1   **Title:** Sex differences in scent-marking in captive red-ruffed lemurs.

2   **Short running head:** Sex differences in scent-marking in red-ruffed lemurs.

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Abstract

Primate chemical communication remains underappreciated, as primates are considered to rely on other sensory modalities. However, various lines of evidence suggest that olfaction plays an important role in primate societies, including the conspicuous scent-marking behavior of many strepsirrhines and callitrichines. Although lemurs typically show scent-marking, little is known about this behavior in red-ruffed lemurs (*Varecia variegata rubra*). We combined behavioral observations and semiochemistry analyses to improve our understanding of scent-marking in two captive troops housed at Dudley and Twycross zoos (UK). We collected olfactory behavioral observations by focusing on two family troops (*N*=7) for 132 h. We investigated the volatile compounds of ano-genital scent-marks using solid-phase microextraction and gas chromatography-mass spectrometry and compared volatile chemical profiles with features of the signaller. Males scent-marked most frequently and predominantly in specific meaningful areas of the enclosure, while within females the occurrence of scent-marking was related to their age. We found behavioral sexual dimorphism, with male predominantly depositing secretions via neck and mandible glands and females via ano-genital glands. We identified a total of 32 volatile components of ano-genital gland secretion, including compounds that have already been found in other mammals as sex pheromones and cues to fitness, in ano-genital scent-marks spontaneously left on filter paper by adult females. Our findings suggest that red-ruffed lemurs might use scent-marking to convey information about sex and female age, with male neck-marking behavior playing defensive territorial functions and ano-genital marking related to socio-sexual communication.

Key-words: Communication, Signalling, Olfaction, Gas Chromatography–Mass Spectrometry, *Varecia variegata rubra*

Introduction

Communication plays a fundamental role within animal societies, especially for species displaying complex social systems. The ultimate goal of communication is to spread information that influences the behaviors of receivers (Seyfarth and Cheney 2003). Animals can use various sensory modalities to transfer their messages to other individuals. In particular, olfactory communication is exhibited by several mammal species (reviewed in Scordato and Drea 2007), such as rodents (*e.g.*, Hurst *et al* 1998; Roberts 2007), but also by reptiles (*e.g.*, Muller-Schwarze 2006) and birds (*e.g.*, Leclaire *et al* 2017). Odor secretions are directly linked to the physiological conditions of senders (Harris *et al* 2018) and can be costly to produce (Scordato and Drea 2007), thus they are expected to deliver a more honest signal compared to other forms of communication (Hasson 1997).

Primates have traditionally been considered to be "microsmatic", relying more upon other sensory modalities than olfaction (Dulac and Torello 2003). Since vision and acoustics are considered to be the main sensory modalities in most primate species little is known about the chemical signals used by non-human primates (Walker 1998). However, several studies support the hypothesis that chemical communication is crucial also for primates (*e.g.*, Porter and Moore 1971; Geissman and Hulftegger 1994; Wedekind *et al* 1995; Wedekind and Füri 1997; Smith *et al* 2001; Jacob *et al* 2002; Hayes *et al* 2004, 2006; Heymann 2006; Knapp *et al* 2006; Laidre 2009; Smith 2006; Scordato *et al* 2007; Setchell *et al* 2010, 2011; Vaglio *et al* 2016). Particularly, it is established that some primates also rely heavily on olfaction in addition to vision and auditory senses; for instance, this is the case of squirrel monkeys (Laska *et al* 2000) and several lemurs (Gould and Overdorff, 2002; Scordato and Drea 2007). Strepsirrhines have retained their olfactory complexity (reviewed in Hayes *et al* 2006) due to morphological constraints that limit the visual signals produced by senders (Scordato and Drea 2007).

