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2 facultatively social mammal

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Glucocorticoid levels predict subsequent social tactic in females of a facultatively social mammal

ABSTRACT

1. Facultatively social species, in which individuals can switch between group- and solitary-living tactics, offer an opportunity to shed light on proximate mechanisms underlying alternative life histories. Promising hormonal mediators of social tactic include glucocorticoids, which control energy allocation and are negatively related to body condition, and testosterone which regulates numerous social behaviours.
2. Here, we investigated hormone profiles associated with social tactic in eight generations of free-living female striped mice (*Rhabdomys pumilio*). Females are group living during the non-breeding season but live solitarily or with female kin in communally-breeding groups during the breeding season.
3. We tested whether females' baseline levels of corticosterone (a glucocorticoid) and testosterone measured during the breeding season differed before and after females left the group (i.e. when they switched from a group-living to solitary social tactic), and whether the hormone levels of females that remained group living differed before and after their nestmates became solitary. We also asked whether seasonal variation in hormone levels was associated with social tactic.
4. During the breeding season, corticosterone levels were lower in solitary than group-living females both before and after solitary females left the group, and did not differ in solitary females before and after leaving. In group-living females, corticosterone was higher after their nestmates became solitary than before they left. Testosterone levels did not differ before and after females became solitary and were not associated with social tactic.
5. Corticosterone was higher during the breeding than the non-breeding season in group-living females but did not differ between the seasons in solitary females. Testosterone levels were higher in both social tactics during the non-breeding season.
6. We conclude that baseline corticosterone but not testosterone levels are associated with female social tactic, and that corticosterone levels at the start of the breeding season, when all females are group living, may be an indicator of subsequent tactic:

solitary females' corticosterone levels were lower than those of females that remained group living even before the solitary phenotype was expressed. Glucocorticoids might therefore play a modulatory role in social organisation in facultatively social species.

KEYWORDS

alternative reproductive tactics; corticosterone; plural breeder; seasonality; social environment; social organization; social plasticity; socially flexible

INTRODUCTION

Understanding the ecological and physiological mechanisms underlying alternative life histories is a key challenge in evolutionary biology. Facultatively social (or socially flexible) species are those where group- and solitary-living individuals can occur adaptively in a population at the same time, and where an individual can show more than one such social tactic within its lifetime (O'Brien, Tammone, Cuello, & Lacey, 2020; Schradin et al., 2012; Shell & Rehan, 2018). For individuals of facultatively social species, the decision to be group or solitary living is shaped by various evolutionary and ecological factors, including genetic relatedness to group members, reproductive competition and ecological constraints linked to population density (Koenig, Pitelka, Carmen, Mumme, & Stanback, 1992; Randall, Rogovin, Parker, & Eimes, 2005). At a proximate level, it is well established that different social environments elicit variation in the secretion of steroid hormones, such as glucocorticoids and testosterone (Creel, Dantzer, Goymann, & Rubenstein, 2013; Wingfield, Hegner, Dufty, & Ball, 1990). Several studies have compared hormone levels between different social tactics (e.g. Woodruff, Lacey, Bentley, & Kriegsfeld, 2013), but very few have compared hormones before and after individuals switch from one social tactic to another (e.g. Schradin & Yuen, 2011). Elucidating whether changes in hormone level occur before, during or after a tactic switch is necessary to determine whether the expression of social tactic is under hormonal control.

Glucocorticoids (GCs) of the hypothalamic-pituitary–adrenal (HPA) axis regulate energy acquisition and mobilization in all vertebrates, maintaining both homeostasis and the

expression of appropriate responses to environmental challenges (Reeder & Kramer, 2005). By coordinating a suite of physiological and behavioural changes, GCs facilitate transitions between life history stages, including metamorphosis, dispersal, migration, and the differentiation and maintenance of alternative reproductive tactics (ARTs) in a range of taxa (Crespi, Williams, Jessop, & Delehanty, 2013; Oliveira, Canário, & Ros, 2008; Wada, 2008). Baseline GCs are influenced by the social environment and can help individuals to prepare for social challenges (Creel et al., 2013). The presence of a stressor triggers GC secretion, which reallocates energy from physiological processes and behaviours that are not essential to immediate survival to those that aim to resolve the stressful situation (Chrousos, 2009; Reeder & Kramer, 2005). Social stress occurs when an individual's natural capacity to cope with the demands of the social environment is exceeded. This might arise as a consequence of interactions with members of the same or a different social group, including conflicts over resources, or from reproductive competition (Goymann & Wingfield, 2004; Robertson et al., 2017). While GC secretion can help individuals to confront challenges in the short-term, long-term activation of the HPA axis can have deleterious effects (Bartolomucci, 2007). In contrast, positive social interactions can reduce stress through social buffering (Hennessy, Kaiser, & Sachser, 2009). In domestic guinea pigs, *Cavia porcellus*, for example, GC levels were lower in females introduced to novel environments alongside a female group member compared to in females introduced alone (Hennessy, Zate, & Maken, 2008). The social environment can therefore produce or buffer stress, which might shape decisions to be group or solitary living.

Sex steroids such as testosterone, regulated by the hypothalamic-pituitary-gonadal (HPG) axis, play an important role in regulating reproduction and social behaviours such as territoriality, aggression and breeding dispersal. In males, the sex for which most work has been carried out, testosterone levels are higher after dispersal than in philopatric adults (Alberts, Sapolsky, & Altmann, 1992; Holekamp & Smale, 1998; Schradin & Yuen, 2011). In both sexes, androgens assist in protection of the central nervous system (reviewed in Fargo, Foecking, Jones, & Sengelaub, 2009), and can increase skeletal muscle contraction and glucose metabolism (Yoshioka, Boivin, Bolduc, & St-Amand, 2007). Androgens also play an important role in many aspects of female physiology (Staub & DeBeer, 1997). In females, testosterone is synthesised in the ovaries, and also in the brain in response to social stimuli (de Bournonville, McGrath & Remage-Healey, 2020), and androgen receptors are present in many neural and peripheral tissues (Staub & DeBeer, 1997). Female Belding's

ground squirrels (*Uroditellus beldingi*) treated with testosterone at birth were more likely to disperse than were control females (Nunes, Duniec, Schweppe, & Holekamp, 1999). These observations point to the possibility that androgens modulate behaviours related to social organisation in females.

