1 **Sniffing behaviour of Barbary macaques at Affenberg Salem**

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14

15 **Abstract**

16   Olfaction is one of the evolutionarily oldest senses and plays a fundamental role in
17 foraging as well as social interactions across mammals. In primates, however, its role has
18 long been underappreciated, leading to a scarcity of studies on the role of olfaction for
19 primate lives, particularly in Old World monkeys and apes. We therefore observed the
20 sniffing behaviour of semi-free ranging Barbary macaques, *Macaca sylvanus*, at Affenberg
21 Salem, Germany, to assess how frequently macaques use olfaction in which contexts, and
22 how sniffing behaviour is affected by individual attributes such as sex and age. Focal
23 observations of 24 males and 24 females aged 1 to 25 years showed that Barbary macaques
24 sniffed, on average, 5.3 times per hour, with more than 80% of sniffs directed at edible
25 items. Irrespective of the context, younger individuals used olfaction more often than older
26 ones. Females sniffed more often at edible items than males did, while males used olfaction
27 more often in a social context than females did. Sniffs at conspecifics primarily occurred in a
28 sexual context, with 70% of social sniffs directed at female anogenital swellings. Of the 176
29 anogenital inspections recorded during focal follows and ad libitum, 51 involved sniffing the
30 swelling. Notably, olfactory inspections of anogenital swellings were followed by a
31 copulation significantly less often than merely visual inspections, suggesting that anogenital
32 odours provided additional information guiding male mating decisions. In sum, results show
33 that Barbary macaques routinely use olfaction during foraging, but also in a social context.
34 Our study further suggests that odours may guide mating decision, but the role of olfaction
35 in Barbary macaque sexual interactions warrants further investigations.

36 **Introduction**

37   Animals use various sensory modalities to gain information about their social and

38 physical environment. Olfaction, the sense of smell, is one of the evolutionarily oldest senses

39 and represents an important pathway of information transfer that is used in fundamental

40 behaviours such as foraging (e.g. Short-nosed fruit bats, *Cynopterus sphinx*, Zhang et al.,

41 2014), predator avoidance (e.g. black-tailed deer, *Odocoileus hemionus*, Chamaillé-Jammes

42 et al., 2014) or mating (e.g. Harvest mice, *Micromys minutus*, Robert & Gosling, 2004).

43   In primates, optic convergence and stereoscopic vision represent significant parts of

44 their evolution, accompanied by a relative shrinking of the olfactory apparatus (Kay, 2018).

45 Thus, it was long assumed that olfaction is of little relevance in taxa with advanced visual

46 capabilities, resulting in a paucity of olfactory studies in many primate taxa. Many

47 strepsirrhine primates are nocturnal and possess bigger-sized main and accessory olfactory

48 bulbs and less specialized vision than other primate taxa (Barton, 2006). Accordingly, the

49 role of olfaction in their ecology and social lives has been studied more intensely than in

50 other taxa. It is well-established that odour plays an important role in their sociality by

51 providing information about attributes such as sex, age or territory (e.g. Janda et al., 2019)

52 as well as in their foraging behaviour (e.g. Cunningham et al., 2021). However, it has become

53 increasingly evident that also diurnal primates may rely strongly on olfaction in a range of

54 contexts. In New World monkeys, studies in several species have shown that they actively

55 use olfaction in foraging situations, and that the use of olfaction is modulated by the

56 available visual information. White-faced capuchins (*Cebus capucinus*) and spider monkeys

57 (*Ateles geoffroyi*), for instance, were observed to sniff more on fruits that give only vague

58 visual cues about their ripeness (Nevo & Heymann, 2015; Hiramatsu et al., 2009). Moreover,

59 olfaction also plays a role in the sociality of New World monkey species. For instance,

60 anogenital odour of female common marmosets (*Callithrix jacchus*) was shown to vary with

61 fertile stage, and males inspected peri-ovulatory odours more intensely than odour samples

62 from other fertile stages (Kücklich et al., 2019).

63   Catarrhine primates (Old World monkeys and apes) show trichromatic vision in both

64 sexes along with a missing or non-functional vomeronasal organ, only half the number of

65 olfactory receptor genes and relatively smaller olfactory bulbs compared to strepsirrhine

66 primates (Niimura et al., 2018). In this group, the assumption of "microsmatic" primates has

67 persisted the longest. However, a more recent viewpoint suggests that neuroanatomical

68   features like the number of olfactory genes are not always synonymous with olfactory

69   sensitivity (Laska & Galizia, 2001; Laska et al., 2007). Furthermore, the importance of

70   proportionally smaller olfactory bulbs has been questioned, since the mere proportional

71   correlation of body size to organ size and, therefore, function may not apply in olfactory

72   systems (Smith & Bhatnagar, 2004). In addition, the traditionally assumed separation of the

73   main and the accessory olfactory system appears to be vaguer than previously thought

74   (Sipos et al., 1995; Petrulis et al., 1999). Hence, it is not surprising that research has also

75   started to focus on the involvement of olfaction in catarrhine sociality and ecology (e.g.

