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Alternative reproductive tactics in female striped mice: Solitary breeders have lower corticosterone levels than communal breeders

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ABSTRACT

Alternative reproductive tactics (ARTs), where members of the same sex and population show distinct reproductive phenotypes governed by decision-rules, have been well-documented in males of many species, but are less well understood in females. The relative plasticity hypothesis (RPH) predicts that switches between plastic ARTs are mediated by changes in steroid hormones. This has received much support in males, but little is known about the endocrine control of female ARTs. Here, using a free-living population of African striped mice (*Rhabdomys pumilio*) over five breeding seasons, we tested whether females following different tactics differed in corticosterone and testosterone levels, as reported for male striped mice using ARTs, and in progesterone and oestrogen, which are important in female reproduction. Female striped mice employ three ARTs: communal breeders give birth in a shared nest and provide alloparental care, returners leave the group temporarily to give birth, and solitary breeders leave to give birth and do not return. We expected communal breeders and returners to have higher corticosterone, owing to the social stress of group-living, and lower testosterone than solitary breeders, which must defend territories alone. Solitary breeders had lower corticosterone than returners and communal breeders, as predicted, but testosterone and progesterone did not differ between ARTs. Oestrogen levels were higher in returners (measured before leaving the group) than in communal and solitary breeders, consistent with a modulatory role. Our study demonstrates hormonal differences between females following (or about to follow) different tactics, and provides the first support for the RPH in females.

Introduction

Alternative reproductive tactics (ARTs) are discrete reproductive phenotypes selected to maximise fitness in two or more distinct ways in the same sex and population (Gross, 1996). They can be plastic, whereby an individual is able to switch from one ART to another, or they can be fixed for life (Taborsky, 1998). The differentiation and maintenance of ARTs are mediated by changes in the secretion of steroid hormones (reviewed in Oliveira et al., 2008). This idea was first conceptualised in the relative plasticity hypothesis (RPH), which predicts that fixed tactics are regulated by organisational endocrine effects in early development, whereas switches between plastic tactics are regulated by activational endocrine effects in sexually mature individuals (Moore, 1991; Moore et al., 1998). Alternative adult phenotypes of species with fixed ARTs should therefore have similar steroid profiles provided that they experience the same social environment, while steroid levels are predicted to differ between alternative adult phenotypes in species with plastic ARTs (Moore, 1991).

ARTs are expected to evolve when there is pronounced variance in reproductive success within a sex, leading to reproductive competition (Taborsky et al., 2008). Competition for mates is usually more intense in males than in females (Trivers, 1972), which probably explains why ARTs occur more frequently in males (Alonzo, 2008). Nevertheless, females of many species experience intense reproductive competition (Stockley and Bro-Jørgensen, 2011), and an increasing number of female ARTs has been described in recent years. Examples include brood parasitism versus maternal care in ruddy ducks (*Oxyura jamaicensis*) (Reichart et al., 2010) and monandry versus polyandry in horseshoe crabs (*Limulus polyphemus*) (Johnson and Brockmann, 2012). Little, however, is known about the role of hormones in mediating female ARTs (Oliveira et al., 2008).

Glucocorticoids (GCCs) regulate basal metabolism and facilitate appropriate responses to stress (Reeder and Kramer, 2005; Sapolsky et al., 2000). In species with plastic ARTs, bourgeois (dominant) males sometimes have higher GCC levels than males of subdominant tactics (satellite, roamer, sneaker), while in other species the pattern is reversed (Oliveira et al., 2008). This difference might depend on whether

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it is more energetically demanding to occupy a dominant or a subordinate rank (Creel, 2001). Experimental manipulations of GCC levels in species with plastic ARTs can induce males to switch tactics. For example, bourgeois male Great Plains toads (*Bufo cognatus*) and Woodhouse's toads (*Bufo woodhousii*) with experimentally-elevated corticosterone levels were more likely than controls to switch to a satellite tactic (Leary et al., 2006). Given their role in mediating ARTs in males (Oliveira et al., 2008) and transitions between life history stages in both sexes (Crespi et al., 2013; Wada, 2008), GCCs are a promising candidate for regulating female ARTs.

In species with plastic ARTs, bourgeois males typically have higher androgen levels than subordinates, and experimentally increasing androgen levels in subordinate males can induce a switch to the bourgeois tactic (Oliveira et al., 2008). Marine iguanas (*Amblyrhynchus cristatus*), for example, employ three plastic ARTs, with satellite and sneaker males having lower androgen levels than territorial males (Wikelski et al., 2005). Experimentally increasing androgen levels in satellites and decreasing androgens in territorial males can bring about non-adaptive tactic switches (Wikelski et al., 2005). Bourgeois males are more aggressive than subordinates in many species (e.g. Corlatti et al., 2013; Schutz et al., 2010), and the role of androgens in mediating male aggression is well-established (Wingfield et al., 1987). Fewer studies have tested for an association between aggression and androgen levels in females, but most work suggests that female testosterone levels vary in response to intra-sexual competition and are under direct sex-specific selection (Rosvall, 2013). This raises the possibility that testosterone could facilitate responses to intra-sexual competition in females following different tactics.

