Chem. Senses 35: 647–654, 2010

doi:10.1093/chemse/bjq049
Advance Access publication June 7, 2010

Urinary Volatile Molecules Vary in Males of the 2 European Subspecies of the House Mouse and Their Hybrids

C. Mucignat-Caretta[1], M. Redaelli[1], A. Orsetti[2], M. Perriat-Sanguinet[3], G. Zagotto[2] and G. Ganem[3]

[1]Department of Human Anatomy and Physiology, University of Padova, Via Marzolo 3, 35131, Padova, Italy, [2]Department of Pharmaceutical Sciences, University of Padova, Via Marzolo 5, Padova, Italy and [3]Institut des Sciences de l'Evolution de Montpellier, UMR 5554 (UM2, Centre National de la Recherche Scientifique), Laboratoire Génétique et Environnement, C.C. 065, Université Montpellier II, Place E. Bataillon, Montpellier, France

Correspondence to be sent to: Carla Mucignat-Caretta, Department of Human Anatomy and Physiology, University of Padova, Via Marzolo 3, 35131, Padova, Italy. e-mail: carla.mucignat@unipd.it

Accepted April 27, 2010

Abstract

Mice recognize other mice by identifying chemicals that confer a molecular signature to urinary marks. Such molecules may be involved in species recognition, and previous behavioral studies have related divergence of sexual preference between 2 subspecies of the house mouse (*Mus musculus musculus* and *Mus musculus domesticus*) to urinary odors. To characterize the differences between odors of males of the 2 subspecies and their first-generation offspring, the urinary volatile molecules were examined via gas chromatography coupled to mass spectrometry. Seven molecules were present in the samples from mice of at least one group. Their quantity varied among groups: *M. m. domesticus* showed a quantitatively richer panel of odorants in their urine when compared with *M. m. musculus*. The hybrids showed a more complex picture that was not directly related to one or the other parental subspecies. These quantitative differences may contribute to the specificity of the odorant bouquet of the 2 subspecies.

Key words: chemosignals, *Mus musculus*, pheromones, speciation, urine

Introduction

Mice communicate among themselves by emitting chemosignals that convey information about gender, age, hormonal, and social status as well as health conditions (Wyatt 2003; Arakawa et al. 2008). The basic characteristics of chemical communication in mice have been revealed, albeit details are still missing. In laboratory mice, it has been shown that urine contains different types of chemicals that can be used for communication: broadly, they can be divided in to low-molecular weight molecules, mainly represented by odorant components, and proteins (Bigiani et al. 2005). The urine of male mice is characterized by an unusual proteinuria: the major urinary proteins (MUP) are androgen-dependent small proteins that signal the presence of a male, ultimately modifying reproductive physiology in females (Mucignat-Caretta et al. 1995), permitting individual identification (Hurst et al. 2001; Cheetham et al. 2007), and inducing aggressive behavior in other males by acting through vomeronasal receptors (Chamero et al. 2007). Other proteins may be involved in MHC-dependent signaling (Thompson et al. 2007, but see Sherborne et al. 2007).

The urine of male mice is characterized also by a strong smell that results from a blend of different molecules present in variable proportions in the urine of different mice (Novotny et al. 1990; Cavaggioni et al. 2006). Some androgen-dependent substances have been long recognized, among which 2,3-dehydro-*exo*-brevicomin (DHB) and 2-*sec*-butyl-4,5-dihydrothiazole (SBT) (Novotny et al. 1985). Noteworthy, these substances are differently bound and slowly released by MUP in the air (Bacchini et al. 1992; Armstrong et al. 2005; Cavaggioni et al. 2006).

In the laboratory, communication through urinary signals is known to be important for gender and dominance signaling (Hurst and Rich 1999; Gosling et al. 2000) and a number of molecules involved have been identified (Harvey et al. 1989). Also the time elapsed from urine deposition is conveyed by differential release in the air of the

various urinary volatile molecules (Cavaggioni et al. 2006, 2008).

However, very little is known as for the chemistry of species recognition in mice or in mammals in general. Recently, the role of urinary chemosignals on divergence in investigation time as an index of the propensity to mate between different subspecies of mice (*Mus musculus musculus*, hereafter: M; and *M. m. domesticus*, hereafter D) was pointed out in naturally occurring populations of the 2 subspecies and hybrids (Smadja and Ganem 2002; Smadja et al. 2004; Ganem et al. 2008) and was suspected to be involved in incipient speciation between these 2 taxa (Smadja and Ganem 2005, 2008). The present study aimed at identifying volatile candidates that may be involved in signal divergence between the 2 subspecies in urine from mice derived from wild populations and maintained in controlled conditions.