76   Matsumoto-Oda et al., 2007; Poirotte et al., 2017; Vaglio et al., 2021). For instance, rhesus

77   macaques (*Macaca mulatta*) as well as chimpanzees (*Pan troglodytes*) were shown to

78   differentiate between group and non-group members when presented with samples of body

79   odour (Henkel et al., 2015; Henkel & Setchell, 2018). In mandrills (*Mandrillus sphinx*), group

80   members infected with parasites were discovered to have a different faecal odour than non-

81   infected members, whereby healthy individuals avoided the faecal matter of infected

82   conspecifics and avoided grooming them (Poirotte et al., 2017). Both in the wild and in

83   captivity, chimpanzees were observed to sniff at food as well as at conspecifics. While

84   females used olfaction to inspect food more than males did, males were observed to sniff

85   more in a social and sexual context than females, which was attributed to their fission-fusion

86   society and the high level of male competition (Matsumoto-Oda et al., 2007; Jänig et al.,

87   2018). Hence, evidence is accumulating that also catarrhine primates rely on olfaction in a

88   range of contexts, but the number of studies and species investigated remains low, thereby

89   hampering a more general understanding of the role of olfaction in catarrhine lives.

90   To contribute to closing the knowledge gap regarding olfaction in catarrhine species,

91   the present study investigated the natural sniffing behaviour of Barbary macaques (*Macaca*

92   *sylvanus*), an Old World monkey native to northern Africa and Southern Europe. Their diet

93   consists of various plants and their different parts such as seeds, leaves or bark, as well as

94   insects such as grasshoppers or butterflies (Fooden, 2007). Like many other catarrhine

95   species, Barbary macaques live in multi-male, multi-female groups and show a promiscuous

96   mating system (Modolo & Martin, 2007). They are sexually active year-round, but show

97   increased sexual behaviour during the mating season in autumn in winter. Offspring are born

98   in spring and early summer (Paul & Kuester, 1992). Females are philopatric, while males

99   often disperse from the natal group around puberty (Kuester & Paul, 1999). Females show a

100   conspicuous visual fertility signal, the anogenital swelling, which is directly linked to estrogen

101   levels and reaches its maximum size during the most fertile phase (Brauch et al., 2007).

102   Nonetheless, observations of genital inspections by looking, sniffing and touching, as well as

103   mating behaviour suggest that males may have more than visual information available to

104   determine female fertility (Young et al., 2013).

105   Hence, we aimed to investigate the use of olfaction (i.e. sniffing behaviour) in Barbary

106   macaques in different contexts (feeding, social and non-social), and to examine possible

107   variation in the use of olfaction related to sex and age. Similar to results in chimpanzees, we

108   hypothesized that male Barbary macaques sniff more in the social context than females. On

109   the other hand, we expected females to sniff more at edible items, as females are

110   energetically more constrained than males. With regard to age, we expected younger

111   Barbary macaques to sniff more frequently than older individuals irrespective of the context.

112   Infants and juveniles are in a process of learning about their environment and conspecifics

113   surrounding them. Hence, olfaction could be used more often to explore and gain

114   experience in all contexts, as observed e.g. in great apes (Jänig et al., 2018). We also

115   assessed the role of olfaction in inspection behaviour of the female sexual swelling. In

116   particular, we expected olfactory inspections to be more prevalent in younger, more

117   inexperienced males and towards older females, whose fertility status may be uncertain to

118   the respective male.

119

120   **Methods**

121   <u>Study site</u>

122   The study was conducted at Affenberg Salem close to Lake Constance, Germany,

123   which is home to ~ 200 Barbary macaques living in 20 hectares of fenced forest year-round

124   under near-natural conditions. The park is open to visitors from March to November. Visitors

125   are restricted to a path in ~ one third of the enclosure, while the monkeys can roam freely

126   across the entire area (see de Turckheim & Merz 1984 for details on the park). The monkeys

127   feed on food found throughout the park and receive daily supplements of fruits and

128   vegetables. Furthermore, wheat is distributed widely on and around the feeding grounds.

129   Water is accessible at several ponds and water troughs ad libitum. The park is home to three

130   naturally formed social groups, each consisting of 50-70 individuals of both sexes and all age

131   classes. All monkeys are individually identifiable by tattoos and natural markings. To control

132　　population size, about two thirds of the adult females receive hormonal implants (Implanon

133　　NXT).

134

135　　<u>Behavioural observations</u>

136　　　　　Observations of sniffing behaviour were conducted between 17.10.2020 and

137　　06.12.2020, and thus, from the onset of the mating season to its peak (Fooden, 2007). We

138　　observed 48 focal animals from two of the three groups. Focal animals comprised 24 females

139　　and 24 males from ages 1 to 25 and thus covered all age classes, from juveniles (up to 2.5

140　　years of age, N = 6) and subadults (up to 4.5 years of age, N = 9) to adults (5 years and older,

141　　N = 33, supplementary table S1). Out of the 24 females observed, 13 had contraception

142　　implanted. Each focal animal was observed six times á 20 minutes over the study period. Out

143　　of the six observations per focal animal, three were conducted in a feeding context (defined

144　　as at least ten minutes of feeding per protocol) and three were recorded in a non-feeding

145　　context. During the focal protocols all instances of sniffing as well as details about the

146　　targets of sniffing were recorded. Observations were randomly distributed between the

147　　available daylight hours from 08:00 am to 05:00 pm.

