**Group and kin recognition via olfactory cues in chimpanzees (*Pan troglodytes*)**

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**Electronic Supplementary Material 1**

**Pilot Study on Odour Source Preference**

We conducted a pilot study to test which odour source chimpanzees respond most strongly to by presenting three different odour sources (body odour, faeces and urine) of the same individual (a group member) to two groups of chimpanzees at Leipzig Zoo.

**Material and Methods**

*Odour sample collection*

1. *Body odour*

We collected body odour by rubbing small pieces of 100% organic cotton, held with clean forceps (sterilized with 95% ethanol), for 20 s on the head, chest, back and arm of trained animals who presented their body through the bars of the sleeping enclosure. We used the cotton directly in the bioassay without freezing it. As a second source of body odour, we collected a handful of nesting material from individuals of group B that sleep in individual enclosures. These individuals sleep on this nesting material and most likely transfer body odour to it. We collected nesting material in the morning directly after the animals woke up.

2. *Faeces*

We collected faeces directly after defecation from the floor of the sleeping or observation rooms and stored them in 50 ml plastic tubes at -20°C until use.

3. *Urine*

Urine collection was the same as for the main bioassay. We collected urine using disposable pipettes directly after animals urinated on the floor in the sleeping or observation rooms and stored it in 15 ml plastic tubes at -20°C until use. We used new pipettes for each individual and only collected samples that we could assign to an individual. For females, we only collected urine when they showed no sexual swelling and were not menstruating, to avoid hormonal influences of the menstrual cycle on the odour.

*Experimental setup*

We presented three different odour sources (urine, faeces, body odour) collected from the same individual (one group member per group) in one session per group. We presented 15 ml of urine, a piece

of faeces and, as body odour, 3 cotton balls for group A and 1 cotton ball and a handful of nesting material for group B in the same setup as for the main bioassay (three plexiglass boxes installed on the ventilation grid at 1 m intervals and in random order). For logistical reasons, we installed the boxes in the inside enclosure for group A for approximately 7 hours and in the outside enclosure for group B for approximately 3.5 hours.

*Statistical Analysis*

To account for differences in session duration, we used the total duration of each behaviour (*sniffing, nose within 20 cm, licking, present within 50 cm, manipulating*), per individual divided by session duration as response variables. To test whether chimpanzees respond differently towards different odour sources, we used a Linear Mixed Model (LMM, [1]) for each response variable. We included odour source (urine, faeces, body odour), subject sex, subject group and subject age as fixed effects and subject ID as random effect. To test for the possibility that males and females react differently towards different odour sources, we also included the interaction between odour source and subject sex in the model. We did not include random slopes because there was hardly any variation in the fixed effects within the levels of the random effect of subject ID. We fitted all models in R (version 3.4.0, [2]) using function lmer of R-package lme4 (version 1.1-13, [3]).

Subject age was approximately symmetrically distributed and z-transformed (to a mean of zero and a standard deviation of one) to obtain easily interpretable estimates [4]. None of the response variables showed any obvious deviations from the assumptions of normally distributed and homogeneous residuals, based on visual inspection of a qqplot and plotting residuals against fitted values. We checked for model stability by excluding subjects from the data one at a time and comparing the model estimates derived for these subsets of the data with those for the full data set. There was no indication of influential subjects, thus all models were stable. We derived Variance Inflation Factors (VIF, [5]) using function vif in R-package car (version 2.1-5, [6]) applied to a standard linear model excluding individuals one at a time. These did not indicate that collinearity was a problem (for max and mean VIFs see table S1).

We established the significance of the full model compared to the null model (comprising only subject group, subject age and the random effect) using a likelihood ratio test (LRT, R function anova with argument test set to "Chisq", [7,8]). To allow a LRT we fitted the models using Maximum Likelihood (rather than Restricted Maximum Likelihood, [9]). We based P-values for the individual effects on LRTs comparing the full with respective reduced models ([10], R function drop1 with argument 'test' set to "Chisq"). We used the function glht of the R-package multcomp (version 1.4-6, [11]) to conduct pairwise post-hoc comparisons between the levels of the main effect odour source. Sample sizes are indicated in table S1.

