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Scent-marking by male mice under the risk of predation

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The use by predators of scent marks made by potential prey is a largely unexplored potential cost of olfactory signaling. Here we investigate how animals that differ in their investment in scent-marking respond to simulated predation risk, by comparing the willingness to approach and counter-mark the scent marks of a competitor in the presence or absence of predator odor. We aimed to test whether animals that invest heavily in scent-marking, and which may thus be more vulnerable to eavesdropping predators, will either (1) take greater risks to counter-mark the competitor's scent or (2) reduce or abandon scent-marking. Using outbred male laboratory mice, *Mus musculus,* we show that, in the absence of predators, individuals which initially scent-mark at high frequency (high markers) approach the competitor's scent marks more quickly and spend more time in counter-marking than those which initially invest relatively little (low markers). In a sib-sib experimental design, simulated presence of predation risk (urine of ferrets, *Mustela putorius furo*) caused both kinds of individual to approach the competitor's marks more slowly, but high markers approached more quickly than low markers and spent more time in the vicinity of the competitor's marks. Only high markers significantly reduced their overmarking of the competitor's scent. These results suggest (1) that there is a unique danger inherent to scent-marking at high frequencies and (2) that high-marking males were prepared to accept increased costs of intrasexual competition in order to reduce the risk of predation. Further tests using the scent of naked mole-rats, *Heterocephalus glaber,* showed that these effects were not elicited simply by an unfamiliar odor. We discuss reasons for the observed difference in response to predation risk between the groups, and the implications of these results for counter-selection on scent-marking strategies. *Key words:* mouse, olfaction, signal cost, status signals. *[Behav Ecol 12:698–705 (2001)]*

Status signals are expected to be costly to signalers if they are reliable, cheat-proof indicators of quality (Grafen, 1990; Zahavi, 1975). The currencies in which costs are expressed vary widely across signal modalities and taxa. An important potential cost of signal emission is that signalers may inadvertently expose their location to eavesdropping parasites and predators (McGregor, 1993; Stoddard, 1999). For example, dipteran parasitoids use cricket song to locate potential larval hosts (Cade, 1975) and vertebrate predators use frog (Tuttle and Ryan, 1981) and insect vocalizations (Bailey and Haythornthwaite, 1998; Belwood and Morris, 1997; Sakaluk and Belwood, 1984) to localize displaying males. Counter-selection on signalers is thought to induce individual variation in response to predation risk (Endler, 1992). Males may follow alternative signaling strategies in this context, such as adopting silent satellite roles (Cade, 1975) or varying signal form (Bailey and Haythornthwaite, 1998; Stoddard, 1999; Zuk et al., 1993). These strategies may help to reduce vulnerability to predation but may also reduce the benefits associated with signaling, particularly success in intrasexual competition or attractiveness to mates (Belwood and Morris, 1997; Burk, 1982; Endler, 1992).

While the use of olfactory social signals to detect prey is less well documented than that of acoustic signals, recent work has shown that some raptors, such as the kestrel (*Falco tinnunculus,* Viitala et al., 1995) and rough-legged buzzard (*Buteo lagopus,* Koivula and Viitala, 1999), use the scent marks of voles as a cue to likely prey density and that they prefer to hunt in areas with high densities of scent marks. However, we are not aware of any study which has examined individual variations in scent-marking behavior in relation to predation risk. As scent-marking, like acoustic signaling, typically functions both in intrasexual competition between males (Brown and Macdonald, 1985; Gosling, 1982, 1990; Gosling and Roberts, 2001) and in advertising male quality to potential mates (Rich and Hurst, 1999), changes in signaling behavior in response to a higher risk of predation should vary between males of different status.

High-quality males usually scent-mark at high frequency, constructing a dense and often complex system of marks within their territory or area of dominance (Gosling, 1981; Roberts and Lowen, 1997). Since these marks may reveal (indeed signal) predictable patterns of behavior and movement, we suggest that high quality territorial or dominant males will be more vulnerable in the presence of predators than males investing relatively little in scent-marking, such as sympatric nonterritorial or subordinate males. We therefore aimed to test two alternative hypotheses that suggest how males that scent-mark at high frequency might respond to the presence of a predator. First, males that adopt a high-marking strategy may be prepared to take relatively greater risks in signaling than low-marking males (cf. Trivers, 1972; Williams, 1966). Second, they might reduce or abandon scent-marking if the benefits of signaling are outweighed by high predation risk (Belwood and Morris, 1997; Zuk et al., 1993).

