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Possible Coding for Recognition of Sexes, Individuals and Species in Anal Gland Volatiles of *Mustela eversmanni* and *M. sibirica*

Jian-Xu Zhang[1,4], Jia Ni[2], Xue-Jie Ren[2], Lixing Sun[3], Zhi-Bin Zhang[1] and Zu-Wang Wang[1]

[1]State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, [2]Department of Biology, Capital Normal University, Beijing 100083, China and [3]Department of Biological Sciences, Central Washington University, Ellensburg, Washington, WA 98926-7537, USA

[4]Present address: Department of Chemistry, Institute for Pheromone Research, Indiana University, Bloomington, IN 47405, USA

Correspondence to be sent to: Zhi-Bin Zhang, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China. e-mail: zhangzb@panda.ioz.ac.cn

Abstract

With a combination of solvent extraction and gas chromatography–mass spectrometry, we found eight new compounds in the two sympatric *Mustela* species, *M. eversmanni* and *M. sibirica*. These compounds had not been detected by headspace sampling with solvent desorption. Two of the newly detected compounds are nitrogen-containing compounds, indole and *o*-aminoacetophenone and the remaining are sulfur-containing volatiles. By comparing same and opposite sexes between the two *Mustela* species, we found that qualitative differences in the anal gland secretion are most likely to be used to code for information about species, corresponding to the idea of digital coding. In the Siberian weasel (*M. sibirica*), both presence or absence of sex-specific compounds (*Z*-2-ethyl-3-methylthietane only in females) and relative abundance of some compounds between males and females could be used to code for information about sex, corresponding to the idea of digital and analog coding, respectively. In the steppe polecat (*M. eversmanni*), only quantitative differences provided the possibility for inter-sexual communication. Thus coding for information about sex appeared to be digital. Coding for individual information could also be either digital or analog or both through the presence or absence of certain compounds and/or the difference in the relative abundances of certain compounds among individuals. Comparing with other *Mustela* spp., we failed to find a congruence between the chemical composition of anal gland secretions and the phylogenetic relationship among the species in this genus.

Key words: anal gland secretion, volatile compounds, Siberian weasel, *Mustela sibirica*, steppe polecat, *Mustela eversmanni*

Introduction

How information is coded is one of the main questions in the study of animal chemical communication. This question is normally answered through two ways, behavioral and chemical (Sun and Müller-Schwarze, 1998a,b). In mammals, where chemical signals are characteristically complex with multiple components, the chemical analysis approach is particularly desirable and often the first step in deciphering a complex chemical signal (Sun and Müller-Schwarze, 1998a,b).

Small carnivores, especially the genus *Mustela*, are amongst the most intensively studied groups in mammals with respect to chemical communication. Chemical secretions from the anal gland have been chemically analyzed in seven species of *Mustela* (Albone, 1984; Brown and Macdonald, 1985; Zhang *et al.*, 2002b). Because of this, a wealth of information in these species has been accumulated for comparison and, thus, these species are valuable model systems in the study of mammalian chemical communication.

Anal gland volatiles of the following species in the genus *Mustela* have been chemically analyzed: the American mink, *M. vison* (Brinck *et al*., 1978, 1983; Sokolov *et al*., 1980); the stoat, *M. erminea* and the domestic ferret, *M. putorius furo*, in New Zealand (Crump, 1980a,b; Crump and Moore, 1985); the mountain weasel (*M. nivalis*); the European polecat, *M. putorius* (Brinck *et al*., 1983); the steppe polecat, *M. eversmanni*; and the Siberian weasel, *M. sibirica* (Zhang *et al*., 2002a,b). Most of the 18 major volatiles detected in their anal glands are unique to the genus *Mustela* and have not been found in other genera of the family Mustelidae (Gorman *et al*., 1978; Brinck *et al*., 1983; Crump and Moors, 1985). Among them are two nitrogen-containing

compounds, indole (in all *Mustela* species) and *o*-aminoacetophenone (in *M. erminea* only), with the remainder all being sulfur-containing compounds.

The main function of anal glands in mustelids is widely believed to be intraspecific communication. It is also possibly used for interspecific communication as well, because there are qualitative and quantitative differences between species in the chemical composition of anal gland secretions (Brinck *et al.*, 1983). However, few studies are available that compare anal gland volatiles within and between sympatric *Mustela* species and investigate their ecological and evolutionary significance associated with communication (Zhang *et al.*, 2002b). Additionally, the widely used, labor-intensive procedure of headspace sampling with solvent desorption and concentration by liquid gas blow may raise the question of reliability, because proportional distortion and escape of anal gland volatiles are likely (Zhang *et al.*, 2002a,b). For example, using this method we were unable to find indole in the secretions of the American mink (Zhang *et al.*, 2002a), the steppe polecat, or the Siberian weasel (Zhang *et al.*, 2002b). This nitrogen-containing compound is common to *Mustela* species and has also been identified in the anal gland secretion of the American mink (Brinck *et al.*, 1983; Sokolov *et al.*, 1980). It was therefore necessary to conduct a further investigation of anal gland volatiles using a more sensitive sampling method.