**Table S1** Sample sizes and variation inflation factors (max and mean) for Linear Mixed Models testing the effect of different odour sources on response variables. $N_{tot}$ = total sample size; $N_{RE}$ = number of levels per random effect (subject ID)

| Response variable | $N_{tot}$ | $N_{RE}$ | $VIF_{max}$ | $VIF_{mean}$ |
|---|---|---|---|---|
| Sniffing | 45 | 22 | 1.157 | 1.088 |
| Nose within 20cm | 48 | 22 | 1.157 | 1.085 |
| Licking | 26 | 15 | 1.336 | 1.208 |
| Presence within 50cm | 55 | 23 | 1.136 | 1.074 |
| Manipulating | 25 | 14 | 1.334 | 1.215 |

**Results**

Overall, the full model was significant when compared to the null model for *sniffing*, nose within 20 cm, and *manipulating*, and showed a trend for *licking* and *present within 50 cm* (table S2). Since the interaction between odour source and subject sex was not significant for *sniffing*, *nose within 20 cm*, *licking* and *present within 50 cm* (table S3), we fitted a reduced model without the interaction to obtain interpretable P-values for the main effects. The effect of odour source was significant for *sniffing* and *nose within 20 cm*, and the interaction between odour source and sex was significant for *manipulating* (table S4). Pairwise post-hoc comparisons revealed that subjects sniffed significantly longer at urine than faeces (Estimate=0.829, SE=0.225, z=3.691, p=0.001; fig. S1) and body odour (Estimate=0.577, SE=0.222, z=2.604, p=0.050; fig. S1) and that they placed their nose significantly longer within 20 cm of urine than faeces (Estimate=1.002, SE=0.314, z=3.197, p=0.008; fig. S1).

**Discussion**

Chimpanzees showed more olfaction-related behaviour (*sniffing, nose within 20 cm*) towards urine than to faeces and body odour. Extended inspection of urine as compared to faeces might reflect a trade-off between obtaining information about the scent donor and the risk of pathogen infection, because fecal-oral is the predominant transmission route for parasitic infections [12] and faeces are more likely to present an infection risk than urine. There is ample evidence that urine contains social information (e.g. species, group membership, sex or kinship) in strepsirrhines [13], New World monkeys [14–18], and Old World monkeys [19,20], and that it elicits sniffing behaviour and scent marking [21]. Recent experimental studies found that mandrills avoid parasitized conspecific faeces and groom conspecifics infected with fecal-orally transmitted parasites less than non-parasitized conspecifics [22]. Similarly, chimpanzees tend to move away when exposed to visual and olfactory cues of conspecific faeces, probably as a parasite or pathogen infection avoidance strategy [23]. Olfactory and/or visual (since boxes were transparent) cues may have led the chimpanzees in our study to avoid faeces with a potential pathogen infection risk and inspect urine to obtain more information about the odour donor.

Furthermore, we found that males manipulated boxes containing urine and feces more than females, potentially reflecting a higher tactile explorative tendency in males.

Body odours also transmit social information in many species, including catarrhine primates [24–26]. Chimpanzees, like most other catarrhines and in contrast to strepsirrhines [27] and platyrrhines [28], do not possess specialized scent glands. However, they do possess sebaceous, eccrine and apocrine skin glands [29]. These skin glands are aggregated in the armpit to form an axillary organ, which is unique among chimpanzees, humans and gorillas and the prime source of body odour [30,31]. Our results show that chimpanzees did inspect body odour (more than faeces although the difference was not statistically significant), suggesting they perceived the smell of this odour source. However, they sniffed body odour significantly less than urine. This might be because the intensity of the body odour was not high enough to elicit sufficient interest or to provide a discernible signal. An alternative explanation is that body odour might be a less relevant odour source relating to obtaining information about conspecifics when the sender is not present and that it might be more important in close contact social situations, like when inspecting the anogenital region to probe for female fertility [32,33].

