TITLE OF PAPER: Sternal gland scent-marking signals sex, age, rank and group identity in captive mandrills

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ABSTRACT: Mandrills are one of the few Old World primates to show scent-marking. We combined ethological and chemical approaches to improve our understanding of this behavior in three zoo-managed groups. We observed the olfactory behavior performed by adults and adolescents (*N*=39) for 775 h. We investigated the volatile components of sternal scent-marks using gas chromatography-mass spectrometry and compared volatile profiles with traits of the signaler. Males marked more than females and within each sex the frequency of scent-marking was related to age and dominance status, but alpha males scent-marked most frequently and particularly in specific areas at the enclosure boundaries. We identified a total of 77 volatile components of sternal gland secretion, including compounds functioning as male sex pheromones in other mammals, in scent-marks spontaneously released on filter paper by 27 male and 18 female mandrills. We confirmed our previous findings that chemical profiles contain information including sex, male age and rank, and we also found that odor may encode information about group membership in mandrills. Our results support the hypotheses that scent-marking signals the status of the dominant male as well as playing territorial functions but also suggest that it is part of socio-sexual communication.

KEY WORDS: Dynamic Headspace Extraction · Gas Chromatography-Mass Spectrometry · *Mandrillus sphinx* · Olfaction · Pheromones · Signaling

INTRODUCTION

Mammals use a broad range of behavior to communicate and facilitate interaction with conspecifics, involving three major communication channels: olfaction, vision, and sound (Walker 1998). Unlike tactile and visual communication, olfactory communication involves signals which can be detected directly, in the presence of the signaler, or indirectly, from the odors that the signaler leaves behind (Alberts 1992). In addition, odor is linked directly to physiological condition and therefore is expected to be more honest than other types of signal (Hasson 1997). In effect, olfactory communication can involve both passive signals given off by individuals going about their everyday business (Washabaugh and Snowdon 1998) and olfactory cues that can be transmitted with active deliberateness such as in the case of informative breath (Laidre 2009) and scent-marking (Strier 2000). Active scent-marking provides good evidence for olfactory communication, often triggering specific responses such as investigation or countermarking (Kappeler 1998). Although behavior is observable, the message that is chemically communicated is difficult to decipher. Therefore, it is crucial to use detailed behavioral observations supplemented with chemical investigation of odor secretions released by scent-marking to link functional and mechanistic levels.

Scent-marking is a conspicuous behavior of many mammals and other terrestrial vertebrates including lizards (Müller-Schwarze 2006; Roberts 2007). The molecules in scent-marks include species-wide pheromones as well as highly individual odors (Wyatt 2009). Mammals have an enormous variety of specialized scent glands but a common pattern of scent-marking: glandular secretions, otherwise feces or urine, are placed at meaningful places in their territories such as along paths and boundaries (Gosling and Roberts 2001). In general, males tend to mark more than females, and dominant males or territory holders mark most, especially during breeding periods (Roberts 2007). If scent-marks effectively act as honest

signals, then scent-marking might show that the marker is both successful in competition with other animals and has successfully held the territory long enough to mark it and, at the same time, reflect the biological state of the marker, including its social status, health, and nutrition (Gosling and Roberts 2001). Scent-marking, which in some species can have significant costs in energy, time and risk, is important in both territorial and non-territorial species. In group-living species many of the scent marks may be directed at intra-group members as scent-marking also advertises dominance and reproductive status within social hierarchies but the scent marks may also advertise outside the group (Roberts 2007). In addition, scent-marking behavior can differ between related species and also varies between populations of the same species in ways that seem to be linked to habitat and the way habitat affects social structures (Wyatt 2014).

As in other mammals, in primates olfactory information plays a crucial role in a variety of contexts, including foraging, sexual interactions, territorial defense, individual and family recognition, mother-offspring bonding, and cooperative behaviors (Zeller 1987). In particular, scent-marking behavior is relatively common in strepsirrhines and callitrichines, among which is supposed to play different functions such as territoriality, regulation of social and reproductive dominance, mating competition and mate attraction (Heymann 2006), whilst is less commonly reported in catarrhines. Although some research work has been carried out on olfactory communication in strephsirrhines and New World primates, very little information exists for catarrhines. Old World monkeys, apes and humans have traditionally been considered as "microsmatic" (*i.e.* olfactory sense reduced; Negus 1958) with a concomitant increased emphasis on vision (Dominy and Lucas 2001; Zhang and Webb 2003). However, there is increasing recognition of the importance of chemical communication to catarrhines (*e.g.* Porter and Moore 1981; Geissman and Hulftegger 1994; Wedekind et al. 1995;

Wedekind and Furi 1997; Smith et al. 2001; Jacob et al. 2002; Vaglio et al. 2009; Charpentier et al. 2013; Crawford and Drea 2015; Drea 2015), including the first detailed chemical analyses of scent-gland secretions for a non-human catarrhine, the mandrill (Setchell et al. 2010).

Mandrills are the largest cercopithecine monkey and are characterized by exaggerated sexual dimorphism (Setchell et al. 2006a; b). Both male and female mandrills possess open nasopalatine ducts (Charpentier et al. 2013) and a sternal cutaneous gland (Hill 1970). This complex scent gland, which arises on a triangular area in the middle of the chest covered with modified hairs, longer than the surrounding hairs, the follicles of which have wide crateriform openings (Mellen et al. 1981), produces a glandular secretion that they rub vigorously against tree trunks and vertical branches (Feistner 1991). The gland of the alpha male (*i.e.* top-ranking male, featured by the most spectacular coloration, higher testosterone levels and enhanced reproductive success; Dixson et al. 1993; Wickings and Dixson 1992) is more active during the breeding season, when it appears greasy and his long sternal hairs are stained yellow, in contrast to the surrounding white chest fur (Setchell and Dixson 2001). The presumed function of the gland and the associated behavior is scent-marking (Mellen et al. 1981); males mark more frequently than females, whereas both males and females show a positive correlation between the frequency of scent-marking and dominance status (Feistner 1991). Scent-marking may serve a variety of functions in this species (Feistner 1991), which are not mutually exclusive, and include orientation within the home range, also based on observations of zoo animals indicating that scent-marking rates increased when males were introduced to new enclosures at Washington Park Zoo Research Center (Mellen et al. 1981). Previous chemical analyses (Setchell et al. 2010; 2011) suggest that scent-marks signal sex, age, male dominance and possibly individual identity, thus potentially serving to avoid

physical confrontations amongst dominant males and facilitate encounters between adult males and females, besides of individual genetic quality and information against which the receiver can compare its own genotype to assess genetic similarity, thus potentially providing a mechanism underlying mate choice for MHC diverse and MHC dissimilar partners.

