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Odour Preferences and Discrimination in Captive Ringtail Possums (
Pseudocheirus peregrinus   )

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ODOUR PREFERENCES AND DISCRIMINATION IN CAPTIVE RINGTAIL POSSUMS (*PSEUDOCHEIRUS PEREGRINUS*)

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ABSTRACT: Olfactory communication has been shown to be important in marsupials and, specifically, in *Trichosurus vulpecula* and *Petaurus breviceps*. Despite its commonality, little is known about the communication in the ringtail possum, *Pseudocheirus peregrinus*. Therefore, to investigate olfactory communication in the ringtail possum, two experiments were conducted. The odour preference experiment examined the importance of fur, salivary, faecal and urinary odours in ringtail possum olfactory communication. The latency to approach urinary odours was significantly longer than for any other odours. Males sniffed familiar female urine samples for a significantly shorter duration than any other odour samples. Fur samples were manipulated for longer durations than any other odour samples. The discrimination experiment examined the ability of ringtail possums to discriminate between urine samples, and hence obtain socially significant information from such odour sources. The ringtail possums showed that they were able to discriminate between individuals and between sexes. The possible functions of urine, fur and paracloacal gland secretions are discussed.

Very few studies have investigated olfaction as a means of communication in marsupial species, but its importance is not disputed. Information on olfactory communication has mainly been derived from studies on social organisation and ecology of species, but two notable studies concentrating on olfaction are those of Schultze-Westrum (1965) and Biggins (1979) on *Petaurus breviceps* and *Trichosurus vulpecula*, respectively. Only the latter has the support of a detailed field study enabling the information to be related to the free-ranging animal. Consequently, our understanding of the function of odours remains superficial. For example, we still know relatively little about the motivation behind marking behaviour.

The importance of olfaction in marsupials is indicated by their possession of extremely prominent olfactory bulbs which in some instances make up almost half of the forebrain (Johnson, 1977). They also have well-developed vomeronasal organs with extensive olfactory epithelium (Negus, 1958). Marsupials possess a variety of potential

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scent glands and behaviour patterns involving these glands, such as olfactory investigation and marking behaviour (Russell, 1985). All of these features are exhibited by the various possum species, as reviewed by Biggins (1984) and Russell (1985).

The ringtail possum, *Pseudocheirus peregrinus* is common and found in close association with urbanisation, yet there is only one detailed study on its social organisation (Thomson & Owen, 1964). In this study, ringtail possums were shown to be gregarious, nocturnal, and arboreal, and to live in communal nests of mixed sexes and ages but the possums' social interactions were not described.

Tyndale-Biscoe (1973) concluded that the ringtail possum does not communicate olfactorily even though this species possesses labial glands (Biggins, 1979), and two pairs of paracloacal oil glands (Bolliger & Whitten, 1948). Saliva, faeces, and urine are also odorous substances which are readily available and are potential olfactory messages in this species. Both olfaction and auditory modes of communication are likely to be the most important in nocturnal animals but the ringtail possum appears to have a limited vocal repertoire (Biggins, 1984). Therefore, further study of the role of olfactory communication in this species is clearly warranted.

The present study investigated odour preferences and odour discrimination in ringtail possums. The odour preference experiment determined odours that are of importance in ringtail possum olfactory communication, and the behaviours that are elicited by these odours. The odour discrimination experiment examined the ringtail possum's ability to discriminate between odours of apparent social significance using an operant conditioning technique.

MATERIALS AND METHODS

Animals

Six adult hand-reared ringtail possums, (2 male and 4 female), and four adult wild caught ringtail possums (2 male, 2 female) were used as experimental subjects. *Petaurus breviceps* was used as a source of odours for comparison with those from the ringtail possum. This species was chosen because of its potential habitat overlap with ringtail possums in the wild, and because it is a member of the same family (Petauridae).

Housing and Feeding

The ringtail possums were housed in three groups of three (1 male, 2 females) in outdoor metal and timber cages (dimensions

2.1m × 1.8m × 1.1m), and a single male was housed in an indoor 'rabbit' cage (dimensions 0.48m × 0.63m × 0.8m). Black plastic was taped to the cages on the sides opposing the neighbouring cage to reduce odour transfer between the cages and so maintain three relatively separate groups. All possums were maintained on a diet of fruit, vegetables, eucalyptus leaves, native flowers and bread. The food was made available in the evening, or as appropriate for each experiment. Water was provided ad lib.

Odour Preference Experiment

Each test possum was exposed to its own urine, faeces, saliva and dorsal fur and that of familiar and unfamiliar conspecifics of both sexes. One male was not tested against familiar male odour stimuli although he was exposed to all the other odour stimuli. Faeces, saliva and dorsal fur of sugar gliders, and distilled water were used as the controls.

