

Hormonal mediation of a carry-over effect in a wild cooperative mammal

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Summary

1. Recent research has shown that parental investment in one breeding attempt often has a profound negative impact on the level of parental investment in subsequent breeding attempts. However, the mechanistic underpinnings that mediate such carry-over effects are poorly understood.

2. Here, we hypothesise that carry-over effects arise because energetic losses lead to elevated levels of glucocorticoid ‘stress’ hormones which inhibit future investment and thereby maintain energetic homeostasis. We investigate this hypothesis through a detailed investigation of a carry-over effect of (allo-) parental investment in the cooperatively breeding banded mongoose (*Mungos mungo*).

3. Using a combination of non-invasive hormone monitoring and feeding experiments, we demonstrate (i) that high glucocorticoid concentrations prior to breeding predict reduced allo-parental investment; (ii) that energetic losses associated with high alloparental investment lead to an increase in glucocorticoid concentrations during the breeding attempt; and (iii) that elevated glucocorticoid concentrations persist into a time period that is known to affect future investment, although high pup mortality meant that we could not measure effects on subsequent alloparental investment directly.

4. Together, our results provide strong evidence for the hypothesis that carry-over effects on parental investment are mediated by circulating glucocorticoid concentrations. Since an individual’s stress physiology is shaped by early-life and social factors, our findings may help to explain how these factors contribute to individual variation in parental investment and lifetime reproductive success.

Key-words: carry-over effect, cooperative breeding, glucocorticoid, energetic state, parental investment

Introduction

Where investment in energetically costly behaviours leads to decreased body condition, the time required to recover from these energetic costs may affect individual performance in a subsequent season or life-history stage (Harrison *et al.* 2011; O’Connor, Orris & Rossin 2014). These ‘carry-over effects’ contribute to between-individual variation in parental investment and reproductive success in a wide variety of taxa including birds (Norris *et al.* 2004; Inger *et al.* 2010), mammals (Festa *et al.* 1998; Perryman *et al.* 2002) and reptiles (Broderick, Godley & Hays 2001).

While there is growing evidence that energetic constraints prior to reproduction are the underlying cause of these carry-over effects (Norris *et al.* 2004; Harrison *et al.* 2011), the *physiological* mechanisms that drive reductions in downstream parental investment as a function of such energetic constraints remain poorly understood. Glucocorticoid (GC; ‘stress’) hormones are a strong candidate for the mechanism driving carry-over effects because circulating GCs become elevated when energetic output outweighs energetic resources and function to regain energetic homeostasis (reviewed in McEwen & Wingfield 2003). Where carry-over effects occur between breeding attempts (Prince *et al.* 1994; Russell *et al.* 2003; Inger *et al.* 2010), this ‘GC carry-over hypothesis’ posits that the energetic

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costs of parental investment lead to elevated circulating GCs which persist into the next breeding attempt and inhibit further investment.

To establish GC variation as the physiological mechanism mediating carry-over effects of parental investment it must be demonstrated that (i) high parental investment in one breeding attempt leads to decreased investment in the subsequent breeding attempt, (ii) GCs inhibit parental investment, (iii) parental investment leads to elevated GC concentrations, and (iv) elevated GC concentrations persist into a time period that can affect future investment. Two studies [of a bi-parental bird (Ouyang *et al.* 2011) and a cooperatively breeding mammal (Carlson *et al.* 2006a)] have examined the link between investment in parental (or 'alloparental') care and GC concentrations both prior to and during the care period. These studies found that individuals investing most in raising offspring have relatively low GC concentrations prior to the care period, suggesting that GCs may inhibit parental care. These high investors also experienced a corresponding increase in GC concentrations over the period of offspring care, consistent with the hypothesis that GCs mediate carry-over effects. However, no study to date has tested if elevated GCs resulting from high levels of parental investment persist *after* the care period. In this paper, we carry out such a test in an unusually tractable cooperative mammal, the banded mongoose (*Mungos mungo*).

Banded mongooses are small (<2 kg) diurnal herpestids that live in stable cooperatively breeding groups of 10–30 individuals (Cant, Vitikainen & Nichols 2013), and 1–12 adult females give birth synchronously (Hodge, Bell & Cant 2011) to large litters [1–20 pups (Gilchrist 2006)] up to four times a year. Most foraging pups (>4 weeks after birth) form a stable association with a single adult 'escort' who provisions, carries, grooms and protects that pup until it reaches independence at approximately 10 weeks (Gilchrist, Otali & Mwanguhya 2004). Although variation in alloparental investment ('escorting effort') within banded mongoose societies has been linked to individual traits such as age, body condition and dominance status (Cant 2003; Gilchrist 2004; Hodge 2007), there remains striking unexplained variation in alloparental investment both within and between individuals. Previous study of the closely related meerkat (*Suricata suricatta*) has shown that variation in current alloparental investment can be attributable to variation in previous investment (Russell *et al.* 2003). In this paper, we report a similar carry-over effect of alloparental investment in the banded mongoose and carry out a detailed investigation of GC variation to test the GC carry-over hypothesis. Cooperative species are a powerful tool to investigate individual variation in behaviour because we can compare group members that vary widely in (allo-) parental investment while controlling for other potential confounds such as territory quality, season and food availability (Cant & Field 2001).

Our design involved repeated fine-scale longitudinal sampling of hormone concentrations before, during and

after the pup care period, allowing us to quantify within-individual hormonal changes and relate these to measures of alloparental investment from the same periods. Though it is not possible to manipulate hormone levels in the banded mongoose population used in this study because of ethical guidelines from the Uganda Wildlife Authority, we complement our correlative studies with supplementary feeding experiments to test the causal links between hormones, behaviour and energetic state. Using these methods, we addressed four questions: (i) Are there carry-over effects of investment in cooperative pup care, whereby heavy investors in one breeding attempt invest less in the next? (ii) Does an individual's baseline GC concentration *before* a breeding attempt predict its level of investment in care? (iii) Does investment in care lead to an increase in the carer's GC concentrations and is this increase attributable to energetic losses? (iv) Do such rises in GC concentrations in heavy investors persist after a breeding attempt into a time period that can affect investment in subsequent breeding attempts? Together, the answers to these questions provide a test of the GC carry-over hypothesis in a wild cooperative mammal.

Materials and methods

STUDY SITE AND DATA COLLECTION

This study was conducted between June 2000 and April 2012, on the Mweya Peninsula in Queen Elizabeth National Park, Uganda (0°12'S, 27°54'E). Details of vegetation and climate are given elsewhere (Cant, Vitikainen & Nichols 2013). Most individuals in the population were trained to step onto a portable weighing scale allowing weights to be recorded daily before the morning foraging trip (Hodge 2007). All individuals were habituated to the presence of observers at a distance of 2–4 m, enabling the collection of detailed behavioural observations without any measurable effect of observer presence.

