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1 **Elevated glucocorticoid concentrations during gestation predict reduced**

2 **reproductive success in subordinate female banded mongooses**

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14 Dominant females in social species have been hypothesised to reduce the reproductive success of

15 their subordinates by inducing elevated circulating glucocorticoid concentrations. However, this

16 'stress-related suppression' hypothesis has received little support in cooperatively breeding species,

17 despite evident reproductive skews among females. We tested this hypothesis in the banded

18 mongoose (*Mungos mungo*), a cooperative mammal in which multiple females conceive and carry to

19 term in each communal breeding attempt. As predicted, lower-ranked females had lower

20 reproductive success, even among females that carried to term. While there were no rank-related

21 differences in faecal glucocorticoid (fGC) concentrations prior to gestation or in the first trimester,

22 lower-ranked females had significantly higher fGC concentrations than higher-ranked females in the

23 second and third trimesters. Finally, females with higher fGC concentrations during the third

24 trimester lost a greater proportion of their gestated young prior to their emergence from the

25 burrow. Together, our results are consistent with a role for rank-related maternal stress in

26 generating reproductive skew among females in this cooperative breeder. While studies of

27 reproductive skew frequently consider the possibility that rank-related stress reduces the

28 conception rates of subordinates, our findings highlight the possibility of detrimental effects on

29 reproductive outcomes even after pregnancies have become established.

30

32   In animal societies, subordinate females often have lower reproductive success than dominant

33   females. The stress-related suppression hypothesis proposes that dominant females suppress

34   subordinate reproduction through behaviours that lead to chronic elevations in circulating

35   glucocorticoids (GCs) and consequent reproductive down-regulation [1–4]. Notably though,

36   compelling support for this hypothesis remains scarce in cooperatively breeding societies, where

37   reproductive skews among females are frequently apparent [1,2; but see 3,5]. Stress-related

38   suppression might only be necessary, however, in the subset of cooperative breeders in which

39   subordinate females do still attempt to breed, as complete reproductive restraint by subordinates

40   might otherwise obviate the need for dominants to stress their subordinates [3,6,7]. Furthermore,

41   stress-related suppression could actually be difficult to detect using the approach most-commonly

42   employed to test it (comparisons of the average GC levels of dominants and subordinates), if

43   dominants target only a subset of likely breeders and do so only during periods when subordinate

44   reproduction would otherwise be costly to dominants [3,5,6]. These suggestions have led to calls for

45   further tests in cooperatively breeding species in which subordinates do attempt to breed, focussing

46   on those subordinates attempting to breed at the same time as their dominants [3,6].

47   While socially-induced GC elevations have frequently been considered a potential cause of reduced

48   conception rates among subordinates, they also have the potential to compromise the outcomes of

49   established pregnancies. For example, elevated GCs during pregnancy may impact in utero or early

50   post-natal development and affect offspring health, condition, and survival [6,7]. While studies of

51   cooperatively breeding mammals have shown that being subjected to aggression by the dominant

52   female is associated with increased abortion rates among subordinates [3,8], whether rank-related

53   maternal stress compromises reproductive outcomes among subordinates that do manage to carry

54   to term has yet to be investigated. If subordinate reproductive success was reduced as a result of

55   elevated GC concentrations during gestation, one might make three predictions: pregnant females

56   of lower social rank will have (1) reduced reproductive success and (2) elevated GC concentrations

57 during gestation, and (3) females experiencing higher gestational GCs will have reduced reproductive

58 success.

59 Here, we test these three predictions with a detailed investigation of faecal glucocorticoid (fGC)

60 concentrations and reproductive success in female banded mongooses (*Mungos mungo*). Banded

61 mongooses live in stable cooperatively breeding groups comprising a "core" of breeding adults (1–5

62 females and 3–7 males) that reproduce 3–4 times per year, alongside a subset of younger individuals

63 that breed occasionally [9]. Aggression received by pregnant subordinates can result in eviction and

64 abortion [8], but pregnant subordinates do often breed successfully alongside pregnant dominants

65 [9]. The rank-related patterns of reproductive success among females that carry to term have yet to

66 be investigated, along with the role that GCs may play in generating them.

67

68 **Methods**

69 We studied a population of banded mongooses living in Queen Elizabeth National Park, Uganda

70 (0°12'S; 29°53'E) between December 2010 and April 2014. All animals were marked and habituated

71 to close observation (< 5 m). Groups were observed every 1 - 4 days to record all breeding events.