The identification of odorants in a complex mixture is not straightforward (Willse et al. 2005), but can benefit from techniques that improve sampling (Cavaggioni et al. 2006). Solid phase microextraction (SPME) was used to collect odorants from the headspace above the urinary samples, representing the volatiles escaping from the aqueous phase, and allowed detection of low-concentration volatiles (Cavaggioni et al. 2006). Gas chromatography (GC) coupled to flame ionization detection (FID) or mass spectrometry (MS) allowed identification and quantification of urinary compounds in each sample. Urine collected from 4 groups of mice was analyzed: M, D, and their first-generation offspring (hereafter F_1) resulting from reciprocal crosses of males and females of the 2 subspecies. The rationale for testing first-generation hybrids was to further assess the extent of divergence between the 2 subspecies. The results show that both subspecies differ in terms of their odorant output and that the hybrids' odors do not directly refer to parents strains: therefore, urinary molecules may be used to identify *Mus* subspecies.

Materials and methods

Animals

Mice originated from 2 strains of wild-derived mice formerly sampled in Denmark (Jutland), where populations of the 2 subspecies occur. They were obtained from the house mouse genetic repository at the University of Montpellier (http://www.univ-montp2.fr/~genetix/souris.htm). Mice of the *M. m. domesticus* strain (referred to as DDO) have experienced 31 generations of breeding, whereas those of the *M. m. musculus* strain (referred to as MDH) have experienced 23 generations of breeding in the laboratory. The last 9 generations of breeding were aimed to enhance inbreeding. The F_1 hybrids involved in the present study were obtained by reciprocal crossing of males and females of the 2 subspecies strains. F_1M is when the father is M and F_1D when the father is D.

At the time of urine collections the mice were at least 3 months old and no older than 6 months. They were housed in heterosexual pairs and were fed the same laboratory standard pellet (Safe, A04, Augy).

All together 27 male mice were used in this study: 8 D, 7 M, 6 F_1D, and 6 F_1M.

Urine collection

Samples of urine were obtained from the males by a gentle pressure on the mouse belly. Each male was sampled repeatedly at different times and over several days. The urine was collected into tubes kept in ice during the sampling procedure. Once a procedure was completed, the samples were stored at –20 °C until further analysis.

Chemical analysis

Samples were blind-coded and analyzed in a randomized order. The urine was thawed at room temperature immediately before analysis, 50 μL were introduced in a 1.5-mL glass vial closed with a silicone stopper (Agilent) and analyzed according to Cavaggioni et al. (2006). This volume of urine is within the physiological range of urine deposition and does not saturate the binding capacity of the fiber (see Supplementary Figures S1 and S2); under these conditions SPME allows a higher sensitivity than solvent extraction. The polydimethylxiloxane SPME fiber (Supelco), 100 μm thick, after preconditioning (280 °C for 60 min in constant helium flux), was inserted into the vial through the stopper for sampling the headspace at 45 °C for 45 min. The use of this temperature, much lower than the usual SPME loading temperature, was implemented (Cavaggioni et al. 2006) to allow the collection of truly volatile molecules in a reasonable time, mimicking the natural release from a urinary spot voided at 37 °C, while avoiding harsh conditions that could denature MUP (which occurs around 70 °C, Mucignat-Caretta C, unpublished observations), in order to sample the real blend that reached the mice nose for putative subspecies signaling. Afterward, the fiber was desorpted into a gas chromatograph coupled to mass spectrometer (GC/MS, Varian Saturn 2000) for chemical identification or gas chromatograph coupled to flame ionization detector for sample quantitation. Split–splitless injection was performed into a VA-5, 30-m-long capillary column, 0.25 mm diameter, coated with a 0.25 μm phenylmethylpolysiloxane film (Varian). Electron ionization mass spectra were acquired in the *m/z* range 20–800 in full-scan mode. The GC temperature program was: holding for 15 min at 35 °C, 3 min up to 60 °C, 5 min at 60 °C, 10 °C/min up to 150 °C, holding for 1 min, 25 °C/min up to 290 °C, and resting for 20 min. Spectra were analyzed with the resident software. The molecules were identified through comparison with the retention time of standards and with probability matching of mass spectra of the NIST2002 library using the software MS Data Review provided form Varian Inc.

Data analysis and statistics

The areas under the selected peaks were calculated using 2 software for the integration: Chrom Data Review and MS Data review both provided form Varian Inc. The data were compared between-subjects for the factor subspecies (4 levels: D, M, F_1D, and F_1M). We applied the Kruskal–Wallis analysis of variance and differences among groups were assessed with a post hoc test when relevant (Siegle and Castellan 1988).

