

Article

Sexual dimorphism in crowned lemur scent-marking.

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Simple Summary: Primates are typically thought to use hearing and vision more than the sense of smell. However, lemurs show a complex olfactory repertoire which includes conspicuous scent-marking behaviours. We studied two family groups of crowned lemurs at Colchester and Twycross zoos (UK) by combining behavioural observations and chemical analyses of odour secretions released via scent-marking. Male lemurs scent-marked most frequently, showing three types of behaviours: ano-genital marking for applying their scent on to females; head marking for placing their secretions on or near the mark left by another individual; and wrist marking to deposit their mark in specific meaningful areas of the enclosure. Female lemurs displayed only ano-genital marking primarily on feeding devices. We detected a total of 38 volatile compounds in male ano-genital scent-marks and 26 in female ano-genital odour secretions, including many compounds that have been identified in odour profiles of other primates. In conclusion, we found sexual dimorphism in crowned lemurs. In males head and wrist marking behaviours would play defensive territorial functions, while ano-genital marking may be related to socio-sexual communication; female ano-genital marking could be involved in resource defense. This study contributes to improve our understanding of lemur communication.

Abstract: Primates are traditionally considered to have a poor sense of smell. However, olfaction is important for non-human primates as demonstrated by conspicuous scent-marking behaviours in lemurs. We studied two family groups (N=4) of crowned lemurs (*Eulemur coronatus*) housed at Colchester and Twycross zoos (UK) by combining behavioural observations and chemical analyses of scent-marks. We recorded observations of olfactory behaviours for 201 h using instantaneous scan sampling. We investigated the volatile compounds of ano-genital odour secretions using solid-phase microextraction and gas chromatography-mass spectrometry. Males scent-marked most frequently, displaying ano-genital marking for allomarking, head marking for countermarking and wrist marking in specific areas of the enclosure. Females displayed ano-genital marking predominantly on feeding devices. We detected a total of 38 volatile components in all male ano-genital scent-marks and 26 in all female samples of ano-genital odour secretions, including a series of esters, aldehydes, ketones, alcohols, terpenes, volatile fatty acids and hydrocarbons that have been identified in odour profiles of other primates. In conclusion, we found sexual dimorphism in crowned lemurs. Male head and wrist marking behaviours might play defensive territorial functions, while ano-genital marking would be related to socio-sexual communication as chemical mate-guarding. Female ano-genital marking might be involved in resource defense.

Keywords: communication; olfaction; scent-marking; gas chromatography–mass spectrometry; *Eulemur coronatus*

1. Introduction

Communication plays a vital role for many species and is particularly important in those that exhibit complex social systems [1]. Animals display different types of behaviours to relay messages to other individuals, including visual [2], auditory [3] and olfactory [4] modes of communication. The olfactory system is, from an evolutionary perspective, the oldest sensory system and is found in many different species including mammals, reptiles and birds [5]. Olfactory communication has several benefits over other communication forms as chemical signals can be received in the absence of the sender and are then able to persist in the environment [4, 6]. Chemical signals are also thought to be honest indicators of health conditions as they are directly linked to internal hormones and energetically costly to produce [6].

In mammals, including primates, olfactory communication plays an important role in both solitary and social species [5, 7]. Olfaction has functions in foraging [8], territorial defense [9] and reproduction [10]. In particular, scent signals can provide information on individual identity, ranks, social status, age, sex and reproductive status [6, 11, 12] in several mammal species. Signals can come from specialised glands, skin, sweat, urine and faeces, and these can be mixed to provide specific messages [13]. Olfactory signals are complex and can be made up of various types of compounds, including both volatile and non-volatile components such as lipocalin proteins [15]. Scent-marking is a common eye-catching olfactory behaviour observed in many different terrestrial species, including mammals, such as rodents and felines, and reptiles [16]. Mammals tend to leave scent marks, urine or faeces, in meaningful locations, often along boundaries of their territories [17].

Primates have typically been considered to be microsmatic and instead rely mainly on visual and auditory senses [18, 19]. However, there is increasing evidence to suggest that olfaction plays a more important role in many primate species than previously thought [11, 20-22]. Similar to other mammalian species, olfaction has reproductive, social and identification functions in primates. Both strepsirrhine and South American primates show an extensive use of olfactory and scent marking behaviours, while this has been less commonly reported in Asian and African species.

Several species of lemur possess scent-glands; this is the case with ring-tailed lemurs (*Lemur catta*), *Eulemur* species [13], Milne-Edwards sifaka (*Propithecus edwardsi*) and red-ruffed lemurs (*Varecia variegata rubra*) [12]. Furthermore, some species, such as ring-tailed lemurs, have more than one scent-gland type and these can be sexually dimorphic [13]. Scent-marking behaviours are

observed extensively in lemur species, with ring-tailed lemurs often being used as a model species for olfactory communication [6]. Lemur scent-marking has many functions including marking territory, social communication and advertising reproductive status [6]. The frequency of scent-marking has been shown to differ between sexes [12, 23] and when comparing breeding and non-breeding seasons [7, 24, 25] in several lemur species. Chemical analysis of lemur scent-marks has identified a number of different volatile compounds, including over 120 compounds found in ring-tailed lemur scents alone [13, 26].

The crowned lemur (*Eulemur coronatus*) is a small, sexually dimorphic species of lemur found in the northern forests of Madagascar and the only lemur to inhabit the Cap d'Ambre Peninsula [27]. It is the smallest species in the *Eulemur* genus [13] and is currently classified as endangered by the IUCN Red List, with wild populations which are continuing to decline [27]. Crowned lemurs show a polygynous mating system and live in multi-male multi-female groups of up to 15 individuals, but typically groups are made up of 5 or 6 individuals [28, 29]. The species is one of only three *Eulemur* species which exhibit female dominance [29]. Crowned lemurs communicate mainly via vocalisations, but they also have different scent glands which vary between the sexes; it is therefore thought that olfactory communication has a crucial role in their communication [13, 24, 30].

