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# BREEDING BEHAVIOR UNDER TEMPORAL RISK OF PREDATION IN MALE ROOT VOLES (*MICROTUS OECONOMUS*)

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We examined breeding behavior responses of male root voles (*Microtus oeconomus*) to temporal risk of predation by using acute and chronic exposure to predator odor. The 2 series of exposure experiments provided 2 types of temporal patterns of risk: continuous safety with a brief period of risk and sustained risk with a brief period of safety. Male root voles that were acutely exposed to predator odor for 1 h suppressed their breeding behavior, but bred immediately after exposure to control odor for 1 h. Those chronically exposed to predator odor for 20 days maintained behavioral suppression during the 1-h period of exposure to control odor. Acutely exposed males did not change their physiological patterns of breeding, but those chronically exposed to predator odor had reduced testosterone concentration and epididymis index. Our results indicate that breeding behavior in a given situation depends on the overall patterns of risk experienced by male root voles, and the acute and chronic stress responses that affect reproduction are responsible for different behavioral responses to the 2 types of temporal patterns of risk. We also discuss the reasons for conflicting results about breeding suppression of voles between previous studies in the laboratory and the field.

Key words: breeding behavior, corticosterone, *Microtus oeconomus*, predation risk

Temporal variation in predation risk is an unavoidable aspect of most natural environments. During the last decade, however, no studies on the breeding responses of voles to predation risk have considered or explicitly addressed the effects of temporal risk (Jonsson et al. 2000; Koskela and Ylönen 1995; Mappes and Ylönen 1997; Mappes et al. 1998; Wolff and Davis-Born 1997; Ylönen 1989; Ylönen et al. 1992; Ylönen and Magnhagen 1992; Ylönen and Ronkainen 1994), which may underestimate or overestimate the breeding responses to risk expected under field situations and the importance of predation risk in nature (Lima and Bednekoff 1999; Sih et al. 2000).

Lima and Bednekoff (1999) devised a risk allocation hypothesis based on the assumption that prey adaptively allocate their foraging efforts, and thus their exposure to predation risk, across high-risk and low-risk situations. The essence of the hypothesis is that foraging behavior of prey in any given situation depends on the proportion of time that prey spends in high-risk versus low-risk situations. For example, if high-risk situations are infrequent (i.e., prey are acutely exposed to high risk), then they should show moderate feeding activity, but should drastically reduce their feeding during those brief periods of risk. In contrast, if high-risk situations are common (i.e., prey are chronically exposed to high risk), they should maintain a minimum necessary feeding rate, but should drastically increase feeding during brief periods of safety (see also Sih et al. 2000). Like foraging, breeding activity increases individual susceptibility to predation (Magnhagen 1991; Sih et al. 1990). As a result, prey breeding under temporal risk also face the problem of adaptively allocating their breeding efforts across various states of risk. However, so far, how the breeding of voles responds to the temporal variable in risk is unknown.

In the present study, we examined the breeding behavior of male root voles (*Microtus oeconomus*) in response to different temporal patterns of predator risk by using acute and chronic exposure to predator odor. The 2 series of exposure experiments provided 2 types of temporal patterns of risk, continuous safety with a brief period of risk, and sustained risk with a brief period of safety. Specifically, from the risk allocation hypothesis of Lima and Bednekoff (1999), we predicted that male root voles maintained under safety should suppress breeding behavior during an acute period of exposure to risk, but should show breeding activity when returning to safety.

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**TABLE 1.**—Protocol for exposure to odors in experiments testing effects of acute exposure and chronic exposure on breeding behavior in male root voles.

| | Days 1–20 odor exposure | | Day 21 (acute group only) odor exposure (for 1 h) | | Behavioral test | | |
|---|---|---|---|---|---|---|---|
| | | | | | Time of odor exposure in behavioral arena before test | Odor exposure | |
| Group | Odor | Amount (ml/day) | Odor | Amount (ml) | | Odor | Amount (ml) |
| Acute exposure | | | | | | | |
| A-Risk | Control | 10 | Predator | 10 | 3 min | Predator | 20 |
| A-Safe | Control | 10 | Predator | 10 | 1 h | Control | 20 |
| A-Control | Control | 10 | Control | 10 | 1 h | Control | 20 |
| Chronic exposure | | | | | | | |
| C-Safe | Predator | 10 | | | 1 h | Control | 20 |
| C-Control | Control | 10 | | | 1 h | Control | 20 |

Because animals chronically exposed to predation risk may be chronically stressed (Boonstra et al. 1998), which affects breeding function (Boonstra et al. 1998; Fernandez-Guasti et al. 1990, 1991), we predicted that male root voles chronically exposed to risk would remain in continuous breeding suppression during a brief period of safety. In addition, we measured the levels of plasma corticosterone and testosterone to determine potential physiological mechanisms that may be responsible for the responses of breeding behavior to the 2 types of temporal patterns of risk. Finally, we discuss the possible reasons that led to conflicting results about breeding suppression between early laboratory and field studies.

## MATERIALS AND METHODS

*Study site and animals*.—We carried out the study during April–September 2001. We used laboratory-made traps to capture approximately 350 root voles for the study at Haibei Alpine Meadow Ecosystem Research Station, Chinese Academy of Sciences, Menyuan County, approximately 155 km north of Xining, the capital of Qinghai Province, People's Republic of China (37°29′N, 101°12′E). All voles were maintained at 18–22°C under a long-day light cycle (16:8 h light : dark), and were individually housed in standard breeding cages (36 × 20 × 17 cm). The voles were provided with granulated rabbit chow (TK-10, Beijing Feed Processing Plant, Beijing, China) and water ad libitum before and during the experiments. Because of the nonsynchronous estrous cycle of females, it was difficult to obtain sufficient sample sizes of synchronous estrous females during our experiments, so only male root voles were involved in this study. Female root voles were only used as a stimulus to measure breeding behavior of males. All males used in experiments were adults (body mass ≥30 g) and had scrotal testes.