Principal component analysis did not add any significant information, most probably due to the low abundance of some compounds and the scattered presence of some peaks in only some animals within each group, hence it will be omitted from results.

Results

Table 1 shows the 31 peaks detected and identified with a spectra library matching over 70%. Several other peaks were present, but it was not possible to identify them with a sufficient matching (Figure 1).

Seven peaks were present at least in all the mice minus one of a group and hence were considered as candidates for the divergence between the 2 subspecies. These peaks were resolved and identified, and their analysis showed that their distribution differed among groups as detailed below and in Figure 2 and Table 1. The F_1 urine were characterized by a lower number of peaks (13–16) compared to the parents (24–25 peaks).

Two molecules were present in different quantities in the 2 subspecies. The SBT (identified with a standard, with a retention time of 32.741), although present in all D and M mice, was more concentrated in D (K-W test: $H_{3,27} = 16.4$; post hoc test, $P < 0.001$). The sesquiterpene nerolidol was identified by matching (92.10% Nist2002) at a retention time of 40.410 (Supplementary Figure S3). It was detected in all D and in 71.4% of M mice and was more concentrated in D (K-W test: $H_{3,27} = 15.8$; post hoc test, $P < 0.01$).

SBT and Nerolidol levels were also significantly different between D and F_1M (respectively: $P < 0.01$ and $P < 0.01$). SBT was detected in 83.3% of F_1D and 66.7% of F_1M mice, whereas Nerolidol was detected in 83.3% of F_1D and 33.3% of F_1M mice.

The fatty alcohol 2-butyl-1-octanol (OCT) was detected and identified with a standard, at the retention time 41.043, in 87.5% of D, 57.1% of M, 16.7% of F_1D, and 100% of F_1M. Its quantity differed significantly between the 2 F_1 types (K-W test: $H_{3,27} = 10.72$; post hoc test, $P < 0.05$) and between M and F_1M ($P < 0.05$).

DHB was identified with standard at the retention time 16.337. It was detected in all D and M mice, but only in 66.7% of the 2 types of F_1. The quantity of DHB was significantly greater in D than in the 2 types of F_1 hybrids (K-W test: $H_{3,27} = 17.55$; post hoc test, $P < 0.001$), M mice presenting intermediate levels.

Two additional sequiterpenes were identified: alpha- and beta-farnesene. Alpha-farnesene was identified with a standard as having a retention time of 39.806. It was detected in all D mice, in 71.4% of M, 83.3% of F_1D, and 16.7% of F_1M mice, but its abundance was only marginally different among groups. Beta-farnesene was identified by matching (76.60% Nist2002) and had a retention time of 39.637 (Supplementary Figure S3). It was never detected in D mice, while being present in 42.8% of M, 66.7% of F_1M, and 83.3% F_1D. Its quantity was the highest in F_1D and the lowest in D (Figure 2).

The polycyclic aromatic ketone menadione was identified by matching (75.20% Nist2002) and had a retention time of 40.234 (Supplementary Figure S3). It was present in all D and M mice, in 83.8% of F_1D, and 50% of F_1M. It was significantly more abundant in M than in F_1M (K-W test: $H_{3,27} = 12.16$; post hoc test, $P < 0.01$).

Discussion

Earlier studies involving natural populations of the 2 European subspecies of the house mouse indicated divergence of their urinary mate recognition signals (Smadja et al. 2004; Smadja and Ganem 2008). The present study aimed at identifying the chemical patterns of this divergence in mice derived from wild populations and kept under controlled conditions. We analyzed the composition in low–molecular weight substances (hereafter volatiles) of males' urine of the 2 subspecies (M, D) and of male descendants of their reciprocal crosses (F_1M, F_1D). We hypothesized that the odorant molecules involved in signaling subspecies identity may be shared among all the members of the same subspecies, therefore, we identified in the GC/MS of every mouse all the substances that were present in one group and compared their quantity in the voided urine of the other groups. The composition of the odorant blend was qualitatively similar in the various groups. However, a different picture emerged when addressing quantitative differences: all molecules except one varied significantly among groups. For the first time, the odorant output of F_1 hybrids was also evaluated, showing a poorer profile than the male parent species (D or M) in terms of number of peaks, as well as a quantitatively different blend of molecules.

