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Chemical Scent Constituents in the Urine of the Red Fox (*Vulpes vulpes* L.) During the Winter Season

Abstract. *Four volatile chemical compounds have been identified as apparently unique constituents in urines of red foxes (both sexes) during the winter season when mating occurs. Quinaldine was found only in male fox urine. Several other compounds identified are found in other species also. Some or all of these compounds may function in olfactory communication in the red fox.*

Olfactory communication has important socioecological significance in the wild canid species, especially in the red fox (*Vulpes vulpes* L.) (*1*–*5*). Olfactory cues may serve for individual and group recognition, territorial marking, as markers in food scavenging, for sexual recognition and attraction, as indicators of reproductive states, and for possible pheromonal function.

Identified sources of odor in the red fox are the supracaudal gland, the anal sac, and the urine. Of these, only the urine has thus far apparently not been investigated to determine the odor constituents. By a combination of gas chromatography–mass spectrometry (GC-MS) with organic structural methods and synthesis, we have identified four compounds that appear to be unique odor sources in the urines of male and female red foxes: Δ^3-isopentenyl methyl sulfide, 2-phenylethyl methyl sulfide, 6-methyl-Δ^5-hepten-2-one, and *trans*-geranylacetone. 2-Methylquinoline (quinaldine) was found in male, but not female, red fox urine. In addition, several compounds were identified, and these have also been found in urines of other species: benzaldehyde, acetophenone, 4-heptanone, and some C_{10}-terpenes. The availability of these compounds by synthesis or from natural sources will permit controlled studies of their possible endocrine or behavioral (pheromonal) effects. The methodology that has been developed may provide the means for quantitative investigation of seasonal and individual variations in olfactory cues and permit their correlation with biological events.

The chemical constituents of the supracaudal gland (sometimes referred to as the "violet gland" because of its ambrosia-like odor) have been investigated by Albone (*6*, *7*) and by Albone and Flood (*8*). They reported dihydroactinidiolide, 6-hydroxy-2,2,6-trimethylcyclohexanone, and *trans*-4-keto-β-ionone in addition to other (unidentified) terpenoid and fluorescent photolabile sebum constituents. They also found high levels of hydroxysteroid dehydrogenase activity.

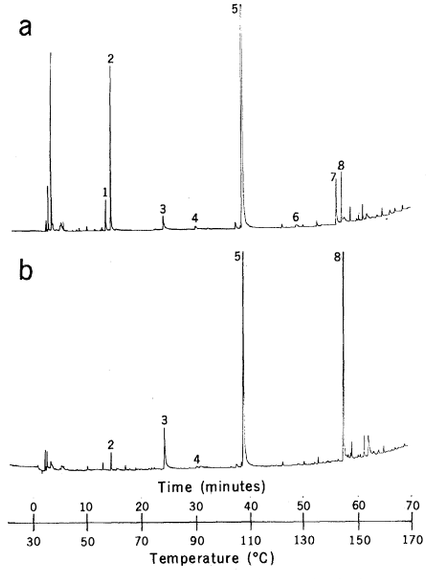

Fig. 1. Gas chromatographic analysis in glass capillary columns of urinary volatile compounds in the red fox (*Vulpes vulpes* L.). (a) Male; (b) female. The constituents are identified by number in Table 1.

The histophysiology and function of the anal sac of the red fox have been described by Spannhof (*9*), who noted seasonal changes in the epithelium of this organ which coincide with variations in locomotor and sexual behavior of the animals. Albone *et al.* (*10*) reported that the predominantly aqueous secretion of the anal sac in the red fox contains high concentrations of odorous volatile fatty acids (C_2 to C_6), ammonia, trimethylamine, 1,4-diaminobutane (putrescine), and 1,5-diaminopentane (cadaverine), all suspected of being microbially produced. They also investigated the bacterial microflora of the sac in studies that were extended by Gosden and Ware (*11*). Albone, Robins, and Patel (*12*) found that 5-aminovaleric acid is a major free amino acid component of the fox anal gland secretion. Although it is generally believed that the anal sac has significance in olfactory communication of the fox, its exact role is not understood.

The red fox uses its urine as the principal means of scent marking (*1*–*5*) and trappers have long used this product in the preparation of fox lures. The strong characteristic odor is said to intensify and change in quality during the breeding season (*13*) when the incidence of marking is said to increase (*2*, *5*). These observations suggest that urine from one or both sexes may have pheromonal activity transmitted through the olfactory sense.

Joffre (*14*) has reported on the significant seasonal variations in the endocrine characteristics (spermatogenetic activity, testicular and prostatic weights, prostate secretions, and plasma testosterone levels) of both the cubs and adult red fox males—phenomena that parallel changes in behavior and in olfactory communication (*5*).

Despite the suspected importance of the urine in scent marking, it is not surprising that the nature of the volatile odor constituents of the red fox urine has not been previously investigated, since collection of uncontaminated urine from wild foxes is difficult. We now present the results of a study of urines of both male and female foxes collected in a natural environment within or near the Acadia National Park (68°W, 44°N) close to Bar Harbor, Maine. The only species of fox in that area is *Vulpes vulpes* L.