Urine and faeces were collected by housing the possums individually in metallic cages with wire floors and a metal tray beneath or by stimulating the possums to urinate into a large metallic funnel. Urine that was free from faecal and food contamination, and faeces free from urine contamination were collected as soon as possible after excretion. The samples were sealed in glass vials or self-sealing plastic bags and refrigerated and used that night or frozen until use. Freezing reduces the loss of volatile odours. Urine and faecal samples could not be standardised for age, or the urine for the concentration of paracloacal gland secretion (i.e., milkiness). However, the variation between samples appeared to be due to an individual rather than a methodological difference.

Saliva was obtained by wiping inside the lips and angle of the mouth with filter paper. Fur samples were collected by wiping the filter paper across the dorsal fur.

Five drops of urine were applied to the test filter papers. The faeces were rubbed across the filter paper to eliminate differences between faecal size. Five drops of distilled water were used to moisten the filter paper discs as this appeared to match the moistness of the urine samples. New vinyl gloves were used for each odour to prevent cross-contamination.

The possums were tested individually in separate rabbit cages (0.48m × 0.63m × 0.8m) which contained a nest box, a branch and a cardboard tray (0.03m × 0.23m × 0.37m).

The odours were presented to the possums on two filter paper discs (Whatman Number 1, 5.5cm diameter) which were placed 10cm away from each other on a paper towel lining the cardboard tray. The

paper towel was replaced after each trial to prevent the odours from contaminating the cardboard tray.

To minimize the effect of the experimenter on the possums' behaviour, a low-light National WVP-F10N System video camera was used. A monitor placed in another room enabled the experimenter to observe the animals' responses. Red lights were used to illuminate the test area. A light dimmer was used to reduce the light intensity to the minimum required by the camera (i.e., 7 lux).

Procedure

The possums were habituated to the rabbit cages over at least two nights prior to testing. One wild female did not adapt, reducing the sample size for this experiment to nine. The possums were then exposed to pairs of clean dry filter paper until their interest in them had waned in order to eliminate the effect of the filter papers as novel objects. Tests were run after sunset when the possums had emerged and commenced feeding. The 24 different odour stimuli were divided into four groups of six (Table 1). These were presented to the possums one at a time. Each group of six was randomised so that the order of presentation was different for each of the two repetitions. A period of 48 hours was allowed between repetitions to prevent the possum from becoming over-familiarised with the odours.

Three measures of behaviour were recorded using a Canon X-07 Handheld Computer.

1. Approach—time measured from when the head of the possum was less than 30cm from the odour sample, as estimated by the investigator, and the possum had orientated toward the sample, and was obviously approaching it.

TABLE 1
Odour Sources Used in the Odour Preference Experiment

Odour Type	Own	Sugar glider*	F male	F female	UF male	UF female
Fur	x	x	x	x	x	x
Saliva	x	x	x	x	x	x
Faeces	x	x	x	x	x	x
Urine	x			x	x	x	x
Water*						

Note. Sugar glider and water samples were used as controls. F = familiar (housed in the same cage), UF = unfamiliar (housed in different cages).

2. Sniff—time recorded when nose was less than or equal to 2cm from the odour sample.
3. Manipulation—time recorded when the possum had made contact with the odour sample with more than the nose.

Approach was not recorded if the possum was accidentally in the odour sample's proximity before focusing its attention on it. Sniffing and manipulation behaviours were always scored separately. It was assumed that the important odours were not volatile and hence required close olfactory investigation.

Odour Discrimination Experiment

The paradigm used in this experiment was based on that of the Wisconsin General Test Apparatus procedure. In this method, the reward is a direct result of the animal's behaviour, providing contiguity between response and reward, and it exploited the possum's natural tendency to manipulate food and other objects with its forepaws. The discrimination apparatus was portable and could be easily removed from the cage as required, thus enabling the investigator to replace the food reward unobtrusively. This apparatus is shown and described in Figure 1. A movable false base made of cardboard was placed in the lower can. Two filter papers (Whatman Number 1, 5.5cm diameter) were moistened with the appropriate odour and attached to the front and back of each can by adhesion of the liquid to the can surface. The cans were washed with pyroneg when the odour stimuli were changed.

To train the possums on the apparatus, food from the possum's daily ration was placed in the top halves of both sets of cans to get them used to taking food out of the cans and to promote the association between the cans and food. Once they had become accustomed to this procedure the lids were placed half on, and then completely over the cans until the possums were removing the lids to obtain their food. Discrimination learning was then commenced.

The Odour Preference Experiment had indicated that urine was a significant odour for the ringtail possum. Consequently, urine samples taken from the ringtail possums were used as the odour stimuli in the discrimination experiment. Table 2 shows the various pairings of the odours tested. The possums were trained using the odour pairing of water and their own urine. The positively reinforced odour was counterbalanced so that for one male and one female, for example, their own urine was positive, while for the other three possums water was the positive stimulus. The urine was collected and handled as for the Odour Preference Experiment.

