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Urinary Olfactory Chemosignals in Lactating Females Show More Attractness to Male Root Voles (*Microtus oeconomus*)

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1. Introduction

Chemical communication among individuals of the same species serves several important functions, including sexual attraction (Achiraman and Archunan 2002, 2005, Brennan and Keverne 2004), interference with puberty, the estrus cycle, and pregnancy (Dominic 1991, Drickamer 1999). Odors also act as social behavior signals, as in territorial marking (Doty 1980, Prakash and Idris 1992), individual identification (Hurst et al 1998, Poddar-Sarkar and Brahmachary 1999), mother-young interactions (Schaal *et al.* 2003), and the initiation of aggression (Mugford and Nowell 1971), in mammals especially. The pheromones that animals secrete externally are used to communicate with conspecific receivers, which react by behavioral response or developmental process (Wyatt 2003).

A comprehensive understanding of mammalian chemical communication requires a combination of bioassay and chemical analysis to identify the pheromonal components involved (Singer *et al.* 1997, Novotny *et al.* 1999a). Gas chromatography-mass spectrometry (GC-MS) analysis is standard in semiochemical research. A systematic approach using gas chromatography to screen for pheromonal compounds from numerous scent sources has been established in mice (*Mus musculus*); volatile compounds that vary with biological characters are tagged as pheromone candidates for further verification by bioassay (Singer *et al.* 1997, Novotny *et al.* 1999a). This approach has identified a large number of pheromones, including 6-hydroxy-6-methyl-3-heptanone, a male pheromone that accelerates puberty in female mice (Novotny *et al.* 1999b); 1-iodo-2-methylundecane, an estrus-specific urinary chemosignal; and 3-ethyl-2, 7-dimethyl octane, a testosterone-dependent urinary sex pheromone in male mice (Achiraman and Archunan 2005, 2006). Pheromone components were recently discovered in the preputial glands of wild rodents, Brandt's vole (*Lasiopodomys brandtii*) (Zhang *et al.* 2007a, b).

Mammals emit chemical signals into their surroundings via urine, feces, saliva, or specialized scent glands (Dominic 1991, Novotny 2003, Hurst and Beynon 2004). Among

these, urine is a major carrier of chemosignals. The odors of most females vary with their reproductive state, and all chemical constituents of urine may vary with the estrous cycle (Michael 1975, O'Connell *et al.* 1981, Rajanarayanan and Archunan 2004). The urine odor of females in estrus is usually more attractive than that of dioestrous females (Drickamer 1999, Vandenbergh 1999).

In many mammals that exhibit spontaneous estrus cycles, the natural estrous cycle is caused by hormonal changes. Thus, endocrine changes may cause excretion of different signals that communicate different reproductive states. Lactation is a period of the reproductive cycle during which hormone levels differ from those of the non-lactating state. Hence, the olfactory cues from lactating-females differ from those of non-lactating females. Several studies of rabbits have indicated that lactating rabbit females emit volatile odor cues that trigger responses in pups, especially specialized motor actions leading to sucking (Coureaud and Schaal 2000, Coureaud *et al.*2001), and the activity of these cues change with advancing lactation (Coureaud *et al.* 2006). Another interesting study indicated that compounds from lactating women might modulate the ovarian cycles of other women (Jacob *et al.* 2004) and increased sexual motivation (Spencer *et al.* 2004). Recently, a published study focused on the ability of mice to discriminate cow urinary odor from prepubertal, preovulatory, ovulatory, postovulatory, pregnancy and lactation (Rameshkumar *et al.* 2008).

The root vole (*Microtus oeconomus*), the only extant Holarctic member of the species-rich genus *Microtus*, is an interzonal small mammal that occurs in wet grasslands of both Arctic and temperate zones (Brunhoff *et al.* 2003). Our studies of social behavior and kin recognition by urine odors have suggested that social rank differences among root voles induce different behavioral patterns (Sun *et al.* 2007a). Male root voles also use a urine association mechanism to achieve discrimination of opposite-sex siblings (Sun *et al.* 2008a). Thus, urine is an important chemosignal source in root voles. Our first objective of this research is to test the hypothesis that urine from lactating and non-lactating females differs in its attractiveness to males. The evidence supported for this hypothesis would indicate that the difference in urine odors could guide preference behaviors of males. The second objective is to identify the chemosignals that cause the male behavioral responses. The individually informational coding forms were confirmed using GC-MS.

2. Materials and methods

2.1 Study animals

Wild root voles were captured from a meadow located in Menyuan County (37°29′ - 37 °45′ N, 101°12′ – 101° 23′ E), China. Laboratory colonies were established at the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. The voles used in this study were housed in clear polycarbonate cages (40×28×15 cm) with wood chip beddings and cotton nesting material. A 14:10 h (light : dark) light cycle was maintained, with the light cycle commencing at 08:00. The laboratory temperature was maintained at 22±2°C, and food (BLARC, China) and water were provided *ad libitum*. The cages were cleaned and the cotton nesting material was replaced once a week. All voles used in this study were F1 – F3 generation offspring of field-captured animals and were housed with their mates until the experiments. In the behavior test, twenty 90 – 120-day-old male voles were used, while ten lactating females 10 – 15 days post partum and ten non-lactating females were used as urine

donors. The females for the non-lactating condition were all in dioestrus. All of the experimental animals were used only once.