Here, we aimed to investigate the endocrine basis of social tactics in free-living female striped mice (*Rhabdomys pumilio*), a diurnal and facultatively social species, by comparing hormone levels before and after females switched social tactic. Striped mouse groups usually consist of 2-4 closely related females, one male and their shared offspring. Group-living females breed plurally and synchronously in the same nest, engaging in alloparental care, including allo-nursing. Nevertheless, intense reproductive competition can occur between female nestmates, based on evidence of infanticide and aggression in captive groups of communally-breeding sisters (Schradin, König, & Pillay, 2010). Under natural conditions, some females leave their natal group and adopt a solitary-living social tactic during the breeding season, probably to avoid this conflict (Schradin et al, 2010). These solitary females can establish their own territory using part of the previous home territory and/or neighbouring areas. In the non-breeding season (December to July), striped mice live in groups with their philopatric adult offspring.

Using field data from eight generations of striped mice, we tested whether baseline corticosterone and testosterone levels in females that would later become solitary differed from those of females that had left, and whether the hormone levels of group-living female whose nestmates had not yet left differed from those of females whose nestmates had already become solitary. Levels of both hormones were expected to be higher before than after females became solitary as a result of greater social stress and reproductive competition when all females were group-living. Corticosterone levels are lower in solitary-breeding than communally-breeding female striped mice, but testosterone levels do not differ between female reproductive phenotypes (Hill, Pillay, & Schradin, 2015a). We therefore predicted that solitary females, which have a more favourable body condition than group-living females (Hill, Pillay, & Schradin, 2015b), would have lower corticosterone levels than group-living females before and after leaving the group. Second, we asked whether hormone levels vary with group size during the breeding season. We expected corticosterone levels to increase with the number of adult female nestmates owing to increased reproductive competition in larger groups, and for the effects to be more pronounced with proximity to parturition. Third,

we compared breeding season hormone levels with those measured during the non-breeding season. We expected corticosterone and testosterone to be elevated during the breeding season to enable females to meet the energetic demands of reproduction (Bonier, Moore, Martin, & Robertson, 2009; Romero, 2002), and we expected to observe a greater increase in breeding season hormone levels in group-living than solitary females. Finally, we tested the prediction that corticosterone levels in solitary-living females would decrease with distance moved from the group. We expected females with hormone profiles more similar to those of group-living females (i.e. those with higher corticosterone levels) to remain in closer proximity to their group.

MATERIALS AND METHODS

Data collection

Fieldwork took place in Goegap Nature Reserve, Northern Cape Province, South Africa (29°41.715 S, 18°01.560 E) from 2006 to 2013. This is an open semi-arid habitat of sandy areas and solitary standing shrubs in which striped mice nest. The site received 200.2 ± 6.88 mm precipitation *per annum* over the study period, mostly falling between April and September (austral winter and spring).

Live traps (26×9×9 cm) baited with bran flakes, sunflower oil and salt were set close to known nest sites just before striped mice became active in the morning and again before they returned to their nests in the late afternoon (the coolest times of the day). Traps were checked 30-45 min after being set, and trapped striped mice were removed and weighed to the nearest gram using an electronic balance. Reproductive status was estimated by assessing whether females' nipples were elongated and pink (suggestive of lactation), visible or not visible. Newly-trapped individuals were provided with aluminium ear-tags (National Band and Tag, Newport, KY) to enable identification, and marked with non-toxic hair dye (Inecto, Pinetown, South Africa), so that they could be recognised during behavioural observations (see below). Juveniles (body mass <30g) were assumed to originate from the nest where they were first trapped and observed interacting with group members, and this assumption was confirmed by molecular analysis for individuals trapped in 2007 and 2008 (Schradin & Lindholm, 2011). Genetic data show that females that spend the night in a shared nest during the breeding season ('nestmates') are close kin (C. Schradin and A.K. Lindholm, unpublished

data). All adult striped mice were fitted with MD-2C radio-collars (Holohil, Canada) during the August to November breeding seasons. Radio-collars weighed 2.5g, representing 5.7% of the body mass of non-gestating female striped mice ($N = 282$ records from 106 females).

We used radio-tracking to determine the location of sleeping sites in shrubs and the identities of adults in a nest. Radio-tracking was carried out 4-5 nights a week during the breeding season using an AR8000 wide-range receiver (AOR, Tokyo, Japan) and an RA-14K antenna (Telonics, Mesa, AZ). Shrubs were scanned from different angles until an individual's unique presence in the shrub was confirmed from her radio frequency. We compared signal intensity at different points around the shrub and any neighbouring shrubs. The open nature of the habitat allowed sleeping sites to be identified with confidence, and these were marked in the field with a unique identifier. Striped mice were assumed to be sharing a nest when their signals were emitted from the same shrub. If an individual was not found in its usual sleeping site, the surrounding area was surveyed until the individual was located. We also radio-tracked each individual once during the day, after it had left the nest to forage, to establish that radio-collars had not been discarded in the nest. Locations of individuals were logged using an eTrex Venture GPS (GARMIN, Olathe, KS), which was accurate to approximately 5m.

