VOLATILE COMPOUNDS IN ANAL GLAND OF SIBERIAN WEASELS (*Mustela sibirica*) AND STEPPE POLECATS (*M. eversmanni*)

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Abstract—The volatile constituents in anal gland secretions of two sympatric *Mustela* species, the Siberian weasel (*M. sibirica*) and steppe polecat (*M. eversmanni*), were studied by the headspace technique, followed by gas chromatography–mass spectrometry (GC-MS) analysis. Nine sulfur-containing compounds were identified. They were 2,2-dimethylthietane, (*Z*)- or (*E*)-2,4-dimethylthietane, (*E*)-2,3-dimethylthietane, 2-ethylthietane, (*E*)-2-ethyl-3-methylthietane, (*Z*)-2-ethyl-3-methylthietane, 2-propylthietane, 3,3-dimethyl-1,2-dithiacyclopentane, and (*Z*)-3,4-dimethyl-2,2-dithiacyclopentane. Among them, (*E*)-2-ethyl-3- methylthietanes, (*Z*)-2-ethyl-3-methylthietanes, and (*Z*)-3,4-dimethyl-1,2-dithiacyclopentane were present in the polecat but not in the weasel. The predominant compound was 2,2-dimethylthietane in the weasel and (*E*)- or (*Z*)-2,4-dimethylthietane in the polecat. These differences were consistent between the two species, regardless of sex and age and, therefore, could possibly be used for species recognition. In the weasel, 2-ethylthietane was found only in the female, and the relative abundance of several compounds was significantly different between males and females. In the polecat, although no sex-specific volatile compounds were found, males and females differed in the relative abundance of several of the compounds. In both species, the relative abundance of some compounds varied with age. We conclude that these volatile compounds can be used to communicate information about species, sex, and age.

Key Words—Anal gland secretion, volatile compounds, Siberian weasel, *Mustela sibirica*, steppe polecat, *Mustela eversmanni*.

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INTRODUCTION

Chemical communication plays a vital role in mammalian social interactions, such as recognition of species, sex, age, dominance status, and physiological and reproductive condition (e.g., Müller-Schwarze, 1974; Brown, 1979; Sun and Müller-Schwarze, 1999). Our current knowledge of animal chemical communication has been mainly garnered through two inseparable approaches: chemical analysis of secretion materials and behavioral bioassays. Chemical analysis of secretions can provide insight into what information is available and how it is coded and, therefore, can narrow or generate new hypotheses for behavioral bioassays (Sun, 1996).

Carnivores, rodents, and ungulates are among the most intensively studied mammalian groups for chemical communication (see Brown and Macdonald, 1985). The chemical composition of the anal gland secretion of small carnivores is of particular interest to researchers. In the *Mustela* genus, chemical analysis has been conducted in the domestic American mink (*M. vison*) (Sokolov et al., 1980; Brinck et al., 1978, 1983), stoat (*M. erminea*) and domestic ferrets (*M. putorius furo*) in New Zealand (Crump, 1980a,b; Crump and Moors, 1985), and mountain weasel (*M. nivalis*) and ferret (*M. putorius*) (Brinck et al., 1983). These efforts have resulted in the identification of 18 major volatile compounds unique to *Mustela*. They are 2-pentylthietane ($C_8H_{16}S$), 3-propyl-1,2-dithiolane ($C_6H_{12}S$), indole (C_8H_7N), and *o*-aminoacetophenone (C_8H_9NO), six isomers of $C_5H_{10}S$, four isomers of $C_6H_{12}S$, and four isomers of $C_5H_{10}S_2$. Among them, two are nitrogen-containing compounds: indole (in all *Mustela* species) and *o*-aminoacetophenone (only in *M. erminea*), and the remaining are all sulfur-containing compounds. These 18 compounds apparently are unique to *Mustela* and have not been found in other genera in the family Mustelidae, such as *Martes martes* (Brinck et al., 1983), *Lutra lutra* (Gorman et al., 1978; Brinck et al., 1983) and *Meles meles* (Brinck et al., 1983). Comparisons of these volatiles from the anal gland secretions of various *Mustela* species show that the presence or absence or relative proportion of isomers of $C_5H_{10}S$, $C_6H_{12}S_2$ and $C_5H_{10}S_2$ is species specific. This provides cues for species recognition among sympatric species.