The overarching aim of this study was to improve our understanding of the olfactory behavioural repertoire, particularly focusing on the different types of scent-marking behaviours, in captive crowned lemurs. We predicted that both scent-marking behaviours and the chemical composition of scent-marks will differ between sexes. We examined variation in scent-marking behaviours and related this to the location of deposition of scent-marks and the sex of the signaller. We also described the chemical composition of male and female ano-genital scent-marks and highlighted promising areas of future research work.

2. Materials and Methods

2.1 Subjects and Housing

We studied two captive groups of crowned lemurs (n=4) housed at Twycross and Colchester zoos. The group at Twycross Zoo consisted of an adult female (aged 8 years at the beginning of the

study period) and an adult male (aged 6 years). The group at Colchester also consisted of an adult female (aged 6 years) and an adult male (aged 6 years). Crowned lemurs are considered sexually mature at 20 months old [31]. The female at Twycross Zoo was contracepted. Both troops were in non-breeding season during the study period.

We carried out behavioural observations and odour sampling from July to November 2019 at Twycross and Colchester zoos. At both institutions the troops lived in heated indoor enclosures with access to outdoor enclosures. The outdoor enclosure at Colchester Zoo was a visitor walkthrough enclosure where crowned lemurs were co-hosted with other lemur species including ring-tailed and red-bellied lemurs.

2.2 Behavioural Data Collection and Analysis

We collected behavioural data by using instantaneous scan sampling [32]. Behaviours were recorded every 30 seconds in one-hour intervals three times per day (once in the morning, once midday, once in the afternoon) over a period of four months. We also used *ad libitum* sampling for recording of olfactory behaviours (**Table 1**) including scent marking (ano-genital marking, head marking, wrist marking) and the location of deposition (*i.e.*, conspecific, countermark, branches, climbing frames, enrichment objects, hatches, ground) (**Table 2**). We recorded a total of 201 hours of observations throughout the study period (132 hours at Twycross Zoo; 69 hours at Colchester Zoo) which included 360 scan samples per individual each day.

Table 1: Ethogram (based on [9, 21], modified).

Behaviour	Description
Scent-Marking: Ano-genital	Individual rubs its genital region against a conspecific, the substrate or object in the enclosure.
Scent-Marking: Head	Individual rubs its head against a conspecific, the substrate or object in the enclosure.
Scent-Marking: Wrist	Individual rubs its wrists or hands against a conspecific, the substrate or object in the enclosure.

Scent-Marking: Allomark	A scent-mark placed directly on the body of another individual.
Scent-Marking: Countermark	A scent-mark placed onto the location another individual scent-marked within 30 seconds.
Sniffing/Licking: Environment	Individual deliberately places its nostrils/tongue within 3 cm from the substrate or object and sniffs/licks
Sniffing/Licking: Conspecific	Individual deliberately places its nostrils/tongue within 3 cm from a conspecific and sniffs/licks.
Self-Licking	Individual uses its tongue to lick an area close to a scent-gland on its own body.

Table 2: Locations and definitions of possible scent-mark deposition.

Deposition Location	Description
Branches	Natural structures in the enclosure consisting of logs, trees and various plant species.
Hatches	Small entrances that can be open or closed, linking the different enclosures together.
Climbing Frames	Man-made wooden structures in enclosure consisting of walkways, ropes and platforms.
Enrichment Devices	Removable objects that were placed daily to promote good welfare.
Ground	The artificial floor of the indoor enclosures and the grassy area of the outdoor enclosure.

We investigated the relationship between sex and olfactory behaviours (including scent-marking). We also investigated the differences in function and deposition location of scent marks. We performed both Mann-Whitney U tests and Kruskal-Wallis tests followed by post-hoc pairwise Mann-Whitney U tests. A significance level of $P < 0.05$ was applied. All statistical analyses were carried out in SPSS (v 26).

2.3 Odour Sampling and Analysis

We collected male odour secretions released via spontaneous scent-marking on brand-new sterile filter paper (12 samples in total) that was fixed to hatches, branches and climbing structures in both the indoor and outdoor enclosures at both Twycross (7 samples) and Colchester (5 samples) zoos. Odour secretions were also collected from the female at Twycross Zoo during a veterinary check, with sterile filter paper rubbed across the ano-genital area 10 times using steady pressure (4 samples). We were not able to collect any odour samples from the female at Colchester Zoo, but we managed to collect odours from ano-genital scent marks from all other study subjects. We also placed control filter paper into the enclosures, veterinary and lab environments to the air in order to identify any volatile compounds that did not derive from crowned lemurs. All samples were placed into sterile vials, sealed and immediately stored at -20°C . We used 10-ml screw-capped clear glass vials (thread 180.D. 22.5 X H 46 mm) closed by teflon-faced rubber septa and seals (1.3 mm thick).

We conducted laboratory analysis at the Rosalind Franklin Science Centre, University of Wolverhampton, UK. We investigated the volatile compounds found in the scent secretions of crowned lemurs by using solid-phase microextraction and gas-chromatography-mass-spectrometry, applying the same methods used in previous work on lemur and mandrill odour signals (reviewed in [33]).