148　　　　　All observations were recorded with a digital video camera (Panasonic HC-V180). If

149　　the recorded subject moved out of sight during filming, the video was either discarded or, in

150　　11 instances, two shorter videos were counted as one. Sniffs were scored from the videos

151　　using the recorded video image as well as commentaries verbally recorded onto the video

152　　during the focal observations. A sniff was defined as the individual bringing its nose at least 3

153　　cm or closer towards an object or touching an object with the hand and then bringing the

154　　hand towards the nose (Zschoke & Thompson, 2014). Each sniff recorded in the videos was

155　　assigned to one of three target categories: food, social (sniffs directed at a conspecific or its

156　　excretions) or other (sniffs directed at the environment, human-made objects and self-sniffs)

157　　following Jänig et al. (2018). For each observed sniff, the target object was specified and

158　　analysed and the behaviour of the monkey after the sniff was noted. All videos were

159　　analysed by one observer (MS), however, five percent of the data were sighted by an

160　　additional observer trained by MS to check for inter-observer reliability. The audio-track of

161　　the video was unavailable to the second coder to avoid bias. By comparing the respective

162　　observed sniffs, the intraclass correlation coefficient (ICC) was calculated and revealed good

163　　reliability (ICC=0.86; Koo & Li, 2016). Besides the video protocols, ad libitum data were

164   collected for every sniff that was observed outside the focal observations for focal and non-

165   focal animals. Furthermore, whenever inspections of female swellings by males were

166   observed, the following data were noted ad libitum: male and female ID, group, date, time,

167   whether visual and/or olfactory inspection occurred and which inspection happened first,

168   and if inspection was followed by a copulation.

169

170   <u>Statistical Analysis</u>

171   Statistical analysis was conducted in R version 4.0.3 (R core team 2020) using

172   Generalized Linear Mixed Models (GLMM) to allow accounting for repeated observations of

173   the same individuals (Bates et al., 2015). We conducted three sets of models that were fitted

174   by using the function "glmer" from the package "lme4" version 1.1- 26 (Bates et al., 2015).

175

176   *Sniffing frequencies*

177   The aim of the first model was to investigate the influence of sex, age and context on

178   sniffing frequencies. Accordingly, the number of sniffs per 20min focal observation and

179   target category was used as the response variable, fitted with a Poisson error distribution (N

180   = 288 observations x 3 target categories = 864). Sex, birth year, context and target were

181   fitted as fixed effects test predictors. Context referred to the observational context 'feeding'

182   or 'non-feeding' in which each protocol was recorded. Target categorized for each sniff

183   whether it was directed at 'food', 'social' or 'other' (as defined above). Group, daytime and

184   Julian day were fitted as fixed effects control predictors. Daytime was coded as morning

185   (until 12:30 pm) or afternoon (after 12:30pm). Julian Day was included to control for the

186   progress of the mating season. Several two-way interactions were included in the model: I)

187   sex and target to address the hypothesis that males sniff more in a social context and

188   females more in a feeding context; II) sex and Julian day, since the observational period

189   started at the early beginning of the mating season and ended at its height. Male and female

190   sniffing behaviour could therefore show different patterns from the beginning to the end of

191   the observation period; III) context and target, because the contexts 'feeding' or 'non-

192   feeding' presumably influence the probable targets of sniffing; IV) birth year and target, to

193   account for the possibility that age affects which objects the monkeys sniff at, and V)

194   daytime and target, as fresh food got distributed every morning at 08:00 and we accordingly

195   expected more sniffs at edible items in the morning than in the afternoon. ID was included

196 as a random effects control predictor. To achieve more reliable p-values (Barr et al., 2013),

197 we fitted random slopes of all predictors showing sufficient variation within ID, i.e. the

198 random slopes of Julian day, context*target, and daytime*target.

199

200 *Olfactory inspection of females*

201 The aim of the second model was to investigate the influence of female and male

202 characteristics and the progression of the mating season on whether male inspections of

203 female sexual swellings included olfaction or not (N = 176 genital inspections). For this

204 purpose, we fitted a GLMM with binominal error distribution using all genital inspections

205 observed during focal observations as well as those recorded ad libitum. Female and male

206 birth year, whether or not the female was contracepted and Julian day were fitted as fixed

207 effects test predictors, while daytime and group were included as fixed effects control

208 predictors. The two-way interaction of female birthyear and male birthyear was included to

209 account for the possibility that an effect of male age on olfactory inspection could be

210 modulated by the age of the male's partner. Female and male ID were included as random

211 effects control predictors. For the random effect of male ID, the random slopes of Julian day,

212 female birth year and contraception were incorporated. For female ID, the random slopes of

213 Julian day and male birth year were included.

214

215 *Genital inspections and copulation*

216 Using focal and ad libitum data, a third GLMM was fitted with binominal error

217 distribution to investigate the influence of female and male characteristics and the

218 occurrence of olfactory inspection on whether or not a genital inspection was followed by a

219 copulation (N = 176 genital inspections). Female and male birth year, contraception (yes/no)

220 and olfactory inspection (yes/no) were fitted as fixed effects test predictors while daytime,

221 group and Julian day were included as fixed effects control predictors. We initially

222 incorporated four two-way interactions: I) female birth year and male birth year; II) female

223 birth year and olfactory inspection; III) Julian day and olfactory inspection and IV)

224 contraception and olfactory inspection. However, the interaction terms were too

225 imbalanced, which caused stability issues (see general model procedures for stability

226 checks), and were therefore removed again from the model. After removing the

227 interactions, the model showed no stability issues. Female and male subject were included

8

228   as random effects control predictors. For the random effect of male ID, Julian day, olfactory

229   inspection, contraception and female birth year were included as random slopes. For female