Findings in intersexual or individual variation in the chemical composition of anal gland secretions may also indicate the possibility for sex and individual recognition within a *Mustela* species. For example, the chromatographic peak profile of the anal gland secretion in the mink is characteristic of the individual and not affected by season (Brinck et al., 1978). In stoats, highly volatile components in the anal gland secretion also demonstrate a stable individual pattern (Erlinge et al., 1982). Sexual dimorphism in the chemical composition of anal gland secretion varies with species. American minks show no specific sex differences over the four seasons (Brinck et al., 1978). Secretions from female stoats during the breeding season, however, contain compounds not present in the male in the breeding season

(Crump, 1980a). This implies that other unidentified compounds may also be involved in sex recognition.

Siberian weasels (*Mustela sibirica fortanieri*) and steppe polecats (*Mustela eversmanni admirata*) distribute sympatrically in farmlands in northern China. They overlap in their activity range and rodent diet (Gao, 1987). Thus, both in-interspecific and intraspecific recognition is important for the two species. Since secretions from the anal gland have already been identified as a source of information for recognition in *Mustela*, chemical analyses of the volatile compounds may allow us to pinpoint the components involved. In this study, we characterized the volatile components of the two *Mustela* species and explored their potential roles by examining species-, sex-, and age-related differences in these compounds.

METHODS AND MATERIALS

Collection and Separation of Volatile Compounds. Anal glands were removed fresh from Siberian weasels (70 males and 50 females) and steppe polecats (40 males and 20 females) captured legally by fur trappers in several farmlands in central northern China in January 2000. They were immediately frozen and transferred to the laboratory and stored at $-20°C$ until use.

For both species, we used the baculum to estimate the age of males. Animals with a baculum weight <250 mg were classified as juveniles and >350 mg as adults (Sheng and Lu, 1976; Gao, 1987). For females, we used body weight to estimate the age. Those weighing >500 g were classified as adults and <350 g as juveniles (Sheng and Lu, 1976; Gao, 1987). We found no juvenile female steppe polecats and, thus, this age group was not included in our study.

Each anal gland was punctured, and secretion material was squeezed into a 1.5-ml vial. Secretions from individuals of the same species, sex, and age group were pooled into larger vials, each containing samples from at least five individuals. These pooled samples were used for chemical analysis.

For each pooled sample, we filtered 150 mg of secretion material and placed it into a 100-ml glass entrainment jar, which was waterbathed at a constant temperature of $55 \pm 1°C$. A headspace sample was collected using Porapak Q (100 ± 2 mg, mesh 80–100). Volatiles trapped on the porous Porapak Q were released by solvent desorption. Porapak Q was packed individually into a glass tube (8 cm long $\times$ 3 mm ID) and kept in place with silanized glass wool plugs. The Porapak Q tube was immersed in ice-water ($0°C$) for better retention of the volatiles. Volatile chemicals from the headspace of the secretion samples were drawn onto the polymer for 10 hr by using Porapak Q-filtered nitrogen gas at a flow rate of 100 ml/min. At the end of entrainment, trapped volatiles were eluted from Porapak Q with 5 ml of redistilled dichloromethane. The dichloromethane solutions were later condensed to 1/8 volume (0.625 ml) by a gentle stream of N_2 gas and were used for the following analysis.

Gas Chromatography–Mass Spectrometry (GC-MS). Analytical GC-MS was performed on a Shimadzu GC-MS QP5050A instrument. The GC was equipped with a 30-m glass capillary column (0.25 mm ID) coated with SE54. Helium was used as the carrier gas at a flow rate of 0.5 ml/min. The temperature of the injector was set at 250°C, and the split ratio was set at 1:10. The oven temperature was programmed as 50°C for 5 min, then increased at 10°C/min to 220°C, and finally held at 220°C for 10 min. Electron impact was used for ionization, which was set at 70 eV and 250°C.

