Chem. Senses 32: 293–303, 2007

doi:10.1093/chemse/bjl058
Advance Access publication January 23, 2007

Putative Chemical Signals about Sex, Individuality, and Genetic Background in the Preputial Gland and Urine of the House Mouse (*Mus musculus*)

Jian-Xu Zhang[1], Xiao-Ping Rao[2], Lixing Sun[3], Cheng-Hua Zhao[1] and Xiao-Wei Qin[1]

[1]State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, 25 Beisihuanxi Road, Haidian District, Beijing 100080, China, [2]Department of Biology, Beijing Normal University, Beijing 100875, China and [3]Department of Biological Sciences, Central Washington University, Ellensburg WA98926-7537, USA

Correspondence to be sent to: Jian-Xu Zhang, State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, 25 Beisihuanxi Road, Haidian District, Beijing 100080, China. e-mail: zhangjx@ioz.ac.cn

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Abstract

To explore whether preputial gland secretions and/or urine from the house mouse (*Mus musculus*) can be used for coding information about sex, individuality, and/or the genetic background of strain [ICR/albino, Kunming (KM), and C57BL/6], we compared the volatile compositions of mouse preputial glands and urine using a combination of dichloromethane extraction and gas chromatography coupled with mass spectrometry (GC–MS). Of the 40 identified compounds in preputial gland secretions, 31 were esters, 2 sesquiterpens, and 7 alcohols. We failed to find any compound unique to a specific sex, individual, or strain. However, many low molecular weight compounds between the sexes, most compounds among individuals, and several compounds among the 3 strains varied significantly in relative ratios. These quantitative differences in preputial gland volatiles (analog coding) are likely to convey information about sex, individual, and the genetic background of mouse strain. We identified 2 new main and male-elevated compounds, 1-hexadecanol ($Z = 3.676$, $P = 0.000$, $N = 19$ in ICR; $Z = 3.576$, $P = 0.000$, $N = 18$) and 1-hexadecanol acetate ($Z = 3.429$, $P = 0.000$, $N = 19$ in ICR; $Z = 3.225$, $P = 0.001$, $N = 18$), which were eluted in GC chromatogram after the 2 sesquiterpens. They might also be potential male pheromones, in addition to the well-known *E*-β-farnesene and *E,E*-α-farnesene. Additionally, a few compounds including 1-hexadecanol also varied with strains and might also code for genetic information. Of the 9 identified volatile compounds in male urine, (*s*)-2-*sec*-butyl-4,5-dihydrothiazole and *R,R*-3,4-dehydro-*exo*-brevicomin are known urine-originated male pheromones from previous studies. We also detected 6-hydroxy-6-methyl-3-heptanone, a male urinary pheromonal compound, which had not been directly detected by GC–MS previously. Chemical analysis shows that the genetically more closely related ICR and KM strains had a higher similarity in the volatile compositions of preputial glands and urine than that between ICR or KM and C57BL/6. *R,R*-3,4-dehydro-*exo*-brevicomin, in particular, was sensitive to genetic shifts and differed in relative abundance among the 3 strains, whereas (*s*)-2-*sec*-butyl-4,5-dihydrothiazole differed between ICR or Km and C57BL/6. Hence, these 2 compounds might code for information about their genetic background.

Key words: house mouse, individual, information coding, *Mus musculus*, pheromone, preputial gland, sex, strain, urine

Introduction

Chemical communication is critical in rodent social interaction. In the house mouse (*Mus musculus*), numerous studies have revealed that preputial glands and urine are sources of a variety of pheromones (Novotny, Ma, Zidek, et al. 1999; Novotny 2003). Preputial glands are often better developed in males than in females and can be enhanced by androgen in some rodents (Brown 1985; Harvey et al. 1989). Among the definitively identified male pheromonal compounds in house mice, 2 preputial gland-originated sesquiterpens, *E*-β-farnesene and *E,E*-α-farnesene, can be excreted with urine to communicate information about sex and dominance and have the effect of estrous induction in grouped females (Harvey et al. 1989; Novotny et al. 1990; Jemiolo et al. 1991; Ma et al. 1999). Five urine-originated pheromonal components, *R,R*-3,4-dehydro-*exo*-brevicomin, (*s*)-2-*sec*-butyl-4,5-dihydrothiazole, 6-hydroxy-6-methyl-3-heptanone, 2,5-dimethylpyrazine, and 2-heptanone, have been found to convey information about sex, dominance, and reproductive status and serve the functions of promoting intermale aggression, inducing puberty, and suppressing estrus (Liebich et al. 1977; Novotny et al.

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1984,1985, 1986; Novonty, Ma, Jemiolo, et al. 1999; Novonty, Ma, Wiesler, et al. 1999; Wiesler et al. 1984; Jemiolo et al. 1985, 1986; Andreolini et al. 1987; Ma et al. 1998). Some compounds such as (*s*)-2-*sec*-butyl-4,5-dihydrothiazole (only in males) and 2,5-dimethylpyrazine (only in females) are sex specific, whereas others are shared by both sexes but differ in relative concentration. *R,R*-3,4-dehydro-*exo*-brevicomin and the 2 sesquiterpens, in particular, are usually considerably higher in males than in females, for which they were initially speculated as male pheromones. Indeed, they were later confirmed by their activities of sexual attractiveness, promotion of estrous cycle and aggression, and induction of the Lee-Boot effect (Jemiolo et al. 1985, 1986, 1991; Novotny et al. 1985, 1986; Schwende et al. 1986).

Other than sex recognition, aggression, and induction of reproduction, the above-mentioned components are poorly understood for their roles in the recognition of individual and genetic background (Schwende et al. 1984; Singer et al. 1997). Also, a previous gas chromatography coupled with mass spectrometry (GC–MS) analysis of preputial gland secretion revealed a large number of volatiles eluded after the 2 sesquiterpens in the gas chromatogram (Ninomiya et al. 1993). The presence of these compounds leads us to questions whether they are potential sex pheromones that have not been characterized and whether their composition may code for information regarding individuality, which is underpinned by the genetic makeup of mouse strain. To answer these questions, we can first conduct chemical analyses and then relate the qualitative and/or quantitative differences in especially volatile compounds to their corresponding behavioral, physiological, and genetic characteristics (Singer et al. 1997; Novotny, Ma, Zidek, et al. 1999; Zhang et al. 2003, 2005). Such approach has led to the discovery of several important pheromones and major histocompatibility complex (MHC) determined chemical signals in the house mouse (Novotny et al. 1984, 1986, 1990; Jemiolo et al. 1986; Harvey et al. 1989; Singer et al. 1997; Novonty, Ma, Jemiolo, et al. 1999). Chemical analysis also allows us to determine whether information in chemical signals is in the digital or analog form. The former refers to information coding using unique compounds (qualitative difference) whereas the latter varying amounts of shared compounds (Sun and Müller-Schwarze 1998a,b, 1999). Both forms have been found in chemical communication in especially rodents and primates (Epple et al. 1979; Singer et al. 1997; Sun and Müller-Schwarze 1998a,b, 1999; Novotny, Ma, Zidek, et al. 1999; Novotny 2003; Zhang et al. 2003, 2005).

