

Pregnancy is detected via odour in a wild cooperative breeder

Supplementary Information

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Details of sample collection

Banded mongooses have two anal glands, either side of the anal opening within the anal pouch. Under anaesthesia, these glands were expressed by applying gentle pressure. Approximately 300 µl of gland secretion was collected from each individual (150 µl from each gland) in 2ml snap-cap glass vials (Fisher scientific) which were cleaned by soaking for several hours in methanol, air drying then soaking in detergent and warm water (1:1000 dilution), rinsing and allowing to air dry again. The anal region was cleaned with cotton wool and a glass vial placed over the gland opening. Secretions were vortexed to mix, labelled and transferred to liquid nitrogen immediately. To avoid contamination, sterile nitrile gloves were worn and changed between individual mongooses. The examiner's fingers never came into contact with the secretion nor the top of the glass vials. Trapping and anaesthetising was conducted by trained field staff and no mongooses were observed to become ill or die as a result of the procedures carried out in this study.

During presentations in the field the same odour sample was occasionally used for multiple presentations if there was a particularly large amount of the sample. Autoclaved cotton swabs were inserted into the deforested sample vial for 15 seconds before removal and the secretion wiped on a clean tile for the presentation. A second clean swab-load of sample could be collected from several samples.

Managing observer bias in odour presentations and scoring

Odour presentations were planned, conducted, and scored by the same researcher. This allowed consistency in the recording of scent marking behaviours (depositing anal marks, faeces, urine, saliva and body odours) and also in the timing of return to foraging behaviour. In order to minimise the potential for observer bias, videos were scored several days after they were taken and the film was muted when being scored. The scorer therefore did not know the state of the odour donor or recipient at the point of scoring (the identities and states of the donor and recipient were stated verbally during the film).

Details and full results of statistical models

General linear mixed effect models (GLMMs) were constructed in R (version 3.0.2) using the lme4 package to test the effect of odour donor pregnancy status on the response of male and female recipients. All models had a Gaussian error structure with an identity link function. For male recipients, explanatory variables included the pregnancy status of the donor and the age of the donor and recipient. For female recipients, explanatory variables included the pregnancy status of the donor and recipient and the interaction between them, in addition to the ages of the donor and recipient. Age was included as banded mongooses have an age-based dominance hierarchy, which may impact on scent composition and marking behaviour. In all models the identity and social group of both donor and recipient were fitted as random effects. Model assumptions (such as normality and homogeneity of residuals and susceptibility to outliers) were checked using the 'plot.merMod' function in lme4. Collinearity of predictors was always below 0.24. Where significant interactions were detected, the Multcomp package was used to perform Tukey post-hoc comparison tests to compare response measures.

Table S1. Results of GLMMs investigating the response of female recipients to anal gland secretion from pregnant and non-pregnant females.

| Response variable | Fixed effect | Estimate | Standard error | t value | p value |
|-----------------------------------|--------------------------------------|---------------|----------------|---------------|---------------|
| Time spent inspecting odour | Intercept | 26.814 | 6.691 | 4.008 | |
| | Donor pregnancy state | -4.306 | 4.479 | -0.961 | 0.339 |
| | Recipient pregnancy state | 11.827 | 4.403 | 2.686 | 0.009 |
| | Donor age | -0.001 | 0.004 | -0.173 | 0.863 |
| | Recipient age | -0.012 | 0.004 | -3.143 | 0.002 |
| Time before returning to foraging | Intercept | 45.863 | 14.864 | 3.086 | |
| | Donor pregnancy state | -7.970 | 9.949 | -0.801 | 0.425 |
| | Recipient pregnancy state | 21.956 | 9.782 | 2.245 | 0.027 |
| | Donor age | 0.0004 | 0.010 | 0.046 | 0.963 |
| | Recipient age | -0.013 | 0.008 | -1.570 | 0.120 |
| Number of scent marks deposited | Intercept | 11.602 | 2.064 | 5.622 | |
| | Donor pregnancy state | -4.507 | 1.717 | -2.626 | 0.010 |
| | Recipient pregnancy state | -2.254 | 1.720 | -1.311 | 0.193 |
| | Donor state * recipient state | 7.727 | 2.392 | 3.231 | 0.0017 |
| | Donor age | -0.0004 | 0.001 | -0.297 | 0.767 |
| | Recipient age | -0.002 | 0.001 | -2.313 | 0.023 |

Female responses to presented odours varied dependent upon the reproductive state of both donor and recipient. Significant effects are highlighted in bold. Analyses based upon the results of 94 odour presentations to 28 individual female mongooses, using 54 female odour donors. Donor pregnancy state was coded as 0 = non-pregnant, 1 = pregnant.

