



Skin and gland but not urine odours elicit conspicuous investigation by female grey short-tailed opossums, *Monodelphis domestica*

IDO ZURI, KEITH DOMBROWSKI & MIMI HALPERN

Department of Anatomy and Cell Biology, State University of New York, Downstate Medical Center

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Chemical communication plays a major role in mammalian behaviour. One mammal with well-developed chemical senses is the grey short-tailed opossum, a small, South American marsupial that has become common in laboratory research. Male and female opossums scent-mark with different body parts, but apparently not with urine, and females enter oestrus following their exposure to male odours. To elucidate the effects of body odours on the behaviour of female opossums, we investigated their ability to discriminate between conspecific odours originating from different body parts. We simultaneously presented female opossums with two cotton balls smeared either with skin and glandular secretions or urine of conspecifics, or with distilled water or blank controls, and we analysed the time females spent investigating each stimulus. Odours of the male suprasternal gland elicited intensive investigation by the test females. Male flank and submandible odours, and female submandible odours, were also attractive to the females. Investigation of male and female urine was comparable to investigation of water and blank controls. We suggest that in this species, which inhabits semiarid areas, urine marking is avoided to reduce dehydration, and consequently urine has lost its biological significance as a chemosignal. On the other hand, glandular and skin secretions, which are more stable than aqueous deposits in dry conditions, allow the opossums to communicate with each other effectively and also to retain body fluids.

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In mammals, a variety of scent glands (Flon et al. 1970; Mykytowycz 1970; Thiessen & Rice 1976), urine (Beauchamp 1973), saliva (Block et al. 1981; Harris & Murie 1982) and vaginal secretions (Johnston 1980) convey significant information from one individual to another. There is ample evidence concerning the broad range of information that these odours carry. For example, chemosignals are used as deterrent signals by territory holders (Gorman & Stone 1990; Richardson 1990; Zuri et al. 1997), and provide information about an animal's sex, physical condition, physiological state, age, social ranking and individual identity (e.g. Mykytowycz 1970). In reproductive contexts, males can determine the status of receptivity in females from their urinary odours (Beach & Gilmore 1949; Doty & Dunbar 1974; Lai et al. 1996),

anogenital secretions (Ziegler et al. 1993) and vaginal secretions (Michael & Keverne 1970; Steel 1984).

Mammalian scent marking, by which the individual advertises its odours, usually occurs in a stereotypic manner, where one part of the body is rubbed against an object, and one or more odours are secreted into the environment (Mykytowycz 1970; Ralls 1971; Thiessen & Rice 1976). Apparently, different parts of the body produce different chemosignals (McClintock 2002). To decode the complexity of odour signals, attention should be given to the role of odours from different body sources in affecting the behaviour and physiology of conspecifics. For example, Johnston et al. (1993) and Tang-Martinez et al. (1993) found that, in the golden hamster, *Mesocricetus auratus*, an individual olfactory 'signature' is provided in signals from specific body sources but not others. Different body odours may have different qualities and effects on conspecifics (Dugmore & Evans 1990; Ferkin & Johnston 1995; Lai et al. 1996; Petrulis & Johnston 1997).

Here we compared the roles of different body odours in a social context in the South American grey short-tailed opossum, a small (80–155 g) marsupial from the family Didelphidae (Fadem et al. 1982). These opossums have

Correspondence: I. Zuri, Department of Anatomy and Cell Biology, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203-2098, U.S.A. (email: ido_zuri@downstate.edu).

complex marking behaviours and they rely heavily on olfaction (Streilein 1982b). Males scent-mark with different parts of their bodies, and especially with the ventral part of the head, flanks and chest, where the sexually dimorphic suprasternal gland is located (Fadem & Cole 1985; Fadem & Schwartz 1986; Poran et al. 1993). Females on the other hand mainly mark with the head and flanks. In surprising contrast to what is observed in many other mammals (Brown 1973; Hart 1974; Hurst 1987; Rozenfeld et al. 1987), urine marking has not been reported in these opossums (Fadem & Cole 1985; Poran et al. 1993).

To date, little is known about the role of body odours in conspecific communication in the grey short-tailed opossum. It is known that females come into oestrus following exposure to male bedding (Fadem 1985; Fadem & Rayve 1985) or to suprasternal glands secretions (Jackson & Harder 2000). It is not clear, however, which of the male odours is responsible for oestrus induction in the females.

In an earlier study, Zuri et al. (2003) reported that female opossums intensively investigate odours of the male suprasternal gland and submandibles, but not male urine. However, female response to two body odours presented simultaneously, and to odours of male flank and female odours, was not tested in that earlier work.

Based on a lack of evidence for urine marking in grey short-tailed opossums (Fadem & Cole 1985), and on our previous findings (Zuri et al. 2003), we hypothesized that urine lost its significance as a chemosignal for female opossums. Since males scent-mark more than females (Fadem & Cole 1985), we also hypothesized that male body odours would be investigated more than female odours, and that odours from the male suprasternal glands would elicit the strongest investigation by the females, since it is the largest scent gland on the male's body surface, and is known to induce female oestrus (Jackson & Harder 2000). We anticipated that our work would shed significant light on the role of urinary excretions in conspecific communication in small mammals from arid niches: the need to conserve body water has not only modified the animals' physiology (Schmidt-Nielsen & Schmidt-Nielsen 1952) but also may have reduced the significance of urinary excretions in conspecific communication.