In this study, we attempted a chemical analysis to explore whether and how information about sex, individuality, and/or genetic background of strain can be potentially coded via pheromones in the mouse. We hypothesized that preputial gland or urine contained information about sex, individuality, and genetic background (strain). This hypothesis necessarily predicts that the chemical composition of the 2 pheromone sources show a detectable sex, individual, and strain specificity. To test this prediction, we used GC and GC–MS analytical methods to compare the chemical compositions of preputial glands and urine from house mice.

Materials and methods

Subjects

Ten male and 10 female ICR and Kunming (KM) albino house mice (8 weeks old) were purchased from Weilitong-Lihua Laboratory Animal Company, Beijing, China. Five male adult C57BL/6 mice were obtained from the Laboratory of Reproductive Biology of the Institute of Zoology, Chinese Academy of Sciences. They were individually kept in plastic cages ($27 \times 12 \times 17$ cm) under the 14:10 h L:D light regime (light on at 6:00 PM) and at the temperature of 21 ± 0.2 °C. Food (standard mouse chow) and water were provided ad libitum. They were used for experiments after 4 weeks of acclimation. All subjects were virgin. Males were scrotal, and females had perforated vaginas. Females' estrous cycles were determined by vaginal smears.

Sample collection and extraction

To collect urine samples, we placed odor donors of 10 ICR, 10 KM, and 5 C57BL/6 male mice individually in a clean plastic mouse cage ($31.8 \times 20.2 \times 31.5$ cm) floored with a wire grid (0.5×0.5 cm) 1 cm above the bottom. We collected urine samples during 8:00 AM–11:00 AM of the dark phase within 4 consecutive days. Once an animal urinated, its urine was absorbed by a glass capillary, transferred into a vial, and stored in ice. The duration of urine collection for each mouse was 20 min. Potentially contaminated urine deposited next to feces was not collected.

Prior to extraction, we thawed each preputial gland at room temperature and collected its yellowish secretion into a clear vial by squeezing. We weighed the secretion and added dichloromethane into the vial in the proportion of 1-mg secretion in 10-µl dichloromethane. After 24 h, we removed the supernatant and stored the remaining solution at 0 °C until GC–MS analysis within 1 week. Preputial glands from 10 male and 9 female ICR, 9 male and female KM, and 5 C57BL/6 mice were used for GC–MS analysis.

To prepare urine samples for comparison between mouse strains, we mixed equal amounts of individual urine samples collected from males of the same strain to obtain 250 µl mixed urine sample for each of the 4 consecutive days when urine samples were collected. This gave us 4 mixed samples for each strain. The volume of individual urine in each mixed urine sample was thus equal to 250 µl divided by the number of individuals used for each strain. To extract urinary compounds, we added 250 µl dichloromethane into the vial containing 250 µl urine sample, stirred it thoroughly, stored it at −20 °C for 24 h, and transferred the lower part into

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another vial for chemical analysis (Novotny, Ma, Wiesler, et al. 1999).

Conditions of GC–MS analysis

Analytical GC–MS was performed on an Agilent Technologies Network 6890N GC system coupled with 5973 Mass Selective Detector with the library NIST 2002. Xcalibur (Windows XP) was used for data acquisition and processing. The GC was equipped with a 30-m glass capillary column (internal diameter 0.25 mm × 0.25 μm film) coated with HP5MS. Helium was used as the carrier gas at the flow rate of 1.0 ml/min. The temperature of the injector was set at 230 °C. The oven temperature was programed as follows: 100 °C (for preputial gland samples) or 50 °C (for urine samples) as the initial temperature, which was increased by 5 °C/min up to 180 °C and then by 1 °C/min up to 220 °C and held for 15 min. Finally, the temperature was increased to 230 °C and held for 10 min for post run to clean the column. Electron impact ionization was used at 70 eV. Transfer line temperature was 280 °C. Scanning mass ranged from 30 to 350 amu. The amount of sample injected was 1 μl every time in a split mode (10:1) for preputial gland secretion samples and a splitless mode for urine samples.

Compound identification

Tentative identification was undertaken by comparing the mass spectra of GC peaks with those in the MS library (NIST 2002) and published literature (Liebich et al. 1977; Novotny et al. 1984; Wiesler et al. 1984; Schwende et al. 1986; Ninomiya et al. 1993; Singer et al. 1997; Novotny, Ma, Jemiolo, et al. 1999; Novonty, Ma, Wiesler, et al. 1999). For esters, in particular, the ion m/z $(14 \times (n + 2) + 5)$ implies their saturated organic acid chain length and m/z $([14 \times \{n + 2\} + 5] - 2)$ indicates their monounsaturated acid chain length. Additionally, m/z $([14 \times \{n + 2\} + 5] - 18)$ or m/z $([14 \times \{n + 2\} + 5] - 2 - 18)$ is the respective accompanied ions. m/z M^+-$([14 \times \{n + 2\} + 5] - 1)$ (M^+ refers to molecular ion) and m/z M^+-$([14 \times \{n + 2\} + 5] - 1) - 2$ (where n is the carbon number in the organic acids) determines alcohol chain length and its double-bond numbers. These MS peaks diminish in intensity as the alcohol chains become more branched or unsaturated. The diagnostic fragments at m/z60, m/z M^+-29, and m/z M^+-43 or at m/z31 and m/z M^+-18 imply fatty acids and alcohols, respectively. The branched, unsaturated low positions of double bonds or *trans* isomers of acetates often have shorter retention times in the gas chromatogram than their straight-saturated chain counterparts separated by a nonpolar GC column. This information is helpful to discriminate between 2 isomers with identical mass spectra. Some compounds were ascertained by synthetic analogs. To characterize the difference between the sexes or strains, we quantitatively compared the GC retention times and MS data of corresponding GC peaks between the opposite sexes within a strain or the same sex between 2 strains.

Data analysis

We used the method developed previously (Sun and Müller-Schwarze 1998b; Zhang et al. 2003) to quantify the absolute and relative abundance of relevant compounds. In our case, we used the peak area as absolute abundance and converted the peak area of a particular compound into the percentage of the summed peak areas of a total of 42 GC peaks.

To determine the variability in volatile composition among individuals, we calculated the relative standard deviation (RSD) using the following formula: RSD = (SD/mean) × 100, where mean and SD are the average of each volatile peak area (in percentage) for all intact males and their standard deviation, respectively (Zhang et al. 2003). The RSD was then compared with the intrasample variation. This method takes the idea of signal-to-noise ratio to show whether a signal contains certain meaningful information (about sex or strain).

To test the significance in difference, we first tested for the normality of the pooled data. For normally distributed data, one-way ANOVA was used followed by Bonferroni post hoc *t*-test. Such data included organ weights and the abundances of some preputial gland compounds numbered as 9, 13, 14, 16, 20, 21, 22, 24–28, 33, 35–38 and 42 in Table 2 and all urinary compounds except compound 6 in Table 4. For non-normal data, we used nonparametric tests. Specifically, Mann–Whitney *U* test was used for the abundance of other compounds in Tables 2 and 4. Comparison of sexual differences in the abundances of the 42 compounds in Table 2 was only conducted between opposite sexes for each strain. Wilcoxon matched-pairs signed-rank test was used for RSDs. All statistical analyses were conducted using SPSS (Version 10.0), and the level of significance was set at $\alpha = 0.05$ for all tests.