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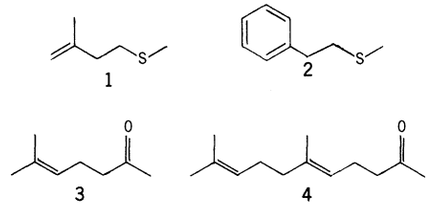
(*15*). which mates during February at this latitude [see (*4*)].

Urine samples were collected during January, February, and March. The animals were trailed soon after sunrise on mornings when snow had just fallen and when the temperature was below freezing (*16*). The tracks were identified with the help of two experienced woodsmen and a field guide (*17*). In addition, the fox urine was recognized by its characteristic "skunky" odor with which one of us (W.K.W.) has been familiar for years, and, as Montague points out (*3*), the scent posts are often smelled before being sighted.

The recency of the urine deposits was judged from the color, which was straw-yellow when fresh but turned orange-brown by noon. The sex of the individuals was inferred from the nature of the urine marks (*3*). Male urine is projected laterally and may be found 20 to 25 cm above the hind paw mark, and obviously one leg has been lifted. The estimated volume varies from 0.5 to 2.0 ml and is frequently sprayed over an area; if the urine misses the post, droplets may be found some distance away (30 cm). In contrast, urine deposited by females is found between and behind the rear paw marks, and greater volumes may be observed. Although females may raise one leg, there is no lateral projection of urine; and, as a rule, females do not urinate on raised objects. Occasionally, we found two marks together and assume that the male urinated on top of the female's mark. We believe that the urine samples originated from at least four males and three females.

Specimens were collected from snow that was not obviously contaminated with other biological material. The frozen urine and the attached snow were picked up with clean stainless steel spatulas and placed into brown glass, wide-mouthed bottles. The caps with Teflon washers were tightly closed, and the specimens were stored frozen; they were transported in Dry Ice. Control samples of snow were gathered before any trails were encountered. Fourteen samples of urine identified as male or female in origin were kept separate and analyzed individually. The volatility profiles were studied by methods established in our laboratory (Indiana University); we used headspace sampling procedures (*18*) and made gas chromatographic analysis (Perkin-Elmer model 3920 gas chromatograph with modified sampling port and detector) of urinary volatiles with glass capillary columns (*19*). The column was a glass capillary (50 m by 0.25 mm, inside diameter) coated with Ucon 50-HB-2000. Compound identifications were carried out with nitrogen-selective thermionic and sulfur-sensitive flame photometric GC detectors and a combined GC-MS (Hewlett-Packard model 5980A dodecapole instrument). All identifications of fox urinary constituents were verified by comparison with authentic samples that were either synthesized in the chemistry department at Indiana University or were obtained from reliable commercial sources.



While the chromatographic analytical results were not quantitative in the absolute sense, they were consistent with each other and permitted meaningful comparisons to be made. Consistent sex differences appeared in the urinary profiles. Typical chromatograms of a male and of a female urine are shown in Fig. 1, and a list of identified components is presented in Table 1. None of these peaks is caused by a contaminant, since blanks of collected fresh snow produced a flat baseline except for a phthalate contaminant eluting beyond the peaks of interest.

Although some of the components are rather common [for example, benzaldehyde, acetophenone, 4-heptanone, and some C_{10}-terpene hydrocarbons are also constituents of normal human urine (*20*)], several major components do not appear to have been found in urines or glandular secretions of other mammalian species.

The strong, unpleasant odor characteristic of the red fox (as perceived by the human olfactory sense) is clearly due to two sulfur-containing compounds, Δ^3-isopentenyl methyl sulfide (**1**) (peak 2, Fig. 1, Table 1) and 2-phenylethyl methyl sulfide (**2**) (peak 6, Fig. 1 and Table 1), both shown to be identical in GC-MS behavior with authentic synthetic specimens. Two terpene-derived ketones, 6-methyl-Δ^5-hepten-2-one (**3**) (peak 3, Fig. 1 and Table 1) and geranylacetone (**4**) (peak 8, Fig. 1 and Table 1), were identified. The terpenes **3** and **4** appear to be more characteristic of the female urine than of the male. It is interesting that the geranylacetone in fox urine showed no detectable *cis* isomer (nerylacetone), which is generally present in varying amounts in synthetic material, even after rigorous purification (*21*). The open-chain terpenic ketones **3** and **4** may have a common biogenesis with the cyclic terpene derivatives reported by Albone (*6*) to be constituents of the supracaudal gland. Cyclization of **4** at the olefinic bonds can be visualized to generate the β-ionone skeleton (*22*); further oxidation

Table 1. Analytical data and structure identification. Relative intensities are shown in parentheses.

