1971; Terman 1973; Massey 1986). Our aim in the present experiment was to investigate whether oestrous cycles of bank vole females change in response to a simulated risk of small mustelid predation.

METHODS

Animals and Apparatus

The female bank voles we used were laboratory-born descendants of wild animals. They were 6–8

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months of age and had successfully given birth to one litter before the experiment. The males originated from the laboratory and field. Those from the field were returned there after the experiment. We obtained six least weasels from the Zoo of Helsinki and a domestic rabbit, *Oryctolagus cuniculus*, as a control, from a farm nearby.

We used 24 female and 28 male bank voles in the experiment. Additionally a number of males, which were housed next to the females during the experiment (see below), were used. All study voles were housed individually in (21 × 21 × 36 cm) cages that had plastic bases and wire-mesh walls and roofs. Sawdust and hay were used as bedding. Laboratory rodent chow (Labfor R36) and water were offered ad libitum and fresh cabbage, potatoes and carrots were provided twice a week throughout the experiment. The amount of pellets consumed by females was weighed during the study.

We randomly assigned females to one of the treatment groups, predation or control. The females in the two groups did not differ in weight at the beginning of the study ($\bar{X} \pm$ SD, predation: 21.75 ± 2.49 g, $N = 12$; control: 21.33 ± 2.50 g, $N = 12$; t-test: $t = 0.41$, $df = 22$, $P = 0.686$). The treatments were conducted in two separate laboratory rooms in similar conditions (20 ± 4°C, light:dark 18:6 h, lights on at 0500 hours). We simulated predation risk by exposing females to the close presence of a (randomly chosen) least weasel for 2–3 h daily during the late evening for 20 days. In the control treatment a rabbit was used to expose the females to novel odours and disturbance. Female cages were situated next to a tube-like arena where a least weasel or a rabbit was allowed to move freely. These arenas (240 × 35 × 30 cm) were made of plywood except for walls that were made of wire-mesh. The floors were covered with sawdust, and food and water were continuously available. The exact location of female cages around the arena was changed at 5-day intervals.

To expose females in both treatments to continuous contact with sexually active males, 'extra' males in their cages were placed on the opposite side of the female cages (so that female cages were situated between the least weasel arena and male cages). Additionally, bedding from male cages (from laboratory stock, outside the experiment) was transferred at 5-day intervals to the cages of females. The 'extra' males were replaced with a new male once or twice during the experiment.

Determination of Oestrous Cycle

The most common method for determining the oestrous cycle in female rodents has been the vaginal smear technique (Whitten & Champlin 1978). However, this method can induce ovulation (MacFarlane & Taylor 1982) or cause abnormally long oestrous cycles at least in some species (Whitten & Champlin 1978). Vaginal smears are also an unreliable indicator of oestrus for the bank vole (Hoffmeyer 1982) and for some *Gerbillurus* species (Dempster & Perrin 1989). In the current experiment we used female receptivity as an indicator of oestrus. Females were considered to be oestrous if they showed lordosis in response to mounting attempts by a male during a 10-min observation period. Lordosis is an immobile posture in which the back is flat or concavely arched. This method does not disturb the natural oestrous cycle and is known to be a reliable indicator of oestrus in voles (Hoffmeyer 1982; Carter et al. 1990).

Receptivity of each female was determined daily between 0900 and 2100 hours during the 20-day experiment. The order of receptivity tests was changed daily so that each female was tested at a different time on each day. We conducted 440 trials on polycarbonate behavioural arenas (19 × 24 × 56 cm) under dim red light in two separate laboratory rooms (one for each treatment). The floors of the arenas were covered with sawdust. The males used in the trials ($N = 28$) were housed individually in standard breeding cages in a separate laboratory room. Scrotal testes were considered as a sign of maturity. Each male was used only once with a particular female during the study, and no male was used in more than one trial a day. The treatment in which a male was used was also changed daily so that every second day the male was used in the predator treatment. If a male did not attempt to mount in a trial, the trial was repeated with a new male. Each male received at least one lordosis during the study.

Before the trial the female, in a wire-mesh cage measuring 10 × 15 × 10 cm, and the male, able to move around but not to enter the female cage, were introduced to the arena. The pair was left undisturbed for 30 min and after that the female was released from her cage and the trial started. If a female showed lordosis during a 10-min observation period the pair was separated immediately and the trial was terminated. After each trial the

female and the male were returned to their own cages.