METHODS

Experimental Animals

Ten adult female grey short-tailed opossums were used as experimental animals. None of the test females were siblings of one another or of the odour donors. The females were 12–18 months old, and had delivered at least one litter. All litters were weaned at least 2 months prior to the beginning of the experiment. Another 10 adult male and eight sexually experienced adult female opossums were used as odour donors. All animals originated from the Southern Foundation for Biomedical Research (San Antonio, Texas, U.S.A.), and were descendents of the 28 founders that were captured in Brazil and Bolivia between 1978 and 1993 (VanderBerg & Robinson 1997).

Previous studies have shown that female grey short-tailed opossums enter oestrus following their exposure to male odours (Fadem & Rayve 1985; Fadem 1987, 1989; Zuri et al. 2003), and that their behavioural receptivity lasts 36 h (Trupin & Fadem 1982). Our preliminary observations (unpublished data) indicated that isolated females do not enter oestrus. Therefore, in the present study, both the donor and test females were held in cages separated from those of the males by at least 3 m, with no direct contact or odour transfer between their cages, and the females were considered to be in a nonoestrous condition. To minimize odour transfer between individual cages, an air-conditioning system provided continuous airflow into and out of the room, and fresh disposable gloves were used on all trials and during animal handling.

The opossums were kept individually in standard plastic cages (42 × 21 × 20 cm) with wood shavings and tissue paper as bedding, and provided with a cylindrical plastic box for nesting. Dry fox food (Milk Specialties Co., New Holstein, Wisconsin, U.S.A.) and water were given *ad libitum*. Fruits were added to the opossum diet twice a week. Animals were kept at 26–28°C and 25–30% RH, with reversed 12:12 h light:dark fluorescent light regime (lights off at 1200 hours) in a room belonging to the Central Animal Service of the Downstate Medical Center in Brooklyn, New York. Since these opossums are nocturnal (Streilein 1982a, b), dim red illumination was provided continuously in their maintenance room by four 5-W red light bulbs, allowing us to monitor the animals during their subjective night.

Experimental Procedures

General procedures

The experiments were conducted from July to December 2002. To minimize possible effects of novelty on the opossum behaviour, the experimental animals were tested in their home cages. The various test odours were presented randomly (Rohlf & Sokal 1981) to the test animals. We also randomized the order of odour presentation to different individuals, and avoided situations where one opossum would be tested repeatedly with odours from the same donor.

Odour collection and presentation

Test odours were collected from the donors between 0900 and 1030 hours before their activity period began. For collection of donors' body secretions, the animals were hand-held for a few seconds by the experimenter and a cotton ball was rubbed gently against a body part and stored in a clean disposable vial. For urine collection, a wire mesh (hole dimension 0.15 × 0.15 cm) was inserted between a standard plastic cage (28 × 17 × 10 cm) and its cover, and the opossum was placed on the wire mesh for 1–2 h. The urine was collected every 20 min and transferred to glass vials and stored at 9°C until the test session began. Animals were returned to their home cages immediately after urine collection. The collection of urine did not prevent contamination of urine material with anal gland secretion and faeces; however, since our aim was to

test the opossums' responses to urine excreted in a natural manner, we did not attempt to isolate the urine components. All tests were conducted on the day of odour sampling. Since previous studies (Streilein 1982a, b; VanderBerg & Robinson 1997) have shown that the activity of grey short-tailed opossums is concentrated during the first several hours of the evening, all tests started 30–60 min after the beginning of the subjective night.

A trial consisted of presentation of two odours to the test opossum. Two-choice presentation is more sensitive than single-presentation and cross-habituation paradigms in determining the ability of animals to discriminate between odours, because it does not require the animal to memorize the odours (Johnston & Peng 2000). The two cotton balls used as test stimuli were presented in a round-robin design. The cotton balls were smeared with one of the three materials: body odours of the opossum donor, urine of the opossum donor, or distilled water (dH₂O). Blank cotton balls were also used as controls. Two cotton balls were placed 15–20 cm apart on one side of the animal's cage. When the test odour was urine or dH₂O, we placed 40 µl of the solution on the top of the cotton ball just before testing the opossum, and presented it with the solution-side directed dorsally. If the test odour was obtained from the donor's body surface, it was already smeared on a cotton ball and ready for use. One person placed the test odours in the test cage, while another person, who videorecorded the animals and analysed their behaviour, was blind to odour placement. All tests were monitored with a digital video camera (Sony DCR-VX2000, 30 frames/s) held at a distance of 2.5 m from the test cage, and analysed later with a video-cassette recorder (JVC SR-VS30U), BTV Pro 5.4.1, and 'Video-analyzer' software.

Test odours

Grey short-tailed opossums frequently mark their environment (Streilein 1982b). Males frequently rub their chests, on which the sexually dimorphic suprasternal gland is located (Fadem & Schwartz 1986), against objects in the environment (Fadem & Cole 1985; Poran et al. 1993). They also scent-mark with the ventral part of their heads and with their flanks (Fadem & Cole 1985; Poran et al. 1993). Therefore, odour samples were taken from the male suprasternal gland, from the skin overlying their flanks and from the skin overlying the ventral part of their mandibles. Male urine was also used as a test odour for the females. Females of this species do not possess a suprasternal gland (Fadem & Schwartz 1986) and scent-mark mainly with their heads and flanks (Fadem & Cole 1985). Therefore, female odours were taken from the skin overlying their flanks, and from the skin overlying the ventral part of their mandibles. Female response to female urine was also tested. In all cases where two male odours or two female odours were being compared, the odours were obtained from the same stimulus animal. Thus, differences in response to two male or two female odours were never the result of individual differences.