We caught 4 male and 5 female steppe polecats (*Mustela eversmanni*) and 1 male and 1 female woolly hares (*Lepus oiostolus*) at Haibei Alpine Meadow Ecosystem Research Station. Polecats are the main predators on voles in the alpine meadow ecosystem on Qinghai-Tibet Plateau (Liu et al. 1994). Polecats were individually housed in metal wire-cages (50 × 45 × 30 cm) and were fed pig viscera and water ad libitum. Hares also were individually housed in the same size wire-cages, and were provided with granulated rabbit chow (TK-10) and water ad libitum. Hares were used to produce control odor. All procedures conformed to guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998).

*General experimental methods*.— Odor of steppe polecats was used to simulate risk of predation. We collected urine and feces every other day in trays under the cages of captive polecats; each tray was washed with 500 ml of water and the washing water was strained through a filter. Filtered solutions that were collected at different times were fully mixed with each other. This mixed solution was used as predator odor throughout experiments. Odor of woolly hares was used as a control.

Exposure to odor was carried out in an exposure cage (36 × 20 × 17 cm) in which sawdust and nest material were sprayed with odor solution as a fine mist. The cage was made of opaque plastic with a wire mesh roof. Behavioral testing was done in a behavioral arena (60 × 25 × 17 cm) made of transparent plastic and a wire mesh roof, in which sawdust was sprayed with odor solution by the same method as exposure cages. Cages and arenas were thoroughly cleaned before exposure and testing.

Two experiments were done to assess effects of acute and chronic exposure to risk. In the acute-risk experiment, male voles were exposed to the control odor for 20 days. On day 21, they were exposed a 2nd time for 1 h to predator odor, and then were paired with females and their behavior was monitored under predator or control odor. In the chronic-exposure experiment, males were exposed to predator odor for 20 days, and on day 21 they were paired and behavior was monitored under control odor. Details of the experiments are given below.

*Acute-risk exposure experiment*.—Each male was placed in an exposure cage. The cage was sprayed daily with 10 ml of control odor for 20 days. At day 21, the male voles were introduced individually into another exposure cage that had been sprayed with 10 ml of predator odor and were exposed to the odor for 1 h. The exposed males were then assigned to 1 of 2 trials: under risk (referred to hereafter as A-Risk; $n = 22$) or under safe (referred to as A-Safe trial; $n = 16$; Table 1) behavior trial. The A-Risk trial was used to examine breeding behavior responses of males that had been exposed to predator odor for 1 h. To keep a consistent risk exposure period of 1 h in the 2 trials, a male and its sexual partner were kept in a behavioral arena that had been sprayed with 20 ml of predator odor, allowed a 3-min period for habituation, and then behavior was tested immediately (see below). Additionally, the A-Safe trial was used to examine breeding behavior responses of males returning to safety after being acutely exposed to predator odor for 1 h. In the trial, the acutely exposed males were introduced individually into the other behavioral arenas with 20 ml of control odor, and kept there for 1 h, and then behavior was tested (see below). For the control ($n = 19$), the experimental routine followed the same protocol as in the A-Safe trial, with the exception that males were exposed to control

odor at all times, including during behavioral testing (Table 1). The control is referred to as the A-Control trial. The 3 trials were run between 0830 and 1130 h in separate rooms.

*Chronic-risk exposure experiment.*—Each individual was placed in an exposure cage sprayed daily with 10 ml of predator odor for 20 days. At day 21, breeding behavior was tested 1 h after the voles were transferred individually into behavioral arenas containing 20 ml of control odor. This trial is referred to as the C-Safe trial ($n = 13$; Table 1) and was conducted to examine behavior responses during a brief safe period in chronically exposed males. For the control ($n = 10$), the experiment routine was the same as in the above trial with the exception that spraying was conducted with control odor. The control is referred to as the C-Control trial. The 2 trials were run between 0830 and 1130 h in separate rooms.

*Breeding behavior observations.*—Males were tested individually with receptive females. We induced receptivity in females used for behavioral tests by subcutaneous injection of estradiol benzoate (0.75 μg/g body weight; Sigma Chemical Company, St. Louis, Missouri) 48 h before testing, and progesterone (15 μg/g; Sigma Chemical) 3 h before testing. Each behavioral test lasted 30 min. Before the start of the test, behavioral arenas were separated into equal-sized sections by a removable partition. A male and a receptive female were kept in opposite sides of the arena for 3 min and then the partition was removed to test behavior. Each receptive female was used only once for each male. We recorded the following activities of males (as in Ronkainen and Ylönen [1994] and Stopka and Macdonald [1998]) during the observation period: general activity (active movement), amicable behavior (grooming female or sitting in contact with female), approaching (approaching female in an attempt to stimulate it), stimulating female behavior (sniffing anogenital area of female and following female in an attempt to mount), avoidance (avoiding or fleeing from female that tries to solicit it), and aggressive behavior (attacking or threatening female). In addition, we recorded the number of males that successfully copulated. Copulation was defined as completing an entire copulatory series from mount to intromission to ejaculation.

To determine whether female behavior under different risk had a confounding effect on male breeding behavior, we also tested the breeding behavior of receptive females in behavioral arenas with predator odor ($n = 9$) and with control odor ($n = 9$). These receptive females and their partners were not used in male behavioral tests in this study. We recorded the following female behaviors: sniffing nose and anogenital area of male, soliciting (biting a male, then moving away), waiting (stopping and turning its head back toward male after soliciting), avoidance (avoiding or fleeing a male), and threat display (refusing male that tried to approach it).

