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INDUCTION OF MARKING BEHAVIOR IN WILD RED FOXES (*Vulpes vulpes* L.) BY SYNTHETIC URINARY CONSTITUENTS

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Abstract—A control aqueous solution containing alcohol and polyethylene glycol, and a test solution with the addition of a mixture of eight volatile synthetic compounds identified in red fox urine, were alternately placed on man-made mounds of fresh snow during January and February, the foxes' courtship season. The foxes preferentially marked those mounds treated with the test solution in the two experimental areas ($P < 0.05$ and <0.0001). It is concluded that one or more of the volatile substances induces mound marking in this species.

Key Words—Red fox, *Vulpes vulpes*, volatile urinary compounds, marking behavior, Δ^3-isopentenyl methyl sulfide.

INTRODUCTION

The red fox is the only small wild canid found in northeastern Maine. The arctic fox (*Alopex lagopus*) occurs much further to the north in Quebec, while the grey fox (*Urocyon cinereoargenteus*) is limited to southwestern portions of Maine but occurs throughout the rest of New England (Burt and Grossenheider, 1976; Montague, 1975).

During winter both male and female foxes, when traveling, may urinate an average of every 30–40 meters on small raised objects (Montague, 1975; Rome, 1978). In the case of three foxes followed, each marking seven or more times, the average distance between marks was 63, 33, and 44 meters with a range of 15–130 meters. At this time of year the urine has a strong skunk-like odor and when fresh is straw-colored but darkens within a few hours to an orange brown (Jorgenson et al., 1978). The absence of other foxes and the

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local leash law for domestic dogs have greatly reduced the chances of incorrect identification of red fox tracks. We have previously exploited these conditions to collect fresh frozen samples of red fox urine and have identified eight odorous volatile compounds (Jorgenson et al., 1978). The major, but previously unknown, sulfur compound, Δ^3-isopentenyl methyl sulfide, has been synthesized (Wilson et al., 1978).

In this report we describe a test situation devised to examine the effect of a solution containing the eight synthetic volatile compounds on the marking activity of wild red foxes. We also present data from several trials in which we obtained positive results.

METHODS AND MATERIALS

It was difficult to test the significance of synthetic compounds under natural conditions because direct observation was virtually excluded by the nocturnal habits of the subjects, and because the presence of an observer within range might disturb them. In addition, the great mobility of the red fox precluded the effective use of observation posts or blinds. Therefore it was essential to choose areas where foxes could be tracked with certainty and their marking patterns observed with a minimum of disturbance of the animals. In addition, the areas chosen had to be ones that the foxes would visit while the test samples were effective.

Study Areas. Two areas on Mount Desert Island, Maine, U.S.A., known to be frequented by red foxes, satisfied these requirements. These areas were studied during January and February when courtship and mating take place at this latitude (44°N) and when sufficient snow had fallen to allow observation of fox tracks. The first area (area 1) contained a pond of about 10 hectares surrounded mainly by coniferous forest. Preliminary observations showed that foxes often traveled the margins of the pond and left characteristic trails on the snow-covered ice. One member of one of the pairs in this area, probably the male, had lost two toes and thus made distinctive footprints. The second area (area 2) was 12 km southeast in Acadia National Park and consisted of hiking trails, mainly through a hardwood forest between a mountain and a brook on which there were several beaver dams. Within this area was a large deer (*Odocoileus virginianus*) carcass which was visited and eaten by foxes. Because of the geographical separation it is unlikely that the same foxes visited both areas during the study.

Test and Control Solutions. The eight odorous volatiles identified in red fox urine were dissolved in one liter of distilled water at the amounts shown in Table 1 for Test Solution. These represent our estimates of the concentrations found in normal male fox urine before dilution with snow or loss from evaporation. In addition, both test and control solutions contained ethanol,

TABLE 1. COMPOSITION OF SOLUTIONS USED

Compound	Formula	Test[a] solution	Control[b] solution
4-Heptanone	$C_7H_{14}O$	10 mg	0
Δ^3-Isopentenyl methyl sulfide	$C_6H_{12}S$	50 mg	0
6-Methyl-5-hepten-2-one	$C_8H_{14}O$	2 mg	0
Benzaldehyde	C_7H_6O	1 mg	0
Acetophenone	C_8H_8O	50 mg	0
2-Phenylethyl methyl sulfide	$C_9H_{12}S$	2 mg	0
2-Methylquinoline	$C_{10}H_9N$	7 mg	0
Geranylacetone	$C_{13}H_{22}O$	7 mg	0
Polyethylene glycol (6000–7500 mol wt)	$H(OCH_2\text{-}CH_2)_nOH$	100 g	100 g
Ethyl alcohol	C_2H_6O	5 g	5 g
Water	H_2O	to 1 liter	to 1 liter

[a] The concentrations of the volatiles in the test solution are estimated values for freshly voided male urine and are approximately three times those found by Jorgenson et al. (1978), thus allowing for loss from evaporation and dilution by snow in the samples they analyzed.
[b] Citronellal, 20 mg, was added to the control for two tests to give it a positive odor.

used as a transfer solvent, and polyethylene glycol (PEG) as an odor fixative. Without the latter compound the odor of the test solution was not detectable to the observers within a few hours of dispensing, whereas with the PEG the odor could be detected with careful sniffing 40 hr later. The PEG also acted as an effective antifreeze and permitted dispensing of the solutions at low temperatures.

Laying the Artificial Trail. Prior to laying a trail marked with synthetic fox urine, sites for marking were identified by numbered labels tied to trees about two meters above the ground, at intervals of 20–40 meters on the pond margin or the hiking trails. These numbered sites were recorded on maps with a scale of 1:4000.