A number of volatiles were identified by several laboratories in the urine of adult male mice (Novotny et al. 1990; Bacchini et al. 1992; Achiraman and Archunan 2002; Cavaggioni et al. 2006; Röck et al. 2006). Two of the most typical male urinary molecules, DHB and SBT, were demonstrated to be involved in intermale aggression and dominance and to attract females if present simultaneously in the male urine (Jemiolo et al. 1985; Novotny et al. 1985). Furthermore, dominant males were shown to present higher concentrations of SBT in their urine than subdominant males (Harvey et al. 1989). More recently, DHB urine concentrations were found to differ between 3 different strains of the house mouse (ICR, KM, and C57BL/6), whereas SBT

Table 1 List of molecules identified in the sample with over 70% matching

		Retention Time	Match	*Domesticus* $N = 8$	*Musculus* $N = 7$	F_1-*Musculus* $N = 6$	F_1-*Domesticus* $N = 6$
1	2-Pentanone	3.635	85.60%	—	7	—	—
2	Hexenal, 2-ethyl-	8.184	79.30%	8	7	—	—
3	2-Heptanone	16.337	Standard	—	3	—	—
4	Brevicomin, 2,3-dehydro-*exo*	30.467	Standard	8	7	4	4
5	1-Octene	31.152	Standard	8	—	—	—
6	Benzoyl bromide	31.300	93.10%	—	6	—	—
7	Acetophenone	31.430	89.30%	8	—	—	—
8	5-Hepten-2-ol, 6-methyl-	31.890	75.00%	7	—	—	—
9	Thiazoline, 2-*sec*-butyl-4,5-dihydro	32.741	Standard	8	7	4	5
10	Monolinurom (herbicide)	33.317	99%	—	—	1	—
11	Formamide, *N*-phenyl-	35.564	82.90%	8	4	—	—
12	4-Decanone	36.008	77.80%	6	—	—	—
13	Indole	37.832	Standard	4	3	—	—
14	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	38.139	71.44%	7	4	—	—
15	Dodecanal	38.626	80.00%	7	3	—	—
16	1-Phenethylamine	39.025	89.40%	—	5	1	4
17	Tert-butyl-4-hydroxy anisole	39.341	90.60%	8	4	—	—
18	Beta-Farnesene	39.637	76.60%	—	3	4	5
19	Alpha-Farnesene	39.806	Standard	8	5	1	5
20	3-Decanone	40.053	74.30%	6	4	—	3
21	Ethylparaben	40.186	86.60%	8	7	—	5
22	Menadione	40.234	75.20%	8	7	3	5
23	Nerolidol	40.410	92.10%	8	5	2	5
24	Octadecene	40.419	Standard	—	—	3	—
25	3-Bromo-pentane	40.669	79.40%	8	7	—	4
26	2-Butyl-1-octanol	41.043	Standard	7	4	6	1
27	Hexanedioic acid, bis(2-methylpropyl) ester	41.180	78.80%	8	5	1	3
28	Oleyl alcohol	41.485	Standard	6	7	—	—
29	Isopropyl myristate	41.968	78.70%	6	5	1	4
30	2,4-bis-(1,1dimethylethyl)phenol	42.217	82.34%	8	7	—	—
31	1-Eicosanol	42.826	82.70%	8	6	1	4

The number of urine samples presenting each molecule is reported in each cell. —, the molecule was not detected in any sample. The number of mice is indicated for each group.

was claimed to differ less between closely related strains (ICR and KM) as compared with more distantly related strains, that is, the latter versus C57BL/6, further suggesting that the relative concentrations of these 2 substances may vary with the genetic background of the mice (Zhang et al. 2007). Finally, a more recent study pointed out the absence of SBT in *M. spicilegus* which is a species closely related to the house mouse (Soini et al. 2009).