*Hormone assay, testis index, and epididymis index.*—Another 48 males were used to assay hormone levels and to test for testis index and epididymis index. These males were assigned to acute-risk exposure (A-Control for $n = 10$, A-Risk for $n = 10$, and A-Safe for $n = 10$) and chronic-risk exposure (C-Control for $n = 9$ and C-Safe for $n = 9$). Experimental protocols were the same as for acute- and chronic-risk exposure experiments, respectively.

Each male used to assay hormone levels was decapitated at day 21 and trunk blood was collected individually within 0.5 min. The blood was centrifuged at 4,000 rpm for 15 min. Separated plasma was frozen and stored at −30°C until analysis. Testosterone was measured in duplicate by radioimmunoassay, by using a commercially available RIA kit (Sigma Chemical). Corticosterone was measured by fluorescence assay as described in Zenker and Bernstein (1958).

We measured wet weights of paired testes and right epididymis for both the acute-and chronic-risk experiments. Testis index was indicated as weight of paired testes/body weight and epididymis index was indicated as weight of right epididymis/body weight. The body weight in the 2 indices was final body weight at the end of trials in acute and chronic exposure experiments.

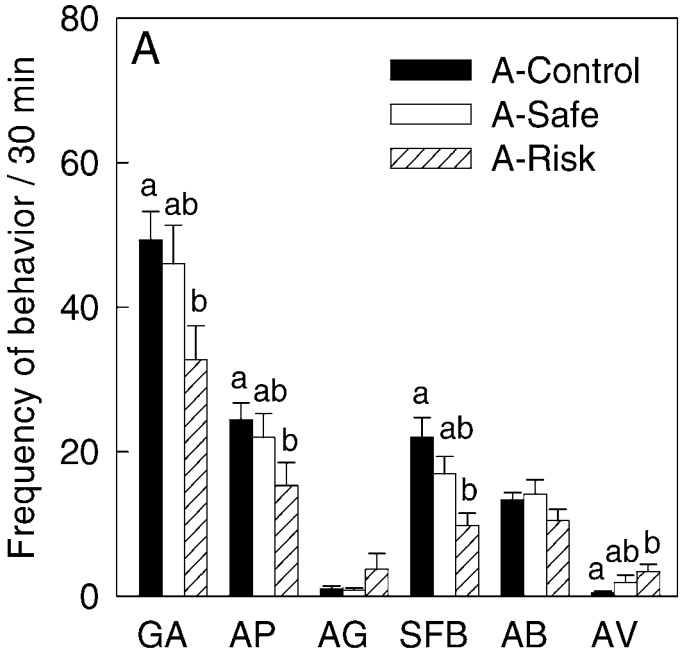
*Statistical analysis.*—Values for variables are given as mean ± *SE* except for percentages. The chi-square test was used to compare percentage of individuals copulating. When the difference in percentages was significant, the chi-square test was used for further pairwise comparison among trials. Because behavioral variables are not normally distributed, we tested every behavioral variable in males and female by using the Kruskal–Wallis *H*-test for acute-risk exposure and Mann–Whitney *U*-test for chronic-risk exposure. In the acute-risk exposure experiment, significant effect was further analyzed by using the Bonferroni method for pairwise multiple comparisons based on the rank-transformed data of behavior variables. Indices of testis and epididymis, and hormone levels as well as initial body mass at the beginning of experiments were tested by using a 1-way analysis of variance for the acute- and chronic-risk exposure. Significant effect was further analyzed by using the Bonferroni method for pairwise multiple comparisons. Effect of acute- and chronic-risk exposure on final male body mass was analyzed by analysis of covariance, where initial body mass was used as a covariate. All mean differences were considered statistically significant if $P < 0.05$. All tests were performed by using SPSS version 10.0 (SPSS Inc. 1999).

## Results

*Breeding behavior.*—Strong effects of acute-risk exposure were found on breeding behavior of males (Fig. 1A). Males in the A-Risk trial had lower general activity ($H = 9.66$, $d.f. = 2$, $P = 0.008$), approaching ($H = 11.04$, $d.f. = 2$, $P = 0.004$), and stimulating female behavior ($H = 11.44$, $d.f. = 2$, $P = 0.003$) and higher frequency of avoidance ($H = 8.32$, $d.f. = 2$, $P = 0.016$) than those in the A-Control trial. No significant differences were found in frequencies of any behaviors between A-Safe and A-Control trials (pairwise comparisons of Bonferroni, $P > 0.05$). In the chronic-risk exposure experiment, breeding behavior of males was affected (Fig. 1B). Males in the C-Safe trial had lower frequency of approaching ($U = 26.50$, $n = 23$, $P = 0.017$) and stimulating female behavior ($U = 22.00$, $n = 23$, $P = 0.008$), and higher avoidance ($U = 13.50$, $n = 23$, $P = 0.001$) than those in the C-Control trial.

A significant effect of the acute-risk exposure was found on proportion of males that copulated (Fig. 2; Pearson's $\chi^2 = 8.45$, $d.f. = 3$, $P = 0.015$). The proportion was lower in the A-Risk trial than in the A-Control and A-Safe trial (Fig. 2; A-Risk = 36.4% versus A-Control = 78.9%, Pearson's $\chi^2 = 7.50$, $d.f. = 1$, $P = 0.006$; A-Risk = 36.4% versus A-Safe = 68.8%, Pearson's $\chi^2 = 3.89$, $d.f. = 1$, $P = 0.045$), but no significant difference was found between the A-Safe and A-Control trials (Fig. 2; Pearson's $\chi^2 = 0.47$, $d.f. = 1$, $P = 0.492$). In the chronic-risk exposure experiment (Fig. 2), the proportion of males copulating was significantly reduced in the C-Safe trial as compared to the C-Control trial (C-Safe = 53.8% versus C-Control = 100%, Pearson's $\chi^2 = 6.24$, $d.f. = 1$, $P = 0.012$).

