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1 **Pregnancy is detected via odour in a wild cooperative breeder**

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6

7

8 **Abstract**

9 Among mammals, scent has long been known to encode oestrus, however in many species detecting
10 pregnancy may also be important in terms of both competition and mate-choice. Here we show,
11 through odour presentation experiments, that pregnancy is discernible via scent by both sexes in the
12 cooperatively breeding banded mongoose. Males spent more time investigating and were more
13 likely to scent mark the odours of non-pregnant females, compared to pregnant females. Females
14 showed increased levels of scent marking when odours were of the same reproductive state as
15 themselves. These results present the first direct demonstration that pregnancy is detectable via
16 scent in wild cooperative breeders. Detecting pregnancy may be particularly important in
17 cooperative breeders as, in addition to the competition between males for receptive mates, there is
18 also intense competition between females for access to alloparental care. Consequently, dominant
19 females benefit from targeting reproductive suppression towards subordinates that represent direct
20 threats, such as pregnant females.

21

22 **Keywords:** scent, pregnancy, reproductive competition, cooperative breeding

23

24 **Introduction**

25 Scent cues are heavily used among mammals and are known to encode information on female
26 reproductive state, with many studies demonstrating that males can detect oestrus [1-3]. However,
27 relatively little is known about whether scent can communicate pregnancy status [4, 5]. Detecting
28 pregnancies may help males to avoid courting pregnant females, while it may help females assess
29 their competitive landscape [6]. This may be particularly beneficial among cooperative breeders,
30 where competition over access to breeding positions, and hence access to alloparental care, is
31 intense in both sexes [7]. Here, per-capita breeding success generally declines when multiple
32 females breed, and dominant females may respond by suppressing subordinates that may be a
33 particular threat, for example those that are pregnant or are likely to become pregnant [7-9]. In

34 addition, dominant males often invest highly in guarding females during fertile periods [7]. Thus in
35 cooperative breeders the communication of pregnancy may benefit both mate-choice and intra-
36 sexual competition.

37

38 Studies investigating olfactory cues to pregnancy in mammals have so far focused on investigating
39 the chemical profiles of female scents before and during pregnancy e.g. [4, 10, 11]. While these
40 studies have discovered chemical differences between pregnant and non-pregnant females, they do
41 not demonstrate whether these changes are detected or acted upon by conspecifics. It is therefore
42 possible that differences in chemical profiles are simply a by-product of hormonal changes that
43 occur during mammalian gestation [4] and are not used to detect pregnancy.

44

45 Here, we investigate behavioural responses to scents of pregnant and non-pregnant female banded
46 mongooses *Mungos mungo*. This species lives in mixed sex groups (mean group size = 29) where a
47 'core' of 1-5 dominant breeders of each sex breed up to 4 times per year, and younger subordinates
48 breed occasionally [12]. Reproduction is synchronised within groups, with all adult females entering
49 oestrus within the same week, and giving birth together, often on the same night [12]. The resulting
50 litter is raised communally by both breeders and non-breeders [12]. Dominant females benefit from
51 increased reproductive success when breeding alongside other females, probably due to reduced
52 levels of infanticide [8]. However, once the number of breeding females exceeds seven, per-capita
53 reproductive success declines due to increased pup mortality [8]. Dominant females in large groups
54 respond by evicting subordinate females, particularly targeting those that are pregnant [8]. Thus the
55 detection of pregnancies could provide a mechanism for assessing the competitive landscape of the
56 group. In addition, synchronous oestrus constrains the number of females a male can guard, so
57 dominant males invest highly in one or two mates per reproductive bout [12]. Pregnancy detection
58 could therefore help males to avoid guarding already-mated females.

59

60 We predict that (1) if males use scent signals within mate-choice they should show heightened
61 responses to non-pregnant females and (2) if females use scent signals within reproductive
62 competition, they should show heightened responses to the odours of females representing direct
63 reproductive threats, in particular pregnant females should show a greater response to odours from
64 other pregnant females.

65

66 **Methods**

67 This study was conducted in Queen Elizabeth National Park, Uganda (0°8'2"S, 29°51'42"E) where a
68 population of wild but habituated banded mongooses have been studied continuously since 1995.
69 Groups are visited by trained observers approximately every two days to collect life history and
70 behavioural data. Detailed descriptions of the population, habitat, and climate are provided by [12].