We introduced a $65\ \mu\text{m}$ polydimethylsiloxane/divinylbenzene solid-phase microextraction syringe needle through the vial septum and the fibre was exposed to the headspace above the sample in the vial for 15 minutes at 40°C . We analysed the adsorbed volatile analytes of all samples by using a 597 5C mass spectrometer (Agilent Technologies, Santa Clara, CA) EI, 70 eV, coupled directly to a 7890B gas chromatograph (Agilent Technologies) equipped with a fused silica HP5-MS UI capillary column (Agilent Technologies) $30\ \text{m} \times 0.25\ \text{mm}$ crossbonded

5%-phenyl-95%-dimethylpolysiloxane, film thickness 0.25 μ m. We maintained the injector temperature at 270°C and transfer line temperature at 280°C. We made injections in splitless mode (purge valve opened after 1 minute) with a constant flow of helium carrier gas of 1 ml per minute. We started the oven temperature program at 45°C for 2 minutes, then raised it by 4°C per minutes to 170 °C and then by 20°C per minute to 300°C.

We assessed any potential contamination from the lab environment through blank analyses of an empty 10 ml vial (Supelco) following the same procedure used for the samples. We also conditioned the fibre at 260°C pre-and post- injection for 5 and 20 minutes respectively to avoid any possible carry-over effects.

We tentatively identified eluted compounds by comparing the experimental spectra with the spectra provided by the mass-spectral library in ChemStation (Agilent Technologies) and NIST (National Institute of Standards and Technology) Database, version MSDF.01.01.2317 (Agilent Technologies). We accepted a probably identification when the minimum matching factor was higher than 80%. If more than one compound was a good match for the same GC peak we considered the chromatographic retention time and compared it with those reported in the literature for the same chromatographic column type to minimize the chances of misidentification. We created a data matrix using the peak area relative to each identified compounds by using the integrated signal of the deconvoluted total ion current (TIC). We analysed all samples in a short period of time to minimize interassay variability. Compounds that appeared in environmental (enclosure or vet) controls or the lab blanks were considered to be contaminants and removed from the results.

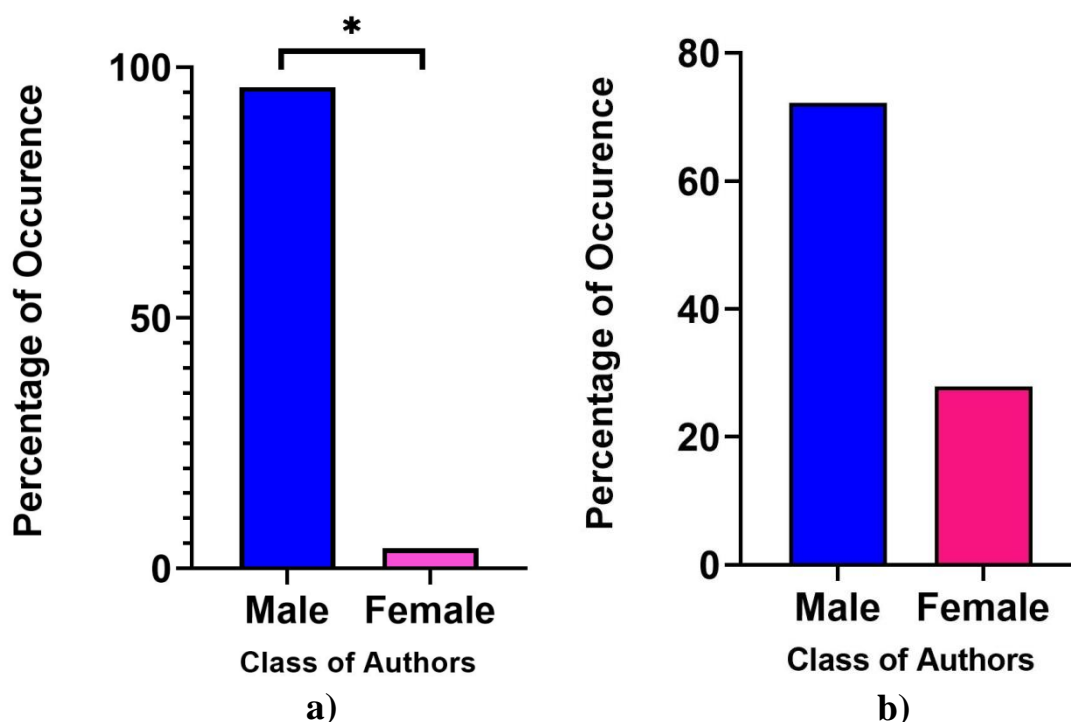
3. Results

3.1. Behavioural Observations

During the study period olfactory behaviours were exhibited significantly more ($U=135.5$; $p<0.001$) by male (83.12%) than female (16.88%) individuals. This included scent marking behaviours and olfactory investigations (sniffing/licking of the environment or a conspecific and self-licking). In particular, scent marking was significantly most commonly exhibited ($U=5218$; $p<0.001$) by males

(93.04%) compared to females (6.96%) (**Figure 1**). A total of 1086 scent marks were recorded throughout the study period.

Figure 1: a) Percentage of occurrence for total scent-marks associated to classes of authors. The difference between the sexes was significant. b) Percentage of occurrence for investigative olfactory behaviours associated to classes of authors.