Progesterone and oestrogen control many aspects of female reproduction (Christensen et al., 2012; Hewitt et al., 2005), and are associated with female–female competition in some species (Goymann et al., 2008; Parn et al., 2008; Rubenstein and Wikelski, 2005) but not in others (Elekonich and Wingfield, 2000; Hay and Pankhurst, 2005; Navara et al., 2006). In female house mice (*Mus musculus*), ovariectomy during gestation brought forward the onset of maternal aggression (Ghiraldi et al., 1993), while an experimental increase of oestrogen levels inhibited maternal aggression (Svare and Gandelman, 1975). To our knowledge, no study has yet tested whether females following alternative tactics differ in levels of progesterone and oestrogen, and tests in males with plastic ARTs are limited to a few teleost species. Progesterone levels are either higher in bourgeois than subdominant males (Cheek et al., 2000; Oliveira et al., 1996) or do not differ (Hourigan et al., 1991; Ros et al., 2003). Oestrogen levels are higher in subdominant than bourgeois male stoplight parrotfish (*Sparisoma viride*) (Cardwell and Liley, 1991), but do not differ between ARTs in saddleback wrasse (*Thalassoma duperrey*) (Hourigan et al., 1991). These studies suggest that the role of progesterone and oestrogen in modulating female ARTs is worth exploring.

Here, for the first time, we ask whether the RPH, which predicts differences in steroid hormones in males that follow plastic ARTs (Moore et al., 1998), also applies to females. The striped mouse (*Rhabdomys pumilio*) is an appropriate model in which to test this because plastic ARTs occur in both sexes. Male striped mice have three ARTs that differ in steroid hormone levels (Schradin et al., 2009b, 2013): 1) philopatric males have very high corticosterone and low testosterone levels; 2) solitary-living roamers have low corticosterone and high testosterone levels; and 3) dominant group-living breeding males have low corticosterone and intermediate testosterone levels. Like males, female striped mice can breed in groups or solitarily. Breeding groups usually comprise 2–4 closely related females and one male (Schradin and Pillay, 2004). Communally-breeding females show alloparental care, including allo-nursing (Schradin and Pillay, 2004; Schubert et al., 2009). Nevertheless, reproductive competition between female nestmates is intense, involving aggression and infanticide (Schradin et al., 2010). Females can avoid reproductive competition by leaving the natal group to nest alone, and solitary and communal females usually co-occur during the breeding season (Schoepf and Schradin, 2012; Schradin et al., 2010). As an alternative to breeding solitarily or communally, gestating females may adopt a third tactic termed 'returner' in which they leave the group to give birth, but later return to it (Hill, D.L., Pillay, N. and Schradin, C., unpublished data). Females can switch between the three phenotypes, which means that tactics are flexible and likely to be regulated by activational endocrine effects.

We tested whether ARTs in free-living female striped mice were associated with differences in baseline levels of steroid hormones. We expected (i) corticosterone levels to be higher in communally-breeding females than in solitary breeders owing to increased social stress and reproductive competition in groups; and (ii) testosterone levels to be higher in solitary breeders than in communal breeders because solitary breeders must defend a territory alone. We focussed on these two hormones because they have been studied in detail in male striped mice (e.g. Schradin et al., 2009b; Schradin and Yuen, 2011). Where additional aliquots of serum were available, we tested for (iii) differences between ARTs in progesterone and oestrogen. The social environment can affect hormone secretion (Wingfield et al., 1990), and so tactic switches that involve a change in social situation (e.g. from communal to solitary breeding) might in turn affect hormone levels. Similarly, returners, which experience a change in social situation from group- to solitary-living and back to group-living within a single tactic, might also show associated changes in hormone levels. These hormonal changes could occur in response to changes in social stress or energetic demands. We therefore tested (iv) whether changes in social situation in solitary breeders and returners were accompanied by changes in hormone levels. Throughout our analyses, we distinguished between females that became solitary while their relatives were still living (and which therefore had the potential to use any tactic) and those that were constrained to live solitarily because their relatives died. Importantly, the two types of solitary breeder experience a similar social environment that is elicited by different mechanisms: solitary breeders with relatives show a true tactic (the outcome of a strategy) that is predicted to be under hormonal control, whereas females without relatives are solitary as a consequence of external stochastic processes. If the decision to follow a solitary tactic is indeed under hormonal control, we would therefore expect (v) solitary breeders with living relatives to differ hormonally from solitary breeders without living kin.

Materials and methods

Fieldwork

We collected data every month during 2006–10 in Goegap Nature Reserve, South Africa (S 29 41.56, E 18 1.60) using methods approved by the Animal Ethics Committee at the University of the Witwatersrand (2004/87/2A, 2005/82/4 and 2007/10/01). The study site receives 180 mm precipitation per annum, mostly falling between April and September (in austral winter and spring; C. Schradin, unpublished data). It is an open habitat of shrubs, in which striped mice nest, and sandy areas.