A food reward was used to reinforce the possum's lid removing
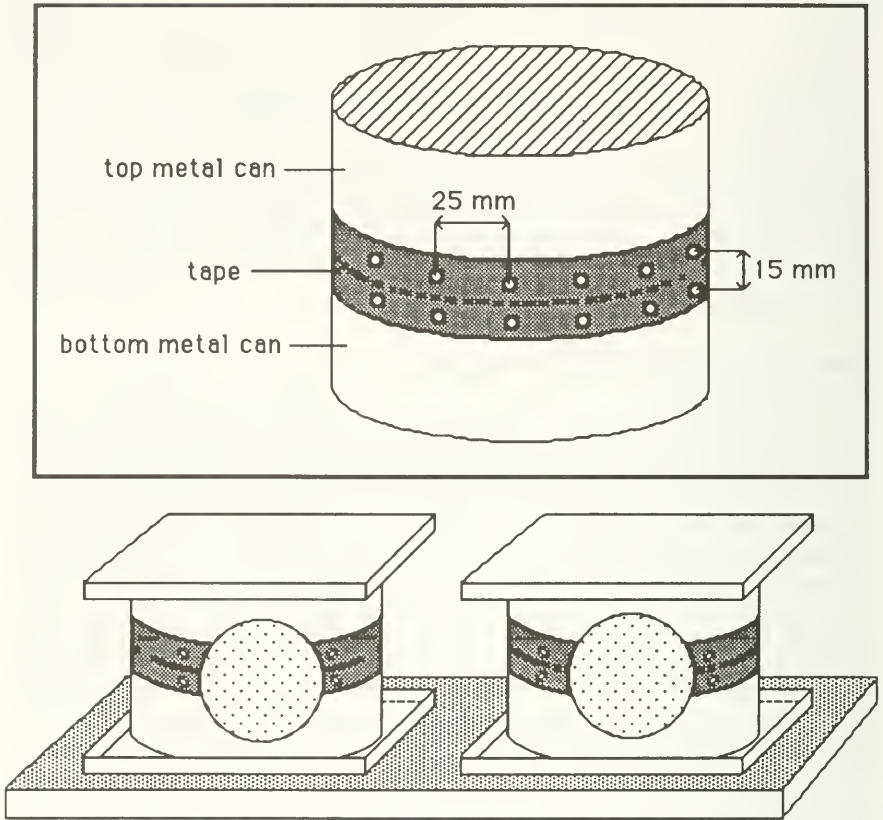
FIGURE 1. Discrimination Apparatus: the bases of two pairs of cans (height 34mm x diameter 75mm) were soldered together. Black tape covered the central join. Two rows of 1.5mm diameter holes were drilled around the circumference of both cans of each pair (inset). Metal bases (90mm x 90mm x 10mm) were attached to a board (13mm x 170mm x 350mm) 125mm apart, and each pair of cans was attached to these bases. Metal lids identical to the bases were placed on top of the cans.

behaviour in response to the positive stimulus. This food was placed on the floor of the upper can of the pair to which the positive odour was attached. The negative odour stimulus was attached to the other pair of cans. A false reward was placed immediately below the floor of the upper can of the pair and so was inaccessible to the possum. The possum's food intake prior to the trials was not reduced as they were sufficiently motivated by hunger at feeding time to perform the de-

TABLE 2
Odour Pairings Used in the Odour Discrimination Experiment

| | Animals tested | | | |
Pair of Odours	Columbine	Mintie	Mars	Scrumpy
Own vs water	x	x	x	x
Own vs UF male	x			
Own vs UF female	x	x		
F1 female vs UF1 female	x			
F2 female vs UF2 female	x			
UF1 female vs UF2 female	x	x	x	x
F1 female vs F2 female	x			
UF female vs UF male				x
F male vs UF male		x		
UF1 male vs UF2 male	x	x	x	x

Note. Only urine samples were used. F1 = familiar animal 1, F2 = familiar animal 2, UF1 = unfamiliar animal 1, UF2 = unfamiliar animal 2.

sired behaviour for a food reward. On completion of the nightly trials the possum was given its normal food ration.

Discrimination Learning

The possums were again tested individually indoors in the rabbit cages using red lights for illumination. The possum's responses were monitored from an adjacent room using the National low-light video camera.

The first possum was tested using massed trials in accordance with past investigations (Friedman & Marshall 1965; Tilley, Doolittle, & Mason, 1966; Jenkins, 1985) which suggested that learning in marsupials is enhanced by this method. However, this was found to be unnecessary and the remaining trials were in the blocked format. Depending on the possum's progress, blocks of 12, 16 or 20 trials were run with a maximum of three blocks per night. Each block of trials tested a different odour pair and there was an interval of 10 min. during which the possum was allowed to eat a native flower. The position of the positive stimulus was altered in accordance with the chance stimulus sequences for discrimination tasks devised by Fellows (1967). A correction method of training was used whereby the possum was able to correct its initial wrong choice and obtain a reward, although only the first choice was scored. The possum was considered to be discriminating when it made $\geq 10/12$, $\geq 13/16$ or $\geq$

16/20 correct responses on two consecutive days for a particular odour stimuli pair. The probability of results such as these occurring by chance alone is 0.019 (Binomial test, SSPS, Inc., 1986).