As there is typically a delay between signal emission and reception in olfactory signaling, the main mechanism through which receivers can positively identify the signaler is thought to be scent-matching, in which competitors or mates match the odor from scent marks with the odor of conspecifics they

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encounter (Gosling, 1982; Gosling and McKay, 1990). It is thus crucial that signalers maintain their scent marks in such a way that maximizes the success of matching (Gosling, 1986; Gosling and Roberts, 2001; Roberts and Lowen, 1997). This is achieved both by replenishing their own scent marks on a regular basis and by counter-marking any scent deposited by competitors within their territory or area of dominance (Rich and Hurst, 1999; Roberts, 1998). In small rodents, males of high status are known to both scent-mark (Desjardins et al., 1973; Hurst, 1990) and counter-mark to a greater extent than individuals of lower status (Hurst, 1990; Johnson, 1973). These trends increase success in maintaining status (Gosling and McKay, 1990; Gosling et al., 2000), deter challenges from poor quality individuals (Gosling et al., 1996a, 1996b; Hurst et al., 1994) and enhance the signaler's attractiveness to mates (Johnston et al., 1997b; Rich and Hurst, 1999).

In this article, we examine how animals of different quality, varying only in the amount they invest in scent-marking, respond to the risk of predation. We present results from an experiment in which we simulate territorial intrusions by transferring between cages the scent marks of male laboratory mice, *Mus musculus,* and test the willingness of males to counter-mark the intruder's scent, either in the presence or absence of the scent of a predator (ferret, *Mustela putorius furo*). We interpret the results in the light of the two proposed hypotheses for the response of individuals at relatively higher risk and discuss the implications of these findings for ideas about the evolution of olfactory signals.

METHODS

Husbandry and techniques

We used an outbred strain of laboratory mice (TO, purchased from B&K Universal Ltd., Hull, UK), in which there is large variation in scent-marking rates between individuals and where this variation arises early in ontogeny (Collins et al., 1996; Gosling et al., 2000). While the use of laboratory mice in an experiment to investigate predation risk might be criticized on the grounds that subjects would not have prior experience of predators, preliminary observations suggested that, at least in this outbred strain, mice responded adaptively: when two tubes (one clean, one containing predator odor) were presented simultaneously, mice avoided the one containing predator scent but freely entered the clean tube. We established breeding pairs which were housed in MB1 cages (45 × 28 × 13 cm) and fed *ad libitum* breeder diet RM3 and water, under a reversed 12:12 light:dark cycle at constant temperature (21 ± 2°C). During the dark phase of the cycle, red lights were used to allow observation without disrupting mouse activity cycles. Sires were removed two weeks after pairing. Twenty litters were obtained, born within a 5-day span. At age 3 days, each litter was culled to a 3:2 M:F sex ratio to control for potential influences of social environment on subsequent competitive strategies (Collins et al., 1997; Mendl and Paul, 1990). Litters were weaned at 3 weeks of age and female sibs were removed. After weaning, males were housed in sibling groups (MB1 cages, maintenance diet RM1) for a further 2 weeks, during which dominance relationships were assessed. We first used two perforated translucent plastic (perspex) dividers to separate the three sibs within their cage for 30 min. Litters were then videotaped from above for 15 min after removal of the dividers, which stimulated agonistic interactions and facilitated the assessment of relationships (Brain and Kamal, 1989). We recorded fighting behavior, biting, persistent following of one individual by another, and submissive or defensive posturing. Dominance status was attributed on the basis of the frequency with which individuals displayed these behaviors to their siblings and the outcome of such interactions.

At 5 weeks of age, the three male sibs were isolated and, during weeks 6 and 8, scent-marking rates of all males were measured. A plastic block (5 × 5 × 4 cm) covered by clean Benchkote absorbent paper was placed in the cage for 1 h. Mice regularly scent-mark novel objects in their environment, enabling us to assess individual variation in the intensity of scent-marking. The 1-h test period has been found to be an appropriate measure of scent-marking variation between individuals while minimizing the degree of overlap between successive deposits (Collins et al., 1997; Gosling et al., 2000). We used ninhydrin to stain the scent marks deposited by males on the paper, and quantified the percent cover of the marks using an overlaid transparent grid. These data were arcsine-transformed before analysis (Sokal and Rohlf, 1995). Mean litter marking rates were calculated and litters were divided into two groups based on these means (10 litters which marked at high rates and 10 which marked at low rates). The marking rate of the high-marking group (mean 34%, range 23–43) was almost three times higher than that of the low-markers (mean 12%, range 4–21; $t_{18} = 8.21$, $p < .001$). We used litter means (the mean of the three sibs' marking rates) because there is typically significantly more variation between than within litters (Collins et al., 1997, unpublished data), and because of our sib-sib experimental design. We divided mice into treatment groups on the basis of their scent-marking rates within litters, rather than dominance status, because our interest was in the response to predation risk specifically in the context of levels of signal investment. In addition, we controlled for dominance status within litters by allocating equal numbers of dominants and subordinates to each group (sibs of intermediate status from each litter were set aside for control trials using mole-rat scent, see below) and by housing males in isolation for 6 weeks before carrying out this experiment. Isolated male mice are known to behave similarly to dominant males (Bronson and Marsden, 1973; Corridi et al., 1993; Parmigiani et al., 1981). We later checked whether these measures had worked, by testing for residual effects of within-litter status on responses to intruder and predator scent, but we found no significant effects ($p > .1$ in all comparisons).