69   Lemur behavioral repertoire comprises both olfactory investigative and scent-marking

70   behaviors. Lemurs show both direct and indirect olfactory investigative behaviors (Drea

71   2015); direct investigations may include behaviors such as sniffing and/or licking a

72   conspecific's skin (palms, soles, eyelids, or nostrils) or genitals, and self-sniffing genitals,

73   while indirect behaviors consist of sniffing and/or licking scent-marks deposited by the

74   signaller. Scent-marking behavior is shown by several terrestrial vertebrates, including

75   mammal and reptile species (Müller-Schwarze 2006; Roberts 2007). Scent-marks may

76   include species-wide pheromones (*i.e.*, chemical substances released by an animal or insect

77   which can affect a conspecific individual; for futher details see Vaglio *et al* 2018) as well as

78   highly individual odors. Scent-marking is a very effective form of communication within

79   habitats that make difficult the detection of visual and auditory signals (Gould and Overdorff

80   2002), which is the case with forests inhabited by lemurs (Sussman *et al* 2003). In particular,

81   this behavior is reasonably common in lemurs and New World monkeys – among which may

82   play several functions, including the reproductive suppression of subordinate females,

83   advertisement of individual "quality", preparing males to assist in the delivery and care of

84   newborn infants, and territorial defence (*e.g.*, Gould and Overdorff 2002; Pochron *et al* 2005;

85   Heyman 2006) – while is less commonly reported in Old World monkeys and apes (*e.g.*,

86   Freeman *et al* 2012). Especially, among strepsirrhines, social complexity may have selected

87   for olfactory complexity in lemurs (delBarco-Trillo *et al* 2012).

88   Mammals have a common pattern of scent-marking: glandular secretions, if not feces or

89   urine, are placed at meaningful places such as along paths and territorial boundaries (Gosling

90   and Roberts 2001). Scent-glands have been observed in various lemur species, including all

91   *Eulemur* species (delBarco-Trillo *et al* 2012), ring-tailed lemurs (Scordato and Drea 2007),

92   red-bellied lemurs (Gould and Overdorff 2002), red-fronted lemurs (Hayes *et al* 2006),

93   Milne-Edward's sifakas (Hayes *et al* 2004), black-and-white and red-ruffed lemurs (Gould

and Overdorff 2002). In particular, red-ruffed lemurs have multiple scent-glands (Gould and Overdorff 2002), composed of neck and mandible glands (male), and anogenital glands (male and female) (Pereira *et al* 1988); indicating that olfactory communication should be significant for this species (Elisa *et al* 2004).

The red-ruffed lemur is a large, frugivorous lemur species (Vasey 2006), which inhabit the residual primary forests of the Masoala Peninsula (Andriaholinirina *et al* 2014). Red-ruffed lemurs have a variable social system; in smaller home ranges their group size is usually between 2-5 individuals, whereas larger home ranges have been known to support between 18-32 individuals (Rigamonti 1993). Although red-ruffed lemur communities are not cohesive units, the home range is communally defended. In addition, only females participate in communal home range defense against females from other groups, which includes agonistic behaviors such as chasing, scent-marking, vocalizing, and even physical contact with members of neighboring communities (Vasey 2005; 2007). Females are dominant to males, winning almost all agonistic encounters with them and rarely showing submissive behavior towards them (Raps and White 1995; Meyer *et al* 1999). Communication is commonly observed as vocalisations, emitting species-specific calls which serve several functions and are transmittable between groups (Macedonia and Taylor 1985); however, also chemical communication is thought to play a crucial role in group dynamics (Elisa *et al* 2004).

The overarching aim of this study is to improve our understanding of the role played by chemical communication, particularly focusing on scent-marking behavior, in red-ruffed lemurs. We predict that red-ruffed lemurs advertise information about their sex, age and rank by using scent-marking. We also anticipate that this study may contribute to further exploring the connection between functional and mechanistic levels of lemur scent-marking (Charpenter *et al* 2010).