RESULTS

Odour Preference Experiment

The data on latency to approach, and duration of manipulating odour samples were analysed using three-way ANOVAS (SSPS, Inc., 1986). These ANOVAS looked at the effects of sex, odour type and odour familiarity on the three behaviours and showed that the type of odour affected both approach ($F(3,382) = 4.286, p = .005$) and manipulation duration (s) ($F(3,382) = 4.141, p = .007$). Examination of the means using the Student-Neuman-Keuls procedure showed that the latency to approach urine samples was significantly longer than for any other odour samples (Figure 2), while fur samples were manipulated significantly shorter than other samples (Figure 3). The manipulation behaviours which occurred included picking the odour sample up with forepaws, and/or chewing or licking the sample briefly. One extended behaviour pattern was performed by one female and two
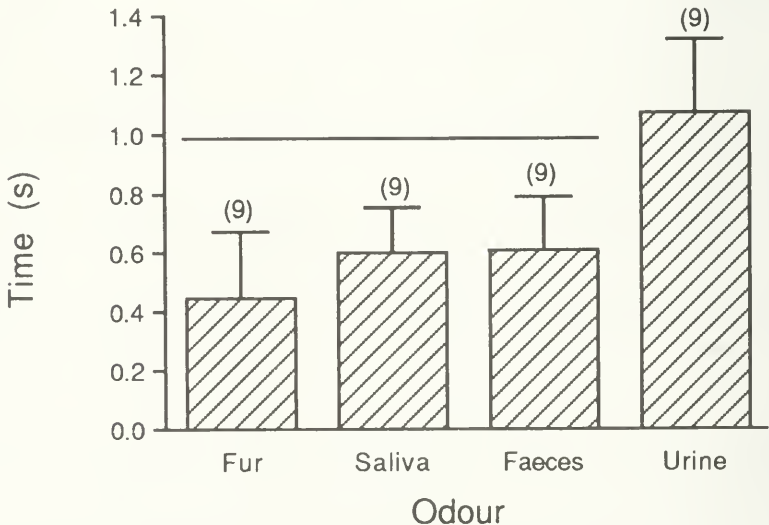
FIGURE 2. Odour Preference Experiment: comparison of the means for the latency (s) to approach odour samples depending on the odour type. The line joins odours for which the means were not significantly different (Student-Neuman-Keuls, $P>0.05$).
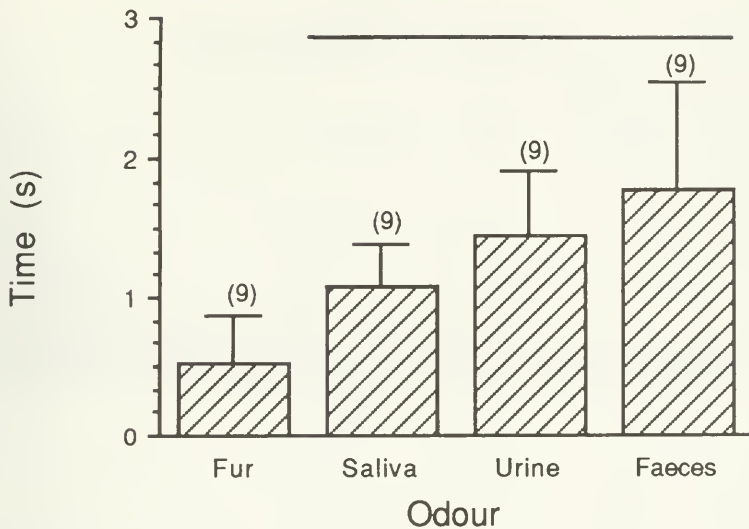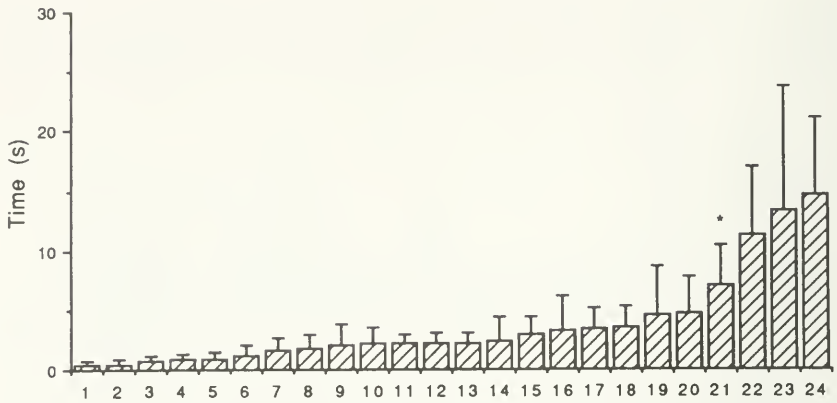
FIGURE 3. Odour Preference Experiment: comparison of means showing the effect of odour type on the duration (s) of manipulating odour samples. The line joins odour types for which the means were not significantly different (Student-Neuman-Keuls, $p > 0.05$).

male possums. Briefly, the paper was picked up with the mouth or forepaws and thrust underneath the possum's body using the hindlegs to help push it behind the possum, or into its tail.