Results

Comparison of preputial gland size between the sexes and strains

Between the sexes, the absolute and relative weights of preputial glands were almost 10 times heavier in males than those in females for both ICR and KM strains. However, no significant difference was found in males among the 3 strains or in females between the ICR and KM strains (Table 1).

Detected and identified compounds

We tentatively identified 40 compounds from the 42 GC peaks with 2 still unknown from preputial glands. Two sesquiterpens (1, 2) were definitively identified in previous studies (Schwende et al. 1986; Harvey et al. 1989; Novotny et al. 1990). Peaks 5, 6, 10, 17, and 24 were identified and verified by synthetic analogs as 1-tetradecanol, *Z*-5-tetradecenol acetate, 1-tetradecanol acetate, 1-hexadecenol, 1-hexadecanol acetate, and 1-octadecanol acetate, respectively. The remaining compounds were tentatively identified as esters (Figure 1).

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Table 1 Comparison of the weights of body and preputial gland between sexes and strains of house mice (mean ± SD)

	ICR mice		KM mice		C57BL/6 mice
	Male (n = 10)	Female (n = 10)	Male (n = 9)	Female (n = 10)	Male (n = 5)
Body weight (g)	37.64 ± 1.18^{a,e}	30.76 ± 1.33^{c,e}	40.69 ± 1.30^{a,b,d}	33.19 ± 1.54^{c,d}	34.06 ± 5.20^{b}
Weight of paired preputial glands (mg)	126.84 ± 21.21^{a}	12.67 ± 3.76^{a}	124.69 ± 13.66^{b}	9.42 ± 6.01^{b}	89.37 ± 62.83
Relative weight of paired preputial glands (mg/g body weight)	3.37 ± 0.57^{a}	0.41 ± 0.13^{a}	3.07 ± 0.38^{b}	0.29 ± 0.19^{b}	2.53 ± 1.40

The means ± SDs in one row marked by the same superscript letters (a–e) are significant at the 0.05 level, using one-way ANOVA followed by Bonferroni post hoc t-test.

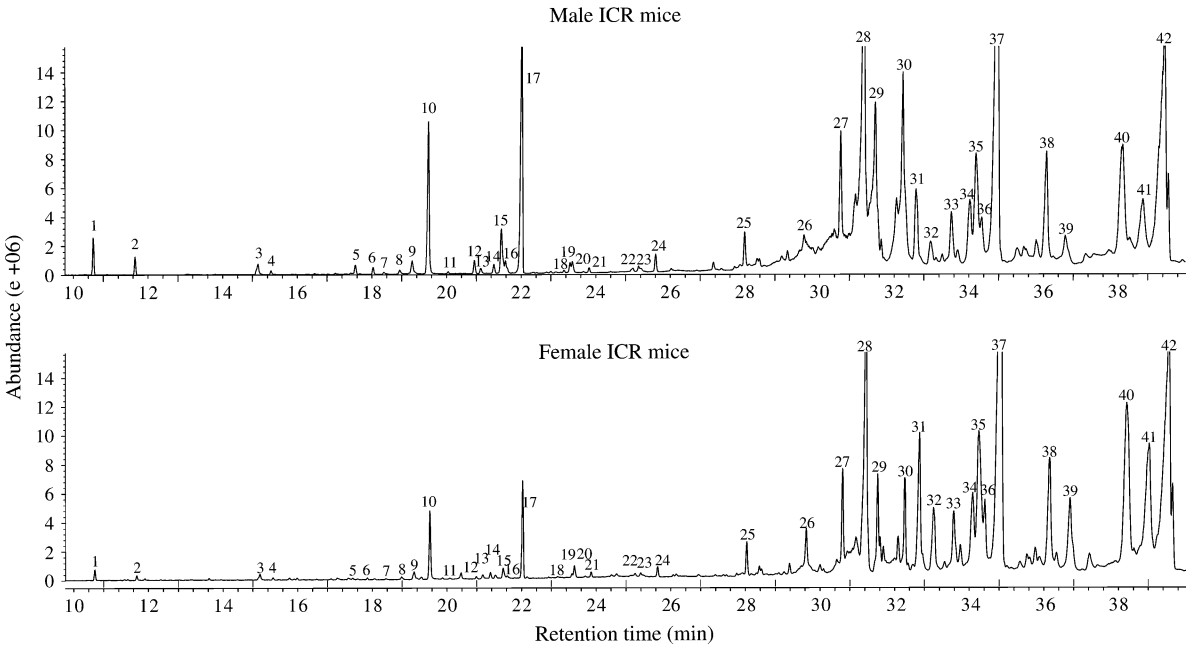

Figure 1 Representative gas chromatogram of the volatile compounds from the preputial gland secretion of male (top panel) and female (bottom panel) ICR house mice. The numbers of GC peaks correspond with peak numbers in Table 2.

We identified peak 9 as 6-hydroxy-6-methyl-3-heptanone in equilibrium with its corresponding lactol (5,5-dimethyl-2-ethyltetrahydrofuran-2-ol), which was implied by the losses of m/z 129(78), m/z 115(85), m/z 97(80), m/z 59(26), m/z 57(100), and m/z 43(68) (Novotny, Ma, Jemiolo, et al. 1999; Novonty, Ma, Wiesler, et al, 1999; Wiesler D, personal communication) (Figure 2).

Intrastrain difference in preputial gland volatile composition

Sexual differences

The 42 detected GC peaks were almost always present in all experimental mice irrespective of their amounts. Among them, 21 GC peaks (1–6, 8–17, 22–24, 27, and 28) were significantly larger and 6 (26, 32, 35, and 39–41) smaller in males than their counterparts in females. The remaining peaks did not display sexual difference in ICR mice (Table 2). Similarly, 18 GC peaks (1–6, 9, 10, 12, 14–18, 24, 25, 28, and

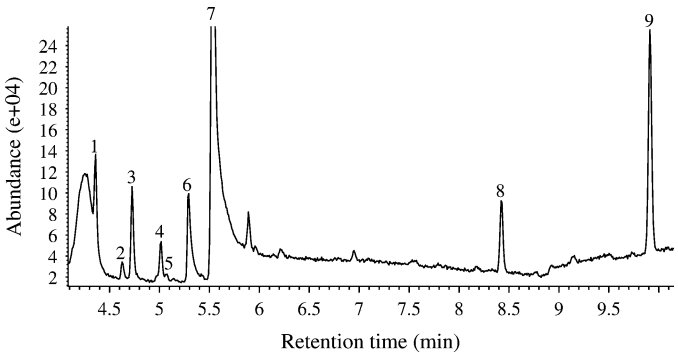

Figure 2 Representative gas chromatogram of the volatile compounds from the urine of male ICR house mice. The numbers of GC peaks correspond with peak numbers in Tables 4.