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Materials and Methods

121

Subjects and Housing

We studied two captive troops of red-ruffed lemurs (*n*=7) housed at Dudley and Twycross zoos (UK). The troop housed at Dudley Zoological Gardens consisted of two related (brothers) adult males (13 years old) and one unrelated adult female (12 years old). The troop housed at Twycross Zoo consisted of one adult male (11 years old), one adult female (12 years old) and their offspring (two 1.5 years old females). Red-ruffed lemurs are considered sexually mature at 2 years old, with first conception approximately one year later (Vasey 2007). Adult females were contracepted, and all individuals in non-breeding season (*i.e.* regarding red-ruffed lemurs in captivity in the Northern Hemisphere breeding usually occurs in December-January with births in April-May; Brockman *et al* 1987).

We carried out behavioral observations and odor sampling from September to November 2016 (Twycross Zoo) and from July to September 2018 (Dudley Zoological Gardens). In both institutions, the troops lived in an indoor enclosure (heated to 28°C) with access to an outdoor enclosure ('visitor walktrough' enclosures).

136

Ethics Statement

This study followed the guidelines for the care and use of captive animals in the UK, involving non-invasive methods for obtaining both behavioral data and odor samples from red-ruffed lemurs. Moreover, the study was conducted in compliance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

142 and approved by the Life Sciences Ethics committee at the University of Wolverhampton

143 (UK) and the Ethics committees at Dudley Zoological Gardens and Twycross Zoo (UK).

144

145   *Behavioral Data Collection and Analysis*

146   We collected behavioral data by instantaneous scan sampling (Altmann 1974), with

147 behaviors recorded at 30-sec intervals over the duration of an hour in three time periods (two

148 during the morning, and one during the afternoon), two days per week, over three months.

149 Alongside the scan sampling we also used *ad libitum* sampling for recording olfactory

150 behaviors **(Table I)**, including both scent-marking (ano-genital marking, neck-marking – that

151 is scent-marking via neck and mandible glands) behavior and locations (comprising of

152 "hatches", "tree branch", "indoor enclosure", "wooden hut", and "climbing frame"). We

153 recorded a total of 132 hours of observations throughout the study period, including 360 scan

154 samples each sampling day on the entire group.

155   We investigated the relationships between individuals and scent-marking behavior in

156 relation to sex and age of senders. We also investigated the role played by different types of

157 scent-marking behavior, and locations of scent-marks within the enclosure. We tested all

158 variables through Shapiro-Wilk test for normality. As data were not normally distributed, we

159 performed non-parametric Kruskall-Walis tests followed by pairwise Mann-Whitney U *post-*

160 *hoc* tests. All tests were carried out using SPSS v.23, and a significance level of $P<0.05$ was

161 applied.

162

163   [*insert Table I here*]

164

165 *Odor Sampling and Analysis*

166   We collected odor secretions spontaneously released via scent-marking by red-ruffed

167   lemurs on brand-new filter paper fixed on hatches, climbing equipment, and tree trunks and

168   branches **(Figure I)**. Unfortunately, we were not able to collect any odor sample from males,

169   while we collected scent-marks deriving from ano-genital marking by all the females (14

170   samples, 3-4 replicates per individual). In addition, we placed control filter paper in the

171   environment to control for the contact with wood (where there may be chemical compounds

172   deriving from the wood, but also algae, microorganisms, etc.) and we exposed control filter

173   paper also to the air during sampling in order to detect any chemical compounds which did

174   not derive from the red-ruffed lemurs. We collected odor samples immediately after scent-

175   mark deposition by red-ruffed lemurs in the outdoor enclosure. We placed all samples and

176   controls into brand-new sterile vials (Supelco) and immediately stored them at -20°C. We

177   used 10-ml screw-capped clear glass vials (thread: 18O.D. 22.5-mm x H 46-mm) closed by

178   teflon-faced rubber septa and seals (1.3-mm thick).

