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# Chemical Composition of Scent Marks in the Ringtailed Lemur (*Lemur catta*): Glandular Differences, Seasonal Variation, and Individual Signatures

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## Abstract

The apocrine and sebaceous scent glands of ringtailed lemurs (*Lemur catta*) appear to serve different social functions. In behavioral experiments, lemurs modulate their responses to scent marks based on the type of odorant, their own physiological state, the signaler's physiological state, and prior social experience. To examine variation in odorant chemistry relative to olfactory behavior, we used gas chromatography and mass spectrometry to analyze over 86 samples of glandular secretion collected over 2 years from 15 adult lemurs. Labial and scrotal secretions contained organic acids and esters, whereas male brachial secretions were composed almost entirely of squalene and cholesterol derivatives. Principal component and linear discriminant analyses revealed glandular, individual-specific, and seasonal variation in chemical profiles but no relationship to the signaler's social status. The chemical composition of the various secretions provides further clues about the function of the different glands: the higher molecular weight compounds in genital and brachial secretions may increase signal longevity and provide lasting information to conspecifics, consistent with a role in advertising resource ownership or reproductive state. Conversely, the lower molecular weight compounds of antebrachial secretions produce ephemeral signals used primarily in social dominance displays and require integration of multiple sensory modalities for effective signal transmission.

**Key words:** gas chromatography mass spectrometry, olfactory communication, principal component analysis, social dominance, squalene, strepsirrhine primate

## Introduction

Chemosignaling is a complex mode of communication critical to mediating social interaction in mammalian taxa (Ralls 1971; Eisenberg and Kleiman 1972; Brown and Macdonald 1985). Whereas asocial species tend to use chemical signals as “bulletin boards” that relay messages in the absence of the signal sender (Alberts 1992), social species frequently integrate concurrent visual, auditory, and behavioral displays into scent deposition (Candolin 2003; Partan and Marler 2005). The functions attributable to the different components of the latter, complex signals may differ but are difficult to tease apart. Most research on olfactory communication in social species addresses the information conveyed via the behavioral cues (e.g., scent marking: Gese 2001; Smith and Gordon 2002), but the importance of isolating and examining the chemical fraction of composite signals is gaining recognition. Although behavioral bioassays can

reveal the information perceived by a signal responder (e.g., Drea et al. 2002; Mateo 2003, 2006), chemical analyses target the range of information potentially available within a chemosignal, including seasonal, sex-related, and individual-specific variation (Belcher et al. 1986, 1990; Smith et al. 2001). Thus, it is necessary to integrate multiple levels of analysis to elucidate signal content and function, particularly in social taxa. The present study forms part of our 3-pronged (observational, experimental, and chemical) approach to understanding olfactory communication in a socially complex strepsirrhine primate, the ringtailed lemur (*Lemur catta*). Here, we use gas chromatography and mass spectrometry (GCMS) to examine the chemical composition of the volatile fraction of *L. catta* scent gland secretions. Specifically, we ask if the volatile profiles of secretory products vary by type of gland and by an individual's reproductive state, identity, or social status.

Ringtailed lemurs are seasonal breeders that live in multi-male–multifemale groups characterized by female philopatry, male dispersal, and intrasexual dominance hierarchies (Jolly 1966). They arguably exhibit the most highly developed olfactory system among primates (Schilling 1979; Epple 1986; Kappeler 1993), possessing a suite of specialized scent glands (see figure 1 in Scordato and Drea 2007) and a variety of scent-marking displays (Montagna and Yun 1962; Jolly 1966; Schilling 1979). The latter incorporate multiple sensory modalities and occur in view and earshot of group members. Both sexes have apocrine and sebaceous gland fields in their genital regions and adopt distinctive handstand postures to deposit glandular secretions on substrates. Males possess 2 additional glands: paired brachial organs are pockets of sebaceous glands on the axillary surface of each shoulder that secrete a brown paste and paired antebrachial organs are apocrine gland fields located on the wrists, adjacent to a keratinized spur, that produce small quantities of clear fluid. Males sometimes mix the secretions of the latter 2 glands in a distinct “shoulder-rubbing” behavior and then deposit this mixture via “wrist marking,” an audible action during which males draw their antebrachial organ and spur across a substrate. Males also impregnate their tail fur with antebrachial and brachial secretions and then waft their tail at opponents during characteristic “stink fights” (Jolly 1966).

Based on observational studies and behavioral bioassays, scent marking in *L. catta* presumably serves to advertise social rank (Kappeler 1990) and reproductive state (Scordato and Drea 2007; Drea and Scordato forthcoming) and to demarcate territories or mediate intertroop spacing (Mertl-Milhollen 1988, 2006). In experimental presentations, the odorants derived from different glands are discriminated by both male and female lemurs (Dugmore et al. 1984; Dugmore and Evans 1990; Scordato and Drea 2007). In preliminary chemical analyses of the 4 types of glandular secretions, Hayes et al. (2004, 2005) showed that some of the glandular secretions differ in composition. Some types of secretion may even convey the individual identity of the signaler (Mertl 1975; Palagi and Dapporto 2006; Scordato and Drea 2007). In addition to gland-specific differences in chemical composition and receiver responses, each type of lemur scent secretion is deposited under different social conditions. Consequently, we have proposed that the different scent glands may serve distinct functions and that, as in other species (Partan and Marler 2005), part of the signal’s information may be conveyed via social cues, independent of, or in conjunction with, chemical content (Scordato and Drea 2007, Drea and Scordato forthcoming).

Here, we use GCMS to determine if variation in the chemical composition of scent gland secretions supports our interpretation of *L. catta* olfactory behavior. Specifically, we test the following 4 predictions. 1) As both male and female *L. catta* respond differently to the odorants produced by the 4 different glands, we predict that each odorant will show a distinct chemical profile. 2) As the attractiveness of odor

cues varies with the reproductive state of the signaler, we predict that the chemistry of secretions will vary seasonally. 3) As male lemurs only differentiate familiar animals, and not strangers, by their dominance status, implicating the potential for individual recognition, we predict that the chemical composition of glandular secretions will reflect individually stable “scent signatures.” 4) Lastly, as dominance status appears to be conveyed by the behavioral component of scent marking, we do not expect chemical profiles to differ reliably based on an individual’s dominance status. To address these predictions, we examine how the volatile profiles of scent secretions vary within and between individuals over time.