Data and Statistical Analysis

Opossums that are presented with a novel stimulus in their cage usually approach the stimulus and touch it with their snout, either sniffing or nuzzling the novel object (Poran et al. 1993). Because of the dim illumination under which the opossums were tested, it was difficult to determine whether the opossum sniffed or nuzzled the cotton ball stimulus; therefore, we included all touches of the cotton ball with the snout as 'investigation' behaviour.

The total time the opossums investigated each cotton ball during a 5-min period was determined, starting from the first snout contact with one of the cotton balls. Data analysis included repeated measures ANOVAs where the sphericity of the data was tested by Mauchly's test. When the data were not spherical, the Huynh-Feldt epsilon correction was applied using procedures in SPSS version 10.0 (SPSS 2000). In the analysis, the test session (the combination of two test odours or one odour and one control) and the source of odour were the within-subjects factors. A one-sample Kolmogorov-Smirnov test of normality was applied to the standardized residuals using SPSS, and in all tests the normality of data was confirmed (one-sample Kolmogorov-Smirnov tests: $P > 0.05$). Post hoc LSD pairwise comparisons (PWC) were conducted to determine whether investigation time of one stimulus differed significantly from that of another.

For analysis, the data were organized into four different matrices allowing us to conduct five separate ANOVAs. Using this method we were able to answer four questions corresponding to our hypotheses. (1) Do conspecific body odours and urine elicit stronger investigation by female opossums than dH₂O? (2) Do male odours elicit stronger investigation by the test females than female odours? (3) Do female opossums discriminate between different odours from the same stimulus male? (4) Do female opossums discriminate between different odours of a strange stimulus female?

Results are presented as means \pm SE, and significant results are indicated when $P < 0.05$.

RESULTS

Female Investigation of Conspecific Odours versus dH₂O

Overall, female opossums discriminated significantly between the different odour stimuli (ANOVA: $F_{1,9} = 14.22$, $P = 0.004$; Fig. 1). There was also a significant effect of the test session on female investigation ($F_{7,63} = 7.62$, $P < 0.001$) and an interaction effect of stimulus*test session ($F_{7,63} = 9.65$, $P < 0.001$).

In general, all male and female skin odours were investigated significantly longer than dH₂O (Fig. 1). Odours from the male suprasternal gland were investigated significantly longer than dH₂O (65.8 ± 13.8 s versus 2.4 ± 1.05 s; pairwise comparison (PWC): $P = 0.002$). Similarly, odours from the skin overlying the submandibles and flanks of males were investigated significantly longer than dH₂O (51.45 ± 38.4 s versus

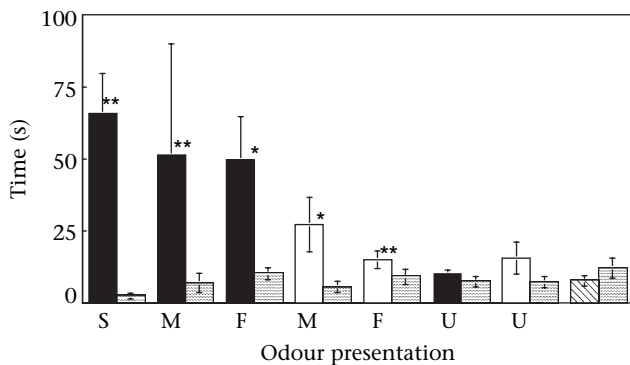


Figure 1. Mean time spent by female opossums investigating skin and urinary odours of male and female opossums, and blank controls, presented simultaneously against distilled water. Each pair of bars represents a single two-choice preference test. S = suprasternal gland, M = submandibular area, F = flank, U = urine. ■: male; □: female; □: distilled water; ▨: blank. * $P < 0.05$; ** $P < 0.01$.

6.96 ± 3.3 s; PWC: $P = 0.007$; 49.8 ± 14.9 s versus 10.15 ± 2.15 s; PWC: $P = 0.002$). Odours from the skin overlying the submandibles and the flanks of strange females elicited significantly longer investigation than dH₂O (27.3 ± 9.5 s versus 5.6 ± 1.9 s; PWC: $P = 0.03$; 15.0 ± 2.99 s versus 9.09 ± 2.65 s; PWC: $P = 0.01$). Neither the odour of urine from either male or female opossums, nor that of blank cotton balls, was investigated significantly longer than that of dH₂O (9.89 ± 1.46 s versus 7.39 ± 1.88 s; PWC: $P = 0.21$; 15.49 ± 5.61 s versus 7.21 ± 1.93 s; PWC: $P = 0.14$; 7.64 ± 1.85 s versus 12.12 ± 3.55 s; PWC: $P = 0.19$; Fig. 1).