179   We conducted laboratory analyses at the Rosalind Franklin Science Centre,

180   University of Wolverhampton (UK). We investigated the volatile components of odor

181   secretions using established solid-phase microextraction (SPME) and gas chromatography-

182   mass spectrometry (GC-MS) and applying the same methods used in our previous work on

183   mandrill odor signals (Setchell *et al* 2010; Vaglio *et al* 2016).

184   We introduced a 65-µm polydimethylsiloxane/divinylbenzene SPME syringe needle

185   through the vial septum and then we exposed the fibre to the headspace above the sample in

186   the vial for 15 minutes at 40°C. We analysed the adsorbed volatile analytes of all samples by

187   using a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) EI, 70 eV,

188   coupled directly to a 7890B gas chromatograph (Agilent Technologies, Santa Clara, CA,

189   USA) equipped with a fused silica HP5-MS UI capillary column (Agilent Technologies,

190   Santa Clara, CA, USA) 30 m x 0.25 mm crossbonded 5%-phenyl-95%-dimethylpolysiloxane,

191   film thickness 0.25 µm. We maintained the injector and transfer line temperatures at 270°C

192   and 280°C, respectively. We made injections in splitless mode (purge valve opened after 1

193   min) with a constant flow of helium carrier gas of 1 mL min-1. We started the oven

194   temperature program at 45°C for 2 min, then raised it by 4°C min-1 to 170°C, and finally by

195   20°C min-1 to 300°C.

196   We assessed potential contamination due to the lab environment through blank

197   analyses of an empty 10-mL vial (Supelco) following the same procedure as for the samples.

198   In addition, we conditioned the fibre at 260 °C pre- and post- injection, for 5 and 20 mins

199   respectively in order to avoid any possible carry-over effects.

200   We tentatively identified eluted compounds by comparing the experimental spectra

201   with the spectra provided by the mass-spectral library in ChemStation (Agilent Technologies,

202   Santa Clara, CA, USA) and NIST (National Institute of Standards and Technology) Database,

203   version MSD F.01.01.2317 (Agilent Technologies, Santa Clara, CA, USA). We accepted a

204   putative identification when the minimum matching factor was higher than 80%. If more than

205   one compound was a good match for the same GC peak then we considered the

206   chromatographic retention time and compared it with the retention time reported in the

207   literature for the same chromatographic column type (El-Sayed 2016) in order to minimize

208   the chance of misidentification. We created a data matrix using the peak area relative to each

209   identified compound by using the integrated signal of the deconvoluted total ion current

210   (TIC). We analysed all samples in a short period of time (approximately 24 hours) to

211   minimize interassay variability. We removed all the contaminants (*i.e.*, any compounds that

212   appeared in the 'environmental controls' and 'lab blanks') from the scent-mark results.

213

214   [*Insert Figure I here: Filter paper attachments*]

215

216   **Results**

217

218   *Behavioral Observations*

219   During the study period olfactory behaviors were exhibited predominantly by males

220   (40.00%), followed by adult females (34.48%) and subadult females (25.52%). These

221   behaviors included both scent-marking (ano-genital marking and neck-marking) and

222   investigative behaviors (sniffing and/or licking an area within the enclosure, sniffing and/or

223   licking a conspecific, self-licking of the ano-genital glands). Scent-marks were most

224   commonly deposited by males (51.55%), followed by adult females (26.80%) and subadult

225   females (21.65%) **(Figure II)**, although differences were not significant between the sexes

226   (U=137.5; p=0.688).

227

228   [*Insert Figure II here: Frequency of marks in relation to sex and age*]

229

230   We observed males (N=3) scent-marking significantly more via neck glands rather

231   than via ano-genital glands (U=12.5; p<0.001). Females (N=4) displayed the opposite

232   behavioral pattern; showing a significantly higher frequency of ano-genital marking rather

233   than neck marking (U=41.5; p=0.022). We found significant differences in neck-marking

234   behavior between individuals, and particularly between males and females (N=7; U=12.5;

235   p=0.003).

