WEASELS’ (*MUSTELA NIVALIS NIVALIS*) PREFERENCE FOR OLFACTORY CUES OF THE VOLE (*CLETHRIONOMYS GLAREOLUS*)

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Abstract. Many studies on life history strategies of small mammals under predation risk are based on assumptions that mammalian predators use scent marking from prey in searching and hunting. This is especially true for small mustelids hunting in the tunnels and cavities of their prey. It is assumed that weasels use the estrous signs of female voles as hunting cues, which exposes such females to a more pronounced risk of predation. We studied the preferences of 57 least weasels (*Mustela nivalis nivalis*) toward odor cues from four different reproductive categories of the bank vole (*Clethrionomys glareolus*). In the first experiment, weasels selected clearly for vole odors over clean bedding in a Y-maze arena. The second experiment demonstrated that there was no difference in weasel preference between estrous and pregnant or lactating females. Thirdly, there was no preference for odors from dominant vs. subordinate males. Finally, we offered weasels a dyadic choice between olfactory signs of six possible combinations of four prey categories: reproductive females, reproductive males, immature females, and immature males. There was no clear preference for any of the categories; only immature males seemed to be preferred less than females of the same age. Our study does not support the hypothesis that the weasel would have clear olfactory preference for some reproductive category of voles. However, in the field, factors like spacing behavior, mobility and acoustic cues of the prey, and conspicuousness of the nest site might affect the behavior of hunting weasels and individual vulnerability of prey.

Key words: bank vole; Clethrionomys glareolus; coevolution; least weasel; Mustela nivalis nivalis; odors; olfactory cues; predation

INTRODUCTION

The continuous survival game between a predator and its prey affects both predator and prey behavior on an ecological and evolutionary time scale. The risk of getting killed and unsuccessful attacks by a predator improve prey behavior to escape predation (Vermeij 1987, Lima and Dill 1990, Lima 1998). In turn the evolutionary arms race improves a predator’s ability to more effectively find and attack its prey.

Mammals are considered to be olfactory communicators (Macdonald and Barrett 1993). Especially in small mammals, cryptic morphology and behavior, and dense habitat, make scent marking the most effective and long lasting means of communication. Voles of boreal habitats actively mark to protect territories and advertise mating quality (Viitala and Hoffmeyer 1985). However, accumulation of odor signals around the nest site might be risky (Banks et al. 2000). Small mustelids, and especially the least weasel (*Mustela nivalis nivalis*), are considered to hunt “with nose” in tunnels and holes in order to encounter the odor signs of their prey (King 1989). These signals might be used both for orientation and searching and as cues for most profitable prey. This information might be especially important for female weasels. Due to their small size, females need to be aware of prey size and the possibility of a successful attack in order to maximize hunting efficiency in their relatively small hunting area, especially when energy demands are high during breeding.

The least weasel is the only “snake-like” predator of the boreal habitats (King 1989), and is the smallest predator specialized in rodent prey in northern Europe (Erlinge 1975). The weasel is the only predator that can effectively use the same world in which its prey lives (i.e., tunnels in the soil and under litter, stones, and tree trunks, and the subnivean space during the snowy winter). Due to its relatively unfavorable body surface or body:volume ratio (Macdonald and Barrett 1993) this tiny predator must be a very efficient hunter, especially in cold climate. Thus we can predict that the weasel has evolved highly specialized hunting behaviors.

Cushing (1984, 1985) showed experimentally that weasels could use differential sexual odors left by deer mice (*Peromyscus maniculatus*) for prey selection. By selecting an estrous female over a nonestrous one, the hunting weasel might get stronger signal for orientation and perhaps also a more profitable meal, including...
pups, than selecting for nonestrous individuals. Cushing's study has been actively cited ever since. Ylönen (1989, 1994) and Ylönen and Ronkainen (1994) introduced the predator-induced breeding suppression hypothesis, which was based on Cushing's findings on selective hunting in weasels and rapidly changing predation risk within and between seasons. They suggested that entering into a breeding condition increases the female's probability of being the object of predation by least weasels. If weasel density and predation risk are high, it might be favorable for the female vole to postpone breeding. This hypothesis has had a number of follow-up experiments on different species with somewhat contradictory results from the laboratory, from enclosures, and from field studies (reviewed by Norr Dahl and Korpimäki 2000, Ylönen 2001). Further, in recent studies it has been shown in male mice (Mus musculus) that active scent marking might increase predation risk, especially for the most actively marking mice (Roberts et al. 2001). Thus we predicted that weasels would select odors of both reproductive males and females over odors of younger, immature individuals.