None of the breeding behavior variables for receptive females differed between pairings with males in safe and risk situations (Fig. 3; $U = 32.00$, $n = 18$, $P = 0.450$ for sniffing nose; $U = 32.50$, $n = 18$, $P = 0.478$ for sniffing anogenital area; $U = 29.50$, $n = 18$, $P = 0.330$ for soliciting; $U = 32.00$,



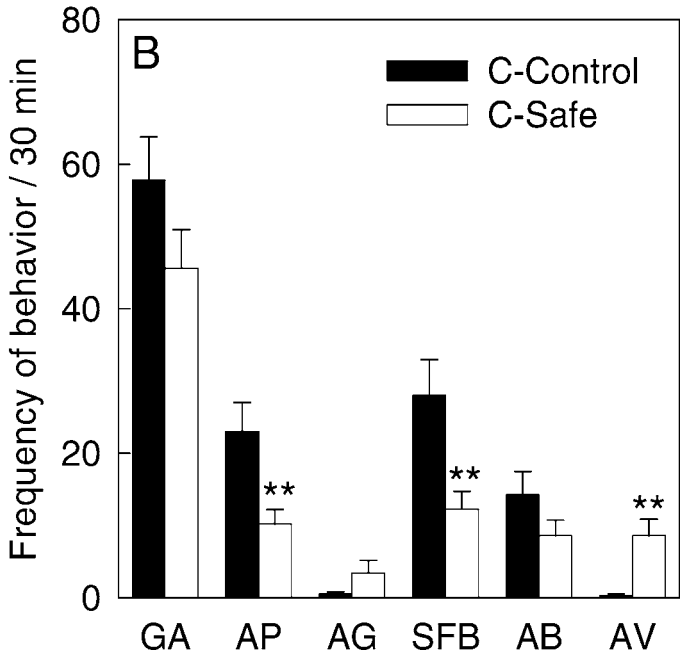




FIG. 1.—Frequencies of breeding behaviors of male root voles for 30 min after A) acute and B) chronic exposure to predator odor, shown as mean + *SE*. A-Safe = behavior tested in brief (1-h) period of safety under control odor after brief (1-h) exposure to predator odor. A-Risk = behavior tested under predator odor after brief (1-h) exposure to the odor. A-Control = behavior tested under control odor after exposure to control odor. C-Safe = behavior tested in brief (1-h) period of safety under control odor after chronic (20-day) exposure to predator odor. C-Control = behavior tested under control odor after chronic exposure to control odor. (Protocols for acute and chronic experiments are described in the text and Table 1.) Behaviors of males: GA = general activity; AP = approach; AG = aggression; SFB = stimulating female behavior; AB = amicable behavior; AV = avoidance. Bars sharing the same letters are statistically equivalent (Bonferroni method for pairwise multiple comparisons based on the rank-transformed data of behavior variables). Asterisks (**) indicate $P < 0.01$.

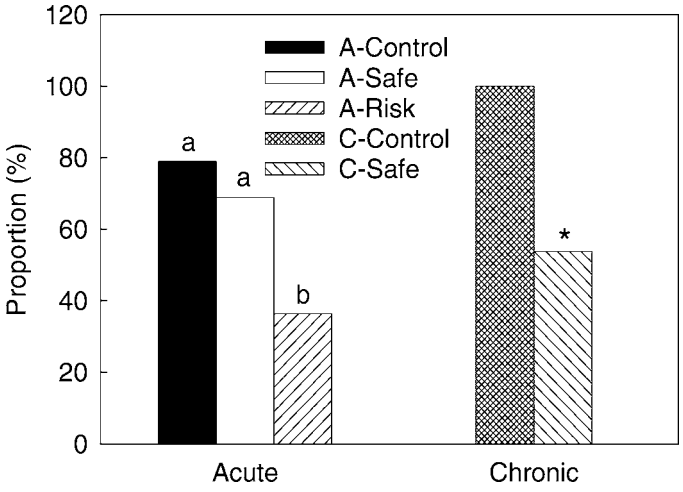



FIG. 2.—Percentages of copulations by males tested with A) acute and B) chronic exposure to predator odor. Abbreviations for groups in experiments are as in Fig. 1 and Table 1. Bars sharing the same letters are statistically equivalent by pairwise comparison with chi-square test. Asterisk (*) indicates $P < 0.05$.

$n = 18$, $P = 0.352$ for wait; $U = 36.00$, $n = 18$, $P = 0.317$ for avoidance; $U = 34.50$, $n = 18$, $P = 0.587$ for threat display).