Sun and Müller-Schwarze (Sun and Müller-Schwarze, 1998a, 1999) postulate two general forms of information coding—digital and analog—corresponding to information coding by presence/absence of chemicals used for communication versus coding by varying amounts of these substances. In the genus *Mustela*, it has been reported (Crump, 1980a) that 2-ethylthietane and 3-ethyl-1,2-dithiacyclopentane are female-specific in stoats and inferred from gas chromatography (GC) profiles that 3-propyl-1,2-dithiacyclopentane seems more abundant in females. Thus, coding for sex information in stoats can be either digital or analog or both. There have also been several valuable attempts to determine how individuality is coded by chemically analyzing anal gland secretions in the mink and stoat (Brinck *et al.*, 1978; Erlinge, *et al.*, 1982; Clapperton *et al.*, 1988). The forms of coding and the generality of these findings might be substantiated by comparing situations in other mustelids, which are yet to be investigated.

In this study, we investigated the chemical composition and coding form—digital or analog or a combination of the two—in two closely related, sympatric species, the Siberian weasel (*M. sibirica fortanieri*) and the steppe polecat (*M. eversmanni admirata*). The two species overlap in ecological niches in North China (Gao, 1987). Specifically, we intended to achieve two research goals in this study. The first was to find previously undetected compounds and to quantify the proportions of anal gland volatiles in the two species by the solvent extract sampling method. The second was to explore the likely forms—digital or analog or both—of coding for information about species, sex and individuality in the two species.

Materials and methods

Collection and extraction

Anal glands along with anuses were removed fresh from steppe polecats (11 males and 10 females) and Siberian weasels (11 males and 11 females) within 12 h of their being captured by legal fur trappers in several farmland areas in central North China in midwinter (January) 2001. They were immediately frozen and then transferred to the laboratory and stored at –20°C until use. For both species, males with a baculum weight >350 mg and females with body wt >500 g were classified as adults and their anal glands were used (Sheng and Lu, 1976; Gao, 1987).

After thawing, we thoroughly cleaned every collected specimen using a 75% solution of ethyl alcohol, squeezed the anal gland sacs and collected the yellowish secretion around the two gland duct openings using a clear glass capillary pipette. We weighed the collected secretion and then immersed the pipette in redistilled dichloromethane in a glass vial in the proportion of 8 mg secretion in 1 ml dichloromethane. After 5 min, we removed the pipette and stored the sealed vials at –20°C for gas chromatography and mass spectrometry (GC–MS) analysis within 1 week.

GC–MS analysis

Analytical GC–MS was performed on a Finnigan GC–MS (Trace 2000 series) instrument. Xcalibur (Windows 2000) was used for data acquisition and processing. The initial area reject threshold was 20 000. The GC was equipped with a 30 m glass capillary column (internal diameter 0.25 mm) coated with DB5. Helium was used as the carrier gas at a flow rate of 1.0 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 follows: 50°C for 5 min initially, increased by 5°C/min up to 200°C and held at 220°C for 5 min and then increased again at 10°C/min up to 290°C and finally held at 290°C for 15 min. Electron impact ionization was used at 70 eV. Transfer line temperature was 250°C. The amount of sample injected was 1 µl every time.

We used parallel GC–MS analyses to compare GC retention times and MS spectra between previous (prepared by headspace trap and solvent desorption) and present samples (solvent extraction) of anal gland secretions from male steppe polecats. We identified the following compounds: (1) 2,2-dimethylthietane; (2) *Z*- or *E*-2,4-dimethylthietane; (3) *E*-2,3-dimethylthietane; (4) 2-ethylthietane; (5) *E*-2-ethyl-3-methylthietane; (7) *Z*-2-ethyl-3-methylthietane; (8) 2-propylthietane; (9) 3,3-dimethyl-1,2-dithiacyclopentane; and (12) *Z*-3,4-dimethyl-2,2-dithiacyclopentane. These compounds have previously been identified in headspace samples of anal gland secretions in steppe polecats (Zhang *et al.*, 2002b).

