Sanderson, JL, Nichols, HJ, Marshall, HH, Vitikainen, EIK, Thompson, FJ, Walker, SL, Cant, MA and Young, AJ

Elevated glucocorticoid concentrations during gestation predict reduced reproductive success in subordinate female banded mongooses

Elevated glucocorticoid concentrations during gestation predict reduced reproductive success in subordinate female banded mongooses

J Sanderson[1], HJ Nichols[2], HH Marshall[1], EIK Vitikainen[1], FJ Thompson[1], SL Walker[3], MA Cant[1], AJ Young[1]

1 Centre for Ecology and Conservation, University of Exeter (Penryn Campus), Penryn, TR10 9FE

2 School of Natural Science and Psychology, Liverpool John Moores University, Liverpool, L3 3AF

3 Chester Zoo Wildlife Endocrinology Laboratory, Caughall Road, Upton-by-Chester, Chester, CH2 1LH

Corresponding Author: M.A.Cant@exeter.ac.uk

Dominant females in social species have been hypothesised to reduce the reproductive success of their subordinates by inducing elevated circulating glucocorticoid concentrations. However, this 'stress-related suppression' hypothesis has received little support in cooperatively breeding species, despite evident reproductive skews among females. We tested this hypothesis in the banded mongoose (*Mungos mungo*), a cooperative mammal in which multiple females conceive and carry to term in each communal breeding attempt. As predicted, lower-ranked females had lower reproductive success, even among females that carried to term. While there were no rank-related differences in faecal glucocorticoid (fGC) concentrations prior to gestation or in the first trimester, lower-ranked females had significantly higher fGC concentrations than higher-ranked females in the second and third trimesters. Finally, females with higher fGC concentrations during the third trimester lost a greater proportion of their gestated young prior to their emergence from the burrow. Together, our results are consistent with a role for rank-related maternal stress in generating reproductive skew among females in this cooperative breeder. While studies of reproductive skew frequently consider the possibility that rank-related stress reduces the conception rates of subordinates, our findings highlight the possibility of detrimental effects on reproductive outcomes even after pregnancies have become established.

Keywords: reproductive conflict; intra-sexual selection; female competition; cooperative breeding

In animal societies, subordinate females often have lower reproductive success than dominant females. The stress-related suppression hypothesis proposes that dominant females suppress subordinate reproduction through behaviours that lead to chronic elevations in circulating glucocorticoids (GCs) and consequent reproductive down-regulation [1–4]. Notably though, compelling support for this hypothesis remains scarce in cooperatively breeding societies, where reproductive skews among females are frequently apparent [1,2; but see 3,5]. Stress-related suppression might only be necessary, however, in the subset of cooperative breeders in which subordinate females do still attempt to breed, as complete reproductive restraint by subordinates might otherwise obviate the need for dominants to stress their subordinates [3,6,7]. Furthermore, stress-related suppression could actually be difficult to detect using the approach most-commonly employed to test it (comparisons of the average GC levels of dominants and subordinates), if dominants target only a subset of likely breeders and do so only during periods when subordinate reproduction would otherwise be costly to dominants [3,5,6]. These suggestions have led to calls for further tests in cooperatively breeding species in which subordinates do attempt to breed, focussing on those subordinates attempting to breed at the same time as their dominants [3,6].

While socially-induced GC elevations have frequently been considered a potential cause of reduced conception rates among subordinates, they also have the potential to compromise the outcomes of established pregnancies. For example, elevated GCs during pregnancy may impact in utero or early post-natal development and affect offspring health, condition, and survival [6,7]. While studies of cooperatively breeding mammals have shown that being subjected to aggression by the dominant female is associated with increased abortion rates among subordinates [3,8], whether rank-related maternal stress compromises reproductive outcomes among subordinates that do manage to carry to term has yet to be investigated. If subordinate reproductive success was reduced as a result of elevated GC concentrations during gestation, one might make three predictions: pregnant females of lower social rank will have (1) reduced reproductive success and (2) elevated GC concentrations

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

Here, we test these three predictions with a detailed investigation of faecal glucocorticoid (fGC) concentrations and reproductive success in female banded mongooses (*Mungos mungo*). Banded mongooses live in stable cooperatively breeding groups comprising a "core" of breeding adults (1–5 females and 3–7 males) that reproduce 3–4 times per year, alongside a subset of younger individuals that breed occasionally [9]. Aggression received by pregnant subordinates can result in eviction and abortion [8], but pregnant subordinates do often breed successfully alongside pregnant dominants [9]. The rank-related patterns of reproductive success among females that carry to term have yet to be investigated, along with the role that GCs may play in generating them.