**Table S2** Results for full-null model comparisons testing the effect of different odour sources on response variables using likelihood ratio tests. Significances and trends are marked in bold

| Response variable | $\chi^2$ | df | p |
|---|---|---|---|
| Sniffing | 12.816 | 5 | **0.025** |
| Nose within 20 cm | 15.106 | 5 | **0.010** |
| Licking | 10.155 | 5 | **0.071** |
| Presence within 50 cm | 10.796 | 5 | **0.056** |
| Manipulating | 14.281 | 5 | **0.014** |

**Table S3** Results for the non-significant interaction of the full models testing the effect of different odour sources on response variables. Degrees of freedom were 2 throughout. Colons represent interactions between fixed effects

| Response variable | Predictor variable | Estimate | SE | CL$_{lower}$ | CL$_{upper}$ | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|
| Sniffing | Odour source (faeces) [1]: Subject sex [2] | 0.071 | 0.495 | -0.925 | 0.202 | 1.098 | 0.685 |
| | Odour source (urine) [1]: Subject sex [2] | 0.410 | 0.485 | -0.567 | 1.404 | | |
| Nose within 20 cm | Odour source (faeces) [1]: Subject sex [2] | 1.403 | 0.679 | 0.033 | 2.784 | 4.015 | 0.134 |
| | Odour source (urine) [1]: Subject sex [2] | 0.542 | 0.663 | -0.799 | 1.880 | | |
| Licking | Odour source (faeces) [1]: Subject sex [2] | 2.316 | 0.927 | 0.156 | 4.210 | 4.373 | 0.112 |
| | Odour source (urine) [1]: Subject sex [2] | 1.577 | 0.813 | -0.528 | 1.905 | | |
| Presence within 50 cm | Odour source (faeces) [1]: Subject sex [2] | 1.775 | 0.796 | 0.178 | 3.381 | 4.971 | 0.083 |
| | Odour source (urine) [1]: Subject sex [2] | 0.329 | 0.712 | -1.104 | 1.755 | | |

[1]: odour source was dummy coded with body odour being the reference level
[2]: reference level = female

**Table S4** Results of Linear Mixed Models testing whether chimpanzees show differential behavioural responses towards different odour sources. Degrees of freedom (df) were 1 except for odour source, where df = 2. Significant effects are marked in bold. Ref = reference level. Colons represent interactions between fixed effects

| Response variable | Predictor variable | Estimate | SE | $CL_{lower}$ | $CL_{upper}$ | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|
| Sniffing | Intercept | -9.266 | 0.353 | -9.982 | -8.554 | [1] | [1] |
| | Odour source (faeces)[2] | -0.253 | 0.221 | -0.699 | 0.202 | 11.730 | **0.003** |
| | Odour source (urine)[2] | 0.577 | 0.222 | 0.130 | 1.033 | | |
| | Subject sex (ref = female) | 0.219 | 0.541 | -0.883 | 1.327 | 0.164 | 0.686 |
| | Subject group (ref = Chimp A) | 1.055 | 0.475 | 0.090 | 2.031 | 4.521 | **0.033** |
| | Subject age[3] | -0.375 | 0.232 | -0.844 | 0.100 | 2.472 | 0.116 |
| Nose within 20 cm | Intercept | -8.454 | 0.416 | -9.298 | -7.615 | [1] | [1] |
| | Odour source (faeces)[2] | -0.364 | 0.309 | -0.984 | 0.268 | 9.009 | **0.011** |
| | Odour source (urine)[2] | 0.638 | 0.309 | 0.016 | 1.268 | | |
| | Subject sex (ref = female) | 0.865 | 0.646 | -0.451 | 2.184 | 1.734 | 0.188 |
| | Subject group (ref = Chimp A) | 0.810 | 0.563 | -0.337 | 1.965 | 1.991 | 0.158 |
| | Subject age[3] | -0.492 | 0.284 | -1.070 | 0.090 | 2.808 | **0.094** |
| Licking | Intercept | -8.328 | 0.651 | -9.532 | -7.014 | [1] | [1] |
| | Odour source (faeces)[2] | -1.327 | 0.587 | -2.528 | -0.135 | 4.489 | 0.106 |
| | Odour source (urine)[2] | -0.090 | 0.496 | -1.075 | 0.878 | | |
| | Subject sex (ref = female) | 0.623 | 0.764 | -0.887 | 2.191 | 0.630 | 0.427 |
| | Subject group (ref = Chimp A) | -0.061 | 0.804 | -1.684 | 1.448 | 0.006 | 0.940 |
| | Subject age[3] | -0.267 | 0.366 | -0.933 | 0.429 | 0.513 | 0.474 |
| Presence within 50 cm | Intercept | -7.120 | 0.420 | -7.969 | -6.272 | [1] | [1] |
| | Odour source (faeces)[2] | -0.105 | 0.346 | -0.796 | 0.605 | 5.391 | **0.067** |
| | Odour source (urine)[2] | 0.653 | 0.333 | -0.013 | 1.326 | | |
| | Subject sex (ref = female) | 0.373 | 0.629 | -0.918 | 1.645 | 0.347 | 0.556 |
| | Subject group (ref = Chimp A) | -0.147 | 0.559 | -1.283 | 1.006 | 0.069 | 0.793 |
| | Subject age[3] | -0.567 | 0.285 | -1.154 | 0.009 | 3.724 | **0.054** |
| Manipulating | Intercept | -6.069 | 0.615 | -7.260 | -4.834 | [1] | [1] |
| | Odour source (faeces)[2] | -3.087 | 0.663 | -4.371 | -1.856 | [1] | [1] |
| | Odour source (urine)[2] | -0.754 | 0.513 | -1.730 | 0.226 | [1] | [1] |
| | Subject sex (ref = female) | -0.185 | 0.933 | -2.226 | 1.801 | [1] | [1] |
| | Subject group (ref = Chimp A) | -1.612 | 0.836 | -3.265 | -0.105 | 3.295 | 0.069 |
| | Subject age[3] | -0.044 | 0.386 | -0.815 | 0.732 | 0.013 | 0.910 |
| | Odour source (faeces) : Subject sex | 3.966 | 1.008 | 2.014 | 6.033 | 8.857 | **0.012** |
| | Odour source (urine) : Subject sex | 2.160 | 0.881 | 0.478 | 3.736 | | |