Data Analysis

One female died and one was lost in the control group during the study. The data from these two females are excluded from all the analyses. Lordosis latency was measured from the start of the test session. As the time of day could have affected the trials, we compared the number of oestruses found between morning (0900–1300), afternoon (1300–1700) and early evening (1700–2100) using Pearson's chi-squared test. The length of every oestrous cycle was determined as the time (in days) between two observed oestruses. If a female did not show oestrus at all or only once during the study period, we considered that during the 20-day study the female did not have any oestrous cycle. That was the case for three females in the predation risk group: two were not in oestrus at any point during the study and one showed oestrus only once (on day 18). When analysing the oestrous cycle length between the treatments, the minimum cycle lengths for these females were estimated as 21 and 18 days, respectively.

Having three out of 12 'abnormal' females in the experimental group and none out of 10 in the control group by chance is not very likely (Fisher's exact test: $P = 0.22$), especially as all females gave birth before the experiment. However, to clarify the significance of these females to the results (will the trend between different treatments remain?), the statistics for variables concerning oestrous cycles are presented both including and excluding (in square brackets) these females.

We used a two-sample *t*-test and paired *t*-test (both two-tailed) when analysing unrelated and related samples, respectively. The sample size in all the analyses is 12 for the predation and 10 for the control group. Variables representing proportions were arcsine squareroot transformed before analyses. Values for variables are presented for untransformed data as the $\bar{X} \pm \text{SD}$. For the statistical analyses we used SPSS for Windows (SPSS 1992).

RESULTS

Females under predation risk had fewer oestrous cycles than did the control females during the 20-day study period (predation: 2.0 ± 1.3 [2.7 ± 0.7]; control: 3.1 ± 0.9; $t = -2.22$, $df = 20$, $P < 0.05$ [$t = -1.18$, $df = 17$, $P > 0.3$]). Consequently, the oestrous cycles of experimental females were longer than those of the control females (predation: 8.9 ± 6.8 days [5.3 ± 1.6]; control: 4.5 ± 2.0 days; $t = 2.12$, $df = 13.23$, $P = 0.05$ [$t = 0.85$, $df = 17$, $P > 0.4$]). The observed oestrous cycle length of control females (4–5 days) is in accordance with earlier reports concerning the oestrous cycle of the bank vole (3–5 days according to Bujalska 1983). Females under predation risk had significantly more abnormally long cycles than did control females: nine out of 12 and one out of 10 females in the experimental and control groups, respectively, had oestrous cycles longer than 5 days during the 20-day study period (Fisher's exact test: $P < 0.005$, [6:3, 1:9, $P < 0.02$]).

The mean proportion of days a female was in oestrus tended to be greater in the control group (0.24 ± 0.07) than in the experimental group but not significantly so (0.17 ± 0.10 [0.22 ± 0.04]; $t = -1.83$, $df = 15.2$, $P = 0.087$ [$t = -0.49$, $df = 17$, $P > 0.6$]). The reason why there was only a tendency for treatments to differ, even though control females had significantly more oestrous cycles than experimental females, is due to three females with long cycles showing receptivity for 2 or even 3 days once in oestrus.

The time of day did not affect the number of oestruses found in the trials (Pearson's χ^2: control: 1.72, $df = 2$, $P > 0.4$; predation = 0.06, $df = 2$, $P > 0.9$). Lordosis latency did not differ significantly between the treatment groups (predation: 107 ± 74 s; control: 79 ± 49 s; *t*-test: $t = 1.00$, $df = 20$, $P > 0.3$). Neither was there any difference in the amount of food consumed between treatments (predation: 73.4 ± 21.6 g; control: 72.1 ± 21.2 g; $t = 0.15$, $df = 20$, $P > 0.9$).

Females lost weight similarly in both treatments during the experiment (predation: mean weight change = -2.2 ± 1.3 g; $t = -5.92$, $df = 11$, $P < 0.001$; control: -2.2 ± 1.9 g; $t = -3.71$, $df = 9$, $P = 0.005$). Thus, at the end of the experiment final weights of the females did not differ between treatments (predation: 19.6 ± 2.8 g; control: 19.4 ± 1.9 g; $t = 0.18$, $df = 20$, $P > 0.8$).