## Materials and methods

### Subjects and housing

The subjects were 15 adult, reproductively intact ringtailed lemurs, including 7 females (mean age  $\pm$  standard error [SE]:  $10.3 \pm 2.5$  years; range: 3–20 years) and 8 males (mean age  $\pm$  SE:  $12.8 \pm 2.5$  years; range: 3–19 years), housed at the Duke Lemur Center (DLC) in Durham, NC. All were healthy, in good condition, and within normal weight ranges (female mean  $\pm$  SE:  $2.2 \pm 0.6$  kg; male mean  $\pm$  SE:  $2.5 \pm 0.8$  kg, excluding males for whom values were unavailable). The animals belonged to one of three semi-free-ranging social groups, each comprising 3–12 individuals of varying ages. Each group occupied a forested enclosure (of 3–6 ha), with access to their own heated indoor housing. Groups free ranged in the outdoor enclosures from mid-March to mid-November and resided indoors when the weather dropped below 4 °C. Two of the groups inhabited adjacent enclosures, allowing their members visual and olfactory contact through a chain-link fence, whereas the third group’s enclosure was located approximately 300 m from the nearest fence boundary of the other 2 groups. Throughout the year, the lemurs foraged on native flora and were supplemented once daily with a diet of Purina Monkey Chow (Monkey Diet PMI Feeds, Inc., St Louis, MO) and assorted fruits and vegetables.

This species is characterized by female dominance over males (Jolly 1966), but our rank assignments treated the sexes separately. From behavioral observation sessions conducted while the animals ranged outdoors, we identified intrasexual dominance relationships for the members of each group (Drea and Scordato forthcoming). These remained stable during the period of study. Because only 2 lemurs occupied mid-ranking positions (one per sex), we classified the remaining subjects as either dominant or subordinate and excluded the 2 mid-ranking animals from the analysis of dominance effects (see below).

### Scent sample collection and preparation

We collected odorants approximately once per month from September 2003 to December 2005. Based on observation of

seasonal patterns in scent-marking behavior (Kappeler 1998; Drea and Scordato forthcoming) and endocrine function (Drea 2007), we selected distinct time periods, a priori, to represent the prebreeding (October), breeding (November–February), and nonbreeding (March–September) seasons of *L. catta* in the northern hemisphere. The last period encompasses late-term pregnancy and lactation. Trained personnel captured and gently restrained the animals while we obtained odorant samples using cotton swabs that we had washed in methanol and pentane prior to use. We collected secretions from 4 glandular sources: female labial glands and male scrotal, antebrachial, and brachial glands, according to previously described procedures (Scordato and Drea 2007). In brief, we used clean forceps to gently rub a cotton swab against the gland fields of the labia or scrotum. We did not collect genital samples if the area contained fresh urine or feces. We collected samples from the antebrachial and brachial glands by first gently squeezing the gland to express its secretions, then using a cotton swab to collect the resulting exudate. We stored all the samples in solvent-washed chromatography vials at  $-80^{\circ}\text{C}$  until analysis.

We extracted the volatile compounds from the cotton swabs following a protocol adapted from Safi and Kerth (2003). We added 500  $\mu\text{l}$  of deionized water and 500  $\mu\text{l}$  of methyl-*tert*-butyl ether to each sample, vortexed the vials for 45 s, and then centrifuged samples for 5 min at 3000 rpm. We removed the solvent fraction with a pipette and placed it into a clean, solvent-washed chromatography vial. We repeated this extraction procedure twice, each time with 500  $\mu\text{l}$  of ether added to the cotton swab. We then placed a 600- $\mu\text{l}$  aliquot of the solvent fraction into a clean, 1-ml conical vial (Kimble) and concentrated those samples over compressed nitrogen to a final volume of 30  $\mu\text{l}$ . We kept all the samples on ice throughout the entire procedure to minimize the loss of low-molecular weight volatiles. We analyzed these samples by GCMS (see below) no more than 2 days after concentration.

#### GCMS analysis and compound identification

We manually injected 1  $\mu\text{l}$  of each concentrated sample into an HP 5890 series II gas chromatograph, attached to a double-focusing JEOL JMS-SX 102A high-resolution mass spectrometer using electron ionization at resolution of 3000, and ran them on an HP-5MS fused silica (5% phenyl)-methylpolysiloxane column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ , Agilent Technologies, Palo Alto, CA). We set the injector temperature at  $280^{\circ}\text{C}$  and the ion source at  $190^{\circ}\text{C}$  and used helium as the carrier gas. Masses were scanned from 50–525 in electron ionization mode in 1 s. We ran the following temperature protocol after a 3-min solvent delay:  $80^{\circ}\text{C}$ – $180^{\circ}\text{C}$  ramped at  $20^{\circ}\text{C}/\text{min}$  (after a 2-min hold at  $80^{\circ}\text{C}$ );  $180^{\circ}\text{C}$ – $320^{\circ}\text{C}$  ramped at  $7^{\circ}\text{C}/\text{min}$  (held at  $320^{\circ}\text{C}$  for 5 min). The entire run lasted 32 min; tests revealed that no compounds eluted after 30 min.

We ran the samples in large batches to minimize potential interassay variability. To verify retention times prior to analyzing each set of samples, we ran a standard mixture of 15  $\mu\text{l}$  each of squalene, farnesol, farnesal, and 7 alkenes (C12–C18; Sigma-Aldrich, St. Louis, MO) in 50 ml of methyl-*tert*-butyl ether. We required that retention times be within  $\pm 2$  s and abundances within 100 units of baseline standard runs. We also ran several samples multiple times throughout the study, which served to verify both instrument consistency and sample preservation over storage time. Finally, we ran a control cotton swab at the end of each batch to ensure that no sample-derived compounds had accumulated in the column.