2.2 Experimental apparatus

The behavior choice maze included an odorant box (30x30x30 cm) and a neutral box (30x30x30 cm) made of organic glass (Fig. 1) and connected by a pellucid organic glass tube (20x7 cm). A switch controlled the passage of experimental voles between the odorant and neutral boxes. A culture dish containing fresh urine was placed in the center of the odorant box as an odor stimulant.
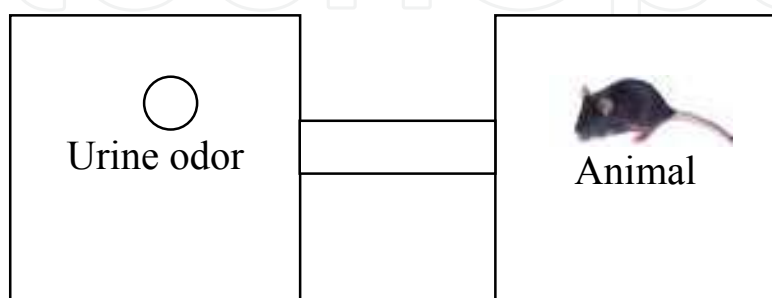
The experimental apparatus used to test behavior choice. The odorant box (30×30×30 cm, left) and neutral box (30×30×30 cm, right) were made of organic glass. The odorant box and the neutral box were connected by pellucidly organic glass tube (20×7 cm). The culture dish containing fresh urine from ether a lactating or a non-lactating vole was put in the center of the odorant box as a stimulant. A switch controlled the movement of voles between the odorant and neutral boxes.

Fig. 1. Experimental apparatus (behavior choice maze)

A vidicon (SONY Version 805E) recorded the behavior of the male voles in the choice maze. The videos were converted to digital data after the experiment.

2.3 Odorant preparation

On the day of a behavioral test, lactating and non-lactating female voles were used as urine donors. The donors were placed on the covers of the clear cages, and two layers of gauze were used to separate feces and urine. We collected the urine samples on clean, absorbent cotton, which was then placed in a culture dish as the odorant. To ensure that only fresh odors were encountered during the trials, urine more than 20 min old was discarded.

2.4 Behavioral test

In this study, behavioral tests were conducted in a room in which the temperature, lighting, and air circulation were identical to those of the breeding room. Subjects with their home cages were moved to testing room 2 h earlier before the behavioral test so they could habituate to the conditions. We tested the responses of male voles to lactating and non-lactating female urine samples. Every test lasted 10 min and was performed between 09:00 and 18:00, during the light phase. Immediately before each trial, we transferred a male subject from its home cage to the clean testing arena. The test animals were allowed to acclimatize in the apparatus for 5 min, then the odor stimulus apparatus was introduced and trial was recorded using a vidicon.

2.5 Statistical analysis of behavior

After the experiment, the videos were converted to digital data. The video files were named by a member of the lab who unfamiliar with the experiment and the videos were then rescored by the original experimenter. Behavioral variables were treated as an index of duration (sec) or frequency. Behavior variables included sniffing and self-grooming. Sniffing, a rhythmic inhalation and exhalation of air through the nose, is a behavior thought to play a critical role in shaping how odor information is represented and processed by the nervous system (Wesson et al 2008). We recorded sniffing behavior when subjects' noses within 0.5cm of the odor stimulation and displayed a rhythmic sniff. In voles, the general pattern of self-grooming consists of a cephalocaudal progression that begins with rhythmic movements of the paws around the mouth and face, over the ears, descending to the ventrum, flank, anogential area, and tail (Ferkin et al. 1996, Leonard and Ferkin 2005, Leonard et al. 2005). According to our knowledge, the root vole in our study generally groomed their anogenital area, head, and flanks (Sun et al., 2005, 2006). Thus, we recorded self-grooming when subjects rubbed, licked or scratched any of these body areas.

All statistical analyses were conducted using SPSS for Windows (version 13.0). We used the Mann-Whitney test to evaluate the hypothesis that male voles displayed different behavioral patterns in the presence of urine cues of lactating and non-lactating females. A difference was regarded as significant at $p<0.05$. All data are reported as mean ± SE.

2.6 Scent sample collection and extraction

Lactating and non-lactating female root voles were used as urine donors. On the day of GC-MS analysis, the females were removed from their home cages and placed on the covers of the clear cages. Food and water were offered. Two layers of gauze were used to separate feces and urine. Urine contaminated by feces was rejected. Urine used for analysis was collected into clean glass vials and immediately stored at -20°C.

Each sample was thawed at room temperature prior to analysis. We used dichloromethane to extract the volatile compounds from the urine samples, mixing 300 μl dichloromethane with 100 μl urine. The mixture was immediately analyzed by GC-MS.

2.7 GC-MS analysis

GC-MS was performed on a Agilent Technologies Network 7890N GC system coupled with a 5975C Mass Selective Detector with the NIST05 Mass Spectral Library (2005 version). Chemstation Software (Windows XP) was used for data acquisition and processing. The GC was equipped with a 30-m glass capillary column (0.25-mm i.d. x0.25-μm film thickness). Helium was used as the carrier gas, at a flow rate of 1.5 ml/min. For each test, 8 μl of extracted dichloromethane were injected into the GC-MS. The temperature of the injector was set at 280°C, and the electron impact ionization (EI) temperature was set at 300°C. The oven temperature was programmed as follows: 50°C initially, increasing 5°C /min to 200°C, and then 1.5°C /min to 230°C, and finally 5°C /min to 250°C, where it was maintained for 6 min. The relative amount of each component was reported as the percentage of the ion current. We identified unknown volatile compounds by probability-based matching using the computer library (NIST05 Mass Spectral Library).