All individuals in the population were marked with either colour-coded collars (7 g) or unique shave patterns to enable identification in the field. Collars and shaves were maintained by trapping individuals every 3–6 months, though individuals in highly habituated groups could have their hair trimmed without capture. One individual within each group was fitted with a radio-collar weighing 27 g (Sirtrack Ltd., Havelock North, New Zealand) with a 20-cm whip antenna (Biotrack Ltd., UK). Individuals were trapped using box traps (67 × 23 × 23 cm; Tomahawk Live Trap Co., Tomahawk, WI, USA) and anaesthetised using either ketamine or isoflurane (details of trapping protocol are given elsewhere; ketamine: Hodge 2007; isoflurane: Jordan *et al.* 2010).

Banded mongoose females often enter oestrus within 2 weeks of giving birth so are commonly pregnant during periods of pup care (Cant 2000; Hodge 2007). As such, to avoid complications that would otherwise arise from pre and postpartum changes in body mass and GCs among females and their impacts on patterns of caring, we focussed our study solely on male carers.

Behavioural observations

Measures of escorting effort were available for 732 male banded mongooses over 122 breeding attempts in 10 social groups. These groups were visited every 1–3 days, and associations between adults and pups were noted. Adults were scored as escorts if they

were seen to be within 30 cm of the same pup for more than 50% of the observation period (Gilchrist 2004). We defined an 'escorting period' as the period between the first and last observation of escorting, and 'escorting effort' as the proportion of days that an individual was recorded as an escort out of the total number of days escorting was recorded by any individual within the group (7–21 observation days per breeding attempt).

Previous studies have shown that this association score is a reliable summary of quantitative nearest-neighbour data and is a strong proxy of individual alloparental investment (Gilchrist 2004). However, in 10 breeding attempts between April 2010 and April 2012, we attained more accurate measures of pup provisioning rates through conducting a series of 20 min pup focals. Observations of pup provisioning were recorded *ad libitum* throughout each focal. The number of food items fed by each adult to each pup was first weighted by total observation time for that pup; these feeding rates were then summed to give a total number of items fed to all pups per hour for each adult. This total provisioning rate was highly correlated with scores of escorting effort (Spearman correlation: $\rho = 0.56$, $n = 129$ observations, $P < 0.001$) and gave a detailed measure of individual alloparental investment.

Within each breeding attempt, we defined group size as the number of individuals aged > 1 year at the start of the escorting period. Litter size was defined as the number of pups seen at pup emergence. Body mass measures were calculated as mean body mass (g) from observations collected in the 30 days prior to the escorting period. Total rainfall in the 30 days prior to the escorting period was included in all analyses as a proxy of food availability (Nichols *et al.* 2012). Individual age was defined as age in months at the start of the escorting period throughout.

FAECAL SAMPLE COLLECTION AND HORMONE ASSAY

A total of 432 faecal samples were available from 82 adult male banded mongooses in five social groups between April 2010 and April 2012. All samples were collected between 6:30 am and 10:00 am and immediately placed on ice in a Thermos flask. They were then transferred to a -20°C freezer within 3 h. Samples were transferred to the UK on ice and again transferred to a -20°C freezer for storage. Banded mongooses commonly use urine or anal gland secretions to place a scent mark over the faeces of other group members (Jordan *et al.* 2010). As this over-marking behaviour may contaminate faecal samples, samples were only collected if they had been watched continually from defecation and it was known that they had not been overmarked. Only half of each faecal deposit was collected to minimise any resulting interference with scent marking signals. Time of collection and time to freezer were included in all analyses where appropriate but were found to be non-significant predictors of faecal glucocorticoid (fGC) concentrations throughout.

Hormones were extracted from faecal samples following thawing and manual homogenisation using a wet-weight shaking extraction adapted from Walker, Waddell & Goodrowe (2002). In brief, 0.5 g of faecal material was combined with 90% methanol, shaken overnight at room temperature and centrifuged for 20 min at 598 g. The methanol fraction was decanted and evaporated to dryness. Faecal extracts were re-suspended in 1 ml methanol and stored at -20°C until analysis.

fGC concentrations were analysed using modified protocol from a previously described enzyme immunoassay (Watson *et al.* 2013). The enzyme immunoassay utilised an antibody (polyclonal corticosterone CJM006 supplied by CJ Munro, University of California, Davis, CA), horseradish peroxidase-conjugated label (prepared according to Munro & Stabenfeldt 1984) and standards (Sigma-Aldrich, Gillingham, UK). The corticosterone antiserum CJM006 was found to cross-react with corticosterone 100%, desoxycorti-

costerone 14.25%, progesterone 2.65%, tetrahydrocorticosterone 0.90%, testosterone 0.64%, cortisol 0.23%, prednisolone 0.07%, 11-desoxycortisol 0.03%, prednisone $< 0.01\%$, cortisone $< 0.01\%$ and estradiol $< 0.01\%$ (Watson *et al.* 2013).

The corticosterone assay was validated for measuring corticosterone metabolites in male banded mongoose faeces by parallelism, accuracy check and ACTH challenge. The intra- and interassay coefficients of variation for the corticosterone assay were 7.52 and 6.33% (C1 and C2) and 8.66 and 10.47% (C1 and C2), respectively. Serial dilutions of male banded mongoose faecal extract yielded a displacement curve parallel to the standard curve (corticosterone: sample % binding = $17.786 + 0.816$, $R^2 = 0.9957$, $F_{1,7} = 1631.26$, $P < 0.001$). There was no evidence of matrix interference in male corticosterone, as addition of diluted faecal extract to standards did not alter the amount expected (Observed = $8.484 + 0.959$ (Expected), $R^2 = 0.997$, $F_{1,7} = 2342.13$, $P < 0.001$). The physiological validity of using this enzyme immunoassay technique to measure faecal glucocorticoid (fGC) metabolite concentrations in banded mongoose faecal samples was established by demonstrating a cause-and-effect relationship between exogenous administration of adrenocorticotrophic hormone (ACTH) (one intramuscular injection of $13\text{ }\mu\text{L}$ of 1 mg mL^{-1} Tetracosactide (Synacthen), $n = 3$ males) and the subsequent excretion of fGC metabolites in the faeces. fGC concentrations were higher in the 2 days following injection than in the 2 days prior to injection (Mann–Whitney; $n = 27$ samples, $P = 0.028$; Fig. 1). Peak fGC elevation occurred $6.56 \pm 0.29\text{ h}$ (mean \pm SE, from three individuals) after ACTH administration.