Female Investigation of Male versus Female Odours

Overall, there was a significant effect of the sex of the odour donor (ANOVA: $F_{1,9} = 14.64$, $P = 0.004$; Fig. 2) on time spent in olfactory investigation by test females. In addition, there was a significant effect of the test session ($F_{11,99} = 5.26$, $P < 0.004$), and a significant interaction effect of the donor's sex*test session ($F_{11,99} = 6.5$, $P < 0.001$). On simultaneous presentations of two non-urinary odours, the females investigated the male odour longer than the female odour. Odours of male suprasternal gland elicited significantly more investigation than flank odours from a strange female (52.8 ± 12.9 s versus 9.07 ± 2.5 s; PWC: $P = 0.005$; Fig. 2). Similarly, odours from male submandibles elicited significantly more investigation than flank odours from a strange female (39.06 ± 8.3 s versus 10.8 ± 2.4 ; PWC: $P = 0.01$). Male flank odours elicited significantly longer investigation by the test females than flank and submandible odours of a strange female (50.9 ± 11.7 s versus 20.7 ± 6.4 s; PWC: $P = 0.004$ and 37.3 ± 5.1 s versus 22.6 ± 6.7 s; PWC: $P = 0.006$; Fig. 2). However, odours from male submandibles and male suprasternal gland did not elicit significantly longer investigation than odours from female submandibles (61.69 ± 11.5 s versus 38.36 ± 8.24 s;

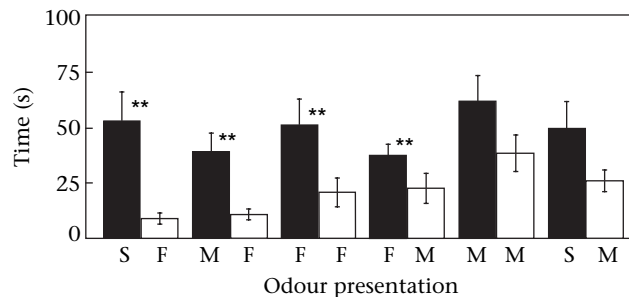


Figure 2. Mean time spent by female opossums investigating skin odours of male and female conspecifics. Each pair of bars represents a single two-choice preference test. S = suprasternal gland, F = flank, M = submandibular area. ■: male; □: female. ** $P < 0.01$.

PWC: $P = 0.11$; 49.45 ± 12.07 s versus 26.02 ± 4.84 s; PWC: $P = 0.07$; Fig. 2).

On simultaneous presentations of two odours, one from the skin surface of a male donor and the other from a strange female's urine, the test females investigated the male odours significantly longer (Fig. 3). Odours of male submandibles and suprasternal gland were investigated significantly longer than urine odours of a strange female (51.07 ± 14.08 s versus 4.6 ± 1.6 s; PWC: $P = 0.009$; 55.8 ± 14.2 s versus 9.4 ± 2.2 s; PWC: $P = 0.009$). Similarly, odours of male flanks were also investigated significantly longer than urine odours from a strange female (48.7 ± 10.7 s versus 12.8 ± 3.6 s; PWC: $P = 0.007$; Fig. 3). Male urinary odours were investigated significantly less than the submandible odours of a strange female (9.4 ± 2.2 s versus 24.8 ± 4.8 s; PWC: $P = 0.009$; Fig. 3). Investigation time of male and of female urine was similar (11.11 ± 2.66 s versus 13.76 ± 3.9 s; PWC: $P = 0.63$; Fig. 3).

Female Investigation of Different Odours from the Same Stimulus Male

Overall, there was a significant effect of the type of stimulus on female investigation (ANOVA: $F_{1,9} = 12.18$, $P = 0.007$; Fig. 4). There was no significant effect of the test session on the female investigation ($F_{5,45} = 1.43$, $P = 0.23$) but there was a significant interaction effect of the test session*type of stimulus ($F_{5,45} = 7.7$, $P < 0.001$). Odours from male suprasternal gland were investigated significantly longer than urinary odours of the same stimulus male (71.9 ± 16.9 s versus 5.4 ± 1.8 s; PWC: $P = 0.003$). In addition, odours from the skin overlying the male submandible were investigated significantly longer than urinary odours from the same stimulus male (53.9 ± 17.04 s versus 7.1 ± 1.6 s; PWC: $P = 0.02$). Similarly, odours from the male flank elicited significantly longer investigation by test females than did urine odours from the same stimulus male (74.3 ± 19.8 s versus 16.6 ± 7.7 s; PWC: $P = 0.01$; Fig. 4).

Females investigated odours of male suprasternal gland significantly longer than odours obtained from the flanks of the same stimulus males (60.6 ± 16.9 s versus 26.8 ± 7.5 s; PWC: $P = 0.02$; Fig. 4). Suprasternal gland

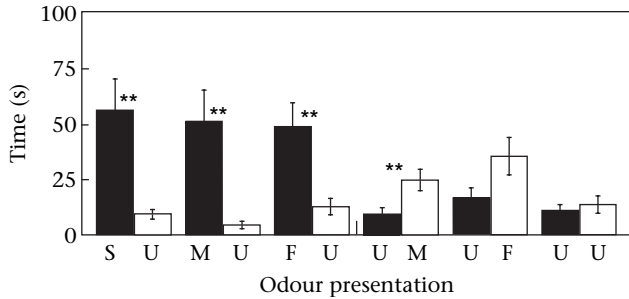


Figure 3. Mean time spent by female opossums investigating skin odours of male and female conspecifics, presented simultaneously with urine from members of the opposite sex. Each pair of bars represents a single two-choice preference test. S = suprasternal gland, U = urine, M = submandibular area, F = flank, ■: male; □: female. $**P < 0.01$.

odours from males elicited more investigation than odours from the submandibles of the same males, but this difference was not significant (51.82 ± 14.66 s versus 25.60 ± 4.17 s; PWC: $P = 0.065$). Investigation of submandible and flank odours of the same stimulus male did not differ significantly (40.16 ± 42.83 s versus 28.83 ± 16.96 s; PWC: $P = 0.37$).