In this study, we combine the chemical investigation of spontaneously released scent-marks with detailed behavioral observations of sternal gland scent-marking in three unrelated groups of captive mandrills living in modern zoo enclosures. We focused on captive groups because the collection and storage of odor secretion samples is more feasible and effective in captivity rather than in the wild. We investigated the features (sex, age, rank, physiological and reproductive status) of the actors performing sternal gland marking, where sternal gland marking was performed, and whether it was associated with other (olfactory and contextual) behavior. Additionally, we investigated the volatile components of odor secretions of sternal gland scent-marks spontaneously released by mandrills and compared chemical profiles with features (sex, age, rank, group identity) of the signaler. These latter analyses both replicate and build on earlier chemical analysis of mandrill secretions (Setchell et al. 2010, 2011). We tested the following hypotheses and predictions in mandrills:

1. Scent-marking signals sex, age and dominance. We predicted that males would mark more than females and the frequency of scent-marking would be related to age and rank within each sex, and that chemical profiles would convey information about sex, age and rank of the signaler.

2. Scent-marking serves a territorial function. We predicted that alpha males would scent-mark most frequently along enclosure boundaries.

3. Scent-marking facilitates orientation within the home range. Here we considered the transfer of a mandrill group hosted at Rome Zoo from one enclosure to another, predicting that the frequency of sternal gland marking would increase initially in the new environment.

4. Scent-marking functions as mediator of both intra- and inter- group interactions. We predicted that chemical profiles would encode information about individual and group identity of the signaler.

5. Scent-marking plays a role in socio-sexual communication. We predicted that chemical profiles would contain compounds which function as sex pheromones and cues to quality in other mammals.

METHODS

Subjects and housing

Subjects

The subjects were 39 mandrills (including adults and adolescents, males and females) belonging to three captive groups at Rome Zoo (Italy), The Tisch Family Zoological Gardens in Jerusalem (Israel) and Colchester Zoological Gardens (UK). We term males 'adolescent' from the age of 4 to 9 years, and 'adult' thereafter when they attain adult body mass, crown-rump length and full expression of secondary sexual traits (Setchell and Dixson 2002; Setchell et al. 2006b). We established dominance rank separately for males and females using dyadic interaction matrices, including all interactions where one individual avoided or fled

when another individual approached. Both male and female dominance ranks were stable during the study period. All females sampled were multiparous. Information was retrieved on sterilization, female cycle stage and possible pregnancy status **(Table 1)**.

----------Table 1----------

Housing

At Rome Zoo, the group was observed from October 2006 to September 2007; the group lived in a 20 m^2 indoor and 320 m^2 outdoor enclosure from October to December 2006, then moved to a new 25 m^2 indoor and 400 m^2 outdoor enclosure from January to September 2007. At the Tisch Family Zoological Gardens in Jerusalem, the group lived in a 50 m^2 indoor and 600 m^2 outdoor enclosure and were observed from January to March 2008. At Colchester Zoological Gardens, the group lived in a 42 m^2 indoor and 991 m^2 outdoor enclosure and were observed from May to July 2008. Similar diets were guaranteed across zoos during the study period.

Zoo enclosures were characterized by adequate (Griede 1989) and comparable environmental conditions. We defined different places from the center ("door" and "wall") to the periphery ("fence" and "glass") of enclosures, including places having a potential role of strategic targets ("pole", "wood", "tree", "tire"). The "glass" represented the boundary between mandrill enclosures and visitors.

Behavioral data collection and analysis

We recorded all olfactory behavior (sternal gland marking and investigative behaviors – *i.e.* sniff and/or lick substrate, sniff and/or lick genitals, sniff body), as well as contextual behavior (attacking, bouncing, crest raise, genital presenting, grooming, mounting, playing,

silent bared-teeth face, threatening, yawning) **(Table 2)**, receivers (target individuals) and substrate (door, fence, glass, land, pole, wall, wood, tree, tire), via all occurrences sampling (Altmann 1974; Martin and Batenson 1986) with a total of 775 h observations. We were always able to see the entire outdoor enclosure and all individuals during our behavioral observations.

----------Table 2----------

Two different kinds of analyses were performed: first, we explored the relationships between actors and sternal gland marking in relation to contextual behaviors and places; then, we investigated sterilization, estrus and pregnancy roles for the 'actor-sternal gland marking' dyad. To achieve these objectives: first, we used contingency tables to compare groups using Pearson's chi-square test and tetrachoric correlation for binary variables; then we used a multiple logistic regression model for repeated measures, with a log link function, accounting for inter- and intra-subject effects, because each participant provided more than one response.

Sternal gland marking is a highly visible, unambiguous behavior where an individual rubs its chest vigorously against tree trunks, branches or other vertical frameworks. This behavior was considered as dependent variable, while actors (male, female), contextual behaviors, receivers (adults, adolescents), places, sterilization status (yes, no), and pregnancy status (yes, no) were entered as predictor variables.

In all tests, the significance level was set to 0.05 and all tests were two-sided. STATA version 9.0 (STATA Corp, College Station, TX) was used for all the analyses on behavioral data.