### 2.8 GC-MS statistical analysis

As a measure of the relative abundance of a compound, the area under each peak was used for quantitative calculations. The relative amount of each component was reported as the percent of the ion current. GC peak areas that were too small to display the diagnostic MS ions of the corresponding compound were recorded as zero.

We visually examined other volatile compounds to identify those that were individual- or lactation-specific. We then quantitatively compared the basic volatile compounds by examining the distribution of the raw data of the 12 basic volatile compounds, using the Kolmogorov-Smirnov test in SPSS for Windows. As all data were normally distributed, we used an independent two-tailed *t*-test to analyze for stage differences in the relative abundances. All statistical analyses were conducted using SPSS for Windows (version 13.0). The critical value was set at $\alpha = 0.05$.

3. Results

3.1 Behavioral differences

The behavioral tests showed that the sniffing frequency of males was greater in response to the urine of lactating than non-lactating females urine (mean ± SE 7.3±0.79 and 5.4±0.58 Num,
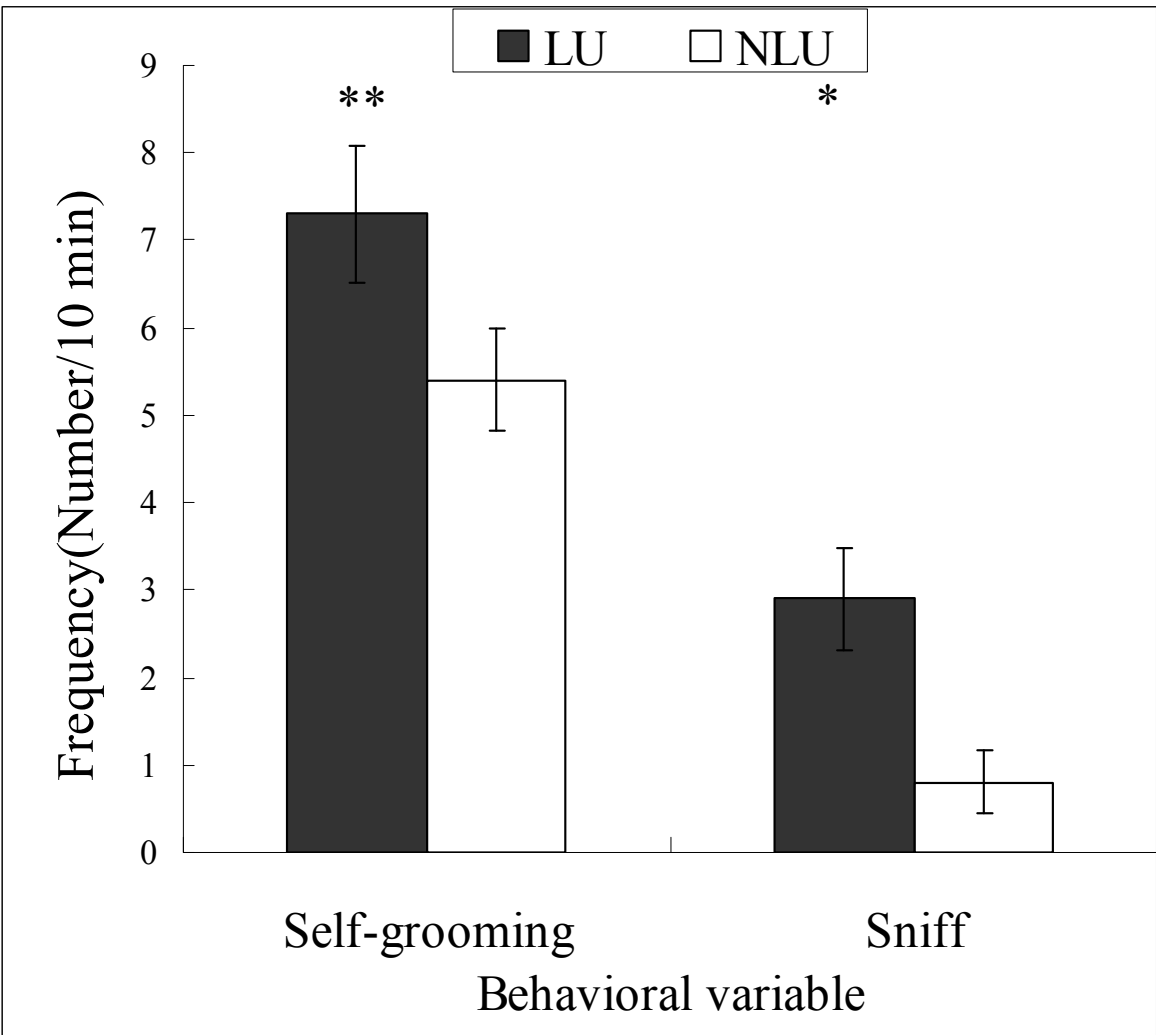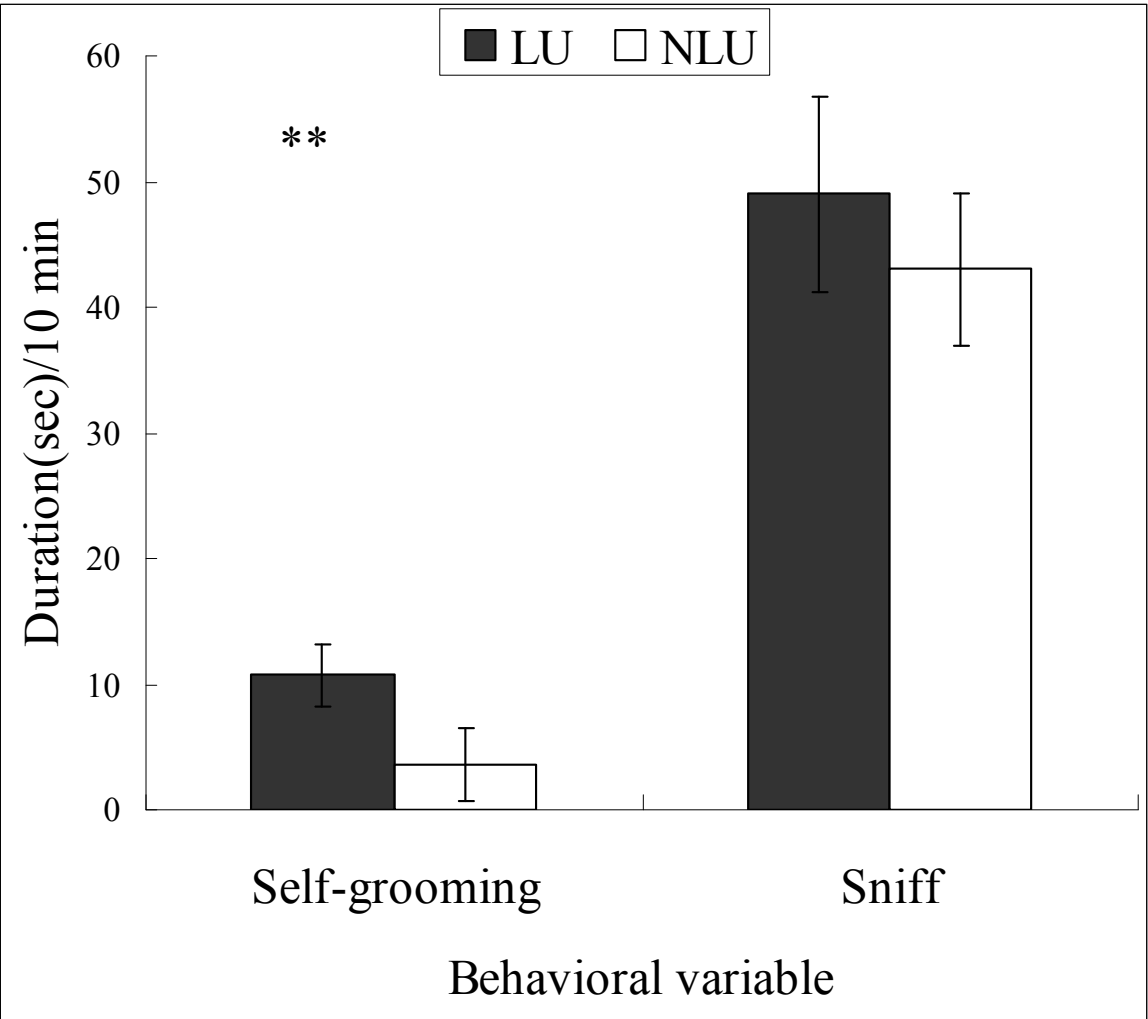
*indicates significant difference (p<0.05); ** indicates significant difference (p<0.01); LU: lactating-female urine; NLU: non-lactating-female urine.*