During trials in which male urine, flank and submandible odours were paired with suprasternal gland odours from the same stimulus male, females investigated the submandible and the flank odours significantly longer than the urine odours (25.6 ± 4.1 s versus 5.4 ± 1.8 s; PWC: $P = 0.001$; 26.8 ± 7.5 s versus 5.4 ± 1.8 s; PWC: $P = 0.02$; Fig. 4).

Female Investigation of Body versus Urine Odours of the Same Strange Female

Overall, there was a significant effect of the female odour stimulus on investigation time by the test females (ANOVA: $F_{1,9} = 14.4$, $P = 0.04$; Fig. 4). Odours from the flanks of female donors were investigated significantly longer than urine odours from the same stimulus females (27.5 ± 8.1 s versus 5.5 ± 2.07 s; PWC: $P = 0.02$). Similarly, test females investigated odours from the skin

overlying the females' submandibles significantly longer than urine odours of the same stimulus females (24.7 ± 8.9 s versus 9.5 ± 2.6 s; PWC: $P = 0.05$). However, investigation of submandible and flank odours of the same stimulus female did not differ significantly (22.46 ± 16.25 s versus 22.11 s; PWC: $P = 0.5$; Fig. 4).

DISCUSSION

In contrast to many other mammals (Janus 1988; Hurst 1990; Gao 1991; Ferkin & Johnston 1995), female grey short-tailed opossums do not respond to conspecific urine by investigating it (Zuri et al. 2003). The failure of urine to elicit strong investigation by conspecifics has been reported so far in two other species, the kangaroo rat, *Dipodomys merriami* (Randall 1985), and the golden hamster, *Mesocricetus auratus* (Johnston & Bullock 2001). In the kangaroo rat, urine of females in any reproductive state does not elicit investigation by males. It has been suggested that in the desert environment occupied by these rodents, females do not advertise their reproductive state with urine but with vaginal discharges for reasons of water conservation (Randall 1985). In the golden hamster, females do not investigate urine of males significantly more than clean vials (Petruelis & Johnston 1997). Furthermore, Johnston & Bullock (2001) reported that male golden hamsters fail to make an association between urine and other body odours of female hamsters, and suggested that urine lost its significance as a chemosignal because of its limited use for individual recognition in the semiarid environment from which golden hamsters originate.

Grey short-tailed opossums occur in the semiarid xeric environment of caatinga, Brazil (Streilein 1982a). We believe that, similar to other desert occupants, these opossums use physiological and behavioural strategies of water conservation to avoid dehydration (Schmidt-Nielsen et al. 1948; Schmidt-Nielsen & Schmidt-Nielsen 1952). This notion had been put forward by Christian (1983), who reported that the renal capabilities of grey short-tailed opossum to concentrate water are well developed and resemble that of insectivores and carnivores from desert habitats.

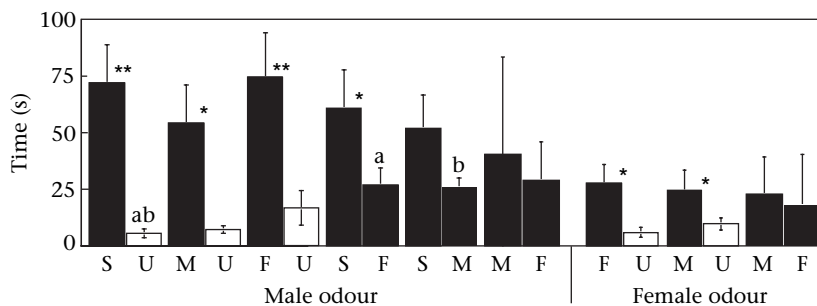


Figure 4. Mean time spent by female opossums investigating skin odours of male and female conspecifics, presented simultaneously with skin odours or urine of the same donor. Each pair of bars represents a single two-choice preference test. S = suprasternal gland, U = urine, M = submandibular area, F = flank. ■: skin or glandular odour; □: urine. Significance levels are presented by asterisks for simultaneous tests and by lower case letters for cross-session tests. a = $*P < 0.05$; b = $**P < 0.01$.

In a previous study, Regnier & Goodwin (1977) demonstrated that lipids and other nonpheromonal materials deposited in scent marks ensure signal release to the environment for prolonged periods. Such a strategy would favour skin and glandular deposits over urine marking and would be advantageous also for grey short-tailed opossums in the arid habitat that they occupy, where aqueous deposits, such as urine, evaporate rapidly. The use of skin lipids for conspecific communication appears to be common in other mammals from arid environments: desert heteromyids typically scent-mark by sandbathing (Randall 1987, 1993), and desert woodrats, *Neotoma lepida lepida*, scent-mark their environs with gland secretions but not with urine (Fleming & Tambosso 1980).