Striped mice were captured using Sherman-style live-traps ($26 \times 9 \times 9$ cm) baited with bran flakes, salt and sunflower oil. Traps were placed in the shade close to a group's nest site in the morning and the late afternoon five days a week, as striped mice are diurnal, and checked 30–45 min after being set. Each group was trapped every two weeks. Females were weighed to the nearest gramme using an electronic balance, and we recorded whether their nipples were pink and elongated (characteristic of lactation), otherwise visible or not visible. Newly-trapped individuals were provided with numbered aluminium ear-tags (National Band and Tag, Newport, KY), and marked with non-toxic hair dye (Inecto, Pinetown, South Africa), so that they could be recognised during behavioural observations at their nest sites (described in Schradin and Pillay, 2004). All adults trapped during

the breeding season were fitted with MD-2C radio-collars (Holohil, Canada). Radio-collars weighed 2.5 g, representing 5.4 ± 0.07% of the body mass of non-gestating adult females ($N = 181$ records from 110 individuals). We assumed that juveniles (body mass <30 g) were born at the nest where they were first trapped and observed interacting with group members. This method was validated using microsatellite markers for 2007 and 2008 (Schradin and Lindholm, 2011). We refer to females that nested together or did so before becoming solitary as 'relatives' because genetic data show that female group members are close kin (C. Schradin and A.K. Lindholm, unpublished data).

We used radio-tracking to determine the identities of all adult striped mice sharing a nest and the date that females left the nest for another. All individuals were radio-tracked 4–5 nights a week throughout the breeding season using an AR8000 wide-range receiver (AOR, Tokyo, Japan) and an RA-14K antenna (Telonics, Mesa, AZ). Nest sites were identified using the homing-in method, which involved approaching potential nest sites from different angles until the source of the radio-signal was located. Individuals were assumed to be nesting together when their signals derived from the same position. Locations were recorded using an eTrex Venture GPS (GARMIN, Olathe, KS; accurate to ~5 m). We continued to radio-track one female from each group outside the breeding season to maintain a record of the groups' movements. Group membership is stable outside the breeding season so transmitters were removed from all other group members at the end of each breeding season (Schoepf and Schradin, 2012; Schradin et al., 2010).

Blood samples were collected between August and November of each year. Traps were set close to nest sites in the morning and monitored from a distance of 5–10 m. All blood sampling took place within 45 min of striped mice becoming active in the morning to reduce the potential effects of circadian rhythms on hormone levels. Trapped females were immediately anaesthetised with diethyl ether (validated in Schradin (2008)), and a blood sample of 100–500 μl (depending on body size) was drawn from the sub-lingual vein as described in Heimann et al. (2009). We recorded the time (s) taken to collect a blood sample from the moment an individual entered the trap (sampling latency, see Measurement of hormone levels). Females were monitored during recovery from anaesthesia and then weighed to the nearest gramme. Blood was left to clot at room temperature (<20 °C) for 1 h, centrifuged to allow the serum to be extracted and then stored at −20 °C.

Determination of parturition date and ART

Striped mice give birth between August and December, in the spring. For each adult female fitted with a radio-collar and for which blood samples were available, we plotted body mass records from July to January against the date that she was weighed. Individual plots were examined for the rise and sudden fall in body mass indicative of gestation and parturition. Parturition was assumed to occur on the median day within each trapping interval (the period between the last time a female was trapped before parturition and the first time she was trapped postpartum) unless we could refine the estimate from observational data. Estimated parturition dates were consistent with the onset of lactation.

Females were classed as nesting 'communally' (sharing a nest with ≥1 adult female) or not nesting communally on the night before they gave birth. Those that were not nesting communally were further classified as: a) those that resumed nesting with their original group ≥1 night after parturition ('returners'); b) those that did not resume nesting with the group although female relatives were still alive ('solitary with relatives'); and c) those whose female relatives had died ('solitary without relatives'). We use the term 'reproductive phenotype' (hereafter 'RP') to refer to the four categories of breeding female (communal breeder, returner, solitary breeder with relatives, solitary breeder without relatives), and 'ART' to describe the first three of these categories, which are predicted to be under hormonal control. We ensured that solitary females were not nesting with unmonitored females by observing the nests of solitary females at dusk when striped mice were returning from foraging, and only assigned a solitary or returner ART to a female if she and all her adult female relatives were fitted with a radio-collar when she gave birth. The date of birth of each female was estimated from the population-specific growth curve described in Schradin et al. (2009c), and we used this to calculate the age of females at blood sampling. We included in the study all females for which blood samples were available and for which RP could be determined ($N = 105$ females from 27 groups; Table 1). Two females provided blood samples and gave birth in two consecutive breeding seasons (both in 2007–08); the remaining 103 individuals bred within a single season.