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Table 2 Comparison of relative abundance of preputial gland volatiles between same sexes of intrastrains or opposite sexes of interstrains in the house mouse (mean ± SD)

GC peak	RT	Compounds	ICR mice		KM mice		C57BL/6 mice	
			Male (n = 10)	Female (n = 9)	Male (n = 9)	Female (n = 9)	Male (n = 5)	Five duplicates of one male samples
1	9.72	*E*-β-farnesene★	0.49 ± 0.15*	0.21 ± 0.09*	0.43 ± 0.14*	0.14 ± 0.06*	0.43 ± 0.46	1.09 ± 0.05
2	10.84	*E,E*-α-farnesene★	0.26 ± 0.09*	0.09 ± 0.05*	0.21 ± 0.08*	0.05 ± 0.03*	0.23 ± 0.27	0.64 ± 0.03
3	14.11	*Z*-7-tetradecen-1-ol	0.28 ± 0.09*	0.12 ± 0.03*	0.30 ± 0.17*	0.14 ± 0.12*	0.19 ± 0.20	0.26 ± 0.02
4	14.50	1-Tetradecanol★	0.07 ± 0.03*	0.03 ± 0.01*	0.07 ± 0.03*	0.04 ± 0.02*	0.04 ± 0.04	0.05 ± 0.007
5	16.73	*Z*-5-tetradecenol acetate★	0.11 ± 0.05*	0.02 ± 0.01*	0.11 ± 0.05*	0.01 ± 0.01*	0.06 ± 0.06	0.13 ± 0.009
6	17.20	1-Tetradecanol, acetate★	0.07 ± 0.03*	0.01 ± 0.008*	0.08 ± 0.03*	0.02 ± 0.01*	0.05 ± 0.04	0.10 ± 0.004
7	17.53	11-Hexadecen-1-ol, (*Z*)-	0.06 ± 0.06	0.02 ± 0.007	0.07 ± 0.1	0.02 ± 0.02	0.01 ± 0.02	0.04 ± 0.01
8	17.95	Branched 1-hexadecanol	0.11 ± 0.10*	0.04 ± 0.01*	0.09 ± 0.05[a]	0.05 ± 0.03	0.03 ± 0.02[a]	0.06 ± 0.01
9	18.26	9-Hexadecen-1-ol, (*Z*)-	0.37 ± 0.11[a]*	0.13 ± 0.04*	0.35 ± 0.12[b]*	0.14 ± 0.11*	0.17 ± 0.14[a,b]	0.31 ± 0.02
10	18.70	1-Hexadecanol★	3.40 ± 0.71[a]*	1.00 ± 0.20*	3.35 ± 0.71b*	0.85 ± 0.27*	1.84 ± 1.02[a,b]	2.84 ± 0.13
11	19.25	1-Pentadecanol acetate	0.04 ± 0.01*	0.02 ± 0.007*	0.04 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.05 ± 0.005
12	19.93	*Z*-9-hexadecen-1-ol acetate	0.19 ± 0.05[a]*	0.05 ± 0.02*	0.18 ± 0.06*	0.05 ± 0.03*	0.11 ± 0.07[a]	0.20 ± 0.01
13	20.10	1-Heptadecanol (branched ?)	0.10 ± 0.03*	0.05 ± 0.01*	0.09 ± 0.02[a]	0.07 ± 0.06	0.05 ± 0.02[a]	0.08 ± 0.01
14	20.44	1-Hexadecanol, acetate (branched)	0.14 ± 0.04*	0.05 ± 0.02*	0.14 ± 0.05*	0.08 ± 0.05*	0.10 ± 0.07	0.22 ± 0.02
15	20.65	Isomer of 11-hexadecen-1-ol, acetate, (*Z*)-	0.75 ± 0.19[a]*	0.21 ± 0.08*	0.77 ± 0.25*	0.20 ± 0.12*	0.46 ± 0.26[a]	0.80 ± 0.07
16	20.76	11-Hexadecen-1-ol, acetate, (*Z*)-	0.12 ± 0.03*	0.06 ± 0.08*	0.12 ± 0.03*	0.03 ± 0.03*	0.13 ± 0.08	0.26 ± 0.02
17	21.23	1-Hexadecanol, acetate★	5.23 ± 1.38*	2.39 ± 0.67*	5.60 ± 0.96*	2.59 ± 1.31*	5.49 ± 2.04	8.15 ± 0.29
18	22.36	1-Heptadecanol, acetate (branched)	0.10 ± 0.18	0.01 ± 0.01	0.04 ± 0.03*	0.02 ± 0.01	0.06 ± 0.04	0.03 ± 0.005
19	22.49	1-Heptadecanol, acetate (branched)	0.09 ± 0.07	0.05 ± 0.02	0.08 ± 0.05	0.07 ± 0.04	0.06 ± 0.009	0.10 ± 0.02
20	22.55	1-Octadecanol	0.18 ± 0.05[a]	0.15 ± 0.04	0.17 ± 0.02[b]	0.14 ± 0.04	0.09 ± 0.05[a,b]	0.10 ± 0.02
21	23.00	1-Heptadecanol, acetate	0.10 ± 0.04	0.13 ± 0.03	0.13 ± 0.04	0.11 ± 0.04	0.09 ± 0.03	0.12 ± 0.01
22	24.17	Dodecyl octanoate	0.10 ± 0.03*	0.07 ± 0.03*	0.11 ± 0.05[a]	0.09 ± 0.04	0.20 ± 0.02[a]	0.20 ± 0.02
23	24.32	*Z*-7-octadecen-1-ol acetate	0.09 ± 0.04*	0.05 ± 0.02*	0.07 ± 0.03	0.05 ± 0.02	0.11 ± 0.04	0.10 ± 0.04
24	24.78	Octadecyl acetate★	0.33 ± 0.07*	0.21 ± 0.04*	0.36 ± 0.05*	0.23 ± 0.07*	0.41 ± 0.08	0.46 ± 0.02
25	27.18	5-Tetradecen-1-ol, octanoate, (*Z*)-	0.75 ± 0.14[a,b]	0.70 ± 0.11	0.96 ± 0.13[a]*	0.70 ± 0.21*	0.99 ± 0.17[b]	0.97 ± 0.09
26	28.83	Tetradecen-1-ol nonanoate	0.49 ± 0.20*	1.00 ± 0.15*	0.58 ± 0.17*	0.85 ± 0.25*	0.45 ± 0.17	0.45 ± 0.10
27	29.81	Tetradecen-1-ol decanoate	2.38 ± 0.24*	1.97 ± 0.44*	2.39 ± 0.45	2.38 ± 0.60	1.97 ± 0.93	2.76 ± 0.12
28	30.45	Tetradecen-1-ol decanoate	11.14 ± 1.69[a]*	7.70 ± 2.22*	13.06 ± 1.53[a]*	8.04 ± 1.43*	10.57 ± 4.60	13.04 ± 0.37
29	30.70	Tetradecanol decanoate	2.63 ± 1.56	5.85 ± 9.75	2.21 ± 0.84	3.63 ± 2.36	5.24 ± 4.31	3.02 ± 0.78
30	31.42	Tetradecen-1-ol palmitate (?)	2.70 ± 1.22	3.23 ± 1.55	2.96 ± 1.48	2.75 ± 0.90	1.72 ± 0.95	2.11 ± 0.31
31	31.78	Tetradecanol dodecanoate	2.68 ± 0.51	5.55 ± 4.94	2.99 ± 0.51	4.59 ± 2.39	2.66 ± 0.63	2.70 ± 0.23
32	32.18	1-Pentadecanol decanoate	1.19 ± 0.27*	2.59 ± 0.68*	1.27 ± 0.23*	2.47 ± 0.53*	1.63 ± 0.85	1.15 ± 0.07
33	32.75	Hexadecen-1-ol decanoate	1.62 ± 0.44[a]	1.64 ± 0.45	1.39 ± 0.44*	2.10 ± 0.54*	1.14 ± 0.30[a]	1.37 ± 0.11