DISCUSSION

Our results indicate that bank vole females may respond to predation risk by changes in their

oestrous cycle. Females exposed to a simulated risk of small mustelid predation had fewer oestrous cycles than did control females exposed to a rabbit. Furthermore, three females under predation risk seemed to suppress their oestrus or at least had remarkably longer oestrous cycle lengths than did control females. The trend in results seems to remain even without these three females. Our results suggest that suppressed oestrus may be the underlying reason for the observed breeding suppression in female voles under risk of small mustelid predation.

One factor that might affect the way we interpret our results is the possible confounding effect of males that were placed on the other side of the female cages to keep females reproductively active. The suppressed oestrus in females may have been direct, via least weasel odour, or indirect, via an effect on males. Males exposed to the predator may have avoided advertising their presence and producing odour cues that would have stimulated females, thus affecting reproductive cycling. However, because we replaced these males with a new male once or twice during the experiment, and additionally, bedding from the male cages (from laboratory stock) was transferred to the females' cages at 5-day intervals during the study, we suggest that this indirect effect is unlikely. Furthermore, in other studies with bank voles, males exposed to mustelid odours were not affected physiologically (Ylönen & Ronkainen 1994) or behaviourally (Ronkainen & Ylönen 1994) while females in the same conditions suppressed breeding.

Crowding is known to cause changes in the oestrous cycles of female mice, and two factors have been suggested to be responsible: stress resulting from crowding and olfactory stimuli which prevent ovulation (reviewed in Brown 1985). Since crowding can cause stress and increased adrenal weights and plasma corticosteroid levels (Christian & Davis 1964), it is possible that anoestrus in grouped females is caused by a stress response. However, the evidence supporting the hypothesis is ambiguous (Brown 1985). The second possible factor, olfactory stimuli from other individuals, is considered a more likely explanation for suppressed oestrus (Reynolds & Keverne 1979). In our study, bank vole females in both treatments were exposed to both olfactory stimuli and disturbance. We found no evidence for significantly different stress levels between the treatments in the variables observed. The amount of food consumed and the changes in body weight, which could indicate stress, did not differ between the treatments. Therefore, since bank voles can recognize their predators by olfactory cues (Jedrzejewski et al. 1993), we suggest that the more likely factor causing the observed changes in oestrous cycles is the olfactory stimuli from the small mustelid predator. However, because voles, unlike mice, are induced ovulators (Richmond & Stehn 1976), the physiological mechanisms that causes anoestrus (generated by olfactory stimuli) in the bank vole and mouse may be different.

Ronkainen & Ylönen (1994) argued that females could respond to high predation pressure behaviourally by refusing to copulate and that could lead to breeding suppression. In contrast our results suggest that the proximate cause of breeding suppression in females is physiological. Since non-receptive females will not permit males to mount them (Witt et al. 1990), lack of copulatory behaviour in earlier studies (Ronkainen & Ylönen 1994; Koskela & Ylönen 1995) could be due to anoestrus in females.

Suppressed oestrus under predation risk may function as an anti-predatory behaviour in voles. Because small mustelids can detect and thus capture oestrous females more readily than anoestrous ones when hunting for prey (Cushing 1985), even short-term changes in the oestrous cycle may give individuals that alter their oestrous period a selective advantage in terms of better survival.

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REFERENCES

Brown, R. E. 1985. The rodents I: effects of odours on reproductive physiology (primer effects). In: *Social*

Odours in Mammals. Vol. 1 (Ed. by R. E. Brown and D. W. Macdonald), pp. 245–344. Oxford: Clarendon Press.

Bujalska, G. 1983. Reproduction. *Acta theriol.*, **28,** *Suppl. 1*, 148–161.

Carter, C. S., Witt, D. M., Manock, S. R., Adams, K. A., Bahr, J. A. & Carlstead, K. 1990. Hormonal correlates of sexual behavior and ovulation in male-induced and postpartum estrus of female prairie vole. *Physiol. Behav.*, **46,** 941–948.

Champlin, A. K. 1971. Suppression of oestrous of grouped mice: the effects of various densities and the possible nature of the stimulus. *J. Reprod. Fert.*, **27,** 233–241.

Chitty, H. & Austin, C. R. 1957. Environmental modification of oestrus in the vole. *Nature, Lond.*, **179,** 592–593.

Christian, J. J. & Davis, D. E. 1964. Endocrines, behavior and population. *Science*, **146,** 1550–1560.