We searched the literature to determine which reference substances should be used for parallel analyses. This led to our identification of 2,2-dimethylthietane (**1**), 2-ethylthietane (**4**), and 3,3-dimethyl-2,2-dithiacyclopentane (**8**), which had been identified in the mink by using a similar method (Brinck et al., 1978, 1983; Sokolov et al., 1980). (The number that follows each compound corresponds to the number in Table 1 below.) The GC profile and mass spectrum of 2-propylthietane (**7**) was compared with that of a commercially available authentic chemical compound. We used the mass spectra available in the literature to aid in the identification of the following compounds: (*Z*)-or (*E*)-2, 4-dimethylthietane (**2**), (*E*)-2,3-dimethylthietane (**3**) (Crump, 1980b), (*E*)-2-ethyl-3-methylthietanes (**5**), (*Z*)-2-ethyl-3-methylthietanes (**6**) (Crump and Moors, 1985), and (*Z*)-3,4-dimethyl-1, 2-dithiacyclopentane (**9**) (Crump, 1980b; Sokolov et al., 1980; Brinck et al., 1983).

We used the method developed by Sun and Müller-Schwarze (1998) to quantify the relative abundance of the nine compounds. We converted the peak area of a particular compound into the percentage of the summed peak areas of all nine compounds in a sample. We then used a two-tailed independent *t* test to test for the difference in these compounds between species, sexes, and age groups. The level of significance was set at 0.05. Because we had four samples for each specific sex or age group, the degree of freedom was 6 for all tests.

RESULTS

Volatile Compounds in Anal Gland Secretions. Typical GC chromatogram profiles of volatiles from anal gland secretions in male steppe polecats and female Siberian weasels are shown in Figure 1. Mass spectral information of the numbered peaks is given in Table 1. Using headspace sampling and subsequent GC-MS analysis, we identified nine volatiles in the anal gland secretion of the two species: 2,2-dimethylthietane (**1**), (*Z*)-or (*E*)-2,4-dimethylthietane (**2**), (*E*)-2, 3-dimethylthietane (**3**), 2-ethylthietane (**4**), (*E*)-2-ethyl-3-methylthietanes (**5**), (*Z*)-2-ethyl-3-methylthietanes (**6**), 2-propylthietane (**7**), 3,3-dimethyl-1,2-dithiacyclopentane (**8**), and (*Z*)-3,4-dimethyl-1,2-dithiacyclopentane (**9**) (Table 1).