STATISTICAL ANALYSES

All statistical analyses were carried out using R 3.0.0 (R Core Team 2013). We used the lme4 package (Bates, Maechler & Bolker 2013) to fit both general linear mixed models (GLMMs) and generalised linear mixed models (GzLMMs). When data were zero-inflated, models were fitted using the glmmADMB package (Fournier *et al.* 2012) and model comparisons were made using likelihood ratio tests. Normally distributed response variables were analysed with an identity link function, and Poisson response variables with log link function. All possible explanatory variables were initially fitted together in a maximal model and then sequentially dropped from the model in order of least significance until only those variables explaining significant variation ($P < 0.05$) remained. All dropped variables were then put back into the

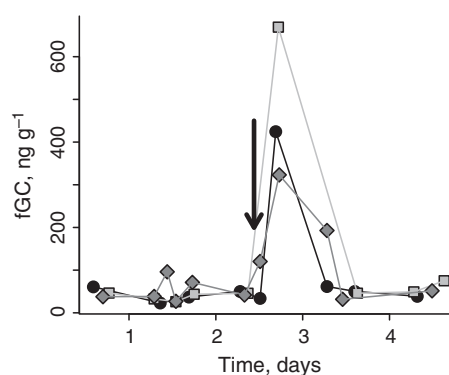


Fig. 1. Faecal glucocorticoid metabolite (fGC) concentrations from male banded mongooses before and after administration of synthetic ACTH (Tetracosactide; Synacthen). Each line represents a single individual. The arrow represents time of ACTH administration.

minimal model to reconfirm their non-significance. Residuals from both maximal and final models were checked for normality and homogeneity of variance to verify that all model assumptions were met. Social group, breeding attempt and individual identities were fitted as random effects (breeding attempt was nested within pack and individual identity was fitted as a crossed random effect) throughout to control for repeated measures.

Are there carry-over effects of investment in cooperative pup care?

A total of 583 measures of change in escorting effort were collected from 149 male banded mongooses over 52 pairs of consecutive breeding attempts from six social groups (see Table S1a, Supporting Information). Change in escorting effort between consecutive breeding attempts was corrected for 'regression to the mean' following (Kelly & Price 2012) and fitted as the response term in a GLMM. Escorting effort in the first of the two breeding attempts was fitted as the main predictor of interest. The time between two consecutive escorting periods was calculated as the number of days between the first observation of escorting in each of the two breeding attempts and factorised into 'short'

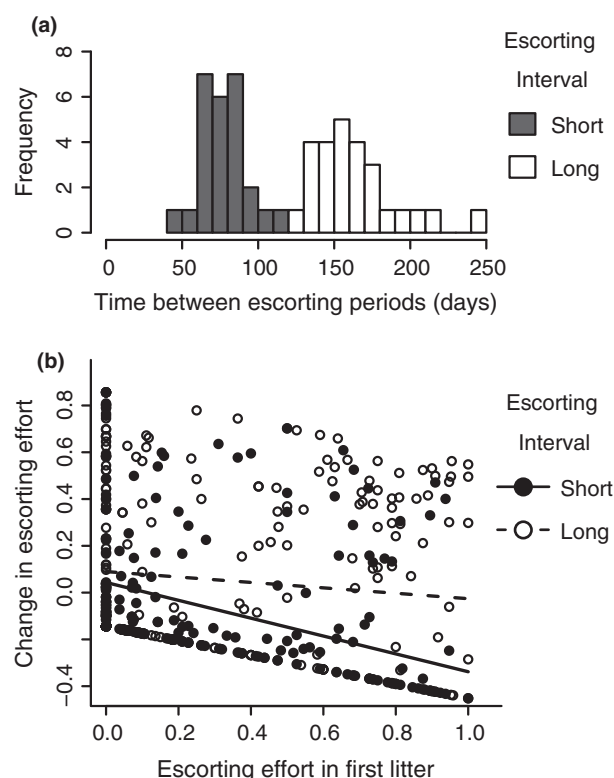


Fig. 2. Individual change in escorting effort across two consecutive breeding attempts as a function of escorting effort in the first breeding attempt. (a) Escorting periods were classified as having 'short' or 'long' escorting intervals when the number of days since the start of the previous escorting period was less than or more than 120 days, respectively ($n = 52$ breeding attempts from six social groups). (b) Breeding attempts with 'long-' and 'short' escorting intervals are shown with open and closed circles, respectively. Dotted and solid lines represent prediction estimates for 'long' and 'short' escorting intervals, respectively; prediction estimates from a GLMM while controlling for significant effects of group size and the number of pups (see Table S2a) ($n = 583$ observations from 52 pairs of consecutive breeding attempts).

(<120 days) and 'long' escorting intervals (>120 days) (Fig. 2a). The interaction between previous escorting effort and escorting interval was included to test whether increased recovery time affected individuals differently dependent on previous escorting effort. Age, group size, the number of pups and rainfall (all relative to the second escorting period) were also fitted as covariates. Escorting periods included in this analysis were limited to those with at least 10 observation days.

Does an individual's baseline GC concentration before a breeding attempt predict its level of investment in care?

In total, 102 provisioning rates were calculated from observations of 56 adult male banded mongooses over 14 breeding attempts in five different social groups (see Table S1b). A total of 100 faecal samples were collected in the 14 days prior to the escorting period (1–4 samples per individual; within-individual [and within breeding attempt] repeatability of fGC concentrations = 0.61). fGC concentrations from these samples were then averaged for each individual within each breeding attempt to give one pre-escort fGC value per individual. Individual provisioning rates in the first 2 weeks of the escorting period were fitted as a Poisson response in a GLMM controlling for zero-inflation. As provisioning rates were calculated by summing provisioning rates which were weighted for observation time per pup (see above), we were unable to use an offset to control for observation time. Rather, we rounded calculated provisioning rates (per hour) to the nearest integer to meet the model assumptions. Mean pre-escort fGC concentration was fitted as the predictor of interest. Small sample size of nonzero values ($n = 25$) meant that models including other fixed effects did not converge, so no other fixed effects were fitted.

Does investment in care lead to an increase in the carer's GC concentrations, and is this increase attributable to energetic losses?

First, we measured 51 changes in body mass from 33 individuals over nine breeding attempts in five social groups (see Table S1c). Morning weights were collected before (1–14 days prior to escorting) and during the escorting period. Measures of change in body mass were then calculated by taking the slope of a regression line of body mass against day (11.2 ± 0.2 measures of body mass per observation; mean \pm SE). Mass change (g day^{-1}) was fitted as the response variable in a GLMM with provisioning rate as the main predictor of interest. Age, rainfall, group size, the number of pups and body mass (prior to the escorting period) were also fitted as covariates. The inclusion of body mass prior to the escorting period as a fixed effect allowed us to test for the effects of pup provisioning on mass change while controlling for the fact that initially heavy individuals were more likely to lose weight than initially lighter individuals (Kelly & Price 2012).

Secondly, we collected 82 measures of fGC concentrations during the escorting period from 20 male banded mongooses over nine breeding attempts in five social groups (1–7 measures per individual) (see Table S1d). fGC during the escorting period was fitted as the response variable in a GLMM with provisioning rate as the main predictor of interest. The interaction between provisioning rate and day (after the first observation of escorting) was fitted to test whether fGC concentrations increased or decreased during the escorting period dependent on investment in care. We also fitted mean pre-escort fGC concentration (ng g^{-1}) (1–14 days prior to escorting; 40 samples; 1–4 per individual) as a fixed effect to control for variation in baseline GC concentrations. Age, body mass, rainfall, group size and the number of pups were also fitted as covariates.