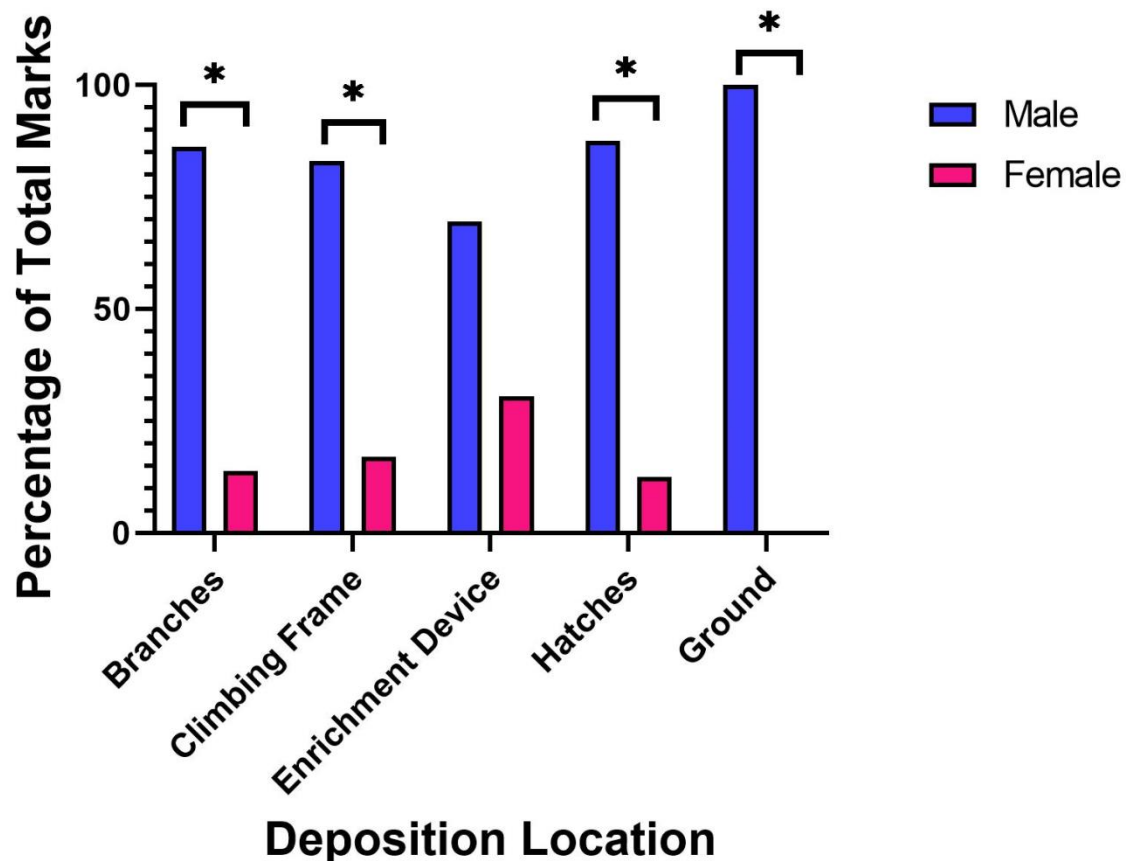


Females exhibited only one type of scent marking (anogenital marking), while males displayed three types of scent marking behaviours (anogenital, head and wrist marking). However, males predominantly ($\chi^2=30.10$; $p<0.001$) displayed anogenital marking (66.18%), followed by head marking (21.61%) and wrist marking (12.21%).

The study subjects deposited scent marks more often in the indoor enclosures (62.78%) but this difference was not significant ($U=328.5$; $p=0.13$). Within enclosures scent marks were most commonly deposited on trees and branches (45.77%) followed by enrichment devices (17.20%). We found a significant difference between ano-genital marking on branches ($U= 7615$; $P= 0.002$), hatches ($U= 7623$, $P<0.001$), the ground ($U= 8375$, $P= 0.002$) and climbing structures ($U= 8284$, $P= 0.033$) between the sexes (**Figure 2**). Scent marks were also deposited on enrichment devices, but we found

no significant differences between the sexes ($U=8641$, $P=0.122$). Furthermore, only males displayed both allomarking and countermarking behaviours.

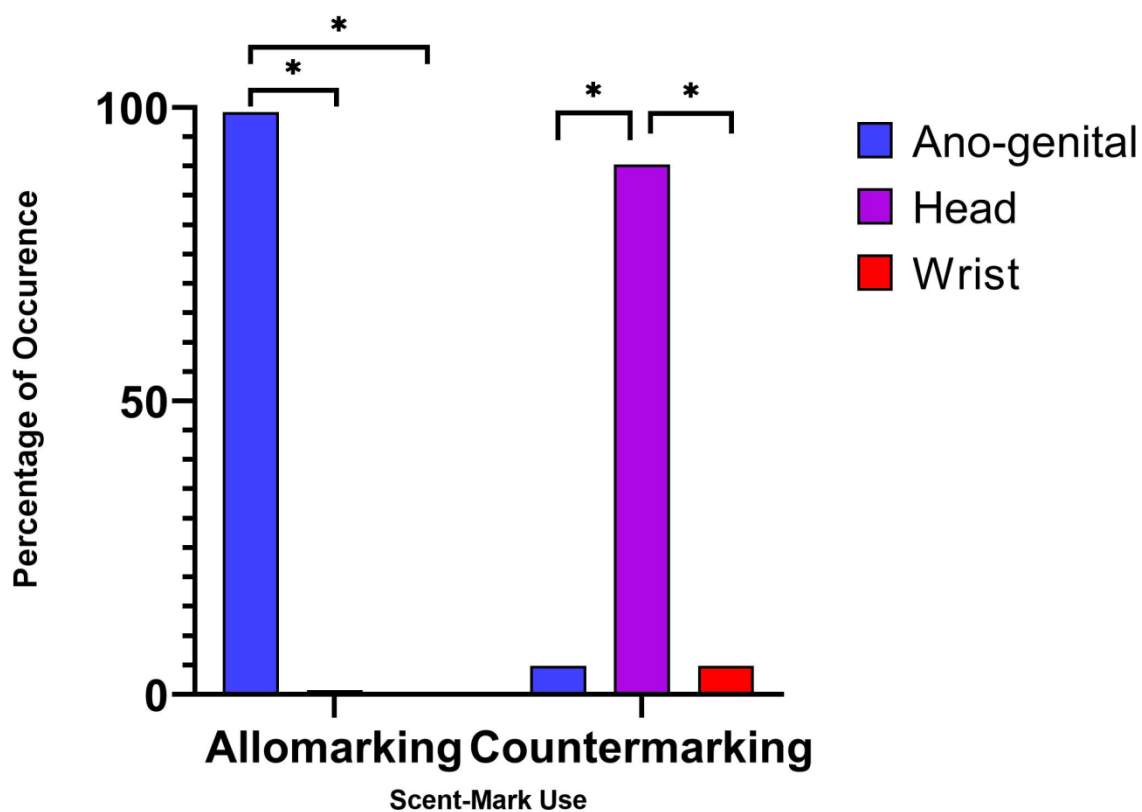
Figure 2: The percentage of total ano-genital scent-marks deposited on each location associated with sex. A * indicates where significant differences between the sexes were found.