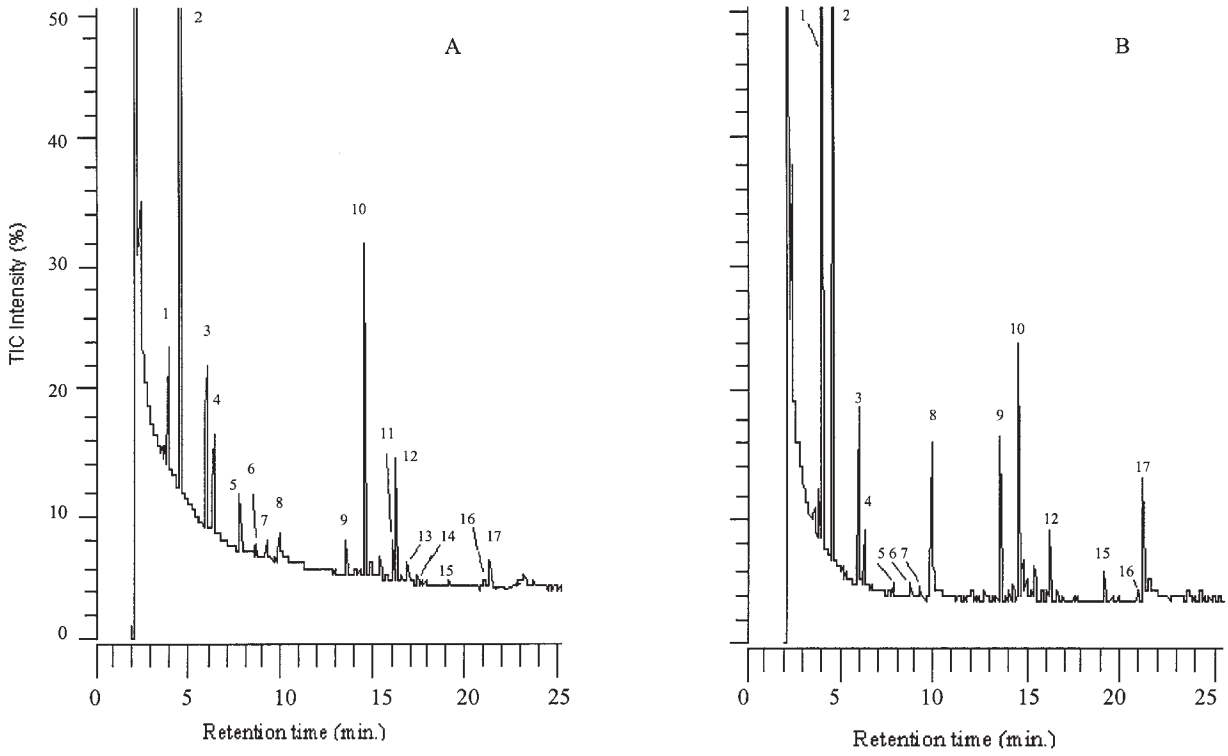

Figure 1 Chromatogram of volatile compounds from the anal gland secretion of male steppe polecats **(A)** and female Siberian weasels **(B)**. The number for each identified peak corresponds with peak numbers in Tables 1–3.

Similarly, indole has been identified in the American mink (Brinck *et al*., 1978, 1983; Sokolov *et al*., 1980). The parallel analysis of solvent-extracted samples of anal gland secretion in American minks led to the identification of (16) indole in steppe polecats and Siberian weasels.

We used the mass spectra available in the literature to aid the identification of the following compounds: (6) 2-isopropylthietane, (10) 3-ethyl-1,2-dithiacyclopentane, (11) *E*-3,4-dimethyl-1,2-dithiacyclopentane, (13) 2-pentylthietane, (14) 3- and (15) 4-propyl-1,2-dithiacyclopentane and (17) *o*-aminoacetophenone (Crump, 1980b; Sokolov *et al*., 1980; Brinck *et al*., 1983; Crump and Moors, 1985).

We used the method developed previously (Sun and Müller-Schwarze, 1998b) to quantify the relative abundance of relevant compounds. That is, we converted the peak area of a particular compound into the percentage of the summed peak areas of all 17 compounds in steppe polecats and 14 compounds in female and 13 in male Siberian weasels in a sample, respectively. The statistical comparisons of the levels of excreted volatiles among two species and two sexes were made using one-way analysis of variance (ANOVA) with the *post hoc* Bonferoni *t*-test. The level of significance was set at $P < 0.05$ for all tests. The statistical analysis was conducted in SPSS v. 6.0.

To determine the variability of the volatile compositions between individuals, relative standard deviation (RSD) was used and calculated using the following formula:

$$\text{RSD} = (\text{SD}/\text{mean}) \times 100$$

where mean and SD are the average of each volatile peak area percentage for all same-sex individuals and their standard deviation, respectively.

Results

Anal gland volatiles

GC–MS analysis of individual samples revealed 17 total ion chromatogram (TIC) peaks in both sexes of steppe polecats. Fourteen TIC peaks (absence of peaks 11, 13 and 14) in females and 13 TIC peaks (one more absent peak: 7) in males of Siberian weasels were detected within 25 min (Fig. 1 and Table 1).