Thirdly, to test whether changes in fGC concentration during the escorting period were attributable to the energetic losses associated with pup care, we provided supplementary food for both escorts and non-escorts during the escorting period while measuring changes in fGC concentrations. In doing so, we were able to experimentally reduce the energetic costs of escorting without affecting other stimuli from the presence of pups (e.g. pup begging calls) that may affect the behaviour and physiology of adults. Specifically, escorts and non-escorts were randomly assigned to one of four treatment groups: (i) fed escorts ($n = 8$), (ii) control escorts ($n = 6$), (iii) fed non-escorts ($n = 6$) and (iv) control non-escorts ($n = 9$). Fed individuals were given 80 g of dried fish for 6 consecutive days on the third week of the escorting period. Measures of fGC concentrations were collected prior to and during supplementary feeding (before: 0–4 days prior to feeding; 1–3 measures per individual; during: days 3–6 of feeding; 1–4 measures per individual) (see Table S1e). Individual mean fGC concentrations before and during supplementary feeding were compared within each treatment using paired t -tests. One fed non-escort was removed from analysis because he began escorting during feeding.

Do such changes to GC concentrations in heavy investors persist after the care period?

A total of 116 measures of body mass were collected from 26 male banded mongooses over eight breeding attempts in five social groups (see Table S1f); 83 measures of fGC concentrations were collected from 31 male banded mongooses over six breeding attempts in four social groups (see Table S1g). These measures of body mass and fGC concentrations were both collected between days 50 and 72 after the first observation of escorting in each breeding attempt (1–7 per individual). The longest escorting period observed in this study lasted 49 days, so these measures are collected in the absence of any current alloparental investment. Thus, for the purposes of this paper, we define the period 50–72 days after first escorting as the 'post-escorting' period. High levels of pup mortality between 2010 and 2012 meant that we could not test directly if elevated GC concentrations in the post-escorting period affected alloparental investment in a second breeding attempt. However, we know from our long-term data that the post-escorting period (as defined here) overlaps with 'pre-escorting' period covered by our samples (14 days prior to first escorting) in 73% of breeding attempts with a short escorting interval. Thus, our post-escorting measurements of GC levels were taken in a time period which typically represents the pre-escorting period of subsequent breeding attempts where carry-over effects occur.

Post-escorting fGC measures were log-transformed to retain normality of errors. Both body mass and log-transformed fGC concentrations were fitted as response terms in LMMs with provisioning rate as the main predictor of interest. The interaction between provisioning rate and day since the first observation of escorting was also fitted in both models to test whether the rate of change in fGC concentrations/body mass was dependent on investment in care. Age, rainfall, group size, the number of pups and length of the escorting period were also fitted as covariates in both models. Mean pre-escorting body mass (g) and mean pre-escorting fGC concentration (ng g^{-1}) were also fitted in the models with body mass and fGC concentrations as the response variables, respectively. Estimates of pre-escorting body mass were only available for 34 of 83 measures of fGCs, so it was not included in analysis of fGC concentrations after the care period to maintain the required sample size (there was no significant relationship between pre-escorting body mass and post-escorting fGCs within these samples: $\chi^2_{(1)} = 0.19$, $P = 0.67$).

Results

ARE THERE CARRY-OVER EFFECTS OF INVESTMENT IN COOPERATIVE PUP CARE?

The carry-over effect of previous investment in escorting on current escorting effort was dependent on the length of time between escorting periods (GLMM: interaction between previous escorting effort and escorting interval; $\chi^2_{(1)} = 13.57$, $P < 0.001$; Fig. 2b). Individuals that had invested heavily in escorting in the first breeding attempt decreased their escorting effort in a second breeding attempt when there was a short interval between escorting periods but not when there was a long interval [ANOVA (change in escorting effort \sim previous escorting effort): short escorting intervals: $F_{(1,307)} = 5.31$, $P = 0.022$; long escorting intervals: $F_{(1,272)} = 1.40$, $P = 0.24$].

The reduction in escorting effort between consecutive breeding attempts was lower for individuals in larger groups, and when fewer pups were present in the second breeding attempt but was unaffected by rainfall or individual age (see Table S2a). The measures of change in escorting effort used in this analysis were adjusted to control for the effect of regression to the mean following (Kelly & Price 2012), which means that the effect seen exceeds that expected by chance and provides strong evidence of a carry-over effect of escorting effort in consecutive breeding attempts with a short escorting interval.

DOES AN INDIVIDUAL'S BASELINE GC CONCENTRATION BEFORE A BREEDING ATTEMPT PREDICT ITS LEVEL OF INVESTMENT IN CARE?

Mean faecal glucocorticoid (fGC) concentrations in the 2 weeks prior to the escorting period predicted individual alloparental investment: individuals with lower fGCs prior to escorting expressed higher provisioning rates during the escorting period (GzLMM: $P = 0.020$; Fig. 3).

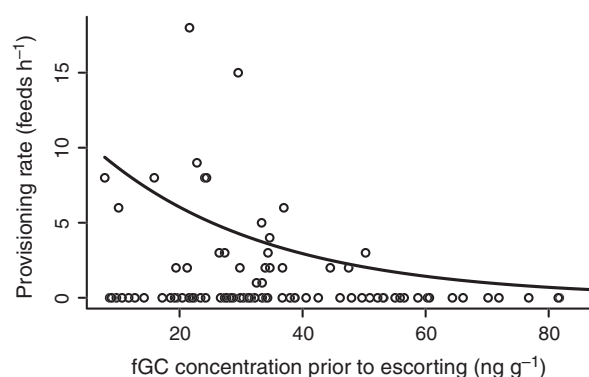


Fig. 3. Individual provisioning rate (feeds h^{-1}) as a function of fGC concentration prior to the start of the escorting period ($n = 102$ observations from 14 breeding attempts). Line shows the back-transformed (log link) predicted trend from a GzLMM controlling for zero-inflation. Points show raw data.

DOES INVESTMENT IN CARE LEAD TO AN INCREASE IN THE CARER'S GC CONCENTRATIONS, AND IS THIS INCREASE ATTRIBUTABLE TO ENERGETIC LOSSES?

Individuals that provisioned offspring at relatively high rates during the escorting period lost more body mass (GLMM: $\chi^2_{(1)} = 6.30$, $P = 0.01$; Fig. 4a) and had higher fGC concentrations (GLMM: $\chi^2_{(1)} = 4.07$, $P = 0.044$; Fig. 4b) over the course of the escorting period compared to individuals that provisioned offspring at relatively low rates. Individuals that were heavier prior to the escorting period also showed greater reductions in body mass (GLMM: $\chi^2_{(1)} = 7.50$, $P = 0.006$) and greater elevations in fGC concentrations (GLMM: $\chi^2_{(1)} = 10.74$, $P = 0.001$) over the course of the escorting period. Individual body mass changes over the course of the escorting period were unaffected by age, rainfall, group size or the number of pups (see Table S2b). Individual fGC concentrations over the escorting period were positively correlated with pre-escorting fGCs and were lower in older individuals, but were unaffected by group size, the number of pups, rainfall or the day of sample collection (see Table S2b).