Experimental design

Each mouse was transferred to its experimental cage 1 week before observations began, when the mice were 12 weeks old. These were large plastic cages (RC1, 56 × 38 × 18 cm) with a plywood inner framework of walls that divided the cage into two sections (Figure 1). One section contained a wooden nestbox and allowed access to food and water, while the other comprised an open area in which two scent-marking blocks were fixed in position (termed the experimental section). The dividing wall between the two sections was pierced by two small holes to allow olfactory contact between sections. Direct access between sections was possible only through a 15 cm transparent plastic tunnel (5 cm in diameter); a plywood wall running alongside the tunnel prevented mice from viewing the experimental section until they emerged from it.

In each of the trials, mice were placed in the nestbox while its two scent-marking blocks were removed and replaced by one clean block and one previously marked by an unfamiliar, ''donor'' male (additional trials were run on the day before observation, without any introduced scent, in order to familiarize males with replacement of the blocks). Donor males were 2 weeks older than the subjects and were bought directly from the supplier at 5 weeks old and housed singly (M2 cages, 20 × 10 × 13 cm). Of 85 potential donors whose scent-marking rates were measured, the half (42 males) which marked

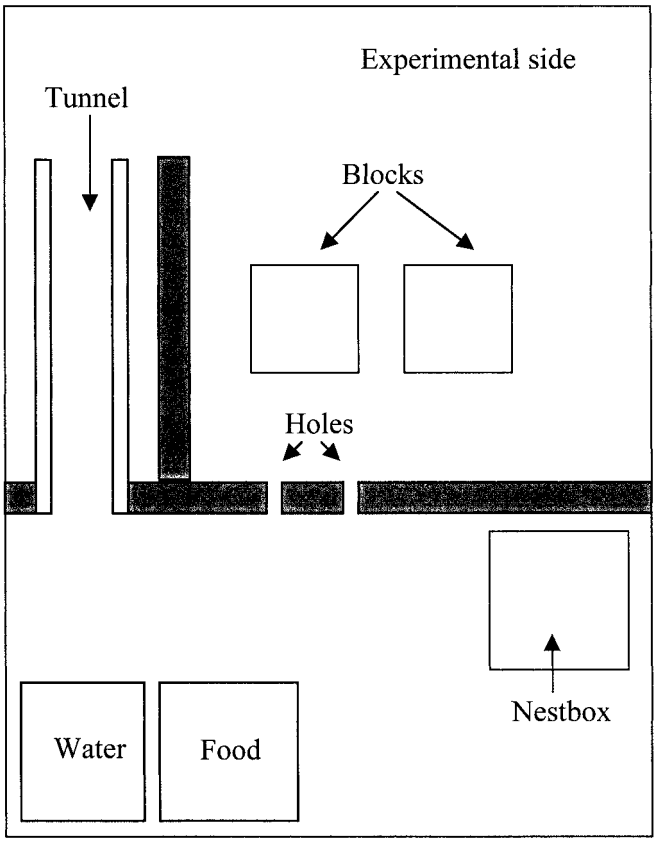

Figure 1
Diagram of the experimental set-up. Shaded areas denote vertical walls. Plastic scent-marking blocks were held in place by means of a drilled hole on the underside and a wooden dowel attached to the substrate. One of the blocks had been scent-marked by an unfamiliar male. Holes were drilled through wall to aid detection of these scent marks by the subject from the nest-box area.

at the highest rates were selected for use in the trials, to ensure that simulated intruders were of high quality and that the introduced blocks were comprehensively scent-marked. Scent marks were collected from donors by introducing scent-marking blocks into their cages for 1 h. They were collected 24 h before use, frozen overnight at −20°C in sealed plastic bags and placed at room temperature for 30 min before use. This procedure minimized the loss of volatile components of the scent marks and its possible effects on subject behavior (Roberts, 1998). Blocks were observed under ultra-violet light before use, to confirm that they had been scent-marked by the donor. The respective positions in the cage of the clean and competitor-marked blocks were alternated between trials.

In addition to the competitor's scent, half the mice in each of the high- and low-marking groups (one sib from each sib pair) were exposed to the odor of a potential predator. Fresh sawdust (replaced daily) from the floor of an open-plan enclosure containing five ferrets was collected on the day of the trials. A standard volume (100 ml) was placed inside the tunnel and sprinkled around the substrate of the experimental section at the same time as the blocks were replaced. To ensure that this was a novel odor, trials in which ferret scent was used were carried out in a different room from those with only competitor scent, and mice were brought into the room immediately before the trial began. Using this sib-sib design, 10 males received only competitor scent and 10 received both competitor and predator scents, in each of the high and low-marking groups.