The GC chromatogram shows nine volatile constituents in the secretion of the steppe polecat (Figure 1A). Compounds **1**–**4** (Table 1) have a molecular mass of
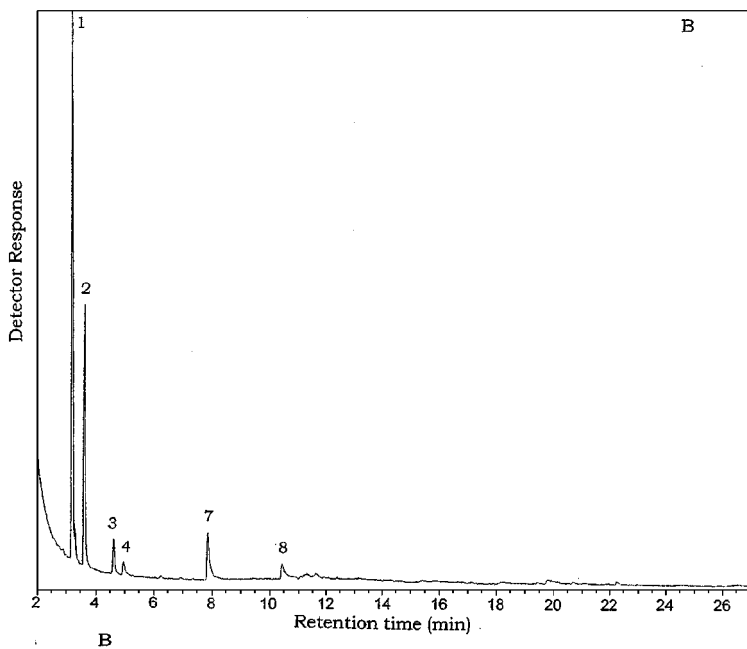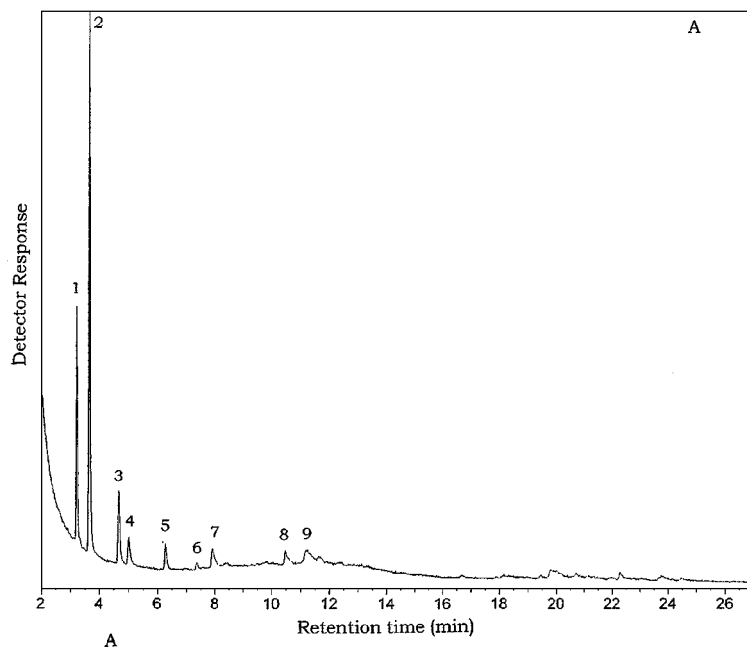
FIG. 1. Gas chromatogram of the anal gland secretion from male adult steppe polecats (*Mustela eversmanni admirata*) (A) and female adult Siberian weasels (*Mustela sibirica fortanieri*) (B).

TABLE 1.   MS DATA AND COMPOUNDS IDENTIFIED IN ANAL GLAND VOLATILES OF
SIBERIAN WEASEL OR STEPPE POLECAT

Compound	Mass spectrum data [m/z values (ion intensities)]	Identification
1	102(48), 87(28), 74(28), 69(25), 68(18), 67(15), 60(25), 56(82), 41(100)	2,2-dimethylthietane
2	102(32), 87(3), 74(1), 73(1), 69(4), 68(2), 67(3), 60(100), 61(16), 59(22), 56(31), 45(26), 41(45)	Z- or E-2,4-dimethylthietane
3	102(30), 87(12), 74(7), 73(7), 69(8), 67(8), 61(15), 60(100), 59(20), 56(28), 41(45)	E-2,3-dimethylthietane
4	102(64), 87(42), 74(49), 73(52), 69(14), 68(24), 67(18), 61(13), 60(100), 59(12), 56(29), 55(50), 45(89), 41(100)	2-ethylthietane
5	116(59), 101(22), 87(59), 74(16), 73(42), 69(27), 68(8), 67(51), 60(39), 59(19), 55(70), 45(77), 41(100)	E-2-ethyl-3-methylthietanes
6	116(37), 86(6), 84(13), 74(100), 70(19), 69(26), 60(2), 59(27), 56(18), 55(45), 45(29), 41(98)	Z-2-ethyl-3-methylthietanes
7	116(55), 88(43), 87(79), 86(26), 74(13), 73(73), 69(45), 67(50), 56(52), 55(51), 41(100)	2-propylthietane
8	134(20), 86(2), 70(10), 69(99), 67(8), 59(14), 57(4), 56(4), 55(13), 53(11), 41(100)	3,3-dimethyl-1,2-dithiacyclopentane
9	134(31), 86(12), 85(13), 70(24), 69(100), 57(42), 55(32), 41(87)	Z-3,4-dimethyl-1,2-dithiacyclopentane