To test whether the observed changes in fGC concentrations across the escorting period were due to effects of

declining energetic state, we conducted supplementary feeding experiments on escorts and non-escorts and examined their effects on fGC concentrations. Supplementary feeding significantly decreased fGC concentrations in escorts ($t_6 = -2.61$, $P = 0.040$; Fig. 4c). There was no significant change in fGC concentrations in any of the other treatment groups (control escorts: $t_5 = -0.63$, $P = 0.55$; fed non-escorts: $t_4 = 0.03$, $P = 0.98$; control non-escorts: $t_6 = 0.36$, $P = 0.73$; Fig. 4c).

DO SUCH RISES IN GC CONCENTRATIONS IN HEAVY INVESTORS PERSIST AFTER THE CARE PERIOD?

Male banded mongooses that had invested heavily in provisioning pups during the escorting period had higher fGC concentrations (GLMM: $\chi^2_{(1)} = 4.23$, $P = 0.039$) and lower body mass (GLMM: $\chi^2_{(1)} = 4.87$, $P = 0.028$; Fig. 5a) in the post-escorting period than individuals that had invested less in pup provisioning. This means that high investment in pup provisioning led to elevated fGC concentrations in a period which is expected to negatively impact investment in the subsequent breeding attempt.

The effect of previous provisioning rate of fGCs after the escorting period was dependent on the number of days

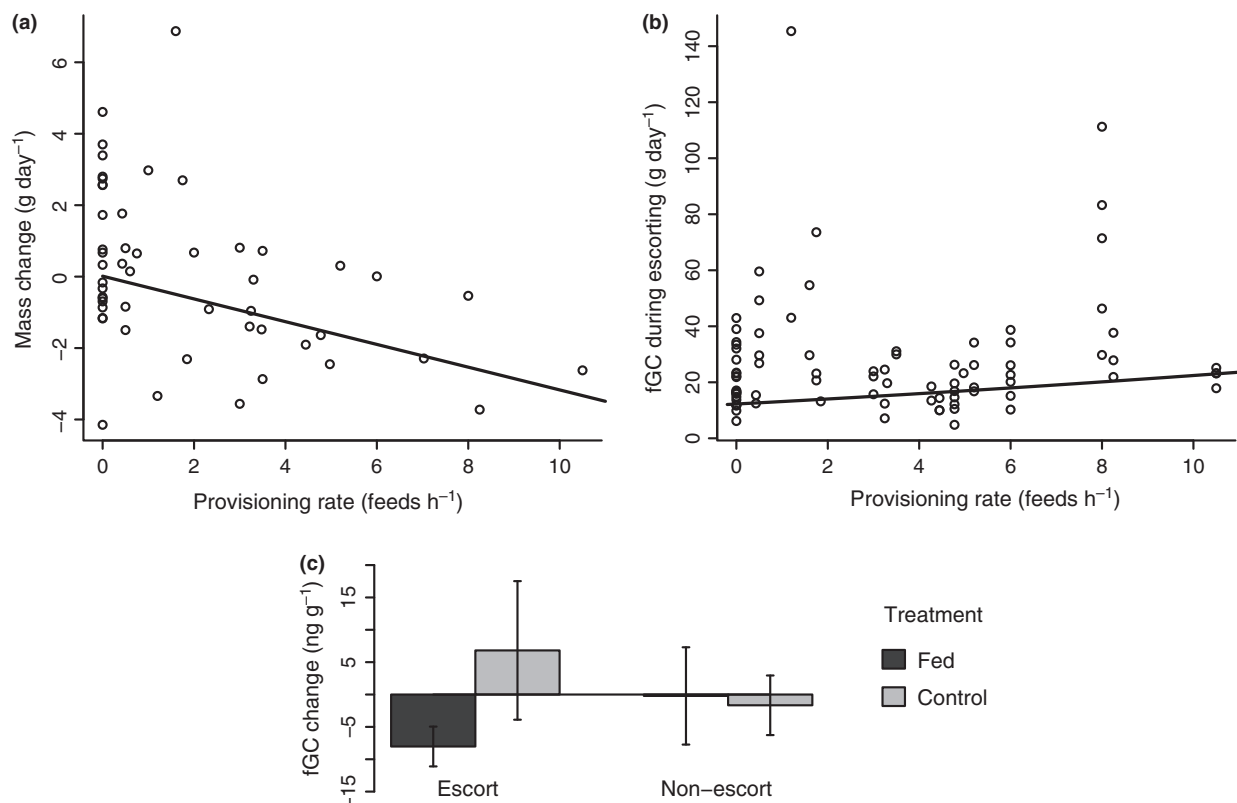


Fig. 4. Relationship between individual provisioning rate and (a) mass change ($n = 51$ observations from nine breeding attempts) and (b) fGCs ($n = 82$ fGC measures from nine breeding attempts) during the escorting period. Lines show the predicted trends from GLMMs while controlling for a significant effects of (a) mass prior to the escorting period (see Table S2b) and (b) age, mass and fGCs prior to the escorting period (see Table S2b). Points show raw data. (c) Effects of supplementary feeding on fGC concentrations in fed escorts ($n = 8$), control escorts ($n = 6$), fed non-escorts ($n = 6$) and control non-escorts ($n = 9$). Mean changes in fGC concentrations \pm SE are shown.

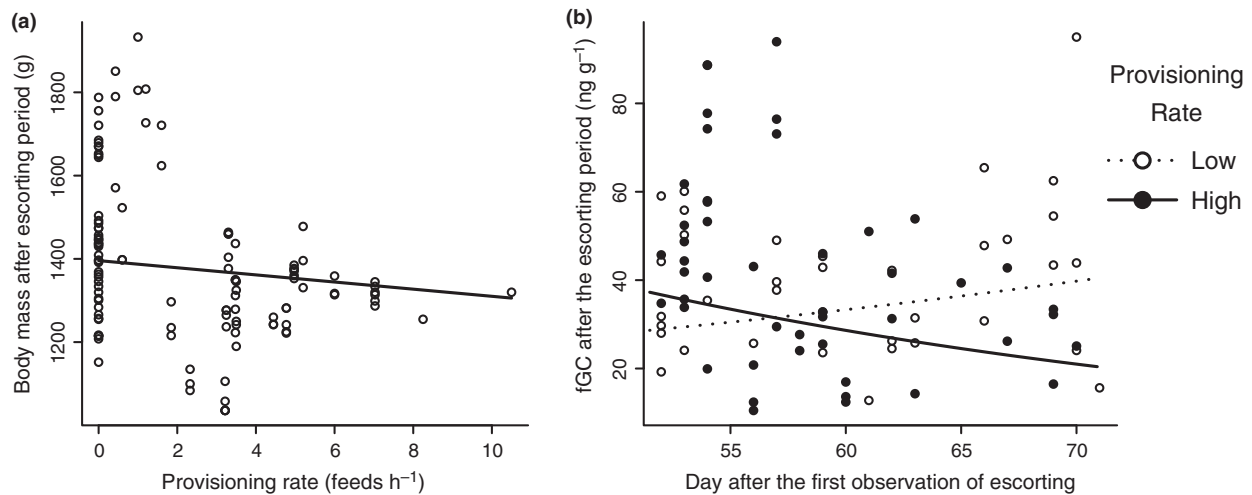


Fig. 5. Relationship between provisioning rate and (a) body mass ($n = 116$ observations from eight breeding attempts) and (b) fGCs ($n = 83$ fGC measures from six breeding attempts) after the escorting period. (a) Solid line shows a predicted trend from an LMM while controlling for significant effects of group size, pre-escorting body mass and the length of escorting period (see Table S2c). Points show raw data values. (b) Solid and dotted lines show predicted trends from a LMM for individuals exhibiting the maximum (6 feeds h^{-1}) and minimum (0 feeds h^{-1}) feeding rates observed in this analysis, respectively, while controlling for a significant effect of group size (see Table S2c). Closed and open points show raw data values from individuals that were and were not observed to feed one or more food items to a pup, respectively (provisioners and non-provisioners).