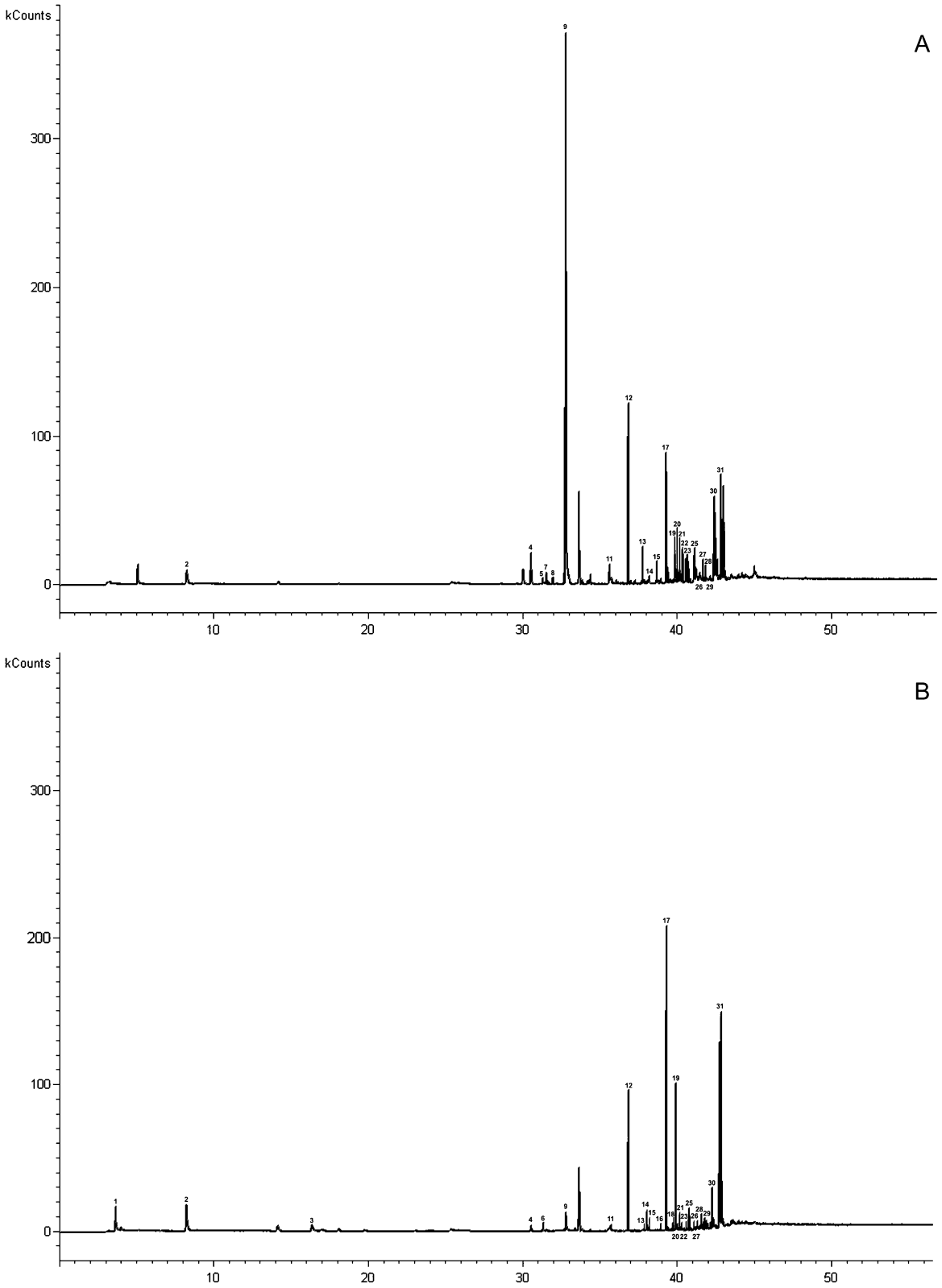

Figure 1 **(A)** Representative chromatogram of urinary volatiles detected in the *Mus mus domesticus* samples. Peak numbers refer to Table 1. **(B)** Representative chromatogram of urinary volatiles detected in the *M. m. musculus* samples. Peak numbers refer to Table 1.