102 ($C_5H_{10}S$). Compounds **1** and **4** have GC and MS data identical to peaks
identified as 2,2-dimethylthietane and 2-ethylthietane by Brinck et al. (1978,
1983) in a parallel analysis of the anal gland secretion of the mink. Compounds
2 and **3** show mass spectra that are superimposable on those of (Z)- or (E)-
2,4-dimethylthietane and (E)-2,3-dimethylthietane found in the domestic ferret
(Crump, 1980b) and mountain weasel (Brinck et al., 1983). GC retention time se-
quence shows that compound **2** is (Z)- or (E)-2,4-dimethylthietane and compound
3 is (E)-2, 3-dimethylthietane. Compounds **5**, **6**, and **7** all have a molecular mass
of 116 ($C_6H_{12}S$) (Table 1). Compound **7** and authentic synthetic 2-propylthietane
have identical GC and MS data, whereas the MS data of compounds **5** and **6** cor-
respond to (E)- and (Z)-2-ethyl-3-methylthietane identified in the stoat (Crump
and Moors, 1985). The GC and MS data of compounds **8** and **9** show that they
both have a molecular mass of 134 ($C_6H_{12}S_2$) (Table 1). The presence of ions at
m/z 41, m/z 69, and m/z 134 and the absence of an ion at m/z 29 indicate that they
are most likely isomers of 3,3-dimethyl-1,2-dithiacyclopentane. The GC and MS
data of compound **8** are similar to those of 3,3-dimethyl-1,2-dithiacyclopentane
(Sokolov et al., 1980). The absence of ions at m/z 102 and 94 in the MS of
compound **9** agrees with that observed by Crump (1980b) for (Z)-3, 4-dimethyl-
1,2-dithiacyclopentane in the ferret.

Interspecific Differences. In the Siberian weasel, the gas chromatograms and mass spectra of anal gland secretions of both sexes and two age groups had six peaks (Figure 1B) corresponding to 2,2-dimethylthietane (peak 1), (*Z*)- or (*E*)-2,4-dimethylthietane (peak 2), (*E*)-2,3-dimethylthietane (peak 3), 2-ethylthietane (peak 4), 2-propylthietane (peak 7), and 3,3-dimethyl-1,2-dithiacyclopentane (peak 8).

(*E*)-2-Ethyl-3-methylthietanes (peak 5), (*Z*)-2-ethyl-3-methylthietanes (peak 6), and (*Z*)-3,4-dimethyl-1,2-dithiacyclopentane (peak 9) were present in the polecat but not in the weasel. Several compounds, although present in both species, showed a consistent difference in relative abundance between the two species. 2,2-Dimethylthietane was the dominant component in the weasel (male: $t = 5.022$, $P = 0.004$; female: $t = 4.038$, $P = 0.006$), while in the polecat (*Z*)- or (*E*)-2,4-dimethylthietane was the dominant component (male: $t = 4.975$, $P = 0.002$; female: $t = 2.455$, $P = 0.048$). For males, 2-ethylthietane occurred only in the polecat but not in the weasel. The polecat also had a higher level of (*E*)-2,3-dimethythietane ($t = 3.272$, $P = 0.017$) and a lower level of 3,3-dimethy-1,2-dithiacyclopentane ($t = 3.490$, $P = 0.012$) than the weasel. For females, the polecat had a higher level of 2-ethylthietane and a lower level of 2-propylthietane ($t = 3.811$, $P = 0.009$) than the weasel (Table 2).

Intraspecific Differences. In terms of *sex difference*, in Siberian weasels, 2-ethylthietane (peak 4) only occurred in females and was undetectable in males. Furthermore, the relative abundance of (*Z*)- or (*E*)-2,4-dimethylthietane (peak 2) and (*E*)-2,3-dimethylthietane (peak 3) was significantly higher in females than in males ($t = 4.954$, $P = 0.003$ and $t = 2.927$, $P = 0.026$, respectively).