Note that for the response variables sniffing, nose within 20 cm, licking and presence within 50cm we ran a reduced model without the interaction odour source*subject sex because the effect of the interaction was not significant. Results for the interaction of the full models are indicated in table S3

[1]: not shown because it has no reasonable interpretation
[2]: odour source was dummy coded with body odour being the reference level
[3]: z-transformed to mean=0 and sd=1; mean ± sd of the original age were for sniffing 22.2±12.4 y, nose within 20 cm 22.9±12.9 y, licking 21.0±14.4 y, presence within 50 cm 23.3±12.9 y, manipulating 19.6±13.4 y
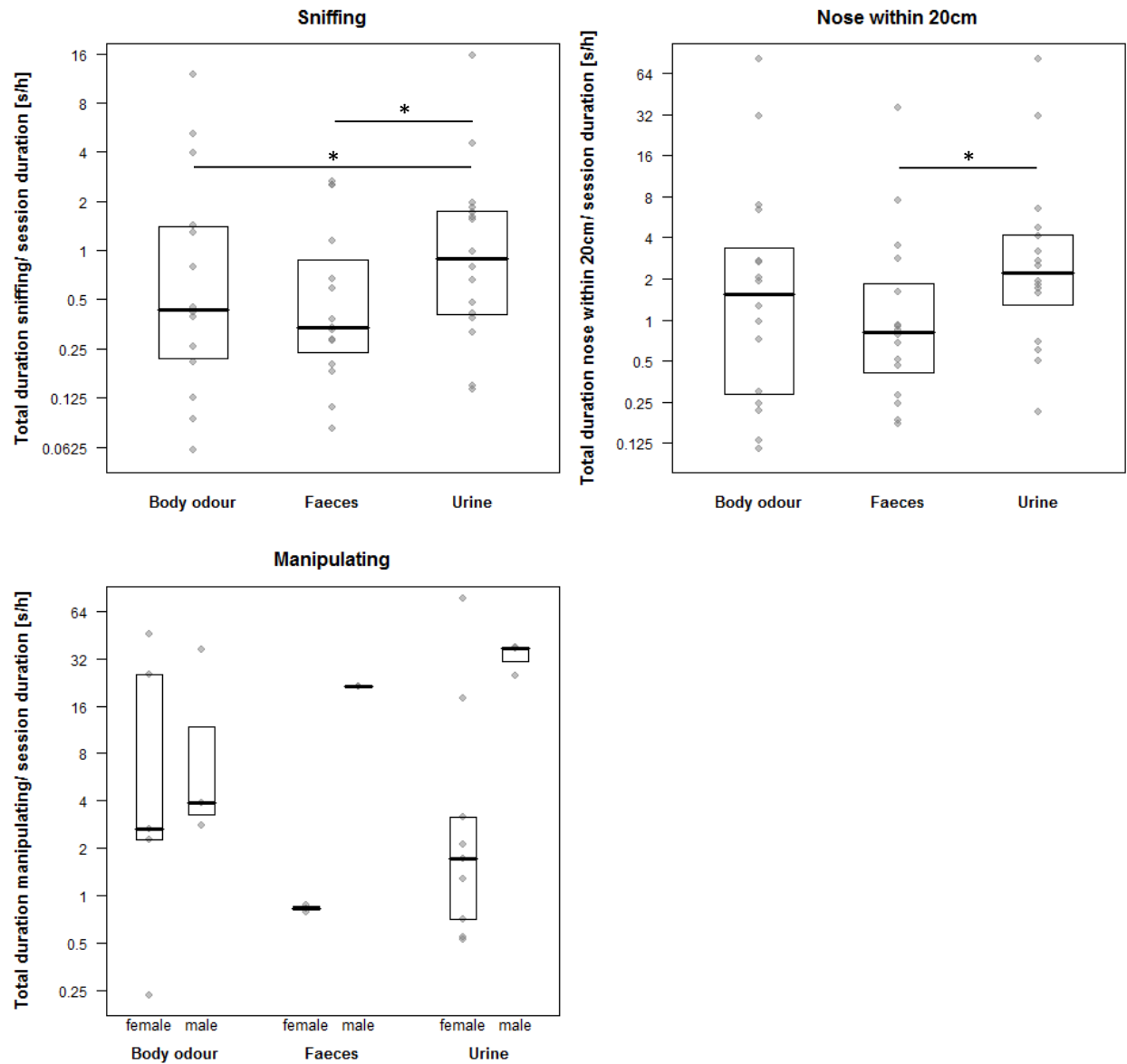
**Fig. S1** Total durations of response behaviours per individual divided by session duration towards different odour sources from one ingroup individual per group. Plots show the median (thick horizontal lines) and quartiles (boxes). Data are presented on a log-scale