The responses of male root voles (Microtus oeconomus) to odors from the urine of lactating and non-lactating females (n=20). The behavior examined were frequency and duration of sniff and self-groom. The sniffing (p<0.05) and self-grooming (p<0.01) frequency of males response to lactating and non-lactating urine indicated significant difference. The self-grooming duration (p<0.01) of males response to lactating and non-lactating urine showed significant difference, but not the sniffing duration (p>0.05).

Fig. 2. Behavioral difference of male voles in response to lactating and non-lactating female urine odor

respectively; N = 10 each; $p = 0.043$). Males' self-grooming frequency was also greater when males were exposed to the urine odor of lactating females, compared to that of non-lactating females (2.9±0.59 and 0.8±0.36 Num, respectively; N = 10; $p < 0.01$), as was the duration of self-grooming behavior (10.7±2.54 and 3.6±2.95 sec, respectively; N = 10; $p < 0.01$; Fig. 2). Thus, the duration and frequency of self-grooming, as well as sniffing frequency, revealed that the urine of lactating females was more attractive than that of non-lactating females to male root voles.

3.2 Volatile constituents of urine

The GC-MS profiles of Table 1 and Figures 3 show the representative volatile compounds contained in the urine of lactating and non-lactating females, with 10 – 20 detectable peaks in each condition. In all, 34 peaks were detected in lactating and non-lactating states. The constituents identified in the urine samples included alkanes, alkenes, alcohols, ketones, benzo-forms, esters, acids, furans, pyrans, and other volatile compounds. Benzenes and
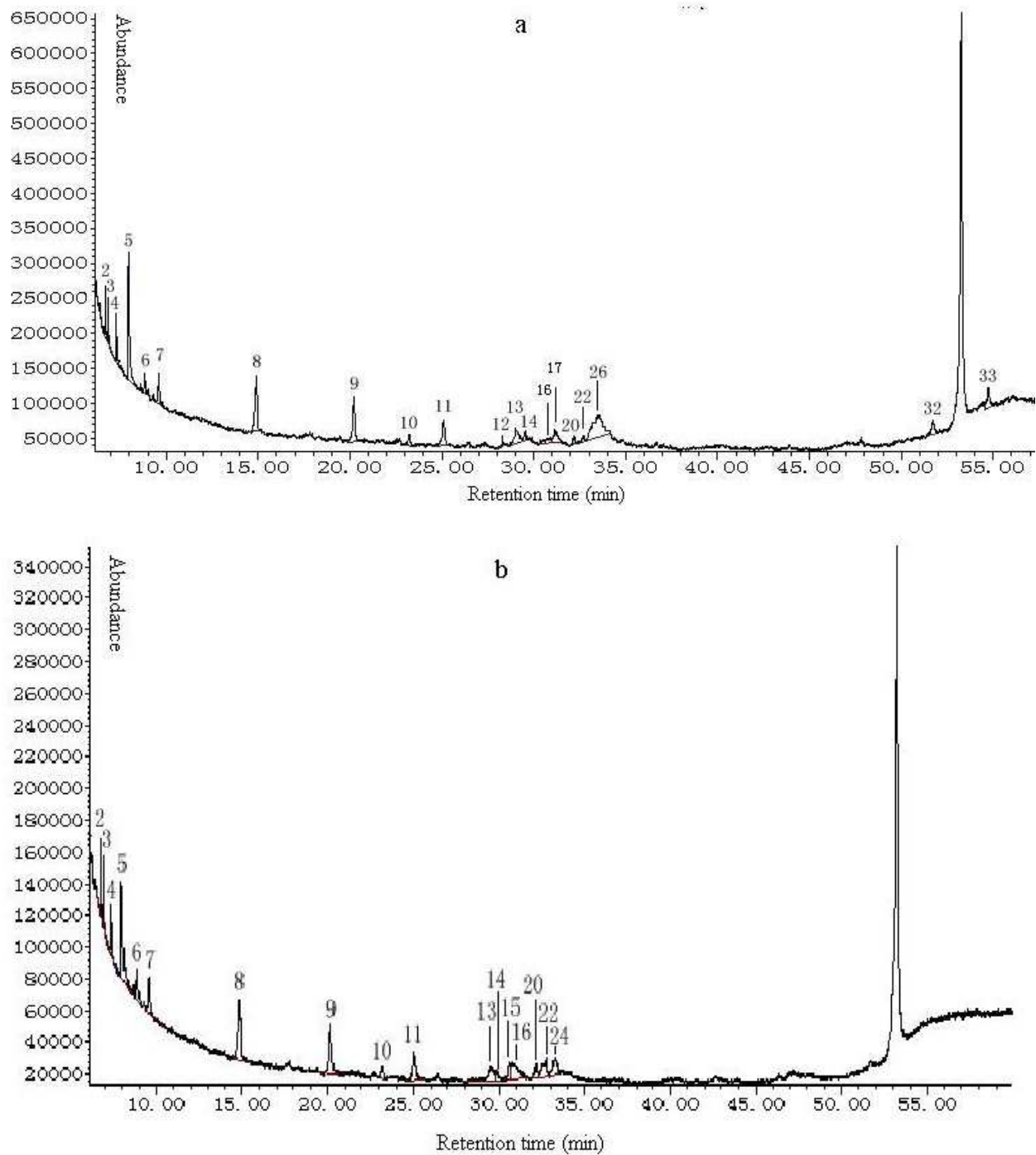

Fig. 3. Total ion chromatogram of urinary volatile compounds identified in (a) lactating and (b) non-lactating female root voles