The JEOL integration software that is associated with the instrument described above allows integration of the chromatogram area, but does not include mass spectral library-searching capabilities; therefore, to identify the major compounds present in our samples, we analyzed a subset of labial, scrotal, and brachial samples on a Shimadzu GCMS-QP2010 (Shimadzu Scientific Instruments, Columbia, MD) equipped with an AOC-20 series autosampler, and GCMS solution workstation software, including the NIST and Wiley libraries to search for matching spectra. The conditions on the Shimadzu GCMS were similar to those used with the JEOL SX-102: the column was a Restek SHR5XLB (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ , Shimadzu), the injector temperature was  $280^{\circ}\text{C}$ , the column was ramped over the same temperature profile as previously used, with helium as the carrier gas at a constant linear velocity of 1 ml/min, and the transfer line was held at  $275^{\circ}\text{C}$  while the ion source was held at  $260^{\circ}\text{C}$ .

#### Data analysis

We present GCMS data from 86 odorant samples: 33 samples of labial secretion (representing 6, 11, and 16 samples obtained during the prebreeding, breeding, and nonbreeding seasons, respectively), 29 samples of scrotal secretion (representing 5, 9, and 15 samples from the same 3 seasons, respectively), and 24 samples of brachial secretion (representing 4, 5, and 15 samples from the same seasons, respectively). Most of the subjects were represented by at least 1 sample from each season, but our seasonal analyses included subjects for which at least 2 seasons were represented. We also performed GCMS on a smaller sampling of antebrachial secretion; however, the results were too inconsistent to include in our analyses.

We identified specific compounds from representative labial ( $n = 3$ ), scrotal ( $n = 3$ ), and brachial ( $n = 2$ ) samples run on a Shimadzu GCMS-QP2010. We integrated the areas under each chromatogram and examined the mass spectrum of each compound for consistency with results obtained from the JEOL GCMS. We then searched spectra using the NIST 2002 mass spectral library. Using a greater than 85% confidence criterion and confirmation by visual inspection and fragment matching, we tentatively identified a portion of the total number of compounds present in the 3 types of

secretions. In cases of disagreement within the database search over the identity of esters, we identified the compounds as “long-chain esters.”

We could not control for the amount of secretion collected, so we did not rely on the absolute abundance of chromatogram peaks in our statistical analyses. Instead, we used the JEOL MS system integration software to express each peak as a percentage of the overall area of a chromatogram. Although we could not positively identify many of the compounds, we were able to identify the components that occurred in multiple samples by matching retention times and mass spectra. Labial and scrotal samples mainly comprised a series of organic acid esters and long-chain organic acids that occurred in three or four different structural isomers (see Results), each appearing as unique peaks within the chromatogram. Because of intersample variation in the number and relative area of different isomeric compounds and the close proximity with which isomers eluted off the column we could not always rely on retention times for peak identification. To ensure accurate identification of compounds, we examined the mass spectra and determined the molecular ion for each component in a chromatogram and combined the relative areas of structural isomers. We thus entered into the statistical analyses only 1 overall percentage for each unique compound. We excluded from the analyses all peaks that comprised less than 0.05% of the total area. We also excluded from our analyses a large compound (molecular weight: 508) that appeared in only 1 run of samples and was probably a contaminant from the column.

To reduce the dimensionality of our data set, we ran principal component analyses (PCAs) on the compounds identified in labial, scrotal, and brachial samples. First, we grouped all the compounds identified across the labial, scrotal, and brachial samples and ran a single PCA to test if the secretions produced by the different glands had unique chemical profiles. Second, we ran separate PCAs on the secretions from each of the 3 types of glands to test for seasonal and interindividual variation in odorant composition. We extracted principal components with eigenvalues  $> 1$  and used these as covariates in the linear discriminant analysis (LDA). We used LDA to group samples by gland of origin, season, and individual and used Wilks'  $\lambda$  tests of group differences as a statistical measure of accurate classification. To examine how the profiles of individual lemurs clustered by rank (using only dominant and subordinate individuals), we used hierarchical clustering with the Ward's minimum variance method. We performed all analyses using the JMP 6 statistical software package (SAS institute, v. 6.0.0).

## Results

### Volatile compounds in glandular secretions

We found differences in volatile chemical composition between the 4 types of glandular secretions of ringtailed

lemurs, as illustrated by the representative chromatograms of scrotal, labial, antebrachial, and brachial secretions (Figure 1a–d). GCMS analysis of antebrachial secretion revealed many fewer compounds than were present in genital secretions, most of which occurred sporadically between samples and at low concentrations. Only squalene ( $C_{30}H_{50}$ ), a high-molecular weight compound (MW = 410), occurred reliably in solvent-extracted antebrachial samples (Figure 1c). Because the apparently high volatility of this gland's secretions affected the reproducibility of our findings, we dropped antebrachial samples in subsequent analyses.

Among the remaining 3 types of glandular secretions for which we obtained consistent solvent extraction data (i.e., labial, scrotal, and brachial), we found a total of 122 distinct compounds: 66 in the labial samples, 52 in the scrotal samples, and 39 in the brachial samples. We assigned 36 of these compounds tentative identifications using the NIST 2002 mass spectral database (Table 1). Genital secretions were most similar in their composition: Both labial and scrotal secretions comprised a series of organic acids and esters, squalene, and cholesterol derivatives. Male brachial secretion primarily comprised squalene, with appreciable amounts of cholesterol derivatives and lanosterol.