We conducted a set of experiments to study hunting preference of weasels for different prey categories. We used female and male bank voles of different age and reproductive categories as odor donators for weasel choice tests and asked the following questions. (1) Does the weasel use odor cues from vole prey for its orientation when hunting? (2) Can the weasel distinguish between estrous cues and other reproductive cues in females? We question that estrus, as a short event, could be a target for differentiation in hunting cues, but reproductive odors as a whole could. (3) Does the intense scent marking of dominant males expose them for weasel predation more than subordinate males? (4) Which functional category in a breeding population is most exposed to weasel predation?

**Material and Methods**

We conducted four sets of experiments to test the use of olfactory cues by weasels in hunting, and to test their preference among reproductive cohorts of voles. The study was carried out at the Lammi Biological Station of the University of Helsinki and at the Konnevesi Research Station of the University of Jyväskylä. The experiments were conducted in the laboratory with wild weasels and weasels born in captivity. They were held singly in cages or in outdoor pens and were fed with chicken or occasionally with voles. All bank voles were raised in captivity so their age was known. Depending on the experiment, reproductive females were either pregnant, in postpartum estrus, or lactating. Reproductive males were stud males with scrotal testes. Immature females and males were kept separate in single sex cages after weaning and had no contacts with the opposite sex before the experiments.

First we conducted a set of trials asking if the choice of a weasel in a Y-maze arena was random or followed an odor cue of a vole (Exp. 1). Next we tested to see if the selected cue in females was estrous odor or any odor cue in a reproductive (pregnant or lactating) female (Exp. 2). Thirdly, we tested preference of weasels to odors of males with different dominance status (Exp. 3). Finally, we repeated the dyadic choice test for a group of weasels with four test groups of bank voles of different age and sex (Exp. 4). Each weasel was only used in one set of experiments to avoid habituation and pseudoreplication. In total, 66 weasels were tested and 64 of these weasels (31 females and 33 males) were used in the analyses.

**Experimental arena**

The arena used in all four experiments resembled the Y-maze arena used in Cushing’s (1984, 1985) original study. The arena was formed from three transparent Perspex plastic tubes of 80 mm inside diameter and lengths of 80, 60, and 60 cm, forming a Y (Perspex, Rotterdam, The Netherlands). The weasel entered the tube of 80-cm length and came to a bifurcation of ~60°. There it had to choose to continue into either of the 60-cm tubes until it reached the target “nest box” of a vole at the end of the tube. The nest boxes were small laboratory cages of 25 × 10 × 10 cm covered with a Perspex roof. The ends of the Perspex tubes were separated from the nest box by a Perspex door with 12 holes of 4-mm diameter to allow airflow. A pipe from a fan box placed 1 m away from the nest box was attached at the rear end of each nest box. The fan (12 V, 0.15 A) caused a weak but continuous air current through both nest boxes to the tubes (cf., Lambin and Mathers 1997). In three trials of Exp. 1 and in the male odor preference experiment (Exp. 3) the fan was not used.

A 2 × 10 × 10 cm wire mesh basket was placed in each nest box. During a trial, baskets were filled with vole bedding with stimulus odor and the airflow to the tube and the weasel cage passed through the bedding and carried the odor to the tube through the holes in the Perspex door.

The entrance to the tube was a wooden box, where the weasel was acclimatized for the trial. The box was separated from the tube by a Perspex door that could be opened from outside the experimentation room with a fishing line. The door had holes to allow the airflow from the arena to the weasel box during the acclimatization period.

Above the arena was mounted a video camera that was connected to a monitor in an adjacent room where the behavior of the weasel could be observed on screen.

**Experiment 1**

In the first experiment the olfactory orientation of the weasel was tested. In the Y-maze arena weasels had to choose between clean wood shavings and wood shavings used as vole bedding. This experiment served as a control, testing if the weasel followed the smell
of a vole rather than selecting randomly between the tubes. The voles used were females in breeding condition. The night prior to the experiment these voles were kept in 25 × 10 × 10 cm cages similar to those used in the arenas. The cages had clean bedding of wood shavings.