Peak No	Retention Time (min)	Compound	Molecular weight	Group
1	6.388	2-pentanone,4-hydroxy-4-methyl	106	Ketone
2	6.715	Ethylbenzene	106	Benzo-
3	6.869	(*D*)xylene	106	Benzo-
4	7.301	Styrene	104	Benzo-
5	7.943	(*Z*)1,3,5-hexatriene,3-methyl	94	Alkene
6	8.841	Benzene, 1-ethyl-2-methyl	120	Benzo-
7	9.585	Decane	142	Alkane
8	14.861	Dodecane	170	Alkane
9	20.179	Tetradecane	198	Alkane
10	23.181	Butylated hydroxytoluene	220	Benzo-
11	25.054	Hexadecane	226	Alkane
12	28.283	Methoxyacetic acid decyl ester	230	Ester
13	29.026	Tetradecanoic acid	228	Acid
14	29.532	Heptadecane	240	Alkane
15	30.626	(*R*)1-H-2benzopyran-1-1,3,4-dihydro-8hydroxy-6-methoxy-3-methyl	208	Pyran
16	30.777	1-H-2benzopyran-1-1,3,4-dihydro-8hydroxy-6-methoxy-3-methyl	208	Pyrane
17	31.249	1-Hexadecanol	242	Alcohol
18	31.344	(*E*)5-octadecene	252	Alkene
19	32.1	2,5-cydohexadion-1-1,2,6-bis[1,1-dimethylethyl]4-ethylidene	232	
20	32.215	3-Ethoxycarbonyl-4-hydroxyguinoline	217	
21	32.53	Etyl-4-oxo-1,4-dihydroquinoline-3-carboxylate	217	
22	32.708	Naphtho[2.3-b]furan-9(4H)-1,4a.5.6.7.8a-hexahycho-3,4a.5-frinethyl-[4aR-{4a,alpha,5,alpha,8a,beta}]	232	Furan
23	33.107	1-Naphthalenecarboxylic acid	217	Acid
24	33.267	(*P*)Hexaclecenoic acid	254	Acid
25	33.312	(*D*)octylacetophenone	232	Benzo-
26	33.531	(*N*)Hexadecanoic acid	256	Acid
27	36.117	Oleyl alcohol	268	Alcohol
28	36.962	(*R*)- [-]-ol-4-methyl-8-hexadecyn-1-ol	252	
29	37.135	(*Z*)7-Hexadecanoic acid, methyl ester	268	Acid
30	42.871	Squalene	410	Alkene
31	50.843	1, 19-Eicosacliene	278	Alkene
32	51.730	Eicosane	282	Alkane
33	54.73	Henicosane	296	Alkane
34	57.918	Docosane	310	Alkane

Table 1. Urinary volatile compounds identified in lactating and non-lactating female root voles

alkanes were predominant in the urine samples. Although the chromatograms showed consistent qualitative differences in each individual chemical profile, 12 basic volatile compounds were present in almost all samples, including ethylbenzene [peak number (PN) 2], (*D*)xylene(PN 3), styrene(PN 4), (*Z*)1,3,5- hexatriene,3- methyl (PN 5), 1-ethyl-2-methyl-benzene (PN 6), decane (PN 7), dodecane (PN 8), tetradecane (PN 9), butylated hydroxytoluene (PN 10), hexadecane (PN 11), heptadecane (PN 14), and 1-H-2-benzopyran-1-1,3,4-dihydro-8 hydroxy-6-methoxy-3-methyl (16).

3.3 Individual-specific constituents

Lactating and non-lactating individuals were numbered 1 – 6 and 7 – 12, respectively. In addition to the presence of 12 basic volatile compounds in urine, 20 constituents were individual-specific, including 2-pentanone, 4-hydroxy-4-methyl (PN 1), methoxyacetic acid decyl ester (PN 12), tetradecanoic acid (PN 13), (*R*)1-H-2benzopyran-1- 1, 3, 4- dihydro-8hydroxy-6-methoxy-3-methyl (PN 15) 1-hexadecanol (PN 17), 2, 5-cydohexadion-1- one, 2, 6-bis [1,1-dimethylethyl] 4 -ethylidene (PN 19), 3-Ethoxycarbonyl-4- hydroxyguinoline (PN 20), Etyl-4- oxo-1,4- dihydroquinoline-3- carboxylate (PN 21), Naphtho [2.3-b] furan-9 (4H) - one, 4a.5.6.7.8a - hexahycho- 3, 4a.5- frinethyl- [4aR- {4a,alpha,5,alpha,8a,beta}] (PN 22), 1-Naphththalenecarboxylic acid,3-intro- (PN 23), (*P*)Hexaclecenoic acid (PN 24), (*D*)octylacetophenone (PN 25), Oleyl Alcohol (PN 27), (*R*)- [-]-ol-4-methyl-8- hexadecyn-1-ol (PN 28), (*Z*)7-Hexadecanoic acid, methl ester (PN 29), Squalene (PN 30), 1, 19-Eicosacliene

Peak No.	Compound	Detected frequency	Individual No.
1	2-pentanone, 4-hydroxy-4-methyl	1	6
12	Methoxyacetic acid decyl ester	1	3
13	Tetradecanoic acid	4	1, 2, 3, 11
17	1-Hexadecanol	4	1, 2, 3, 10
19	2,5-cydohexadion-1-1,2,6-bis[1,1-dimethylethyl]4-ethylidene	2	5, 8
20	3-Ethoxycarbonyl-4-hydroxyguinoline	5	3, 6, 8, 10, 11
22	Naphtho[2.3-b]furan-9(4H)-1,4a.5.6.7.8a-hexahycho-3,4a.5-frinethyl-[4aR-{4a,alpha,5,alpha,8a,beta}]	4	3, 5, 8, 11
23	1-Naphthalenecarboxylic acid	1	8
24	(*P*)hexaclecenoic acid	5	1, 5, 8, 9, 10
25	(*D*)octylacetophenone	1	12
27	Oleyl Alcohol	1	1
28	(*R*)- [-]-ol-4-methyl-8-hexadecyn-1-ol	1	7
29	(*Z*)7-hexadecanoic acid, methyl ester	1	5
30	Squalene	1	4
31	1, 19-Eicosacliene	1	1
32	Eicosane	2	3, 4
33	Henicosane	2	3, 10
34	Docosane	1	3

Table 2. Individual-specific urinary volatile compounds identified in female root voles

(PN 31), Eicosane (PN 32), Henicosane (PN 33), Docosane (PN 34). These volatile compounds were presented randomly in lactating and non-lactating female urine, with each sample including one to six individual-specific volatile compounds that could identify individual females. Table 2 lists the individual urinary odor characters.