### Glandular differences

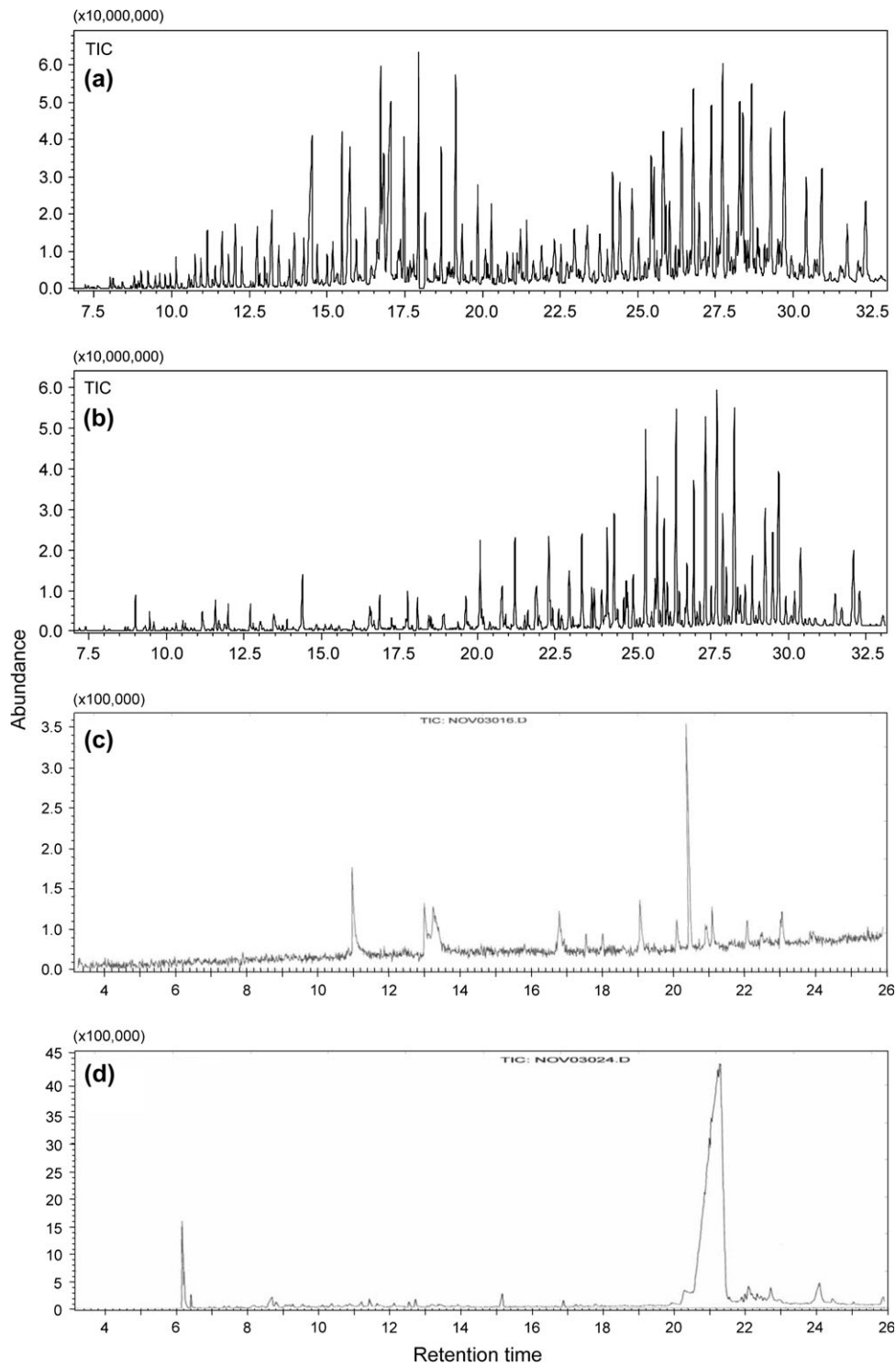
We reduced the 122 compounds present in the 86 labial, scrotal, and brachial gland secretions into 26 principal components that had eigenvalues  $> 1$ . LDA analysis of these principal components revealed significant differences between the chemical composition of the 3 different secretions (Wilks'  $\lambda = 0.002$ ,  $P < 0.0001$ ; Figure 2). Although the secretions from female and male genital glands were most similar in their content (with only 1 labial sample being misclassified as a scrotal sample), classification accuracy was at 98.8%.

### Seasonal differences

We extracted 20, 18, and 10 principal components with eigenvalues  $> 1$  from labial, scrotal, and brachial samples, respectively. LDA performed separately on these principal components showed seasonal effects for all 3 glands: 94.1% of labial, 93.1% of scrotal, and 91.6% of brachial samples were classified to the correct seasonal category (labial samples: Wilks'  $\lambda = 0.023$ ,  $P = 0.003$ ; scrotal samples: Wilks'  $\lambda = 0.019$ ,  $P = 0.006$ ; brachial samples: Wilks'  $\lambda = 0.136$ ,  $P = 0.047$ ; Figure 3).

### Interindividual differences

Using the same principal components as in the prior analysis, LDA accurately classified 94.1% of labial and 96.5% of scrotal samples to the correct odorant donor (i.e., the signal sender), regardless of season; however, statistical significance was achieved only for labial secretions (Wilks'  $\lambda = 3.09 \times 10^{-5}$ ,  $P = 0.004$ ; Figure 4a), with borderline



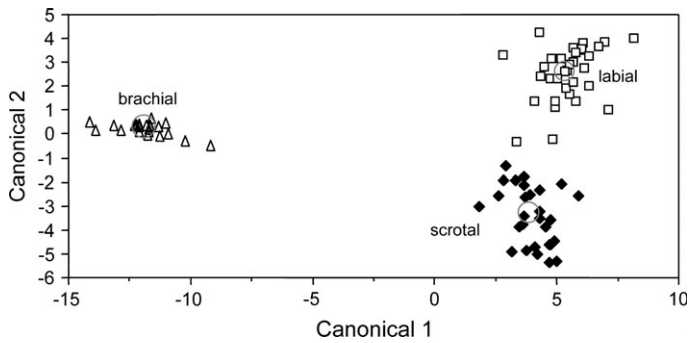
**Figure 1** Representative chromatograms of the secretions produced by the (a) labial glands of adult, female ringtailed lemurs and the (b) scrotal, (c) antebrachial, and (d) brachial glands of adult male ringtailed lemurs. Shown are examples drawn from both GCMS protocols using (a, b) a Shimadzu GCMS-QP2010 and (c, d) an HP 5890 series II gas chromatograph.