71

72 *Odour collection*

73 Banded mongooses are prolific scent markers, engaging in conspicuous anal marking, urination and
74 defecation at latrine sites [13]. Previous work has found that anal marking plays a key role in within-
75 group communication and intrasexual competition [13], so for this study we focused on anal gland
76 secretions (AGS).

77

78 AGS were collected from females in 4 social groups between April and July 2015 following [14]. We
79 obtained 111 samples (63 pregnant and 48 non-pregnant but non-oestrus) from 54 individual
80 females that were each sampled 1-3 times. In brief, animals were trapped in baited Tomahawk traps
81 and anaesthetized using isoflurane [13]. Pregnancy status was determined by an ultrasound scan 7-
82 14 days after behavioural oestrus and AGS was collected in a clean 2ml snap-cap glass vial and was
83 transferred immediately to liquid nitrogen. Further details are provided in the Supplementary
84 Information.

85

86 *Odour presentations*

87 A total of 142 odour presentations were conducted from July to August 2015 on 32 males and 28
88 females from two well-habituated social groups. Recipients were presented with freshly defrosted
89 AGS samples from pregnant or non-pregnant females. AGS samples were spread upon a clean
90 ceramic tile using an autoclaved cotton swab, and presented directly to the recipient individual
91 following [14]. Presentations were conducted when the recipient was foraging at least 1m away
92 from other mongooses. Responses were filmed using a handheld camera and scored after the field
93 session. Three measures of response to odour presentations were considered (1) the time before
94 returning to foraging behaviour (2) the time spent inspecting the odour (within 30 cm), and (3) the
95 number of scent marks deposited on or around the odour. Previous research on banded mongooses
96 and other species suggests that direct over-marking can obliterate the original scent and is therefore
97 likely to function in competition [13, 15, 16]. For presentations to female recipients, who may use
98 scent cues for intra-sexual competition, we recorded the number of marks deposited directly on top
99 of an odour. For presentations to male recipients, we recorded the number of marks deposited
100 within 30cm of the odour as vicinity marking is thought to function within mate-acquisition, rather
101 than competitive interactions [15]. The three measures of scent marking behaviour are not
102 fully independent of one another, both scent marking and time spent inspecting an odour
103 correlate with the time taken to return to foraging in male and female datasets. For full
104 details of this correlation see supplementary material table S4. Donors and recipients were
105 sexually mature adults (aged >12 months for females and >24 months for males [12]). Recipient
106 females were presented to within seven days of an ultrasound scan confirming their reproductive
107 state. Where individuals were presented to multiple times, a minimum of 48 hours lapsed between
108 presentations to prevent habituation to the protocol. Recipients were presented with odours from
109 non-neighbouring groups to avoid confounding results with previous information on the
110 reproductive state of odour donors.

111

112 General linear mixed effect models (GLMMs) were constructed in R (version 3.0.2) using the lme4
113 package [17] to test the effect of odour donor pregnancy status on the response of male and female
114 recipients. Where significant interactions were detected, the Multcomp package [18] was used to
115 perform Tukey post-hoc comparison tests compare response measures. All models were fit with
116 Gaussian assumptions as response variables conformed to normal distributions. For full model
117 details and outputs see Supplementary Information, Tables S1-3.

118

119 **Results and Discussion**

120 Pregnancy appears discernible by scent in the banded mongoose, with both sexes responding
121 differently to odours from pregnant and non-pregnant females. In line with our first prediction,
122 males spent longer investigating non-pregnant odours (GLMM: $t = -2.282$, $p = 0.029$, Figure 1a) and
123 took longer to return to foraging (GLMM: $t = -2.454$, $p = 0.019$, Figure 1b), suggesting that odours
124 encode information relevant to mate-choice. Detecting pregnancy via scent is likely to be beneficial
125 to males, as it could prevent them from wasting time and energy mate-guarding pregnant females.