3.4 Differences in urinary volatile compounds of lactating and non-lactating females

Quantitative analyses revealed that the relative amount of the basic volatile compounds did not differ significantly between lactating and non-lactating stages. A quality comparison of the identified volatile compounds revealed that (*E*)5-octadecene (PN18) and (*N*)Hexadecanoic acid (PN 26) (Fig. 4) were specific to the lactating stage.
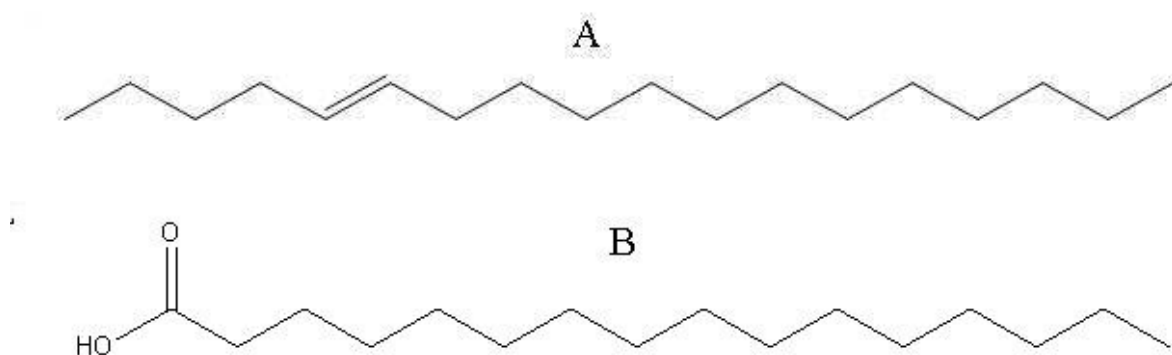
A: (*E*)5-octadecene, B: (*N*)hexadecanoic acid

Fig. 4. Molecular structural of two putative lactating pheromones.

4. Discussion

Among mammals, the olfactory information of urine is complex. The olfactory information of urine is about sex, health conditions, relatedness, individual personality, reproductive and social status (Gheusi *et al*. 1997, Hurst and Beynon 2004, Hurst 2004, Penn and Potts 1998). Our studies about urine odor demonstrated that the olfactory information of urine from parents is about relatedness and the retention of maternal odor of the female offspring lasted for 40 d after post weaning (Sun *et al*. 2007b) and of the male offspring lasted for 10 d after post weaning (Sun *et al*. 2008b) in root voles. During the breeding season, female voles may be pregnant, lactating, pregnant and lactating, or neither pregnant nor lactating. The female voles may be in different states of sexual receptivity, including heightened receptivity during postpartum estrus or moderate receptivity when they are not pregnant or lactating (Ferkin *et at*. 2004). The phenomenon of postpartum estrus presents very common in root vole. The female root voles can copulate with males within 24h after parturition (unpublished data).

The chemical constituents of mammalian urine vary with the estrous cycle (Michael 1975, Rajanarayanan and Archunan 2004, Achiraman and Archunan 2006), and the urine odor of estrous females is usually more attractive to males than that of diestrous or non-estrous females (Drickamer 1999, Dominic 1991, O'Connell *et al*. 1981; Vandenbergh 1999, Rajanarayanan and Archunan 2004). Since the natural estrous cycle is induced by hormonal changes, the variation in urinary volatiles may be influenced by the endocrine system. In

present study, correspondingly, behavioral observations showed that the male reactions to lactating female urine differed from to non-lactating females. Therefore, our results supported the view that the variation in urinary volatiles might be influenced by the endocrine system. Male root voles engaged in more sniffing behavior and both more frequent and longer duration in self-grooming behavior when respond to lactating than to non-lactating urinary odors. Sniffing and self-grooming behaviors facilitate sexual interactions between opposite-sex conspecifics (Moffatt and Nelson 1994, Ferkin *et al*. 2001, Ferkin and Leonard 2005, Pierce *et al*. 2005). Thus, the increase of sniffing behavior in males in response to lactating female urinary odor reflects a sexual motivation. Prairie voles (*Microtus ochrogaster*) use self-grooming behavior to enhance sexual communication when met with opposite-sex individuals (Ferkin *et al*. 2001). For male root voles, self-grooming may increase the detection of their scent cues by conspecifics and may attract females (Zhao *et al*. 2003). Our behavioral results showed that the urine of lactating females is more attractive to males than that of non-lactating females.

We detected 34 kinds of volatile compounds in the urine of lactating and non-lactating female voles, which are fewer than the number of volatiles in the urine of male voles (Boyer *et al*. 1988) and mice (Schwende *et al*. 1986). A low number of volatile compounds have also been reported in the urine of the California mouse (*Peromyscus californicus*) (Jemiolo *et al*. 1994) and *Mus musculus* (Achiraman and Archunan 2006). Thirty-four kinds of volatile components those we found are distributed among basic, individual-specific, and lactation-specific volatile compounds. The basic volatile compounds included a strikingly high number of alkanes and benzo- forms. These volatile compounds were present in all individuals, suggesting that they are probably common metabolic final products in vole urine.