significance being achieved for scrotal secretions (Wilks'  $\lambda = 0.0003$ ,  $P = 0.073$ , n.s.; Figure 4b). By contrast, LDA accurately assigned only 66.7% of brachial samples to their

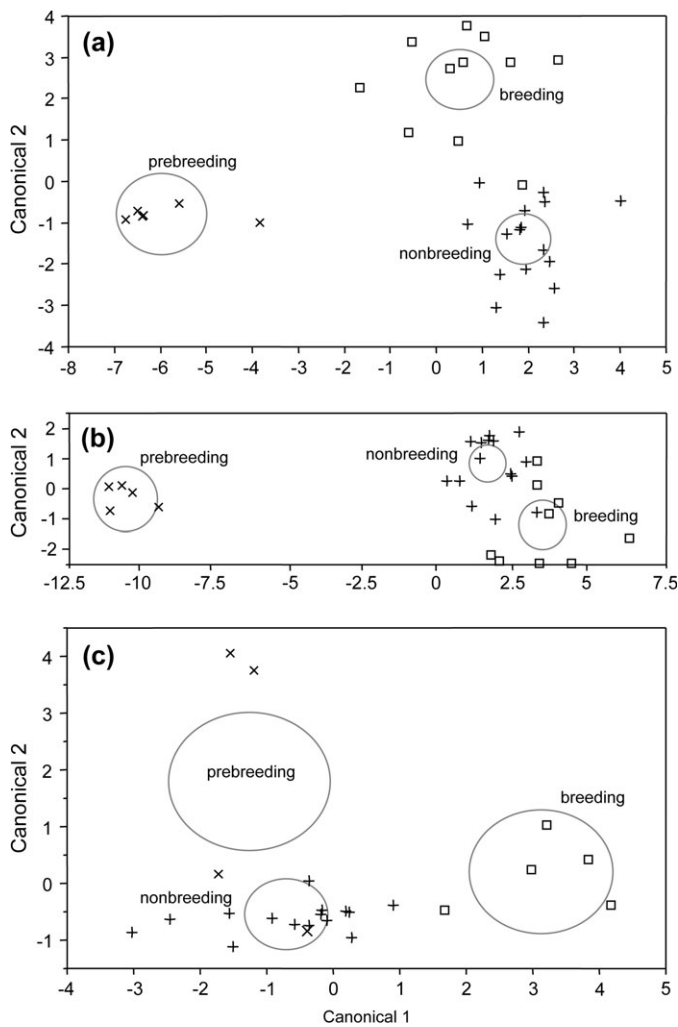
rightful donor, thereby revealing no significant effect of individual identity on the grouping of 24 brachial samples (Wilks'  $\lambda = 0.013$ ,  $P = 0.116$ , n.s.; Figure 4c).

**Table 1** Compounds present in the various glandular secretions of ringtailed lemurs and tentatively identified using the NIST 2002 mass spectral database

Molecular weight	Labial	Scrotal	Brachial
186	Undecanoic acid		
196	Cyclotetradecene	Cyclotetradecene	
200	Dodecanoic acid, 1-tridecanol	Tridecanol	
204	Alpha-farnesene	Alpha-farnesene	
204	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-, (E)-	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-, (E)-	
210		Pentadecene	
214	Tridecanoic acid	Tridecanoic acid	
224	Hexadecene	Hexadecene	
228	Tetradecanoic acid	Tetradecanoic acid	
240		Long-chain alcohol	
242	Pentadecanoic acid	Pentadecanoic acid	
256	<i>n</i> -Hexadecanoic acid	<i>n</i> -Hexadecanoic acid	<i>n</i> -Hexadecanoic acid
270	Heptadecanoic acid	Heptadecanoic acid	
282	Oleic acid	Oleic acid	Oleic acid
284	Octadecanoic acid	Octadecanoic acid	Octadecanoic acid
298	Nonadecanoic acid		
312	Eicosanoic acid	Long-chain ester	
326	Octanoic acid, tridecyl ester	Octanoic acid, tridecyl ester	
340	Octanoic acid, tetradecyl ester	Octanoic acid, tetradecyl ester	
354	Octanoic acid, pentadecyl ester	Long-chain ester	
368	Octanoic acid, hexadecyl ester	Octanoic acid, hexadecyl ester	
382	Octanoic acid, heptadecyl ester	Long-chain ester	
384			Cholesterol derivatives
386	Cholesterol derivatives	Cholesterol derivatives	Cholesterol derivatives
388	Cholestanol	Cholestanol	Cholestanol
394			Octacosane
396	Tetradecanoic acid, dodecyl ester	Tetradecanoic acid, dodecyl ester	
410	Squalene	Squalene	Squalene
424	Tetradecanoic acid, tetradecyl ester	Tetradecanoic acid, tetradecyl ester	
426			Lanosterol
438	Long-chain ester	Long-chain ester	
452	Long-chain ester	Tetradecanoic acid, hexadecyl ester	
466	Long-chain ester	Long-chain ester	
480	Hexadecanoic acid, hexadecyl ester	Hexadecanoic acid, hexadecyl ester	
508	Long-chain ester	Long-chain ester	



**Figure 2** Glandular differences in the composition of ringtailed lemur secretions. LDA comparing the composition of the secretions from the labial (square), scrotal (diamond), and brachial (triangle) glands of ringtailed lemurs.



