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Female Marmoset Monkeys (*Callithrix jacchus*) Can Be Identified from the Chemical Composition of their Scent Marks

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Abstract

The present study analyzed 42 organic solvent extracts of scent mark pools from five dominant female common marmosets by gas chromatography (GC) and combined GC and mass spectrometry. We determined whether there were qualitative or quantitative differences between the chemical composition of scent marks from individual females. Gas chromatography and mass spectral analysis detected the same 162 chemicals in 86% (36/42) of scent mark pools from five dominant females. This near identical chemical composition of scent marks suggested there were few, if any, qualitative differences between the chemical composition of scent marks from individual females. Instead, quantitative differences in scent may provide the key factor distinguishing individual females. Using the relative concentration of highly volatile chemicals detected by GC in scent marks, linear discriminant analysis classified scent mark pools to their correct donor ~91% of the time. Such highly reliable statistical matching of scent to donor suggested that each individual female common marmoset has a unique ratio of highly volatile chemicals in their scent marks which may permit individual identification of females from odors in their scent alone.

Introduction

Chemical signaling of individual identity is widespread among solitary and social species (Halpin, 1986). Individual chemical signatures are communicated by many scent sources, including urine [squirrel monkey, *Saimiri sciureus* (Laska and Hudson, 1995)], feces [rabbit, *Oryctolagus cuniculus* (Mykytowycz, 1973)], vaginal secretions [golden hamster, *Mesocricetus auratus* (Johnston *et al.*, 1993)], secretions from specialized glands [ring tailed lemur, *Lemur catta* (Dugmore and Evans, 1990)] or general body odors [virginia opossum, *Didelphis viriniana* (Holmes, 1992)]. Odors specific to individuals are important in mammals for regulating social interactions in several contexts, including mate recognition (prairie vole, *Microtus ochrogaster*), mate choice (brown lemming, *Lemmus trimucronatus*), reproductive success (golden hamster), mother–infant relationships (virginia opossum) and territorial defense [mouse, *Mus musculus* (Coopersmith and Banks, 1983; Newman and Tang-Halpin, 1988; Gosling and McKay, 1990; Holmes, 1992; Tang-Martinez *et al.*, 1993)]. In at least one species, the common marmoset, odor cues specific to certain individuals may regulate the reproductive physiology of conspecifics (Barrett *et al.*, 1990, 1993; Smith and Abbott, 1995; Abbott *et al.*, 1997). The present study chemically analyzed scent marks from female common marmosets to assess quantitative and/or qualitative differences between scent marks that may distinguish individual females.

The callitrichids (marmosets and tamarins) are small, arboreal, social primates that live in extended family groups in the neotropics (Rylands, 1993). Marmosets have a well-developed odor communication system with behavioral and morphological adaptations to effect efficient deposition and reception of scent material (Epple *et al.*, 1993). Both male and female common marmosets possess specialized glandular areas in the ano-genital region comprised of large sebaceous and apocrine glands in the external skin of the genitalia (circumgenital gland) (Sutcliffe and Poole, 1978). Marmosets mix secretions from the ano-genital glands with secretions from non-specialized circumanal skin glands, a few drops of urine and possibly feces and genital discharge on the substratum to form a circumgenital scent mark (Sutcliffe and Poole, 1978). The frequency of scent marking in common marmosets is significantly modified by social and physiological context, in addition to environmental features (Epple, 1970; Sutcliffe and Poole, 1978; Epple *et al.*,

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1993; Smith and Abbott, 1998; Lazaro-Perea *et al.*, 1999). Chemicals in the marmoset circumgenital scent mark have been implicated as playing major roles in regulating social interactions.

We have previously used behavioral bioassays to demonstrate that circumgenital scent marks (and organic solvent extracts of these scent marks) from female common marmosets contain chemical cues permitting discrimination between familiar and unfamiliar conspecifics (Smith *et al.*, 1997). These results provided indirect evidence for the existence of individual odor signatures in common marmosets. The chemical signaling of individuality may be important to marmosets in several contexts, including regulating reproductive physiology, mother–infant recognition, territory defense and maintenance of social dominance hierarchies (Epple *et al.*, 1993; Abbott *et al.*, 1997). To obtain direct evidence of whether qualitative and/or quantitative differences between the chemical composition of scent marks from individual females would enable individual discrimination, we analyzed 42 organic solvent extracts of scent mark pools from five individual females by gas chromatography (GC) and combined GC and mass spectrometry (GCMS).