236   We found a significant difference (N=7; Z =-5.675, p<0.001) in scent-marking

237   behaviors between inside and outside locations, with 81.73% of scent-marks occurring in the

10

238   outdoor enclosure. Moreover, scent-marks were most commonly deposited near, or upon, the

239   hatches leading to the indoor and off-show enclosure (18.27%). However, scent-marks were

240   also deposited on tree branches next to the path of the walk-through, all climbing frames in

241   the walk-through, a hunt providing shelter within the walk-throug, and upon furniture in the

242   indoor enclosure.

243   We also found a preference of location when considering the type of scent-marking

244   performed; "hatches" were used most commonly overall for neck-marking (81.82%)

245   compared to ano-genital marking (18.18%), whereas "climbing frame" was used more for

246   ano-genital marking (71.43%) than neck-marking (28.57%).

247   We found significant differences in areas used for neck-marking, most commonly

248   upon "hatches" (X^2=23.152; p<0.001), and upon "tree branches" (X^2=9.456; p=0.009).

249   Deposition of neck scent-marks upon "hatches" was significantly different between males

250   and females (U=04.5; p<0.001). Neck-marking on "tree branches" showed a difference

251   between males and females (U=30.0; p=0.029), and between adult and subadult females

252   (U=30.0; p=0.029).

253   We found significant differences in areas used for ano-genital marking, with most

254   frequent occurrences upon "hatches" (X^2=11.748; p=0.003) and "climbing frame"

255   (X^2=13.119; p<0.001). Deposition of ano-genital marks upon "hatches" was significantly

256   different between adult and subadult females (U=84.0; p=0.037). Ano-genital marks upon

257   "climbing frames" were also significantly different between adult and subadult females

258   (U=35.0; p<0.001).

259

260   *Odor secretions*

261   We identified a total of 32 individual compounds from the analysis of 14 filter paper samples

262   of female ano-genital secretions. These compounds included a range of naturally occurring

263   odorous volatile compounds such as hydrocarbons, terpenes, terpene alcohols and ketones.

264   Tentative identifications are listed in **table II**, while typical chromatograms (1 from the blank

265   control and 1 from a female lemur ano-genital scent-mark) are shown in **figure III**. There

266   was variation in the number and abundance of the compounds observed from sample to

267   sample across different individuals. However, six compounds (benzaldehyde, 2-ethyl-1-

268   hexanol, p-cresol, cis-p-mentha-2,8-dien-1-ol, 2-pinen-4-one, pentadecane) were present in

269   all samples. We were not able to analyse the ratios of individual components in order to

270   compare the volatile profiles with features of the signaller (for instance, adult *vs* subadult

271   females) due to the small amount of filter paper samples.

272

273   [*Insert Table II here. Secretion compounds*]

274   [*Insert Figure III here. Chromatographs*]

275

276   **Discussion**

277   Primates rely on olfactory communication in several contexts, including foraging,

278   territorial defense, individual and family recognition, mate choice and mother-offspring

279   bonding (Zeller 1987). Although very little is known about Old World primates, research has

280   been accumulating on chemical communication in strephsirrhines and New World monkeys;

281   particularly, semiochemical data are accessible for few non-human primate species, including

282   various strepsirrhines [galago (Crewe et al 1979); lemurs (delBarco-Trillo *et al* 2011, 2012;

283   Hayes *et al* 2004, 2006; Palagi and Dapporto 2006; Scordato *et al* 2007), owl monkeys

284   (Macdonald *et al* 2008), marmosets and tamarins (Epple *et al* 1993; Smith *et al* 2001),

285 macaques (Curtis *et al* 1971) and mandrills (Setchell *et al* 2010, 2011; Vaglio *et al* 2016)]. In

286 this study we focused on scent-marking behavior, via both behavioral and chemical

287 approaches, in two troops of zoo-managed red-ruffed lemurs.