Methods

We studied a population of banded mongooses living in Queen Elizabeth National Park, Uganda (0°12'S; 29°53'E) between December 2010 and April 2014. All animals were marked and habituated to close observation (< 5 m). Groups were observed every 1 - 4 days to record all breeding events. We ran generalised linear mixed models (GLMMs) using the lme4 package [10] in R v3.1.1 [11] with Poisson and binomial data fitted with log and logit link functions, respectively. Female, social group, and litter identities were included as random intercepts in all models to control for repeated measures.

Pregnancy can be detected at around 40 days by swelling of the abdomen [12] and birth can be detected by a sudden decrease in female body size [13]. Females were captured during pregnancy to estimate the number of foetuses each carried by palpation [12]. We assigned maternity using a

combination of phenotypic and microsatellite data; full details are given in [14]. Analyses of reproductive success were limited to communal litters in which at least one pup emerged.

We collected 218 faecal samples from 35 females prior to and during gestation (2.5 ± 0.3 samples per female pregnancy, mean ± SE; number of samples collected per time period: pre-gestation = 59 samples, first trimester = 57 samples, second trimester = 45 samples, third trimester = 54 samples). Full details of sample collection and hormone analysis including validations are given in [15]. In brief, all samples were collected between 6:30am and 10:00am and stored on ice [15]. Hormones were extracted from faecal samples using a wet-weight extraction (adapted from [16]) and then analysed using an enzyme immunoassay.

1. Do lower ranking female experience reduced reproductive success?

We calculated three measures of reproductive success for each female recorded as having given birth: (i) the number of foetuses, (ii) the number of emergent offspring, and (iii) the proportion of foetuses surviving to emergence. We fitted each of these three measures as a response variable in a GLMM. Rank (determined by ranked age following [17]) was fitted as a fixed effect as were female age, group size, rainfall (month prior to conception), and pre-conception body mass [13] to control for other factors which may lead to variation in reproductive success.

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

We fitted fGC concentrations as a response variable in a GLMM with rank as the main predictor of interest. As GC concentrations may vary within a breeding attempt, we also fitted an interaction between rank and stage of pregnancy (pre-gestation; first trimester; second trimester; third

trimester) as well as fixed effects of female age, group size, rainfall, and pre-conception body mass to control for other factors which may contribute to fGC variation.

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

We fitted the number of emergent offspring and the proportion of foetuses surviving to emergence as response variables in two separate GLMMs with fGCs during the third trimester as the predictor of interest. We focused this analysis on fGCs in the third trimester because that is when we saw the clearest difference in fGCs between low- and high-ranking females.

Results

Lower-ranking females that carried to term experienced lower reproductive success than higher-ranking females, both when measured as the number of assigned offspring ($\chi^2_{(1)}$ = 4.18, P = 0.041, figure 1a) and the proportion of foetuses surviving to emergence ($\chi^2_{(1)}$ = 4.29, P = 0.038, figure 1c). There was no effect of rank on the number of foetuses carried by a female ($\chi^2_{(1)}$ = 0.027, P = 0.87). We found a significant interaction between female rank and pregnancy stage on fGC concentrations: lower-ranking females did not differ from higher-ranking females prior to conception or during the first trimester but had elevated fGCs during the second and third trimesters ($\chi^2_{(1)}$ = 4,18, P = 0.041, figure 2). Females experiencing higher fGC concentrations during the third trimester had fewer assigned offspring than those with lower GCs ($\chi^2_{(1)}$ = 5.26, P = 0.022, figure 1b) and a lower proportion of their foetuses survived to emergence ($\chi^2_{(1)}$ = 4.07, P = 0.044, figure 1d). Full model outputs are included in supplementary material (S1).