*Hormones, indices of testis and epididymis, and body mass.*—In the acute-risk exposure experiment, corticosterone concentration of males in the A-Risk trial was significantly increased as compared to the A-Control trial (Fig. 4A; $F = 5.26$, *d.f.* = 2, 27, $P = 0.012$), but testosterone concentration

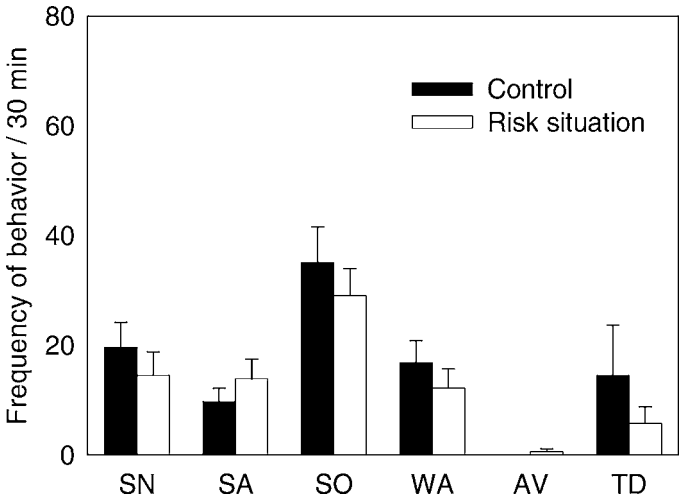



FIG. 3.—Frequencies (over 30 min) of breeding behaviors of receptive female root voles in control (safe) and risk situations, shown as mean + *SE*. Behaviors of females: SN = sniffing nose of males; SA = sniffing anogenital area of males; SO = soliciting; WA = wait; AV = avoiding male; TD = threat display (as described in the text). Differences were not significantly different between experiments for any behaviors.

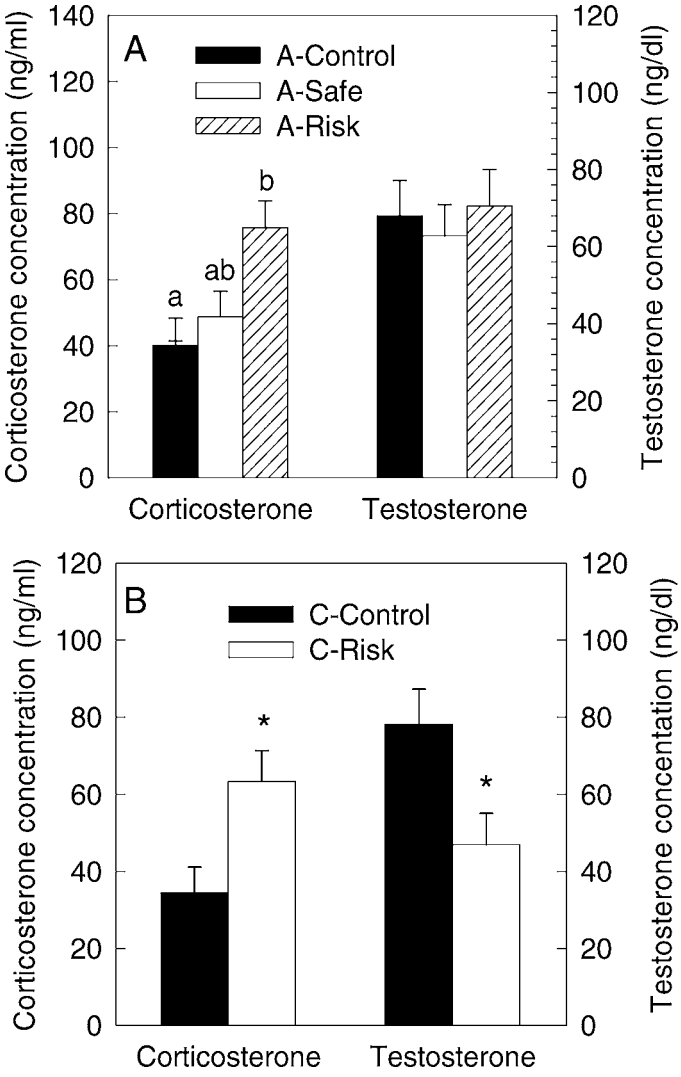



**FIG. 4.**—Concentrations of corticosterone (ng/ml) and testosterone (ng/dl) for A) acute and B) chronic exposure to predator odor in male root voles, shown as mean + *SE*. Abbreviations for experimental groups are as in Fig. 1 and Table 1. Bars sharing the same letters are statistically equivalent (Bonferroni method for pairwise multiple comparisons). Asterisk (*) indicates $P < 0.05$.