Using parallel GC–MS analysis of previous samples (prepared by headspace trap and solvent desorption) of male steppe polecats and comparing their retention times and MS data with the previous results (Zhang *et al*., 2002b), we identified nine compounds: (1) 2,2-dimethylthietane; (2) *Z*- or *E*-2,4-dimethylthietane; (3) *E*-2,3-dimethylthietane; (4) 2-ethylthietane; (5) *E*-2-ethyl-3-methylthietane; (7) *Z*-2-ethyl-3-methylthietane; (8) 2-propylthietane; (10) 3,3-dimethyl-1,2-dithiacyclopentane; and (12) *Z*-3,4-dimethyl-2,2-dithiacyclopentane. Comparing their retention times and MS data with the GC–MS data from the solvent-extracted samples of polecats and the weasels, we identified

Table 1 GC–MS data and identified compounds in anal gland volatiles of the steppe polecat (*M. eversmanni*) and Siberian weasel (*M. sibirica*)

Peak no.	Retention time	Mass spectrum data [*m/z* values (ion intensities)]	Mol. wt	Formula	Identification
1	4.00	102 (100), 87 (35), 74 (36), 69 (38), 68 (25), 67 (16), 60 (15), 56 (92), 41 (85)	102	$C_5H_{10}S$	2,2-dimethylthietane
2	4.60	102 (55), 87 (7), 74 (2), 73 (1), 69 (7), 68 (3), 67 (4), 60 (100), 61 (12), 59 (17), 56 (30), 45 (18), 41 (27)			*Z*-or *E*-2,4-dimethylthietane
3	5.90	102 (50), 87 (5), 74 (3), 73 (2), 69 (6), 67 (3), 61 (15), 60 (100), 59 (20), 56 (23), 41 (28)			*E*-2,3-dimethylthietane
4	6.30	102 (100), 87 (45), 74 (70), 73 (48), 69 (15), 68 (32), 67 (20), 61 (8), 60 (20), 59 (22), 56 (27), 55 (47), 45 (62), 41 (72)			2-ethylthietane
5	7.80	116 (58), 101 (3), 87 (8), 74 (100), 73 (4), 69 (18), 68 (2), 67 (8), 60 (2), 59 (13), 55 (25), 45 (22), 41 (57)	116	$C_6H_{12}S$	*E*-2-ethyl-3-methylthietane
6	8.7	116 (100), 101 (70), 86 (2), 84 (4), 74 (23), 70 (10), 69 (40), 60 (10), 59 (40), 56 (25), 55 (40), 45 (35), 41 (77)			2-isopropylthietane
7[b]	9.20	116 (26), 101 (2), 88 (2), 87 (5), 86 (7), 74 (47), 73 (12), 69 (17), 67 (12), 56 (8), 55 (20), 44 (100), 41 (40)			*Z*-2-ethyl-3-methylthietane
8	9.88	116 (100), 101 (36), 88 (17), 87 (66), 86 (2), 74 (3), 73 (45), 69 (32), 67 (53), 55 (45), 41 (46)			2-propylthietane
9	13.5	134 (35), 105 (2), 101 (5), 88 (1), 86 (2), 70 (8), 69 (100), 67 (5), 59 (10), 57 (9), 56 (5), 55 (12), 53 (5), 41 (51)	134	$C_5H_{10}S_2$	3-ethyl-1,2-dithiacyclopentane
10	14.5	134 (40), 105 (1), 101 (1), 94 (1), 88 (1), 85 (1), 70 (10), 69 (100), 67 (4), 59 (7), 57 (2), 56 (2), 55 (16), 53 (3), 41 (48)			3,3-dimethyl-1,2-dithiacyclopentane
11[a]	16.05	134 (46), 105 (10), 101 (6), 87 (1), 85 (3), 70 (10), 69 (100), 67 (6), 59 (5), 57 (7), 56 (1), 55 (8), 53 (3), 41 (75)			*E*-3,4-dimethyl-1,2-dithiacyclopentane
12	16.23	134 (23), 101 (1), 94 (1), 88 (1), 86 (2), 70 (8), 69 (100), 67 (5), 59 (7), 57 (2), 56 (2), 55 (15), 53 (4), 41 (47)			*Z*-3,4-dimethyl-1,2-dithiacyclopentane
13[a]	16.92	144 (32), 115 (100), 101 (29), 87 (57), 81 (28), 73 (40), 67 (28), 60 (40), 55 (63), 45 (37), 41 (48)	144	$C_8H_{16}S$	2-pentylthietane
14[a]	17.39	148 (58), 115 (1), 106 (3), 101 (3), 83 (68), 69 (15), 67 (3), 64 (7), 57 (13), 55 (100), 41 (46)	148	$C_6H_{12}S_2$	3- or 4-propyl-1,2-dithiacyclopentane
15	19.06	148 (50), 115 (35), 114 (10), 106 (8), 101 (5), 83 (88), 81 (7), 74 (18), 69 (22), 67 (6), 64 (5), 57 (7), 56 (35), 55 (100), 45 (20), 43 (25), 42 (26), 41 (23)			4- or 3-propyl-1,2-dithiacyclopentane
16	21.02	117 (100), 90 (33), 89 (25), 73 (16), 69 (12), 63 (7), 59 (7), 58 (6), 55 (7), 49 (5), 39 (6)	117	C_8H_7N	indole
17	21.13	135 (85),120 (100), 92 (42), 65 (28)	135	C_8H_9NO	*o*-aminoacetophenone