**Figure 3** Seasonal patterns in the composition of the scent gland secretions of ringtailed lemurs. LDA comparing secretions derived during the prebreeding (times symbol), breeding (square), and nonbreeding (plus symbol) seasons from the (a) labial, (b) scrotal, and (c) brachial glands.

### Status differences

Hierarchical cluster analysis on the principal components of labial, scrotal, and brachial secretions divided labial and scrotal samples into 3 main clusters each and brachial samples into 2 clusters (Figure 5a–c). Samples derived from dominant and subordinate animals were distributed throughout these clusters, such that no particular rank-related pattern emerged for any type of odorant.

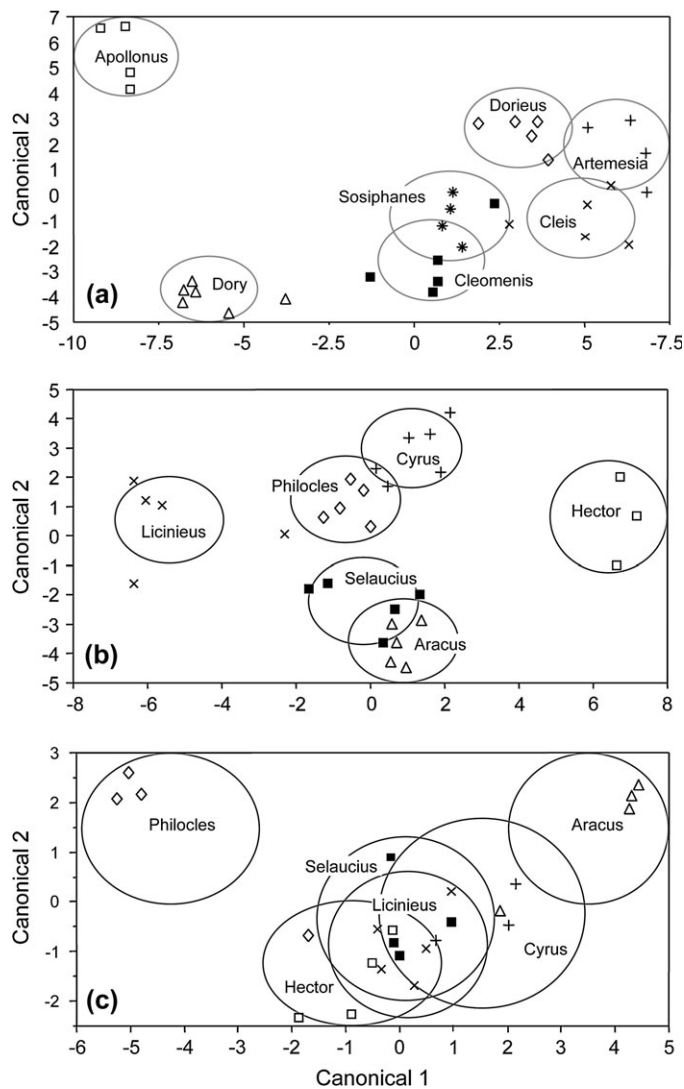
### Discussion

We found distinctive patterns in the chemical composition of the 4 different types of glandular secretions of ringtailed lemurs, in accord with our earlier behavioral findings on conspecific response to the different types of odorants (Scordato and Drea 2007). Likewise, we found seasonal variation in the chemical profiles of the labial, scrotal, and brachial secretions, consistent with seasonal differences in endocrine function (Drea 2007), scent-marking frequencies (Drea and Scordato forthcoming), and olfactory interest (Scordato and Drea 2007), supporting our prior interpretation that scent marking serves to advertise reproductive state and modulate intrasexual competition. Moreover, the secretions of male and female genital glands showed stable, individual-specific patterns, validating our interpretation that individual identity is encoded specifically within those scent marks that appear to function as long-lasting signals of resource ownership. Finally, as predicted, we found no evidence of rank-related differences in the chemistry of glandular secretions, supporting our prior suggestion that signal transmission is additionally dependent on the social context or the visual and auditory cues incorporated into signal deposition.

### Glandular differences

Our analyses of the glandular secretions of ringtailed lemurs revealed distinct differences in their chemical composition, largely consistent with preliminary findings from prior chemical studies (Hayes et al. 2004, 2005). Nevertheless, in contrast to Hayes et al. (2005), we suggest that semiochemical differences between the antebrachial and brachial secretions do exist, and we offer a possible explanation for why such differences escaped earlier detection.

Hayes et al. (2005) analyzed spectra from 10 antebrachial and brachial samples obtained from 10 different animals but found few early eluting compounds and no significant differences between odorant type. We suggest that this lack of differentiation is the result of 1) the volatility of the antebrachial secretions, such that components are lost at ambient temperatures, and 2) the natural contamination of antebrachial secretions with brachial secretions, as a consequence of the mixing of the 2 exudates by the male lemur. In our initial chromatograms of antebrachial samples, squalene, the primary component of brachial secretion, was frequently the only identifiable compound. We have since



**Figure 4** Individual-specific patterns of scent gland secretions in ringtailed lemurs, showing scent signatures in (a) labial and (b) scrotal secretions but not in (c) brachial secretions. Names and unique symbols represent the individual animals.