230   ID, Julian day, olfactory inspection and male birth year were included as random slopes.

231

232   *General Model Procedures*

233   For all models, covariates were z-transformed to a mean of zero and a standard

234   deviation of one before running the models to facilitate interpretation of model coefficients

235   and model convergence (Schielzeth, 2010). Variance Inflation Factors (VIF) were computed

236   using the function 'vif' of the package 'car' (Fox & Weisberg, 2019) to check for collinearity

237   between the predictors (Quinn & Keough, 2002). With largest VIFs of 1.08 (model 1), 1.3

238   (model 2) and 1.1 (model 3), no collinearity issue could be detected in either model. All three

239   models were tested for over- and underdispersion, with resulting dispersion parameters of

240   0.59 (model 1), 0.91 (model 2), and 0.62 (model 3). As the first and the third model were

241   moderately underdispersed, the computed p-values should be considered conservative.

242   Model stabilities were assessed by excluding levels of random effects one at a time. With the

243   exception of model 3 when fitted with interactions, none of the models showed stability

244   issues. A Likelihood Ratio Test (LRT) was used to determine the effects of the test predictors

245   on the response variables by comparing a null model without fixed effects test predictors to

246   the respective full model containing all predictor (Dobson, 2002; Forstmeier & Schielzeth,

247   2011). If the full-null model comparison was significant ($p < 0.05$) or a trend ($p < 0.1$), the p-

248   values of the individual predictors were afterwards determined by using the function

249   "drop1" from the package "lme4" version 1.1-26 (Bates et al., 2015). To facilitate

250   interpretation of the main terms, non-significant interactions were removed from the

251   models.

252

253   **Results**

254   In total, 511 sniffs were observed across all focal observations (96 hours total

255   observation time), with 1.78 ± 1.70 (mean ± SD) sniffs per individual per

256   20 min observation period (corresponding to 5.34 sniffs per hour). Females were observed

257   to sniff a total of 314 times (mean ± SD: 2.18 ± 2.9 per individual and observation period)

258   and males 197 times (mean ± SD: 1.37 ± 1.81 per individual and observation period, see Tab.

259   1). With 83% of sniffs the vast majority of sniffs observed during focal observations was

9

260   directed at food items (423/511), while 8% (40/511) and 9% (47/511) were directed at social

261   or other targets, respectively (Tab. 1).

262

263   Table 1: Number of sniffs observed at different targets for 48 focal animals in 96 hours of focal

264   observations.

265

	Food	Social	Other	Sum
Male	150	29	18	197
Female	274	11	29	314
Sum	423	40	47	511

266

267   49 different identifiable edible items (see supplementary table S2) were observed to

268   be olfactorily inspected by the monkeys. When the target was a conspecific, 70% (28/40) of

269   sniffs were directed at a female swelling (26 by adult males, 2 by adult females). Eleven of

270   the social sniffs were directed at an infant (1 by an adult male, 7 by adult females and 3 by

271   another infant). One sniff was directed at the bottom of a juvenile male by a five-year old

272   male. Of the 47 sniffs at 'other' targets, 27 were self-sniffs, almost exclusively directed at

273   their own hand after scratching themselves, 14 were directed at the environment (e.g. tree

274   branch or ground) and 6 at human-made objects (e.g. bottle cap, camera trap).

275   In addition, 136 sniffs were observed ad libitum, of which 70 were directed at food,

276   44 at conspecifics and 22 at other targets, with distributions across sex and age classes

277   similar to data recorded during focal observations. Sniffs observed ad libitum included 18

278   cases of infants sniffing at their mother's, or in one case their grandmother's, mouth while

279   these were eating.

280

281   <u>Sniffing frequencies</u>

282   The suite of test predictors had a significant effect on the number of sniffs per

283   individual per observation (LRT, $\chi2 = 111.8$, df = 12, P < 0.001). Sniffing frequencies differed

284   between the sexes, whereby the effect of sex depended on the target of the sniff. In

285   particular, more sniffs were observed for females at food and 'other' targets compared to

286   males. Males, on the other hand, sniffed slightly more often at social targets than females

287   (see Tab. 1 & 2, Fig. 1). Furthermore, younger individuals sniffed significantly more often

288   than older individuals (Tab. 2, Fig. 2).

289

10

290   Table 2: Results of the GLMM investigating sniffing frequency (number of sniffs) per focal animal,
291   observation period and target category as response variable with Poisson error distribution. Values in
292   parenthesis indicate trait levels relative to the respective reference level. Values for the non-
293   significant interactions (daytime*target, context*target and birth year*target) represent values
294   before removal of the respective terms from the model. SE = standard error. χ^2 and P values are
295   derived from Likelihood Ratio Tests to determine the significance of the individual test predictors.

term	Estimate	SE	χ^2	P
Intercept	-0.042	0.259	*	*
context (non-feeding)	-1.255	0.204	31.479	<0.001
group (2)	0.111	0.268	0.159	0.690
sex (male)	-0.368	0.267	*	*
Julian day	-0.308	0.097	9.103	0.003
daytime (morning)	0.862	0.164	*	*
target (other)	-2.630	0.568	*	*
target (social)	-3.545	0.591	*	*
birth year	0.297	0.137	4.502	0.034
target*sex			7.368	0.025
target (other)*sex (male)	0.159	0.785		
target (social)*sex (male)	1.780	0.678		
daytime*target			15.085	<0.001
daytime(morning)*target (other)	-2.592	0.775		
daytime(morning)*target (social)	-0.994	0.455		
context*target			2.480	0.289
context (non-feeding)*target (other)	1.342	0.765		
context (non-feeding)*target (social)	-0.739	0.703		
birth year*target			0.602	0.740
birth year*target (other)	0.193	0.346		
birth year*target (social)	0.25	0.402		
sex (male)*Julian day	-0.193	0.198	-0.974	0.329