[a]Absent in both sexes of *M. sibirica*.

[b]Present only in females of *M. sibirica*.

these nine compounds in the two *Mustela* species (Table 1 and Fig. 2).

Through parallel analysis of the anal gland secretion from the American mink with the same sample preparation method as used in this study, we identified peak 16 as indole in *M. eversmanni* and *M. sibirica*, since the retention time and MS data were the same as those of indole in American minks (Sokolov *et al.*, 1980; Brinck, *et al.*, 1983) (Table 1 and Fig. 2)

Analysis of the mass spectrum implied that peak 6 is the fourth isomer of 2-propylthietane. The loss of *m/z* 101 and *m/z* 74 suggested the presence of methyl and propyl, respectively; in addition, the absence of *m/z* 87, 88 implied the absence of ethylene. This suggested that the compound was 2-isopropylthietane, which has been identified in ferrets, *M. putorius* forma *furo* (Crump and Moors, 1985) (Table 1 and Fig. 2).

In peak 9, the loss of *m/z* 101, *m/z* 69 and *m/z* 55 implied the presence of HS, HS_2 and CH_3S_2, respectively, which implied a dithiacyclopentane ring. In addition, the loss of *m/z* 105 implied an ethyl. These data suggested 3-ethyl-1,2-dithiacyclopentane (Crump, 1980a) (Table 1 and Fig. 2).

Peak 11 had a similar MS spectrum to peak 12 and a shorter retention time than *Z*-3,4-dimethyl-1,2-dithiacyclo-

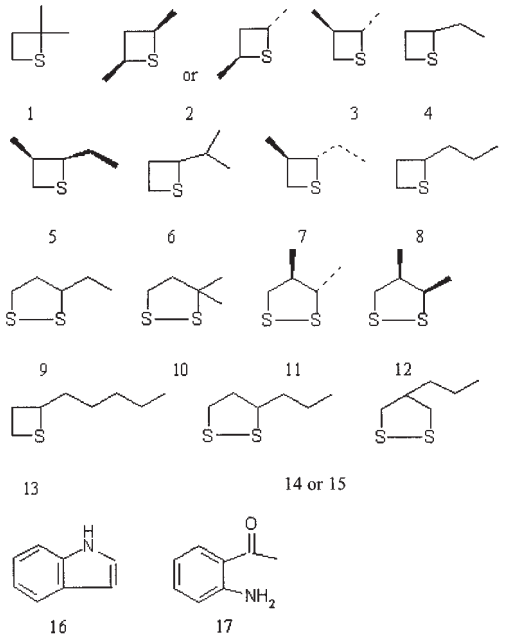

Figure 2 Structures of volatile compounds from the anal gland secretion of steppe polecats and Siberian weasels. The number for each structure corresponds with peak numbers in Figure 1 and Tables1–3.

pentane. This led us to identify peak 11 as *E*-3,4-dimethyl-1,2-dithiacyclopentane, as was identified by Crump (Crump, 1980b) in the ferret (Figs 1 2 and Table 1).

In peak 13, the loss of *m*/*z* 115, *m*/*z* 101, *m*/*z* 87 and *m*/*z* 73 implied ethyl, propyl, butyl and pentyl. These data implied an *n*-pentyl. Comparison of the mass spectrum with that of peak 8 (2-propylthietane) revealed a thietane structure with a pentyl side-chain. Peak 13 was identified as 2-pentyl-thietane (Crump, 1980a) (Table 1 and Fig. 2).

The GC and MS data for the peaks 14 and 15 showed that they both had a mol. wt of 148 and were similar to those of 3-propyl-1,2-dithiacyclopentane, as identified by Crump (Crump, 1980a) in stoats. The loss of *m*/*z* 115, *m*/*z* 83 and *m*/*z* 69 implied HS, HS_2 and CH_3S_2, which implied a dithiacyclopentane ring. In addition, the loss of *m*/*z* 106 implied a propyl. These data suggested that the two peaks were 4- or 3-propyl-1,2-dithiacyclopentane (Crump, 1980a) (Table 1 and Fig. 2).