Materials and methods

Subjects

Scent marks for chemical analysis were provided by five adult female common marmosets (*Callithrix jacchus*) that had held dominant status (rank 1) for at least 6 months prior to the study. The females were housed either as female pairs ($n = 1$, one dominant, one subordinate) or as trios ($n = 4$) of two females (one dominant, one subordinate) and a male. The marmosets used in this study were captive born and were housed at the Institute of Zoology, London [see Abbott *et al.* for details of animal husbandry (Abbott *et al.*, 1988)].

Determination of female rank

Details of social group formation are provided by Abbott and Barrett *et al.* (Abbott, 1984; Barrett *et al.*, 1990). Briefly, social groups were formed by placing between two and four unrelated females and between two and four unrelated males in a large observation room (2.9 × 2.2 × 1.7 m) and allowing the animals to form intra-sex behavioral dominance hierarchies. Behavioral observations were made daily on each group, for 45 min between 0900 and 1000 and 45 min between 1600 and 1700 for the first 10 days following group formation. All submissive and dominant behavioral interactions were recorded [excluding interactions involving feeding and drinking (Abbott, 1984)] and dominance matrices were constructed. A dominance hierarchy was established within each sex ~3 days following group formation. Among females the hierarchy comprised a dominant female (rank 1) and one or two subordinate females (rank 2 and below). The most dominant individual (rank 1) received least aggression and most submission and the subordinate individuals received most aggression and least submission. Once the group was established it was transferred to a home cage (100 × 50 × 75 cm) and given access to an exercise cage (2 × 1 × 2 m) every 5–7 days. The sizes of social groups used in the current study (two or three animals in each) were reduced from their original sizes (between four and eight animals) because marmosets had been removed for use in additional research studies.

Determination of ovulatory function

Frequently only dominant females ovulate in social groups of common marmosets (Abbott *et al.*, 1988). To standardize the ovarian hormone environment during odor collection circumgenital scent marks were collected from dominant females exclusively during the luteal phase of the ovarian cycle, when plasma progesterone concentrations commonly exceed 50 ng/ml. Ovulatory function was confirmed from concentrations of plasma bioactive luteinizing hormone (bLH) and progesterone in twice weekly blood samples. Ovulation (±1 day) can be reliably estimated as the day prior to plasma progesterone concentrations exceeding 10 ng/ml (Harlow *et al.*, 1983). Methods pertaining to blood sampling in common marmosets and the measurement of concentrations of plasma bLH and progesterone were described previously by Abbott *et al.* (Abbott *et al.*, 1988).

Collection of circumgenital scent marks

The technique and collection-device used for scent mark collection were previously described by Smith *et al.* (Smith *et al.*, 1997). Briefly, marmosets were trained, using positive reinforcement techniques, to circumgenitally scent mark an apparatus designed for collecting scent marks [see Smith *et al.* for details of the procedures used to train marmosets to scent mark (Smith *et al.*, 1997)]. Marmosets deposited scent marks on frosted Pyrex tubes (7.5 × 1 cm) that lay in an aluminum foil lined groove running the length of a wooden perch (48 cm long, 4 cm diameter). The foil minimized contamination of the Pyrex collection tubes by the wooden perch. The frosted collection tubes had a well, 0.5 cm deep, that ran along half the length of the tube. It was intended that the well would mimic the feeding sites that marmosets typically mark as well as aiding the collection of drops of urine deposited as part of the scent mark (Coimbra-Filho and Mittermeier, 1976, 1978; Sutcliffe and Poole, 1978). The wooden perch fitted with collection tubes was designed to be attached inside the home cage of the scent donor in place of the regular wooden perch that was normally positioned in the cage. The equipment used to collect and process the scent material was thoroughly cleaned prior to use and stored in an airtight, foil lined plastic container for a maximum of 24 h prior to use [see Smith *et al.* for details of the procedures used to clean the equipment (Smith *et al.*, 1997)].

Pooled samples of 15 circumgenital scent marks were

collected from each of the five subject females, approximately once per 2–3 weeks (coincident with the luteal phase of the ovarian cycle), over a 22 week period. All scent marks were collected between 0700 and 0900 and a pool of 15 scent marks was usually collected from an individual female within 10 min. Between five and 10 scent mark pools were collected from each female marmoset (a total of 42 scent mark pools). Scent marks were not collected if females were under veterinary supervision.