The data on sniffing duration (s) were analysed using a two-way ANOVA which revealed a two-way interaction between sex and odour ($F(23,384) = 1.635$, $p = .034$). An examination of the means showed that there were no significant sex differences in sniffing duration. One-way ANOVAS were used to investigate the differences in sniffing separately for the two sexes and only a significant effect for males ($F(23,168) = 1.602$, $p = .048$) was revealed. However, a Student-Neuman-Keuls test failed to reveal any significant difference between means. Mann-Whitney U (two-tailed) tests examined the effect of a individual's sex on its sniffing behaviour. The only significant difference was that males ($\overline{X} = 7.000 \pm 9.502$ s; $n = 8$) (Figure 4A) sniffed familiar female urine for a longer duration than did females ($\overline{X} = 0.2 \pm 0.422$ s; $n = 10$) (Figure 4B) ($U = 13$; $P = .008$).

Odour Discrimination Experiment

Five hand-reared possums (2 males and 3 females) were trained to discriminate between pairs of odours. The results (Table 3A) show
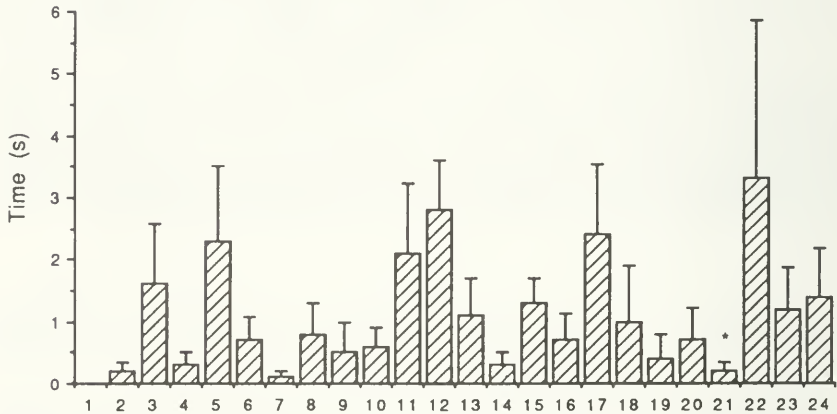
FIGURE 4. Odour Preference Experiment: Comparison of the means showing the effect of the odour sample on sniffing duration (s) (n = 7-10) in A: males and B: females. *Indicates significant difference between sexes for sniffing duration (Mann-Whitney U (two-tailed) tests). Bars represent standard errors. Key: 1 - familiar male fur, 2 - own faeces, 3 - unfamiliar male fur, 4 - familiar female fur, 5 - familiar female saliva, 6 - sugar glider fur, 7 - own fur, 8 - familiar female faeces, 9 - unfamiliar female fur, 10 - own saliva, 11 - unfamiliar male saliva, 12 - sugar glider faeces, 13 - own urine, 14 - unfamiliar female saliva, 15 - familiar male faeces, 16 - familiar male urine, 17 - unfamiliar male faeces, 18 - water, 19 - familiar male saliva, 20 - sugar glider saliva, 21 - familiar female urine, 22 - unfamiliar female urine, 23 - unfamiliar female faeces, 24 - unfamiliar male urine.

TABLE 3A
Discrimination Test Results

Discrimination	Possum	Sex	Trials to Criterion	No. of Errors
Own vs water				
	Columbine	male	54*	16
	Mars	male	28	3
	Mintie	female	24	3
	Scrumpy	female	24	1
	Mouse	female	24	2
UF1 female vs UF2 female				
	Columbine	male	28	5
	Mars	male	24	2
	Mintie	female	24	1
	Scrumpy	female	36	6
	Mouse	female	24	2
UF1 male vs UF2 male				
	Columbine	male	24	4
	Mars	male	32	4
	Mintie	female	28	3
	Mouse	female	36	6
	Scrumpy	female	no attempts	

Note. For this test, each possum was tested. F1, F2, UF1, UF2 as for Table 2. Criterion = $\geq 10/12$, $\geq 13/16$, or $\geq 16/20$ correct on two consecutive nights.
Massed trials were used on the first night.

that they were able to discriminate between water and their own urine, between the urine of two unfamiliar females, and between the urine of two unfamiliar males, except for Scrumpy who made no attempts to discriminate between the latter odour pair. Other discrimination trials were also run for individual possums (Table 3B). Columbine discriminated between his own urine and that of an unfamiliar male and an unfamiliar female, between familiar and unfamiliar female urine, and between familiar female urine samples. Mintie discriminated between unfamiliar and familiar female urine samples, and Scrumpy discriminated between unfamiliar female and unfamiliar male urine samples.

Observations

A number of behaviours which were assumed to be involved in olfactory communication were observed during the course of this study.