Odor sampling and investigation

We collected scent-marks spontaneously released by mandrills on sterile filter paper fixed on vertical poles or tree trunks and branches. In addition, we exposed control filter paper to the air in the indoor enclosure during sampling, to identify any volatile compounds in the air that did not derive from the mandrills. We transferred all samples and controls to separate sterile vials, froze them in liquid nitrogen immediately, and stored them at -80°C. In total we obtained 44 filter paper samples from 25 individuals (1-2 replicates per individual).

We carried out laboratory analyses of odor in the Mass Spectrometry Center, Florence University, Italy. We subjected all samples to dynamic headspace extraction (DHS) followed by gas chromatography-mass spectrometry (GC-MS) analysis. We placed samples into 10 ml screw capped vials, closed by teflon-faced rubber septa and seals (Supelco, Bellefonte, PA, USA). We passed purified nitrogen (50 ml min^{-1}) through the system for 20 min at 50°C and adsorbed the entrained volatiles on an adsorbent cartridge trap filled with XLTenax Tm (Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany), maintained at 15°C within a Gerstel DHS device. The volatile compounds were subsequently thermally desorbed and transferred to the GC system using a thermal desorption unit (Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany). We carried out desorption at 300°C for 10 min under a helium flow (30 ml min^{-1}) and cryofocused the analytes in a programmable temperature vaporizer injector (Gerstel CIS 4) maintained at −40°C with liquid carbon dioxide. We injected the volatile components into the GC capillary column by heating the CIS 4 injector to 300°C at 720°C min^{-1}. We carried out blank analyses using an empty 10 ml vial (Supelco, Bellefonte, PA, USA) to assess possible environmental contamination. We purged the adsorbent traps at 300°C for 10 min after each analysis using the thermal desorption unit (TDU) apparatus to avoid any possible carry-over effects.

We analyzed the adsorbed volatile analytes of both types of sample using a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) EI, 70 eV, coupled directly to a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a fused silica HP 5-MS capillary column (Agilent Technologies, Santa Clara, CA, USA) 30 m x 0.25 mm crossbonded 5%-phenyl-95%-dimethylpolysiloxane, film thickness 0.25 μm. We maintained the injector and transfer line temperatures at 270°C and 280°C, respectively. We made injections in splitless mode (purge valve opened after 2 min) with a constant flow of helium carrier gas of 1.5 ml min^{-1}. We started the oven temperature program at 45°C for 2 min, then raised it by 4°C min^{-1} to 170°C, by 7°C min^{-1} to 300°C, and finally by 20°C min^{-1} to a final temperature of 320°C.

Mass spectra were acquired within the m/z interval 31-350 at a scan speed such to obtain 3.5 scans sec^{-1}. If the chemical standards of the detected compounds were not available, we tentatively identified the eluted compounds by using the Automated Mass-spectral Deconvolution and Identification System (AMDIS), Version 2.68 (Stein 1999) and comparing the EI deconvoluted experimental spectra with those of the NIST and Wiley mass spectral databases, Version 8.0 and Version 7.0 respectively (Agilent Technologies, Santa Clara, CA, USA); the putative identification was preliminary accepted only if the minimum matching factor was higher than 70%. To minimize the chance of mis-identification and when more than one compound had a good matching for the same GC peak, the chromatographic retention time was considered; the experimental retention index were calculated from the retention data of *n*-alkanes standard solution (analyzed in the same condition) and compared with those reported in the literature for the same chromatographic column type (El-Sayed 2012). Even if we cannot completely exclude a mis-identification for the molecules whose chemical standard was not in our availability, all the proposed compounds, satisfying the

previously listed characteristic and excluding those not deriving from the animals (as explained later), were used for classification. The overall alignment procedure of the chromatograms and the extraction of the purified mass spectra were obtained using the R package flagme (Robinson and Romoli 2014). The data matrix was created using the peak area relative to each determined compound using the integrated signal of the deconvoluted total ion current (TIC). Due to the different ranges of peak area for each compound in the analyzed samples, scaling of data was necessary: this was done on the obtained matrix prior to data analysis by means of scaling procedure. This procedure was chosen so that each considered compounds become equally important (van den Berg et al. 2006).We analyzed all samples in a short period of time to minimize inter-assay variability. We used controls and blanks to identify compounds that did not derive from the animals and remove these from the filter paper results. We also carefully removed potential environmental and industrial contaminants to avoid the risk of obscuring meaningful biological patterns (Drea et al. 2013).

We used established methods in chemometrics to evaluate differences among the samples in volatile profile (Massart et al. 1997; Varmuza and Filzmoser 2009; Schleyr et al. 1998). In particular, we applied hierarchical cluster analysis to the obtained data matrix. Euclidean distances were calculated and Ward's minimum variance method was used to examine the data structure. The Support Vector Machine (SVM)classification model with a radial kernel was carried out to test whether we could classify samples based on sex, age and rank of animals. The data-set was randomly split in two parts: the first half was used to train the models using a 10-fold cross-validation, whilst the second half was used to test the models. We could not test female rank, because we were able to collect only scent-marks released by dominant females, nor individual identity of signaler, because of the small number of sampled individuals.

R version 3.1.1 (R Core Team 2014), with Debian 7.6 (wheezy), kernel Linux 3.2.0-4-amd64, a 2.20GHz CoreDuo CPU and 4GiB memory workstation, was used for all the analyses on chemical data.

RESULTS

Sternal gland scent-marking behavior

In the group (*N*=9) housed at Rome sternal gland marking was almost always performed by the dominant male on 'glass' (95.0%) and often when visitors were present (*ad libitum* data). In the group (*N*=16) housed at Jerusalem sternal gland marking was performed more frequently (45.5%) by the dominant male on 'wood'. The dominant female also scent-marked (27.3%) on 'wall' and 'glass' at the enclosure boundaries. In the group (*N*=14) housed at Colchester sternal gland marking was performed by adult dominant and subordinate males with a preference (50.0%) for two specific places ('tower' and 'tire') having a potential role of strategic targets.