126 Male banded mongooses also deposited more scent marks around the odours of non-pregnant
127 females (GLMM: $t = -3.275$, $p = 0.002$, Figure 1c). Increased scent marking by males may function
128 in intra-sexual competition, whereby males that invest highly in scent marking are more effective
129 mate-guards [19]. Alternatively, scent marking may be involved in female-choice, as has been
130 demonstrated in other mammals [20]. Despite being mate-guarded while in oestrus, banded
131 mongoose females often refuse the mating attempts of their guards and 68% of pups are fathered
132 by a male not observed to guard the female [21]. Scent marking in the vicinity of receptive females
133 may therefore serve to advertise males to potential mates.

134

135 Female banded mongooses responded differently to pregnant and non-pregnant odours in their
136 over-marking response depending on their own pregnancy status (GLMM: $t = 3.231$, $p = 0.0017$,

137 Figure 2), suggesting that they can detect pregnancy in other females. In line with our prediction,
138 pregnant odours received more over-marks from pregnant recipients than from non-pregnant
139 recipients (Tukey: $z = 3.338$, $p = 0.004$). Similarly, non-pregnant recipients marked the odours of
140 non-pregnant females significantly more than they did the odours of pregnant females (Tukey; $t = -$
141 2.811 , $p = 0.025$). The finding that females show heightened over-marking when odours were from
142 females in the same reproductive state suggests that scent marking may be related to intra-sexual
143 competition, whereby the scents of potential competitors are over-marked in order to obliterate
144 their scent [13, 15].

145

146 The finding that pregnant females inspected scents for longer (GLMM: $t = 2.686$, $p = 0.009$) and
147 took longer to return to foraging (GLMM: $t = 2.245$, $p = 0.027$) than non-pregnant females
148 suggests that detecting the reproductive state of others could be particularly important when
149 pregnant. Indeed, evictions are most common when dominant females are pregnant [8]. We also
150 found that younger females spent longer inspecting odours (GLMM: $t = -3.143$, $p = 0.002$) and
151 deposited more scent marks around odours (GLMM: $t = -2.313$, $p = 0.023$) than older females,
152 possibly as younger subordinate individuals are more likely to be targeted for eviction and their
153 litters are more vulnerable to infanticide than those of dominants [22]. Furthermore, abortion and
154 reabsorption of pregnancies are known to occur in the banded mongoose [8] and, as in other
155 mammals [9], these may be adaptive strategies for mothers who find themselves out-competed or
156 out of synchrony with other breeders. Detecting pregnancies may therefore help females to avoid or
157 respond to reproductive competition.

158

159 In many territorial species, reproductive threats come not just from within the social group, but also
160 from competing social groups [9]. In the banded mongoose, neighbouring groups engage in frequent
161 aggressive encounters over territory, often resulting in severe injury and deaths [23]. As we

162 presented odours from individuals that recipients are unlikely to be familiar with, it is possible that
163 scents may be used to assess the competitive landscape between social groups. For example,
164 knowing the reproductive status of females in other groups could allow individuals to time
165 aggressive encounters to periods of vulnerability, such as when pups are present (young pups are
166 most likely to be present when females are non-pregnant and non-oestrus). In addition, mating
167 between groups sometimes occurs during aggressive encounters [23]. Through inspecting scent
168 marks, males may be able to assess potential inter-group mating opportunities. Future work
169 investigating the timing of inter-group interactions will shed light on these possibilities.

170

171 **Author contributions**

172 JM conceived the study, collected data and conducted analyses. HJN and JM wrote the paper. HJN
173 supervised data collection and analyses. MAC coordinated the field project. All authors gave final
174 approval for publication and agree to be accountable for the all aspects of the work.

175

176 **Data accessibility**

177 Data are available in Dryad (doi:10.5061/dryad.0ss0k). Temporary link:

178 <http://datadryad.org/review?doi=doi:10.5061/dryad.0ss0k>.