For many mammals, urinary excretions and body odours play a part in conspecific sexual recognition (McClintock 2002 and references therein). The ability to determine the sex of another individual from a distance is especially important for solitary animals, as it allows them to follow odours to members of the opposite sex, and to avoid agonistic confrontation with same-sex conspecifics. Streilein (1982a, b) reported that grey short-tailed opossums are nocturnal and probably nomadic, that they encounter each other infrequently, and that they are intolerant of one another except for brief periods when females are receptive. Thus, it would be advantageous for females to determine the sex of another individual from a distance, to avoid same-sex confrontations, and to remain in the vicinity of males on days of behavioural oestrus, when they are more docile (Trupin & Fadem 1982; unpublished data). Our present study indicates that conspecific skin odours are important for sexual recognition by female opossums.

Secretions from the skin overlying the submandibles of strange females elicited strong investigation by test females, comparable to investigation of male submandible and suprasternal gland odours. It seems likely, therefore, that female mandibular secretions have an important, as yet unspecified, role in conspecific communication in this species. Similar investigation by females of female flank and submandible odours suggest that these two body parts may be important for same-sex olfactory communication.

To date, only a few studies have investigated the differential role of body odours in behavioural responses of conspecifics. The flank secretions of male golden hamsters elicit increased flank and vaginal marking in female hamsters, while male urine and faeces induce decreased markings in the females (Petruilis & Johnston 1997). In addition, individual recognition is determined by only certain body odours in this species (Johnston et al. 1993; Tang-Martinez et al. 1993). In Djungarian hamsters, *Phodopus campbelli*, the odours of urine and of the midventral gland, but not odours of the sacculi (oral corner) or faeces, provide females with information about conspecific sexual identity (Lai et al. 1996). In dogs, *Canis familiaris*, urine deposits are the strongest sexual attractant for both sexes compared with other body odours (Dunbar 1977).

In grey short-tailed opossums, odours of male suprasternal glands elicit significantly more investigation by females than do flank odours, whereas odours from male

mandibles elicit investigation similar to that observed for flank odours. Differences in odour investigation might reflect differences in the quantities of the chemosignal released by each body part. However, the failure of some odours (e.g. urine) to elicit intensive investigation by females, supports our suggestion that odour quality plays a major role in chemical communication in this species. In the opossums, the male suprasternal gland is a large, distinct gland that continuously deposits oily material over the male sternum, and is frequently used for scent marking (Poran et al. 1993). Although investigation of suprasternal gland odours elicited more investigation than odours from the male submandible, this difference just missed significance. This failure to reach significance may be due to commingling of suprasternal gland secretions with mandible odours during grooming. Similar investigation of the male submandible and flank odours by females during simultaneous presentations suggests that secretions of these two body parts are equally attractive to females, and less attractive than odours from the suprasternal gland.

Previous studies have shown that those chemosignals most frequently used for scent marking elicit stronger responses in conspecifics than do other body odours (e.g. Thiessen et al. 1970; Davies & Bellamy 1974; Hart 1974; Dunbar 1977; Kruuk et al. 1984; Hurst 1990). Similarly, female opossums are significantly most attracted to male suprasternal gland excretions, which are most commonly used for scent markings (Poran et al. 1993).

There is no doubt that chemical communication plays a major role in the nocturnal activity of grey short-tailed opossums. With the paucity of data on their behaviour in nature, laboratory observations under controlled conditions provide significant information to allow us to understand conspecific communication in this species. We have shown that odours of different body sources elicit different amounts of investigation by females, and that urine is not a salient chemosignal in this species.

Acknowledgments

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References

- Beach, F. A. & Gilmore, R. W. 1949. Response of male dogs to urine from females in heat. *Journal of Mammalogy*, **30**, 391–392.
- Beauchamp, G. K. 1973. Attraction of male guinea pigs to conspecific urine. *Physiology & Behavior*, **10**, 589–594.
- Block, M. L., Volpe, L. C. & Hayes, M. J. 1981. Saliva as a chemical cue in the development of social behavior. *Science*, **211**, 1062–1064.