* not presented because of having a very limited interpretation

296

297   Of the control predictors, context, Julian day and the interaction between daytime

298   and target significantly affected sniffing frequencies (see Tab. 2). In particular, we observed

299   more sniffs during feeding than during non-feeding focal follows, earlier in the season, and

300   at food items more frequently in the morning than in the afternoon. None of the other

301   predictors had a significant influence on sniffing frequencies (Tab. 2).
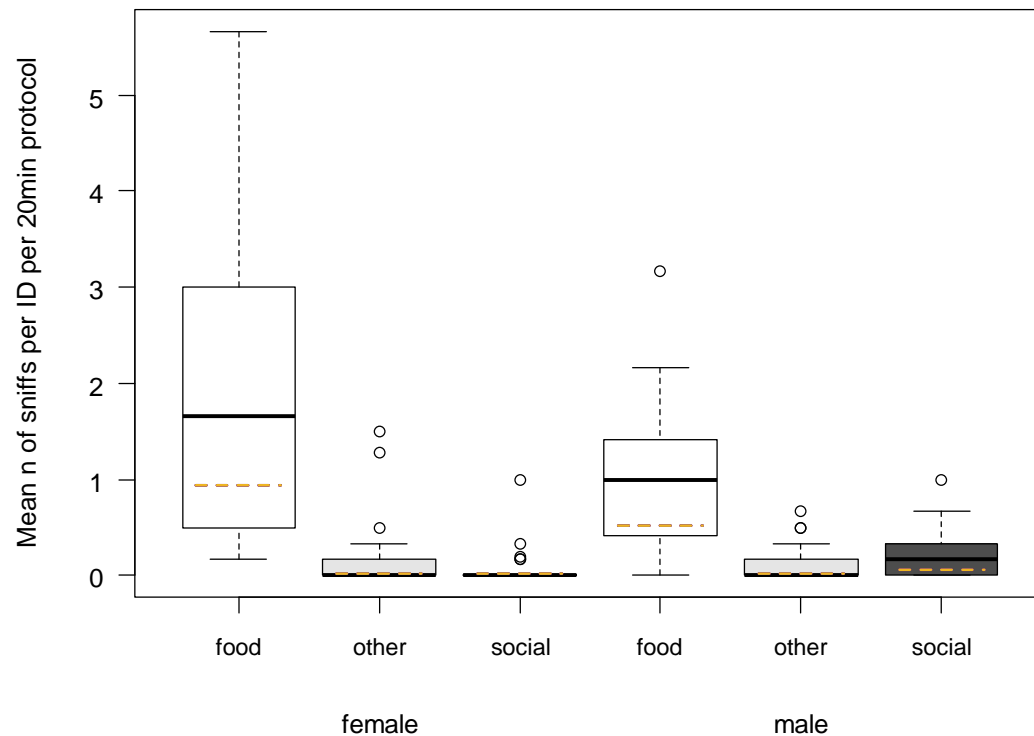
302

303

Figure 1: Mean number of sniffs per individual per 20 min focal observation for both sexes divided into the target contexts food, other and social. Boxes represent medians and first and third quartiles. The dashed lines represent the model estimates when all other predictors are at their average.
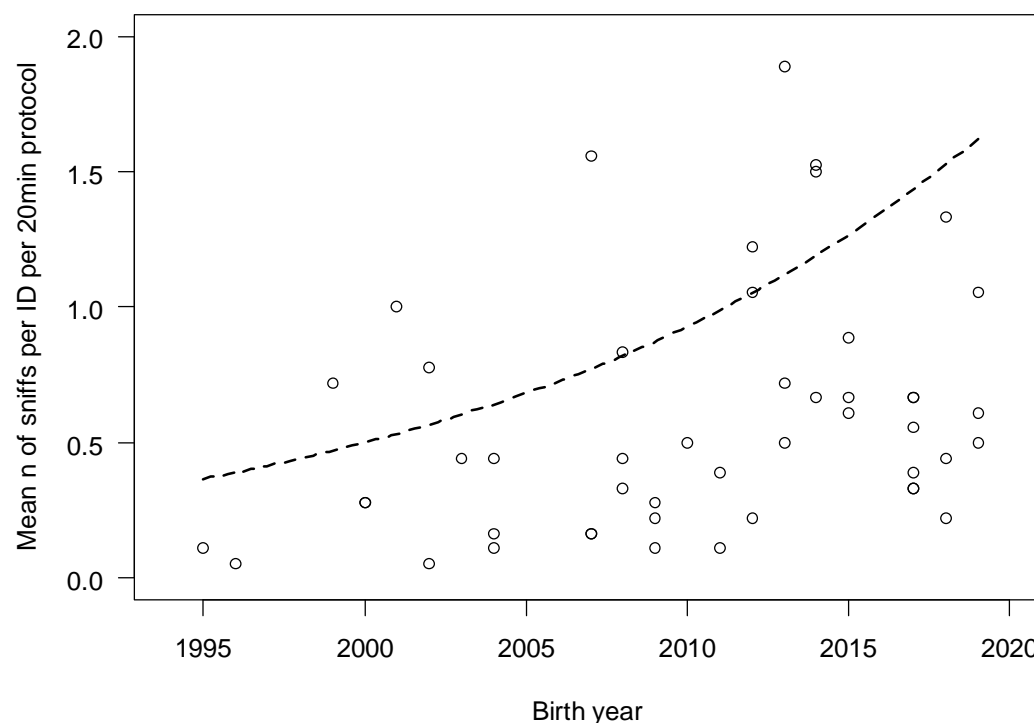

307

Figure 2: Mean number of sniffs per individual per 20 min focal observation across birth years. The dashed lines represent the model estimate when all other predictors are at their average.