since escorting began (GLMM: $\chi^2_{(1)} = 4.23$, $P = 0.039$; Fig. 5b). Individuals that were observed provisioning pups showed decreasing fGC concentrations over time, but individuals that were not observed provisioning pups showed no change in fGCs [ANOVA (fGC ~ day): provisioners: $F_{(1,43)} = 5.64$, $P = 0.022$; non-provisioners: $F_{(1,36)} = 0.15$, $P = 0.70$]. This result shows that elevated GC concentrations do persist after the care period, but they also decline over time. fGC concentrations after the escorting period were higher in larger groups, but there was no significant effect of age, body mass, length of escorting period, pre-escorting mean fGC concentration, rainfall or the number of pups (see Table S2c). Body mass measures after the escorting period were higher in larger groups, with a shorter escorting period and with higher pre-escorting body mass, but there were no effects of age, group size, rainfall or the number of pups (see Table S2c).

Discussion

Our findings provide support for the hypothesis that carry-over effects of (allo-) parental investment are mediated by variation in GC concentrations. Banded mongooses showed significant carry-over effects of alloparental care when individuals had a short time to recover between breeding attempts. Individuals with low fGC concentrations prior to the care period were more likely to provision pups, and individuals that invested most in alloparental care of offspring showed elevated fGC concentrations during the care period. The elevated fGC concentrations observed in high investors persisted after the care period, consistent with the idea that GCs serve a homeostatic function to inhibit further offspring care after periods of

high parental investment. Finally, the elevated fGC concentrations observed in individuals that provisioned offspring declined with time since the end of the care period. This result complements our finding that carry-over effects are absent when there is a longer interval between breeding attempts and supports our general hypothesis that variation in parental effort attributable to previous investment is mediated by circulating GCs.

The methods used in this study were limited to correlations between hormones and behaviour; this lack of hormonal manipulation means that we are unable to state explicitly that GCs inhibit alloparental care in banded mongooses. However, the function of GCs to inhibit reproduction is common across vertebrate taxa (Balm 1999; Tilbrook, Turner & Clarke 2000; Moore & Jessop 2003), suggesting that GC inhibition of provisioning behaviours is likely to occur in this system. For example, a study of house sparrows (*Passer domesticus*) found that parents with low GCs prior to the breeding season have high provisioning rates, and these high provisioners experience a GC increase during the breeding season (Ouyang *et al.* 2011). These findings closely match the results described in the current study and indicate that GC mediation of carry-over effects on parental care may extend to non-cooperative species.

Supplementary feeding of provisioners during the care period led to reduced fGC concentrations in these individuals, supporting our hypothesis that high GCs during the care period are attributable to energetic losses. That supplementary feeding increased daily weight gain in non-escorts but left their GC concentrations unchanged is consistent with this conclusion; non-escorts are less likely to experience a net resource deficit (and a consequent rise in

circulating GC concentrations; McEwen & Wingfield 2003) during the escorting period, leaving it not unexpected that feeding non-escorts left their fGC concentrations unchanged.

Though we have focused on carry-over effects on post-natal parental investment (i.e. provisioning rates), we are aware that carry-over effects due to energetic constraints can affect a range of pre-natal reproductive traits such as clutch size (Ebbinge & Spaans 1995), timing of breeding (Sorensen *et al.* 2009), size of eggs (Sorensen *et al.* 2009) or the likelihood of pregnancy (Cook *et al.* 2004). Recent evidence suggests that state-dependent variation in circulating GC concentrations can also inhibit pre-natal parental investment through its effects on follicle development (Vitousek *et al.* 2010), egg-laying date (Goutte *et al.* 2011) and circulating concentrations of luteinising hormone (Goutte *et al.* 2010). Again, this is highly indicative of a widespread role for GCs in mediating reproductive carry-over effects. Whether energetic constraints and associated elevated GC concentrations affect pre- or post-natal parental investment will depend on species-specific costs and benefits. The benefits of making facultative adjustments to provisioning rates may be especially apparent for helpers within cooperatively breeding species as they have no control over the presence of young within the group. Furthermore, compensatory increases in provisioning by other helpers mean that the costs of reduced provisioning by a single helper can be very low (Wright & Dingemanse 1999; Koenig & Walters 2011). By contrast, the absence of carers in non-cooperative systems means that any decrease in provisioning can have large impacts on offspring survival. Hence, non-cooperative breeders may benefit from using GC signalling of energetic state to delay breeding or reduce the number of offspring, rather than reducing their investment in care.

Studies of non-cooperative species have highlighted carry-over effects as drivers of variation in reproductive success between individuals (Harrison *et al.* 2011). However, it remains unknown if carry-over effects generate similar variation in the success of reproductive attempts in cooperatively breeding species. The amount of care that a banded mongoose pup receives during the escorting period has large effects on its survival, growth and fecundity (Hodge 2005). Reductions in alloparental effort attributable to carry-over effects of previous investment may therefore have detrimental consequences for the fitness of pups. Compensatory increases in care effort from other helpers may buffer these effects (Komdeur 1994; Hatchwell 1999; Wright & Dingemanse 1999), but complete compensation might not be expected and indeed there may be circumstances where it is not possible. For example, while in large cooperative groups, small compensatory responses by many carers may be sufficient to offset reductions in care by any given individual, in small groups such buffering may be more difficult to achieve. If this is the case, the implications of carry-over effects for offspring fitness may be greater in smaller cooperative groups and might indeed

contribute to the increased extinction rates often documented in such species at low population densities (i.e. Allee effects; Courchamp, Clutton-brock & Grenfell 1999).