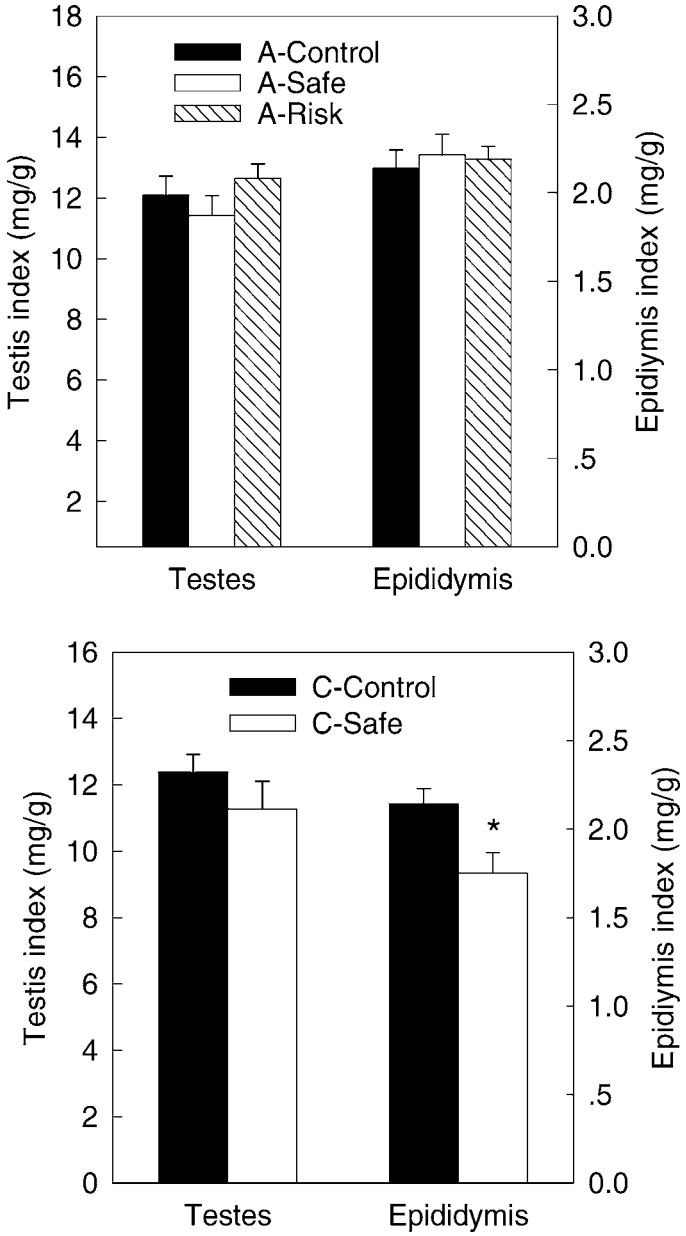



**FIG. 5.**—Testis index and epididymis index: weight of paired testes divided by body weight (mg/g) and weight of right epididymis divided by body weight (mg/g) for A) acute and B) chronic exposure to predator odor in male root voles (mean + *SE*). Abbreviations for experimental groups are as in Fig. 1 and Table 1. Asterisk (*) indicates $P < 0.05$.

($F = 0.20$, *d.f.* = 2, 27, $P = 0.822$) was not affected. In the chronic-risk exposure experiment, males in the C-Safe trial had higher corticosterone concentration and lower testosterone concentration than the C-Control trial (Fig. 4B; $F = 7.62$, *d.f.* = 1, 18, $P = 0.013$ for corticosterone; $F = 6.40$, *d.f.* = 1, 18, $P = 0.021$ for testosterone).

The acute-risk exposure experiment did not affect the testis index (Fig. 5A; $F = 1.07$, *d.f.* = 2, 27, $P = 0.357$) or epididymis index (Fig. 5A; $F = 0.148$, *d.f.* = 2, 27, $P = 0.863$). However, males chronically exposed to predation risk (in the C-Safe trial) had a lower epididymis index than those in the C-Control trial (Fig. 5B, $F = 7.17$, *d.f.* = 1, 18, $P = 0.015$).

Body mass of males did not differ in the beginning between groups for the acute or chronic experiments ($F = 0.19$, *d.f.* = 2, 54, $P = 0.826$ for acute-risk exposure; $F = 0.79$, *d.f.* = 1, 21, $P = 0.384$ for chronic-risk exposure; Table 2). However, for the chronic-risk experiment, body mass of males in the C-Safe trial was significantly reduced by the end of the trial compared to those in the C-Control trial ($F = 5.15$, *d.f.* = 1, 20, $P = 0.034$; Table 3), but for the acute-risk experiment, no significant difference was found in body mass by the end of the trials ($F = 1.18$, *d.f.* = 2, 53, $P = 0.314$; Table 3).

## DISCUSSION

Our results demonstrate that male root voles have different responses to acute and chronic exposure to predation risk. In

**Table 2.**—Body mass of male root voles in acute- and chronic-risk exposure experiments. Conditions of experimental trials are shown in Table 1.

| Trial | Initial body weight ($\bar{X} \pm SE$) | Final body weight ($\bar{X} \pm SE$) |
|---|---|---|
| A-Safe | 39.33 ± 0.81 | 39.49 ± 0.86 |
| A-Risk | 40.12 ± 0.94 | 39.88 ± 1.02 |
| A-Control | 40.05 ± 1.01 | 40.78 ± 1.11 |
| C-Safe | 38.76 ± 1.09 | 35.75 ± 0.96 |
| C-Control | 40.21 ± 1.19 | 38.95 ± 1.09 |

**Table 3.**—Results of analysis of covariance for effect of acute- and chronic-risk exposure on the final weight of male root voles when initial weight is used as a covariate.

| Source of variation | *d.f.* | *MS* | *F* | *P* |
|---|---|---|---|---|
| Acute-risk exposure | | | | |
| Initial weight | 1 | 864.438 | 211.068 | 0.000 |
| Trial | 2 | 4.850 | 1.184 | 0.314 |
| Error | 53 | 4.096 | | |
| Chronic-risk exposure | | | | |
| Initial weight | 1 | 5.929 | 0.485 | 0.494 |
| Trial | 1 | 62.991 | 5.152 | 0.034 |
| Error | 20 | 12.226 | | |

the acute-risk exposure experiment, as predicted, male root voles responded to the acute risk by showing decreases in general activity, approach, stimulating female behavior, and proportion of copulations, and an increase in avoidance of females soliciting them (Figs. 1A and 2A; A-Risk versus A-Control). These changes should contribute to the suppression of breeding behavior of males. Additionally, as predicted, male root voles that had already experienced an acute risk did not suppress breeding behavior when returning to a safe situation (Figs. 1A and 2A; A-Safe versus A-Control). Thus, male root voles have different breeding behavior in response to the presence versus absence of risk in the acute-risk exposure.

In contrast, male root voles that were chronically exposed to predation risk still suppressed breeding behaviors during the brief period of safety (Figs. 1B and 2B; C-Safe versus C-Control). Although we did not test for the behavior under a condition of continuous-risk exposure, it is very possible that male root voles could not breed under the chronic-risk exposure situation. This result thus shows that breeding behavior in chronically stressed males remains continuously suppressed, even during a brief period of safety.