288   Red-ruffed lemurs, as the well-studied ring-tailed lemurs, are characterised by a

289 female-dominated society. In ring-tailed lemurs males scent-mark more than females

290 (Pochron *et al* 2005) and female age is positively correlated to scent-marking frequency

291 (Kappeler 1990; Gould and Overdorff 2002; Pochron *et al* 2005). Similarly, in our study,

292 males scent-marked significantly more than any female and adult females showed the highest

293 frequency of scent-marking within females. Therefore, our findings support the prediction

294 that scent-marking would differ between individuals based on their sex, as found in other

295 lemur species such as sifakas (Pochron et al. 2005), and age, as found in ring-tailed lemurs

296 (Kappeler 1990; Gould and Overdorff 2002; Pochron et al. 2005).

297   Neck-marking was exhibited by all study subjects, but significantly more by males. In

298 contrast, females exhibited ano-genital scent-marking significantly more than males. This

299 supports the hypothesis of sexual dimorphism in red-ruffed lemur scent-marking, as already

300 observed by Vasey (2003). The preferences shown by males for neck-marking "hatches" and

301 ano-genital marking "tree branches", and by adult females for ano-genital marking "climbing

302 frames", confirm behavioral sexual dimorphism. These observable preferences also suggest

303 that scent-marking behaviors might play different roles in males and females, as observed in

304 other primates, including ring-tailed lemurs (Scordato and Drea 2007), black-and-white

305 ruffed lemurs (Pereira *et al* 1988) and mandrills (Vaglio *et al* 2016).

306   Our results also support the hypothesis that scent-marking might have a territorial

307 function in this species (Pereira *et al* 1988). In particular, males scent-marked mostly specific

308 meaningful places, by using neck-marking for hatches (small openings allowing access from

309   outdoor to indoor enclosure; *i.e.*, potential role of territorial defense) and ano-genital marking

310   for tree branches and climbing equipment (areas of frequent transit by individuals; *i.e.*,

311   potential role of social communication). This also implies that scent-marks released via neck-

312   marking and ano-genital marking might communicate different messages to the receivers by

313   conveying information about distinct features of the senders. Previous studies have indicated

314   information regarding sex to be conveyed in scent-marks from ring-tailed lemurs (Hayes *et al*

315   2004, Scordato *et al* 2007), but absent in odorants from sifakas (Hayes *et al* 2004, 2006).

316   Although scent-marking behavior is observable, it is challenging to decipher the

317   message which is chemically communicated. Therefore, the chemical investigation of odor

318   secretions released by scent-marking is critical to understand the message transferred by this

319   behavior. Since we used spontaneously released scent-marks, we were able to investigate

320   odor secretions released by scent-marking and thus corresponding to the exact message sent

321   by red-ruffed lemurs.

322   A total of 32 compounds were tentatively identified within the ano-genital secretions

323   released by female study subjects (excluding environmental and lab contaminants as well as

324   co-eluted compounds). This low amount of volatile compounds in comparison to other

325   female lemur ano-genital marks (for example, ring-tailed lemurs and sifakas; Hayes *et al*

326   2004, Scordato *et al* 2007) might be explained by the fact that breeding *versus* non-breeding

327   season (Scordato & Drea 2007) and chemical contraception (Crawford *et al* 2011) can have

328   significant impacts on semiochemical signals in lemurs. For instance, in ring-tailed lemurs

329   (Crawford *et al* 2011) genital odorants of adult contracepted females were proved altered,

330   including decreased richness, modified relative abundances, and minimized individual

331   chemical distinctiveness of their volatile chemical profiles.