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Table 2 Continued

GC peak	RT	Compounds	ICR mice		KM mice		C57BL/6 mice	
			Male (n = 10)	Female (n = 9)	Male (n = 9)	Female (n = 9)	Male (n = 5)	Five duplicates of one male samples
34	33.23	Isomers of hexadecyl decanoate	1.72 ± 0.62	2.25 ± 0.58[a]	1.41 ± 0.42*	3.13 ± 0.89[a]*	1.45 ± 0.33	1.32 ± 0.22
35	33.40	Hexadecen-1-ol decanoate	2.89 ± 0.74*	3.75 ± 0.94*	3.06 ± 0.66	3.69 ± 0.61	2.42 ± 1.03	2.09 ± 0.16
36	33.55	Unknown	0.65 ± 0.18	0.78 ± 0.22	0.56 ± 0.11[a]*	0.80 ± 0.16*	0.94 ± 0.51[a]	0.67 ± 0.11
37	33.99	Hexadecyl decanoate	21.94 ± 2.06[a]	21.09 ± 2.40	23.41 ± 3.63	22.71 ± 1.78	25.58 ± 2.84[a]	22.93 ± 0.79
38	35.36	Branched hexadecyl undecanoate	4.59 ± 1.10	4.49 ± 0.96	3.99 ± 0.80*	5.14 ± 1.12*	4.33 ± 1.26	3.61 ± 0.18
39	35.90	Hexadecyl undecanoate	1.54 ± 0.45*	3.34 ± 1.00*	1.61 ± 0.53*	2.63 ± 0.74*	1.19 ± 2.46	1.11 ± 0.09
40	37.44	Branched hexadecyl dodecanoate	6.20 ± 1.40*	9.45 ± 2.10*	5.29 ± 0.95[a]*	9.72 ± 0.96*	7.00 ± 1.14[a]	5.85 ± 0.11
41	37.98	Unknown	2.84 ± 0.76[a]*	6.24 ± 1.54*	2.71 ± 0.60[b]*	5.86 ± 0.56*	3.93 ± 1.02[a,b]	2.88 ± 0.24
42	38.56	Hexadecyl dodecanoate	19.26 ± 3.67	13.08 ± 2.45	17.20 ± 2.55*	13.69 ± 2.17*	16.31 ± 2.96	15.55 ± 1.48

The same superscript letters (a or b) indicate that the data in same row have significant differences ($P < 0.05$) between strains for the same sexes, whereas the data in same row are marked by asterisks have significant differences ($P < 0.05$) between sexes of the same strains, using one-way ANOVA followed by Bonferroni post hoc t-test for compounds 9, 13, 14, 16, 20, 21, 22, 24–28, 33, 35–38 and 42, and Mann–Whitney U test for other data. The compounds marked by pentacles are definitively identified. RT, retention time.

42) were significantly larger and 9 (26, 32–34, 36, and 38–41) smaller in males than their counterparts in females in KM mice (Table 2).

Fifteen male-elevated peaks (1–6, 9, 10, 12, 14–17, 24, and 28) and 5 female-elevated peaks (26, 32, and 39–41) were shared by ICR and KM mice. Of these, most compounds that were higher in males than in females were early eluting in the gas chromatogram (prior to peak 26) with low molecular weights. Two major semivolatile compounds, 1-hexadecenol (16C:OH) and 1-hexadecanol acetate (16C:Ac), occurred in higher concentrations in males than in females as did the 2 previously identified male pheromones, *E*-β-farnesene and *E,E*-α-farnesene (Table 2).

Individual differences

In the males of all 3 strains and females of the ICR and KM mice, most volatile compounds in preputial gland displayed extremely high interindividual RSDs. Most volatile compounds from preputial gland had higher interindividual than intraindividual RSDs. The exceptions were compounds 19, 22, and 23 in male C57BL mice and compound 26 in female ICR mice, which did not have significantly different RSDs (Table 3).

Interstrain differences in preputial gland and urinary volatile composition

In preputial gland, 2 compounds (25 and 28) in males and 1 (34) in females differed in quantity between ICR and KM mice. Twelve compounds differed significantly in quantity between C57BL and ICR males (7–10, 12, 13, 15, 20, 22, 25, 33, and 37) or between C57BL and KM males (8, 9, 10, 13, 20, 22, 29, 36, and 40) (Table 2).

In the urinary volatiles of ICR and KM males, 2 compounds (5 and 8) varied in relative concentration. Seven (2, 3, 5, 6, 7, 8, and 9) displayed significant quantitative differences between C57BL and ICR and between C57BL and KM males (Table 4).

Discussion

Among the 42 compounds detected from preputial glands in house mice, only *E*-β-farnesene and *E,E*-α-farnesene had been definitively identified previously (Novotny, Ma, Zidek, et al. 1999; Novotny 2003; Ninomiya et al. 1993). Our study added 6 new compounds to the list of those definitively identified out of the 42 detected volatiles. From urine, we identified 10 volatiles, 3 of which (*R,R*-3,4-dehydro-*exo*-brevicomin, (*s*)-2-*sec*-butyl-4,5-dihydrothiazole, and 6-hydroxy-6-methyl-3-heptanone) are known important pheromonal components (Liebich et al. 1977; Wiesler et al. 1984; Novotny et al. 1985, Jemiolo et al. 1986; Novonty, Ma, Jemiolo, et al. 1999; Novonty, Ma, Wiesler, et al. 1999). 6-Hydroxy-6-methyl-3-heptanone, in particular, has recently been confirmed as a male urinary pheromone, which can accelerate puberty in female mice (Novonty, Ma, Jemiolo, et al. 1999; Novonty, Ma, Wiesler, et al. 1999). Interestingly, a large quantity of 6-hydroxy-6-methyl-3-heptanone was detected by our GC–MS system in the dichloromethane extract. In previous GC–MS analyses,

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Table 3 Individual variation (RSD) of relative abundance of the preputial gland volatiles of 3 strains of house mice