The MS data for peak 17 were identical with *o*-amino-acetophenone, which was found only in the stoat (Crump, 1980a; Brinck *et al*., 1983) (Table 1 and Fig. 2).

Intraspecific differences

Sex differences

In the steppe polecat, seven compounds showed quantitative differences between the two sexes. Compounds 1, 9 and 17 were all higher in females, whereas compounds 2, 3, 12 and 13 were all higher in males. However, no sex-specific compounds were observed (Table 2). In the Siberian weasel, compound 4 was detected in 7 out of 11 females, but not in any males. Also, the relative abundances of compounds 9, 10, 15 and 16 were significantly higher in females than in males. Furthermore, peak 17 was more abundant in males than in females (Table 2).

Individual differences

First, the presence or absence of some compounds in different combinations may qualitatively characterize different individuals. In the steppe polecat, some males did not have compounds 6, 7, 13, 14, 15, 16 or 17 and some females did not have compounds 6, 7, 8, 14 or 15 (Table 3). In the Siberian weasel, some males lacked compounds 3, 4, 5, 6, 7, 12, 15, 16 or 17 and some females lacked compounds 3, 4, 5, 6, 7, 8, 12, 15, 16, 17 or 18 (Table 3).

Secondly, most compounds had extremely high RSDs. Generally, RSDs between duplicate GC–MC experiments are <10. With the exception of RSDs of compounds 2, 3 and 15, which in male steppe polecats were 16.91, 18.42 and 16.27, respectively, the RSDs of the remaining compounds ranged from 33.45 to 207.89 (Table 2, $n = 10$ or 11). This implied that some volatiles of anal gland secretions in both *Mustela* species may be quantitatively different among different individuals (Table 3).

Interspecific differences

In males, four compounds (7, 11, 13 and 14) were present only in the polecat and were absent in the weasel. For the remaining 11 compounds found in both species, nine differed significantly in quantity. Compounds 1, 9 and 17 were significantly lower and compounds 2, 3, 4, 5,10, 12, 15 and 16 higher in male polecats than in male weasels. Compounds 6 and 8 showed no significant differences between males of the two species (Table 2)

In females, three compounds (peaks 11, 13 and 14) were present only in the polecat and were absent in the weasel. However, only compound 9 was lower in female polecats than in female weasels and other compounds common to the two species (1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 16 and 17) showed no significant differences in quantity (Table 2).

Between male polecats and female weasels, compounds 11, 13 and 14 were unique to the polecat. Compounds 1, 9 and 17, though present in both, were less abundant and compounds 2 and 3 were more abundant in the polecat than in the weasel (Table 2).

Between female polecats and male weasels, compounds 7, 11, 13 and 14 were not found in male weasels. Female steppe polecats had a higher quantity in peaks 3, 4, 5, 10 and 16, and a lower quantity in peaks 1 and 17 (Table 2).

Discussion

Besides the previously identified nine volatiles in the two *Mustela* species, which were 2,2-dimethylthietane (1), *Z*- or *E*-2,4-dimethylthietane (2), *E*-2,3-dimethylthietane (3), 2-ethylthietane (4), *E*-2-ethyl-3-methylthietane (5), *Z*-2-ethyl-3-methylthietane (7), 2-propylthietane (8), 3,3-dimethyl-

Table 2 Sex differences and inter-specific variation in relative abundance of anal gland volatiles of the steppe polecat and the Siberian weasel

