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Sniffing Behaviours in Guenons

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Abstract
In Old World monkeys (OWM), the olfactory sense is thought to be less important than in other primate taxa. However, during the last decade experimental studies have shown that OWM possess much better olfactory capabilities than suspected. Here, we investigate for the very first time sniffing behaviours in three guenon species (Diana, de Brazza's and owl-faced monkeys) held in the Leipzig Zoo, Germany. We recorded frequencies and contexts of sniffing. The sniff index was used to allow comparisons across species. It was found that individuals sniffed 6.12 ± 7.69 times per hour. Most sniffing occurred in food-related contexts. Furthermore, Diana monkeys sniffed less often than de Brazza's and owl-faced monkeys. Thus, natural selection may drive the differences in using the olfactory sense in closely related primate species.

Introduction

During primate evolution, the ratio between total brain volume and olfactory bulb volume decreased from Strepsirhini (wet-nosed primates) to Haplorhini (dry-nosed primates) [Stephan et al., 1970; Barton, 1998; Meisami and Bhatnagar, 1998] and most of the living Old World monkeys (OWM) do not possess scent glands [Stoddart, 1990; Feistner, 1991]. Although the anatomical evidence for a relative reduction in olfactory structures amongst OWM is undeniable, the inference that this indicates a functional reduction in olfactory abilities in the entire suborder is currently debated [Heymann, 2006].

Sniffing is the behaviour most obviously related to olfaction and the perception of chemical signals. While olfactory communication has been extensively studied in

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Strepsirhini and New World monkeys [Laska and Hudson, 1995; Bolen and Green, 1997; Scordato and Drea, 2007; Sündermann et al., 2008; Charpentier et al., 2010], studies in OWM are rare. Most have concentrated on behavioural experiments to determine olfactory threshold levels, to test the ability for individual or kin recognition or to distinguish food items solely by olfactory cues [Laska et al., 2000; Clarke et al., 2009; Laidre, 2009; Setchell et al., 2010; Freeman et al., 2012; Hepper and Wells, 2012].

Frequencies or contexts of sniffing behaviours have been studied in species that belong to the tribe of the Papionini and in one great ape. Male chacma baboons *(Papio ursinus)* sniff at female genitals during mate choice [Clarke et al., 2009], mandrills *(Mandrillus sphinx)*, drills *(M. leucophaeus)* and olive baboons *(P. anubis)* sniff during foraging at the mouth of conspecifics [Laidre, 2009], chimpanzees *(Pan troglodytes)* sniff in various social and food-related contexts [Matsumoto-Oda et al., 2007], and the males sniff on the substrate while patrolling along the borders of their territories [Mitani and Watts, 2005; Herbinger et al., 2009].

To enhance our knowledge about the importance of the olfactory sense in OWM, we studied three species of the genus *Cercopithecus* – namely, Diana monkeys *(C. diana)*, de Brazza's monkeys *(C. neglectus)* and owl-faced monkeys *(C. hamlyni)*. Guenons have been residing in Africa since the Pliocene (from ca. 5 to 2 million years ago). Their main radiation occurred within the last 1 million years and they are suspected still to be in an active stage of speciation [Glenn and Cords, 2002]. Currently, the number of known species varies from 26 to 36 [Butynski, 2002] depending on the taxonomic classification used. Although most species seem to form small one-male units with 2–4 adult females, the genus also exhibits strikingly different forms of social organisation as an adaptation to the various habitats in which they reside [Fleagle, 1998]. When sexual maturity is achieved, usually females remain philopatric, while males emigrate from the natal troop to live solitarily until they take over their own group of females [Zuberbühler, 2002].

Diana monkeys inhabit the upper strata of West African rainforests. In their natural habitat they form highly social large multi-male multi-female groups comprising up to 50 individuals. As a strategy against predators such as crowned eagles *(Stephanoaetus coronatus)*, leopards *(Panthera pardus)*, chimpanzees and humans, they associate sympatrically with other monkey species [Höner et al., 1997; Noë and Bshary, 1997; Buzzard, 2010]. Diana monkeys further are known to possess a large repertoire of vocalisations [Zuberbühler, 2000; Arnold et al., 2008].

De Brazza's monkeys exhibit the largest sexual dimorphism of any guenon species. They reside in central Africa and inhabit temporarily flooded forests, swampy and gallery forests and the lower and middle strata of tropical rainforests [Leutenegger and Lubach, 1987; Geissmann, 2003; King, 2008]. Although they are regarded as the only socially monogamous guenon species [Bouchet et al., 2012], in most habitats they form small multi-male multi-female groups with 6–10 individuals [Leutenegger and Lubach, 1987; King, 2008].

Owl-faced monkeys are the most inconspicuous of the three species in both behaviour and appearance. They prefer bamboo forests as a habitat in the eastern Democratic Republic of the Congo and Rwanda [Rowe, 1996; Hart et al., 2012]. They are exceedingly rare, and almost no information on their behaviours, ecology and social organisation is available. Fuentes [1999] argued that the species forms small groups of 10 members or less, with 1 or 2 males and multiple females.