In the group housed at Rome, translocation of the group from an old enclosure to a new one did not result in a significant increase of the frequency of sternal gland marking (Pearson's chi-square test: χ^2=0.115; p=0.734) in the first period spent within the new enclosure **(Figure 1)**.

----------Figure 1----------

Across groups, sternal gland marking behavior was performed significantly more (96.55%; Pearson's chi-square test: χ^2=48.014; p<0.0001) by males and proportionally to rank and age

(Figure 2), with a significant predominance (70.11%; Pearson's chi-square test: χ^2=31.278; p<0.0001) towards specific places at the periphery of the enclosure ('fence' and 'glass') rather than places at the center of the enclosure ('door' and 'wall') or with a potential role of strategic targets ('pole', 'wood', 'tire' and 'tower') **(Figure 3)**. Sternal gland marking was significantly associated (Pearson's chi-square test: χ^2=6.67; p<0.0001) with sniff and/or lick substrate but not with any other behavior.

----------Figure 2----------

----------Figure 3----------

The independent variables 'sex' and 'place' significantly influenced 'sternal gland marking' (multiple logistic regression - sex: z=–4.99; p<0.0001; multiple logistic regression - place: z=–3.03; p=0.002). In such a context, a significant association also occurs between the independent variables 'sex' and 'place' (multiple logistic regression: z=–5.33; p<0.0001). However, we found no relationship between 'physiological status' (*i.e.* 'estrus', 'no estrus', 'pregnancy', 'no pregnancy', 'sterilization') and 'sternal gland marking'.

Chemical profiles of sternal gland scent-marks

We identified a total of 77 distinct peaks in 49 filter paper samples of mandrill sternal gland secretions that were not present in the control swab. These compounds included a series of hydrocarbons and fatty alcohols, organic aliphatic acid esters and carboxylic acid, aldehydes and ketones. Tentative identifications are listed in **Table 3**, and typical chromatograms (one from the blank control and one from a mandrill scent-mark) are shown in **Figure 4**. 19

compounds were present in all 49 samples, and approximately one third of compounds (25/77) were present in >90% of samples.

----------Table 3----------

----------Figure 4----------

Across groups, according to the SVM model, we can classify the samples on the basis of sex, age and rank. Results **(Table 4)** show the accuracy of the predictions model for both train- and test-set.

----------Table 4----------

Hierarchical cluster analysis shows differences among individuals belonging to Jerusalem, Rome, and Colchester groups **(Figure 5)**.

----------Figure 5----------

DISCUSSION

Scent-marking is used by many strepsirrhines and New World monkeys (*e.g.* Gould and Overdorff 2002; Pochron et al. 2005; Heyman 2006) but is traditionally considered to be less important in Old World primates (*e.g.* Freeman et al. 2012). We know remarkably little about the nature of the chemical signals employed by non-human primates, although several studies suggest that chemical communication is of importance to these species (Dapporto 2008; Geissman and Hulftegger 1994; Hayes et al. 2004; Hayes et al. 2006; Heymann 2006; Jacob et al. 2002; Knapp et al. 2006; Palagi and Dapporto 2006; Porter and Moore 1971; Wedekind

et al. 1995; Wedekind and Furi 1997; Scordato et al. 2007; Setchell et al. 2010; Smith 2006; Smith et al. 2001).

In this research work the sternal gland scent-marking behavior was considered, via behavioral and chemical approaches, through the study of three unrelated groups of captive mandrills characterized by adequate housing in modern enclosures (Griede 1989) and comparable environmental and dietary conditions. This study focused on captive groups because the collection and storage of odor secretion samples is more feasible and effective in captivity rather than in the wild.

Our behavioral results **(Figure 2)** support the following predictions, in line with evidence reviewed in the introduction: (i) both adult female and male mandrills mark the substrate with their sternal glands, but alpha males scent-mark most frequently; and (ii) within each sex, frequency of scent-marking is related to age and dominance status, although males mark more than females. In addition, our findings support the hypothesis that scent-marking may have a territorial function in this species. In particular, alpha males scent-marked mostly specific places on the enclosure boundaries or, otherwise, places with a potential role of strategic targets **(Figure 3)**.

On the contrary, our data do not support the hypothesis that scent-marking facilitates orientation within the home range. In particular, at Rome Zoo, the translocation of the group to a new enclosure did not result in a significant increase of the frequency of sternal gland marking in the first period spent within the new enclosure **(Figure 1)** as expected by the hypothesis of facilitation of the orientation within the home range based on a similar transfer of a group hosted at Washington Park Zoo Research Center (Feistner 1991; Mellen et al.

1981). A more critical test of the orientation function of scent-marking would require detailed studies in the wild. There are alternative reasons which might explain the decrease of scent-marking in the new enclosure. For instance, mandrills may exhibit neophobia when moving into a new enclosure and therefore their activity patterns could be substantially reduced as they hesitantly explore a new enclosure.

Mandrills frequently marked glass in the presence of visitors. Among other mammals, including strepsirrhines (Watson et al. 1999) and New World monkeys (Bassett et al. 2003), some types of scent-marking may serve as displacement activities which reduce physiological arousal in stressful situations. However, although authors (Fornasieri and Roeder 1992; Nash and Chilton 1986; Schilling 1979) proposed that strepsirrhines may scent-mark in response to unfamiliar environments as a self-calming mechanism, our results regarding the translocation of the group to a new enclosure **(Figure 1)** suggest that mandrills do not show scent-marking as a stressor behavioral indicator when exposed to unfamiliar environments.