To confirm that all group members were identified and to avoid sampling bias, we carried out behavioural observations when striped mice were returning to nest sites at dusk. Individuals were observed from approximately 10m for 30 min using binoculars, noting individual identities (based on marking patterns) and any affiliative or aggressive interactions. Groups were observed on rotation throughout the year, except that trapping and observations were not carried out on the same day to minimise disturbance.

Determining social tactic, gestation status and age

We used the radio-tracking data to identify females that permanently left their natal group (i.e. those that stopped nesting with adult female group members at night) and did not return ('solitary-living females') and those that remained group living, sharing a nest with ≥ 1 adult female ('group-living females'). Female striped mice often leave the natal nest for several nights to give birth, and then resume nesting with their original group ('returners'; Hill et al., 2015b). These females do not differ in corticosterone and testosterone profiles from other group-living females (Hill et al. 2015a). Similarly, we found no difference in breeding season

corticosterone ($\beta = 0.07 \pm 0.14$, $t_{103.0} = 0.53$, $P = 0.595$) or testosterone ($\beta = -0.07 \pm 0.18$, $t_{123.7} = 0.39$, $P = 0.701$) between group-living females whose only nestmate(s) had left and would later return (corticosterone: $N = 16$ samples from 14 females; testosterone $N = 20$ samples from 16 females) and those whose nestmates were present (corticosterone: $N = 93$ samples from 54 females; testosterone: $N = 110$ samples from 57 females). Returners and females whose nestmates temporarily left were thus treated here as group living; a group-living female could therefore spend nights alone under these circumstances. We ensured that females classed as solitary were not nesting with unmonitored females by observing and trapping at the sites of new nests. Females can also nest alone owing to mortality of nestmates; the hormone profiles of these females were examined in a previous study (Hill et al., 2015a). In the present study we focussed solely on individuals whose adult female relatives were living when blood was sampled (see below). Distances between former group nests and new solitary nests were estimated using the distance tool in MAPSOURCE 6.12 (GARMIN).

To determine a female's gestation status, we plotted her body mass records against the date, and examined the plot for the rise and abrupt fall in body mass indicative of gestation and parturition. Parturition was assumed to occur on the median date within the interval between a female's last body mass measurement antepartum and her first measurement post-partum. Estimated parturition dates were checked against records of reproductive status. The date of birth of each female was modelled from her body mass at first capture as a juvenile using a population-specific growth curve (Schradin, Schneider, & Yuen, 2009).

Blood sampling

Blood samples were collected several mornings a week between August 2006 and December 2013. Each group was sampled on rotation for 1-3 days throughout this period. Sampling took place within 60 min of striped mice becoming active in the morning to minimise effects of circadian rhythms on circulating hormone levels (e.g. Dallman et al., 1987). Traps were set close to known nest sites and monitored from 5-10m. Trapped individuals were immediately removed from the trap and anaesthetized with diethyl ether. A blood sample of 100-500 μ l (depending on body size) was drawn from the sub-lingual vein. We recorded the time taken to collect a blood sample from the moment an individual entered the trap. All samples assayed for corticosterone were collected ≤ 180 s from the individual entering the trap; within this timeframe, there was no effect of sampling duration on corticosterone level ($\beta = -$

0.001±0.001, $t_{269,6} = 1.03$, $P = 0.306$; $N = 287$ samples from 110 females). Striped mice were monitored as they recovered from anaesthesia, weighed and then released at the site of capture. Blood samples were brought immediately to a laboratory next to the study site, where they were left to clot for 1 h at room temperature, centrifuged to allow the serum to be extracted and then stored at -20°C.

Hormone assays

Blood samples were analysed for corticosterone and testosterone using commercial Enzyme-Linked Immuno-Sorbent Assay (ELISA) kits (Immuno-Biological Laboratories, Hamburg). The cross-reactivity of the corticosterone assay was 7.4% with progesterone, 3.4% with deoxycorticosterone, 1.6% with 11-dehydrocorticosterone, 0.3% with cortisol and 0.3% with pregnenolone. The cross-reactivity of the testosterone assay was 3.3% with 11β-hydroxytestosterone and 19-nortestosterone, 0.9% with androstenedione, 0.8% with 5α-dihydrotestosterone and <0.1% with cortisol. Validation of both hormones is described in Schradin (2008). Intra- and inter-assay variabilities in hormone levels were estimated using pools of striped mouse serum so that values from the same pools could be compared directly. Intra-assay variability for corticosterone from different pools was 9.9% (8 samples) and 12.2% (10 samples). For testosterone sampled from different pools, intra-assay variability was 5.3% (10 samples) and 8.8% (10 samples). Inter-assay variability was 8.1% (10 assays) for corticosterone and 12.2 and 13.7% (13 and 11 assays respectively) for testosterone. All blood samples were run in duplicate, providing a mean hormone concentration for each sample, and samples with a coefficient of variation $\geq 10\%$ were repeated. All values fell within the standard curves of the assays.

Hormone records

Hormone records were labelled 'before' or 'after' depending on when the blood sample was collected in relation to females becoming solitary. Table 1 shows the numbers of blood serum samples and individuals assayed for each hormone, season and year. A given breeding season yielded 1.85±0.12 (mean ± SE) corticosterone samples per female ($N = 94$ female and season combinations from 92 females) and 2.11±0.15 testosterone samples per female ($N = 93$ seasons from 91 females). A given non-breeding season provided 1.59±0.11 corticosterone samples ($N = 83$ seasons from 71 females) and 1.71±0.13 testosterone samples ($N = 97$ seasons from 82 females) per female. We assessed whether 'trap happiness' or 'trap shyness' could bias the distribution of samples between the 'before' and 'after' groups, and found no

association between the timing of sampling (before or after females became solitary) and whether or not the sample came from a recaptured female (Pearson's Chi-squared test with Yates' continuity correction: $\chi^2_1 = 2.08$, $P = 0.149$, $N = 221$ samples taken during the breeding season). Numbers of samples and individuals analysed in relation to social tactic and season are given in Fig. 1. Many hormone records (49.6%) also contributed to a companion study, Hill et al. (2015a).