Preparation of scent material for chemical analysis

Methods used to prepare scent material for chemical analysis were modified from protocols established by previous authors to chemically analyze scent material from callitrichid primates (Yarger *et al.*, 1977; Belcher *et al.*, 1988). Each pooled sample in the current study contained 15 scent marks from one individual. Scent marks were collected when a scent collection tube was marked once, or at most twice (on occasion marmosets would mark the same tube twice in quick succession). The marked collection tube was removed from the foil lined groove in the wooden perch using clean forceps and dropped into a glass test tube (16 × 2 cm) containing 2 ml extraction solvent (1:3 v/v methanol: dichloromethane mix). A clean Pyrex collection tube was put in the wooden perch in place of the removed tube for further scent collection. The test tube containing extraction solvent mix and scent marked collection tube was vortexed gently for 1 min. The collection tube was removed from the test tube using a clean glass rod, discarded and the process repeated until 15 circumgenital scent marks from an individual donor had been extracted into the solvent mix. The 2 ml volume of solvent-extracted scent mark was reduced to 20 µl under a stream of nitrogen in a filtered hood and 10 µl of methanol were added. The 30 µl volume was stored at –20°C under nitrogen for a maximum of 1 month until chemical analysis.

Control samples

A control sample (total $n = 20$) was obtained approximately once per week over the 22 week study period (control samples were not collected during weeks 5 or 16 of the study). To prepare a control sample the complete collection device (i.e. wooden perch fitted with frosted Pyrex tubes) was placed in a soiled marmoset cage from which all animals had been removed 5 min previously. At regular intervals over a 10 min period (approximately the time taken to collect a pool of 15 scent marks) a collection tube was removed from the wooden perch and extracted in 2 ml of extraction solvent. A clean collection tube replaced the removed tube. This process was repeated until 15 tubes had been removed from the wooden perch and placed in the extraction medium. The 2 ml control solvent volume was treated as described above for a scent mark solvent extract.

GC and combined GCMS analysis

Chemical analysis was performed on a 5890 gas chromatograph (Hewlett Packard) fitted with a flame ionization detector (FID) and a WCOT OV351 column (dimensions 30 m × 0.32 mm, with a film thickness of 0.15 µm) (Phase Sep, UK). A sample volume of 2 µl was injected onto the column using split injection by an autosampler. Injector temperature was 260°C and detector temperature was 270°C. The temperature of the GC was held at 70°C for 5 min, followed by an increase of 2°C/min to 270°C. The final temperature of 270°C was held for 40 min. A control sample was run approximately every fifth sample. Accurate peak measurements (and hence relative concentrations) were obtained from the GC data for highly volatile chemicals detected prior to 54 min. A second 5890 gas chromatograph was linked via a direct interface to a VG70-250SE mass spectrometer (VG Analytical, Manchester, UK) with an interface and re-entrant temperature of 250°C. Electron impact, with an electron energy of 70 eV was used to ionize the analytes. The ion source temperature was 180°C throughout. Repetitive magnetic scanning, over the mass range 450–25, was performed using 0.5 s per mass decayed.

Data analysis

Quantitative data generated by FID were processed and analyzed by a Hewlett Packard ChemStation linked to the gas chromatograph. For all 42 samples the relative retention time of each chemical detected was calculated with respect to the last eluting chemical that was present in all samples (identified as a squalene derivative with a mean elution time ± SEM of 143 ± 0.61 min). In addition, the relative concentration of each chemical detected was computed by dividing the peak area for the chemical (as computed by the ChemStation) by total peak area for all chemicals detected (as computed by the ChemStation). The chemicals present in each pooled scent mark extract were compared by expanding all GC traces on the ChemStation into eight 20 min GC portions. Each GC portion measured 27.75 × 21.5 cm. GC traces were overlaid using the ChemStation and the pattern of peaks examined. Peak parameters assessed included height, shape and spacing in conjunction with relative retention times.