Measurement of hormone levels

Serum was analysed for total corticosterone, testosterone, progesterone and oestrogen levels using commercial Enzyme-Linked Immuno-sorbent Assay (ELISA) kits from IBL (Immuno Biological Laboratories, Hamburg). All measurements fell within the standard curves of the assays. Table 1 shows the number of serum samples assayed for the four hormones per breeding season (2006–10). The focus of our studies has always been on corticosterone and testosterone (e.g. Schradin, 2008; Schradin et al., 2009b), and this was also the case in the present study. Where additional aliquots were available, progesterone and oestrogen were analysed, resulting in a smaller sample size for those two hormones (Table 1). Progesterone and oestrogen assay kits were validated for the range of hormone levels found in females from the study population. Validation of corticosterone and testosterone kits for striped mouse serum is described in Schradin (2008). Serial dilution of two striped mouse sample pools each for progesterone and oestrogen (this study) and for testosterone and corticosterone (Schradin, 2008) closely followed the standard curves. Intra and inter-assay variability was estimated using several pools from striped mice with low (L), intermediate (I) and high (H) hormone values. Intra-assay variability was 4.0% (based on 2 samples from a L corticosterone pool), 9.4% (2 samples, L), 9.9% (8 samples, L) and 12.2% (10 samples, I) for corticosterone, 5.3% (10 samples, I), 8.8% (10 samples, I) and 24.8% (7 samples, L) for testosterone, 3.7% (7 samples, H), 7.3% (8 samples, H), 8.3% (2 samples, H) and 9.8% (9 samples, L) for progesterone, and 8.3% (6 samples, I) for oestrogen. Inter-assay variability was 8.1% (10 assays, I), 17.2% (3 assays, L) and 20.0% (4 assays, L) for corticosterone, 12.2% (13 assays, I), 13.7% (11 assays, I), 15.3% (4 assays, L) and 16.6% (4 assays, H) for testosterone, and 14.7% (2 assays, L) and 18.0% (3 assays, H) for progesterone. A single oestrogen assay was carried out.

To reduce variation in progesterone levels as a result of the stage of gestation, we assayed progesterone from females whose body mass and reproductive records suggested that they were not gestating at the time of sampling. We did not assay progesterone in females without living relatives due to the small sample size. For corticosterone, only blood samples collected with a sampling latency ≤180 s were assayed to avoid a potential stress response, and there was no effect of sampling latency on log-transformed corticosterone levels (ng/ml) within this range (Linear Mixed effects Model: $\beta = -0.002 \pm 0.002$ (mean slope ± standard error), $t_{174.4} = 1.17$, $P = 0.245$, controlling for random intercepts of individual identity, group identity and year; see Table 1 for N). Sampling latency did not influence log-transformed levels of testosterone ($\beta = -0.0005 \pm 0.002$, $t_{225.5} = 0.31$, $P = 0.760$; sampling latency range: 78–260 s, 80.1% of samples collected within 180 s), oestrogen ($\beta = 0.002 \pm 0.002$, $t_{20.7} = 1.10$, $P = 0.286$; sampling latency range: 104–180 s, 86.7% of samples <180 s) or progesterone ($\beta = -0.001 \pm 0.006$, $t_{12.8} = 0.09$, $P = 0.928$; sampling latency range: 115–225 s, 90.0% of samples <180 s).

Table 1
The numbers of groups, focal females and blood samples assayed for four steroid hormones. Focal females are females that gave birth while they and their female relatives were fitted with a radio-collar, and which provided a blood sample. Numbers of individuals sampled for each hormone are given in brackets.

Breeding season	No. of focal groups	No. of focal females	Corticosterone	Testosterone	Progesterone	Oestrogen
2006	6	14	13	11	0	0
2007	9	20	22(16)	25(19)	6	8
2008	14	32	75(28)	91(29)	12(8)	15(11)
2009	9	24	29(18)	51(21)	2	6(5)
2010	9	17	40(16)	51(17)	0	1
total	27[a]	105[a]	179(90)	229(95)[a]	20(16)[a]	30(25)

[a] Some groups and individuals were sampled over multiple years; totals give the number of unique individuals and groups across all years.

Statistical analysis

Data were analysed in R version 3.1.1. (R Development Core Team, 2014) using the lme4 (Bates et al., 2014) and car (Fox and Weisberg, 2014) libraries. Females switch ARTs and so we tested whether hormone levels were associated with the reproductive phenotype used on the closest parturition date to blood sampling. To take into account fluctuations in circulating hormone levels over the reproductive cycle (e.g. Barkley et al., 1979), which might also vary with RP, we determined the number of days between blood sampling and parturition ('parturition latency', which was a negative number before parturition (day 0) and positive after parturition). We noted which RP a female used on day 0 and whether or not her female relatives were still living when blood was sampled. Females whose closest RP was 'solitary without relatives' but whose relatives were living when blood was sampled ($N = 2$) were discarded.

We modelled the effects of RP on corticosterone and testosterone in Linear Mixed effects Models (LMM) fitted using restricted maximum likelihood (REML) such that

$$y_j = \mu + \mathrm{RP} + \mathrm{PL} + \mathrm{PL}^2 + \mathrm{RP} \times \mathrm{PL} + \mathrm{RP} \times \mathrm{PL}^2 + mass_j + age_j + id + group + year + \varepsilon \quad (1)$$

where y is the log-transformed blood serum level of corticosterone or testosterone taken on sampling date j; μ is the overall mean; RP is a fixed factor with four levels (communal, returner, solitary with living relatives, solitary without living relatives) indicating the reproductive phenotype used on the closest parturition to sampling date j; PL (parturition latency) is a covariate of the number of days between parturition and j; PL^2 is the quadratic term of parturition latency; *mass* and *age* are covariates of body mass and age on date j; *id*, *group* and *year* are random intercepts of individual identity, natal group identity and year of blood sampling to account for repeated measures within the same individuals, groups and years, and ε is the error term. All continuous explanatory variables were mean-centred to improve the interpretability of the results and reduce collinearity between linear and polynomial terms of PL.