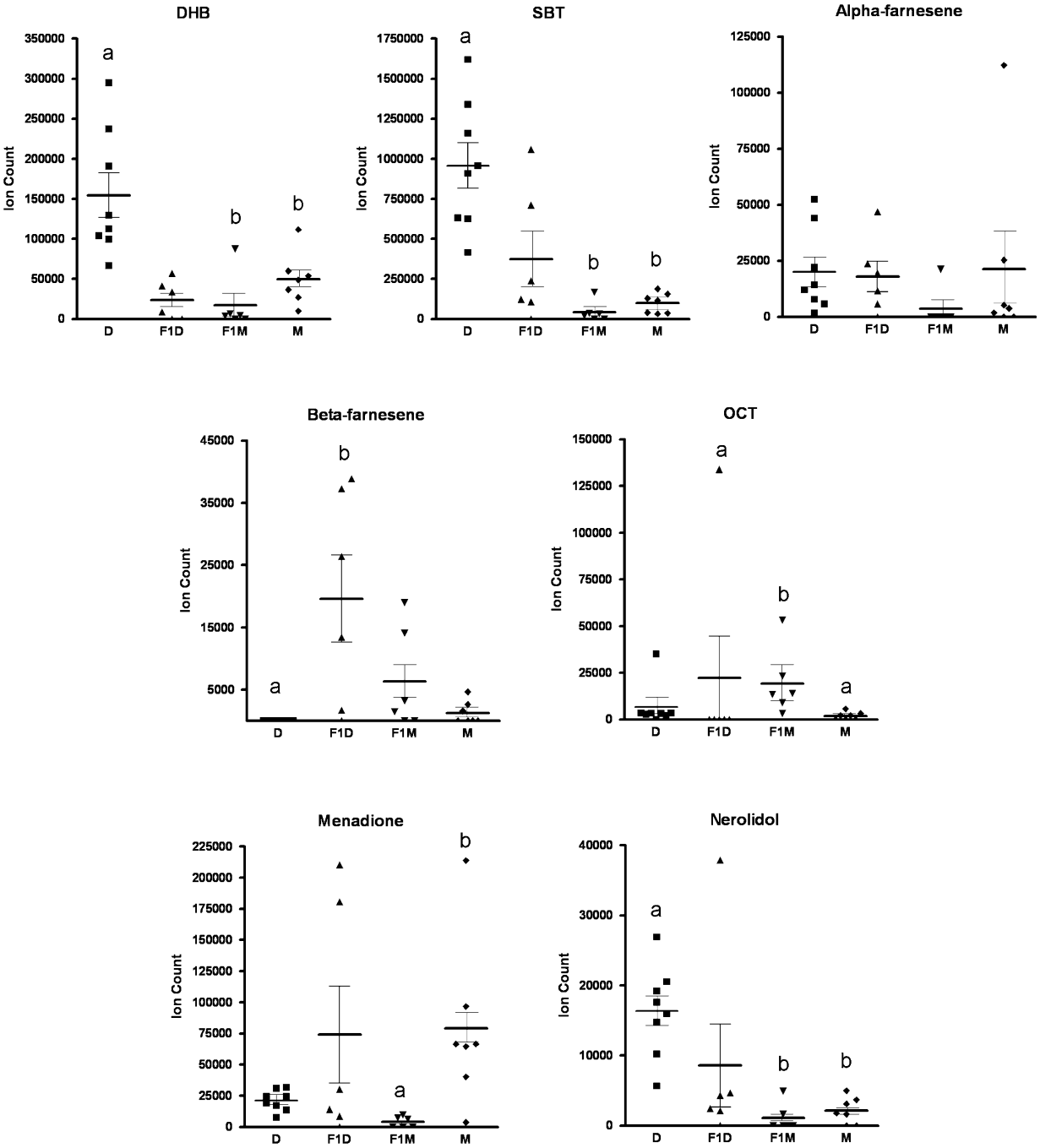

Figure 2 Quantitative comparisons of the peak areas of 7 urinary volatiles in the 4 groups of mice (D, *Mus mus domesticus*, M, *M. m. musculus*; F_1D, F_1M, first-generation hybrids of D × M). Scattered dot plots, standard error mean, and mean as central line are shown. Different letters (a and b) on the plots correspond to significant differences.

The present study shows that the urine of D males is the most concentrated in DHB and SBT. Furthermore, SBT is more present in the urine of D males as compared with M and F_1M, and DHB is more concentrated in D than in the 2 F_1 types of urine. Behavioral dominance as well as higher aggressiveness was repeatedly reported for D males as compared with M males (Thuesen 1977; Van Zegeren and Van Oortmerssen 1981; Munclinger and Frynta 2000) and may relate to higher concentrations of SBT in D mice. Our mice were kept in heterosexual pairs and were not subjected to challenges from other males; hence, the quantity of molecules measured here might be considered as "basal" levels and appear to be higher in mice belonging to subspecies D than those of subspecies M. Furthermore,

our results suggest that SBT might be part of the molecular blend that may specifically characterize the odorant signals of the 2 subspecies through quantitative differences. Different concentrations of the 2 pheromones promoting intermale aggression, dominance, and female attraction suggest that the relative fitness (reproductive success) of males of the 4 categories may differ and that D males may be more successful in situations where the different types of males interact. Interestingly, SBT presents a slow and relatively long lasting (more than 24 h) release from urinary spots (Cavaggioni et al. 2006) and hence is expected to advertise the male presence for a relatively long period.

The 2 farnesene isomers are also known to have an important role in intermale aggression and female attraction (Harvey et al. 1989; Ma et al. 1999; Zhang et al. 2007). Alpha-farnesene is the only molecule for which we did not detect significant variation among groups. As far as beta-farnesene is concerned, surprisingly, we did not detect this molecule in any of our D males' urine, although it was present in the 3 other groups. An earlier report suggested that frozen samples of urine may tend to lose their farnesenes (Cavaggioni et al. 2006). However, this does not seem to apply to our study because all the urine samples were treated in the same way and still some contained either both isomers or at least one. Moreover, the presence of beta-farnesene in urine was shown to vary within and between individuals (Kayali-Sayadi et al. 2003), which is consistent with our findings for all molecules, but may not suffice to explain its absence in all D individuals tested in the present study.

Nerolidol was identified here and was present in significantly higher concentrations in D than in M mice. To our knowledge, this molecule was reported to be present in temporal excretions of African and Asian elephants (Goodwin et al. 2002). It may also participate to subspecies recognition in the house mouse.