Table 1. Age and sex composition of the study groups (n = 17 individuals)

	Number of individuals	Adults		Non-adults	
		males	females	males	females
Diana monkeys	4	0	3	0	1
De Brazza's monkeys	8	1	3	4	0
Owl-faced monkeys	5	1	3	1	0

To date, there is no information on sniffing behaviours in any of the 3 study species. Here, we present the first investigation into the frequencies and contexts of sniffing in guenons. Since de Brazza's and owl-faced monkeys scent-mark using a secretion from a sternal gland [Gautier and Gautier, 1977; Geissmann, 1987; Loireau and Gautier-Hion, 1988], we suspect them to rely, in general, more on the olfactory sense. Therefore, they also should sniff more frequently than the Diana monkeys.

Methods

Study Site and Study Groups

Most guenons' natural habitats are the middle and higher strata of tropical rainforests (see Introduction) where direct observations on sniffing are almost impossible to gain. Therefore, we decided to study guenons living in the Leipzig Zoo, Germany. The 17 individuals (table 1) lived in three groups in enclosures of 24–28.5 m^3. Non-adults (infants and juveniles) were defined as such after Leutenegger and Lubach [1987]. All were weaned but still co-housed with their mothers. Fresh food (fruit, vegetables, bread and corn) was provided 5 times a day and water was available ad libitum.

Data Collection

The data presented here were collected during spring 2011 when the outdoor enclosures were still closed. We observed each group for an equal number of hours during the morning and afternoon. Since the entire indoor enclosure could be viewed, continuous observations of all individuals per group were possible. In total, the groups were observed for 90 h (Diana monkeys: 30 h; de Brazza's monkeys: 30 h; owl-faced monkeys: 30 h). Sniffing behaviours of all the members of a group were recorded simultaneously via all-occurrence sampling [Martin and Bateson, 2007]. We defined an ethogram of the recorded sniffing behaviours and three different contexts (food, social and other) when they occurred (table 2). During grooming or when mothers carry infants the nose naturally approaches the other monkeys closer than 3 cm, therefore such periods were excluded from data collection.

Data Analysis

To allow a comparison between groups (i.e., that represent species here) of different sizes, we calculated sniff indices (SI). The total number of sniffs recorded per observation session was summed up and divided by the hours of observation and the number of individuals per group [Matsumoto-Oda et al., 2007]. As the data were not normally distributed, we used non-parametric statistics to calculate exact probability tests [Mundry and Fischer, 1998]. We used Kruskal-Wallis tests to test differences in sniffing between and within species as well as post hoc Mann-Whitney U (MWU) tests (referred to in R version 2.13.2 as 'Wilcoxon test') to examine the source of any significant result. To account for multiple testing, we set a Bonferroni-corrected α value at 0.017 [Sachs, 2004].

Table 2. Definitions of sniffing behaviours and contexts in captive guenons

Categories of sniffing	
Sniff	Nose moved to within ≤3 cm of the object
Grab-sniff	Object taken by hand and moved to within ≤3 cm of the nose
Touch-sniff	Object touched by hand, and only the hand moved to within ≤3 cm of the nose
Contexts when sniffing occurs	
Food	Sniffing food items
Social	Sniffing conspecifics or faeces and urine of conspecifics
Other	Sniffing own body (self-check) or inedible items

Table 3. Absolute numbers of recorded sniffs in the three study groups and contexts (total observation time: 90 h)

	Food	Social	Other	Total
Diana monkeys	23	12	19	54
De Brazza's monkeys	127	44	52	223
Owl-faced monkeys	155	10	112	277
Total	305	66	183	554

Results

Sniffing Behaviours across Species

In total, we recorded 554 sniffs (table 3) during 90 h (SI ± SD = 6.12 ± 7.69 times per hour). Due to the small sample size, absolute numbers of sniffs were not compared with the help of statistics. However, there seems a tendency that Diana monkeys sniffed less often than the other two species. Furthermore, most sniffs occurred in a food-related context, but the three species also sniffed in social and other contexts.

SI across Age and Sex Classes, Species and Contexts

Here we descriptively present SI that exemplify the frequencies of sniffing in form of sniffs per hour. In all three species, non-adults seem to sniff more frequently than adult females. The highest SI, however, was shown by the adult male owl-faced monkey, who sniffed approximately 3 times per hour (SI = 3.07), while the 3 female Diana monkeys sniffed a mean of 0.23 times per hour (SI = 0.23; table 4).