Although behavior is observable, the message that is chemically communicated is difficult to decipher. Therefore, the chemical investigation of odor secretions released by scent-marking is crucial to understand the function of this behavior. Comparing this study with previous work (Setchell et al. 2010), we used the same chemical method for the whole set of samples (*i.e.* we subjected all the samples to DHS extraction - which provides a high concentration factor for volatiles - followed by GC-MS, rather than some samples to DHS extraction and other samples to SPME extraction) as well as a different software to identify the eluted compounds (i.e. we used AMDIS and hence compared the EI deconvoluted experimental spectra with those of the NIST and Wiley mass spectral databases, rather than directly comparing the experimental spectra with those of the database), and spontaneously released

scent-marks (*i.e.* we investigated odor secretions released by sternal gland marking and thus corresponding to the exact message sent by mandrills, rather than glandular secretions collected by sedated individuals). As a consequence, we were able to detect a consistent pattern of differentiation between the sexes and a perfect discrimination between adolescent and adult male mandrills.

As in our previous work (Setchell et al. 2010), many of the compounds identified were volatile hydrocarbons that have also been identified in GC-MS odor profiles for other mammals, including primates. For example, generic hydrocarbons and pentadecane have been found in *Lemur catta* (Hayes et al. 2004; Knapp et al. 2006), whereas acetic acid and benzaldehyde have been found in marmosets (Smith et al. 2001). As in lemurs (Scordato et al. 2007) and tamarins (Epple et al. 1988), some compounds, including squalene, were relatively high-molecular weight hydrocarbons which may act as a fixative that slows the release of more volatile compounds, as suggested for major urinary proteins in mice (Hurst et al. 1998). In addition, some compounds - including benzaldehyde, dodecanal, dodecane, heneicosane, heptadecane, heptanal, hexanal, hexadecane, naphthalene, nonadecane, nonal, octadecane, pentadecane, tetradecanal, tridecane and undecanal - function as male sex pheromones (reviewed in El-Sayed 2012) in other mammals (*e.g.* African wild dogs, Asian short-clawed otters, black-backed jackals, Bengal tigers, brown rats, Campbell's dwarf hamsters, Canadian river otters, cheetahs, desert hamsters, European badgers, European otters, European pine martens, European rabbits, gray wolfs, ferrets, giant pandas, leopards, lions, red foxes, reindeer, Siberian hamsters, sika deer) or even function as cues to quality (reviewed in Wyatt 2014) in other vertebrates (for example, in the crested auklet, a seabird with citrus scent based

on decanal and octanal produced by both sexes in the breeding season, concentration appears to correlates in males with social rank).

We confirmed our previous findings (Setchell et al. 2010) that volatile profiles contain information including: (i) sex, as in other mammals (Wyatt 2014), including ring-tailed lemurs (Hayes et al. 2004; Scordato et al. 2007) and owl monkeys (MacDonald et al. 2007), but not sifakas (Hayes et al. 2004; 2006); (ii) male age, as in elephants (Rasmussen et al. 2002); (iii) male rank, as in other mammals, including European rabbits (Hayes et al. 2003) and mice (reviewed in Gosling and Roberts 2001), but not in other primates, such as ring-tailed lemurs (Scordato et al. 2007). In addition, we found a significant signal of group identity in the volatile profiles. These results suggest that odor may encode information about group membership in mandrills, as demonstrated for other mammals, including naked mole-rats (O'Riain and Jarvis 1997), hyenas (Theis et al. 2012) and fur seals (Stoffel et al. 2015). However, these findings should be regarded as preliminary because they are based on only three groups and few replicates for each individual. The study of a larger sample of groups, ideally in the wild, would be critical to figure out if odour secretions really are indicative of an individual's group in mandrills.

Differences in odor profiles according to sex, age, male rank, family and genetic quality are also accentuated by behavior and the quantity of secretion produced. As shown by our behavioral results and reported by Feistner (1991), male mandrills scent-mark more than females, adult males mark more than adolescent males, and dominant males mark more than subordinate males. Males also have more active scent-glands than females, adult males have more active glands than adolescent males, and dominant males are the most active of all (Setchell and Dixson 2001).Such male signals may help to attract females, by allowing

females to recognize the species identity and then to choose a male using chemosensory cues to his quality (which reflect factors such as his social status, diet, reproductive state, and health), and mediate male interactions, by allowing males to recognize the group membership and then to avoid confrontation and physical aggression between rival males (Wyatt 2014). Unlike acoustic and visual signals, odor has the advantage of informing recipients in the absence of the signaler (Alberts 1992).

CONCLUSION

In conclusion, given the hypothesis that in mandrills scent-marking may serve more than one function (*i.e.* the different types of glands, sebaceous and apocrine, may produce secretions with different functions), particularly both territorial and social functions (Feistner 1991), the present study supports the hypothesis that scent-marking may have a territorial function in this species. Moreover, our results suggest that the male scent-marking behavior is involved in sexual communication whereas the female sternal gland marking might act as a multi-modal -visual and chemical- signal (signals with multiple components and modalities are widespread across the animal kingdom; Laidre and Johnstone 2013). In addition, our findings highlight that mandrill volatile profiles may encode information about group identity of the signaler besides of sex, age and male dominance status as already shown by our previous work (Setchell et al. 2010). These findings contribute to our understanding of the link between functional and mechanistic levels of the sternal-gland marking behavior in mandrills. Furthermore, the similarity of our findings to those found in other vertebrates (in particular mammals, including primates that are more distantly related to humans) provides a new evidence to the biochemical convergence of odor signals across the animal kingdom already stated by other authors (reviewed in Wyatt 2014) and supports our previous suggestion

(Setchell et al. 2010) that Old World primates are not as microsmatic as previously assumed.

Future research work should examine information perceived by the recipient, for example looking for evidence of behavioural or physiological responses mediated by scent-marks via bioassay tests (Wyatt 2014). In addition, we focused on the volatile components of mandrill odor, but we are currently investigating the non-volatile profile of such odor secretions. Chemical signals are mixtures of both volatile and non-volatile compounds, with high molecular weight compounds which may prolong the life of volatile signals in scent-marks(Alborne 1984; Belcher et al. 1990; Hurst and Beynon 2004).