Peak no.	Standard percentage of GC profile areas of volatile compounds in the steppe polecat (mean ± SD)%		Standard percentage of GC profiles area of volatile compounds in the Siberian weasel (mean ± SD)%	
	Males (n = 11)	Females (n = 10)	Males (n = 11)	Females (n = 11)
1	8.22 ± 3.61^{ace}	29.20 ± 21.55^{af}	49.31 ± 19.65^{cf}	36.22 ± 18.09^{e}
2	42.47 ± 7.18^{ace}	17.10 ± 9.35^{a}	10.06 ± 6.67^{c}	13.81 ± 8.03^{e}
3	7.49 ± 1.38^{ace}	4.05 ± 2.02^{af}	2.05 ± 1.23^{cf}	3.38 ± 3.57^{e}
4	5.35 ± 1.79^{c}	7.69 ± 8.55^{f}	0.65 ± 0.69^{cf}	6.29 ± 11.57
5	4.21 ± 2.41	2.16 ± 2.34	0.05 ± 0.09	2.20 ± 4.28
6	0.27 ± 0.22	0.22 ± 0.20	0.28 ± 0.27	0.32 ± 0.43
7	1.29 ± 0.75	0.71 ± 0.67	–	0.74 ± 1.53
8	3.11 ± 2.30	2.57 ± 2.13	3.37 ± 3.37	2.20 ± 2.38
9	2.58 ± 1.30^{ace}	5.28 ± 2.36^{ad}	4.78 ± 3.21^{bc}	9.92 ± 6.34^{bde}
10	12.01 ± 5.17^{ce}	7.35 ± 6.57	2.65 ± 2.41^{bc}	7.28 ± 4.72^{be}
11	1.93 ± 2.00	6.95 ± 10.93	–	–
12	3.75 ± 1.52^{ac}	1.32 ± 1.41^{af}	0.21 ± 0.27^{cf}	2.74 ± 4.93
13	0.97 ± 0.76^{a}	0.10 ± 0.14^{a}	–	–
14	1.31 ± 2.11	0.64 ± 0.94	–	–
15	1.06 ± 1.23^{c}	2.18 ± 3.57	0.22 ± 0.29^{bc}	0.75 ± 0.77^{b}
16	3.69 ± 3.04^{c}	7.55 ± 5.97^{f}	1.18 ± 1.12^{bcf}	6.34 ± 7.45^{b}
17	0.29 ± 0.79^{ace}	4.92 ± 4.79^{af}	25.19 ± 23.24^{bcf}	7.82 ± 6.84^{be}

The areas of undetectable compounds are taken as zero.

The means in a row marked by the same superscript letters (a–f) are significant at the 0.05 level, using one-way ANOVA and the *post hoc t*-test.

1,2-dithiacyclopentane (9) and *Z*-3,4-dimethyl-2,2-dithiacyclopentane (12) (Zhang *et al.*, 2002b), eight new compounds were detected: 2-isopropylthietane (6), 3-ethyl-1,2-dithiacyclopentane (10), *E*-3,4-dimethyl-1,2-dithiacyclopentane (11), 2-pentylthietane (13), 3- (14) or 4-propyl-1,2-dithiacyclopentane (15), indole (16) and *o*-aminoacetophenone (17). These compounds have been previously identified in other *Mustela* species, but not in the steppe polecat and the Siberian weasel, with the exception of 4-propyl-1,2-dithiacyclopentane (Crump, 1980b; Sokolov *et al.*, 1980; Brinck *et al.*, 1983; Crump and Moors, 1985; Zhang *et al.*, 2002b). It appears that most of the newly detected compounds in this study are relatively low in concentration or volatility. For example, the two nitrogen-containing compounds, indole (16) and *o*-aminoacetophenone (17), both have a high mol. wt despite their high abundance. Also, the concentrations in male Siberian weasels of 2-isopropylthietane (6) and 2-ethylthietane (4) are low, despite their higher volatility. This indicates that the failure to detect these compounds could be ascribed to the insensitivity of headspace sampling with solvent-desorption.

In a chemical information coding study, Sun and Müller-Schwarze (Sun and Müller-Schwarze, 1998a, 1999) first proposed the idea of digital and analog coding to distinguish the two forms of information coding: presence/absence of chemicals versus varying amounts of shared chemicals. This distinction has greatly clarified and simplified our discussion on information coding in mammals. The presence in steppe polecats and absence in Siberian weasels of peaks 11, 13 and 14 could be used as cues for species discrimination. These differences might be sufficient for discrimination between the two species and, thus, the coding for species information could be most likely digital. Besides digital coding, analog coding by varying the amounts of some shared compounds might also be used for inter-specific communication in some *Mustela* species (Brinck *et al.*, 1983), but we did not find the common differences in quantity amongst opposite- and/or same-sexes of the two *Mustela* species. Only the compounds with stable and common differences might code for the difference between species.

Volatiles from the anal gland among species of *Mustela* are similar and most of the compounds, especially the sulfur-containing compounds, are genus-specific. These compounds have not been found in other genera of family Mustelidae and other families in the order Carnivora (Brinck *et al.*, 1983; Albone, 1984; Raymer, 1984; Brown and Macdonald, 1985; Buesching *et al.*, 2002a,b). There is a substantial amount of evidence for the positive correlation between relatedness and similarity in the chemical composition in the anal gland secretions between conspecific individuals in beavers (*Castor canadensis*) (Sun and Müller-Schwarze, 1998a,b), lions (*Panthera leo*) (Anderson

Table 3 Individual variation in relative abundance of anal gland volatiles of the steppe polecat and the Siberian weasel