Statistical analysis

Chemicals for which the relative concentration in the scent mark was <0.01% were omitted from the analysis because of unreliable quantification at such low relative amounts. There was little variability in the chemicals detected in the scent marks from the five female marmosets. All female scent marks contained the same, or nearly the same, chemicals. Combined GC and GCMS ascertained that chemicals detected after 54 min sometimes co-eluted, whereas chemicals detected prior to 54 min did not co-elute (see Results). A maximum of 98 chemicals eluted prior to

54 min (defined as highly volatile chemicals). Statistical analysis was only performed for highly volatile chemicals eluting prior to 54 min, excluding six chemicals that appeared to reflect contamination, since the latter were also identified in control material that did not include any scent marks (see Results). Analysis therefore involved 42 scent mark pools and 92 highly volatile chemicals detected in each scent mark.

Stepwise linear discriminant analysis (LDA) was used to test the hypothesis that the relative concentrations of chemicals in the circumgenital scent mark were unique for each individual female. Ten pairwise LDA were performed on data from each possible pairing of females using the StatSoft statistical package (StatSoft, 1991). For each female pair a discriminant function was formed from the information on the relative concentrations of the chemicals in the scent marks. Between four and nine scent mark pools were used from each female to complete each discriminant function (see below). Wilk's λ was calculated for each discriminant function as a measure of the function's discriminatory power. Wilk's λ statistic was converted to a standard F value and the corresponding P value determined (Rao, 1951). The effectiveness of the discriminant function to correctly classify a scent mark to the correct scent donor was tested using a 'jack-knife technique' (Afifi and Clarke, 1996). Briefly, a discriminant function was formed for each pair of females using information from all possible scent marks from the two females minus one. The discriminant function was then used to assign a donor to the remaining scent mark. This process was repeated for all scent marks from the female pair.

Results

Chemical analysis

Scent mark extracts

A total of 162 chemicals were detected by GC in the scent marks of five dominant female marmosets, as illustrated by the individual exemplified in Figure 1. Each of the 162 chemicals had a relative concentration in a scent mark of ≥0.01%. Of the 42 pooled samples of scent marks, 86% (36/42) comprised the same 162 chemicals, as illustrated by the two dominant females in Figure 2. The remaining six pooled samples contained a reduced number of these 162 chemicals (range 112–144 chemicals). The latter six pooled samples were not from any particular individual or sub-group of donor females and included female 401W (one pooled sample), female 408W (three pooled samples) and female 272W (two pooled samples). This considerable similarity in chemical composition of scent marks from five marmoset females was confirmed by MS (Table 1).

Control samples

A total of six chemicals were identified in the 20 control samples as probable contaminants. No more than three of the six probable contaminants were found in any single control sample. Probable contaminants were identified as three impurities originating from the extraction solvents (methanol and/or dichloromethane) and three chlorinated chemicals [GC code nos 62, 79 and 95, identified as 2,4-dichloro-3,5-dimethylphenol, 4-chloro-3-methylphenol and phenyl-4-(chloromethyl)phenylether, respectively]. The chlorinated chemicals probably originated from cleansing disinfectants. None of these six chemicals were considered in any quantitative analyses of marmoset scent.

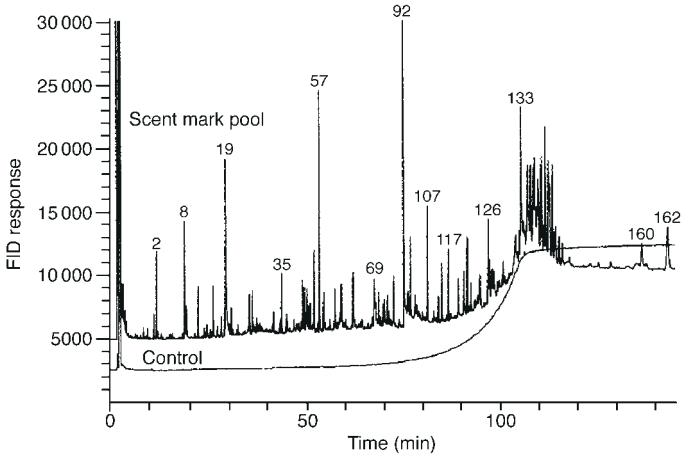

Figure 1 GC trace of a scent mark pool from a dominant marmoset (401W) and a control solvent extract (see Materials and methods). A subset of peaks are labeled with the GC code number.