Females predominantly ($\chi^2=14.76$; $p=0.002$) marked enrichment devices (40.32%) followed by branches/trees (35.48%). Female also deposited scent marks on climbing structures and hatches, but no allomarking or countermarking behaviours were observed.

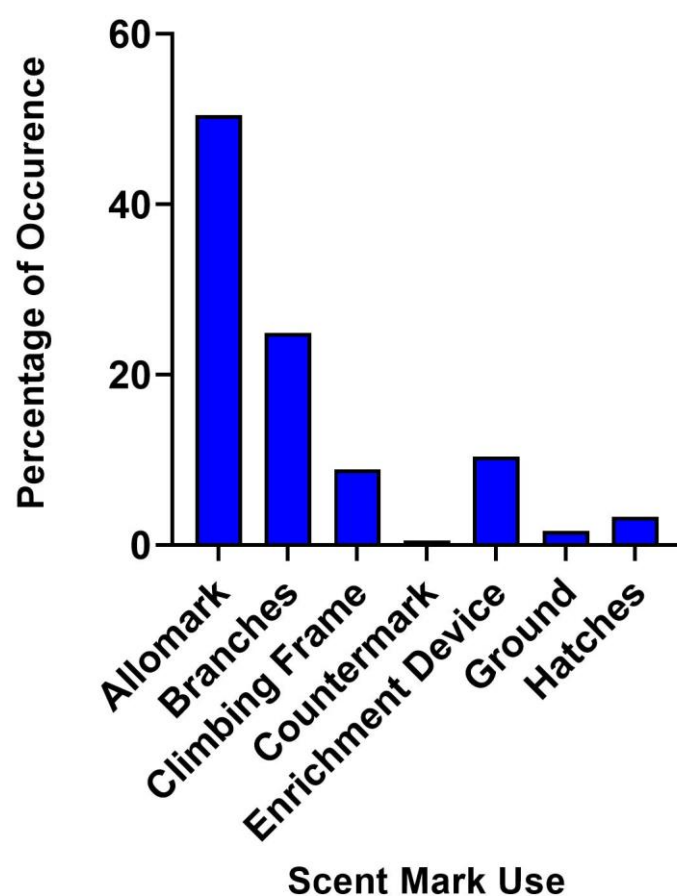
We found a preference in scent mark type in relation to both allomarking and countermarking responses by males (**Figure 3**). Allomarking was most commonly observed in relation to anogenital marking (98.58%) compared to head marking (1.42%) and wrist marking (0%), whereas countermarking was most common via head marking (90.32%) compared to anogenital marking (4.84%) and wrist marking (4.84%).

Figure 3: Percentages of occurrence for male allomarking and countermarking associated with scent-mark type.



We found a significant difference ($\chi^2 = 90.44$; $p < 0.001$) in the use of male anogenital scent marks (Figure 4). Allomarking was the most frequent (50.45%) followed by deposition on branches/trees (24.86%) and enrichment devices (10.34%). Males also deposited anogenital marks on climbing structures, hatches, the ground and occasionally as a countermark.

Figure 4: Percentages of occurrence for the use of male ano-genital marking. Allomarking accounted for the majority of ano-genital marking, followed by deposition on branches. Differences between the uses were found to be significant.



We found that the use of head marking by males was predominantly ($\chi^2= 39.94$; $p < 0.001$) used for countermarking (38.36%), followed by marking the ground (24.66%) and branches (13.01%). Males also deposited head marks on climbing structures, hatches, enrichment devices and also used head marks as a form of allomarking. We found that wrist marks were mainly ($\chi^2=15.42$; $p=0.001$) deposited on branches (51.47%), followed by climbing structures (23.53%) and hatches (20.59%). In addition to this, wrist marking was occasionally used as a countermarking response.

3.2. Odour secretions

We found a total of 38 distinct peaks in 12 paper samples of male ano-genital scent-marks and 26 distinct peaks in 4 paper samples of female ano-genital odour secretions that were present in all paper samples but not in the controls. In total we found 56 volatile components, as 8 compounds (hexanal, nonanal, decanal, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, benzaldehyde, 1-octen-3-ol, D-limonene, 2-ethyl- 1-hexanol) were found in both male and female paper samples (Table 3). We could tentatively identify only 42 compounds, while 14 compounds were classified as “unknown hydrocarbons” (Table 3). These compounds included a series of organic aliphatic acid

esters, acetate esters, aldehydes, ketones, alcohols, terpenes, volatile fatty acids and hydrocarbons.

Figure 5 shows typical chromatograms used to compare control and lemur odour samples.