Our results contrast with previous findings that, in cooperatively breeding meerkats (*Suricata suricatta*), provisioning effort is positively correlated with GC concentrations prior to the care period (Carlson *et al.* 2006a). However, there are some clear differences in provisioning behaviours between meerkats and banded mongooses which may explain this disparity; meerkats do not form the stable pup-escort relationships that are seen in banded mongoose societies. This lack of stable pup-adult associations means that meerkat helpers may be able to modulate their alloparental investment throughout the care period given daily fluctuations in energetic state. Hormone measures prior to the pup care period may not therefore be representative of hormone concentrations when the (hour by hour) decision regarding how heavily to invest in pup care is being made. For example, babysitting effort (care of young in the den) in meerkats is negatively correlated with GC concentrations when measured on the day (i.e. in the early morning prior to the babysitter being left) but not when measured prior to the breeding attempt and related to an individual's long-term contribution to babysitting (Carlson *et al.* 2006b). Though it is possible that banded mongoose escorts alter their provisioning rates on a daily basis similar to that suggested of meerkat helpers, pup-escort relationships tend to be stable throughout the pup care period (Gilchrist 2004), and so the decision to invest heavily in pup care through the escorting period is likely to be made at pup emergence – which is when we see the GC association.

Together, the results of this study provide evidence for the hypothesis that GCs may be the general mechanism through which state-dependent carry-over effects are mediated in vertebrates. However, limitations of the current study (i.e. the absence of hormonal measures in consecutive breeding attempts) mean that further study is required to fully demonstrate such a mechanism. Our focus on the hormonal mechanisms regulating alloparental investment may help to explain why there is so much within-group variation in helping effort in cooperatively breeding societies, even among individuals of the same age, sex, condition, relatedness and dominance class (Cant & Field 2001; Griffin & West 2003; Cornwallis, West & Griffin 2009; Bergmüller, Schürch & Hamilton 2010). Our results corroborate previous findings that GCs modulate parental investment (Goutte *et al.* 2010, 2011; Vitousek *et al.* 2010) and suggest that different social and environmental factors may interact with previous investment to affect GC concentrations and associated current or future parental investment. For example, positive social interactions can decrease circulating GC concentrations (Sachser, Dürschlag & Hirzel 1998; Thorsteinsson & James 1999; DeVries, Glasper & Detillion 2003), which suggests that GC-mediated carry-over effects may be attenuated in individuals experiencing positive social interactions.

Furthermore, where carry-over effects are mediated by changes to GC concentrations, the magnitude of the carry-over effect will be greater in individuals with amplified reactivity of the hypothalamic-pituitary-adrenal (HPA) axis (i.e. individuals that show a greater increase in circulating GCs in response to a stressful stimulus). Where research has shown that adult HPA reactivity is affected by early-life factors such as maternal stress and parental investment (Anisman *et al.* 1998; Emack *et al.* 2008; Love & Williams 2008; Banerjee *et al.* 2012), our findings indicate that these early-life factors may have larger effects on patterns of parental investment and lifetime reproductive success than previously described.

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Data accessibility

All data analysed in this study are available online in supporting information (see Table S2).

References

- Anisman, H., Zaharia, M.D., Meaney, M.J. & Merali, Z. (1998) Do early-life events permanently alter behavioral and hormonal responses to stressors? *International Journal of Developmental Neuroscience*, **16**, 149–164.
- Balm, P.H.M. (ed.) (1999) *Stress Physiology in Animals*. Sheffield Academic Press, UK.
- Banerjee, S.B., Arterbery, A.S., Fergus, D.J. & Adkins-Regan, E. (2012) Deprivation of maternal care has long-lasting consequences for the hypothalamic-pituitary-adrenal axis of zebra finches. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **279**, 759–766.
- Bates, D., Maechler, M. & Bolker, B. (2013) *lme4: Linear Mixed-Effects Models Using Eigen and S4 classes*. R Package Version 0.999999-2. <http://lme4.r-forge.r-project.org/>.
- Bergmüller, R., Schürch, R. & Hamilton, I.M. (2010) Evolutionary causes and consequences of consistent individual variation in cooperative behaviour. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **365**, 2751–2764.
- Broderick, A.C., Godley, B.J. & Hays, G.C. (2001) Trophic status drives interannual variability in nesting numbers of marine turtles. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **268**, 1481–1487.
- Cant, M.A. (2000) Social control of reproduction in banded mongooses. *Animal Behaviour*, **59**, 147–158.
- Cant, M.A. (2003) Patterns of helping effort in co-operatively breeding banded mongooses (*Mungos mungo*). *Journal of Zoology*, **259**, 115–121.
- Cant, M.A. & Field, J. (2001) Helping effort and future fitness in cooperation animal societies. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **268**, 1959–1964.
- Cant, M.A., Vitikainen, E. & Nichols, H.J. (2013) Demography and social evolution of banded mongooses. *Advances in the Study of Behavior*, **45**, 407–446.
- Carlson, A.A., Manser, M.B., Young, A.J., Russell, A.F., Jordan, N.R., McNeilly, A.S. *et al.* (2006a) Cortisol levels are positively associated with pup-feeding rates in male meerkats. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **273**, 571–577.
- Carlson, A.A., Russell, A.F., Young, A.J., Jordan, N.R., McNeilly, A.S., Parlow, A.F. *et al.* (2006b) Elevated prolactin levels immediately precede decisions to babysit by male meerkat helpers. *Hormones and Behavior*, **50**, 94–100.
- Cook, J.G., Grande, L., Johnson, B.K., Cook, R.C., Riggs, R.A., Bryant, L.D. *et al.* (2004) Effects of summer-autumn nutrition and parturition date on reproduction and survival of elk. *Wildlife Monographs*, **155**, 1–64.
- Cornwallis, C.K., West, S.A. & Griffin, A.S. (2009) Routes to indirect fitness in cooperatively breeding vertebrates: kin discrimination and limited dispersal. *Journal of Evolutionary Biology*, **22**, 2445–2457.
- Courchamp, F., Clutton-brock, T. & Grenfell, B. (1999) Inverse density dependence and the Allee effect. *Trends in Ecology & Evolution*, **14**, 405–410.
- DeVries, A.C., Glasper, E.R. & Detillion, C.E. (2003) Social modulation of stress responses. *Physiology & Behavior*, **79**, 399–407.
- Ebbinge, B.S. & Spaans, B. (1995) The importance of body reserves accumulated in spring staging areas in the temperate zone for breeding in Dark-bellied Brent Geese *Branta B. bernicla* in the high Arctic. *Journal of Avian Biology*, **26**, 105–113.
- Emack, J., Kostaki, A., Walker, C.-D. & Matthews, S.G. (2008) Chronic maternal stress affects growth, behaviour and hypothalamo-pituitary-adrenal function in juvenile offspring. *Hormones and Behavior*, **54**, 514–520.
- Festa, M., Gaillard, J.M., Jorgenson, J.T., Festa-bianchet, M. & Gaillard, J. (1998) Mass- and density-dependent reproductive success and reproductive costs in a capital breeder. *American Naturalist*, **152**, 367–379.
- Fournier, D., Skaug, H., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M. *et al.* (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. <http://glmmadmb.r-forge.r-project.org/>.
- Gilchrist, J.S. (2004) Pup escorting in the communal breeding banded mongoose: behavior, benefits, and maintenance. *Behavioral Ecology*, **15**, 952–960.
- Gilchrist, J.S. (2006) Reproductive success in a low skew, communal breeding mammal: the banded mongoose, *Mungos mungo*. *Behavioral Ecology and Sociobiology*, **60**, 854–863.
- Gilchrist, J.S., Otali, E. & Mwanguhya, F. (2004) Why breed communally? Factors affecting fecundity in a communal breeding mammal: the banded mongoose (*Mungos mungo*). *Behavioral Ecology and Sociobiology*, **57**, 119–131.
- Goutte, A., Angelier, F., Chastel, C.C., Trounev, C., Moe, B., Bech, C. *et al.* (2010) Stress and the timing of breeding: glucocorticoid-luteinizing hormones relationships in an arctic seabird. *General and Comparative Endocrinology*, **169**, 108–116.
- Goutte, A., Clément-Chastel, C., Moe, B., Bech, C., Gabrielsen, G.W. & Chastel, O. (2011) Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes. *Journal of Experimental Biology*, **214**, 2005–2013.
- Griffin, A.S. & West, S.A. (2003) Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science*, **302**, 634–636.
- Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. & Bearhop, S. (2011) Carry-over effects as drivers of fitness differences in animals. *Journal of Animal Ecology*, **80**, 4–18.
- Hatchwell, B.J. (1999) Investment strategies of breeders in avian cooperative systems. *American Naturalist*, **154**, 205–219.
- Hodge, S.J. (2005) Helpers benefit offspring in both the short and long-term in the cooperatively breeding banded mongoose. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **272**, 2479–2484.
- Hodge, S.J. (2007) Counting the costs: the evolution of male-biased care in the cooperatively breeding banded mongoose. *Animal Behaviour*, **74**, 911–919.
- Hodge, S.J., Bell, M.B.V. & Cant, M.A. (2011) Reproductive competition and the evolution of extreme birth synchrony in a cooperative mammal. *Biology Letters*, **7**, 54–56.
- Inger, R., Harrison, X.A., Ruxton, G.D., Newton, J., Gudmundsson, G.A., Mclwaine, G. *et al.* (2010) Carry-over effects reveal reproductive costs in a long-distance migrant. *Journal of Animal Ecology*, **79**, 974–982.
- Jordan, N.R., Mwanguhya, F., Kyabulima, S. & Cant, M.A. (2010) Scent marking within and between groups of wild banded mongooses. *Journal of Zoology*, **280**, 72–83.
- Kelly, C. & Price, T.D. (2012) Correcting for Regression to the Mean in Behavior and Ecology. *American Naturalist*, **166**, 700–707.
- Koenig, W.D. & Walters, E.L. (2011) Brooding, provisioning, and compensatory care in the cooperatively breeding acorn woodpecker. *Behavioral Ecology*, **23**, 181–190.
- Komdeur, J. (1994) Experimental evidence for helping and hindering by previous offspring in the cooperative-breeding Seychelles warbler *Acro-*