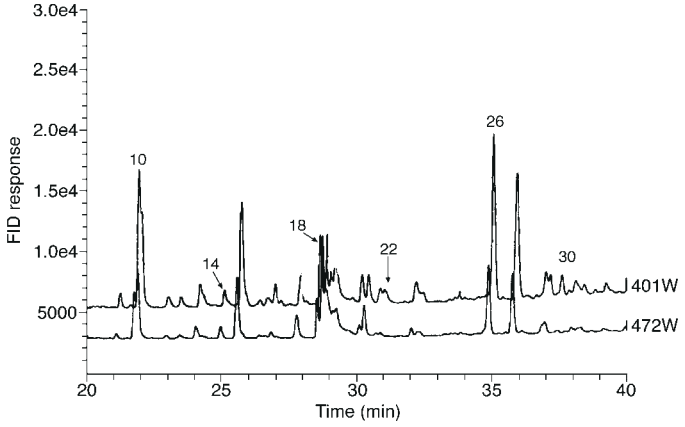

Figure 2 A 20 min section of two individual GC traces of scent mark pools from dominant female marmosets 272W and 472W. Data from the two females are compared on the same trace to illustrate comparable chemical composition of scent marks from different females. A subset of peaks are labeled with the GC code number.

Stepwise linear discriminant analysis

Wilk's λ was significant for each discriminant function for all except one of the 10 possible female pair comparisons (Table 2). Using the computed discriminant functions, ~91%

Table 1 Chemicals in the scent mark for which a positive identification was made are shown together with the corresponding code number

GC code no. for each peak detected	Chemical identification
1	Hydrazoic acid
4	2,5-Dimethylpyrazine
5	2,3-Dimethylpyrazine
6	Dimethyl trisulphide
7	Trimethylpyrazine
8	Acetic acid
9	Furfural
10	Pyrrole
11	Benzaldyhyde
12	Propanoic acid
13	2-Methylpyrrole
14	5-Methylfurfural
15	Cyclopent-2-en-1,4-dione
16	2,5-Dimethyltetrahydrofuran
18	Butanoic acid
19	Trimethylbenzene
20	1-Methyl-2-pyrrolidine
21	2-Methylbenzoxazole
22	Pentanoic acid
23	2-Methyl-2-propenoic acid
24	2(5*H*)-Furanone
25	Methylphenylacetate
26	Acetamide
27	Propanamide
29	Trimethylbenzene
30	1-Furfuryl pyrrole
31	Isomer of trimethyl cyclopentanone
32	2-Cyanopyridine
33	Dimethylsulphone
34	Phenylisocyanide
35	3-Hydroxy-2-methyl-4-pyrone
36	2-Acetylpyrrole
37	Solvent impurity
38	*n*-Decanol
41	Phenol
42	*p*-Methoxybenzaldyhyde
43	(1*H*)-Pyrrole-2-carboxaldehyde
44	2-Pyrrolidinone
46	Maleimide
47	1,2-Dimethyl-4-ethenylbenzene
48	1,1-Dimethylurea
49	4-Methylphenol
50	*n*-Undecanol
51	2-Piperidinone
56	*N*-Cyclopentylidene methylamine
57	2-Hydroxy-5-methylacetophenone
58	3-Phenylpyridine
59	4-Oxo-2-pyrrolidinone
60	2-Methyl-5-phenylpyridine
61	2,3-Dihydro-3,5-dihydroxy-6-methylpyran-4-one
62	2,4-Dichloro-3,5-dimethylphenol
63	4-Methyl-5-thiazole ethanol
64	3-Methylsuccimimide
65	Solvent impurity
69	4,9-Dimethylnaptho[2,3-*b*]thiophene
71	3-Pyridinol
73	Phenylacetonitrile
75	Succimimide
76	Lactone
77	Skatole
78	Pyrazole
79	4-Chloro-3-methylphenol
80	Dodecanoic acid
81	3-Aminopyridine
82	Phenylacetic acid
86	2-Phenylphenol
87	3,4-Dimethoxyphenylmethylpropanoate
88	2-Methoxy-1,1-biphenyl
89	1,3-Diphenylpropan-2-one
90	Benzamide
91	Methylnaphthyridine isomer
92	Phenylacetamide
93	Tetradecanoic acid
94	2-Ethylquinoline
95	Phenyl-4-(chloromethyl)phenylether
97	1,2-bis(*p*-methoxyphenyl)ethane
98	3-Methylquinoline
99	4-Methoxybenzoic acid
101	α-Lactone
102	Hexadecanoic acid
103	Nicotinamide
104	2-Methoxyhydroquinone
107	4-Methoxyphenylacetic acid
110	Hydroquinone
113	5-Methyl-2,4-imidazolidinedione

of scent marks were classified to the correct scent donor using data on the relative concentration of highly volatile chemicals in the scent mark (Table 3).