FUNDING

This work was supported by the Ministry of Education, Universities and Research, Italy (Doctoral Scholarship for S.V.); the In.Ser. spa, Turin (sample collection); and the Department of Biology, Florence (laboratory analysis).

ACKNOWLEDGEMENTS

We would like to thank Sara Fontani, Stefano Tognetti, Annalisa Antonelli and Michela Pirredda for helping with data collection. We would also like to thank all staff members of Rome Zoo (especially Yitzhak Yadid), Ostrava Zoo (especially Jan Pluhacek and Jana Kalnova), The Tisch Family Zoological Gardens in Jerusalem (especially Shmulik Yedvab and Noam Werner), and Colchester Zoological Gardens (especially Rebecca Perry). Furthermore, we would like to thank Bert de Boer for his endorsement, alongside Elena Baistrocchi and Paolo Cavicchio for their useful contacts. Finally, we are deeply in debt to Jo

Setchell for her invaluable suggestions, Durham University's Primatology Group members for their constructive comments, and Brunetto Chiarelli for his encouragement. On top of that, we also thank an anonymous reviewer for constructive comments.

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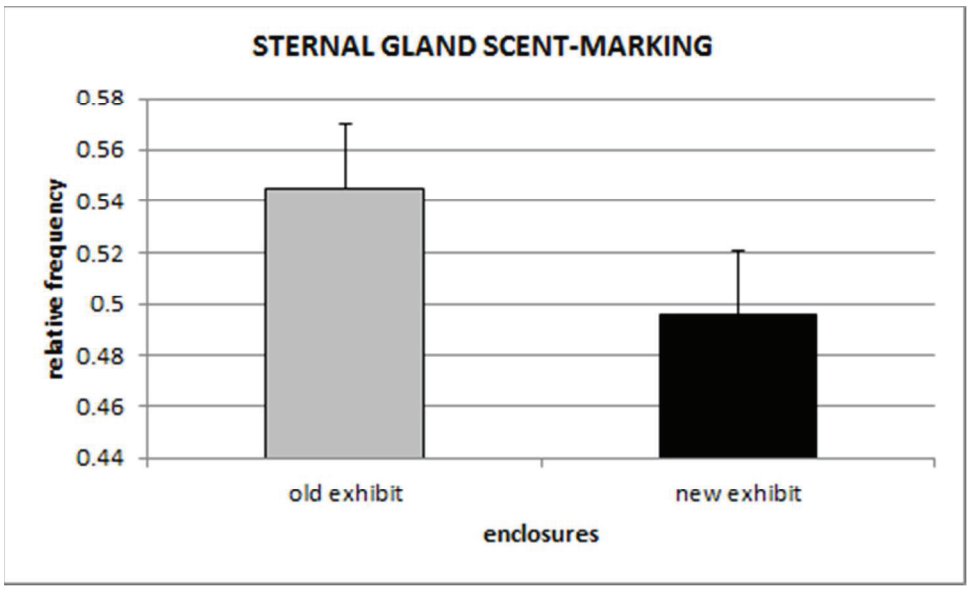
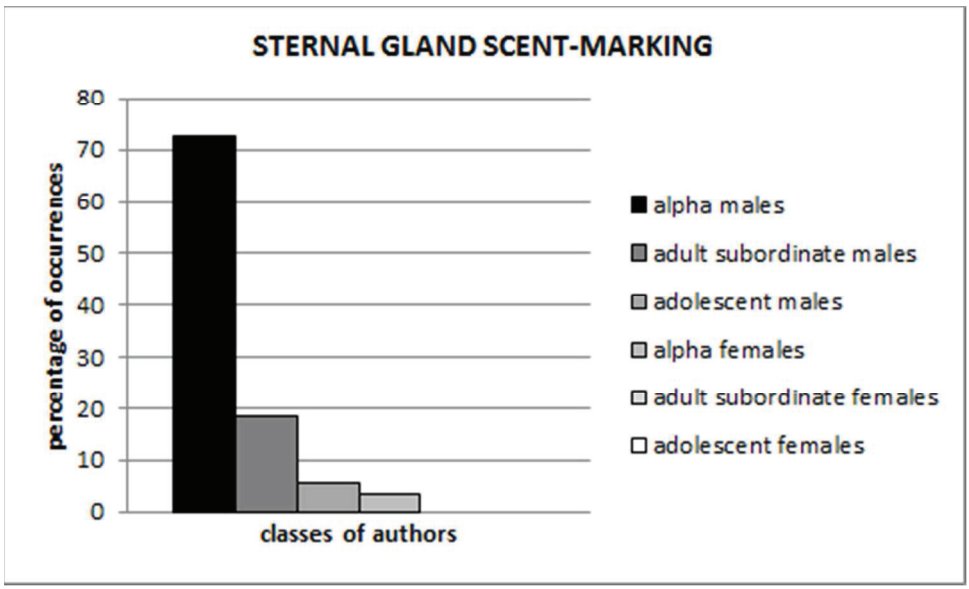
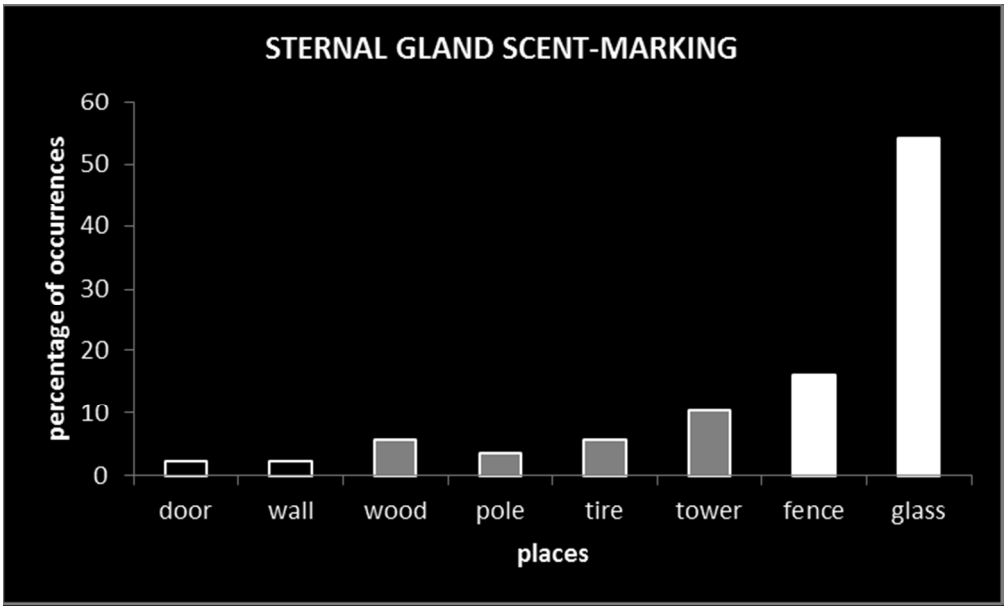