332   Volatile hydrocarbons have previously been identified in odorants deriving from ring-

333   tailed lemurs and sifakas (Hayes *et al.* 2004; Scordato *et al.* 2007) as well as from Old World

334   monkeys such as mandrills (Setchell *et al* 2010; Vaglio *et al* 2016) and olive baboons (Vaglio

335   *et al* in preparation). In particular, high-molecular weight volatile hydrocarbons might act as

336   a fixative which slows the release of more volatile compounds, as suggested for major

337   urinary proteins in mice (Green 2016; Hurst *et al* 1998). The compounds benzaldehyde, p-

338   cresol (also known as p-methylphenol), hexanal and geranylacetone are commonly

339   encountered in mammal scent markings (*e.g.*, lions, wild dogs, wolves, mice, red foxes)

340   (Osada *et al* 2015; Roberts *et al* 2010; Soso & Koziel 2016). The compound benzaldehyde

341   has already been found in gland secretions released by marmosets (Smith *et al* 2001), and

342   functions as sex pheromone in other mammals (reviewed in El-Sayed 2016) and also as cue

343   to genetic quality (reviewed in Wyatt 2014) in other vertebrates (*e.g.*, in the crested auklet, a

344   seabird with citrus scent based on decanal and octanal produced by both sexes during the

345   breeding season, concentration correlates with rank in males). Thus, benzaldehyde might

346   serve as pheromone and play a role in signalling individual quality also in red-ruffed lemurs.

347   In addition, ethyl-phenol occurs in rat urine as mate attraction signal and also in beaver urine

348   as part of a multicomponent signal of range occupation (reviewed in Apps *et al* 2015). The

349   compound 2-pine-4-one (also known as verbenone) is a bark beetle antiaggregation

350   pheromone (Lindgren & Miller 2002), which has similarly been found in other insects (*i.e.*,

351   bees and butterflies) and is also naturally occurring in plants (reviewed in Bakthavatsalam

352   2016). 2-ethyl-1-hexanol and cis-p-metha-2,8-dien-1-ol both appear to be associated with

353   fragrancies. Finally, other compounds, such as α-pinene, are known to derive from plants;

354   therefore, they might be a by-product and potentially vary with the environmental context but

355   could also contribute to the message communicated by red-ruffed lemurs through scent-

356   marking (for instance, convey information about group identity).

357

Conclusions

In conclusion, the present study supports the hypotheses (Smith *et al* 2015) of sexual dimorphism and of more than one function served by scent-marking in red-ruffed lemurs. We suggest that scent marking could serve a function in intergroup spacing and intrasexual competition for both sexes, as might be expected in a female-dominant species.

In particular, male neck-marking might have a defensive territorial function while ano-genital marking might play a role in socio-sexual communication in this lemur species. Furthermore, our findings suggest that odor secretions released via ano-genital-marking might convey information about the age of female signallers. Additionally, the similarity of red-ruffed lemur's volatile chemical profiles to those found in other vertebrates would support our previous suggestion (Setchell *et al* 2010) that non-human primates are not as microsmatic as traditionally considered.

Since this study is based on seven animals living in two captive family troops it can only be considered a preliminary work for the red-ruffed lemur species. Future research work should focus on a larger sample size, record behaviors consistently throughout the day, and investigate the odor secretions released by adult non-contracepted females and also by male scent-marks. In addition, it would be crucial to study the perception by the recipient, for instance looking for evidence of behavioral or physiological responses facilitated by scent-marks via bioassay tests (Wyatt 2014). Also, more detailed analysis of the ratios of individual components could form the basis of further studies. Finally, although we focused on the volatile profile of red-ruffed lemur odor, we also recognize the significance of non-volatile components of odor secretions, as high-molecular weight compounds may extend the persistence of volatile signals in scent-marks (Alborne 1984; Belcher *et al* 1990; Hurst and

381 Beynon 2004).

382

398

399 **Conflicts of interest**

400   The authors declare that they have no conflict of interest.

401

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Table I. Ethogram (based on Scordato and Drea, 2007, and Vaglio *et al* 2016, modified).