179

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182

183 **Competing interests**

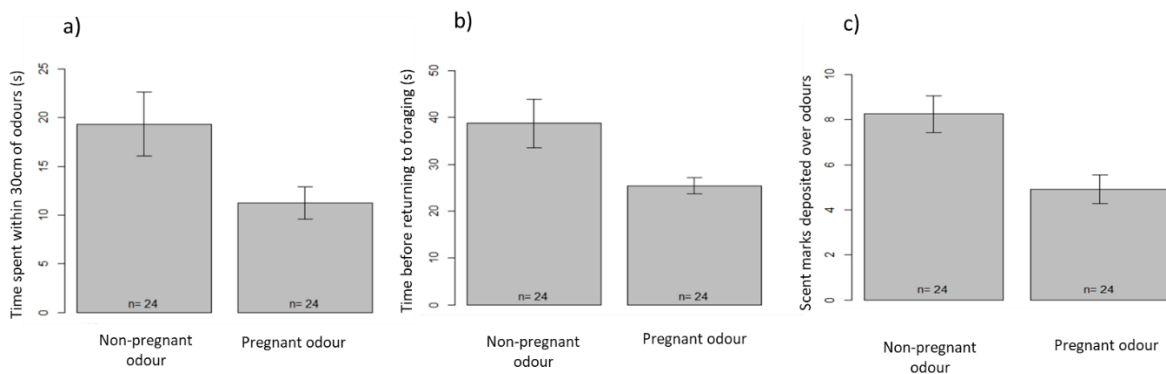
184 None

185

186 **Ethical statement**

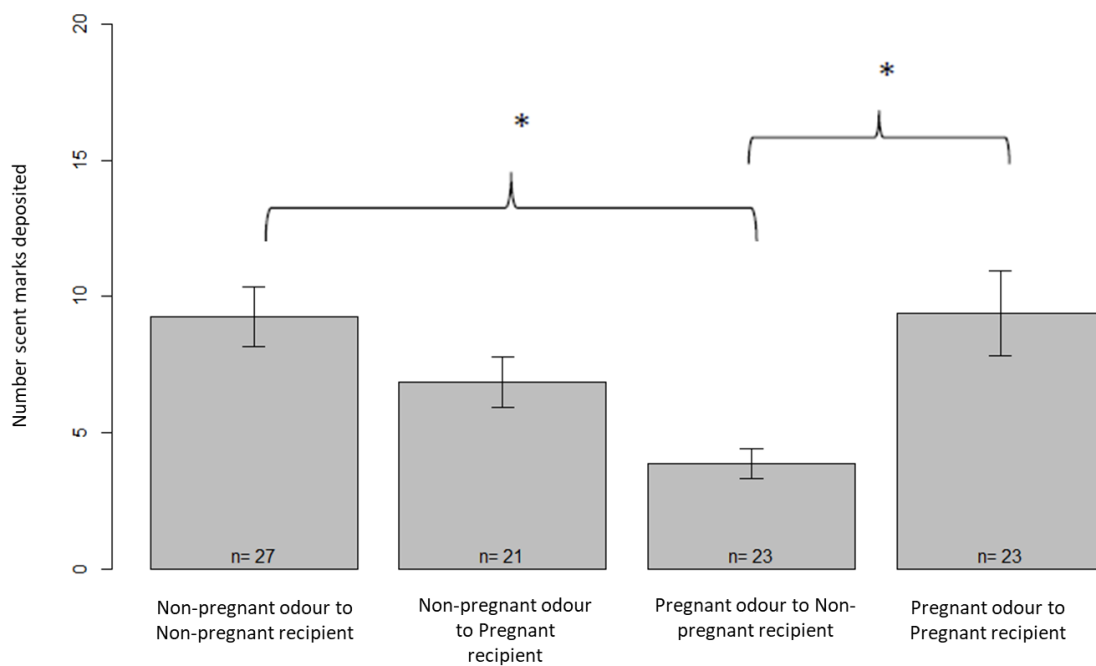
187 Research was approved by the Uganda National Council for Science and Technology and Uganda
 188 Wildlife Authority (EDO/35/01), and procedures were approved by the University of Exeter's Ethical
 189 Review Committee.

190



191

192 Figure 1: Differences in the response of males to the odours of pregnant and non-pregnant females
 193 in relation to (a) the length of time spent within 30 cm of the odour (b) the length of time before
 194 returning to foraging and (c) the number of scent marks deposited within 30cm of the odour. Error
 195 bars show standard error.



Female to Female Presentation Format

196

197 Figure 2: The number of scent marks deposited by pregnant and non-pregnant recipients on scents
198 from pregnant and non-pregnant donors. Brackets and asterisks illustrate significant differences
199 between conditions at either end of the bracket. Error bars show standard error.

200

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