TABLE 2. COMPARISON OF ANAL GLAND VOLATILES BETWEEN SEXES AND CONSPECIES IN SIBERIAN WEASEL AND STEPPE POLECAT[a]

Component	Adult Siberian weasel		Adult steppe polecat	
	Males	Females	Males	Females
1	$78.37 \pm 26.33^{\dagger\dagger}$	$55.68 \pm 21.22^{\dagger\dagger}$	15.99 ± 3.88	14.14 ± 5.09
2	$11.06 \pm 3.67^{\dagger\dagger}$	$26.74 \pm 5.86^{**\dagger}$	47.92 ± 16.75	39.01 ± 15.47
3	$1.97 \pm 0.52^{\dagger}$	$4.41 \pm 0.96^{*}$	7.78 ± 2.07	6.83 ± 3.01
4		$2.13 \pm 0.29^{**\dagger}$	3.92 ± 0.78	4.97 ± 1.59
5			3.54 ± 0.54	$10.07 \pm 2.27^{*}$
6			1.19 ± 0.09	$4.12 \pm 1.04^{*}$
7	7.83 ± 2.31	$8.51 \pm 2.72^{\dagger\dagger}$	5.12 ± 1.69	$2.89 \pm 0.98^{*}$
8	$10.42 \pm 2.89^{\dagger}$	$2.53 \pm 1.02^{*}$	2.44 ± 0.19	$4.15 \pm 0.88^{*}$
9			3.82 ± 1.07	$10.77 \pm 3.00^{*}$

[a] Mean of percent peak area (%) $\pm$ SD, $N = 4$. †:$P < 0.05$; ††:$P < 0.01$ between the two species of the same sex (marked only in the weasel data); *:$P < 0.05$; **:$P < 0.01$ between the two sexes of the same species (marked only in the female data).

Furthermore, 3,3-dimethyl-1,2-dithiacyclopentane (peak 8) was more abundant in males than in females ($t = 3.27$, $P = 0.017$; see Table 2).

In the steppe polecat, five compounds showed quantitative differences between the two sexes. (E)- and (Z)-2-Ethyl-3-methylthietanes (peaks 5 and 6), 3,3-dimethyl-1,2-dithiacyclopentane (peak 8), and (Z)-3,4-dimethyl-1,2-dithiacyclopentane (peak 9) were all higher in females ($t = 2.990$, $P = 0.034$; $t = 2.371$, $P = 0.046$; $t = 2.450$, $P = 0.043$; and $t = 3.318$, $P = 0.012$, respectively), whereas 2-propylthietane (peak 7) was higher in males ($t = 2.762$, $P = 0.038$). However, no sex-specific compound was observed (Table 2).

In terms of *age difference*, in male Siberian weasels, adults had a higher level of 2-propylthietane (peak 7) and 3,3-dimethyl-1, 2-dithiacyclopentane (peak 8) than juveniles ($t = 2.978$, $P = 0.034$ and $t = 3.097$, $P = 0.026$, respectively). In female weasels, adults had a higher level of (Z)- or (E)-2,4-dimethylthietane (peak 2), (E)-2,3-dimethylthietane (peak 3), and 2-propylthietane (peak 7) than juveniles ($t = 2.608$, $P = 0.041$; $t = 2.990$, $P = 0.032$ and $t = 3.582$, $P = 0.014$, respectively). Juveniles, however, had a higher level of 3,3-dimethyl-1, 2-dithiacyclo pentane (peak 8) than adults ($t = 3.049$, $P = 0.026$).

In male steppe polecats, adults had a lower level of 2-ethylthietane (peak 4) and (E)-2-ethyl-3-methylthietanes (peak 5) in comparison with those in juveniles ($t = 2.976$, $P = 0.383$ and $t = 2.901$, $P = 0.0371$, respectively; Table 3).

DISCUSSION

Our results show that all volatiles detected in the anal gland secretion of the Siberian weasel and steppe polecat were sulfur-containing compounds, as previously reported in other *Mustela* species (Sokolov et al., 1980; Crump, 1980a,b; Brinck et al., 1983; Crump and Moors, 1985). A unique aspect that has not been previously reported, however, is that four isomers of $C_5H_{10}S$ occurred in either Siberian weasels or steppe polecats. Whether this similarity in the anal gland secretions is due to their close phylogenetic relationship or to the similarity in microbial communities is yet unknown.