310

311   <u>Olfactory inspection of females</u>

312   Data on male inspections of sexual swellings, collected ad libitum as well as from the

313   recorded observations, showed that out of 176 observed visual inspections, 51 were

314   accompanied by an olfactory inspection of the swelling. The full-null comparison of the

315   model investigating which parameters affected the occurrence of olfactory inspections

316   revealed only a weak trend (LRT, χ2 =16.111, df= 10, P=0.09). Solely the interaction between

317   female and male birth year significantly influenced the probability of an olfactory inspection

318   (Tab. 3).

319

320   Table 3: Results of the binomial GLMM investigating the probability of an olfactory inspection during
321   genital inspections. Values in parenthesis indicate trait levels relative to the respective reference
322   level. SE = standard error. χ^2 and P values are derived from Likelihood Ratio Tests to determine the
323   significance of the individual test predictors.

	Estimate	SE	χ2	P
Julian day	-0.176	0.217	0.571	0.450
male birth year	0.648	0.290	*	*
female birth year	-0.421	0.224	*	*
contraception (yes)	0.774	0.754	1.050	0.306
daytime (morning)	0.292	0.403	0.499	0.480
group (2)	-0.744	0.599	1.478	0.224
male birth year*female birth year	0.417	0.200	4.512	0.034

* not presented because of having a very limited interpretation

324

325   <u>Genital inspections and copulation</u>

326   Whether or not genital inspections were followed by a copulation was significantly

327   affected by the suite of test predictors (model excluding interactions, LRT, χ2 =22.142, df=4,

328   P<0.001). In particular, olfactory inspection of the swelling significantly decreased the

329   probability for copulation after inspection (Tab. 4). In fact, only 2 out of the 51 visual *and*

330   olfactory inspections were followed by a copulation, while copulation followed genital

331   inspection in 44 of the 125 solely visual genital inspections. Furthermore, the female birth

332   year also impacted the copulation probability, with copulations more likely after inspections

333   of younger females than older females (Tab. 4).

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335

13

336 Table 4: Results of the binomial GLMM investigating the probability of a copulation following a
337 genital inspection. Values in parenthesis indicate trait levels relative to the respective reference level.
338 SE = standard error. χ² and P values are derived from Likelihood Ratio Tests to determine the
339 significance of the individual test predictors.

	Estimate	SE	χ2	P
Julian day	-0.508	0.301	2.212	0.137
olfactory inspection (yes)	-3.287	1.014	21.987	<0.001
male birth year	0.101	0.291	0.111	0.739
female birth year	0.874	0.345	5.221	0.022
contraception (yes)	-0.490	0.747	0.393	0.531
daytime (morning)	-0.492	0.466	0.919	0.338

340

341 **Discussion**

342   This study significantly contributes to understanding the sniffing behaviour of Barbary
343 macaques and thereby, Old World monkeys in general. In particular, we could show that
344 most sniffs occurred when assessing edible items, with females sniffing more at food than
345 males did. Males used olfaction in the social context more than females did, and sniffing
346 generally decreased with increasing age. With an average of 5.3 sniffs per hour and
347 individual, Barbary macaques sniffed at similar rates as some guenon species (*Cercopithecus*
348 *diana, neglectus* and *hamlyni*) observed in captivity, which were reported to sniff, on
349 average, 6.1 times per hour and individual (Zschoke & Thomson, 2014). In contrast, sniffing
350 rates reported for four species of great apes, also observed in captivity, were considerably
351 lower (0.2 to 0.4 sniffs per hour and individual, Jänig et al., 2018).

352   In our study, the vast majority of sniffs was directed at food, which parallels findings
353 for great apes (Jänig et al., 2018), guenons (Zschoke & Thomson, 2014), mandrills and olive
354 baboons (Laidre, 2009). Species that depend mostly on fruits and other plant parts as their
355 diet were suggested to steadily rely on olfactory cues to find and identify ripe food (e.g.,
356 Nevo & Heymann, 2015), get information about the nutritional value (Dominy et al., 2001),
357 determine food safety and notice possible contamination (Sarabian et al., 2020). Hence, it
358 comes as no surprise that an almost exclusively vegetarian species like the Barbary macaque
359 shows frequent olfactory assessments of edible items. The collected data, however, did not
360 provide suitable information to assess why certain items were sniffed at and others not.
361 While food ripeness can be implied for all produce fed at the park, nutritional value, safety
362 and contamination with, e.g., soil or faeces, are more complicated to assess for an observer.
363 Additionally, in an environment with a regular feeding routine, primates may learn to rely on

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364 food safety and habituate to the produce available, which could lead to a decrease in the

365 importance of olfactory inspection (Laska et al., 2007). Captive squirrel monkeys (*Saimiri*

366 *sciureus)* and spider monkeys (*Ateles paniscus*) were described to rely mostly on visual cues

367 when assessing familiar food items whereas novel food was more likely to prompt olfactory

368 inspections (Laska et al., 2007). In line with this suggestion, we observed a disproportional

369 number of sniffs at hay and a chestnut, both of which were only rarely available to the

370 monkeys, but whether Barbary macaques really sniffed more frequently at novel or rare

371 food items could not be addressed as we did not systematically collect data on which types

372 of food and other plants were available how often. However, it should be kept in mind that

373 seasonality and varying geographical availability of food sources could potentially lead to a

374 different exhibition of olfactory inspection of food at other times or in other Barbary

375 macaque populations.