Table S2. Tukey test outputs testing the interaction between recipient and odour donor pregnancy state

| Comparison groups | Estimate | Std. Error | z value | P value |
|-----------------------------------|---------------|--------------|---------------|--------------|
| non to preg - non to non | -2.006 | 1.560 | -1.286 | 0.571 |
| preg to non - non to non | -4.598 | 1.636 | -2.811 | 0.025 |
| preg to preg - non to non | 0.826 | 1.590 | 0.520 | 0.954 |
| preg to non - non to preg | -2.592 | 1.754 | -1.478 | 0.450 |
| preg to preg - non to preg | 2.832 | 1.716 | 1.650 | 0.349 |
| preg to preg - preg to non | 5.423 | 1.601 | 3.388 | 0.004 |

The pregnancy status of donors is given first in the comparison groups. 'non' = non-pregnant, 'preg' = pregnant. Significant effects are highlighted in bold.

Table S3. Output of GLMMs testing the responses of males to anal gland secretion from pregnant and non-pregnant females.

| Response variable | Fixed effects | Estimate | Standard Error | T value | P value |
|-----------------------------------|------------------------------|----------------|----------------|---------------|--------------|
| Time spent inspecting odour | Intercept | 20.921 | 9.342 | | |
| | Donor age (increasing) | 0.004 | 0.007 | 0.565 | 0.575 |
| | Donor pregnancy state | -10.217 | 4.478 | -2.282 | 0.029 |
| | Recipient age | -0.003 | 0.004 | -0.895 | 0.376 |
| Time before returning to foraging | Intercept | 45.438 | 13.868 | | |
| | Donor age (increasing) | 0.001 | 0.011 | 0.120 | 0.905 |
| | Donor pregnancy state | -16.456 | 6.705 | -2.454 | 0.019 |
| | Recipient age | -0.004 | 0.005 | -0.796 | 0.430 |
| Number of scent marks deposited | Intercept | 6.794 | 2.547 | | |
| | Donor age (increasing) | 0.002 | 0.002 | 1.162 | 0.252 |
| | Donor pregnancy state | -3.940 | 1.203 | -3.275 | 0.002 |
| | Recipient age | -0.0004 | 0.001 | -0.368 | 0.715 |

Analyses are based upon the results of 48 presentations to 32 individual males, using 26 female odours. Donor pregnancy state was coded as 0 = non-pregnant, 1 = pregnant. Original models also included the interactions between reproductive state and donor age, however these were sequentially removed due to non-significance. Significant effects are highlighted in bold.

Table S4: Output of models testing the correlations between time before return to foraging and scent marking behaviours

| Model testing | Fixed effect | estimate | SD | t value | p value |
|---|-------------------|----------|-------|---------|------------|
| Correlations with time before return to foraging in the Female data set | Intercept | 2.842 | 6.163 | 0.461 | |
| | Marking frequency | 5.008 | 0.639 | 7.836 | 7.45 e-12 |
| | Intercept | 6.962 | 3.545 | 1.964 | |
| | Contact time | 1.829 | 0.113 | 16.164 | 9.358 e-29 |
| Correlations with time before return to foraging in the Male data set | Intercept | 12.133 | 4.957 | 2.448 | |
| | Marking frequency | 3.031 | 0.627 | 4.832 | 1.48 e-05 |
| | Intercept | 13.634 | 2.809 | 4.854 | |
| | Contact time | 1.225 | 0.133 | 9.217 | 4.158 e-12 |

Female analyses based on the same 94 odour presentations and male analyses based on the same 48 presentations analysed previously