In this first experiment we used vole odor from either bank voles (Clethrionomys glareolus) or field voles (Microtus agrestis). In the bank vole trial, we used five male and four female weasels, while in the field vole trial six female and six male weasels were used. In this control experiment we used odors from both vole species, voles being the preferred weasel prey, because the test was solely intended to show whether or not the weasels followed an odor cue. Furthermore, if weasels showed a preference for one species over the other, this information was important for another experiment where weasels were hunting free ranged bank voles and field voles under field conditions in outdoor enclosures (Sundell et al. 2003).

After the arena had been prepared for the trial prior to testing (bedding in the nest boxes, weasel in the entrance box, fan turned on in some cases), the weasel was kept in an acclimatization box for 10 min without any disturbance. Then the door to the tube was opened and the weasel was allowed to enter the arena. All weasels slowly approached the tube and selected one of the tubes at the bifurcation. The test ended when the weasel reached the end of the Y-tube and sniffed the holes at the door separating the tube from the nest box. After the trial all parts of the arena were cleaned with water and 70% alcohol and dried.

Experiment 2

In the second experiment we tested whether weasels discriminate odors of estrous females, as suggested in Cushing’s (1984) work, from odors of reproductive females beyond the short estrous period (female either pregnant or lactating). For estrous females we used females in postpartum estrus, one day after delivery of pups. In the other group, females were either visibly pregnant or had pups older than four days in their nest. In total 15 weasels (8 females, 7 males) were tested as described for the Exp. 1.

Experiment 3

In the third experiment we tested if weasels prefer odor from a dominant male bank vole over that of a subordinate male. In this experiment we used 11 weasels (6 females, 5 males). Rozenfeld and Rasmont (1991) and Horne and Ylönen (1996, 1998) showed that dominance between males of a pair could be estimated by means of their urine marking pattern (Horne and Ylönen 1996). Two males were placed in an arena of 60 × 40 cm, with a wall 40 cm high. The arena was separated by a wall with the lower 5 cm made of wire mesh. This was to allow tactile and olfactory contact between the pair of males. Under the arena there was brown packing paper. Males were kept in the arena overnight (12 h) and removed in the morning. A quantitative analysis of the intensity of urine marking along the whole or part of the area yielded a dominance rank for both males (Horne and Ylönen 1996, 1998). We divided each half of the arena in 5 × 5 cm squares, 48 on each side of the arena, and counted the number of squares marked by the male during the night. Males were ranked into one of six classes according to their marking intensity. We used pairs of males in the trial whose urine marking values (UMV = dominance rank) differed by at least three ranks. After the dominance trial the males spent the night in their cage as described in Exp. 1, and the bedding was used in the weasel preference trials the next day.

Experiment 4

In this experiment, instead of comparing only two reproductive categories as in Cushing’s (1984, 1985) work, we tested the preference rank of individual weasels toward odors from four different reproductive and age cohorts. This experiment differs from the others as each weasel had to make six choices instead of one. All weasels used here were different from those used in the other trials to avoid any learning effects. We used as vole odor donators: (1) lactating females (mass 20–36 g) with the pups in the nest; (2) reproductive “stud” males (mass 19–26 g) with large scrotal testes; (3) immature females (mass 15–18 g) with no sexual experience, not in reproductive activity, and with closed vagina; and (4) immature males (mass 15–19 g) with no sexual experience and with small testes.

Each weasel was tested with each possible combination of odors from the four vole groups. Thus there were six trials for each weasel. Maximum rank for the most preferred category given by each weasel was three. No more than two tests with each weasel were carried out during the same day. The order of vole categories was changed randomly for each weasel to avoid possible learning by the weasel during the trials. The odorous bedding of a number of voles of each reproductive category was prepared overnight as in other experiments. Odor samples from the four different vole categories were selected for each day’s trials.

In total, 12 weasels were used. However for the data analysis only 10 (4 females, 6 males) could be used as two weasels did not cooperate through the whole set of six trials; they stayed in the entrance box in the later tests.

Results

Experiment 1: Do weasels follow odor cues of voles?

These control experiments showed that weasels respond to the odor cue coming from either of the tubes. All nine weasels chose the odor stimulus of bank voles over clean wood shavings. This includes three cases where the fan was not used. In the trials with weasel
selecting between field vole odor and clean bedding 10 out of 12 selected the vole odor ($\chi^2 = 5.3, P = 0.021$). Thus in total, 19 weasels out of 21 selected an odor cue over clean bedding ($\chi^2 = 13.7, P < 0.001$; Fig. 1).