Trials began when the nest-box door was removed. Individuals were then observed for the following 30 min. During this time, the latency to passing through the tunnel and time spent in the experimental section were recorded (one low-marker did not pass through the tunnel when exposed to both competitor and predator odor). In addition, the use of the scent-marking blocks was noted, including the latency to visit each of the blocks, and the scent-marking rate and time spent on them (the last two measures were divided by the time spent in the experimental section, to control for time spent elsewhere). Comparisons between the time spent on each of these blocks indicated the degree of interest in the competitor's scent.

Scent-marking rates were measured in two ways. First, direct measurement was taken by scoring the percent coverage on the clean block, using the same methods as previously described. This measured the amount that males marked in the immediate vicinity of the introduced marks (counter-marking, Johnston et al., 1997b; Rich and Hurst, 1999). Second, time spent on the competitor-marked block was used to estimate the amount of over-marking of the competitor's scent by the subject. This could not be determined directly, as it is known from other studies that rodents often place scent marks on top of, or overlapping, competitor's marks (Ferkin, 1999; Johnston et al., 1997a). We verified that this was a reasonable surrogate measure of direct overmarking rate by the following analyses. First, we compared the amount of time spent on clean blocks with the resulting direct measure of marking rate on the same block during the familiarization trial, in which neither competitor nor predator odor was present. Time spent on the block was found to be positively related to the measurement of scent-marking rate (Pearson $r = .377$, $n = 40$, $p = .018$). Second, and more importantly, the same relationship was found to be much stronger during experimental conditions, when odors of both competitor and predator were present ($r = .643$, $n = 19$, $p < .01$). Last, we also found a positive relationship between the time spent on the competitor-marked block and the scent-marking rate as measured on the clean block (Figure 2; $r = .782$, $n = 19$, $p < .001$). Here, we present both measures of the amount of counter-marking in our analyses in order to compare the response to the competitor's scent between the two groups of mice in the presence or absence of predator scent.

To ensure that the recorded responses were not simply due to the introduction of an unfamiliar scent, we conducted further trials in which the third male sibling (of intermediate status, not used above) was exposed to the scent of the naked mole-rat, *Heterocephalus glaber,* rather than the predator. In addition to the competitor's scent, the mole-rat scent was introduced in the form of soiled sawdust and scattered in the tunnel and floor of the cage in the same way as the predator scent. The design was identical in all other respects to the trials described above.

Analysis

Data were recorded using Psion Organisers (LZ64) and Observer for Windows v.3, and analyzed using SPSS v.7 for Windows. Data were checked for normality before analysis. Measures of latency were not normally distributed and we thus used nonparametric tests to compare latencies between sibs (Wilcoxon signed ranks tests) and between high- and low-marking groups (Mann-Whitney U tests). In other comparisons, we used either paired or independent t tests. We used Levene's test for equality of variance before carrying out t tests and where these are unequal the degrees of freedom were adjusted accordingly; outliers (more than two standard deviations from the mean) were discounted. Scent-marking rates

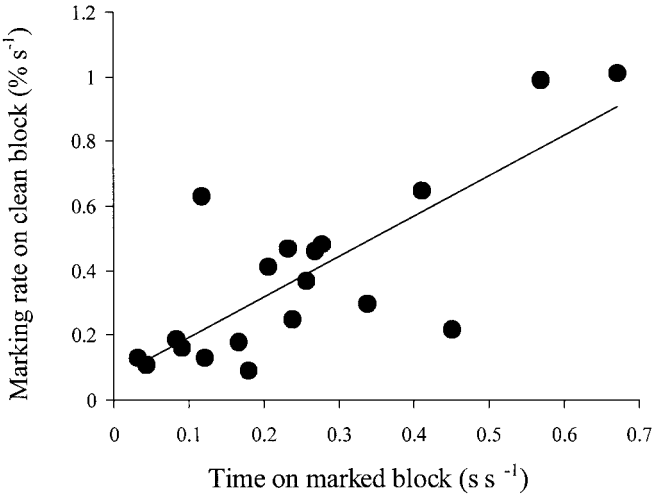

Figure 2
Relationship between time spent on the competitor-marked block and scent-marking rate on the clean block. The relationship is highly significant ($r = .782$, $n = 19$, $p < .001$) suggesting that time spent on the marked block is strongly indicative of the individual's marking rate on this block. The data come from mice which were exposed to both competitor and predator scent in the experiment described. Both variables are divided by time spent on the experimental side of the apparatus in order to control for time spent elsewhere.

(which were skewed towards zero) and other proportional data were arcsine-transformed before analysis. All tests are two-tailed.

Ethical note

Following Huntingford (1984), predation risk was simulated using predator scent in preference to using live predators. Pilot experiments were carried out before data recording began to ensure that mice were able to move freely throughout the cage and use the feeding and drinking stations in the presence of the predator scent. During determination of dominance relationships, litters were observed during videotaping to check for injuries and mice would have been separated if injuries occurred (within-litter aggression typically occurs at low levels compared to adult mice). However, no injuries were seen. Cardboard tunnels were provided to offer routes of escape from dominant sibs and for environmental enrichment.