Table 3: Volatile compounds present in filter paper samples from male **(a)** and female **(b)** ano-genital odour secretions identified tentatively using ChemStation (Agilent Technologies) and NIST (version MSD F.01.01.2317) mass spectral databases, listed in order of retention time. Compounds in **bold font** were found in both male and female samples.

a) Male ano-genital scent-marks.

Molecular weight (Da)	Compound
100.089	Hexanal
106.078	Benzene, 1,3-dimethyl-
114.104	Heptanal
106.042	Benzaldehyde
128.120	1-Octen-3-ol
126.104	5-Hepten-2-one, 6-methyl-
138.104	Furan, 2-pentyl-
136.125	D-Limonene
130.000	2-Ethyl- 1-Hexanol
126.104	2-Octenal, (E)-
142.136	Nonanal
-	<i>Unknown hydrocarbon 01</i>
426.407	Carbonic acid, decyl hexadecyl ester
156.151	Decanal
184.119	Undecane, 2,6-dimethyl-
182.203	Cyclohexane, 2-butyl-1,1,3-trimethyl-
-	<i>Unknown hydrocarbon 02</i>
-	<i>Unknown hydrocarbon 03</i>
356.329	Carbonic acid, decyl undecyl ester
-	<i>Unknown hydrocarbon 04</i>
-	<i>Unknown hydrocarbon 05</i>
184.219	Dodecane, 4-methyl-
-	<i>Unknown hydrocarbon 06</i>
-	<i>Unknown hydrocarbon 07</i>
-	<i>Unknown hydrocarbon 08</i>
-	<i>Unknown hydrocarbon 09</i>
-	<i>Unknown hydrocarbon 10</i>
198.235	Tridecane, 6-methyl-
-	<i>Unknown hydrocarbon 11</i>
-	<i>Unknown hydrocarbon 12</i>
-	<i>Unknown hydrocarbon 13</i>

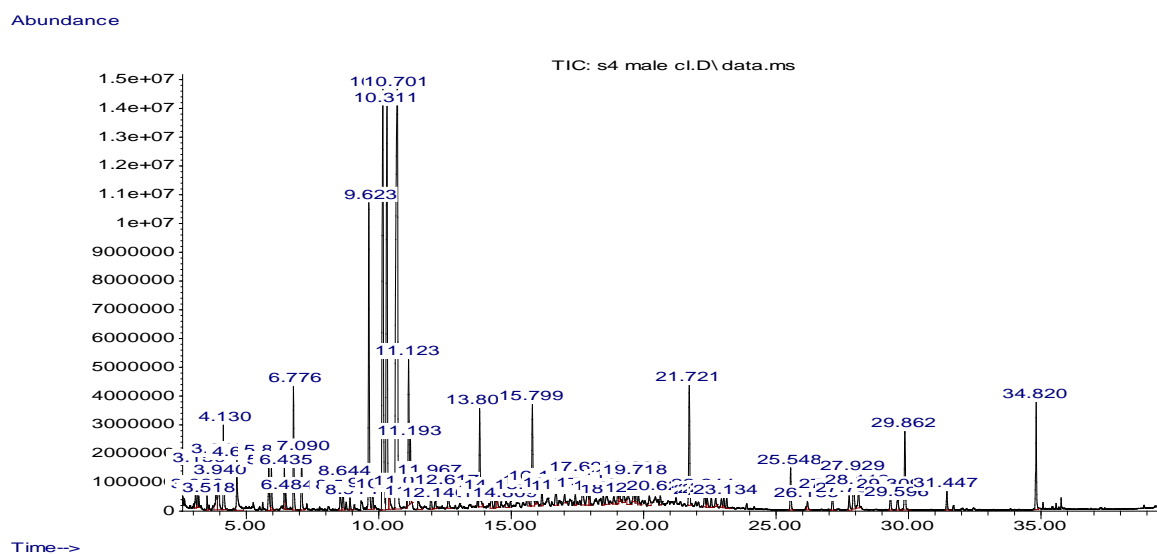
248.114	2-Bromo dodecane
-	<i>Unknown hydrocarbon 14</i>
286.214	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate

b) Female ano-genital scent-gland odour secretions.

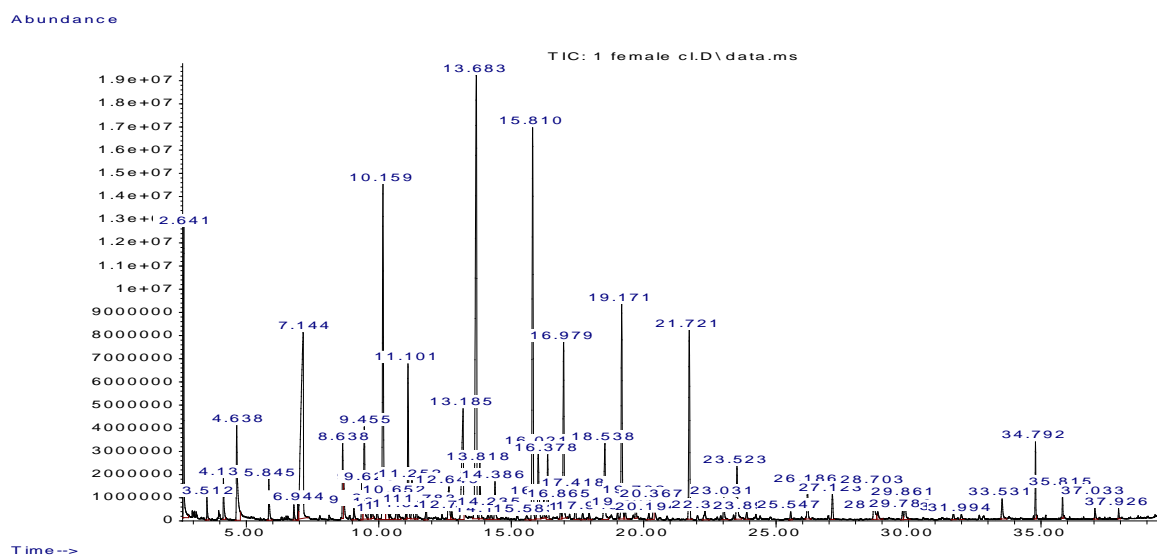
Molecular weight (Da)	Compound
100.089	Hexanal
118.099	Ethanol, 2-butoxy-
106.042	Benzaldehyde
128.120	1-Octen-3-ol
94.042	Phenol
128.120	3-Octanone
136.125	.beta.-Myrcene
107.063	4-Cyanocyclohexene
136.125	D-Limonene
130.000	2-Ethyl- 1-Hexanol
108.058	Benzyl alcohol
136.158	.alpha.-Pinene
136.125	.beta.-Ocimene
156.151	7-Octen-2-ol, 2,6-dimethyl-
154.136	Linalool
142.136	Nonanal
122.037	Phenol, 4-ethyl-
154.136	Terpinen-4-ol
154.136	α -Terpineol
152.047	Methyl salicylate
156.151	Decanal
196.143	Linalyl acetate
158.167	1-Decanol
196.146	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate
286.214	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
214.099	Benzene, 1,1'-[1,2-ethanediylbis(oxy)]bis-