**Experiment 2: Are there differences in female reproductive odor signs for hunting weasels?**

There was no difference in the preference of weasels between females in postpartum estrus and females that were either pregnant or lactating. Seven weasels chose the odor of an estrous female and eight chose the odor of a pregnant or lactating female ($\chi^2 = 0.67, P = 0.796$; Fig. 2).

**Experiment 3: Are dominant males more susceptible to weasel predation?**

Weasels did not show any preference between dominant and subordinate males; six chose odor from subordinate males and five chose odor from dominant males ($\chi^2 = 0.09, P = 0.763$; Fig. 2).

**Experiment 4: Preference of the weasel for age and sex groups**

There was no clear difference in weasel preference between four sex and age groups of bank voles: reproductive females, reproductive males, immature females, and immature males (One-way ANOVA $F_{3,39} = 2.79, P = 0.054$). The only tendency was for immature males to be chosen less often than immature females (One-way ANOVA with Tukey’s; difference between immature females and immature males $P = 0.03$, all other comparisons $P > 0.26$; Fig. 3).

**DISCUSSION**

The least weasel is a vole specialist accounting for up to 80–90% vole mortality during cyclic declines of vole populations (Norrdahl and Korpimäki 1995). In his pioneering study on olfactory hunting preference of the weasel, Cushing (1984, 1985) showed that all nine weasels he used chose the odor of estrous females over diestrous ones. The present study is, however, the first to test the assumptions of olfactory hunting preferences of weasels over a broad scale of prey groups and using each weasel only once to avoid pseudoreplication (cf., Derting 1989, Pekkarinen and Heikkilä 1997).

We showed unambiguously that weasels use their olfactory sense in making a choice in preference trials. In experiments examining the preferences of weasels between a vole cue and clean bedding, 19 weasels out of 21 selected the vole odor. However, we did not find any clear preference among reproductive categories of rodents; i.e., that weasels use differential signs of reproduction as hunting cues, which would increase costs of sexual signalling in either females (Cushing 1984,
1985) or males (Roberts et al. 2001). Our results suggest that odors of potential prey are recognized by a hunting weasel and used in searching for prey. In our dyadic choices the odor cues were independent of sex and age.

In the first experiment, we showed that the least weasels used their olfactory sense in choosing a search path. Further, we falsified the assumption that estrus alone would act as a strong evolutionary target for hunting weasels. Estrus is an event of short duration, and it is followed by a gestation period of several weeks before the next estrus can occur after birth of pups in species with postpartum estrus. Our study shows that the least weasel does not distinguish females in estrus from pregnant or lactating females. We suggest that reproductive odors as such can form a cue for any hunting mammalian predator, especially as they tend to accumulate at and near the nest site and make it more conspicuous for olfactory hunting (Banks et al. 2000). Sharpe and Millar (1990) suggested that the accumulation of signs for predators would be the reason for frequent nest relocations in female deer mice (Peromyscus maniculatus). Further, many boreal small mammals are “continuous” breeders; except for the first breeding, estrous events are connected with lactation and followed immediately by a new pregnancy. Thus estrous signs are mixed with state-specific odors of lactation and pregnancy. This, together with being bound to nest and pups, might well make reproductive odors as a whole signs to be used as hunting cues by mammalian predators such as weasels.

We expected also dominant, mobile, and actively marking males to be more attractive to weasels than the subordinate males with less scent marking (Norrdahl and Korpimäki 1998, Roberts et al. 2001). However, we found no evidence for this. Only immature males with undescended and undeveloped testes, and probably small preputial glands, were the least preferred when four age and sex categories were compared. Under field conditions, however, it might well be that it is not the quality of the odor but the quantity of scent marking that increases the risk of predation for actively marking individuals. This was indicated by the study of Roberts et al. (2001), where actively marking mice reduced their marking behavior under simulated predation risk.

We have to ask how well our laboratory study can answer questions on weasel hunting strategies in the field (Ylönen and Wolff 1999, Norrdahl and Korpimäki 2000). In all experiments, we used scent absorbed bedding from an individual vole’s nest box. This does not necessarily mean active marking with social or sexual odors. In particular, male marking might be much more intense in the field with advertisement of sexual competence and own quality (Rozenfeld and Rasmont 1991, Horne and Ylönen 1996). Thus future studies should use the odor left during active marking outside the nest.

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LITERATURE CITED