Peak no.	Steppe polecat (RSD)		Siberian weasel (RSD)	
	Males (n = 11)	Females (n = 10)	Males (n = 11)	Females (n = 11)
1	43.91 (0)	73.80 (0)	39.84 (0)	49.93 (0)
2	16.91 (0)	54.68 (0)	66.29 (0)	58.16 (0)
3	18.42 (0)	49.88 (0)	59.96 (1)	105.57 (2)
4	33.45 (0)	111.18 (0)	105.63 (4)	184.01 (3)
5	57.24 (0)	108.33 (0)	184.4 (8)	194.81 (8)
6	81.27 (3)	90.83 (4)	96.44 (4)	133.81 (6)
7	58.24 (1)	94.37 (2)	– (11)	207.89 (7)
8	73.95 (0)	32.76 (2)	100.00 (0)	108.29 (3)
9	50.48 (0)	44.66 (0)	67.25 (0)	64.02 (0)
10	43.01 (0)	89.39 (0)	90.68 (0)	64.82 (0)
11	103.63 (0)	157.21 (0)	–	–
12	40.37 (0)	106.82 (0)	129.02 (5)	179.58 (3)
13	78.69 (2)	139.02 (0)	–	–
14	160.99 (2)	145.86 (1)	–	–
15	16.27 (5)	163.54 (1)	131.74 (6)	102.60 (4)
16	82.28 (1)	79.07 (0)	95.31 (2)	117.53 (2)
17	270.79 (5)	97.44 (0)	92.27 (2)	87.44 (1)

The figures in parentheses indicate the numbers of individuals from which that compound was undetectable.

and Vulpius, 1999) and badgers (*Meles meles*) (Buesching *et al*., 2002a). One would expect that this relationship might also occur above the species level. That is, the closer the two species are related phylogenetically, the more similar the composition of the anal gland secretion might be. This, however, turns out not to be the case in *Mustela* and other related genera. For example, ferrets (*M. purorius* forma *furo*) are closely related to and can interbreed with the European and steppe polecats, but we cannot determine their phylogentic relationship with stoats and weasels by comparing the volatiles in the anal gland secretion (Brinck *et al*., 1983; Crump and Moors, 1985). The steppe polecat shares 14 volatile compounds with the Siberian weasel and 11 with the stoat, but only eight with ferrets and three or four with the European polecat (Brinck *et al*., 1983; Schildknecht and Birkner, 1983; Crump and Moors, 1985). Two other factors will have to be considered before we can determine whether there is a correlation between phylogenetic relationship and similarity in the anal gland secretion in mustelids. One is the effect of microbial community (Zhang *et al*., 2002b) and the other is the role of high mol.-wt compounds and odor-binding proteins.

Our results also demonstrate that the anal gland secretion contains a wealth of information characteristic of sexes and individuals in the two *Mustela* species. In the Siberian weasel, both the presence or absence of sex-specific compounds (*Z*-2-ethyl-3-methylthietane only in females) and relative abundance of some compounds between males and females could be used to code for information about sex. Thus, the coding for information about sex could be either digital or analog or both. In steppe polecats, however, only quantitative differences provide the possibility of analog coding for inter-sexual communication. Some studies have also shown that other carnivores use either or both of the two forms to code for sex information. For example, in red foxes (*Vulpes vulpes*), quinaldine is male-specific in the urine (Jorgenson *et al*., 1978). In stoats, inter-sexual communication might be based on the qualitative differences (digital) in 2-ethylthietane and 3-ethyl-1,2- dithiacyclopentane and quantitative differences (analog) in 3-propyl-1,2-dithiacyclopentane (Crump, 1980a). Female ferrets have high concentrations of 2,3-dimethylthietane and/or 3,4-dimethyl-1,2-dithiacyclopentane, while males have high concentrations of indole (Clapperton *et al*., 1988). In lions, there are no sex-specific compounds, but 2-butanone is higher and acetone is lower in the urine of males versus females (Andersen and Vulpius, 1999).

Coding for individual information may take either or both of these two forms: presence or absence of some compounds (digital) and/or difference in the relative abundance of some compounds (analog) among individuals. The two *Mustela* species in this study might take both forms, analog and digital coding, to code for individual information. In ferrets, Clapperton *et al*. (Clapperton *et al*. 1988) identified an inter-individual variation in the combination of five compounds of anal gland secretions (analog) and also noted consistent qualitative differences (digital) in the color of anal gland secretions of different individuals, but without supporting GC–MS data. In lions, no two individuals had an identical compound composition in urine; therefore, it is possible that the compound composition of a scent mark alone could provide information about individuality (Andersen and Vulpius, 1999), corresponding to the digital coding. In female marmoset monkeys (*Callithrix jacchus*), there are few qualitative differences in the chemical composition of circumgenital scent marks among individuals. Instead, quantitative differences might be the key for distinguishing individuals (Smith *et al*., 2001) and such coding for individuality would be digital.

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