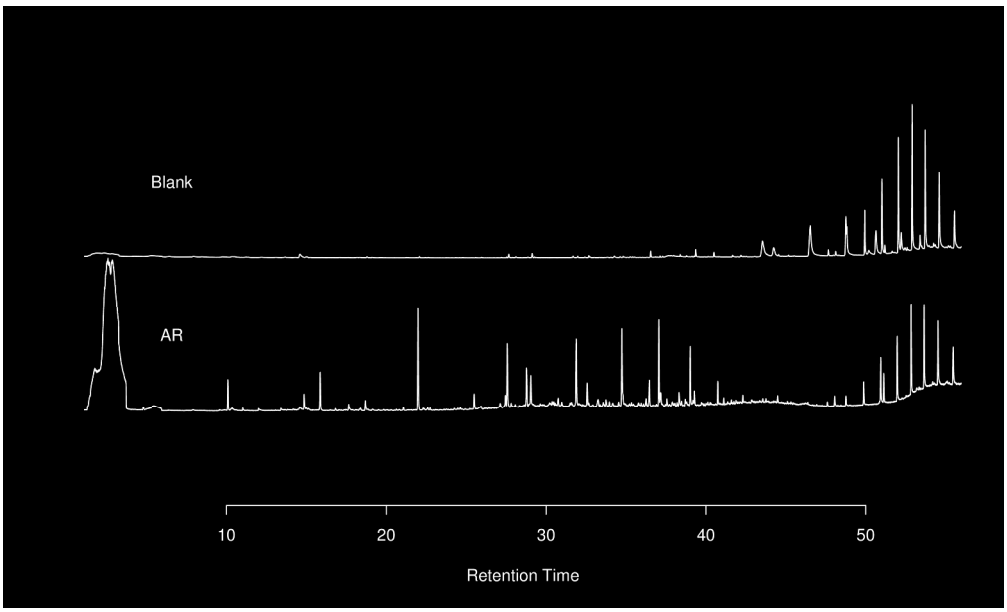
Relative frequency (occurrences/hour) of sternal gland scent-marking in the old and new enclosure at the Rome Zoo.
127x76mm (96 x 96 DPI)



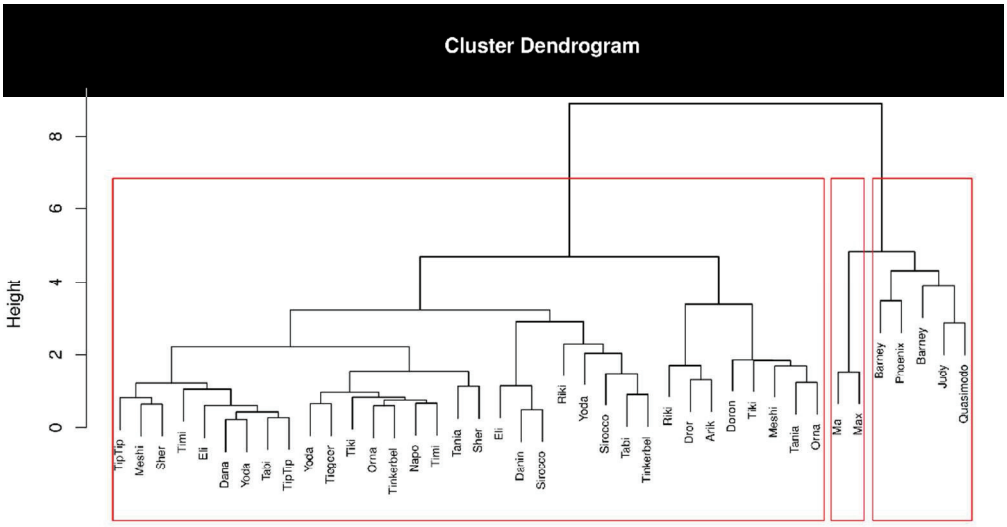
Percentage of occurrences associated to classes of authors (based on sex, age and rank of mandrills) that scent-marked.
127x76mm (96 x 96 DPI)



Percentage of occurrences of scent-marking based on substrate, arranged in order from the center ("door" and "wall") to the periphery ("fence" and "glass") of enclosures.
198x119mm (96 x 96 DPI)



Example TICs of a control (top) and a sample of scent-mark spontaneously released by a male mandrill (bottom).
793x476mm (96 x 96 DPI)



Hierarchical cluster analysis. The y-axis indicates the "distance" among different sub-groups, while the connections show were the successive split/join take place. Red boxes correspond to individuals belonging to Jerusalem, Rome and Colchester groups (from left to right clusters respectively).
305x169mm (96 x 96 DPI)

Table 1 – Composition of study groups at the beginning of data collection.

Zoological Garden	Infants and Juveniles	Females of breeding age	Adolescent and adult males	Total
Rome	7	5	4	16
Jerusalem	7	10	6	23
Colchester	8	7	7	22
Total	22	39		61

Table 2 – Ethogram (based on Mellen 1981 & Setchell and Wickings 2005, modified).