**References**

1. Baayen H. 2008 *Analyzing Linguistic Data: A Practical Introduction to Statistics using R*. 1st edn. Cambridge: Cambridge University Press.

2. R Core Team. 2017 *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

3. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**. (doi:10.18637/jss.v067.i01)

4. Schielzeth H. 2010 Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution* **1**, 103–113. (doi:10.1111/j.2041-210X.2010.00012.x)

5. Field A. 2009 *Discovering Statistics Using SPSS*. 3 Pap/Cdr. Sage Publications Ltd.

6. Fox J, Weisberg S. 2011 *An {R} Companion to Applied Regression*. Second Edition. Thousand Oaks CA: Sage.

7. Dobson AJ. 2002 *An introduction to generalized linear models*. 2nd edn. Boca Raton: Chapman & Hall/CRC.

8. Forstmeier W, Schielzeth H. 2011 Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse. *Behav Ecol Sociobiol* **65**, 47–55. (doi:10.1007/s00265-010-1038-5)

9. Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS. 2009 Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* **24**, 127–135. (doi:10.1016/j.tree.2008.10.008)

10. Barr DJ, Levy R, Scheepers C, Tily HJ. 2013 Random effects structure for confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language* **68**, 255–278. (doi:10.1016/j.jml.2012.11.001)

11. Hothorn T, Hornik K. 2013 *exactRankTests: Exact Distributions for Rank and Permutation Tests*. See http://CRAN.R-project.org/package=exactRankTests.

12. Cohen J, Powderly W, Opal SM, editors. 2017 *Infectious Diseases, 2-Volume Set*. 4 edition. London: Elsevier.

13. Palagi E, Dapporto L, Borgognini Tarli S. 2005 The neglected scent: on the marking of urine in *Lemur catta*. *Behavioral Ecology and Sociobiology* **58**, 437–445.

14. Candland DK, Blumer ES, Mumford MD. 1980 Urine as a communicator in a New World primate, *Saimiri sciureus*. *Animal Learning & Behavior* **8**, 468–480. (doi:10.3758/BF03199636)

15. Epple G, Alveario MC, Belcher AM, Smith IAB. 1987 Species and subspecies specificity in urine and scent marks of saddle-back tamarins (*Saguinus fuscollis*). *International Journal of Primatology* **8**, 663–680.