The model used to analyse log-transformed progesterone and oestrogen levels was the same as Eq. (1) except that we did not test for interactions between RP and PL on either hormone, nor for the fixed effects of mass and age on progesterone because of the small sample size. Progesterone was sampled after parturition only and so we did not fit a quadratic term for PL. Generalised Variance Inflation Factors adjusted for the degrees of freedom for the fixed effects in the full models were ≤2.37 for the four hormones.

Solitary breeders experience a change in social situation when they leave the natal group. We tested whether this is associated with a change in hormone levels in solitary breeders with living relatives using the following LMM:

$$y_j = \mu + SS_j + mass_j + age_j + id + group + year + \varepsilon \quad (2)$$

where y is the log-transformed blood serum level of corticosterone, testosterone or oestrogen on sampling date j, and *SS* (social situation) is a two-level fixed factor indicating whether blood sampling took place before or after the sampled female became solitary. Solitary breeders' progesterone levels were not analysed in Eq. (2) because sample size was small.

Returners experience a similar change in social situation from living in a group to giving birth alone and returning to the group. To test whether these changes are accompanied by changes in hormone levels, we compared log-transformed corticosterone, testosterone and progesterone levels between returners that had been sampled before the temporary solitary stage, while nesting alone and after re-joining the group. The LMM used was the same as Eq. (2) except that *SS* was a three-level fixed factor (before, during time alone, after) for corticosterone and testosterone, and a two-level factor (during, after) for progesterone; samples from gestating females (before) were not assayed for progesterone. We did not control for body mass and age on progesterone levels in Eq. (2) because of small sample size, and did not consider the effects of changes in social situation in returners on oestrogen levels because 8 of 9 samples were collected before females left the group. Where paired samples were available (2008–10), we ran a paired t-test to compare hormone levels in returners before they became temporarily solitary and after they re-joined the group.

We found no significant heterogeneity of variance across the four female RPs for parturition latency or body mass. We report parameter estimates and degrees of freedom from Type II ANOVA Wald chi-square tests, assuming significance where $P < 0.05$. Multiple comparisons were carried out using Tukey contrasts with *P*-values adjusted using a single-step method from the multcomp package (Hothorn et al., 2014). Statistical tests are two-tailed. Means are least-squares means ± SE expressed on the original response scale using the lsmeans package (Lenth, 2014).

Results

Were corticosterone levels associated with reproductive phenotype?

Breeding season corticosterone levels in female striped mice were lower in solitary breeders with relatives than in communal breeders, returners and solitary breeders without relatives, but there was no difference in corticosterone between any of the other reproductive phenotypes (Fig. 1A, Table 2, overall effect of RP: $\chi^2_3 = 18.53$, $P < 0.001$). Corticosterone levels increased with body mass ($\chi^2_1 = 16.19$, $P < 0.001$) but did not vary with age ($\chi^2_1 = 0.23$, $P = 0.629$). Corticosterone did not increase in the days leading up to parturition or decrease after it (linear term of PL: $\chi^2_1 = 0.76$, $P = 0.383$; quadratic term: $\chi^2_1 = 0.05$, $P = 0.816$; sampling range: 99 days before parturition to 97 days after). The relationship between corticosterone levels and parturition latency did not vary with RP (RP × PL linear term: $\chi^2_3 = 0.357$, $P = 0.949$, quadratic term: $\chi^2_3 = 1.22$, $P = 0.748$). A second ANOVA examining females only after they became solitary showed that corticosterone levels were lower in solitary breeders with living relatives (881 ± 192 ng/ml; $N = 17$ samples from 11 females) than in females that were solitary because their relatives had died (2006 ± 391 ng/ml, $N = 19$ samples from 10 females; $\chi^2_1 = 13.91$, $P < 0.001$, controlling for body mass) in spite of the similar social environments.