Behavioral category	Behavior	Description
Olfactory behaviors	Sternal gland marking	Animal rubs sternal area in an anterior-posterior motion on an object; chin is usually raised
	Sniff and/or lick substrate	Deliberate placing of the nostrils within 3 cm of substrate and sniffs and/or licks the substrate.
	Sniff and/or lick genitals	Deliberate placing of the nostrils within 3 cm of a female's anogenital area and sniffs and/or licks the female's anogenital area.
	Sniff body	Deliberate placing of the nostrils within 3 cm of a female's body area (excluding the anogenital area) and sniffs the female's body area.
Contextual behaviors	Attacking	Any physical contact between two individuals accompanied by loud vocalizations is considered an attack, such as grabbing hair, hitting and biting
	Bouncing	Individual grips a surface (usually chain link front of enclosure or climbing structure) with all four limbs and shakes that surface vigorously; or, while grasping the surface with front limbs, the hind feet "bounce" on the surface two or three times in rapid succession
	Crest raise	A momentary erection of the sagittal crest
	Genital presenting	Direct rear end towards another individual, usually while looking back at the animal. Performed upon individuals of the opposite and/or same sex
	Grooming	Animal picks through the fur of another individual using fingers, tongue and/or lips (*allo-grooming*). Individual picks through own fur using fingers and occasionally tongue, lips or teeth (*auto-grooming*)
	Mounting	(dorso-ventral position) Mounting individual grasps the other anterior to the pelvic region with hands. Mounter's feet remain on the substrate. Mounter usually exhibits pelvic thrusting. Intromission may or may not occur
	Playing	Engaging in relaxed chasing, biting, wrestling that is almost always accompanied by a relaxed, open-mouthed play face (teeth are usually covered)
	Silent bared-teeth face	Mouth retracted horizontally and

	vertically at the corners so the canines are partly exposed. Mouth remains closed in the centre and the incisors are covered by the lips and only partly visible, resulting in a figure-eight shape
Threatening	There are two distinctive types of threats: the *head bob* and the *threat rush*. Head bob involves "threatener" staring at its opponent and jerking the head forward and down. Occasionally the "threatener" slaps the ground with a hand while head bobbing. Fur on neck and shoulders is erect and crest is raised. A grunting vocalization often accompanies a head bob. Threat rush involves rapid quadrupedal locomotion toward the individual being threatened, but actual contact is not made
Yawning	An animal opens its mouth and exposes all of its canines

Table 2 – Volatile compounds present in filter paper samples from mandrill sternal gland secretions identified tentatively using the NIST (v.08) and Wiley (v.05) mass spectral databases, listed in order of retention time. Compounds in bold font were found in our previous work based on swab and hair samples (Setchell et al. 2010).

Molecular weight (Da)	Compound
186.334	1-Dodecanol
130.228	**1-Hexanol, 2-ethyl-**
262.387	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol
290.397	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester
194.313	5,9-Undecadien-2-one, 6,10-dimethyl-
276.371	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
281.477	9-Octadecenamide, (Z)-
296.488	**9-Octadecenoic acid, methyl ester, (E)-**
60.052	Acetic acid
106.122	Benzaldehyde
134.175	Benzaldehyde, 2,5-dimethyl-
194.227	**Benzoic acid, 4-ethoxy-, ethyl ester**
182.218	Benzophenone
156.265	Decanal
184.318	Dodecanal
170.335	Dodecane
212.415	Dodecane, 2,6,10-trimethyl-
242.398	Dodecanoic acid, 1-methylethyl ester
204.263	Ethanol, 2-(2-butoxyethoxy)-, acetate
138.164	Ethanol, 2-phenoxy-
296.574	Heneicosane
240.468	Heptadecane
114.186	Heptanal
226.441	**Hexadecane**
282.547	Hexadecane, 2,6,10,14-tetramethyl-
270.451	**Hexadecanoic acid, methyl ester**
100.159	Hexanal
298.504	Isopropyl Palmitate
204.351	Junipene
204.308	Lilial
204.351	Longicyclene
156.266	Menthol
128.171	**Naphthalene**
268.521	Nonadecane

142.239	**Nonanal**
254.494	**Octadecane**
340.584	Octadecanoic acid, butyl ester
128.212	**Octanal**
252.392	**Oxacycloheptadec-8-en-2-one**
212.415	**Pentadecane**
268.5209	Pentadecane, 2,6,10,14-tetramethyl-
254.494	Pentadecane, 2,6,10-trimethyl-
226.441	Pentadecane, 2-methyl-
226.4412	Pentadecane, 3-methyl-
286.407	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester
216.317	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester
410.718	**Squalene**
212.372	Tetradecanal
198.388	Tetradecane
198.345	Tridecanal
184.361	Tridecane
170.292	Undecanal
156.308	**Undecane**
-	Unknown_01
-	Unknown_02
-	Unknown_03
-	Unknown_04
-	Unknown_05
-	Unknown_06
-	Unknown_07
-	Unknown_08
-	Unknown_09
-	Unknown_Hydrocarbon_A
-	Unknown_Hydrocarbon_B
-	Unknown_Hydrocarbon_C
-	Unknown_Hydrocarbon_D
-	Unknown_Hydrocarbon_E
-	Unknown_Hydrocarbon_F
-	Unknown_Hydrocarbon_G
-	Unknown_Hydrocarbon_H
-	Unknown_Hydrocarbon_I
-	Unknown_Hydrocarbon_R01
-	Unknown_Hydrocarbon_R02
246.303	[1,1':3',1''-Terphenyl]-2'-ol
270.451	i-Propyl tetradecanoate
200.361	n-Tridecan-1-ol
170.207	o-Hydroxybiphenyl

Table 3 – Accuracy of the predictions model for both train and test set.

	TRAIN	TEST
Rank	76.47	85.29
Sex	64.71	82.35
Age	70.59	70.59