16. Laska M, Hudson R. 1995 Ability of Female Squirrel Monkeys (*Saimiri sciureus*) to Discriminate between Conspecific Urine Odours. *Ethology* **99**, 39–52. (doi:10.1111/j.1439-0310.1995.tb01087.x)

17. Ueno Y. 1994 Olfactory Discrimination of Urine Odors from 5 Species by Tufted Capuchin (*Cebus apella*). *Primates* **35**, 311–323.

18. Ueno Y. 1994 Responses to urine odor in the tufted capuchin (*Cebus apella*). *Journal of Ethology* **12**, 81–87. (doi:10.1007/BF02350052)

19. Demaria C, Roeder J-J. 1989 Responses to Urinary Stimuli in Pigtailed (*Macaca nemestrina*) and Stumptailed (*Macaca arctoides*) Macaques. *Primates* **30**, 111–115.

20. Rigaill L, Suda-Hashimoto N, Ducroix L, Mouri K, Furuichi T, Garcia C. 2017 Testing for Links Between Female Urine Odor and Male Sexual Behaviors in Japanese Macaques (*Macaca fuscata*). *International Journal of Primatology* **38**, 823–837. (doi:10.1007/s10764-017-9980-y)

21. Epple G. 1978 Studies on the nature of chemical signals in scent marks and urine of *Saguinus fuscicollis* (Callitricidae, primates). *Journal of Chemical Ecology* **4**, 383–394. (doi:10.1007/BF00989496)

22. Poirotte C, Massol F, Herbert A, Willaume E, Bomo PM, Kappeler PM, Charpentier MJE. 2017 Mandrills use olfaction to socially avoid parasitized conspecifics. *Science Advances* **3**, e1601721. (doi:10.1126/sciadv.1601721)

23. Sarabian C, Ngoubangoye B, MacIntosh AJJ. 2017 Sarabian et al. 2017 Avoidance of biological contaminants through sight, smell and touch in chimpanzees. *R. Soc. open sci* **4**, 170968.

24. Henkel S, Lambides AR, Berger A, Thomsen R, Widdig A. 2015 Rhesus macaques (*Macaca mulatta*) recognize group membership via olfactory cues alone. *Behavioral Ecology and Sociobiology* **69**, 2019–2034. (doi:10.1007/s00265-015-2013-y)

25. Weiß BM, Kücklich M, Thomsen R, Henkel S, Jänig S, Kulik L, Birkemeyer C, Widdig A. 2018 Chemical composition of axillary odorants reflects social and individual attributes in rhesus macaques. *Behavioral Ecology and Sociobiology* **72**, 65. (doi:10.1007/s00265-018-2479-5)

26. Pause BM. 2017 Human chemosensory communication. In *Springer Handbook of Odor*, pp. 129–130. Springer.

27. Schilling A. 1979 Chapter 11 - Olfactory Communication in Prosimians. In *The Study of Prosimian Behavior* (eds GA Doyle, RD Martin), pp. 461–542. Academic Press. (doi:10.1016/B978-0-12-222150-7.50016-7)

28. Epple G. 1974 Primate pheromones. In *Pheromones* (ed MC Birch), pp. 366–385. New York: Elsevier.

29. Montagna W, Yun JS. 1963 The skin of primates. XV. The skin of the chimpanzee (*Pan satyrus*). *American Journal of Physical Anthropology* **21**, 189–203. (doi:10.1002/ajpa.1330210211)

30. Montagna W. 1972 The Skin of Nonhuman Primates. *American Zoologist* **12**, 109–124.

31. Wysocki CJ, Preti G. 2004 Facts, fallacies, fears, and frustrations with human pheromones. *Anatomical Record A* **218A**, 1201–1211. (doi:10.1002/ar.a.20125)

32. Clarke PMR, Barrett L, Henzi SP. 2009 What role do olfactory cues play in chacma baboon mating? *American Journal of Primatology* **71**, 493–502. (doi:10.1002/ajp.20678)

33. Rigaill L, Higham JP, Lee PC, Blin A, Garcia C. 2013 Multimodal Sexual Signaling and Mating Behavior in Olive Baboons (*Papio anubis*). *American Journal of Primatology* **75**, 774–787. (doi:10.1002/ajp.22154)