72 We ran generalised linear mixed models (GLMMs) using the lme4 package [10] in R v3.1.1 [11] with

73 Poisson and binomial data fitted with log and logit link functions, respectively. Female, social group,

74 and litter identities were included as random intercepts in all models to control for repeated

75 measures.

76

77 Pregnancy can be detected at around 40 days by swelling of the abdomen [12] and birth can be

78 detected by a sudden decrease in female body size [13]. Females were captured during pregnancy to

79 estimate the number of foetuses each carried by palpation [12]. We assigned maternity using a

80 combination of phenotypic and microsatellite data; full details are given in [14]. Analyses of

81 reproductive success were limited to communal litters in which at least one pup emerged.

82

83 We collected 218 faecal samples from 35 females prior to and during gestation (2.5 ± 0.3 samples

84 per female pregnancy, mean ± SE; number of samples collected per time period: pre-gestation = 59

85 samples, first trimester = 57 samples, second trimester = 45 samples, third trimester = 54 samples).

86 Full details of sample collection and hormone analysis including validations are given in [15]. In brief,

87 all samples were collected between 6:30am and 10:00am and stored on ice [15]. Hormones were

88 extracted from faecal samples using a wet-weight extraction (adapted from [16]) and then analysed

89 using an enzyme immunoassay.

90

91 *1. Do lower ranking female experience reduced reproductive success?*

92 We calculated three measures of reproductive success for each female recorded as having given

93 birth: (i) the number of foetuses, (ii) the number of emergent offspring, and (iii) the proportion of

94 foetuses surviving to emergence. We fitted each of these three measures as a response variable in a

95 GLMM. Rank (determined by ranked age following [17]) was fitted as a fixed effect as were female

96 age, group size, rainfall (month prior to conception), and pre-conception body mass [13] to control

97 for other factors which may lead to variation in reproductive success.

98

99 *2. Do lower ranking females experience elevated fGCs during gestation?*

100 We fitted fGC concentrations as a response variable in a GLMM with rank as the main predictor of

101 interest. As GC concentrations may vary within a breeding attempt, we also fitted an interaction

102 between rank and stage of pregnancy (pre-gestation; first trimester; second trimester; third

103   trimester) as well as fixed effects of female age, group size, rainfall, and pre-conception body mass

104   to control for other factors which may contribute to fGC variation.

105

106   *3. Do females with higher fGCs during gestation have reduced reproductive success?*

107   We fitted the number of emergent offspring and the proportion of foetuses surviving to emergence

108   as response variables in two separate GLMMs with fGCs during the third trimester as the predictor

109   of interest. We focused this analysis on fGCs in the third trimester because that is when we saw the

110   clearest difference in fGCs between low- and high-ranking females.

111

112   **Results**

113   Lower-ranking females that carried to term experienced lower reproductive success than higher-

114   ranking females, both when measured as the number of assigned offspring ($\chi^2_{(1)}$ = 4.18, *P* = 0.041,

115   figure 1a) and the proportion of foetuses surviving to emergence ($\chi^2_{(1)}$ = 4.29, *P* = 0.038, figure 1c).

116   There was no effect of rank on the number of foetuses carried by a female ($\chi^2_{(1)}$ = 0.027, *P* = 0.87).

117   We found a significant interaction between female rank and pregnancy stage on fGC concentrations:

118   lower-ranking females did not differ from higher-ranking females prior to conception or during the

119   first trimester but had elevated fGCs during the second and third trimesters ($\chi^2_{(1)}$ = 4,18, *P* = 0.041,

120   figure 2). Females experiencing higher fGC concentrations during the third trimester had fewer

121   assigned offspring than those with lower GCs ($\chi^2_{(1)}$ = 5.26, *P* = 0.022, figure 1b) and a lower

122   proportion of their foetuses survived to emergence ($\chi^2_{(1)}$ = 4.07, *P* = 0.044, figure 1d). Full model

123   outputs are included in supplementary material (S1).

124

125 **Discussion**

126 Our findings are consistent with the hypothesis that subordinate female banded mongooses exhibit

127 reduced reproductive success as a result of rank-related maternal stress during gestation. Lower-

128 ranked females had lower reproductive success than higher-ranked females (despite conceiving

129 litters of the same size), both when measured as the proportion of foetuses surviving to emergence

130 and the number of emergent offspring. Whereas higher- and lower-ranked females had similar fGC

131 concentrations prior to gestation and during the first trimester, lower-ranked females showed

132 significantly elevated fGC concentrations during the second and third trimesters. These results

133 highlight the possibility that stress-related suppression of subordinate reproduction arises through

134 gestational effects that compromise offspring survival either during the latter stages of pregnancy or

135 soon after birth (prior to emergence from the burrow). Accordingly, females that experienced higher

136 fGC concentrations during the third trimester had fewer emergent pups and a lower proportion of

137 foetuses surviving to emergence.