Our results suggest that root vole urine contains a wealth of information that codes for sex and individuality, which is consistent with previous behavioral tests (Zhao 1997, Zhao *et al*. 2002, 2003, Sun *et al*. 2007a,b). The information concerning sex and individuality, as proposed by Sun and Müller-Schwarze (1998a, b) for beavers and exemplified further by three *Mustela* species (Zhang *et al*. 2003, 2005), may be coded by analog components and/or digital forms. The two general forms of information coding-digital and analog-corresponding to information ceding by presence/absence of chemicals used for communication versus coding by varying amounts of these chemicals. In present study, we found 18 individual-specific volatile compounds that code individual information (Table 3), with information for each individual being coded by one to six volatile compounds, which are distributed uniquely and randomly. This indicates that root vole urinary odor information is in digital form.

Urinary volatile compounds that covary in quality or quantity with biological characters may be considered putative pheromones (Singer *et al*. 1997, Novotny *et al*. 1999a). Based on qualitative differences in female root vole urine, we hypothesize that (*E*)5-octadecene and (*N*)hexadecanoic acid are potential lactating pheromones. The molecular weight of these two putative pheromones is less than 300, and they have fewer than 20 carbons. Pheromones usually contain 5 – 20 carbon atoms and must be volatile to reach the receiver (Dominic 1991). The molecular weight of the urinary compound 1-iodoundecane, an estrus-specific compound in bovines, is less than 300 (Rameshkumar *et al*. 2000). Likewise, the

preovulatory urine of female Asian elephants that is involved in attracting males contains a specific compound, *(Z)*7-dodecene-1-ylacetate, with 13 carbons and a molecular weight of 300 (Rasmussen *et al.* 1997). Hence, (*E*)5-octadecene and (*N*)hexadecanoic acid have the physical properties of putative urinary chemosignals.

Individual No.	Individual-Specific Compound Number	Shape simulation
1	13, 17, 24, 27, 31	■□▶◀▫
2	13, 17	■□
3	12, 13, 17, 20, 32, 33, 34	▓■□♦ ◙°☼
4	30, 32	●◙
5	19, 22, 24, 29	▪━▶○
6	1, 20	░♦
7	28	◊
8	19, 20, 22, 23, 24	▪♦━▲▶
9	24	▶
10	17, 20, 24, 33	□♦ ▶°
11	13, 20, 22	■♦━
12	25	▼

Individual-specific volatile compounds were shape-coded to express individual urinary odor characters clearly.
1 ░ 12 ▓ 13 ■ 17 □ 19 ▪ 20 ♦ 22 ━ 23 ▲ 24 ▶ 25 ▼ 27 ◀ 28 ◊ 29 ○ 30 ● 31 ▫ 32 ◙ 33 ° 34 ☼

Table 3. Individual information coded by individual-specific volatile compounds

(*N*)hexadecanoic acid is a long-chain fatty acid. Long-chain fatty acid pheromones are found in a variety of other vertebrates, including the male ferret (*Mustela furo*) (Clapperton *et al.* 1988) and leopard gecko (*Eublapharis macularius*) (Mason and Gutzke 1990). Some straight-chain fatty acids are common components of insect pheromone blends (Elsayed 2005). Such convergent uses of the same type of compound in different species indicate that these volatile compounds may possess chemical properties typical of pheromones, such as volatility, which allows them to convey airborne cues over a distance.

Hexadecanol and hexadecyl acetate have been reported to be among the major components of preputial gland secretions in both sexes of mice (Zhang *et al.* 2007a) and are pheromonal components in many insects, as well as in the bank vole (*Clethrionomys glareolus*) (Brinck and Hoffmeyer 1984; Wyatt 2003). Zhang *et al.* (2008) found that hexadecanol and hexadecyl acetate attract males in a dose-dependent manner. These investigations support the suggestion that (*N*)hexadecanoic acid, which is similar to hexadecanol and hexadecyl acetate, is a pheromonal compound.

In summary, we found that individual identification is coded in digital form. We confirmed that the urine of lactating females was more attractive to male root voles than that of non-lactating females and suggested that the presence of two putative pheromones was the effective component. In future studies, we plan to verify pheromonal activity via bioassay and application of synthetic chemosignals.

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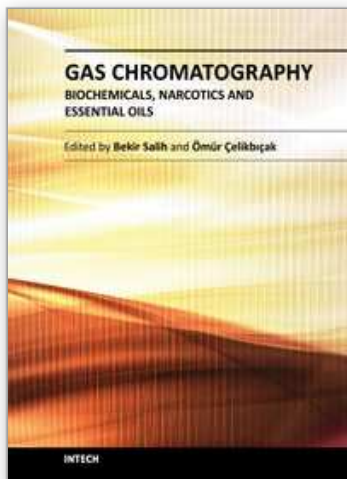
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Edited by Dr. Bekir Salih

Gas Chromatography involves the study of various vaporizable molecules in chemistry and the other related research fields. This analytical method has a number of features and advantages that make it an extremely valuable tool for the identification, quantification and structural elucidation of organic molecules. This book provides detailed gas chromatography information to applications of biochemicals, narcotics and essential oils. The details of the applications were briefly handled by the authors to increase their comprehensibility and feasibility. This guide should be certainly valuable to the novice, as well as to the experienced gas chromatography user who may not have the enough experience about the specific applications covered in this book. We believe this book will prove useful in most laboratories where modern gas chromatography is practiced.

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