RESULTS

Effects of predator odor on mouse movement

Presence of predator odor influenced the response of male mice to the competitor's odor in a number of ways. In both high- (Wilcoxon signed ranks test, $T = 0$, $n = 10$, $p < .01$) and low-marking subjects ($T = 0$, $n = 10$, $p < .01$), males which encountered predator odor took longer to pass through the tunnel than their sibs which did not encounter predator odor (Figure 3a). In addition, low-marking mice spent less time than their sibs beyond the tunnel (on the experimental side of the arena) when they encountered predator scent (Figure 3b; paired t test, $t_9 = 5.04$, $p = .001$). However, there was no comparable difference among high-marking males ($t_7 = 0.612$, ns). Comparing between groups, high-markers passed more quickly through the tunnel than low-markers, both when predator scent was present (Mann-Whitney test, $U = 18$, $n_1 = n_2 = 10$, $p < .02$) and absent ($U = 14$, $n_1 = n_2 = 10$, $p < .01$). High- and low-marking males spent similar amounts of time (around 80%) on the experimental side when predator odor was absent (independent samples t test, $t_{18} = 1.96$, ns). When it was present, however, high-markers spent more time there than low-markers ($t_{18} = 3.19$, $p < .01$).

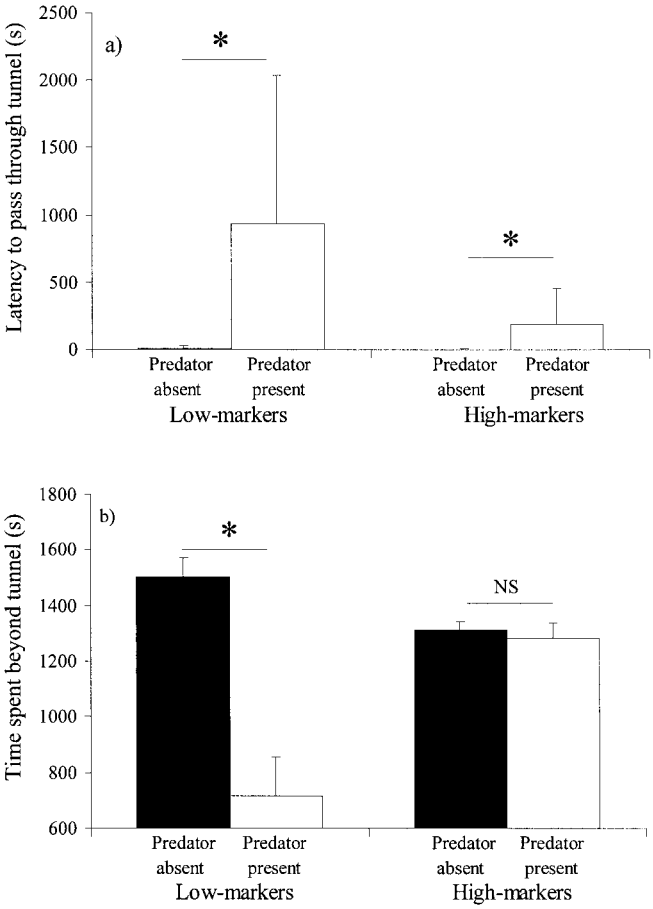

Figure 3
Latency to the first journey through the tunnel (a) and time spent beyond the tunnel on the experimental side of the arena (b) in high- and low-marking mice. Both groups took longer to pass through the tunnel when predator scent was present than when it was absent. Unlike low-markers, presence of predator scent did not affect the time spent by high-markers on the experimental side, where the scent marks of a competitor were present. Latency data are medians + interquartile range, paired Wilcoxon tests; data on time spent are means + SE, paired t tests; *$p < .01$.

Overmarking the competitor's scent

Both high- ($T = 2$, $n = 9$, $p < .02$) and low-marking ($T = 0$, $n = 10$, $p < .01$) males which encountered predator scent took longer to visit the competitor-marked block for the first time than their sibs which did not encounter predator scent (Figure 4a). Comparing between groups, high-markers were always quicker than low-markers to visit the marked block, whether predator scent was absent ($U = 8$, $n_1 = n_2 = 10$, $p = .001$) or present ($U = 17$, $n_1 = 9$, $n_2 = 10$, $p < .05$).