376

377 Influences of sex and age

378   The significant interaction between sex and age on sniffing probability supports our

379 hypothesis that male Barbary macaques sniff more often in a social context than females do.

380 This imbalance in social sniffs was also observed in other primate species such as

381 chimpanzees (Jänig et al., 2018; Matsumota-Oda et al., 2007) or owl monkeys (*Aotus*

382 *nancymaae*; Spence-Aizenberg, 2017). In chimpanzees, the fission-fusion dynamics of a high-

383 competition multi-male multi-female group were suggested to enhance olfaction in the

384 social context (Jänig et al., 2018; Matsumota-Oda et al., 2007). For Barbary macaques, which

385 also live in multi-male multi-female groups, almost all social sniffs of adult males were

386 observed to be directed at female sexual swellings and only one at another adult male.

387 Olfaction thus may play a role in sexual competition for fertile females by, for example,

388 providing information about the ovarian cycle in addition to the visual signal of swelling size.

389 However, it does not seem that olfaction is important for males to gather direct information

390 about other males. Female Barbary macaques, on the other hand, sniffed more at food than

391 males. This corresponds to observations of female chimpanzees (Matsumota-Oda et al.,

392 2007). Moreover, female primates of various species tend to be more wary of contamination

393 risks for parasites or bacteria through food that is rotten or spoiled with faeces (e.g.

394 Japanese macaques: Sarabian et al., 2015; grey mouse lemurs: Poirotte et al., 2019; long-

395 tailed macaques: Sarabian et al., 2020; mandrills: Poirotte et al., 2019), as evident from more

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396   olfactory assessment of contaminated food, followed by food manipulation (Sarabian et al.,

397   2020). This behaviour was rewarded by lower infection rates of females (Poulin, 1996; Rolff,

398   2002; Poirotte et al., 2019), suggesting that more visual or olfactory inspections, even

399   though potentially costly, maximize the long-term fitness and therefore reproductive success

400   of females. Similar mechanisms might apply to Barbary macaques, but a systematic analysis

401   of parasite occurrence in both sexes would be needed to assess this possibility.

402   Also our hypothesis of young individuals sniffing more often than older ones was

403   supported for Barbary macaques. These findings agree with the consensus that young

404   animals inspect their environment more closely than older individuals since they are still in

405   the process of learning to evaluate food, conspecifics or their general environment. Since the

406   decrease of sniffing events with age in Barbary macaques appears to be quite linear, it is

407   likely that it is primarily caused by increased experience and not by a loss of olfactory

408   capability in old age. 18 out of 22 social sniffs that were observed ad libitum for infant

409   monkeys were directed at the feeding mother's, or in one case the grandmother's, mouth. In

410   each case the infants appeared to observe the eating behaviour and tried to inspect the item

411   visually. They were not chased off and the mothers reacted with indifference to the

412   inspection attempt of their infant. This 'muzzle-muzzle' behaviour has been observed in

413   different mammals and may enable the individuals to smell the breath of their conspecifics

414   while they are eating (e.g. mice: Renn, 2004; Norway rats: Noble et al., 2001; mandrill, drills

415   and baboons: Laidre, 2009). In this way they may gather information on which food has been

416   deemed safe and valuable to eat by their conspecific.

417

418   <u>Olfaction in sexual interactions</u>

419   Most social sniffs occurred in a sexual context and were directed at the female

420   anogenital swelling. However, not every inspection observed included olfaction. Hence, we

421   tried to assess which circumstances led to an olfactory inspection and whether an olfactory

422   inspection had an influence on the outcome of the sexual interaction. The model on the

423   occurrence of olfactory inspections provided only weak evidence for an effect of individual

424   characteristics affecting the likelihood to sniff at a sexual swelling during inspection,

425   potentially pointing to an interplay of female and male age effects on olfactory inspections.

426   It has to be noted though, that we did not track changes in female swelling size or other

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427   transient individual attributes, and thus could not assess whether the salience of the visual

428   fertility signal affected whether or not males additionally used olfaction to inspect females.

429   However, if an olfactory inspection of the swelling occurred, this almost exclusively

430   led to no subsequent copulation. This may indicate that, although swelling size closely

431   reflects female ovarian function (Brauch et al., 2007; Tschoner, 2015), an olfactory cue may

432   provide additional information that deters males from engaging in costly mating behaviour.