GC peak	ICR mice		KM mice		C57BL/6 mice	
	Male (n = 10)	Female (n = 9)	Male (n = 9)	Female (n = 9)	Male (n = 5)	Five duplicates of one male samples
1	30.61	42.86	32.56	42.86	106.98	4.59
2	34.62	55.56	38.10	60.00	117.39	4.69
3	32.14	25.00	56.67	85.71	105.26	7.69
4	42.86	33.33	42.86	50.00	100.00	14.00
5	45.45	50.00	45.45	100.00	100.00	6.92
6	42.86	80.00	37.50	50.00	80.00	4.00
7	100.00	35.00	142.86	100.00	200.00	25.00
8	90.91	25.00	55.56	60.00	66.67	16.67
9	29.73	30.77	34.29	78.57	82.35	6.45
10	20.88	20.00	21.19	31.76	56.67	4.58
11	25.00	35.00	50.00	100.00	50.00	10.00
12	26.32	40.00	33.33	60.00	63.64	5.00
13	30.00	20.00	22.22	85.71	40.00	12.50
14	28.57	40.00	35.71	62.50	70.00	9.09
15	25.33	38.10	32.47	60.00	56.52	8.75
16	25.00	133.33	25.00	100.00	61.54	7.69
17	26.39	28.03	17.14	50.58	37.16	3.56
18	180.00	100.00	75.00	50.00	66.67	16.67
19	77.78	40.00	62.50	57.14	15.00	20.00
20	27.78	26.67	11.76	28.57	55.56	20.00
21	40	23.08	30.77	36.36	33.33	8.33
22	30.00	42.86	45.45	44.44	10.00	10.00
23	44.44	40.00	42.86	40.00	36.36	40.00
24	21.21	19.05	13.89	30.43	19.51	4.35
25	18.67	15.71	13.54	30.00	17.17	9.28
26	40.82	15.00	29.31	29.41	37.78	22.22
27	10.08	22.34	18.83	25.21	47.21	4.35
28	15.17	28.83	11.72	17.79	43.52	2.84
29	59.32	166.67	38.01	65.01	82.25	25.83
30	45.19	47.99	50.00	32.73	55.23	14.69
31	19.03	89.01	17.06	52.07	23.68	8.52
32	22.69	26.25	18.11	21.46	52.15	6.09
33	27.16	27.44	31.65	25.71	26.32	8.03
34	36.05	25.78	29.79	28.43	22.76	16.67
35	25.61	25.07	21.57	16.53	42.56	7.66
36	27.69	28.21	19.64	20.00	54.26	16.42
37	9.39	11.38	15.51	7.84	11.10	3.45

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Table 3 Continued

GC peak	ICR mice		KM mice		C57BL/6 mice	
	Male (n = 10)	Female (n = 9)	Male (n = 9)	Female (n = 9)	Male (n = 5)	Five duplicates of one male samples
38	23.97	21.38	20.05	21.79	29.10	4.99
39	29.22	29.94	32.92	28.14	20.67	8.11
40	22.58	22.22	17.96	9.88	16.29	1.88
41	26.76	24.68	22.14	9.56	25.95	8.33
42	19.06	18.73	14.83	15.85	18.15	9.52
Mean ± SD	37.05 ± 29.35	39.77 ± 31.27	33.99 ± 22.78	45.76 ± 26.65	53.75 ± 36.80	10.70 ± 7.70
Z values	5.645	5.540	5.570	5.579	5.540	
P values	0.000	0.000	0.000	0.000	0.000	

RSD was calculated using the formula RSD = (SD/mean) × 100, where mean and SD are the average of each volatile peak area (in percentage) for all intact males and their SD, respectively. Wilcoxon matched-pairs signed-rank test for RSDs between same compounds of each individual group and duplicate data. Significance was set at $P < 0.05$.

Table 4 Comparison of relative abundance of urinary volatiles between strains of male house mice (mean ± SD)

GC peak no.	Retention times (min)	Compounds	ICR mice	KM mice	C57BL/6
1	4.36	*Z*-5,5-dimethyl-2-ethylidenetetrahydrofuran	4.09 ± 1.20	2.80 ± 1.55	3.50 ± 2.03
2	4.62	2-Heptanone	1.15 ± 0.18^{a}	1.53 ± 0.28^{b}	0.49 ± 0.15^{a,b}
3	4.72	5-Hepten-2-one	6.09 ± 0.90^{a}	7.25 ± 1.39^{b}	3.83 ± 0.29^{a,b}
4	5.01	*E*-5,5-dimethyl-2-ethylidenetetrahydrofuran	1.40 ± 0.47	0.77 ± 0.33	0.91 ± 0.39
5	5.07	*Z*-2-pentenyl acetate	0.44 ± 0.12^{a}	0.86 ± 0.08^{a,b}	0.62 ± 0.10^{b}
6	5.29	Dimethyl sulfone	7.04 ± 2.05^{a}	7.53 ± 1.95^{b}	21.34 ± 2.51^{a,b}
7	5.53	6-Hydroxy-6-methyl-3-heptanone and 5,5-dimethyl-2-ethyltetrahydrofuran-2-ol	55.77 ± 3.74^{a}	53.30 ± 9.56^{b}	16.81 ± 6.16^{a,b}
8	8.43	*R,R*-3,4-dehydro-*exo*-brevicomin	5.60 ± 0.67^{a,b}	9.62 ± 1.73^{a,c}	22.75 ± 1.34^{b,c}
9	9.92	(*S*)-2-*sec*-butyl-4,5-dihydrothiazole	18.43 ± 1.90^{a}	16.35 ± 2.80^{b}	29.77 ± 1.55^{a,b}

The means in a row marked by the same superscript letters (a–c) are significant ($P < 0.05$), using one-way ANOVA and the post hoc t-test with exception of compound 6 using Mann–Whitney U test.

however, only a few cyclic vinyl ethers (the degradation products of 5,5-dimethyl-2-ethyltetrahydrofuran-2-ol) were detected, and a silylation agent had to be used to detect the lactol in mouse urine (Novonty, Ma, Jemiolo, et al. 1999; Novonty, Ma, Wiesler, et al. 1999).

We found that ICR males were 8–10 times as heavy in the size of preputial gland, 5–10 times as high in the concentrations of *E*-β-farnesene and *E,E*-α-farnesene in urine (Zhang JX, Soini HA, Bruce KE, Wiesler D, Novotny MV, unpublished data), and 2–3 times (current study) as high in the percentage of the sesquiterpens as were ICR females. Based on our results, preputial gland, in particular, appeared to contain a wealth of information about sex and individuality in all 3 strains of laboratory mice. Of the 42 detected compounds, few were absent from all samples. Nor was there any sex-specific compound detected in the preputial glands. Their varying amounts speak for the use of predominant analog, rather than digital, coding for these 2 types of information (Sun and Müller-Schwarze 1998a,b, 1999). We did find the 2 previously identified male pheromones, *E*-β-farnesene and *E,E*-α-farnesene, and 2 new major compounds, 1-hexadecanol and 1-hexadecanol acetate. Together with most of other minor low molecular weight volatiles, they were all significantly higher in relative concentration in males than in females. Thus, the preputial glands should contain other sex-related chemical signals besides the 2 known sesquiterpens. In particular, the 2 major semivolatile compounds, 1-hexadecanol and 1-hexadecanol acetate, might be important potential male pheromones that have not been identified previously. Both were present only in intact males but absent from preputial gland-removed mice (Zhang JX, Liu YJ, Zhang JH, unpublished

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data). Recently, we have found that adding either or both of the 2 compounds enhances the attractiveness of the castrated urine to females (Zhang JX, Rao X-P, Sun L, Zhao C-H, Qin X-W, unpublished data). Hence, they must be new preputial gland-originated pheromones. In addition, other 2 urine-originated pheromonal compounds, (*s*)-2-*sec*-butyl-4,5-dihydrothiazole (male-specific urinary metabolite) and *R,R*-3,4-dehydro-*exo*-brevicomin (male-elevated urinary metabolite) (Wiesler et al. 1984; Novonty et al. 1985; Jemiolo et al. 1986; Harvey et al. 1989), were also detected in our study.