The presence of predator scent did not influence the amount of time that low-markers spent on the competitor-marked block (Figure 4b; $t_8 = 0.002$, ns). In contrast, high-markers that encountered predator scent spent significantly less time on the competitor-marked block than their sibs that did not encounter predator scent ($t_9 = 5.03$, $p = .001$). These

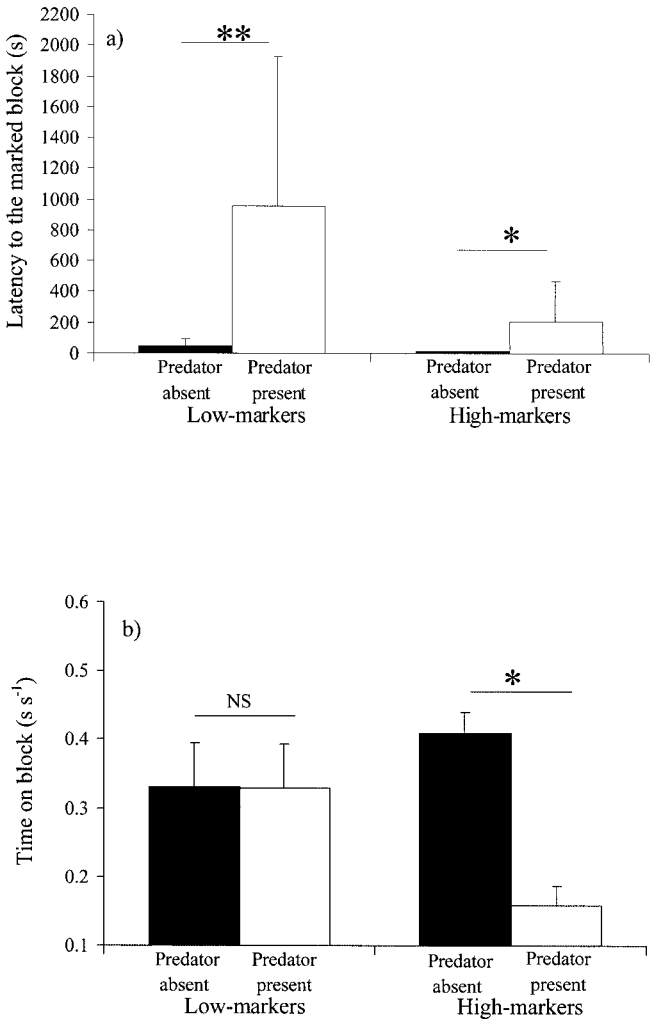

Figure 4
Latency to the first visit to the competitor-marked block (a) and time spent on it (b) in high and low-marking mice. Both groups were slower to visit the competitor's marks in the presence of predator scent. In the presence of predator scent, high-markers, but not low-markers, spent less time on the marked block than their sibs that did not encounter predator scent. Time spent on the competitor-marked block is divided by time spent in the experimental section, to control for differences in time spent elsewhere. Data are (a) medians + interquartile range, paired Wilcoxon tests; (b) means + SE; paired *t* tests; $*p < .05$; $**p < .01$.

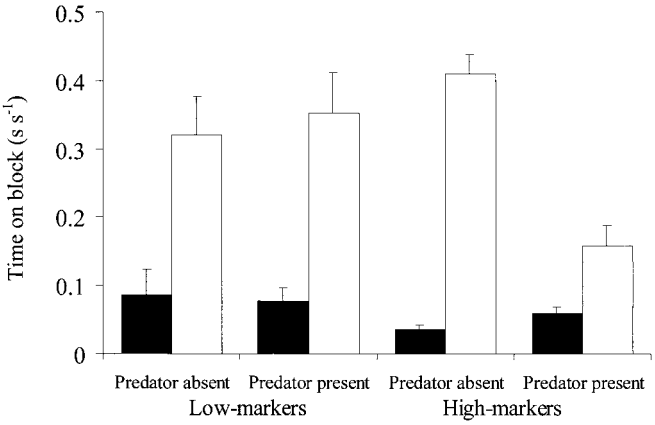

Figure 5
Time spent on the clean and competitor-marked blocks when predator odor was present or absent. In either condition, both high and low-marking mice spent more time on the marked (open bars) than the clean block (filled bars; paired *t* tests, $p < .01$). Time spent on the blocks was divided by time spent on the experimental side of the arena, to control for time spent elsewhere. Data are means + SE.

results indicate that the presence of predator scent caused high-markers, but not low-markers, to reduce their investment in overmarking. As a result of this difference in response, high-markers spent significantly less time on the competitor-marked block than low-markers in the presence of predation risk ($t_{11.54} = 2.77$, $p < .02$), even though there was no difference between the two groups when predator scent was absent ($t_{18} = 1.40$, ns).

Scent-marking on the clean block

Patterns in the amount of time spent on the clean block were different from those on the marked block, but, since mice spent (on average) six times longer on the marked block (Figure 5), clean block marking rates are less representative of the response to the two stimuli presented. Mice spent less time on the clean block both when predator odor was present (Figure 5; high-markers, $t_9 = 5.02$, $p = .001$; low-markers, $t_8 = 3.93$, $p < .01$) and absent (high-markers, $t_9 = 16.91$, $p < .0001$; low-markers, $t_9 = 4.40$, $p < .01$). Despite this, the clean block enabled direct measurement of scent-marking rates during the experiment. Low-marking mice that encountered predator scent marked at higher rates on the clean block than their sibs that did not encounter predator scent (Table 1). In contrast, the presence of predator scent did not change marking rates of the high-marking sibs on the clean block. There was no difference between the high- and low-marking groups in the total amount of marking recorded on the clean block dur-

Table 1
Scent-marking rates on the clean block

	Predator absent	Predator present	*t*	df	*p*
Low-markers	0.0058 ± 0.0022	0.019 ± 0.0049	3.14	7	<.02
High-markers	0.012 ± 0.0022	0.0093 ± 0.0029	0.71	9	NS

Low-marking mice exposed to predator odor scent-marked at higher rates than their sibs that were not, but there was no difference in high-markers (paired *t* tests, * = $p < .05$). Marking rates were divided by time spent on the experimental side to control for time spent elsewhere. Data are means + SE; NS, not significant.