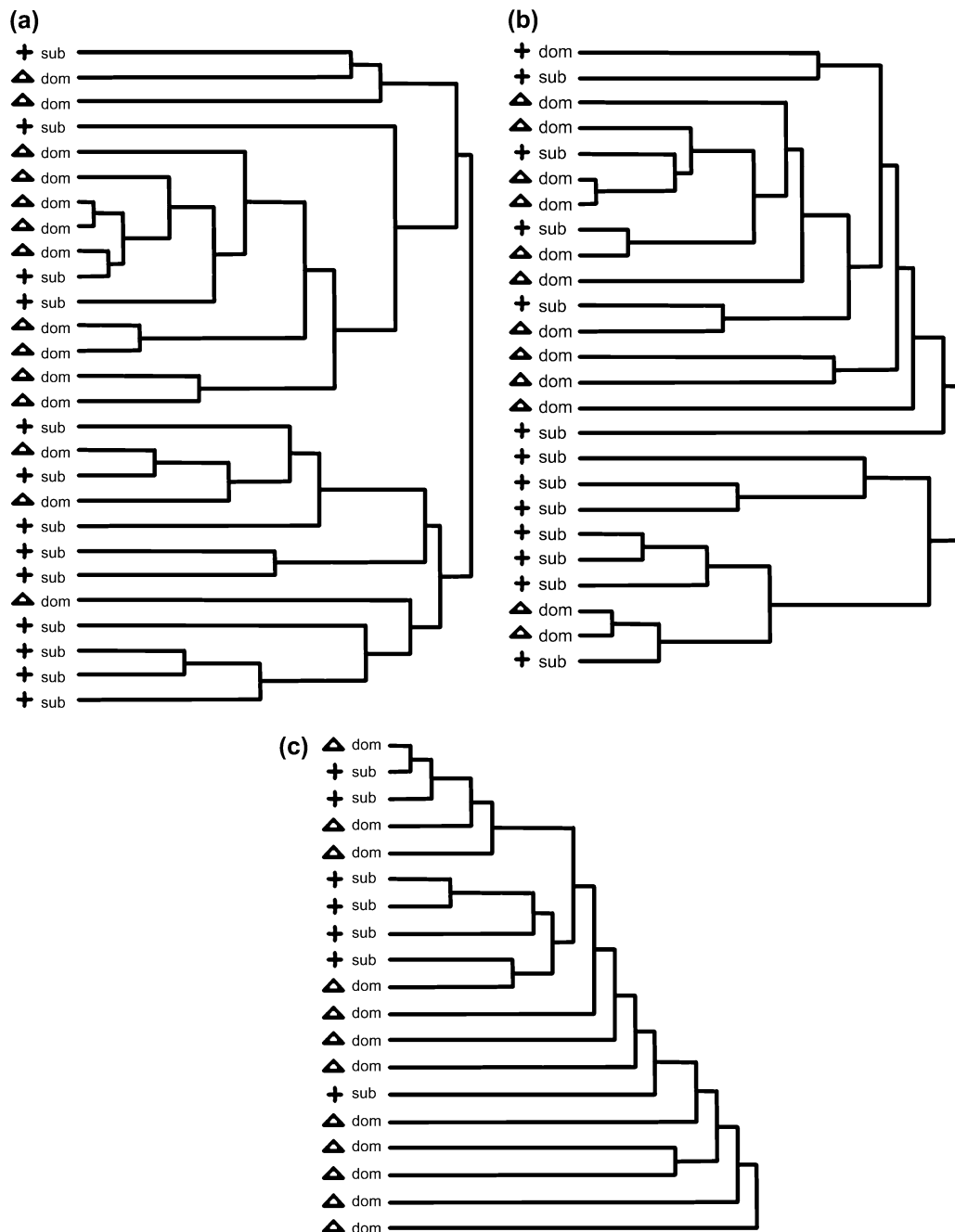
adopted the technique of cleaning the antebrachial organ before expressing its secretions. Moreover, in collaboration with Tom Goodwin (Hendrix College), we are analyzing the secretions from all 4 glands using solid-phase dynamic extraction (SPDE) techniques (Goodwin et al. forthcoming). Preliminary results from SPDE–GCMS assays show the antebrachial chromatogram to comprise many lower molecular weight volatiles (Scordato ES, Chen JC, Jackson SR, Weddell ME, Goodwin TE, Drea CM, unpublished data). Thus, consistent with the histological differences between the 2 glands (Montagna and Yun 1962) and the qualitative differences in their exudates (see figure 1 in Scordato and Drea 2007), we view antebrachial and brachial secretions as quite distinct and suggest that a combination of analytical techniques may be necessary to detect their chemical differences.

We found the labial and scrotal secretions to be chemically complex and more similar to each other than either was to antebrachial or brachial secretion. Although there were compounds unique to each type of genital secretion, the primary shared compounds were greasy, less volatile organic acids and esters. The brachial gland produced a similarly greasy mixture; however, its chemical profile was relatively simple, comprising almost entirely squalene, lanosterol, and several forms of cholesterol. Squalene, in particular, is a high-molecular weight compound that is widespread in mammalian glandular secretions and often functions as a fixative or waterproofing agent (Yarger et al. 1977; Albone 1984; Russell 1985; Rosell 2002). We suggest that the preponderance of squalene in brachial secretions points to a possible function for shoulder rubbing, namely, to mix squalene with antebrachial secretion, thereby “fixing” the latter volatile components to the substrate and increasing longevity of the scent mark. In this regard, squalene may serve a function in lemurs similar to that of 2-phenoxyethanol in rabbits (Hayes et al. 2003) or major urinary proteins in mice (Hurst et al. 1998).

#### Seasonal differences

The present chemical analyses also confirm seasonal differences in the volatile profiles of the labial, scrotal, and brachial secretions, complementing prior findings that male and female lemurs show seasonal patterns in scent marking (Kappeler 1990; Drea and Scordato forthcoming) and differentiate between odors derived from conspecifics in breeding versus nonbreeding condition (Palagi et al. 2003; Scordato and Drea 2007). These seasonal differences in both odorant composition and attractiveness seemingly correspond, in both sexes, with seasonal changes in reproductive hormones (Drea 2007).

Accurate signaling of physiological condition is widespread across mammalian taxa and is critical to reproductive success in *L. catta*. Female lemurs are sexually receptive for 24–48 h and cycle a maximum of 3 times per year (Jolly 1966; Evans and Goy 1968; Van Horn and Resko 1977); yet, they exhibit limited visual signs of ovulation. Thus, odor cues may provide a barometer of female reproductive state (Kappeler 1998; Palagi et al. 2003; Drea and Scordato forthcoming). Like males, other females also assess the breeding condition of female conspecifics (Palagi et al. 2003; Scordato and Drea 2007), possibly to influence the timing of their own estrus cycles (Pereira 1991) and/or to regulate intrasexual competition over resources (Mertl-Millhollen 2006) and potential mates (Parga 2006), as might be expected of a female dominant species (Scordato and Drea 2007). Our sampling protocol incorporated odorant collection during the peak breeding season (in early November), when the majority of females conceive; however, had we been able to enhance or confirm sampling at precisely the time of ovulation, we suspect that the seasonal differences detected might have been even more pronounced.