TABLE 3B
Discrimination Trials Run with Individual Possums

Discrimination	Possum	Sex	Trials to Criterion	No. of Errors
Own vs UF female				
	Columbine	male	28	4
	Mintie	female	28	3
Own vs UF male				
	Columbine	male	24	1
F female vs UF1 female				
	Columbine	male	28	5
F female vs UF2 female				
	Columbine	male	44	7
F1 female vs F2 female				
	Columbine	male	24	3
UF female vs UF male				
	Scrumpy	female	24	0
UF male vs F male				
	Mintie	female	24	1

Note. F1, F2, UF1 and UF2 as for Table 2.

1) When the possums were extremely frightened such as when the wild animals were handled, they exuded a viscous creamy coloured secretion from their cloaca. Its suggested source is the paracloacal glands.

2) At least three variations of micturition were observed:—

i) Casual micturition appeared to be normal micturition without any special marking function (c.f. Ewer, 1968). The possum moved to a specific location and eliminated the urine where it stood without any postural changes. This behaviour was generally performed by females or subadults and occurred in the same location on the floor of the cage each night. Occasionally though, micturition was followed by a short urine dribble (or cloacal drag) where a trail of very milky urine was sometimes deposited. On drying, a white powdery mark often remained.

ii) Urine dribbling (or cloacal dragging). It was difficult to determine the difference between urine dribbling and cloacal dragging. The behaviour appeared to be a combination of the two. A distinction may be made when the quantity of urine in comparison with paracloacal gland secretion is minimal. Russell (1986) also found it difficult to differentiate between the two behaviours in *Tarsipes rostratus*.

The behaviour is an example of dispersive scent marking, although it may also have a passive marking function. Contact of the feet and other bodily areas with the substrate would result in the passive transfer of this urine to these areas. To perform this behaviour the hind-quarters were lowered with the tail at approximagely a 45° angle to the substrate. The protruding cloaca touched the substrate as the possum waddled forward at various speeds along a branch or the substrate. As the possum proceeded, urine was dribbled out of the cloaca. Occasionally, faeces were also dropped along this line. These urine trails were usually a sigmoid pattern especially when they were performed on the cage floor. The urine varied in colouration from clear to milky. Viscosity also increased along this gradient and the quantity of urine eliminated varied. Males particularly, marked the same branch or same location several times in succession, and frequently throughout the night. This behaviour increased in frequency when the possums were excited, disturbed, or thwarted by the investigator, or were investigating a cage odourised by another possum. The possums were generally aroused when performing this behaviour. This behaviour was performed when the possums were placed in novel cages, such as the rabbit cages but was not performed in response to novel objects in home cages. On drying, a persistent white powdery mark usually remained. Although not quantitatively measured, the males appeared to urine dribble significantly more than the females when in their home cages. However, when isolated in the rabbit cages the females also frequently 'urine dribbled.' Urine marking, and milky urine were not observed prior to the possum's sexual maturation.

iii) Urine squirting. This micturition pattern is also an example of dispersive marking. Clear to milky urine was ejected in short frequent bursts as the possums ran erratically about the cage to escape from human contact. Occasionally they also secreted pure secretion which remained coating the cloaca.

3) On standing, a white precipitate settled out in urine samples. If the urine was shaken or aerated it changed from greenish-brown to reddish-brown. Bolliger and Whitten (1940) report the same observation in reference to *T. vulpecula*.

4) Defecation occurred frequently and at any time or place, including the nest box, and with no special associated behaviour pattern except when possums practised coprophagy. Defecation is an example of passive marking. Defecation also occurred when the possums were highly aroused such as when they were caught and restrained.

5) Sniffing rarely occurred when possums in their home cage met. However sometimes naso-nasal, naso-cloacal, and pouch sniffing, as well as sniffing the base of the tail occurred, usually initiated by

males. Males would also sniff where a female had urine dribbled, the site where a female had been sitting, and at casual micturition sites. The possums would also sniff other parts of a foreigner's body such as the feet. Naso-nasal sniffing was usually a reciprocated behaviour and would extend to sniffing the corners of the mouth and ears, although it was brief. The recipients of the other forms of sniffing usually responded aggressively by hitting out and attempting to bite.

Males became intensely interested in females at certain times of the year (presumably during oestrus) and correspondingly the frequency of their sniffing increased. Sniffing frequency was also high when the possums met a foreigner or 'familiar' individuals from which they had been isolated for several days. Female-female interactions involved naso-nasal sniffing while male-female interactions involved sniffing of the female's cloaca and sometimes the pouch area.

6) Air and substrate sniffing were frequently observed. When sniffing the air, the possums would often sit or stand on their hind legs, body upright, with forelegs held close to their body, and nose extended. On other occasions the possums appeared to be sniffing the air (head up), or substrate (head almost touching substrate), with all four feet on the ground. They did this as they moved about the cage. At times when the possums seemed intensely interested in an odour, they appeared to touch the substrate with the filtrum of their noses while moving their heads back and forth over this area. A wet mark was usually left on the substrate following these incidents.