On late afternoons, following significant ($\geq$5 cm) snowfalls, small uniform mounds of snow (roughly 20 × 20 × 20 cm. cube) were made near each numbered site but reasonably remote from tree trunks or other natural scent posts such as rocks, grass tussocks, or conifer seedlings. The observers, wearing snowshoes and traveling single file, made mounds in the undisturbed snow about one meter to one side of their snowshoe track. A clean shovel was used and care was taken not to contaminate the blade. On each of the mounds was placed 10–15 ml of either the test or the control solution. To minimize cross contamination, one person handled the test solution and another the control mixture, and to simplify record keeping under the adverse conditions of failing light and freezing temperatures, the control preparation was always

placed on the odd-numbered sites and the test on the even. The same alternating pattern was adhered to in subsequent trials to exclude any error caused by lingering odors. We believe, therefore, that this departure from strict randomization was justified and that any bias was minimized by the large number of observations and by the apparent random manner in which the foxes joined and left the test routes. Usually trials were not conducted more frequently than once in two weeks, and for each trial 50–100 fresh mounds were prepared. There were 3 trials in area 1, and 4 in area 2. The average number of mounds per trial in area 1 was 96 and in area 2 it was 58. On completion of a trial, any marked mounds were removed. The remainder were covered by additional snowfalls before the next trial.

Reading the Response. On the two to three mornings after setting up each test, the mounds were examined, and records of fox tracks, urine or fecal marks were made on magnetic tape and on the maps. The marks were checked for the characteristic skunk-like odor and for indications of the sex and number of foxes involved. Evidence that the mound had been investigated or just encountered when traveling at some speed was noted.

DISCUSSION

Direct urine or fecal marks on the mounds appeared to be the most useful criteria and the most easily analyzed. Figure 1 shows an example of a marked mound. Data from all trials when any such marks were found are presented in Table 2. These results show that the synthetic compounds induced an increase in marking activity which was significant in both study areas. They also show that we have devised a method suitable for testing substances that stimulate investigation and overmarking.

The introduction of citronellal as a positive odor appeared to have no effect on marking activity. Foxes passed it within 3 meters for a total of 20 times in two trials without any evidence of marking, avoidance, or approach. In both of these trials, the synthetic solution was marked (2 and 5 times).

The sex of the marking fox was not determined with certainty, since the foxes were never actually observed marking. There were definitely three cases in which footprints characteristic of a squatting posture and a large compact urine volume were both observed; hence, the marking was most likely done by a female fox. The majority of the marking appeared to involve leg raising, however, and therefore was probably done by male foxes. One of the three squatting urinations appeared to be part of a double marking by a male and female fox.

The question of whether or not the synthetic urine induced marking or merely investigation of the scented mounds is a difficult one to answer with the data accumulated thus far. The reason for this is that there were very few cases

FIG. 1. Fox tracks leading to a marked snow mound in area 2.

(2) in which the mounds were investigated but not marked. Analysis of the next best criterion for investigation, i.e., the number of mounds that were passed (within 3 meters) in test versus control mounds, shows that 88 test mounds and 95 control mounds were passed. These data indicate that the test mounds were not passed with any greater frequency than the control mounds.

Unless subsequent trials occur in which investigation without marking is much more frequent, it would seem that investigation of scented mounds is almost invariably followed by marking. The one possible exception to this might be in cases where insufficient (1/7 volume) scent is available as occurred during an early trial. Here, 6 scented mounds were approached (passed over) but not marked.

It is interesting that half of the marking occurred on the first night (10/20) and that half occurred on the subsequent two nights. This "delayed" marking occurred during the coldest weather when the typical skunk-like scent was still faintly discernable to us after 2–3 days; and it occurred at sites not passed on the first night. With overnight temperatures close to 0° C the skunk-like odor could not be smelled by the observers on the following day, but some residual odor was present. No marking was observed under these conditions.

The converse of this situation, in which the scented material may have

TABLE 2. EFFECT OF SYNTHETIC VOLATILE URINARY COMPOUNDS ON MARKING OF MOUNDS BY FOXES IN TWO AREAS

	Marked	Not marked	χ^2	P
Area 1				
Test	4[a]	139		
Control	0	143	4.06	<0.05
Area 2				
Test	16[b]	95		
Control	1	110	14.33	<0.0001
Combined				
Test	20	234		
Control	1	253	17.93	<0.00001

[a]Included 1 fecal mark.
[b]Included 2 fecal marks.

been too "intense," also occurred after a particularly cold blizzard. On this occasion 21 trail sites without mounds were marked on the first night, while only 1 of 21 passed mounds (within 3 m) was marked. On subsequent nights 5 additional scented mounds were marked.

Using this method we will examine the individual compounds and combinations thereof for the capacity to induce marking responses. Already, we have some evidence that the two sulfur compounds together are active in this regard, so they could be the source of the odor that is characteristic for this species.

With similar methods, it should be possible to identify those substances which elicit marking in other species living in cold climates. The work of Heimberger (1959) with captive animals indicates that there may be some overlap between species and that nonspecific substances such as trimethylamine may interfere. Henry (1977), however, was not able to confirm the latter finding but put forward the interesting hypothesis that foxes mark useless food remnants to save the time needed to investigate them at subsequent encounters.

Our method may also be used to study the odors which are specific to classes or individuals, when it would be necessary to observe identified animals of known age, sex, and social status. This would involve a large amount of work, and there would be the risk of disrupting the community. With no further refinement of the present method, however, it should be possible to collect and analyze vixen urine just prior to mating and identify the compounds which attract males. From our records of the date and the nature of the deposits already analyzed (Jorgenson et al., 1978) we conclude that the samples from females were probably from vixens during early pregnancy.

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