Discussion

Our findings are consistent with the hypothesis that subordinate female banded mongooses exhibit reduced reproductive success as a result of rank-related maternal stress during gestation. Lower-ranked females had lower reproductive success than higher-ranked females (despite conceiving litters of the same size), both when measured as the proportion of foetuses surviving to emergence and the number of emergent offspring. Whereas higher- and lower-ranked females had similar fGC concentrations prior to gestation and during the first trimester, lower-ranked females showed significantly elevated fGC concentrations during the second and third trimesters. These results highlight the possibility that stress-related suppression of subordinate reproduction arises through gestational effects that compromise offspring survival either during the latter stages of pregnancy or soon after birth (prior to emergence from the burrow). Accordingly, females that experienced higher fGC concentrations during the third trimester had fewer emergent pups and a lower proportion of foetuses surviving to emergence.

Rank-related differences in reproductive success among female mammals commonly occur due to differences in conception rates, either because subordinate females exercise reproductive restraint or because their ability to conceive is compromised by active interference by dominant females [18,19]. In contrast, we have demonstrated a rank-related difference in reproductive success within females that carry to term. As there was no observable rank-related variation in litter size *in utero*, this rank-related difference in reproductive success could well have arisen from pre-natal developmental impacts on offspring survival either during late pregnancy or during the early post-natal period. A role for rank-related maternal stress during late gestation in generating these effects on offspring survival would be consistent with experimental evidence that late-gestational GC elevations can inhibit offspring development [4,20]. In the absence of experimental evidence of a role for maternal GC elevations, however, it is also possible that alternative mechanisms, such as

early post-natal infanticide [21], play a role in generating the observed rank-related variation in offspring survival from detection as a foetus to emergence from the burrow.

The stress-related suppression hypothesis posits that elevated GC concentrations observed in lower ranking females are a result of aggression from dominant females. However, conspicuous aggression among female banded mongooses is rare outside of eviction events [9]. As such, the elevated GC concentrations observed here may not be a product of overt aggression. Our findings cannot be attributed instead to simple age effects, in which younger females struggle to meet the resource-demands of gestation (and hence exhibit differential GC elevations), as our analyses control for variation in absolute age and attribute variation in both reproductive success and gestational GC concentrations to variation in rank *per se*. However, the gestational GC elevations of lower-ranked females could arise at least in part from energetic differences during gestation. For example, subordinates may be competitively excluded from resources by dominant females. Alternatively, as intra-sexual conflict among females may frequently be resolved without overt physical conflict, these GC elevations could also reflect responses to more subtle rank-related outcomes, such as social isolation [22]. Either way, our findings highlight the possibility that stress-related suppression of subordinate reproduction may occur in the absence of conspicuous aggression.

Ethical Statement

All research was carried out under permit from Uganda Wildlife Authority (UWA) and Uganda National Council for Science and Technology (UNCST). All methods used received ethical approval from UWA, UNCST, and the Ethical Review Committees of the University of Exeter and Chester Zoo.