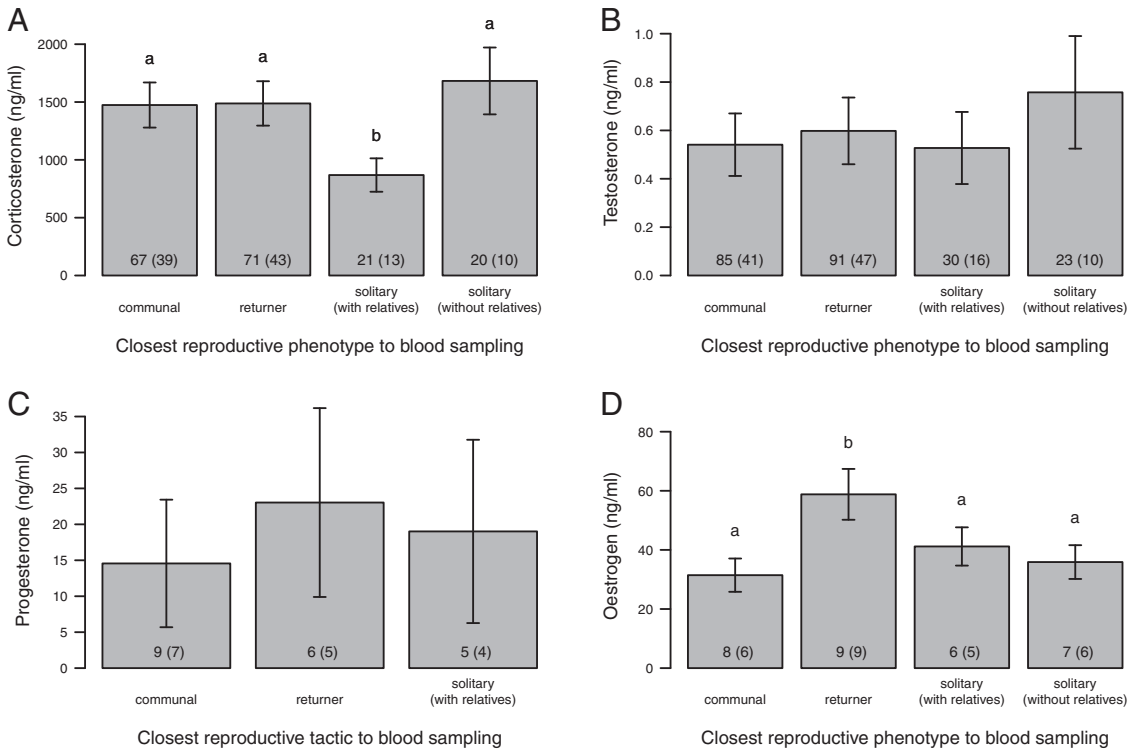

Fig. 1. Corticosterone (A), testosterone (B), progesterone (C) and oestrogen (D) levels in female striped mice with different reproductive phenotypes. Means are least-squares means ± 1SE extracted from Linear Mixed effects Models. Different lower case letters indicate significant differences ($P < 0.05$). Values inside bars show the number of hormone samples with the number of unique individuals in brackets.

Did corticosterone levels change with females' social situation?

In solitary females with living relatives, there was no difference in corticosterone levels before (816 ± 364 ng/ml, $N = 4$ samples from 4 females, 7.1 ± 2.05 days before becoming solitary) and after (1044 ± 290 ng/ml, $N = 17$ samples from 11 females, 27.8 ± 5.44 days after) females became solitary ($\chi^2_1 = 0.66$, $P = 0.416$). A separate ANOVA revealed that corticosterone levels were not associated with social situation in returners ($\chi^2_2 = 0.44$, $P = 0.801$, controlling for body mass). Pairwise Tukey comparisons based on the latter model did not detect a difference in corticosterone levels before (1478 ± 175 ng/ml, $N = 35$ samples from 26 females) and during returners' solitary period (1666 ± 293 ng/ml, $N = 13$ samples from 13 females sampled 1.5 ± 1.32 days postpartum; $\beta = 0.12 \pm 0.18$, $Z = 0.65$, $P = 0.788$), during females' time away from the group and after returning to the group (1494 ± 217 ng/ml, $N = 23$ samples from 19 females; $\beta = 0.11 \pm 0.20$, $Z = 0.54$, $P = 0.849$), nor before returners became solitary and after they returned to the group ($\beta = 0.01 \pm 0.15$, $Z = 0.07$, $P = 0.997$).

Were testosterone levels associated with reproductive phenotype?

Testosterone levels were not associated with RP ($\chi^2_3 = 1.77$, $P = 0.621$; Table 2, Fig. 1B), body mass ($\chi^2_1 = 1.86$, $P = 0.173$), age ($\chi^2_1 = 2.41$, $P = 0.120$) or parturition latency (linear term: $\chi^2_1 = 0.57$, $P = 0.452$; quadratic term: $\chi^2_1 = 1.00$, $P = 0.318$; sampling range: 99 days before parturition to 97 days after). There was no interaction between RP and parturition latency (RP × PL linear term: $\chi^2_3 = 2.03$, $P = 0.566$; RP × PL quadratic term χ^2_3: 1.25, $P = 0.740$).

Did testosterone levels change with females' social situation?

Testosterone levels did not differ in females with living relatives before (0.51 ± 0.27 ng/ml, $N = 6$ samples from 6 females taken 6.4 ± 1.71 days before becoming solitary) and after (0.46 ± 0.17 ng/ml, $N = 24$ samples from 15 females, 35.2 ± 4.93 days after) they became solitary ($\chi^2_1 = 0.08$, $P = 0.781$). Testosterone levels in returners showed a trend towards an association with social situation ($\chi^2_2 = 5.15$, $P = 0.076$). Pairwise comparisons based on this model suggested

Table 2
Linear Mixed effects Models testing for associations between females' reproductive phenotypes and circulating hormone levels (ng/ml, log-transformed). All models controlled for random intercepts of individual identity, group identity and year. Estimates were calculated using Tukey contrasts with *P*-values adjusted for multiple testing using a single-step method. We did not measure progesterone in solitary females without relatives owing to a small sample size (NT, not tested). Significant contrasts are in bold.