In this study, receptive females under risk did not change their breeding behaviors due to manipulation by injections of estradiol benzoate and progesterone (Fig. 3), which indicates that the results observed in males are not confounded by differences between females. Additionally, because we gently handled voles before and during experiments, we think that potential influences of moving the voles during experiments on behavior was minimized.

Considerable evidence exists that a high level of plasma glucocorticoid caused by chronic stress can directly and indirectly affect secretion of testosterone (Bambino and Hsueh 1981; Charpenet et al. 1981; Rivier and Vale 1984). Boonstra et al. (1998) also reported that chronic exposure to predation risk reduced concentration of circulating testosterone in snowshoe hares (*Lepus americanus*) and inhibited reproduction. Our results in the experiment of chronic-risk exposure support these findings. That is, plasma level of corticosterone was increased significantly and testosterone concentration and epididymis index were decreased significantly in chronically stressed male root voles (Figs. 4B and 5B). This result suggests that the chronic exposure for male vole roots lead to a dysfunction of their hypothalamic–pituitary–gonadal axis by chronically activated hypothalamic–pituitary–adrenal axis, which in turn is responsible for the suppression of breeding behavior in the brief period of safety. Nevertheless, in the acute-risk exposure experiment, although male root voles had high corticosterone levels in the A-Risk trial (Fig. 4A), plasma testosterone concentration and indices of testis and epididymis were not influenced (Figs. 4A and 5A). This result indicates that high corticosterone caused by the acute-risk exposure does not affect the function of the hypothalamic–pituitary–gonadal axis. Retana-Marquez et al. (1998) also found that acute administration of corticosterone did not alter sexual behavior and testosterone concentration of male rats. Suppression of behavior in the A-Risk trial may be related to some neurotransmitters or neuropeptides in the central nervous system (Brotto et al. 1998; Gorzalka et al. 1998; Retana-Marquez et al. 1998; Sirinathsinghji et al. 1983). Thus, male root voles acutely exposed to risk were able to show breeding activity when returned to safety (in A-Safe). These changes in physiological parameters expose a potential physiological mechanism responsible for various responses of breeding behavior to the acute- and chronic-risk exposures.

Early laboratory studies have shown that voles suppress breeding when they perceive high predation risk (Koskela and Ylönen 1995; Mappes and Ylönen 1997; Ylönen 1989; Ylönen et al. 1992; Ylönen and Magnhagen 1992; Ylönen and Ronkainen 1994). However, the picture is not conclusive because most field studies using predator odors have not shown any effect (Jonsson et al. 2000; Mappes et al. 1998; Wolff and Davis-Born 1997). Although the present study only focuses on male root voles, our results can explain the difference in breeding suppression between early laboratory and field studies.

A common experimental protocol in early laboratory studies was to spray predator odor daily in the surrounding vole cages (Mappes et al. 1998; Mappes and Ylönen 1997; Ylönen and Ronkainen 1994) or in cages (Ylönen 1989) during the entire experimental period (10–20 days). Voles in these studies, like the males in our chronic-risk exposure, are confined to cages and cannot escape from risk. Consequently, they may be chronically stressed, which in turn affects their physiological patterns of breeding. Although corticosterone levels were not measured in these laboratory studies, body mass of those voles was decreased by the end of the experiments (Koskela and Ylönen 1995; Mappes et al. 1998; Mappes and Ylönen 1997), as shown by our study (Tables 2 and 3). This result is likely due

to a deleterious effect of chronic stress on individual energy mobilization (e.g., stimulating the release of energy substrates from muscle, fat tissue, and liver, and inhibiting a variety of body processes not required for short-term survival—Boonstra et al. 1998; Johnson et al. 1992). This further supports our speculation above that a chronically stressful condition brought on by the protocol of early laboratory studies might have led to the breeding suppression of those voles. The risk scenario in these studies provides an ecologically relevant assay only if predators are abundant and prey experience continuous risk with a brief period of safety. Accordingly, these early laboratory studies overestimate the effect of predation risk where prey normally experience brief, infrequent exposure to risk in nature.

In field studies that have not found breeding suppression with predator odors, a common experimental protocol is to spray predator odors over large enclosures or plots either twice per week (Jonsson et al. 2000; Wolff and Davis-Born 1997) or daily (Mappes et al. 1998) to simulate a constant high risk in nature. However, this may be an unrealistic protocol. First, voles in the field can avoid predator odor by using burrows and refuges as well as emigrating (Fuelling and Halle 2004; Jonsson et al. 2000; Wolff and Davis-Born 1997). Additionally, the concentration of predator odor may decrease as time elapses because of rainfall and wind. Finally, the habitat has a large heterogeneity, which affects the ability to assess risk levels (Lima and Dill 1990). Thus, voles in these field studies, like males in the A-Safe trial, cannot always be exposed to predation risk and suppress breeding for an entire experimental period. Fuelling and Halle (2004) also have demonstrated that, when the time a predator spends on a particular patch depends on prey density and patch habitats of prey have considerable heterogeneity, voles may not suppress breeding for the entire season, but only for the limited time interval of high predation pressure. Thus, the experimental protocol in these field studies underestimates the effects of predation risk where prey experience continuously high risk in nature.

To our knowledge, we present the 1st experiment applying the risk allocation hypothesis of Lima and Bednekoff (1999) to breeding behavior of mammals. Our study suggests that breeding behavior in a given situation depends on the overall patterns of risk experienced by male root voles and also validates the predictions mentioned above in the introduction. Accordingly, our study supports the risk allocation hypothesis of Lima and Bednekoff (1999). We suggest that investigators consider temporal variation in predation risk when exploring and explaining the breeding response of animals to predation risk. Otherwise, laboratory or field studies may overestimate or underestimate the effect of predation risk on breeding in nature.