7) Autogrooming, unlike allogrooming which was rare and very brief (at least out of the nest box), was frequently observed, and occurred both in and out of the nest box. Washing and grooming of the face and hands usually followed feeding but could occur at any time. The possum would alternatively lick its two forefeet and claws and then repeatedly rub them forwards over the sides of its face and towards the nose. All areas of the body were groomed. Face, rump and flanks were scratched and combed with the syndactylous claws and forefeet which were then licked. These grooming behaviours were similar to those recorded in *Tarsipes rostratus* (Russell, 1986) and *T. vulpecula* (Biggins, 1984). Allogrooming was observed on a few occasions. On each occasion a particular male was involved and appeared to initiate the interaction. Only once did the recipient (a female) reciprocate.

10) When the possums were returned to their home cage after a period away (e.g., a week but *not* less than 4 days) they were always repulsed from occupied nests if they attempted to enter them immediately. Thomson and Owen (1964) reported a similar incident occurring in the field.

11) Occasionally the possums chewed bark off the branches they were sitting on. They then either ate this bark (or so it appeared),

dropped it on to the ground, or returned to the nest box with it. Consequently these areas of branch, as well as the dropped pieces of bark, would have traces of saliva on them. These areas may also carry a visual message.

DISCUSSION

The odour preference results showed that the latency to approach the urine samples was significantly longer than that for any other samples, but that males spent more time sniffing familiar female urine samples. The latter information suggests that urinary odours are not aversive. Some information about the donor may be obtained from a distance. Not only is urine an odorous eliminatory product but also the odorous secretions from the paracloacal glands are voided with the urine (Bolliger & Whitten, 1948; Biggins, 1979). A variety of factors has been shown to alter the odoriferous qualities of the eliminatory products and scent gland secretions. These include diet and physiological state, which is affected by age, sex, reproductive state, and dominance status (Biggins, 1979). Specialised exocrine glands are under hormonal control and hence may indicate an animal's hormonal state. Analysis of the paracloacal gland secretions of *T. vulpecula* showed that secretions of adult males, adult females, juveniles, and socially stressed males were all different, especially with respect to the volatile secretions (Biggins, 1979).

The male response to female urinary odours suggests that they recognise females from their own nesting group and may focus their attention on these urinary odours so that they can assess the female's reproductive state. It is likely to be the male that seeks out the female, and continuous checking would ensure that he secured a mating. Other marsupials such as macropodids (Kaufmann, 1974a,b, 1975; Croft, 1981 a,b) and dasyurids (Croft, 1982) also reportedly use urinary cues to assess the female's reproductive state.

The importance of urine and the paracloacal gland secretions in ringtail possum communication was supported by marking behaviours involving micturition, such as urine dribbling and urine squirting, which are dispersive methods of urine distribution promoting the spread of the secretions.

The male ringtail possums performed urine dribbling (or cloacal dragging) more than the females. In other species, such as *T. vulpecula* (Winter, 1977; Biggins, 1979) and *P. breviceps* the same pattern is found. In the present study females increased their level of marking when placed individually in the rabbit cages. In both sexes marking occurred when the possums were aroused, especially in response to interference by the investigator. When male and female

Sminthopsis crassicaudata are housed together only the male marks, but when separated, both sexes mark (Ewer, 1968). Female ringtail possums also used a 'latrine' for micturition when males were present. Female *Dasyuroides byrnei* are also known to defecate and micturate in specific locations of the cage rather than throughout the cage as the males do (Aslin, 1974). Therefore, although marking in the ringtail possums may have been a response to the investigator, in females it may also have been due to the lack of male odour.

Urine squirting was not sexually differentiated, and has been observed in *T. vulpecula* (Winter, 1977). In the present study the urine usually contained large quantities of paracloacal gland secretion and frequently pure secretion was also voided. This behaviour always occurred when the possums were highly aroused. Increased arousal as a causal factor for scent marking involving micturition and defecation, is in agreement with the hypothesis that scent marking has evolved from autonomic responses (Morris, 1956; Kleiman, 1966). Its function appears to be one of familiarising the animal with its environment and reassuring it in unknown situations. Therefore the paracloacal secretion may indicate fear, and as it was so pungent it may have been used to rapidly disseminate the possum's own odour, saturating the immediate environment with it, and masking any other odours, thus enhancing the possum's confidence as suggested for *T. vulpecula* (Biggins, 1979, 1984). Also, because of its pungency and because it was frequently performed when being caught, it may function to ward off predators. This has also been suggested by Thomson and Owen (1964), but has generally been disputed.

Fur samples were manipulated significantly less than any of the other samples. Due to nest sharing, the fur odours were likely to represent a group odour. Self-anointing with salivary odours by autogrooming, followed by close contact with the other possums in the nest, would result in the transfer and mixing of these odours and hence the development of a group odour. The possums micturated and defecated in their nests. This would also result in passive marking of those sharing the nest. Fur sniffing occurs in encounters between unfamiliar *Gymnobelideus leadbeateri* and may involve detection of community specific odours (Smith, 1980). The rejection of ringtail possums on reintroduction to their home cage was immediate and may have been on the basis of the missing group odour.