Data accessibility

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

Competing interests

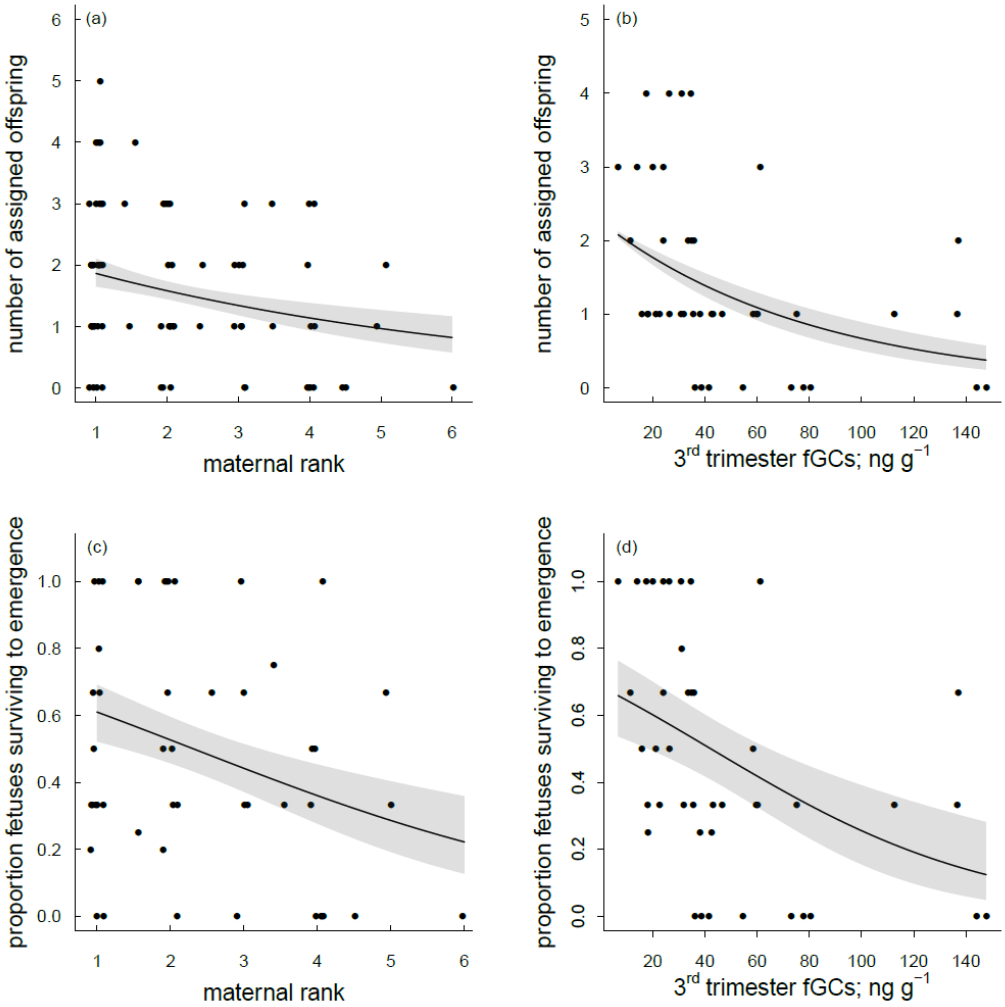
We have no competing interests.

Author's contributions

JS conceived the study, designed the study, organised and carried out fieldwork, organised hormone analyses, carried out parentage assignment and all statistical analyses, and drafted the manuscript; HN carried out genetic analyses and commented on the manuscript; HM, EV, and FT all organised and carried out fieldwork and commented on the manuscript; SW managed hormone analysis and advised on collection of faecal samples; MC managed fieldwork and supervised the design of the study, analysis and drafting of manuscript; AY supervised design of the study, analysis and drafting of manuscript.

Acknowledgements

We thank F. Mwanguhya, S Kyabulima, K. Mwesige, and R. Businge for assistance in the field, and K. Edwards, R. Purcell, and V. Norton for their help with physiological analyses. Research was funded by a NERC Grant (NE/J010278/1) to MAC and AJY and an ERC Grant (309249) to MAC. AJY was supported by a BBSRC David Phillips research fellowship.



194

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

198

199

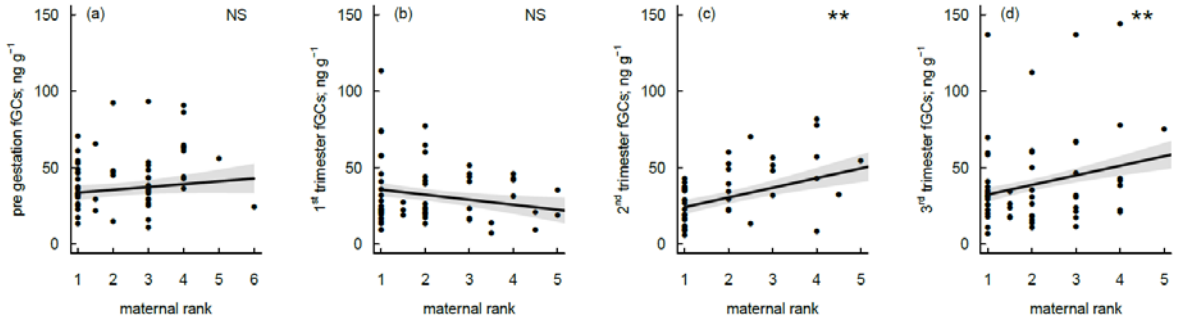


Figure 2. Female fGC concentrations vary during gestation dependant on maternal rank. Dots show raw values and lines and shaded areas show predicted estimates and confidence intervals from a GLMM. Significance values from post-hoc testing of the effect of maternal rank on fGC concentrations (a) within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