Analog coding for information about sex has also been found in other mammalian species such as the preputial glands of Brandt's voles (*Lasiopodomys brandti*) (Zhang JX, Rao X-P, Sun L, Zhao C-H, Qin X-W, unpublished data) and ferrets (*Mustela furo*) urine (Zhang et al. 2005). In stoats (*Mustela erminea*), however, a combination of analog and digital coding is used because the putative compounds involved in intersexual communication show both qualitative (2-ethylthietane and 3-ethyl-1,2-dithiacyclopentane) and quantitative differences (3-propyl-1,2-dithiacyclopentane) (Crump 1980). This seems also the case for the steppe polecat (*Mustela eversmanni*) and the Siberian weasel (*Mustela sibirica*) (Zhang et al. 2003). For the house mouse, our current and previous results indicate that voided urine containing preputial secretion and urinary metabolites can code for sex information in both analog and digital manners.

The large individual variation of shared volatile compounds detected in preputial glands in our study supports the idea that the volatiles might code for information regarding individuality in house mice in the analog form. In addition to the discovery by Hurst et al. (2001) where the major urine proteins (nonvolatiles) are involved in individual recognition in the house mouse, we show here for the first time that volatiles might also be involved in such recognition. A similar coding method seems to be used in female marmoset monkeys (*Callithrix jacchus*) as well, where most differences in the chemical constituents of circumgenital scent among individuals are quantitative (Smith et al. 2001). Analog coding for individuality is, however, not universal. In ferrets, for example, digital coding by varying the combination of presence/absence of 5 compounds in anal gland secretions seems to be predominant (Clapperton et al. 1988). So too are lions (*Panthera leo*), where no 2 individuals have an identical set of compounds in urine. A unique combination of presence/absence seems to be used in coding information about individuals (Andersen and Vulpius 1999). In the urine of red foxes (*Vulpes vulpes*), quinaldine is male specific (Jorgenson et al. 1978), and thus the coding is digital.

ICR and KM mice are both derived from Swiss mice (*Mus m. domesticus*) and outbred strains. Although KM mice were genetically contaminated by several genes unique to *Mus m. castaneus*, a local subspecies (*Mus m. castaneus*) in Kunming, Yunnan province, China, ICR and KM mice are morphologically and genetically much more similar to each other than to C57BL/6 (Zhao et al. 1994; Kikkawa et al. 2001). This was reflected in our study: the volatiles from both preputial gland and urine were more similar between ICR and KM mice than between either of them and C57BL/6. The differences in the volatiles between strains were only in degree (relative amount) rather than in kind (unique compound). That is, coding for the corresponding genetic background of strain might be analog too. How the scent compounds code for information regarding phylogenic/genetic distances of mammals can be reflected by some previous work. Above the species level, scent gland constituents are found to have a high similarity among some genera, such as 3 genera of skunks (Wood et al. 2002), but distinctly different between other genera (Brinck et al. 1983; Buesching et al. 2002). At the species level, quantitative differences in scent compounds appear to be more common. For instance, anal gland volatiles of the *Mustela* genus have a high similarity among species and might code for specific information in the analog manner (Zhang et al. 2003, 2005). It is expected that analog coding should be even more common below the species level. For example, the urinary volatiles of the 2 inbred mouse strains JF1/Ms and MSM/Ms (both derived from *Mus m. molossinus*) are much more similar in quantity between each other than between either of them and CAST/Ei (derived from the subspecies *Mus m. castantus*), and the differences can be discriminated by ICR albino mice (Zhang JX, Soini HA, Bruce KE, Wiesler D, Novotny MV, unpublished data). More surprisingly, the urinary odors of congenic strains differing only in single genes in the MHC loci can be discriminated. Chemical analysis shows that no compounds are unique to any MHC genotype in the urine. Such discrimination is clearly based on the different ratios of some compounds shared by different strains (analog coding) (Singer et al. 1997). In our current study, data from chemical analysis show that both preputial gland secretion and urine metabolites might contain communicative information associated with subspecies and strains in the house mouse.

The chemical conjecture that the preputial gland and urinary metabolites might communicate the information about strains has been exemplified by the behavioral responses of 2 subspecies of house mice (namely, *Mus m. domesticus* and *Mus m. musculus*) (Smadja and Ganem 2002). In the beaver (*Castor canadensis*), the difference of chemical constituents of anal gland secretion between individuals increases with their genetic difference (Sun and Müller-Schwarze 1998b). Of particular interest in our study was the well-known male pheromone compound *R,R*-3,4-dehydro-*exo*-brevicomin. It appears to be sensitive to genetic variation because it varies quantitatively among the 3 mouse strains (Jemiolo et al. 1985, 1986; Novotny et al. 1985). We also found that this compound differed quantitatively among male JF1/Ms, MSM/Ms, CAST/Ei, and ICR mice (Zhang JX, Soini HA, Bruce KE, Wiesler D, Novotny MV, unpublished data). These results suggest that this compound might be important in communicating genetic information in the analog

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manner in addition to sexual attractiveness and induction of physiology when synergistically coupled with (*s*)-2-*sec*-butyl-4,5-dihydrothiazole. Behavioral tests are, however, still needed for confirmation.

In conclusion, the prediction derived from our hypothesis that preputial gland and/or urine can code for sex, individuality, and genetic background of strain in house mice was supported. Furthermore, coding for the information about sex, individuality, and genetic background of strain mainly, if not entirely, takes the form of analog coding. The ecological and evolutionary significance of analog coding, however, needs further investigation.

Acknowledgements

We express our gratitude to Dr Donald Wiesler for his elucidation of MS spectra and to Jinhua Zhang for his care of our mice. The work was supported by grants from International Partnership Project of CAS Innovative Researches (CXTDS2005-4), Chinese NSF (30670268), and Ministry of Science and Technology (2005BA529A05).

References

Andreolini F, Jemiolo B, Novotny M. 1987. Dynamics of excretion of urinary chemosignals in the house mouse (*Mus musculus*) during the natural estrous cycle. Experientia. 45:998–1002.

Andersen KF, Vulpius T. 1999. Urinary volatile constituents of the lion, *Panthera leo*. Chem Senses. 24:179–189.

Brinck C, Erlinge S, Sandell M. 1983. Anal sac secretion in mustelids: a comparison. J Chem Ecol. 9:727–745.

Brown RE. 1985. The rodents: II. In: Brown RE, Macdonald DW, editors. Social odors in mammals. Vol. 1. Oxford: Oxford University Press. p. 345–457

Buesching CD, Waterhouse JS, Macdonald DW. 2002. Gas-chromatographic analyses of the subcaudal gland secretion of the European badger (*Meles meles*) part I: chemical differences related to individual parameters. J Chem Ecol. 28:41–56.

Clapperton BK, Minot EO, Crump DR. 1988. An olfactory recognition system in the ferret, *Mustela furo* L. (Carnivora: Mustelidae). Anim Behav. 36:541–553.

Crump DR. 1980. Thietanes and dithiolanes from the anal gland of the stoat (*Mustela erminea*). J Chem Ecol. 6:341–347.