Discussion

The study identified 162 chemicals in solvent-extracted scent marks from dominant female common marmosets, of which 112–162 were present in the scent marks of all females. The relative concentrations of volatile chemicals in the scent marks were unique for each female. In this regard individual specific ratios of chemicals in the scent mark might impart an individual odor profile to each female's scent marks and thus provide a basis for individual discrimination. With human subjects, at least, the ratio in which chemicals are present in a scent mixture can determine the final odor perceived by an individual (Berglund *et al*., 1973).

Two previous studies investigating the chemical composition of scent marks from related callitrichid species, male saddle-back tamarins and female cotton-top tamarins, detected considerably smaller numbers of chemicals in scent marks than were found in the present study [16 and 13 chemicals, respectively (Yarger *et al*., 1977; Belcher *et al*., 1988)]. The 16 chemicals detected in the scent marks from male saddle-back tamarins were present in all scent marks analyzed in the study. As was found in the present study, the

Table 2 Female pair comparisons and the number (percentage) of scent marks correctly assigned to donor by linear discriminant analysis, the number of chemicals included in each discriminant function for each pair comparison and the probability value for each discriminant function obtained from Wilk's λ

Pair comparison	Female identification	No. of scent marks correctly classified to each female in the pair comparison	Significance value for discriminant function obtained from Wilk's λ
1	487W	7/8 (88%)	0.007
	401W	9/10 (90%)	
2	487W	7/8 (88%)	0.06
	272W	7/9 (78%)	
3	487W	7/8 (88%)	0.0001
	472W	9/10 (90%)	
4	487W	8/8 (100%)	0.0001
	408W	5/5 (100%)	
5	401W	8/10 (80%)	0.0001
	272W	9/9 (100%)	
6	401W	9/10 (90%)	0.0001
	472W	8/10 (80%)	
7	401W	10/10 (100%)	0.0001
	408W	10/10 (100%)	
8	272W	9/9 (100%)	0.0001
	472W	10/10 (100%)	
9	272W	8/9 (89%)	0.01
	408W	4/5 (80%)	
10	472W	8/10 (80%)	0.0001
	408W	5/5 (100%)	

Table 3 Mean percentage of scent marks correctly assigned to each donor female marmoset from all possible pairwise comparisons of females employed in linear discriminant analysis

Female identification	Mean percentage of scent marks correctly classified
487	91
401	90
272	92
472	88
408	92.5
All females combined	Overall mean (± SEM) 90.8 ± 3

ratio of these 16 chemicals in the scent marks of the saddle-back tamarins was unique for each male (Smith *et al.*, 1985). In contrast to the consistent chemical composition of scent marks from female common marmosets (present study) and male saddle-back tamarins (Smith *et al.*, 1985), scent marks from another closely related callitrichid, the cotton-top tamarin, varied considerably in chemical composition between individual animals (Belcher *et al.*, 1988). Of the 13 chemicals detected in female cotton-top scent marks only three appeared consistently in all samples. Two of the three chemicals (*p*-methoxybenzaldyhyde and squalene) were also detected in the scent marks of female common marmosets and male saddle-back tamarins. Results from the current study and those of Yarger *et al.* and Belcher *et al.* (Yarger *et al.*, 1977; Belcher *et al.*, 1988) suggest that the chemical signaling of individual identity within the callitrichid family is mediated via a wide variety of mechanisms and should not be considered synonymous processes at any level.