Figure 5: Example chromatogram from (a) male crowned lemur, ano-genital scent-mark; and (b) female crowned lemur, ano-genital scent-gland odour secretions.

a) Male ano-genital scent-mark.



b) Female ano-genital scent-gland odour secretions.



4. Discussion

Primates have traditionally been considered “microsmatic” [34-35] (*i.e.*, having a reduced olfactory sense [36]), with more reliance on other sensory modalities such as vision [37-39]. This is particularly the case for catarrhines; however, several studies suggest that odour may play a crucial role in catarrhine species [*e.g.* 37-48]. Strepsirrhines are known to rely on olfactory communication in numerous contexts, such as foraging, territorial defense, kin and group member recognition, as well as reproductive functions [49-53]. Importantly, studies have recently accumulated on chemical

communication in non-human primates; therefore, semiochemical data are now accessible for several species, including various strepsirrhines (galagos [54]; lemurs [55-57], owl monkeys [58], marmosets and tamarins [59-61]) but also catarrhines (macaques [62], mandrills [11, 63, 64] and baboons [65]). In this study we focused on scent-marking behaviour, by combining behavioural observations with odour sampling, in two troops of zoo-housed crowned lemurs.

We found that males displayed significantly more olfactory behaviours than females, including both investigative and scent-marking behaviours. Males sniffed females' genitals significantly more than *vice versa* and males often then allomarked females after sniffing them. Rates of sniffing of conspecifics and genital sniffing were also found to be higher in male ring-tailed lemurs compared to females [65]. We also found that males scent-marked significantly more than females, which supports our prediction that scent-marking in individuals varies by sex. Similarly this has been seen in other lemur species such as sifakas [66], ring-tailed lemurs [25, 66], red-bellied lemurs (*Eulemur rubriventer*) [23] and red-ruffed lemurs [12].

Females only displayed ano-genital marking while males displayed three different types of scent-gland marking (ano-genital, head and wrist marking), as already reported by other authors [24]. This demonstrates sexual dimorphism in scent-marking in crowned lemurs. However, despite the presence of three types of scent-glands, ano-genital marking was the most commonly observed form of scent-marking and was shown by both sexes. This is similar to what has been found in other primate species, including the mongoose lemur (*Eulemur mongoz*) [67] and both moustached (*Saguinus mystax*) and saddle-back tamarins (*Saguinus fuscicollis*) [68].

In addition to dissimilar types of scent-marking behaviours, both the frequency of scent-marking and the differences in deposition location between the sexes indicate sexual dimorphism in crowned lemur scent-marking. Females marked enrichment devices most frequently, and interestingly only marked feeding devices and no other enrichment types. This could suggest a role of scent-marking in female resource defense, which has been proposed to occur in both female Verreaux's sifaka (*Propithecus verreauxi*) [69] and ring-tailed lemurs [70]. Furthermore, the remarkable differences between the depositions of scent-marks from different male glands indicate that odour secretions may provide different signals and thus male scent-marking behaviours may have different functions. In particular, male ano-genital marking was deposited most frequently on

conspecifics (allomarking), while countermarking and deposition on branches were most frequently observed for head marking and wrist marking respectively.

Allomarking from males via ano-genital glands was the most frequent scent-marking behaviour observed throughout the study period. Importantly, this behaviour was only ever directed from males to females. Allomarking has been observed in other primate species, such as saddle-back tamarins [68, 71], owl monkeys (*Aotus nancymae*) [72] and other Eulemur species including red-bellied lemurs [23], mongoose lemurs [67], common brown lemurs (*Eulemur fulvus*) and black lemurs (*Eulemur macaco*) [73]. Male allomarking could be a form of chemical mate-guarding or have a role in reinforcing social bonds as suggested in mongoose lemurs [67] and saddle-back tamarins [71]. Sometimes male allomarking also caused agonistic behaviour directed from the female to the male, which was also observed by other authors [24].

We found a total of 38 volatile compounds present in all male samples of odour secretions released via ano-genital scent-marking, and 26 volatile compounds shown in all female samples of ano-genital scent-gland odour secretions. These low amounts of compounds in comparison to ano-genital marks by other lemur species (*e.g.*, ring-tailed lemurs and sifakas; [26, 59]) could be due to the female non-breeding season [6] and contraception [74] as these factors can alter significantly the lemur genital odorants.