Table 1. Numbers of blood samples analysed for each hormone and year during the breeding season ('BS'; August to November, inclusive) and non-breeding season ('NBS'). Numbers of individuals ($N = 135$ overall) are given in brackets where different from the number of records ($N = 667$ overall).

	Corticosterone			Testosterone		
	NBS	BS	Total	NBS	BS	Total
2006	0	13	13	1	10	11
2007	8	17(13)	25(18)	9(8)	17(14)	26(17)
2008	32(16)	70(25)	102(28)	37(18)	72(26)	109(26)
2009	28(15)	29(18)	57(21)	39(19)	42(18)	81(22)
2010	24(17)	31(12)	55(18)	33(17)	36(11)	69(18)
2011	12(4)	6(5)	18(8)	17(6)	14(9)	31(12)
2012	2	7	9	6(5)	5	11(9)
2013	26(18)	1	27(18)	23(20)	0	23(20)
Total	132(71)	174(92)	306(121)	165(82)	196(91)	361(121)

14 individuals provided samples in consecutive years (2 individuals in consecutive breeding seasons). 271 samples were assayed for both hormones. 331 records from the 2006-10 breeding seasons ($N = 156$ for corticosterone and 175 for testosterone) were included in Hill et al. (2015a).

Statistical analyses

Data were analysed in R version 4.0.3. (R Development Core Team, 2020). Linear Mixed effects Models (LMMs) were carried out using the lme4 (Bates, Maechler, Bolker, & Walker, 2015) and lmerTest (Kuznetsova, Brockhoff, & Christensen, 2017) packages. Planned contrasts between levels of significant interactions were assessed using emmeans (Lenth, 2020). Our study used two approaches: an analysis of all available samples (Table 1) and an analysis of paired samples to examine within-individual changes in hormone levels between tactics. All statistical tests are two-tailed and estimated marginal means $\pm$ SE are presented.

Did corticosterone levels vary with testosterone levels?

First, we tested for a relationship between corticosterone and testosterone levels for all 271 blood samples assayed for both hormones using the following LMM:

$$y_{i,j} = \mu + \text{testo}_{i,j} + id + group + year + \varepsilon \quad (1)$$

where y is the log-transformed corticosterone level for focal female i from blood sample j , μ is the overall mean (intercept) and testo is the testosterone level of female i from sample j . In all LMMs, id , $group$ and $year$ are random intercepts of individual identity, group identity and year of blood sampling respectively, and ε is the error term.

Did hormone levels differ before and after females left the group?

Based on all samples collected during the breeding season (August to November), we tested whether hormone levels differed between females that eventually left their group *versus* those that always remain group-living, and whether hormone levels changed before and after females became solitary both in those females that became solitary and in their nestmates.

We fitted the following LMM:

$$y_{i,k} = \mu + ST_i + \text{timing}_k + ST_i \times \text{timing}_k + \text{mass}_{i,k} + \text{age}_{i,k} + id + group + year + \varepsilon \quad (2)$$

where y is the log-transformed hormone level for focal female i on blood sampling date k , ST is a fixed factor representing the final social tactic (two levels: group or solitary living) of female i , timing is a fixed factor to indicate the social tactic of female i on sampling date k (two levels: before or after females became solitary. Where female i became solitary, timing indicates whether the sample was taken before or after her own departure; where i did not become solitary, timing indicates whether the sample was taken before or after the first departure of one or more of i 's adult female nestmates. In groups where all females remained group living, timing was coded as 'before'); mass and age are covariates of body mass and age of female i on date k .

In addition, where repeated samples were available from the same individual both before and after she became solitary ($N = 5$ females for corticosterone and 6 for testosterone) or, in the case of group-living females, before and after relatives became solitary ($N = 6$, testosterone

only), we carried out paired t-tests of the differences in hormone levels based on the closest available sample before and after the change in social tactic.

Did hormone levels vary with temporal proximity to leaving the group?

To investigate whether hormone levels varied with time before females become solitary, we analysed blood samples taken before females became solitary using the following LMM:

$$y_{i,k} = \mu + \text{proximity to leaving} + \text{mass}_{i,k} + \text{age}_{i,k} + \text{id} + \text{group} + \text{year} + \varepsilon \quad (3)$$

where *proximity to leaving* is the fixed effect of the absolute number of days between blood sampling on date *k* and females becoming solitary. Where female *i* became solitary, *proximity to leaving* is the number of days between sampling and female *i*'s own departure; where *i* remained group living, *proximity to leaving* is the number of days between sampling and the departure of one or more of *i*'s adult female nestmates. Solitary and group-living females were analysed in separate LMMs. Analyses were repeated using samples taken after females became solitary.

Did hormone levels vary with the number of female nestmates?

Next, focussing on females that remained group-living, we tested whether hormone levels varied with the number of females with which a gestating focal female was sharing a nest in the following LMM:

$$y_{i,k} = \mu + \text{no.nestmates}_{i,k} + DP_i + \text{no.nestmates}_{i,k} \times DP_i + \text{mass}_{i,k} + \text{age}_{i,k} + \text{id} + \text{group} + \text{year} + \varepsilon \quad (4)$$

where *no. nestmates* is the number of adult females nesting with *i* on sampling date *j* and *DP* is the number of days on sampling date *j* before female *i*'s parturition.

Did hormone levels differ between the breeding and non-breeding season?