Olfactory encoding for individual identity has been observed in a range of mammalian species. Chemical analysis of scent secretions from some mammals has identified quantitative differences in the relative concentrations of chemicals in the odors from individual animals. For example, the anal sac secretion of the Indian mongoose, *Herpestes auropunctatus*, contains six fatty acids, the relative concentrations of which are unique for each individual animal (Gorman, 1976). The biological activity of fatty acids in the mongoose anal sac secretion was confirmed by the ability of test animals to distinguish between synthetic mixtures containing different ratios of fatty acids (Gorman, 1976). In some species the composition of scent from different individuals varies by both quantitative and qualitative parameters [e.g. European badger, *Meles meles*, wolves, *Canis lupus* and mice, *Mus musculus* (Raymer *et al.*, 1985; Novotny *et al.*, 1990; Buesching and Macdonald, 2000)], whereas in other species qualitative and not quantitative differences are the major distinguishing factors [e.g. shrew, *Crocidura russula* (Cantoni *et al.*, 1996)]. Although the current study demonstrated clear quantitative differences between scent from individual female marmosets, there might also be qualitative differences between individual scents that were not detected due to the methodological techniques employed. For example, potential qualitative (or quantitative) differences between trace components in the scent mark would not have been detected in the current study, since chemicals were only included in the analyses if their relative concentration in the mark was ≥0.01%. New World primates demonstrate extremely high levels of olfactory sensitivity (Laska *et al.*, 2000). In the light of the importance of odor to marmoset socio-ecology it is not unlikely that the marmoset olfactory system would have evolved to receive and process trace compounds in scent mixtures.

Quantitative differences between relative concentrations of chemicals in scent secretions have been shown to discriminate attributes of a scent donor other than individual identity. For example, the hormonal status of an individual can be determined by quantitative analysis of the scent secretion [e.g. *Iguana iguana* (Alberts *et al.*, 1992)] or from analysis of both quantitative and qualitative parameters [e.g. *C. lupus* and *M. musculus* (Raymer *et al.*, 1986; Schwende *et al.*, 1986)]. Quantitative differences in scent

mark composition have also been shown to distinguish scent from animals of different social status [e.g. white-tailed deer, *Odocoileus virginianus* (Gassett *et al*., 1996)]. Phylogenetic analyses of scent secretions have identified quantitative differences in the absence of qualitative differences between scent from different sub-species [e.g. *Saguinus fuscicollis* (Smith *et al*., 1985)]. In contrast to the latter example, scent from the anal sac of three different mongoose species (*Helogale parvula*, *Crossarchus obscurus* and *Suricatta suricatta*) were shown to vary both quantitatively and qualitatively (Decker *et al*., 1992).

The present study identified quantitative differences in the chemical composition of scent marks from individual female common marmosets, but the extent to which marmosets use this quantitative information to distinguish individuality is not known. Future studies will need to focus on behavioral responses to synthetic scent marks that mimic the individual specific ratio of chemicals in natural scent marks to verify the functional relevance of quantitative differences to animal discrimination of individual identity. Epple *et al*. assessed the discriminatory response of saddle-back tamarins to synthetic scent marks, the chemical composition of which mimicked those identified in the organic solvent extracts of scent marks of two male conspecifics (Epple *et al*., 1979). Test animals, however, only exhibited discriminatory responses to the natural marks from the two individual males and not to the synthetic versions. The latter results suggest that saddle-back tamarins may use olfactory information in addition to that signaled by chemicals in organic solvent extracts to discriminate individual scent marks (a possibility that cannot be ruled out for common marmosets). Indeed, we have previously shown that common marmosets direct significantly reduced amounts of investigatory behavior toward organic solvent-extracted marks compared with natural marks (Smith, 1994). This latter result suggests that chemicals in addition to those extracted in a solvent extract are necessary for the full communicatory stimulus of the marmoset scent mark, as was the case for the saddle-back tamarin.

The scent mark of the common marmoset is a complex mixture of scents from several sources (Sutcliffe and Poole, 1978). It is therefore not surprising that the solvent-extracted scent marks contained a relatively large number of chemicals and that these chemicals ranged from highly volatile chemicals such as hydrazoic acid to non-volatile proteins (Smith, 1994). Many of the chemicals identified in the scent mark of the female common marmoset feature in the scent secretions of other animals [e.g. aromatic nitrogen-containing chemicals in white-tailed deer, sulfur-containing chemicals in hog-nosed skunk, *Conepatus mesoleucus*, fatty acids, in lion, *Panthera leo*, lactone in striped hyaena, *Hyaena hyaena* (Buglass *et al*., 1990; Wood *et al*., 1993; Gassett *et al*., 1996; Andersen and Vulpius, 1999)] and have proven pheromonal effects [e.g. 2,5-dimethylpyrazine (Novotny *et al*., 1990)]. Certain biochemical properties might render some compounds more effective chemical signals than others and thus favor their evolution as olfactory signals. For example, chemicals with high odor potency [e.g. sulfur-containing chemicals (Novotny *et al*., 1974)] and low odor thresholds [e.g. pyrazines (Barlin, 1982)] might be highly effective odor signals since only a small amount of compound is required to cause an effect in recipient animals. The biochemical pathway by which a compound is formed might also influence its evolution as a chemical signal. For example, aromatic nitrogen-containing compounds can be readily formed from the microbial degradation of proteins. In the light of the constant source of urinary proteins in the marmoset ano-genital area, together with its rich microbial flora (Nordstrom *et al*., 1989), it is not surprising that a variety of aromatic nitrogen-containing compounds were identified in the marmoset scent mark.