- cephalus sechellensis*. *Behavioral Ecology and Sociobiology*, **34**, 175–186.
- Love, O.P. & Williams, T.D. (2008) Plasticity in the adrenocortical response of a free-living vertebrate: the role of pre- and post-natal developmental stress. *Hormones and Behavior*, **54**, 496–505.
- McEwen, B.S. & Wingfield, J.C. (2003) The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, **43**, 2–15.
- Moore, I.T. & Jessop, T.S. (2003) Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Hormones and Behavior*, **43**, 39–47.
- Munro, C. & Stabenfeldt, G. (1984) Development of a microtitre plate enzyme immunoassay for the determination of progesterone. *Journal of Endocrinology*, **101**, 41–49.
- Nichols, H.J., Bell, M.B.V., Hodge, S.J. & Cant, M.A. (2012) Resource limitation moderates the adaptive suppression of subordinate breeding in a cooperatively breeding mongoose. *Behavioral Ecology*, **23**, 635–642.
- Norris, D.R., Marra, P.P., Kyser, T.K., Sherry, T.W. & Ratcliffe, L.M. (2004) Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **271**, 59–64.
- O'Connor, C.M., Norris, D.R., Crossin, G.T. & Cooke, S.J. (2014) Biological carry-over effects: linking comment concepts and mechanisms in ecology and evolution. *Ecosphere*, **5**(3), art28, DOI <http://dx.doi.org/10.1890/ES13-00388.1> [Correction added 19 August 2014 after first online publication: reference details updated.]
- Ouyang, J.Q., Sharp, P.J., Dawson, A., Quetting, M. & Hau, M. (2011) Hormone levels predict individual differences in reproductive success in a passerine bird. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **278**, 2537–2545.
- Perryman, Q.L., Donahue, M.A., Perkins, P.C. & Reilly, S.B. (2002) Gray whale calf production 1994–2000: are observed fluctuations related to changes in seasonal ice cover? *Marine Mammal Science*, **18**, 121–144.
- Prince, P.A., Rothery, P., Chroxall, J. & Wood, A.G. (1994) Population dynamics of black-browed and grey-headed Albatrosses *Diomedes melanophrys* and *D. chrysostoma* at Bird Island, South Georgia. *Ibis*, **136**, 50–71.
- R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN: 3-900051-07-0. <http://www.R-project.org>.
- Russell, A.F., Sharpe, L.L., Brotherton, P.N.M. & Clutton-Brock, T.H. (2003) Cost minimization by helpers in cooperative vertebrates. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 3333–3338.
- Sachser, N., Dürschlag, M. & Hirzel, D. (1998) Social relationships and the management of stress. *Psychoneuroendocrinology*, **23**, 891–904.
- Sorensen, M.C., Hipfner, J.M., Kyser, T.K. & Norris, D.R. (2009) Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. *Journal of Animal Ecology*, **78**, 460–467.
- Thorsteinsson, E.B. & James, J.E. (1999) A Meta-analysis of the effects of experimental manipulations of social support during laboratory stress. *Psychology & Health*, **14**, 869–886.
- Tilbrook, A., Turner, A. & Clarke, I. (2000) Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences. *Reviews of Reproduction*, **5**, 105–113.
- Vitousek, M.N., Mitchell, M.A., Romero, L.M., Awerman, J. & Wikelski, M. (2010) To breed or not to breed: physiological correlates of reproductive status in a facultatively biennial iguanid. *Hormones and Behavior*, **57**, 140–146.
- Walker, S.L., Waddell, W.T. & Goodrowe, K.L. (2002) Reproductive endocrine patterns in captive female and male red wolves (*Canis rufus*) assessed by fecal and serum hormone analysis. *Zoo Biology*, **21**, 321–335.
- Watson, R., Munro, C., Edwards, K.L., Norton, V., Brown, J.L. & Walker, S.L. (2013) Development of a versatile enzyme immunoassay for non-invasive assessment of glucocorticoid metabolites in a diversity of taxonomic species. *General and Comparative Endocrinology*, **186**, 16–24.
- Wright, J. & Dingemanse, N.J. (1999) Parents and helpers compensate for experimental changes in the provisioning effort of others in the Arabian babbler. *Animal Behaviour*, **58**, 345–350.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Table S1. (a–g) All data collected during the course of this study.

Table S2. (a–c) Coefficient estimates and *P*-values from mixed models