To test for differences across species and contexts, SI were compared using statistics. Figure 1 illustrates differences across species and between contexts for each of the three species. The SI significantly differed across species (Kruskal-Wallis test: $\chi^2 = 21.77$, d.f. = 2, $p < 0.001$). Diana monkeys sniffed less often than de Brazza's (MWU test: W = 705, $p < 0.001$) and owl-faced monkeys (MWU test: W = 165, $p < 0.001$), while de Brazza's and owl-faced monkeys did not differ from each other in their SI (MWU test: W = 417, $p > 0.017$). Diana monkeys showed no difference in sniffing between contexts (Kruskal-Wallis test: $\chi^2 = 1.79$, d.f. = 2, $p > 0.017$). De Braz-

Table 4. Mean SI ± SD across species and different age and sex classes

	Males	Females	Non-adults
Diana monkeys	0	0.23±0.46	0.90±1.69
De Brazza's monkeys	1.17±2.30	1.11±1.29	2.81±3.27
Owl-faced monkeys	3.07±7.77	1.37±1.47	2.07±2.59

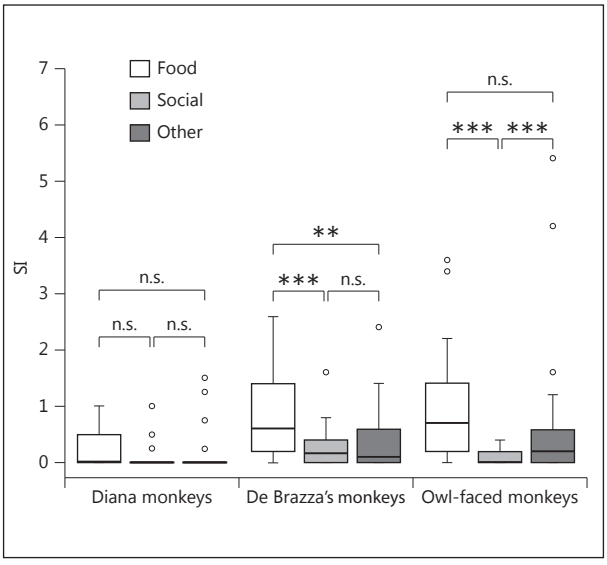

Fig. 1. SI in different contexts (food, social and other). Bold lines illustrate the median, the areas above the lines show the 3rd quartile and the areas below the lines show the 1st quartile of the boxplots. The vertical bars range from the minimal to the maximal values. Dots above the boxplots represent outliers. ** $p < 0.01$; *** $p < 0.001$; n.s. = non-significant $p > 0.017$ (Bonferroni-corrected α value).

za's monkeys (Kruskal-Wallis test: $\chi^2 = 12.67$, d.f. = 2, $p < 0.017$) sniffed more frequently in food-related than in social (MWU test: $W = 671$, $p < 0.001$) and other contexts (MWU test: $W = 629$, $p < 0.01$). Owl-faced monkeys (Kruskal-Wallis test: $\chi^2 = 27.09$, d.f. = 2, $p < 0.001$) sniffed more often in food-related (MWU test: $W = 777$, $p < 0.001$) and other contexts (MWU test: $W = 663$, $p < 0.001$) than in social contexts.

Discussion

Here we provide the first systematic insight into sniffing behaviours and sniffing frequencies of guenons. All individuals of the three studied species showed sniffing in food-related, social and other contexts. The data set presented here is not large enough to draw final conclusions. However, concerning age and sex differences it seems that sniffing is more frequent in infants than in adult females. Probably, primate infants are more curious and explore their environment more intensively via olfaction.

More than 50% of the sniffs were related to food. This is in line with other OWM species such as mandrills, drills, olive baboons [Laidre, 2009], pig-tailed [Laska et al., 2003] and rhesus macaques [Marks et al., 1988], in which olfaction is important when investigating food.

The guenons sniffed rarely in social contexts (12.1% of all recorded sniffs). This does not necessarily mean that important information is not gained via 'social sniffs'. For instance, male baboons may have to sniff only once to check females for reproductive state [Clarke et al., 2009], and the same may apply to guenons. Sniffing that occurred in other contexts such, as sniffing the substrate, seems to be more prevalent in de Brazza's and owl-faced monkeys than in Diana monkeys. Maybe, in the wild, these 2 guenon species scent-mark and therefore sniff the boundaries of their territories to check for non-group members, as chimpanzees do [Mitani and Watts, 2005; Herbinger et al., 2009], while Diana monkeys mark their territories with the help of vocal signalling [Zuberbühler, 2002].

In the across-species comparison, the SI of Diana monkeys was significantly smaller than that of de Brazza's and owl-faced monkeys ($p < 0.001$). Since in the latter two species males possess scent glands [Geissmann, 1987], the SI may be a good indicator for the presences of scent glands in OWM.

On average, all guenons studied here sniffed 6.12 times per hour (SI ± SD = 6.12 ± 7.69). In a pilot study with captive great apes, chimpanzees sniffed approximately once in 2 h (SI ± SD = 0.6 ± 0.8) and gorillas sniffed almost once per hour (SI ± SD = 0.9 ± 1.2). It seems that in guenons, the olfactory sense is of higher relevance than in chimpanzees and gorillas; however, more data on sniffing behaviours from OWM are necessary for a well-founded comparison across species.

Since sniffing varied significantly in the studied guenons, we suspect that natural selection has driven the use of the olfactory sense in phylogenetically closely related OWM. We further suggest that our study might encourage future research on sniffing behaviours to enhance our understanding of the evolution and importance of the olfactory sense in the living primates.

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