- Brown, R. E.** 1973. The fire hydrant effect: stimuli eliciting urine marking in the rat (*Rattus norvegicus*). *Bulletin of the Ecological Society of America*, **54**, 44.
- Christian, D. P.** 1983. Water balance in *Monodelphis domestica* (Didelphidae) from the semiarid caatinga of Brazil. *Comparative Biochemistry and Physiology*, **74A**, 665–669.
- Davies, V. J. & Bellamy, D.** 1974. Effects of female urine on social investigation in male mice. *Animal Behaviour*, **22**, 239–241.
- Doty, R. L. & Dunbar, I.** 1974. Attraction of beagles to conspecific urine, vaginal and anal sac secretion odors. *Physiology & Behavior*, **12**, 825–833.
- Dugmore, S. J. & Evans, C. S.** 1990. Discrimination of conspecific chemosignals by female ringtail lemurs, *Lemur catta* L. In: *Chemical Signals in Vertebrates 5* (Ed. by D. W. MacDonald, D. Müller-Schwarze & S. E. Nalynczuk), pp. 360–366. Oxford: Oxford Science.
- Dunbar, I. F.** 1977. Olfactory preferences in dogs: the response of male and female beagles to conspecific odors. *Behavioral Biology*, **20**, 471–481.
- Fadem, B. H.** 1985. Evidence for the activation of female reproduction by males in a marsupial, the gray short-tailed opossum (*Monodelphis domestica*). *Biology of Reproduction*, **33**, 112–116.
- Fadem, B. H.** 1987. Activation of estrus by pheromones in a marsupial: stimulus control and endocrine factors. *Biology of Reproduction*, **36**, 328–332.
- Fadem, B. H.** 1989. The effects of pheromonal stimuli on estrus and peripheral plasma estradiol in female gray short-tailed opossums (*Monodelphis domestica*). *Biology of Reproduction*, **41**, 213–217.
- Fadem, B. H. & Cole, E. A.** 1985. Scent-marking in the grey short-tailed opossums (*Monodelphis domestica*). *Animal Behaviour*, **33**, 730–738.
- Fadem, B. H. & Rayve, R. S.** 1985. Characteristics of the oestrous cycle and influence of social factors in gray short-tailed opossums (*Monodelphis domestica*). *Journal of Reproduction and Fertility*, **73**, 337–342.
- Fadem, B. H. & Schwartz, R. A.** 1986. A sexually dimorphic suprasternal scent gland in the gray short-tailed opossums (*Monodelphis domestica*). *Journal of Mammalogy*, **67**, 205–208.
- Fadem, B. H., Trupin, G. L., VanderBerg, J. L. & Hayssen, V.** 1982. Care and breeding of the gray, short-tailed opossum (*Monodelphis domestica*). *Laboratory Animal Sciences*, **32**, 405–409.
- Ferkin, M. H. & Johnston, R. E.** 1995. Meadow voles, *Microtus pennsylvanicus*, use multiple sources of scent for sex recognition. *Animal Behaviour*, **49**, 37–44.
- Fleming, A. S. & Tambosso, L.** 1980. Hormonal and sensory control of scent-marking in the desert woodrat (*Neotoma lepida lepida*). *Journal of Comparative and Physiological Psychology*, **94**, 564–578.
- Flon, H., Gerstner, R., Mitchell, O. G. & Feldman, A.** 1970. Salivary glands of heteromyid rodents, with a summary of the literature on rodent submandibular gland morphology. *Journal of Morphology*, **131**, 179–194.
- Gao, Y.** 1991. Behavioural responses of rats to the smell of urine from conspecifics. *Animal Behaviour*, **42**, 506–508.
- Gorman, M. L. & Stone, R. D.** 1990. Mutual avoidance by European moles *Talpa europaea*. In: *Chemical Signals in Vertebrates 5* (Ed. by D. W. MacDonald, D. Müller-Schwarze & S. E. Nalynczuk), pp. 367–377. New York: Oxford University Press.
- Harris, M. A. & Murie, J. O.** 1982. Responses to oral gland scents from different males in Columbian ground squirrels. *Animal Behaviour*, **30**, 140–148.
- Hart, B. L.** 1974. Environmental and hormonal influences on urine marking behavior in the adult male dog. *Behavioral Biology*, **11**, 167–176.
- Hurst, J. L.** 1987. The function of urine marking in a free-living population of house mice *Mus domesticus* Ratty. *Animal Behaviour*, **35**, 1433–1442.
- Hurst, J. L.** 1990. Urine marking in populations of wild house mice *Mus domesticus* Ratty. I. Communication between males. *Animal Behaviour*, **40**, 209–222.
- Jackson, L. M. & Harder, J. D.** 2000. Evidence for spontaneous postlactation estrus in gray short-tailed opossums (*Monodelphis domestica*). *Biology of Reproduction*, **62**, 1823–1827.
- Janus, C.** 1988. The development of responses to naturally occurring odours in spiny mice *Acomys cahirinus*. *Animal Behaviour*, **36**, 1400–1406.
- Johnston, R. E.** 1980. Responses of male hamsters to odors of females in different reproductive states. *Journal of Comparative and Physiological Psychology*, **94**, 894–904.
- Johnston, R. E. & Bullock, T. A.** 2001. Individual recognition by use of odours in golden hamsters: the nature of individual representation. *Animal Behaviour*, **61**, 545–557.
- Johnston, R. E. & Peng, M.** 2000. The vomeronasal organ is involved in discrimination of individual odors by males but not by females in golden hamsters. *Physiology & Behavior*, **70**, 537–549.
- Johnston, R. E., Derzie, A., Chiang, G., Jernigan, P. & Lee, H. C.** 1993. Individual scent signatures in golden hamsters: evidence for specialization of function. *Animal Behaviour*, **45**, 1061–1070.
- Kruuk, H., Gorman, M. & Leitch, A.** 1984. Scent-marking with the subcaudal gland by the European badger, *Meles meles* L. *Animal Behaviour*, **32**, 899–907.
- Lai, S. C., Vasilieva, N. Y. & Johnston, R. E.** 1996. Odors providing sexual information in Djungarian hamsters: evidence for an across-odor code. *Hormones and Behavior*, **30**, 26–36.
- McClintock, M. K.** 2002. Pheromones, odors, and vasanas: the neuroendocrinology of social chemosignals in human and animals. In: *Hormones, Brain and Behavior Vol. 1* (Ed. by D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach & R. T. Rubin), pp. 797–870. New York: Academic Press.
- Michael, R. P. & Keverne, E. B.** 1970. Primate sex pheromones of vaginal origin. *Nature*, **225**, 84–85.
- Mykytowycz, R.** 1970. The role of skin glands in mammalian communication. In: *Advances in Chemoreception. Vol. 1. Communication by Chemical Signals* (Ed. by J. W. Johnston Jr, D. G. Moulton & A. Turk), pp. 327–360. New York: Appleton-Century-Crofts.
- Petruilis, A. & Johnston, R. E.** 1997. Causes of scent marking in female hamsters (*Mesocricetus auratus*): specific signals or classes of information. *Journal of Comparative Psychology*, **111**, 25–36.
- Poran, N., Tripoli, R. & Halpern, M.** 1993. Nuzzling in the gray short-tailed opossum II: familiarity and individual recognition. *Physiology & Behavior*, **53**, 969–973.
- Ralls, C.** 1971. Mammalian scent marking. *Science*, **171**, 443–449.
- Randall, J. A.** 1985. Role of urine in coordinating reproduction in a desert rodent (*Dipodomys merriami*). *Physiology & Behavior*, **34**, 199–203.
- Randall, J. A.** 1987. Sandbathing as a territorial scent-mark in the bannertail kangaroo rat (*Dipodomys spectabilis*). *Animal Behaviour*, **35**, 426–434.
- Randall, J. A.** 1993. Behavioural adaptations of desert rodents (Heteromyidae). *Animal Behaviour*, **45**, 263–287.
- Regnier, F. E. & Goodwin, M.** 1977. On the chemical and environmental modulation of pheromone release from vertebrate scent marks. In: *Chemical Signals in Vertebrates* (Ed. by D. Müller-Schwarze & M. M. Mozell), pp. 115–133. New York: Plenum.
- Richardson, P. R. K.** 1990. Scent marking and territoriality in the aardwolf. In: *Chemical Signals in Vertebrates 5* (Ed. by