138

139 Rank-related differences in reproductive success among female mammals commonly occur due to

140 differences in conception rates, either because subordinate females exercise reproductive restraint

141 or because their ability to conceive is compromised by active interference by dominant females

142 [18,19]. In contrast, we have demonstrated a rank-related difference in reproductive success within

143 females that carry to term. As there was no observable rank-related variation in litter size *in utero*,

144 this rank-related difference in reproductive success could well have arisen from pre-natal

145 developmental impacts on offspring survival either during late pregnancy or during the early post-

146 natal period. A role for rank-related maternal stress during late gestation in generating these effects

147 on offspring survival would be consistent with experimental evidence that late-gestational GC

148 elevations can inhibit offspring development [4,20]. In the absence of experimental evidence of a

149 role for maternal GC elevations, however, it is also possible that alternative mechanisms, such as

150　early post-natal infanticide [21], play a role in generating the observed rank-related variation in

151　offspring survival from detection as a foetus to emergence from the burrow.

152

153　The stress-related suppression hypothesis posits that elevated GC concentrations observed in lower

154　ranking females are a result of aggression from dominant females.　However, conspicuous

155　aggression among female banded mongooses is rare outside of eviction events [9]. As such, the

156　elevated GC concentrations observed here may not be a product of overt aggression. Our findings

157　cannot be attributed instead to simple age effects, in which younger females struggle to meet the

158　resource-demands of gestation (and hence exhibit differential GC elevations), as our analyses

159　control for variation in absolute age and attribute variation in both reproductive success and

160　gestational GC concentrations to variation in rank *per se*. However, the gestational GC elevations of

161　lower-ranked females could arise at least in part from energetic differences during gestation. For

162　example, subordinates may be competitively excluded from resources by dominant females.

163　Alternatively, as intra-sexual conflict among females may frequently be resolved without overt

164　physical conflict, these GC elevations could also reflect responses to more subtle rank-related

165　outcomes, such as social isolation [22]. Either way, our findings highlight the possibility that stress-

166　related suppression of subordinate reproduction may occur in the absence of conspicuous

167　aggression.

168

169　**Ethical Statement**

170　All　research　was　carried　out　under　permit　from　Uganda　Wildlife　Authority

171　(UWA)　and　Uganda　National　Council　for　Science　and　Technology　(UNCST).　All

172　methods　used　received　ethical　approval　from　UWA,　UNCST,　and　the　Ethical

173　Review Committees of the University of Exeter and Chester Zoo.

174 **Data accessibility**

175 All data analysed in this study are available online in the supplementary material (S2).

176

177 **Competing interests**

178 We have no competing interests.

179

180 **Author's contributions**

181 JS conceived the study, designed the study, organised and carried out fieldwork, organised hormone

182 analyses, carried out parentage assignment and all statistical analyses, and drafted the manuscript;

183 HN carried out genetic analyses and commented on the manuscript; HM, EV, and FT all organised

184 and carried out fieldwork and commented on the manuscript; SW managed hormone analysis and

185 advised on collection of faecal samples; MC managed fieldwork and supervised the design of the

186 study, analysis and drafting of manuscript; AY supervised design of the study, analysis and drafting of
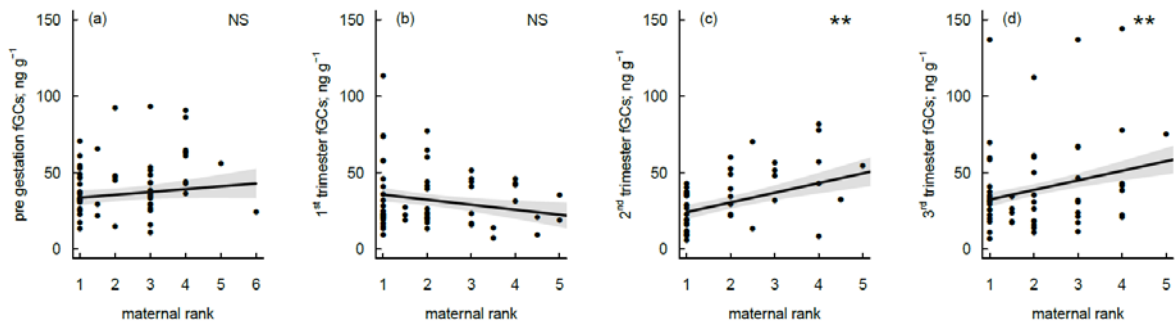
187 manuscript.