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## Literature Cited

Animal Care and Use Committee. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Journal of Mammalogy 79:1416–1431.

Bambino, T. H., and A. J. W. Hsueh. 1981. Direct inhibitory effect of glucocorticoids upon testicular luteinizing hormone receptor and steroidogenesis in vivo and in vitro. Endocrinology 108:2142–2148.

Boonstra, R., D. Hik, G. R. Singleton, and A. Tinnikov. 1998. The impact of predator-induced stress on the snowshoe hare cycle. Ecological Monographs 79:371–394.

Brotto, L. A., B. B. Gorzalka, and L. A. Hanson. 1998. Effects of housing conditions and 5-$HT_{2A}$ activation on male rat sexual behavior. Physiology and Behavior 63:475–479.

Charpenet, G., et al. 1981. Effect of chronic intermittent immobilization stress on rat testicular androgenic function. Endocrinology 109:1254–1258.

Fernandez-Guasti, A., G. Roldan-Roldan, and K. Larsson. 1991. Anxiolytics reverse the acceleration of ejaculation resulting from enforced intercopulatory intervals in rats. Behavioral Neuroscience 105:230–240.

Fernandez-Guasti, A., G. Roldan-Roldan, and A. Saldivar. 1990. Pharamacological manipulation of anxiety and male rat sexual behavior. Pharmacology, Biochemistry, and Behavior 35:263–267.

Fuelling, O., and S. Halle. 2004. Breeding suppression in free-ranging grey-sided voles under the influence of predator odor. Oecologia 138:151–159.

Gorzalka, B. B., L. A. Hanson, and L. A. Brotto. 1998. Chronic stress effects on sexual behavior in male and female rats: mediation by 5-$HT_{2A}$ receptors. Pharmacology, Biochemistry, and Behavior 61:405–412.

Johnson, E., T. C. Kamilaris, G. P. Hrousos, and P. Wold. 1992. Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. Neuroscience and Biobehavioral Reviews 16:115–130.

Jonsson, P., E. Koskela, and T. Mappes. 2000. Does risk of predation by mammalian predators affect the spacing behaviour of rodents? Two large-scale experiments. Oecologia 122:487–492.

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

Lima, S., and P. A. Bednekoff. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. American Zoologist 6:649–659.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68:619–640.

Liu, J. K., J. P. Su, W. Liu, X. Wang, H. Y. Nie, and Y. M. Li. 1994. Effects of food and predation on population density and space behavior of root voles. Pp. 409–420 in Symposium proceedings in commemorating 100th birthday of Professor Zhen Chen (China Zoological Society, eds.) Science and Technology Press, Beijing, China (in Chinese, English abstract).

Magnhagen, C. 1991. Predation risk as a cost of breeding. Trends in Ecology and Evolution 6:183–186.

Mappes, T., E. Koskela, and H. Ylönen. 1998. Breeding suppression in voles under predation risk of small mustelids: laboratory or methodological artifact? Oikos 82:365–369.

Mappes, T., and H. Ylönen. 1997. Reproductive effort of bank vole females in a risky environment. Evolutionary Ecology 11:591–598.

RETANA-MARQUEZ, S., H. BONILLA-JAIME, AND J. VELAZQUEZ-MOCTEZUMA. 1998. Lack of effect of corticosterone administration on male sexual behavior of rats. Physiology and Behavior 63:367–370.

RIVIER, C., AND W. VALE. 1984. Corticotropin-releasing factor acts centrally to inhibit growth hormone secretion in the rat. Endocrinology 114:2409–2411.

RONKAINEN, H., AND H. YLÖNEN. 1994. Behaviour of cyclic bank voles under risk of mustelid predation: do females avoid copulation? Oecologia 97:377–381.

SIH, A., J. KRUPA, AND S. TRAVERS. 1990. An experimental study on the effects of predation risk and feeding regime on the breeding behavior of the water strider. American Zoologist 135:284–290.

SIH, A., R. ZIEMBA, AND K. C. HARDING. 2000. New insights on how temporal variation in predation risk shapes prey behavior. Trends in Ecology and Evolution 15:3–4.

SIRINATHSINGHJI, D. J. S., L. H. REES, J. RIVIER, AND W. VALE. 1983. Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat. Nature 305:232–235.

SPSS INC. 1999. SPSS Base 10.0 user's guide. SPSS Inc., Chicago, Illinois.

STOPKA, P., AND D. W. MACDONALD. 1998. Signal interchange during breeding in the wood mouse (*Apodemus sylvaticus*): the concept of active and passive signaling. Behaviour 135:231–249.

WOLFF, J. O., AND R. DAVIS-BORN. 1997. Response of gray-tailed voles to odors of a mustelid predator: a field test. Oikos 79:543–548.

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

YLÖNEN, H., B. JĘDRZEJEWSKA, W. JĘDRZEJEWSKI, AND J. HEIKKILÄ. 1992. Antipredatory behaviour of *Clethrionomys* voles—"David and Goliath" arms race. Annales Zoologici Fennici 29:207–216.

YLÖNEN, H., AND C. MAGNHAGEN. 1992. Antipredatory behaviour: a "hot spot" of evolutionary ecology of the nineties. Annales Zoologici Fennici 29:179–182.

YLÖNEN, H., AND H. RONKAINEN. 1994. Breeding suppression in the bank vole as antipredatory adaptation in a predictable environment. Evolutionary Ecology 8:658–666.

ZENKER, N., AND D. E. BERNSTEIN. 1958. The estimation of small amounts of corticosterone in rat plasma. Journal of Biological Chemistry 231:695–701.

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