	Corticosterone			Testosterone			Progesterone			Oestrogen		
	$\beta \pm$ SE	*Z*	*P*	$\beta \pm$ SE	*Z*	*P*	$\beta \pm$ SE	*Z*	*P*	$\beta \pm$ SE	*Z*	*P*
Returner vs communal	0.01 ± 0.09	0.10	>0.999	0.08 ± 0.14	0.60	0.928	0.46 ± 0.49	0.94	0.608	0.63 ± 0.16	4.00	**<0.001**
Solitary with relatives vs communal	−0.53 ± 0.14	3.72	**0.001**	−0.02 ± 0.19	0.11	0.999	0.27 ± 0.69	0.39	0.920	0.27 ± 0.14	1.86	0.244
Solitary without relatives vs communal	0.13 ± 0.16	0.85	0.824	0.29 ± 0.24	1.22	0.605		NT		0.13 ± 0.17	0.76	0.873
Solitary with relatives vs returner	−0.54 ± 0.14	3.88	**<0.001**	−0.11 ± 0.19	0.57	0.938	−0.19 ± 0.66	−0.95	0.954	−0.36 ± 0.14	2.57	**0.049**
Solitary without relatives vs returner	0.12 ± 0.15	0.84	0.831	0.21 ± 0.23	0.90	0.798		NT		−0.49 ± 0.16	3.04	**0.012**
Solitary without relatives vs solitary with relatives	0.66 ± 0.18	3.67	**0.001**	0.31 ± 0.27	1.18	0.632		NT		−0.14 ± 0.17	0.83	0.841

that returners had higher testosterone levels before (0.70 ± 0.20 ng/ml, $N = 44$ samples from 31 females) leaving the group than after returning to it (0.45 ± 0.15 ng/ml, $N = 29$ samples from 22 females), but this was not statistically significant after adjusting for multiple testing ($\beta = 0.37 \pm 0.18$, $Z = 2.10$, $P = 0.088$). There was no difference in testosterone levels before and during (0.48 ± 0.17 ng/ml, $N = 18$ samples from 16 females, 1.3 ± 1.16 days postpartum) returners' time away from the group ($\beta = 0.32 \pm 0.21$, $Z = 1.49$, $P = 0.293$), nor during their time away from the group and after returning to it ($\beta = 0.05 \pm 0.23$, $Z = 0.23$, $P = 0.970$). In returners for which paired samples were available, females had higher testosterone levels before leaving the group (1.46 ± 0.23 ng/ml, sampled 13.9 ± 3.02 days antepartum) than after returning to it (0.78 ± 0.21 ng/ml, sampled 10.4 ± 1.09 days postpartum; $t_6 = 3.37$, $P = 0.015$).

Were progesterone levels associated with alternative reproductive tactic?

Circulating progesterone levels were not associated with ART ($\chi^2_2 = 0.890$, $P = 0.641$, Table 2; Fig. 1C) nor the number of days since parturition ($\chi^2_1 = 2.01$, $P = 0.156$; range: blood sampled 1-35 days after breeding) in non-gestating female striped mice.

Did progesterone levels change when returners temporarily became solitary?

Returners had lower progesterone levels during their time away from the group (11.8 ± 10.49 ng/ml, $N = 2$ samples from 2 females, sampled 2.5 ± 1.50 days postpartum) than after they had returned to it (51.0 ± 36.50 ng/ml, $N = 4$ samples from 3 females, 18.0 ± 3.51 days postpartum; $\chi^2_1 = 8.98$, $P = 0.003$).

Were oestrogen levels associated with reproductive phenotype?

Circulating oestrogen levels in female striped mice were associated with RP ($\chi^2_3 = 18.48$, $P < 0.001$, Table 2, Fig. 1D) but were not influenced by body mass ($\chi^2_1 = 1.76$, $P = 0.184$), age ($\chi^2_1 = 0.22$, $P = 0.637$), or latency to parturition (linear term: $\chi^2_1 = 2.21$, $P = 0.137$; quadratic term: $\chi^2_1 = 1.63$, $P = 0.202$; range: blood sampled 48 days before parturition to 39 days postpartum). Oestrogen levels were higher in returners than in all other reproductive phenotypes, which did not differ from each other (Table 2, Fig. 1D).

Did oestrogen levels change with solitary breeders' social situation?

Oestrogen levels did not differ in females with living relatives before (49.9 ± 22.4 ng/ml, $N = 3$ samples from 3 females, 34.0 ± 7.51 days before becoming solitary) and after (44.3 ± 8.67 ng/ml, $N = 6$ samples from 6 females, 34.0 ± 4.05 days after) they became solitary ($\chi^2_1 = 0.16$, $P = 0.690$).

Discussion

We found that alternative reproductive tactics were associated with differences in baseline levels of steroid hormones in female striped mice, as reported previously in males of this species (Schradin et al., 2009b). Solitary breeding females with living relatives (i.e. those that followed a true solitary tactic rather than being constrained by the death of their relatives to rear young alone) had lower levels of the stress hormone corticosterone compared to communal breeders, returners and solitary breeders whose relatives had died. Returners had the highest levels of oestrogen, which is important in female reproduction. As most returners were sampled before leaving the group, we propose that oestrogen plays a role in modulating the returner tactic. There were no differences in corticosterone or oestrogen between the other classes of female, and testosterone and progesterone were not associated with reproductive phenotype. This is, to our knowledge, the first study to demonstrate hormonal differences between plastic ARTs in females.