Behavior	Description
Scent-Marking; Neck / Mandible	Individual rubs neck region against substrate or upon an item within the enclosure
Scent-Marking; Ano-genital	Individual rubs genital region against substrate or upon an item within the enclosure
Sniffing / Licking; Environment	Individual deliberately places nostrils or tongue within 3cm from substrate or an item within the enclosure and sniffs/licks
Sniffing / Licking; Conspecific	Individual deliberately places nostrils or tongue within 3cm from a conspecific and sniffs/licks
Self-Licking	Individual uses tongue to lick an area near a scent gland on their own body

Table 2 – Volatile compounds present in filter paper samples from female lemur ano-genital secretions identified tentatively using ChemStation and NIST mass spectral databases (v. MSD F.0101.2317). Compounds in bold font were found in all samples.

Retention Time (mins)	Tentative Compound ID	Molecular Weight
3.906	Hexanal	100
6.057	5-methyl-3-hexanone	114
7.413	Alpha-pinene	136
8.077	1-isopropyl-4-methylenebicyclo[3.1.0]hex-2-one	134
8.268	**Benzaldehyde**	106
8.623	3,7,7-trimethyl-1,3,5-cycloheptatriene	134
9.096	Phenol	94
9.269	6-methoxy-5-hepten-2-one	126
10.720	**2-ethyl-1-hexanol**	130
12.362	**p-Cresol**	108
12.553	cis-Verbenol	152
13.385	**cis-p-Mentha-2,8-dien-1-ol**	152
14.104	1,7,7-Trimethylbicyclo[2.2.1]hepta-2-one	152
14.536	L-Pinocarveol	152
14.791	trans-Verbenol	152
15.605	p-Ethyl-phenol	122
15.928	Terpinen-4-ol	154
16.415	Alpha-Terpineol	154
16.615	Myrtenol	152
17.047	**2-Pinen-4-one**	150
18.252	Carvone	150
19.217	p-Mentha-1,8-dien-3-one	150
23.283	4,7,7-Trimethylbicyclo[4.1.0]hept-3-ene-2-one	150
23.443	Tetradecane	198
25.094	Geranylacetone	194
25.899	Isomethylionone	206
26.513	**Pentadecane**	212
30.871	2,6,10-Trimethylpentadecane	254
32.208	Heptadecane	240

32.372	2,6,10-Trimethylhexadecane	268
34.446	n-Tetracosane	338
34.591	2,6,10,14-Tetramethylhexadecane	282

Fig. I. Sterile filter paper attached to popular scent-marking locations using plastic cable ties. (a) hatches, (b) climbing frame equipment, (c) tree branches.

Fig. II. Percentage of occurrence for total scent-marks associated to classes of authors. Males scent-marked the most (51.55%), followed by adult females (26.80%) and subadult females (21.65%), however differences were not statistically significant.
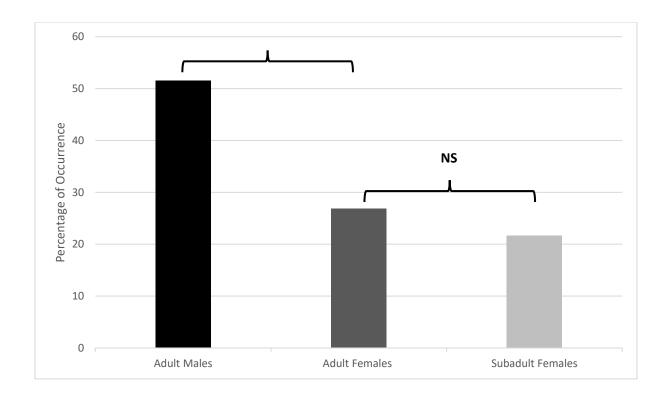
Fig III. Example chromatograms from (a) one adult female ano-genital odour secretions, showing contaminants and meanigful biological compounds; and (b) the control sample, showing contaminants. Red arrows indicate the six meanigful biological compounds which were found in all samples: (a) benzaldehyde; (b) 2-ethyl-1-hexanol; (c) p-cresol; (d) cis-p-mentha-2,8-dien-1-ol; (e) 2-pinen-4-one; (f) pentadecane.