**Figure 5** Variation by social status in the composition of lemur scent gland secretions. Cluster analysis of **(a)** labial, **(b)** scrotal, and **(c)** brachial secretions show no relationship to the social rank of the sample donor.

### Interindividual differences

Male lemurs discriminate a signaler's dominance status when presented with odorants derived from familiar, but not unfamiliar, conspecifics (Scordato and Drea 2007); thus, we predicted that the volatile profiles of glandular secretions would reflect characteristics unique to each individual but would not reflect the signaler's social status. Both these predictions were supported. In particular, labial and, to some degree, scrotal secretions showed stable individual profiles

over the course of this study; however, brachial secretions did not.

Because both sexes disperse genital marks throughout the territory, with core areas receiving disproportionately more scent marks, previous authors have suggested that lemur genital marks function in territorial demarcation and intergroup spacing (Mertl-Milhollen 1988; Kappeler 1998; Mertl-Milhollen 2006). Our chemical data support that interpretation: Consistent with the demands of long-term,

intergroup communication, genital secretions comprised almost entirely large, semivolatile organic acids and esters. The individual-specific “signatures” contained within genital scent marks may facilitate scent matching by potential intruders (Gosling 1982). These olfactory bulletin boards, that persist in the absence of the signal sender, mirror the function of chemical signals in asocial mammals (e.g., Ralls 1971; Alberts 1992; Johnston and Jernigan 1994).

In contrast to genital marks, antebrachial marks likely function as relatively short-term, context-dependent signals, consistent with the greater volatility of these secretions and the elaborate behavioral displays associated with wrist marking. We do not know yet if antebrachial secretions reflect individual identity, but we found no evidence of individual signatures in the brachial secretions with which they are sometimes associated (brachial secretions are not deposited without first being mixed with antebrachial secretions). Although the absence of individuality in brachial secretions would be consistent with a primarily fixative function, our negative findings contrast with those recently reported by Palagi and Dapporto (2006).

This discrepancy over brachial signatures may reflect procedural differences between the 2 studies. On the one hand, Palagi and Dapporto (2006) obtained fewer samples for some individuals and for others, included samples obtained concurrently from the glands on the left and right shoulders. In both cases, individual differences would be more likely to emerge. The risk of using LDA on a small sample is exemplified by our preliminary chemical analyses performed on a subset of scrotal samples (Drea and Scordato forthcoming). Compared with the present analysis, which is based on a larger representation of samples per individual, our preliminary analysis achieved statistical significance for individual signatures. On the other hand, it is possible that by concentrating samples in solvent, we extracted a comparatively greater amount of squalene and cholesterol or failed to capture molecules not soluble in methyl-*tert*-butyl ether. The high concentration of squalene, which often accounted for as much as 97% of the chromatogram area, effectively could have swamped lower concentration, earlier eluting compounds (see, e.g., Knapp et al. 2006), thereby masking potential individual variation. Palagi and Dapporto (2006) did not provide a sample chromatogram, but it is possible that their protocol did not produce similar swamping. Additional research is needed to clarify the function of brachial rubbing and to assess the information content of antebrachial marks in *L. catta*.

#### Status differences

Social rank was not reflected in the volatile profiles of glandular secretions in ringtailed lemurs. Although some species, such as rabbits (Hayes et al. 2003) and voles (Kruczek 1997), have compounds in their scent marks that distinguish dominant animals from subordinates, social status in primates is

not typically considered to be an intrinsic quality of the individual, rather, it reflects a dynamic relationship influenced by the outcome of repeated interaction with familiar conspecifics. Nevertheless, Belcher et al. (1986) found that saddleback tamarins discriminated between natural scent marks of unfamiliar dominant and subordinate males—a result that may owe to intrinsic qualities of the secretion; however, the same finding could obtain from rank-related differences in the amount of secretion deposited or from covariance with reproductive state. To our knowledge, these factors have not been teased apart. In lemurs, however, it appears that the behavioral context of scent marking, rather than the chemical content of the mark, may be the critical element for signaling social standing. Male wrist marking and female genital marking are displayed most frequently by dominant animals (Kappeler 1990; Palagi et al. 2004, Drea and Scordato forthcoming). Social status is further reinforced by differential countermarking behavior, which is critical to intrasexual competition in both sexes (Kappeler 1998; Scordato and Drea 2007).

#### Conclusions

In summary, the chemical matrices of ringtailed lemur scent marks are gland specific and differentially contain information about the individual identity and physiological state of the signal sender, but not about the signaler’s social status. We suggest that olfactory communication in *L. catta* represents a multimodal system, similar in complexity and function to the chemical signals of rodents and other asocial taxa, in which different scent glands are used to convey different information. This is the first study to provide a comparative analysis of seasonal and interindividual variation across *L. catta* scent glands.

As evidenced by the divergent conclusions reached in our study and that of, for example, Palagi and Dapporto (2006) with regard to individual signatures, differences in sample collection and GCMS preparation protocols can produce disparate results, as no single analytical method can detect all molecular weight ranges in a complex chemosignal with equal clarity and resolution. Additionally, GCMS cannot detect nonvolatiles such as proteins; however, these compounds have been shown to play a critical communicatory role in many species (e.g., Belcher et al. 1990; Hurst et al. 1998; Nevison et al. 2003). Current SPDE–GCMS analyses are underway to address some of these procedural differences (Scordato SE, Chen JC, Jackson SR, Weddell ME, Goodwin TE, Drea CM, unpublished data).

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