188

189 **Acknowledgements**

194
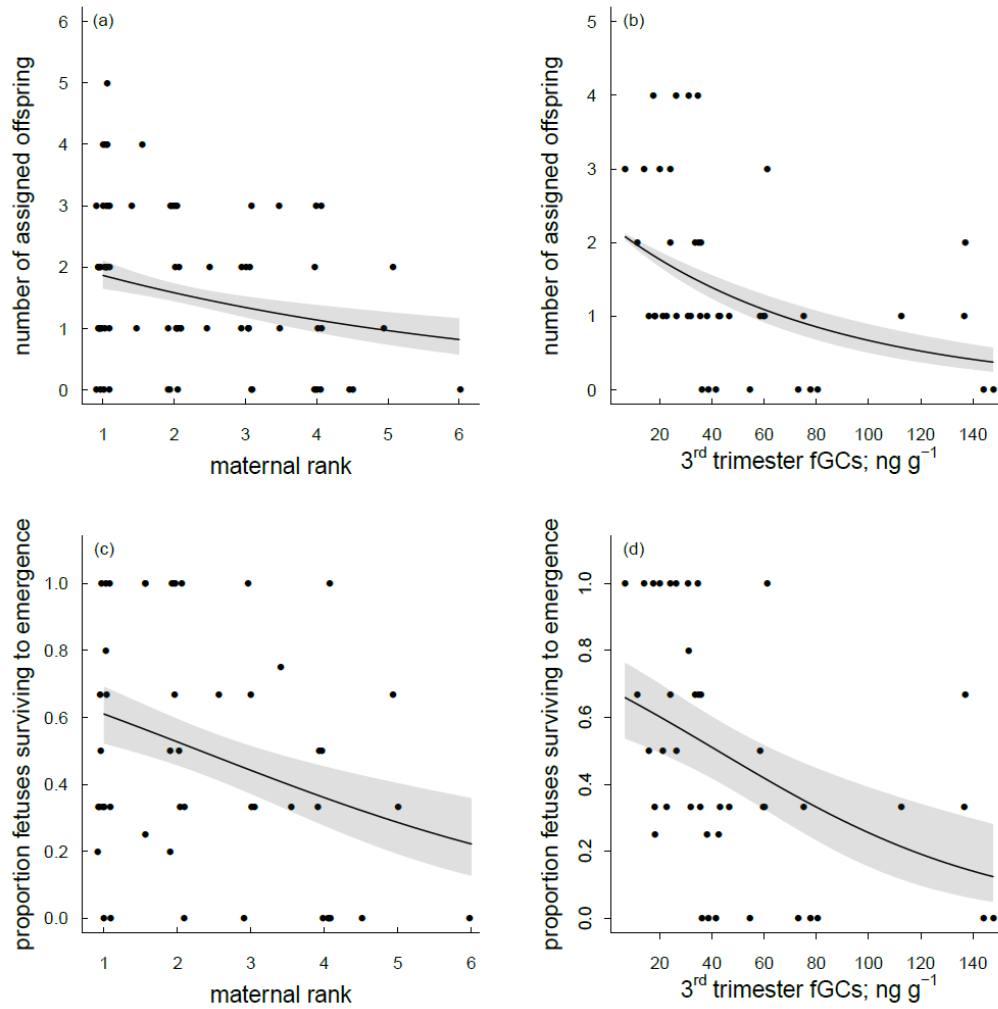
Figure 1. (a, c) Maternal rank and (b, d) gestational fGC concentrations predict female reproductive success. Points show raw values and lines with shaded regions show predicted trends with confidence intervals from GLMMs.

200

201

Figure 2. Female fGC concentrations vary during gestation dependant on maternal rank. Dots show raw values and lines and shaded areas show predicted estimates and confidence intervals from a GLMM. Significance values from post-hoc testing of the effect of maternal rank on fGC concentrations (a) within a pre-gestation phase and (b-d) during 3 trimesters where 'NS': $p > 0.05$; '**': $p < 0.001$.

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