Peak No.	Compound No. (text)	M.W.*	Formula	Significant *m/e*†	Compound	Presence in fox	
						Male	Female
1		114	$C_7H_{14}O$	71 (100), 43 (50), 114 (45), 41 (20)	4-Heptanone	+	+
2	**1**	116	$C_6H_{12}S$	61 (100), 116 (71), 68 (27), 67 (18) 101 (11)	Δ^3-Isopentenyl methyl sulfide	++	+
3	**3**	126	$C_8H_{14}O$	108 (100), 43 (64), 69 (59), 41 (41), 111 (36)	6-Methyl-Δ^5-hepten-2-one	+	++
4		106	C_7H_6O	106 (100), 105 (98), 77 (55)	Benzaldehyde	+	+
5		120	C_8H_8O	105 (100), 77 (53), 120 (44), 51 (8), 106 (8)	Acetophenone	++	++
6	**2**	152	$C_9H_{12}S$	104 (100), 61 (87), 91 (83), 152 (74)	2-Phenylethyl methyl sulfide	+	+
7		143	$C_{10}H_9N$	143 (100), 128 (20), 115 (19), 142 (17), 144 (13), 101 (8)	2-Methylquinoline	++	−
8	**4**	194	$C_{13}H_{22}O$	69 (100), 43 (82), 151 (65), 136 (60) 107 (52), 93 (32), 125 (30), 41 (28)	Geranylacetone	+	++

*Molecular weight. †Mass to charge.

could generate the *trans*-4-keto-β-ionone found by Albone in the supracaudal gland. Isoe, Hyeon, and Sakan (*22, 23*) have shown that β-ionone and dihydroactinidiolide can be formed in a photochemically induced reaction of β-carotene with oxygen (*24*).

An interesting hypothesis could be made of the possible biogenesis of Δ^3-isopentenyl methyl sulfide (**1**) from isopentenyl pyrophosphate and methionine via a *S*-methylsulfonium intermediate. Thus, Δ^3-isopentenyl pyrophosphate (*25*), the first essential reactive isoprenoid intermediate in the long chain of terpene biogenesis leading to sterols and steroid hormones, could be the source of a powerful and fairly persistent olfactory marker, **1**, present in concentrations that could signal an increase of steroid hormone synthesis in preparation for the mating season in the fox. Alternatively, it could be that the steroid requirements have been met and that precursors are available to be diverted to other substances to advertise that fact.

It remains to be determined whether the concentrations of these volatile components (**1** to **4**) of the red fox urines undergo individual and seasonal changes that correlate with the already reported endocrine and behavioral changes characteristic of the mating period. The availability of synthetic mixtures simulating the natural ratios of these known volatiles will also make possible controlled behavioral studies with foxes in their natural habitat.

There is a close chemical relation between the thio ether **1** and mustelan (2,2-dimethylthietane), the malodorous substance from the anal gland of the mink (*Mustela vison*) and the polecat (*Mustela putorius*), and its companion compounds, 3,3-dimethyl-1,2-dithiolane and diisopentyl disulfide (*26*), all of apparent isoprenoid origin. A component (3-methyl-1-butanethiol) of the scent of the striped skunk (*Mephitis mephitis*) contains also (*27*) a putative isoprene unit, although in this case the presence of unbranched thiols and disulfides might suggest other than terpene origin.

The presence of 2-methylquinoline (quinaldine) in male red fox urine, but not detectable in female urine, is an interesting sex-related difference. Albone (*7*) refers without citation to an old report of the occurrence of 2-methylquinoline in skunk scent. This quinoline derivative could have originated from tryptophan or other indole derivative by way of a ring-enlargement, a reaction with well-known biochemical precedent.

The red fox, considered the least social of the wild canids (*1*), depends more than other canid species on olfactory means of communication. Whether any of the compounds reported above may have pheromonal activities is not yet known.

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Command Neurons in *Pleurobranchaea* Receive Synaptic Feedback from the Motor Network They Excite

Abstract. *Command neurons that cause rhythmic feeding behavior in the marine mollusc* Pleurobranchaea californica *have been identified in the cerebropleural ganglion (brain). Intracellular stimulation of single command neurons in isolated nervous systems, semi-intact preparations, and restrained whole animals causes the same rhythmic motor output pattern as occurs during feeding. During this motor output pattern, action potentials recorded intracellularly from the command neurons occur in cyclic bursts that are phase-locked with the feeding rhythm. This modulation results from repetitive, alternating bursts of excitatory and inhibitory postsynaptic potentials, which are caused at least in part by synaptic feedback to the command neurons from identified classes of neurons in the feeding network. Central feedback to command neurons from the motor network they excite provides a possible general physiological mechanism for the sustained oscillation of neural networks controlling cyclic behavior.*

Command neurons are single nerve cells that elicit a recognizable motor component of behavior (*1*). After they were described by Wiersma (*2*), command neurons were studied in several animal groups, including crustaceans (*3*), insects (*4*), molluscs (*5*), and mammals (*6*). On the basis of motor responses to electrical stimulation, these cells have been presumed to serve as simple, one-way relay pathways that convey instructions for specific movements from higher to lower motor "centers" (*6, 7*). This hypothesis has not been tested, however, owing to the paucity of studies involving recordings from command

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