However, clean filter paper was also frequently manipulated (times not recorded). Therefore, the manipulation behaviour may not have been in response to the odour itself, but a stereotyped response as a result of boredom. This same behaviour has been recorded in opossums (Glickman & Sroges, 1966). With long term confinement or inferior living areas, stereotyped behaviour which tends to block attention to environmental stimulation occurs and decreases the mag-

nitude of the animal's responses (Hediger, 1955). The behaviour re-
ferred to here, was frequently performed by the male which had been
housed in the rabbit cage for an extended period, thus supporting this
idea. However, this behaviour is also related to nest building which
was stifled due to a lack of available nesting materials.

Although saliva and faeces did not appear to be significantly in-
vestigated in the context of the odour preference experiment, their
importance may have been underestimated due to the experimental
conditions. The presence of labial glands (Biggins, 1979) and naso-
nasal sniffing, and the exudation of paracloacal gland secretions
along with the faeces indicate that these odour sources may be of
communicatory significance to the ringtail possum. Further studies
are required to elucidate the importance of oral secretions, and faeces
in olfactory communication in this species.

The limited responses to the odour samples in the Odour Prefer-
ence Experiment may have been due to the conditions under which
the experiment was conducted. Jenkins (1985) found that *Aepyprym-
nus rufescens* would not respond to odour stimuli when placed in test
cages. These cages appeared to induce high levels of anxiety. One
wild female, particularly, showed signs of such extreme agitation that
she could not be tested. Also, the frequent interference by the investi-
gator changing the odour samples maintained the possums in a con-
stant state of arousal, hence possibly inhibiting or reducing their re-
sponses.

The choice of a sniffing attraction test may also have affected the
results as Johnston (1981) has found that two choice preference tests
were less subject to variability. Preference tasks also provide finer
distinctions and hence greater accuracy (Wellington, Beauchamp, &
Smith, 1981). The discrimination method used in the present study
enables two odours to be compared and may enable fine distinctions
to be made. Consequently, this may be a more appropriate method for
testing the salivary and faecal odours.

Five possums were successfully trained in the discrimination ex-
periment to discriminate between urinary odours of individual ring-
tail possums, thus showing that they were able to discriminate be-
tween sexes and individuals using urinary cues. Therefore urine, or
the paracloacal gland secretions it carries is a socially significant
odour for ringtail possums.

A variety of constituents in urine may have acted as cues for
discrimination purposes. As mentioned above, urine samples vary
with diet, hormonal state and associated factors. The quantity of
paracloacal gland secretion also varies with the individual's motiva-
tional state. These differences would enable a possum to discriminate
between individuals. However, whether these possums can recognise
individuals is yet to be determined.

It is evident that male and female urinary odours are significantly different from each other and that the differences are identifiable by both males and females. This was indicated in the odour preference experiment, and by the differential reactions of the males and females to the odours in the odour discrimination experiment. For example, one male became extremely excited when presented with female urine samples, and mouthed them, but immediately urine dribbled when presented with male odours. Other evidence such as male interest in female micturition sites, urine dribbles and the female cloacal region further indicate that female urine contains female specific odours. Gas chromatography traces of ringtail possum paracloacal secretions (Biggins, 1979) confirm the apparent difference in odoriferous properties between male and female secretions.

One female refused to participate when the discrimination was between two foreign male odours. She was later found to be carrying a pouch young. This suggests that females are able to distinguish between male and female odours, and emphasizes that it is essential to assess all the possible factors affecting an animal's responses.

The odour samples and even the water samples were occasionally mouthed, indicating the use of the vomeronasal organ in obtaining the relevant information. The vomeronasal organ appears to be sensitive to liquid-borne olfactory stimuli (Wysocki, Wellington, & Beauchamp, 1980) and is a receptor for odours of low volatility (Powers, Fields, & Winans, 1979). The mouthing behaviour and the fact that the possums closely sniffed the odour samples in both the odour preference and discrimination experiments indicated that the important factors were nonvolatile substances. Also, the odour samples frequently dried before the possum had made its choice, but this did not appear to alter the animal's performance, further suggesting that the essential constituents for discrimination were not volatile.

No definitive conclusion can be made as to the function of the paracloacal glands, but it is evident that these glands are used when the possums are frightened or highly aroused. Therefore it may be a submissive signal, or have an anti-predatory function. Urine may carry community specific information, and information on the identity, sex and age of an animal. Unfortunately, it is impossible to put the information in perspective without the support of a field study providing detailed information on olfactory communication. Consequently, this has been the biggest limitation in this study. However, this is the first study to have successfully used the powerful technique of operant conditioning to investigate olfactory communication in a marsupial. It has shown that the ringtail possum exhibits odour preferences and is able to discriminate between conspecific urinary odours, demonstrating that olfaction is an important mode of communication in this species.

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