Our study also evidenced quantitative variation of menadione between the groups. Although the presence of this molecule in our laboratory-reared mice probably relates to their food (menadione is vitamine K, supplemented in laboratory diet), its variation among groups is puzzling and may indicate metabolic differences between the different groups of mice.

Interestingly, patterns of variation of the fatty alcohol OCT suggest that odors of the 2 type of hybrids may be distinguishable on the basis of different concentrations of this molecule. We know that urine of the 2 types of F_1 can be discriminated by a parental nose (Ganem G, unpublished data). However, whether OCT may be involved in discrimination between these F_1 hybrids remains to be tested with bioassays.

The comparison of the odorant content of hybrids versus parental types of urine does not allow us to propose a clear pattern of parental influence. Nevertheless, we may tentatively point out that: 1) Hybrids issued from D type father present similar levels of SBT to their fathers although not similar levels of DHB; 2) Hybrids issued from M father have similar levels of Nerolidol as their fathers although not similar levels of Menadione; 3) Hybrids with a D father present the highest levels of beta-farnesene although not detected in the urine of their fathers. These preliminary results strongly suggest that laboratory-obtained hybrid genomes may be valuable in studies aiming to unravel the possible genetic determinism of pheromone production.

Our study reveals both between- and within-group variations in volatile urinary compounds and suggests that quantitative differences in some of these molecules might be involved in discrimination between the 2 European subspecies of the house mouse and hence might play a role in sexual isolation (Smadja and Ganem 2008). However, mice urine also contains nonvolatile informative components such as the MUP which were shown to differ between strains, populations, and species (Sampsell and Held 1985; Robertson et al. 2007). Urinary MUP are excreted with a variety of volatile ligands bound in their core binding pocket (Bacchini et al. 1992) may act as pheromones (Mucignat-Caretta et al. 1995) and slow pheromone releaser (Armstrong et al. 2005). The present data show that M urine is poorer in volatiles than D urine. A different study reported that M male urine present a higher MUP content compared with D males (Stopkova et al. 2007). In addition, M mice were less effective in aggression toward intruders (Thuesen 1977). These differences may be related to the lesser quantity of volatiles in M mice, whereas D males advertise more strongly their presence with odorants to intruders or to females.

Future studies should test the involvement of our candidate molecules in subspecies signaling and clarify the role of other nonvolatile molecules in the subspecific signal blend in wild population of mice.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/.

Funding

This work was supported by the University of Padova, the University of Montpellier II, and the Centre National de la Recherche Scientifique (CNRS) no. ISEM 2010-030.

References

Achiraman S, Archunan G. 2002. Characterization of urinary volatiles in Swiss male mice (*Mus musculus*): bioassay of identified compounds. J Biosci. 27:679–686.

Arakawa H, Blanchard DC, Arakawa K, Dunlap C, Blanchard RJ. 2008. Scent marking behavior as an odorant communication in mice. Neurosci Biobehav Rev. 32:1236–1248.

Armstrong SD, Robertson DH, Cheetham SA, Hurst JL, Beynon RJ. 2005. Structural and functional differences in isoforms of mouse major urinary proteins: a male-specific protein that preferentially binds a male pheromone. Biochem J. 391:343–350.

Bacchini A, Gaetani E, Cavaggioni A. 1992. Pheromone binding proteins of the mouse, Mus musculus. Experientia. 48:419–421.

Bigiani A, Mucignat-Caretta C, Montani G, Tirindelli R. 2005. Pheromone reception in mammals. Rev Physiol Biochem Pharmacol. 154:1–35.

Cavaggioni A, Mucignat-Caretta C, Redaelli M. 2008. Mice recognize recent urine scent marks by the molecular composition. Chem Senses. 33: 655–663.

Cavaggioni A, Mucignat-Caretta C, Redaelli M, Zagotto G. 2006. The scent of urine spots of male mice, Mus musculus: changes in chemical composition over time. Rapid Comm Mass Spectrom. 20:3741–3746.

Chamero P, Marton TF, Logan DW, Flanagan K, Cruz JR, Saghatelian A, Cravatt BF, Stowers L. 2007. Identification of protein pheromones that promote aggressive behaviour. Nature. 450:899–902.

Cheetham SA, Thom MD, Jury F, Ollier WE, Beynon RJ, Hurst JL. 2007. The genetic basis of individual-recognition signals in the mouse. Curr Biol. 17:1771–1777.

Ganem G, Litel C, Lenormand T. 2008. Variation in mate preference across a house mouse hybrid zone. Heredity. 100:594–601.