1. Creel, SR, Creel, N & Monfort, S 1996 Social stress and dominance. *Nature* **379**, 212.

2. Creel, SR 2001 Social dominance and stress hormones. *Trends Ecol. Evol.* **16**, 491–497.

3. Young, AJ, Carlson, AA, Monforts, SL, Russell, AF, Bennett, NC & Clutton-brock, TH 2006 Stress and the suppression of subordinate reproduction in cooperatively breeding meerkats. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 12005–10.

4. Wasser, SK & Barash, DP 1983 Reproductive suppression among female mammals: implications for biomedicine and sexual selection theory. *Q. Rev. Biol.* **58**, 513–38.

5. Young, AJ, Monforts, SL & Clutton-brock, TH 2008 The causes of physiological suppression among female meerkats: a role for subordinate restraint due to the threat of infanticide? *Horm. Behav.* **53**, 131–9.

6. Seckl, JR & Meaney, MJ 2004 Glucocorticoid programming. *Ann N Y Acad Sci* **1032**, 63–84.

7. Meaney, MJ, Szyf, M & Seckl, JR 2007 Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends Mol. Med.* **13**, 269–277.

8. Cant, MA, Hodge, SJ, Bell, MBV, Gilchrist, JS & Nichols, HJ 2010 Reproductive control via eviction (but not the threat of eviction) in banded mongooses. *Proc. Biol. Sci.* **277**, 2219–2226.

9. Cant, MA, Vitikainen, E & Nichols, HJ 2013 Demography and Social Evolution of Banded Mongooses. *Adv. Study Behav.* **45**, 407–446.

10. Bates, D, Maechler, M & Bolker, B 2013 lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-2.

11. R CORE Team, 2013 R: A Language and Environment for Statistical Computing.

12. Cant, MA 2000 Social control of reproduction in banded mongooses. *Anim. Behav.* **59**, 147–158.

13. Hodge, SJ, Bell, MBV & Cant, MA 2011 Reproductive competition and the evolution of extreme birth synchrony in a cooperative mammal. *Biol. Lett.* **7**, 54–6.

14. Sanderson, JL, Wang, J, Vitikainen, EIK, Cant, MA & Nichols, HJ 2015 Banded mongooses avoid inbreeding when mating with members of the same natal group. *Mol. Ecol.* **24**, 3738-3751

15. Sanderson, JL, Young, AJ, Hodge, SJ, Kyabulima, S, Walker, SL & Cant, MA 2014 Hormonal mediation of a carry-over effect in a wild cooperative mammal. *Funct. Ecol.* **28**, 1377–1386.

16. Walker, SL, Waddell, WT & Goodrowe, KL 2002 Reproductive endocrine patterns in captive female and male red wolves (Canis rufus) assessed by fecal and serum hormone analysis. *Zoo Biol.* **21**, 321–335.

17. Nichols, HJ, Bell, MBV, Hodge, SJ & Cant, MA 2012 Resource limitation moderates the adaptive suppression of subordinate breeding in a cooperatively breeding mongoose. *Behav. Ecol.* **23**, 635–642.

18. Cant, MA & Young, AJ 2013 Resolving social conflict among females without overt aggression. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **368**, 20130076.

19. Young, AJ 2009 The causes of physiological suppression in vertebrate societies: a synthesis. In *Reproductive Skew in Vertebrates* (eds R. Hager & C. Jones), pp. 397–438. Cambridge University Press.

20. Sheriff, MJ & Love, OP 2013 Determining the adaptive potential of maternal stress. *Ecol. Lett.* **16**, 271–280.

21. Cant, MA, Nichols, HJ, Johnstone, RA & Hodge, SJ 2013 Policing of reproduction by hidden threats in a cooperative mammal. *Proc. Natl. Acad. Sci. U. S. A.* , 1–7.

22. Hawkley, LC, Cole, SW, Capitanio, JP, Norman, GJ & Cacioppo, JT 2012 Effects of social isolation on glucocorticoid regulation in social mammals. *Horm. Behav.* **62**, 314–23.