Table 2
Response to an unfamiliar, non-predator scent

Variable	Mole-rat scent	Predator absent			Predator present		
	Means ± SE	Means ± SE	*t*	*p*	Means ± SE	*t*	*p*
High-markers							
Time on experimental side (s)	1371 ± 80.5	1352 ± 91.1	0.28	NS	1133 ± 103.4	3.93	<.02
Marking rate on unmarked block (%)	27.6 ± 3.9	22.0 ± 4.0	1.15	NS	4.2 ± 2.0	4.98	<.01
Time on marked block (s)	277.0 ± 19.9	509.6 ± 80.8	2.43	NS	141.0 ± 27.4	−4.5	<.02
Low-markers							
Time on experimental side (s)	1239 ± 120.5	1536 ± 128.8	1.47	NS	785 ± 245.9	2.75	=.05
Marking rate on unmarked block (%)	16.0 ± 7.3	12.0 ± 4.2	0.39	NS	5.20 ± 1.9	1.58	NS
Time on marked block (s)	237.2 ± 56.7	383.0 ± 129.8	1.18	NS	215.4 ± 74.5	−0.21	NS

The scent used was urine from the naked mole-rat, *Heterocephalus glaber.* Ten males from each group were exposed to mole-rat scent and are compared with five siblings which encountered predator scent and five which did not, using paired *t* tests; NS, not significant.

ing the experiment (low-markers, 10.7 ± 2.5%, high-markers, 13.3 ± 2.3%; $t_{18} = 1.06$, ns), but differences became apparent when we controlled for the amount of time spent near the blocks, on the experimental side of the arena. As expected, high-markers marked at higher rates on the clean block than low-markers, at least in the absence of predator scent ($t_{17} = 2.17$, $p < .05$). However, the fact that the two groups responded differently to predator scent produced the somewhat surprising result that, under the unusual conditions of predator scent being present, low-markers in fact marked at higher rates on the clean block than high-markers ($t_{17} = 2.27$, $p < .05$).

Effects of unfamiliar, nonpredator scent

The response of mice to the presence of unfamiliar mole-rat scent, compared with the presence or absence of predator scent in the above trials, is shown in Table 2. When mole-rat scent was present, mice behaved more similarly to their sibs that did not encounter predator scent than to those that did. Thus males that encountered mole-rat scent behaved similarly to their sibs that did not encounter predator scent in terms of time spent beyond the tunnel, marking rate on the clean block or time spent on the competitor-marked block (in either high- or low-marking groups).

In contrast, males that encountered mole-rat scent behaved differently, in several ways, to their sibs that encountered predator scent (at least in high-markers). Thus high-markers that encountered mole-rat scent spent more time on the experimental side and on the competitor-marked block, and they scent-marked at a higher rate on the clean block, than their sibs that were exposed to predator odor. Latency data for high and low-markers were pooled to satisfy sample size requirements of Wilcoxon tests. Males exposed to mole-rat scent were slower to pass through the tunnel ($T = 8$, $n = 10$ pairs, $p < 0.05$) and to visit the marked block ($T = 8$, $n = 10$, $p < .05$) than their sibs that did not encounter predator scent. These effects were the only indications that mole-rat scent influenced subject behavior. Nonetheless, these delays in passing through the tunnel and reaching the marked block were far smaller when compared with sibs exposed to predator scent (tunnel: $T = 0$, $n = 10$ pairs, $p < .01$; block, $T = 1$, $n_1 = 10$, $n_2 = 9$, $p < .01$).

Taken together, these results suggest that mole-rat scent was perceived as being far less threatening than the predator scent, despite the two scents being equally unfamiliar. Furthermore, there was no indication that mole-rat scent affected behavior in any way other than initial investigation of the odor; notably (unlike the predator odor), it had no effect on subsequent levels of scent-marking.

DISCUSSION

Several studies have demonstrated effects on foraging (Epple et al., 1993; Jedrzejewski et al., 1993; Kotler et al., 1992), spacing (Abramsky et al., 1996), avoidance (e.g., Gorman, 1984; Jedrzejewski et al., 1993; Robinson, 1990; Stoddart, 1976) and defensive (Hendrie et al., 1998) behavior in rodents exposed to the scent of a predator, but none have investigated effects on scent-marking. Here we have shown that animals that differ in investment in scent-marking, a conspicuous status signaling behavior, also differ in their response to a perceived increase in predation risk.