Three compounds [two isomers of 2-propylthietane and (Z)-dimethyl-1,2-dithiacyclopentane] occurred only in steppe polecats. 2,2-Dimethylthietane was dominant in the Siberian weasel, while (Z)- or (E)-2,4-dimethylthietane was most abundant in the steppe polecat. The two species also differed significantly in the relative abundance of 2-ethylthietane, (E)-2,3-dimenthythietane, 3,3-dimethy-1,2-dithiacyclopentane, and 2-propylthietane for males or females or both. These consistent qualitative and quantitative differences between the two sympatric species provide unambiguous recognition cues between them.

Nitrogen-containing indole, detected previously in other *Mustela* species, was not found in the two species we studied. This can probably be attributed to the headspace sampling technique: our sampling apparatus may fail to trap indole,

TABLE 3. COMPARISON OF ANAL GLAND VOLATILES BETWEEN AGE GROUPS OF SIBERIAN WEASEL AND STEPPE POLECAT[a]

Compound	Male Siberian weasel		Female Siberian weasel		Male steppe polecat	
	Juveniles	Adults	Juveniles	Adults	Juvenile	Adult
1	74.25 ± 30.01	78.37 ± 26.33	64.7 ± 19.88	55.68 ± 21.22	19.38 ± 4.19	15.99 ± 3.88
2	9.90 ± 2.87	11.06 ± 3.67	16.29 ± 7.77	26.74 ± 5.86*	38.39 ± 9.88	47.92 ± 16.75
3	1.82 ± 1.03	1.97 ± 0.52	2.42 ± 0.49	4.41 ± 0.96*	6.87 ± 3.01	7.78 ± 2.07
4			2.52 ± 0.72	2.13 ± 0.29	8.94 ± 3.11	3.92 ± 0.78*
5					6.33 ± 1.83	3.54 ± 0.54*
6					1.53 ± 0.67	1.19 ± 0.09
7	3.07 ± 1.35	7.83 ± 2.31*	3.53 ± 0.81	8.51 ± 2.72*	3.72 ± 0.68	5.12 ± 1.69
8	5.44 ± 1.46	10.42 ± 2.89*	6.82 ± 1.08	2.53 ± 1.02*	3.86 ± 0.94	2.44 ± 0.19
9					5.01 ± 1.82	3.82 ± 1.07

[a] Mean of percent peak area (%) ± SD, $N = 4$. *: $P < 0.05$.

which is less volatile than the nine compounds we identified. In fact, we only found three sulfur-containing compounds (2,2-dimethylthietane, 2-ethylthietane, and 3,3-dimethyl-1,2-dithiacyclopentane) in the anal gland secretion of the mink using our sampling method (unpublished data). In contrast, Brinck et al. (1983) detected indole, in addition to these three compounds in solvent extracts from mink secretions. Thus, the headspace technique appears to be insensitive for detecting relatively heavy volatiles.

Intraspecific communication is suggested to be the main function of the anal gland secretion in mustelids (Erlinge et al., 1982; Brinck et al., 1983). Our study demonstrates that there is a wealth of information contained in the anal gland secretion that can be used for age and sex recognition in the two *Mustela* species. It seems that the coding for sex information takes two forms, either by the presence or absence of sex-specific compounds, such as 2-ethylthietane in female Siberian weasels, and/or by the difference in relative abundance of some compounds between males and females. We failed to find any age-specific compounds.

The sex or age difference in the composition of secretions is generally regulated by hormones (Ebling, 1977). For example, female-specific compounds are only found in stoats during the breeding season (Crump et al., 1980a). In ferrets, production of anal gland secretion is suppressed during estrus (Crump et al., 1980b). Quinaldine is only found in the urine of male red foxes, *Vulpes vulpes* (Jorgenson et al., 1978), and sex hormones significantly affect some volatile constituents in the anal gland secretion of the wolf, *Canis lupus* (Raymer et al., 1985). The hormonal difference between males and females or between adults and juveniles may be responsible for the qualitative and quantitative differences in the volatile compounds in our study.

One obvious caveat is that not all volatiles in anal gland secretions were detected due to methodological limitations (e.g., indole). Additionally, nonvolatiles may also play a significant role (e.g., Sun and Müller-Schwarze, 1998). Whether these nine volatiles are exclusively involved in these recognitions should be ultimately confirmed through behavioral bioassay in the future.

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