In considering the functional significance of individual-specific odors of dominant female common marmosets it has been shown that individual-specific odors from dominant females regulate hypothalamic–pituitary–gonadal function in subordinate females (Barrett *et al*., 1990). In captive social groups of adult common marmosets subordinate females do not usually ovulate (Abbott, 1984). Anovulatory subordinate females removed from their social group and housed singly commence ovulatory cycles within ~10 days of single cage housing unless they are maintained in olfactory contact with their dominant female. In the latter case, time to onset of the first ovulation is extended to ~30 days (Barrett *et al*., 1990, 1993). The period of anovulation following removal from the social group is not extended beyond that of control animals (i.e. 10 days) if the isolated females were maintained in scent contact with an unfamiliar dominant female (Smith and Abbott, 1995). As scent from a familiar, but not an unfamiliar, dominant female maintains anovulation in subordinate females, reproductive suppression in female marmosets cannot be mediated via a primer pheromonal effect. Instead, Abbott *et al*. suggest that classical conditioning might maintain anovulation in subordinate females (Abbott *et al*., 1997). They also propose that early harassment by the dominant female during group formation (unconditioned stimulus) provokes anovulation (unconditioned response) in subordinate females. Subordinate females subsequently learn to associate individual characteristics of the dominant female (such as her unique odor profile) with harassment. Eventually the individual-specific characteristics of the dominant female become the conditioned stimuli eliciting a conditioned response of anovulation in subordinate females. The importance of odor for initiating reproductive suppression in marmosets is confirmed by the observation that while anosmic females exhibit behavioral subordination during group formation they continue to ovulate (Abbott *et al*., 1993). Results from the current study are compatible with a classical conditioning model (Abbott *et al*., 1997),

since we have shown that scent marks from individual dominant female marmosets have unique chemical compositions and have the potential to impart individual-specific odor to the scent mark. Implicit in the classical conditioning model is the assumption that subordinate females have the neural substrates from which to form a relatively long-lasting memory (i.e. up to 30 days) of complex odor profiles. Common marmosets maintain memory traces for visual stimuli for up to 1 year, suggesting that they might also possess the neural physiology to maintain olfactory memories for extended periods of time (L. Scott, personal communication). Mammalian olfactory learning can involve both the accessory olfactory bulb [mice (Lloyd-Thomas and Keverne, 1982)] and the main olfactory bulb [ewes (Levy *et al.*, 1995)]. Marmosets possess an accessory olfactory system together with a functional vomeronsasal organ, in addition to the main olfactory system (Harrison, 1987; Taniguchi *et al.*, 1992). Since both the accessory and main olfactory epithelium have neural connections to the hypothalamus, the neural substrates for olfactory learning and subsequent neuroendocrine responses could involve either site (Scalia and Winans, 1976). Since highly volatile chemicals distinguished the scent from individual dominant females (current study), olfactory learning of the dominant female's scent profile might primarily involve the main olfactory bulb: volatile chemicals tend to be received by the main olfactory system (Meredith, 1991). Alternatively, the volatile chemicals distinguishing scent from individual dominant females might be attached to non-volatile carrier proteins in the scent mark (Singer, 1991), implicating involvement of the accessory olfactory bulb in olfactory memory formation and the neuroendocrine events leading to anovulation (non-volatile cues are received primarily via the vomeronasal organ). A further understanding of the olfactory system and neural pathways mediating olfactory learning in marmosets might help to refine our understanding of the chemical components in common marmoset scent marks relevant to distinguishing individual identity.

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