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

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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.

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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
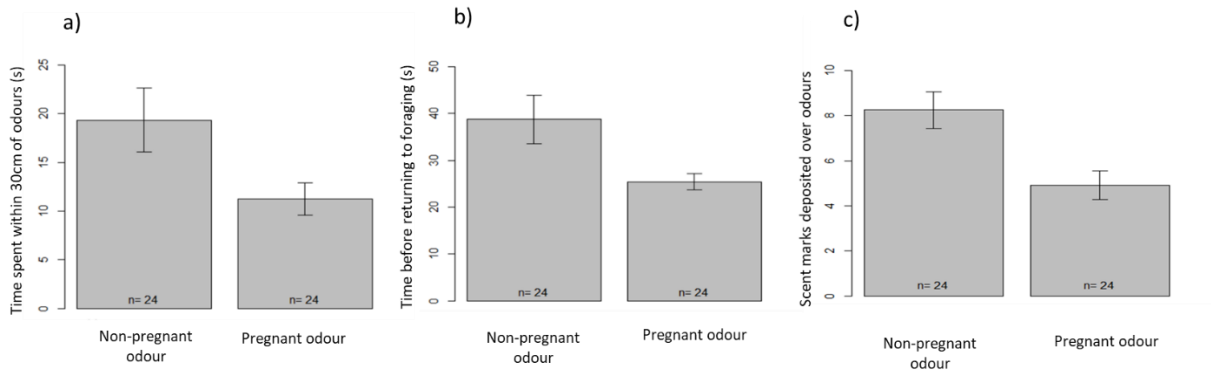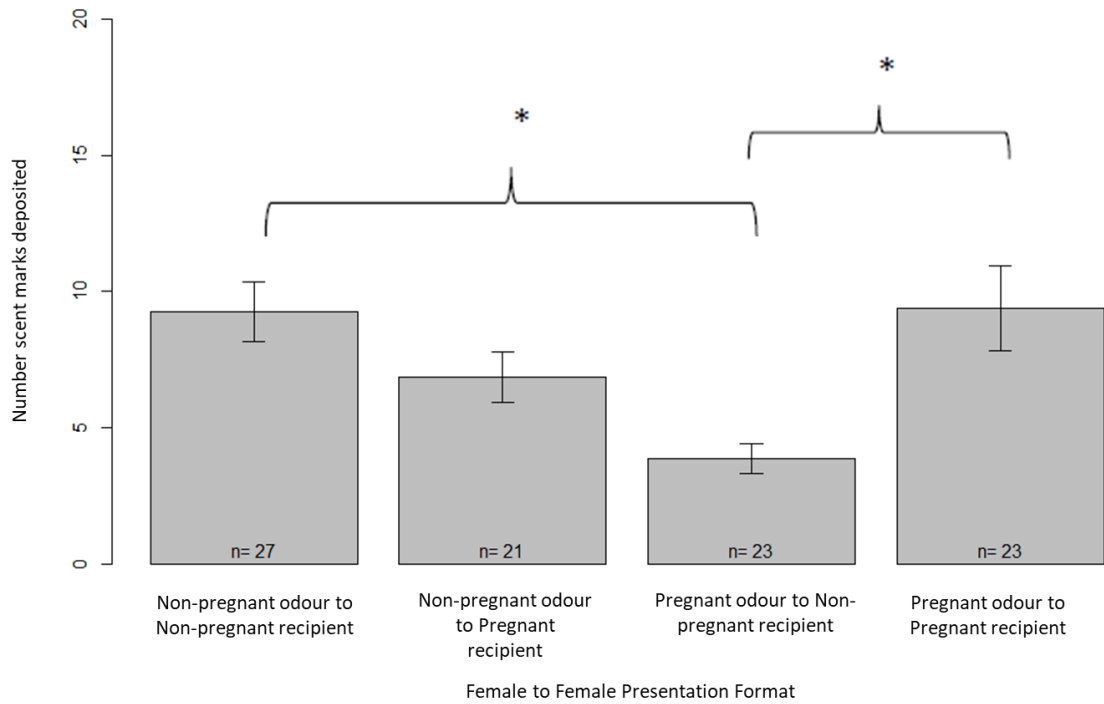
189   Review Committee.

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.

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.

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