Cushing, B. S. 1984. A selective preference by least weasels for oestrous versus dioestrous urine of prairie deer mice. *Anim. Behav.*, **32,** 1263–1265.

Cushing, B. S. 1985. Estrous mice and vulnerability to weasel predation. *Ecology*, **66,** 1976–1978.

Dempster, E. R. & Perrin, M. R. 1989. The estrous cycle and induction of estrous behavior in four species of hairy-footed gerbils (genus *Gerbillurus*). *J. Mammal.*, **70,** 809–811.

Erlinge, S. 1975. Feeding habits of the weasel *Mustela nivalis* in relation to prey abundance. *Oikos*, **26,** 378–384.

Hoffmeyer, I. 1982. Responses of female bank voles (*Clethrionomys glareolus*) to dominant vs subordinate conspecific males and to urine odors from dominant vs subordinate males. *Behavl neural Biol.*, **36,** 178–188.

Jedrzejewski, W., Rychlik, L. & Jedrzejewska, B. 1993. Responses of bank voles to odours of seven species of predators: experimental data and their relevance to natural predator–vole relationships. *Oikos*, **68,** 251–257.

Korpimäki, E., Norrdahl, K. & Rinta-Jaskari, T. 1991. Response of stoats and least weasels to fluctuating prey abundances: is the low phase of the vole cycle due to Mustelid predation? *Oecologia* (*Berl.*), **88,** 552–561.

Korpimäki, E., Norrdahl, K. & Valkama, J. 1994. Reproductive investment under fluctuating predation risk: microtine rodents and small mustelids. *Evol. Ecol.*, **8,** 357–368.

Koskela, E. & Ylönen, H. 1995. Suppressed breeding in the field vole (*Microtus agrestis*): an adaptation to cyclically fluctuating predation risk. *Behav. Ecol.*, **6,** 311–315.

MacFarlane, J. D. & Taylor, J. M. 1982. Nature of estrus and ovulation in *Microtus townsendii* (Bachman). *J. Mammal.*, **63,** 104–109.

Massey, A. 1986. Variable responses to high density of female *Mus musculus*: a merger of two population regulation hypotheses. *Oecologia* (*Berl.*), **69,** 140–143.

Reynolds, J. & Keverne, E. B. 1979. The accessory olfactory system and its role in the pheromonally mediated suppression of oestrus in grouped mice. *J. Reprod. Fert.*, **57,** 31–35.

Richmond, M. & Stehn, R. 1976. Olfaction and reproductive behavior in microtine rodents. In: *Mammalian Olfaction, Reproductive Processes, and Behavior* (Ed. by R. L. Doty), pp. 197–217. New York: Academic Press.

Ronkainen, H. & Ylönen, H. 1994. Behaviour of cyclic bank voles under risk of mustelid predation: do females avoid copulations? *Oecologia* (*Berl.*), **97,** 377–381.

SPSS/Norusis, M. J. 1992. *SPSS for Windows. Advanced statistics.* Release 5. Chicago: SPSS Inc.

Tapper, S. 1979. The effect of fluctuating vole numbers (*Microtus agrestis*) on a population of weasels (*Mustela nivalis*) on farmland. *J. Anim. Ecol.*, **48,** 603–617.

Terman, C. R. 1973. Reproductive inhibition in asymptotic populations of prairie deermice (*Peromyscus maniculatus bairdii*). *J. Reprod. Fert., Suppl. 19*, 457–463.

Whitten, W. K. 1959. Occurrence of anoestrous in mice caged in groups. *J. Endocrinol.*, **18,** 102–107.

Whitten, W. K. & Champlin, A. K. 1978. Pheromones, estrus, ovulation, and mating. In: *Methods in Mammalian Reproduction* (Ed. by J. C. Daniel), pp. 403–417. London: Academic Press.

Witt, D. A., Carter, C. S., Chayer, R. & Adams, K. 1990. Patterns of behaviour during postpartum oestrus in prairie voles, *Microtus ochrogaster. Anim. Behav.*, **39,** 528–534.

Ylönen, H. 1989. Weasels *Mustela nivalis* suppress reproduction in cyclic bank voles *Clethrionomys glareolus*. *Oikos*, **55,** 138–140.

Ylönen, H. & Ronkainen, H. 1994. Breeding suppression in the bank vole as antipredatory adaptation in a predictable environment. *Evol. Ecol.*, **8,** 658–666.