Epple G, Golob NF, Smith AB 3rd. 1979. Odour communication in the tamarin *Saguinus fuscicollis* (Callitrichidae): behavioral and chemical studies. In: Ritter FJ, editor. Chemical ecology: odour communication in animals. Amsterdam: Elsevier/North Holland Biomedical Press. p. 117–130.

Harvey S, Jemiolo B, Novotny M. 1989. Pattern of volatile compounds in dominant and subordinate male mouse urine. J Chem Ecol. 15:2061–2071.

Hurst JL, Payne CE, Nevison CM, Marie AD, Humphries RE, Robertson DHL, Cavaggioni A, Beynon RJ. 2001. Individual recognition in mice mediated by major urinary proteins. Nature. 414:631–634.

Jemiolo B, Alberts J, Sochinski-Wiggins S, Harvey S, Novotny M. 1985. Behavioural and endocrine responses of female mice to synthetic analogues of volatile compounds in male urine. Anim Behav. 33:1114–1118.

Jemiolo B, Harvey S, Novotny M. 1986. Promotion of the Whitten effect in female mice by the synthetic analogs of male urinary constituents. Proc Natl Acad Sci USA. 83:4576–4579.

Jemiolo B, Xie T-M, Novotny M. 1991. Socio-sexual olfactory preference in female mice: attractiveness of synthetic chemosignals. Physiol Behav. 50:1119–1122.

Jorgenson JW, Novotny M, Carmack M, Copland GB, Wilson SR, Whitten WK, Katona S. 1978. Chemical scent constituents in the urine of the red fox (*Vulpes vulpes* L.) during the winter season. Science. 199:796–798.

Kikkawa Y, Miura I, Takahama S, Wakana S, Yamazaki Y, Moriwaki K, Shiroishi T, Yonekawa H. 2001. Microsatellite database for MSM/Ms and JF1/Ms, molossinus-derived inbred strains. Mamm Genome. 12:750–752.

Liebich HM, Zlatkis A, Bertsch W, Van Dahm R, Whitten WK. 1977. Identification of dihydrothiazoles in urine of male mice. Biomed Mass Spectrom. 4:69–72.

Ma W, Miao Z-S, Novotny M. 1998. Role of the adrenal gland and adrenal-mediated chemosignals in suppression of estrus in the house mouse: the Lee-Boot effect revisited. Biol Reprod. 59:1317–1320.

Ma W, Miao Z, Novotny MV. 1999. Induction of estrus in grouped female mice (*Mus domesticus*) by synthetic analogs of preputial gland constituents. Chem Senses. 24:289–293.

Ninomiya K, Nnohara I, Toyoda T. 1993. The pattern of volatile compounds in incubated and fresh preputial fluid of male mice. Zool Sci. 10: 537–542.

Novotny MV. 2003. Pheromones, binding proteins and receptor responses in rodents. Biochem Soc Trans. 31:117–122.

Novotny M, Harvey S, Jemiolo B. 1990. Chemistry of male dominance in the house mouse (*Mus domesticus*). Experientia. 46,109–113.

Novotny M, Harvey S, Jemiolo B, Alberts J. 1985. Synthetic pheromones that promote inter-male aggression in mice. Proc Natl Acad Sci USA. 82: 2059–2061.

Novotny M, Jemiolo B, Harvey S, Wiesler D, Marchlewska-Koj A. 1986. Adrenal-mediated endogenous metabolites inhibit puberty in female mice. Science. 231:722–725.

Novotny M, Ma W, Jemiolo B, Wiesler D, Harvey S, Xu F, Xie T-M, Carmack M. 1999. A unique urinary constituent, 6-hydroxy-6-methyl-3-heptanone, is a pheromone that accelerates puberty in female mice. Chem Biol. 6: 377–383.

Novotny M, Ma W, Wiesler D, Zidek L. 1999. Positive identification of the puberty-accelerating pheromone of the house mouse: the volatiles associating with the major urinary protein. Proc R Soc Lond B. 266: 2017–2022.

Novotny M, Ma W, Zidek L, Daev E. 1999. Recent biochemical insights into puberty acceleration, estrus induction and puberty delay in the house mouse. In: Johnston RE, Müller-Schwarze D, Sorenson P, editors. Advances in chemical communication in vertebrates. New York: Plenum Publishers. p. 99–116.

Novotny M, Schwende FJ, Wiesler D, Jorgenson JW, Carmack M. 1984. Identification of a testosterone-dependent unique constituent of male mouse urine: 7-exo-ethyl-5-methy-6, 8-dioxabicyclo[3.2.1]-3-octane. Experientia. 40:217–219.

Schwende FJ, Jorgenson JW, Novotny M. 1984. A possible chemical basis for the histocompatibility-related mating preference in mice. J Chem Ecol. 10:1603–1615.

Schwende FJ, Wiesler D, Jorgenson JW, Carmack M, Novotny M. 1986.Urinary volatile constituents of the house mouse, *Mus musculus*, and their endocrine dependency. J Chem Ecol. 12:277–296.

Smadja C, Ganem G. 2002. Subspecies recognition in the house mouse: a study of two populations from the border of a hybrid zone. Behav Ecol. 13:312–320.

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Smith TE, Tomlinson AJ, Mlotkiewicz JA, Abbott DH. 2001. Female marmoset monkeys (*Callithrix jacchus*) can be identified from the chemical composition of their scent marks. Chem. Senses. 26:449–458.

Singer AG, Beauchamp GK, Yamazaki K. 1997. Volatile signals of the major histocompatibility complex in male mouse urine. Proc Natl Acad Sci USA. 94:2210–2214.

Sun L, Müller-Schwarze D. 1998a. Anal gland secretion codes for family membership in the beavers. Behav Ecol Sociol. 44:199–208.

Sun L, Müller-Schwarze D. 1998b. Anal gland secretion codes for relatedness in the beaver, *Castor canadensis*. Ethology. 104:917–927.

Sun L, Müller-Schwarze D. 1999. Chemical signals in the beaver. In: Johnson RE, Müller-Schwarze D, Sorensen P, editors. Advances in chemical signals in vertebrates. New York: Kluwer Academic/Plenum. p. 281–288.

Wiesler D, Schwende FJ, Carmack M, Novotny M. 1984. Structural determination and synthesis of a chemical signal of the male state and a potential multipurpose pheromone of the mouse, *Mus Musculus*. J Org Chem. 49:882–884.

Wood WF, Sollers BG, Dragoo GA, Dragoo JW. 2002. Volatile components in defensive spray of the hooded skunk (*Mephitis macroura*). J Chem Ecol. 28:1865–1870.

Zhang J, Ni J, Ren X, Sun L, Zhang Z, Wang Z. 2003. Possible coding for recognitions of sexes, individuals and species in anal gland volatiles of *Mustela eversmanni* and *M. sibirica*. Chem Senses. 28:371–378.

Zhang J, Soini H, Bruce K, Wiesler D, Woodley S, Baum M, Novotny M. 2005. Putative chemosignals of the ferret (*Mustela furo*) associated with individual and gender recognition. Chem Senses. 30:727–737.

Zhao G, Bao S, Zhang D, Zhang R, Jin M. 1994. Genetic characterization of Kunming house mice. Exp Anim Shanghai. 1:1–5. Chinese.

Accepted December 26, 2006

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