Comparisons between the response of mice to the presence or absence of predator scent and the unfamiliar but benign scent of the naked mole-rat showed that subjects differentiated between the two unfamiliar scents and responded much more strongly to the predator scent. Similar discrimination has been found in meadow voles (*Microtus pennsylvanicus*) in tests between odor of weasels (*Mustela erminea*) and guinea pigs (*Cavia porcellus*; Parsons and Bondrup-Nielsen, 1995), and in bank voles (*Clethrionomys glareolus*) between several mustelid predators and rabbits (*Oryctolagus cuniculus*; Jedrzejewski et al., 1993). There would therefore appear to be some qualitative component of the ferret scent that produces the significant aversive response, rather than a reaction to unfamiliar smells per se. Indeed, this type of fine-tuned identification of predators by rodents is not restricted to their scent: several species exhibit particular responses to owl calls but not to the same calls played backwards (Hendrie et al., 1998). These effects must point to innate recognition of the predator odor or its specific properties, since our subjects had not been exposed to this scent before (nor, presumably, had their ancestors of many previous generations). A similar, apparently innate, response has recently been reported in water voles, *Arvicola terrestris,* to the odor of American mink, *Mustela vison* (Baretto and Macdonald, 1999). While such apparently adaptive responses might have disappeared during domestication, this process cannot have produced them.

Both high- and low-frequency scent-marking males were slower to respond to a simulated territorial intrusion by a competitor (approaching the competitor's scent marks) in the presence of predator scent than siblings that were not exposed to predator scent. However, only high-marking males under predation risk reduced the amount of time they spent overmarking the intruder's scent. This appears to support our

second hypothesis for the response of high-frequency signaling males: that the potential costs of predation cause these males to reduce their rates of scent-marking.

The hypothesis that high-markers would take greater risks in signaling than low-markers is not supported, as low-markers in fact increase their signaling investment in the presence of predation risk when high-markers reduce it. Aside from scent-marking itself, however, high-markers were generally more apt to take risks than low-markers, being much quicker to pass through the tunnel and approach the competitor's marks for the first time and spending more time in the vicinity of this simulated intruder's scent (in the experimental section). They thus entered more readily and spent more time in areas of the cage that contained predator scent. Although in our experimental design there was no possibility of the individuals meeting, each of these behaviors would otherwise tend to increase the chances of the subject encountering and challenging the intruder. High-marking males therefore continue to behave as we would expect of territorial or dominant individuals of high competitive ability, with the exception of behavior specifically associated with counter-marking. These findings suggest that there is a unique danger inherent to scent-marking for males which typically mark at high frequencies. If predators do make use of variable densities of scent marks as a cue to likely prey density and foraging success rates (cf. Viitala et al., 1995), then high-marking individuals will become more vulnerable to predation than those which mark their territory or range at lower frequencies. The benefits to reduction in marking rate are therefore relatively larger to high- than low-frequency markers and our observation that high- but not low-markers reduce their level of counter-marking supports this idea.

Our finding that low-marking males scent-marked at higher rates when they encountered predator odor was unexpected, but intriguing. More information is needed about the social strategies of low-markers to fully explain why they did not decrease their levels of investment in a way similar to that observed in high-markers. However, one explanation is that if scent-marking by high-quality animals helps to maintain dominance, but this effect is diminished under predation risk because they reduce signaling rates, then predation risk may cause low-markers to opportunistically increase scent-marking investment. This would enhance their relative competitiveness, and the likelihood of their achieving higher status, at a critical time. In the current context, this would be adaptive if there is competition for refuge sites, as is known to be the case in wild rodents, in which dominants have more ready access to safer habitat sub-types (Koivunen et al., 1998; see also Gray and Hurst, 1997). Low-markers may thus increase their signaling effort in order to have a better chance of securing such access. Alternatively, they may adopt a different mating strategy from high-markers which makes them less susceptible to predation, perhaps varying in the extent to which they patrol territories, challenge competitors or search widely for mates.

This study has shown that males of different quality vary in their response to predator odors and that, as in acoustic signaling systems, predation risk may cause counter-selection on olfactory signaling behavior. Our observations, in a closely-controlled model system, suggest useful avenues for investigation in both captive and wild contexts. Further study is required to investigate the consequences of differential scent-marking responses on male fitness, particularly in view of the possibly different mating strategies of high and low-markers. In addition, we need to determine the time period over which the described effects are sustained. It may be that they are essentially short-term responses to the threat of elevated predation risk. On the other hand, the reduction in the large difference between marking rates of high- and low-quality individuals might represent a predator-induced shift in individual behavior, towards more continuous levels of marking which may have been expressed ancestrally in a more natural, predator-rich environment. In either case, the results demonstrate the potential of predation risk as a powerful agent of counter-selection on olfactory signaling behavior.

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