We tested whether hormone levels differed between the breeding and non-breeding season in females that eventually became solitarily and those that remained group living using:

$$y_{i,k} = \mu + ST_i + \text{season}_k + ST_i \times \text{season}_k + \text{mass}_{i,k} + \text{age}_{i,k} + \text{id} + \text{group} + \text{year} + \varepsilon$$

(5)
This differs from (2) only in that *timing* is replaced with the fixed effect of *season* (breeding or non-breeding season) on sampling date *k*.

Did hormone levels of solitary females vary with distance moved from the group nest?

In females that became solitary, we tested whether there was a relationship between hormone level and the distance moved from the group nest using:

$$y_{i,k} = \mu + DM_i + mass_{i,k} + age_{i,k} + id + group + year + \varepsilon \quad (6)$$

where *DM* is the fixed effect of the distance moved by female *i* from the group nest to her new sleeping site. We expected females with lower corticosterone levels to move further from their natal groups than those with higher corticosterone.

Linear Mixed effect Models

Gestating striped mice had higher levels of corticosterone (1369 ± 128 ng/ml, $N = 77$ samples from 54 females) than non-gestating females (1002 ± 76 ng/ml, $N = 209$ samples from 88 females; $\beta = 0.31 \pm 0.08$, $t_{261.9} = 3.89$, $P < 0.001$). However, when body mass and age were taken into account, this effect was no longer significant ($\beta = 0.13 \pm 0.08$, $t_{270.2} = 1.50$, $P = 0.135$). We therefore controlled for body mass but not gestation status (to reduce potential collinearity between explanatory variables) in all LMMs. There was no association between gestation status and testosterone levels ($\beta = -0.05 \pm 0.10$, $t_{353.4} = 0.49$, $P = 0.623$, $N = 360$ samples from 120 females).

Continuous explanatory variables were mean-centred in LMMs to aid interpretability. Non-significant fixed effects ($P \geq 0.05$) were eliminated from models, removing the interaction term and then the main effects in order of least significance. We report results estimated using Restricted Maximum Likelihood for all fixed effects tested (where these were not significant, values are given for the last model in which they were included). Random effects were retained in all models to account for repeated measures. Where an interaction between factors (i.e. $ST \times timing$ in (2) and $ST \times season$ in (5)) was significant, we explored the nature of the interaction by testing all six pairwise contrasts arising from it, corrected for false discovery rate. Degrees of freedom for pairwise contrasts were calculated using the Kenward-Roger method.

RESULTS

Did corticosterone levels vary with testosterone levels?

There was no relationship between corticosterone and testosterone levels ($\beta = -0.02 \pm 0.02$, $t_{263,2} = 0.73$, $P = 0.465$, $N = 271$ samples from 106 females during the breeding and non-breeding seasons).

Did corticosterone levels differ before and after females left the group during the breeding season?

Females that went on to live solitarily had lower corticosterone levels than females that remained group-living, regardless of whether samples were taken before or after females left the group (Table 2, Figure 1a). There was an interaction between social tactic and the timing of sampling: in group-living females, corticosterone was higher after their female relatives left to become solitary than before, but corticosterone levels of females that would go on to leave the group did not differ from those of females that were already solitary living (Table 2, Figure 1a). Similarly, a paired test showed that solitary females' corticosterone levels did not change before (1146 ± 291 ng/ml) and after (916 ± 87 ng/ml) they left the group ($t_4 = 0.73$, $P = 0.505$; $N = 5$ individuals). Corticosterone levels increased with increasing body mass and decreased with age (Table 2).

Did corticosterone levels vary with temporal proximity to leaving the group?

For females that would later become solitary, there was a tendency for corticosterone levels to decrease as the date they left approached ($\beta = 0.01 \pm 0.005$, $t_{2,3} = 2.80$, $P = 0.090$; $N = 12$ samples from 9 females 1-83 days before leaving), but this was not statistically significant. There was no association between corticosterone levels and the number of days since leaving the group in solitary females ($\beta = 0.001 \pm 0.004$, $t_{5,7} = 0.35$, $P = 0.738$; $N = 15$ samples from 11 females 4-79 days after leaving). In females that remained group living, corticosterone levels were not associated with the number of days before their nestmates left the group ($\beta = 0.003 \pm 0.006$, $t_{2,1} = 0.56$, $P = 0.627$; $N = 17$ samples from 11 females 2-108 days before leaving) or after their nestmates left ($\beta = -0.001 \pm 0.004$, $t_{21,0} = 0.26$, $P = 0.801$; $N = 24$ samples from 12 females 1-94 days after leaving).

Did corticosterone levels vary with the number of female nestmates?

In group-living females during the breeding season, corticosterone levels did not vary with the number of adult female nestmates ($\beta = 0.01 \pm 0.05$, $t_{45.0} = 0.24$, $P = 0.816$; $N = 49$ samples from 38 females with 0-7 adult female nestmates on the sampling date, 1-23 days before parturition; note that group-living females, defined in Materials and Methods, could temporarily nest alone). There was no interaction between the number of female nestmates and days to parturition in gestating females ($\beta = -0.01 \pm 0.01$, $t_{44.0} = 1.27$, $P = 0.211$). Corticosterone levels increased as parturition approached ($\beta = -0.06 \pm 0.01$, $t_{48.8} = 4.98$, $P < 0.001$).

Did corticosterone levels differ between the breeding and non-breeding season?

During the breeding season, corticosterone levels were higher in group-living females than in those that were or would become solitary, but there was no difference during the non-breeding season (Table 3, Figure 1b). In females that remained group living, corticosterone levels were higher during the breeding season than during the non-breeding season, but there was no seasonal difference in females that became solitary (Table 3, Figure 1b).

Did testosterone levels differ before and after females left the group?