The volatile chemical profile of male ano-genital odour secretions encompassed several hydrocarbons. Volatile hydrocarbons have previously been identified in odorants deriving from strepsirrhines such as ring-tailed lemurs [2], red-ruffed lemurs [12] and Coquerel's sifakas (*Propithecus verreauxi coquereli*) [56], and catarrhines such as mandrills (*Mandrillus sphinx*) [11, 63] and olive baboons (*Papio anubis*) [65]. In addition, we found several unknown hydrocarbons which were high-molecular-weight, less volatile hydrocarbons that might act as a fixative which slows the release of more volatile compounds, as seen in cotton-top tamarins (*Saguinus oedipus*) [75]. Male scent-marks also included methyl ketones, which are found as putative semiochemicals in aye-ayes [76], red-ruffed lemurs [12] and olive baboons [65], and volatile fatty acids, which contribute to baboon vaginal odour [65] and human body odour originating from the apocrine sweat glands [77].

On the other hand, the volatile chemical profile of female ano-genital odour secretions comprised a lower amount, but more diverse range, of components. In particular, we identified the

compound phenol which has been found in vaginal odour secretions of several primate species (*e.g.*, ayes-eyes [76], red-ruffed lemurs [12], hamadryas baboons (*Papio hamadryas*) [78] and olive baboons [65]) and also serves as the locust phase change pheromone produced by gut bacteria [79]. Furthermore, ethyl phenol occurs in mammal urine as part of a multicomponent signal of mate attraction or range occupation (reviewed in [80]) and in ano-genital scent-marks by red-ruffed lemurs [12]. Finally, other compounds, such as α -pinene and α -terpineol, have also been identified in ruffed lemur ano-genital scent-marks [12] but are known to derive from plants and flowers; they could therefore be a by-product and vary with the environmental context.

A total of 8 compounds were identified in both male and female odour samples. Among them, benzaldehyde could play a role in signalling individual quality in crowned lemurs. This compound is considered a crucial putative semiochemical occurring at all ancestral nodes leading to both urine and glandular markers in many strepsirrhine species [81]. In particular, it has been found in scent-gland secretions released by several primates (*i.e.*, ayes-eyes (*Daubentonia madagascariensis*) [76], red-ruffed lemurs [12], common marmosets [81], capuchin monkeys [82], emperor tamarins [60, 83], Weddell's saddleback tamarins [60, 83], owl monkeys [72], mandrills [11, 63] and olive baboons [65]) and it has also been reported acting as a cue to genetic quality and as a pheromone in non-primate mammals and other vertebrates (reviewed in [84]). Moreover, hexanal and 1-octen-3-ol are encountered in scent-marks in several non-primate mammals (*e.g.*, lions (*Panthera leo*), African wild dogs (*Lycaon pictus*), gray wolves (*Canis lupus*), house mice (*Mus musculus*), red foxes (*Vulpes vulpes*)) (reviewed in [84]) and in ano-genital odour secretions in primates (*e.g.*, aye-eyes [76], red-ruffed lemurs [12], ring-tailed lemurs [26], Coquerel's sifakas [56], common marmosets [82], olive baboons [65]). Lastly, decanal is a key molecule of the citrus scent of seabirds (*e.g.*, crested auklet (*Aethia cristatella*) and its concentration seems to correlate with male social rank; 2-ethyl-1-hexanol appears to be associated with fragrances and has been found in ruffed lemur ano-genital scent-marks [12]; while D-limonene derives from leaves and flowers and is used by male insects to display successfully and attract females [85].

Overall, our chemical investigation of ano-genital odour secretions supports the hypothesis of sexual dimorphism in crowned lemur scent-marking and, more specifically, suggest that ano-genital marking might convey information about the sex and the quality of crowned lemurs. Short-range

odour signals may be important in species and sex recognition; particularly, female mammals may choose male partners using chemosensory cues to their quality, which reflects factors such as their social status, richness of diet, reproductive state, health and further environmental conditions (reviewed in [84]). Male crowned lemurs might then use ano-genital allomarking to mate guard adult reproductive females and thus advertise themselves as competitors to other males (male-male indirect competition; *i.e.* intra-sexual selection) or even as potential mates to females (female choice; *i.e.* inter-sexual selection).

5. Conclusions

In conclusion, this study supports the hypotheses [87] of sexual dimorphism and of more than one function served by scent-marking in crowned lemurs. Our findings suggest that scent marking could play a role in intergroup spacing and intrasexual competition in this lemur species. In particular, male head and wrist marking would serve a defensive territorial function, with head marking specialized for countermarking, while male ano-genital marking would play a role in socio-sexual communication as chemical mate-guarding of female conspecifics or in reinforcing social bonds between males and females; conversely, female ano-genital marking could be involved in resource defense. Furthermore, our semiochemical investigation suggests that ano-genital odour secretions might convey information about both the sex and the quality of individuals.

This study improves our understanding of the role played by scent-marking in lemur communication. However, since we focused on only four animals living in two captive family troops, this study must be considered a preliminary work for crowned lemurs. Future research should focus on a larger sample size and involve sampling of odour secretions released by male head and wrist marking. In addition, further studies could carry out more detailed analyses of the ratios of individual volatile components, as well as examine non-volatile components such as high-molecular-weight compounds which may extend the persistence of volatile signals in scent-marks [88-89]. Finally, it would be crucial to study the perception by recipients; for instance, looking for evidence of behavioral or physiological responses facilitated by scent-marks via bioassay tests [90].

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