- D. W. MacDonald, D. Müller-Schwarze & S. E. Natynczuk), pp. 378–386. New York: Oxford University Press.
- Rohlf, F. J. & Sokal, R. R.** 1981. *Statistical Tables*. 2nd edn. New York: W.H. Freeman.
- Rozenfeld, F. M., Boulangé, E. L. & Rasmont, R.** 1987. Urine marking by male bank voles (*Clethrionomys glareolus* Schreber, 1780; Microtidae, Rodentia) in relation to their social rank. *Canadian Journal of Zoology*, **65**, 2594–2601.
- Schmidt-Nielsen, B., Schmidt-Nielsen, K., Brokaw, A. & Schneiderman, H.** 1948. Water conservation in desert rodents. *Journal of Cellular and Comparative Physiology*, **32**, 331–360.
- Schmidt-Nielsen, K. & Schmidt-Nielsen, B.** 1952. Water metabolism of desert mammals. *Physiological Reviews*, **32**, 135–166.
- SPSS.** 2000. SPSS 10.0 for the Macintosh, Chicago: SPSS.
- Steel, E.** 1984. Effect of the odour of vaginal secretion on non-copulatory behaviour of male hamsters (*Mesocricetus auratus*). *Animal Behaviour*, **32**, 597–608.
- Streilein, K. E.** 1982a. Ecology of small mammals in the semiarid Brazilian caatinga. I. Climate and faunal composition. *Annals of Carnegie Museum*, **51**, 79–107.
- Streilein, K. E.** 1982b. Behavior, ecology, and distribution of South American marsupials. In: *Mammalian Biology in South America* (Ed. by M. A. Mares & H. H. Genoways), pp. 231–250. Philadelphia: University of Pittsburgh.
- Tang-Martinez, Z., Mueller, L. L. & Taylor, G. T.** 1993. Individual odours and mating success in the golden hamster, *Mesocricetus auratus*. *Animal Behaviour*, **45**, 1141–1151.
- Thiessen, D. & Rice, M.** 1976. Mammalian scent gland marking and social behavior. *Psychological Bulletin*, **83**, 505–539.
- Thiessen, D. D., Lindzey, G., Blum, S. L. & Wallace, P.** 1970. Social interactions and scent marking in the Mongolian gerbil (*Meriones unguiculatus*). *Animal Behaviour*, **19**, 505–513.
- Trupin, G. L. & Fadem, B. H.** 1982. Sexual behavior of the gray short-tailed opossum (*Monodelphis domestica*). *Journal of Mammalogy*, **63**, 409–414.
- VanderBerg, J. L. & Robinson, E. S.** 1997. The laboratory opossums (*Monodelphis domestica*) in laboratory research. *Institute for Laboratory Animal Research National Academy of Sciences News*, **38**, 4–12.
- Ziegler, T. E., Eppler, G., Snowdon, C. T., Porter, T. A., Belcher, A. M. & Kuderling, I.** 1993. Detection of the chemical signals of ovulation in the cotton-top tamarin, *Saguinus oedipus*. *Animal Behaviour*, **45**, 313–322.
- Zuri, I., Gazit, I. & Terkel, J.** 1997. Effect of scent-marking in delaying territorial invasion in the blind mole-rat *Spalax ehrenbergi*. *Behaviour*, **134**, 867–880.
- Zuri, I., Su, W. & Halpern, M.** 2003. Conspecific odor investigation by gray short-tailed opossums (*Monodelphis domestica*). *Physiology & Behavior*, **80**, 225–232.