Breeding season testosterone levels did not differ between females that went on to leave the group and those that would remain group-living, and did not depend on whether samples were taken before or after females became solitary (Table 2, Figure 1c). Similarly, testosterone levels did not change in solitary females where paired samples were available before (1.00 ± 0.36 ng/ml) and after (0.83 ± 0.15 ng/ml) leaving the group (paired t-test: $t_5 = 0.42$, $P = 0.693$, $N = 6$ individuals) nor in group-living females before (0.58 ± 0.35 ng/ml) and after (0.52 ± 0.17 ng/ml) their relatives left the group ($t_5 = 0.22$, $P = 0.836$; $N = 6$ individuals). Testosterone levels were not related to body mass or age (Table 2, Table 3).

Did testosterone levels vary with proximity to leaving the group?

Breeding season testosterone levels in females that would later leave the group were not related to the number of days until they left ($\beta = 0.005 \pm 0.01$, $t_{12.9} = 0.45$, $P = 0.662$; $N = 19$ samples from 10 females 1-83 days before leaving), but decreased in females that were already solitary with the number of days since leaving ($\beta = -0.01 \pm 0.004$, $t_{8.0} = 2.43$, $P = 0.041$; $N = 24$ samples from 17 females 4-92 days after leaving). In females that remained group living, testosterone levels were not related to the number of days before nestmates left

the group ($\beta = 0.0003 \pm 0.01$, $t_{13.0} = 0.049$, $P = 0.962$; $N = 21$ samples from 14 females 30-108 days before females left) or after nestmates left ($\beta = -0.0005 \pm 0.01$, $t_{16.7} = 0.56$, $P = 0.585$; $N = 22$ samples from 11 females 1-94 days before females left).

Did testosterone levels vary with the number of female nestmates?

In gestating group-living females, testosterone levels did not vary with the number of female nestmates ($\beta = -0.08 \pm 0.06$, $t_{58.7} = 1.34$, $P = 0.186$; $N = 60$ samples from 47 females with 0-7 adult female nestmates 1-23 days before parturition), the number of days before the focal female gave birth ($\beta = 0.01 \pm 0.02$, $t_{59.4} = 0.50$, $P = 0.622$) nor an interaction between the two ($\beta = -0.01 \pm 0.01$, $t_{57.3} = 1.01$, $P = 0.318$).

Did testosterone levels differ between the breeding and non-breeding season?

Testosterone levels were lower during the breeding season (0.70 ± 0.12 ng/ml) than the non-breeding season (0.94 ± 0.16 ng/ml), and seasonal variation was not associated with social tactic (Table 3, Figure 1d).

Did hormone levels of solitary females vary with distance moved from the group nest?

Distances moved to establish a new nest were available for 24 females, of which 19 and 20 were assayed for breeding season levels of corticosterone and testosterone respectively. Eight females became solitary because their female nestmates moved away and 16 females became solitary by moving away from the group nest. The distances moved by the latter 16 females showed a right skewed distribution with a median of 45 (range 8-180)m and a raw mean of 54.67 ± 10.30 m. Females that became solitary because their female nestmates moved away were scored in analyses as moving 0m.

There was a trend towards declining corticosterone levels with distance moved from the nest ($\beta = -0.01 \pm 0.003$, $t_{15.5} = 1.97$, $P = 0.068$; $N = 32$ samples from 19 females for which samples were available before or after moving; range 0-180m; elimination of the non-significant age variable in distance analyses resulted in inclusion of 5 samples from 3 females with missing birth dates that were not retained in other LMMs). Females that became solitary because they moved away tended to have lower corticosterone levels (886 ± 170 ng/ml, $N = 19$ samples from 13 females) than females that became solitary because their nestmate(s) moved away (1371 ± 336 ng/ml, $N = 13$ samples from 6 females; $\beta = -0.43 \pm 0.18$, $t_{3.7} = 2.46$, $P = 0.074$). Before females left the nest there was no relationship between corticosterone level and the

distance they later moved ($\beta = -0.003 \pm 0.003$, $t_{11,0} = 1.18$, $P = 0.262$; $N = 13$ samples from 10 females taken 2-83 days before becoming solitary; range 0-180m). After females left the nest, corticosterone declined with increasing distance moved from the group nest ($\beta = -0.01 \pm 0.004$, $t_{13,7} = 3.32$, $P = 0.005$; $N = 19$ samples from 13 females taken 4-79 days after becoming solitary; range 0-92m). Testosterone levels were not related to distance moved ($\beta = -0.0005 \pm 0.004$, $t_{11,8} = 0.12$, $P = 0.911$; $N = 43$ samples from 21 females; range 0-180m). There was no difference in testosterone levels between females that became solitary because they moved away (0.49 ± 0.17 ng/ml, $N = 31$ samples from 15 females) or their nestmates moved away (0.66 ± 0.34 ng/ml, $N = 12$ samples from 6 females; $\beta = 0.26 \pm 0.35$, $t_{4,1} = 0.74$, $P = 0.499$).

DISCUSSION

Changes in social organisation, known as social flexibility, occur to maximise individual fitness, but little is known about the physiological mechanisms that modulate an individual's social tactic. Solitary-breeding female striped mice have lower baseline levels of corticosterone than communally-breeding females but do not differ from them in testosterone levels (Hill et al., 2015a). Here we show that the hormone levels of females that became solitary did not differ before and after they left the group. As corticosterone levels were lower in females that would become solitary than in group-living females both before and after solitary females left the group, low breeding season corticosterone levels appear to be a consistent physiological trait in females that are or will become solitary. Interestingly, the corticosterone levels of group-living females whose female kin had left the group were higher than those of females whose relatives had not yet left, which may reflect increased reproductive effort and / or competition later in the breeding season (e.g. Bonier et al., 2009). In sum, corticosterone but not testosterone levels were associated with alternative social tactic in female striped mice, and solitary females differed consistently from group-living females even before the switch to solitary living had occurred.