Goodwin TE, Brown FD, Counts RW, Dowdy NC, Fraley PL, Hughes RA, Liu DZ, Mashburn CD, Rankin JD, Roberson RS, et al. 2002. African elephant sesquiterpenes. II. Identification and synthesis of new derivatives of 2,3-dihydrofarnesol. J Nat Prod. 65:1319–1322.

Gosling LM, Roberts SC, Thornton EA, Andrew MJ. 2000. Life history costs of olfactory status signalling in mice. Behav Ecol Sociobiol. 48: 328–332.

Harvey S, Jemiolo B, Novotny M. 1989. Pattern of volatile compounds in dominant and subordinate male mouse urine. J Chem Ecol. 15:2061–2072.

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

Hurst JL, Rich TJ. 1999. Scent marks as competitive signals of mate quality. In: Johnson RE, Muller-Schwarze D, Sorenson P, editors. Advances in chemical communication in vertebrates. New York: Plenum. p. 209–226.

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

Kayali-Sayadi MN, Bautista JM, Polo-Díez LM, Salazar I. 2003. Identification of pheromones in mouse urine by head-space solid phase micro-extraction followed by gas chromatography-mass spectrometry. J Chromatogr B. 796:55–62.

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

Mucignat-Caretta C, Caretta A, Cavaggioni A. 1995. Acceleration of puberty onset in female mice by male urinary proteins. J Physiol. 486:517–522.

Munclinger P, Frynta D. 2000. Social interactions within and between two distant populations of house mouse. Folia Zool. 49:1–6.

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 U S A. 82: 2059–2061.

Robertson DH, Hurst JL, Searle JB, Gündüz I, Beynon RJ. 2007. Characterization and comparison of major urinary proteins from the house mouse, Mus musculus domesticus, and the aboriginal mouse, Mus macedonicus. J Chem Ecol. 33:613–630.

Röck F, Mueller S, Weimar U, Rammensee HG, Overath P. 2006. Comparative analysis of volatile constituents from mice and their urine. J Chem Ecol. 32:1333–1346.

Sampsell BM, Held WA. 1985. Variation in the major urinary protein multigene family in wild-derived mice. Genetics. 109:549–568.

Sherborne AL, Thom MD, Paterson S, Jury F, Ollier WER, Stockley P, Beynon RJ, Hurst JL. 2007. The genetic basis of inbreeding avoidance in house mice. Curr Biol. 17:2061–2066.

Siegle S, Castellan NJ. 1988. Nonparametric statistics for the behavioral sciences. 2nd ed. Singapore: McGraw-Hill.

Smadja C, Catalan J, Ganem G. 2004. Strong premating divergence in a unimodal hybrid zone between two subspecies of the house mouse. J Evol Biol. 17:165–176.

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.

Smadja C, Ganem G. 2005. Asymmetrical reproductive character displacement in the house mouse. J Evol Biol. 18:1485–1493.

Smadja C, Ganem G. 2008. Divergence of odorant signals within and between the two European subspecies of the house mouse. Behav Ecol. 19:223–230.

Soini H, Wiesler D, Koyama S, Féron C, Baudoin C, Novotny M. 2009. Comparison of urinary scents of two related mouse species, Mus spicilegus and Mus domesticus. J Chem Ecol. 35:580–589.

Stopkova R, Stopka P, Janotova K, Jedelsky PL. 2007. Species-specific expression of major urinay proteins in the house mice (Mus musculus musculus and Mus musculus domesticus). J Chem Ecol. 33:861–869.

Thompson RN, McMillon R, Napier A, Wekesa KS. 2007. Pregnancy block by MHC class I peptides is mediated via the production of inositol 1,4,5-trisphosphate in the mouse vomeronasal organ. J Exp Biol. 210:1406–1412.

Thuesen P. 1977. A comparison of the agonistic behaviour of the Mus musculus L. and Mus musculus domesticus Rutty (Mammalia and Rodentia). Vidensk Dan Naturhist Foren. 140:117–128.

Van Zegeren K, Van Oortmerssen GA. 1981. Frontier disputes between the West- and East-European house mouse in Schleswig-Holstein, West Germany. Z Säugetierkunde. 46:363–369.

Willse A, Belcher AM, Preti G, Wahl JH, Thresher M, Yang P, Yamazaki K, Beauchamp GK. 2005. Identification of major histocompatibility complex-regulated body odorants by statistical analysis of a comparative gas chromatography/mass spectrometry experiment. Anal Chem. 77:2348–2361.

Wyatt TD. 2003. Pheromones and animal behaviour. Oxford: Cambridge University Press.

Zhang JX, Rao XP, Sun L, Zhao CH, Qin XW. 2007. Putative chemical signals about sex, individuality, and genetic background in the preputial gland and urine of the house mouse (Mus musculus). Chem Senses. 32:293–303.