433   This may particularly be the case for older females in which an inspection – whether or not it

434   involved olfaction – resulted in fewer copulations than in younger females. It would have

435   been interesting to assess whether female age and olfactory inspection interacted in their

436   effects on copulation probabilities but unfortunately the very low number of olfactory

437   inspections that did lead to copulation did not allow us to address this. The same applies to a

438   potential effect of hormonal contraception. Our results provide no indication that female

439   contraception affected either the likelihood of males to sniff at the swelling or the likelihood

440   of a copulation after inspection, but again we were not able to test whether an effect of

441   olfactory inspection on copulatory behaviour may have differed between contracepted and

442   non-contracepted females. More detailed observations on, e.g., whether a copulation was

443   ejaculatory or not, or if males later returned to inspect and/or copulate with the respective

444   female may help to better understand the role of olfaction in Barbary macaque mating

445   interactions. This, however, would require more systematic focal observations of sexual

446   interactions that were beyond the scope of this study. Thus, our analyses should be seen as

447   a starting point for further research on olfaction in sexual interactions.

448   In conclusion, Barbary macaques routinely used olfaction in different contexts and its

449   use was modulated by individual attributes such as sex and age. These finding are in line

450   with current research in other (catarrhine) primates and the growing evidence about the

451   importance of olfaction across primate species. Subsequent research is needed to

452   thoroughly interpret sniffing behaviour at food or conspecifics in the light of visual or other

453   available sensory information, with this study functioning as a basis and offering starting

454   points for future studies. As such, this study represents a first step towards understanding

455   the use and importance of olfaction in the lives of Barbary macaques, and thereby

456   contributes to a better understanding of the role of olfaction for primates in general.

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469 **Ethics statement**

470 This study was purely observational and in accordance with the legal requirements of

471 Germany, all national and institutional guidelines for the care and use of animals.

472

473 **Conflict of Interest**

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

475

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579   Table S1: Overview observed sniffs for all 48 focal animals. For each monkey, the total number of
580   sniffs that was observed during the six focal observations as well as a breakdown of the sniffs that
581   occurred in the context food, social or other are listed. Additionally, the respective birthyear, sex and
582   group are noted. All females marked with an asterisk were contracepted during the study period.
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Birthyear	ID	Sex	Group	Sniffs Total	Food	Social	Other
1995	M1	Male	1	2	2	0	0
1996	F1	Female	1	1	1	0	0
1999	M2	Male	2	13	13	0	0
2000	M3	Male	2	5	4	1	0
2000	F2	Female	2	5	5	0	0
2001	F3	Female*	2	18	18	0	0
2002	F4	Female*	2	14	12	0	2
2002	F5	Female*	1	1	1	0	0
2003	F6	Female*	2	8	8	0	0
2004	M4	Male	2	3	1	0	2
2004	M5	Male	1	2	1	0	1
2004	M6	Male	1	8	8	0	0
2007	M7	Male	1	3	3	0	0
2007	F7	Female*	2	28	28	0	0
2007	F8	Female*	2	3	2	0	1
2008	M8	Male	2	6	5	1	0
2008	M9	Male	2	8	0	6	2
2008	F9	Female*	1	15	12	2	1
2009	M10	Male	1	2	2	0	0
2009	F10	Female*	2	4	4	0	0
2009	F11	Female*	1	5	4	1	0
2010	M11	Male	1	9	7	2	0
2011	M12	Male	2	7	6	0	1
2011	F12	Female	1	2	2	0	0
2012	M13	Male	2	4	3	1	0
2012	F13	Female*	2	19	18	0	1

2012	M14	Male	1	22	19	3	0
2013	M15	Male	2	9	9	0	0
2013	M16	Male	2	13	13	0	0
2013	F14	Female*	2	34	34	0	0
2014	M17	Male	1	12	7	4	1
2014	F15	Female*	1	27	21	6	0
2014	F16	Female	1	32	23	0	9
2015	M18	Male	2	11	10	1	0
2015	F17	Female*	2	12	12	0	0
2015	F18	Female	1	16	16	0	0
2017	M19	Male	2	10	5	2	3
2017	M20	Male	1	12	11	1	0
2017	M21	Male	1	7	6	1	0
2017	F19	Female	2	12	2	1	9
2017	F20	Female	2	5	4	1	0
2017	F21	Female	1	6	6	0	0
2018	M22	Male	1	8	2	2	4
2018	F22	Female	2	4	2	0	2
2018	F23	Female	2	24	23	0	1
2019	M23	Male	2	11	6	4	1
2019	M24	Male	1	9	6	0	3
2019	F24	Female	1	19	16	0	3

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593 Table S2: Identified edible items focal animals sniffed at and the respective number of observed
594 sniffs. Not included were unidentifiable objects (e.g. stems).
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Item	No. Sniffs
Potato	30
Leave	26
Carrot	23
Pineapple	22
Apple	21
Salad	17
Tomato	16
Leek	15
Grape	14
Hay	11
Popcorn	11
Parsley	10
Capsicum	8
Escallion	8
Beechnut	7
Chestnut	7
Stems	7
Celery	6
Orange	6
Seed	6
Tree branch	6
Mushroom	5
Broccoli	4
Cucumber	4
Fennel	4
Mango	4
Basil	3
Brussel sprout	3
Cabbage	3
Chinese cabbage	3
Honey melon	3
Vitelotte	3
Banana	2

Item	No. Sniffs
Black raddish	2
Pear	2
Pomegranate	2
Walnut	2
Wheat	2
Avocado	1
Beetroot	1
Chives	1
Clover	1
Eggplant	1
Fern	1
Gras	1
Pellet	1
Red cabbage	1
Thyme	1
Bark	1

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