During the non-breeding season, when striped mice are group living, corticosterone levels did not differ between females that would later become solitary and those that would remain group living. As solitary females secreted lower levels of corticosterone than their nestmates before becoming solitary, we propose that differential secretion of corticosterone is a

potential mediator of alternative social tactic in female striped mice. In a separate study, breeding season corticosterone levels of solitary females fell below those of group-living female striped mice after females left the group to take up an experimentally vacated territory, but did not differ from group-living females' levels before leaving (Schoepf & Schradin, 2013). This agrees with the present study in the magnitude and direction of the difference, but not in when the difference occurred. This probably reflects differences between the studies in the relative importance of internal (hormonal) and external (e.g. population density) cues eliciting departure from the group. For example, if corticosterone levels respond positively to population density, we would expect to see a decline in corticosterone level only after an unpredictable reduction in density, as reported by Schoepf and Schradin (2013) in their experiment, whereas individuals might have more opportunity to prepare physiologically for leaving the group where environmental cues are more informative (i.e. no manipulation, the present study). Consistent with this idea, we observed a trend towards a decrease in corticosterone levels in our study as the date of leaving the group approached. GC levels did not vary with the number of females in the group unlike in the ring-tailed lemur, *Lemur catta* (Pride, 2005). Together these findings suggest that changes in a female's corticosterone levels occur gradually between the start of the breeding season and her departure from the group, and are likely to play a role in determining social tactic rather than being entirely a consequence of following different tactics. Nevertheless, we cannot exclude the possibility that the observed association between baseline corticosterone levels and social tactic is caused by a shared, unmeasured factor. Experimental manipulations of corticosterone level would provide valuable insight.

Solitary females' levels of corticosterone during their group-living stage were lower than those of females that remained group living. Before becoming solitary the two classes of females experienced similar social environments and the same population density, and so the hormonal difference might reflect an individual-level difference in susceptibility to social and reproductive stress, which could be tested by examining differences in the stress response. In social species, females might leave the group either voluntarily or, as in banded mongooses, *Mungos mungo*, another plural breeder, through eviction by more dominant or aggressive group members (Cant, Hodge, Bell, Gilchrist, & Nichols, 2010). In striped mice, the largest (Hill et al., 2015b) and thus most competitive females (Schradin, 2004) leave the group. In addition, no physical aggression between female group members nor injuries in females that became solitary have been observed in the field (based on 20 years of field

studies). By contrast, fights occur and female infanticide is common in captive-held groups where females cannot become solitary (Schradin et al., 2010). Under natural conditions, females appear to leave the group before aggression occurs, which might represent a tactic to avoid escalation of conflict and infanticide. These observations, and the lower levels of corticosterone in females that will later become solitary compared to those that will remain, offer correlative evidence that females are not evicted but leave voluntarily. As lower baseline corticosterone levels indicate a more favourable physical state in many species (Bonier, Martin, Moore, & Wingfield, 2009), solitary females might thus be better equipped to forego the benefits of group living, such as huddling and alloparental care, allowing them to profit from reduced infanticide risk. Body mass and corticosterone can also interact to influence survival, a proxy for fitness, in this species (Vuarin, Pillay, & Schradin, 2019) in support of the Cort-Fitness Hypothesis (Bonier et al., 2009). An individual's probability of surviving the non-breeding season decreases with increasing corticosterone levels for striped mice with low but not high body mass (Vuarin et al., 2019). In yellow-bellied marmots, *Marmota flaviventris*, another socially flexible species, females with high GCs have lower reproductive success (Pinho, Ortiz-Ross, Reese & Blumstein, 2019) and less-social individuals live longer lives (Blumstein, Williams, Lim, Kroeger, & Martin, 2018). Further studies should test the hypothesis that survival and lifetime reproductive success are greater in solitary than group-living females.

Higher baseline corticosterone levels in the breeding season than the non-breeding season have been reported in most studies of birds, reptiles and amphibians (Romero, 2002), and in a growing number of mammals (Kenagy & Place, 2000; Vera, Zenuto, & Antenucci, 2013). In female mammals, GCs play an important role in the onset and maintenance of lactation (Talwalker, Nicoll, & Meites, 1961), and mediate a range of other reproductive functions (reviewed in Brann & Mahesh, 1991). In our study, corticosterone was indeed higher during the breeding than the non-breeding season in group-living females. This difference arose after their female relatives left the nest, but there was no seasonal difference in females that became solitary. Elevated GC levels signify high energetic demands (reviewed in Reeder & Kramer, 2005), consistent with breeding (Romero, 2002). The higher corticosterone levels in group-living females after their nestmates became solitary could result from a change in social structure or in workload. As milk production increases with body mass in mice (König, Riester, & Markl, 1988), and heavier females are more likely to become solitary (Hill et al.,

2015b), departure of solitary females could place increased caring demands on those that remain to feed and provide warmth for offspring.

Testosterone levels were not associated with social tactic, and did not differ before and after females became solitary in solitary females themselves or in their nestmates. High testosterone levels suppress parental care in males (Wingfield, Lynn, & Soma, 2001), reduce the expression of certain maternal behaviours (Rosvall, 2013; Svare, 1980; Veiga & Polo, 2008), and disrupt ovulation in female birds (Rutkowska, Cichon, Puerta, & Gil, 2005). If testosterone were to modulate female social tactic, females with higher testosterone levels would potentially incur a net fitness cost from reduced maternal care. Consistent with this, we recorded lower testosterone levels during the breeding season than the non-breeding season in females following both tactics. We conclude that social tactic in female striped mice is not modulated by testosterone. While two steroid hormones were the focus of our study, certain peptide hormones merit investigation as potential correlates or even modulators of female social tactic. Vasopressin is associated with alternative reproductive tactics in male striped mice (Schoepf, Kenkel & Schradin, 2015), and oxytocin is associated with female social behaviour and alternative social strategies between species (Carter & Roberts 1997; Olazábal & Sandberg, 2020). The roles of oxytocin and vasopressin in explaining variation in social tactics within females of socially flexible species remains unexplored.

Our study provides evidence that differences in corticosterone levels are associated with alternative social tactic in female striped mice. During the breeding season, solitary females had consistently lower levels of corticosterone, which may indicate more favourable body condition and competitive ability, compared to group-living females. Higher corticosterone in group-living than solitary-living females indicates higher energetic stress, a potential consequence of reproductive competition, in group-living females. The observation that corticosterone levels can predict subsequent social tactic contributes to our understanding of how alternative life histories differ at a physiological level and provides support for the role of GCs in mediating social organisation.

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AUTHORS' CONTRIBUTIONS

CS and DH conceived the ideas and methods and collected data; DH analysed the data and led the writing of the manuscript. NP and CS contributed critically to the drafts and approved the final version of the manuscript.

DATA AVAILABILITY

Data deposited in the Dryad Digital Repository:
<https://doi.org/10.5061/dryad.rv15dv475> (Hill, Pillay & Schradin 2020).

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Fig. 1 Estimated marginal mean corticosterone (a, b) and testosterone (c, d) levels in female striped mice during the breeding season (a, c), and the breeding and non-breeding seasons (b, d). a and c show breeding season hormone levels before members of the group became solitary in females that would remain group living and females that would later become solitary, and after members of the group became solitary in group-living and solitary females. b and d show hormone levels during the non-breeding season (when all individuals were group living) in females that would remain group living and in females that would later switch to solitary living, and during the breeding season in females that remained group living and females that were or went on to become solitary living. Marginal means ± 1 SE were estimated from Linear Mixed effects Models accounting for body mass and age, and random effects of year, group and individual identity (Tables 2 and 3). All 6 possible pairwise contrasts shown in each panel were tested; different lower-case letters denote significant differences ($P < 0.05$) after adjusting for multiple testing. Values inside bars indicate the number of hormone samples and (in brackets) the number of unique individuals.

Table 2. General Linear Mixed effects Models to test for differences in hormone profiles during the breeding season between females that became solitary compared with those that remained group living (*final social tactic*, where group living is the reference level), and before and after females became solitary (*timing*, where ‘before’ is the reference level). A status of ‘before’ for a female that became solitary indicates that she was group living when the blood sample was taken; a status of ‘before’ for a female that remained group living indicates that none of her nestmates had become solitary when the blood sample was taken. All models controlled for individual identity, group identity and year (random intercepts). Models are based on 155 and 196 samples, 81 and 91 individuals, 25 groups and 8 and 7 years for corticosterone and testosterone respectively; sample sizes for each level of social tactic and timing are presented in Figures 1a and c. Non-significant fixed effects that were not involved in significant interactions were eliminated from models sequentially; significant effects are shown in bold.

	β	<i>s.e.</i>	d.f.	<i>t</i>	<i>P</i>
Corticosterone					
Intercept	6.909	0.161	69.5	43.05	<0.001
Final social tactic	0.363	0.165	131.1	2.20	0.030
Timing	-0.150	0.198	140.1	0.76	0.449
Body mass	0.028	0.006	92.8	4.81	<0.001
Age	-0.001	<0.001	144.5	2.27	0.025
Final social tactic $\times$ timing	0.554	0.238	148.3	2.32	0.022
Testosterone					
Intercept	-0.276	0.159	5.9	1.74	0.134
Final social tactic	0.032	0.147	150.9	0.22	0.827
Timing	0.022	0.152	176.7	0.14	0.888
Body mass	0.012	0.007	192.5	1.75	0.082
Age	-0.001	0.001	178.4	1.58	0.115
Final social tactic $\times$ timing	0.050	0.305	183.4	0.16	0.871

Table 3. General Linear Mixed effects Models to test for differences in hormone profiles between females that became solitary compared with those that remained group living in a given year (*final social tactic*, where ‘group living’ is the reference tactic), and between females sampled during the breeding (August to November) and the non-breeding seasons (*season*, where ‘breeding season’ is the reference level). All models controlled for individual identity, group identity and year (random intercepts). Models are based on 287 and 361 samples, 110 and 121 individuals, 36 groups and 8 years for corticosterone and testosterone respectively; samples sizes for each level of social tactic and season are given in Figure 1b and 1d. Non-significant fixed effects were eliminated from models sequentially; significant effects are shown in bold.

	β	<i>s.e.</i>	d.f.	<i>t</i>	<i>P</i>
Corticosterone					
Intercept	6.776	0.122	82.6	55.36	<0.001
Final social tactic	0.438	0.128	155.8	3.42	0.001
Season	0.185	0.155	263.8	1.19	0.236
Body mass	0.024	0.005	199.8	5.20	<0.001
Age	-0.001	<0.001	156.0	3.51	0.001
Final social tactic $\times$ season	-0.559	0.166	277.2	3.37	0.001
Testosterone					
Intercept	-0.203	0.162	8.4	1.25	0.244
Final social tactic	0.040	0.122	98.3	0.33	0.742
Season	0.241	0.087	339.5	2.77	0.006
Body mass	0.008	0.006	210.2	1.29	0.199
Age	-0.001	<0.001	177.4	1.48	0.142
Final social tactic $\times$ season	0.028	0.208	348.0	0.14	0.891