In male striped mice, baseline levels of testosterone are higher in solitary than in group-living individuals (Schoepf and Schradin, 2013; Schradin et al., 2009b; Schradin and Yuen, 2011), but no difference in testosterone levels has been observed between ARTs (this study) or social tactics (Schoepf and Schradin, 2013) in female striped mice. The influence of testosterone on female phenotypes is not well understood (Staub and DeBeer, 1997), but levels of testosterone within females are usually higher in species and situations where reproductive competition is more pronounced (Chapman et al., 1998; Ketterson et al., 2005; Langmore et al., 2002; Møller et al., 2005). Reproductive competition in female striped mice occurs primarily when females are caring for young (Schradin et al., 2009a, 2010). High levels of testosterone suppress parental care in males (Wingfield et al., 2001 but see Trainor and Marler, 2001), and decrease the expression of certain maternal behaviours (Gandelman, 1973; O'Neal et al., 2008), including pup defence (Svare, 1980). This suggests that female tactics associated with higher testosterone levels would potentially incur a net fitness cost owing to reduced maternal care if testosterone were to modulate female ARTs. This may explain why no association was found. Consistent with this, dominant breeding male striped mice, which must balance paternal care with defending a territory and harem, had lower testosterone levels than solitary-living roamer males, which invade dominant breeders' territories to seek matings, and provide no paternal care (Schradin et al., 2009b). In our study, returners' testosterone levels did, however, decrease between leaving the group and returning to it postpartum. This cannot be explained by a change in returners' reproductive state because testosterone levels did not vary with the number of days before or after parturition. Instead, this might reflect differences in the social environment: perhaps returners experienced greater aggression before leaving the group than after returning to it. Our findings suggest that baseline levels of testosterone do not differ between female ARTs in this species but that testosterone levels within a tactic might be influenced by aspects of the social environment.

Among female striped mice with living relatives, solitary breeders had lower baseline levels of corticosterone than group-living females (communal breeders and returners). Corticosterone levels did not differ before and after females became solitary, which raises the possibility that hormonal differences were present in these females even before they left the nest. Interestingly, an experimental field study showed a trend towards lower corticosterone levels in group-living male striped mice that later became solitary (i.e. sampled before leaving the group) than in males that remained permanently group-living (Schoepf and Schradin, 2013). Schoepf and Schradin (2013) did not detect a difference in corticosterone levels between females sampled before leaving the group and those that were permanently group-living, although corticosterone levels were significantly lower after leaving the group than ~9 days before leaving it in both sexes. Whether the switch to a solitary ART might be elicited by a decrease in corticosterone while individuals are still group-living is a promising area for future research.

Males following alternative reproductive tactics can differ in energy expenditure as a result of differences in aggressive or courtship behaviour (e.g. Cummings and Gelineau-Kattner, 2009; Scantlebury et al., 2008; Schradin et al., 2009b). GCCs activate energy stores to meet increased behavioural and metabolic demands, so high GCC levels are likely to indicate energetically demanding situations (Reeder and Kramer, 2005). The higher corticosterone levels we observed in communal breeders and returners compared to solitary breeders (corrected for body mass) could therefore imply that the former tactics are more energetically demanding than solitary breeding. Further studies could test this by comparing energy expenditure between female tactics. Another factor that could influence GCC levels is the availability and quality of food (Kitaysky et al., 1999; Lewanzik et al., 2012). However, differences in food availability are unlikely to have driven the difference in corticosterone levels in our study because communal and solitary

breeders from a given group occupied neighbouring territories with access to the same food plants.

A further possibility is that high levels of corticosterone in group-living females are a consequence of social stress arising from reproductive competition or other interactions within the natal group. Indeed, female aggression and infanticide, indicators of reproductive competition in this species, occurred more frequently in communally-breeding groups of striped mice than in male–female pairs (Schradin et al., 2010). However, in tuco-tucos, *Ctenomys sociabilis*, a plurally-breeding rodent, corticosterone levels were higher in solitary than in group-living females (Woodruff et al., 2013). This might reflect differences in the physical and social environments occupied by the two species. Similarly, corticosterone levels can be higher in bourgeois than in subdominant males in some species, while in other species, including male striped mice (Schradin et al., 2009b), the inverse is true (Oliveira et al., 2008). In summary, studies in female striped mice suggest that living in a group and breeding communally is stressful and potentially more energetically demanding than solitary-living and breeding.

Nevertheless, if social stress from reproductive competition in group-living females were the only explanation for high corticosterone levels, then we would expect to find low corticosterone in all classes of solitary-breeding female striped mice. By contrast, we found that solitary breeders whose female relatives had died did not differ in corticosterone levels from group-living females. Moreover, corticosterone levels were lower in solitary breeders with living relatives than in those without relatives even though they experienced similar social environments. This may reflect differences in their coping abilities. By regulating energy availability, elevated GCC levels are likely to increase the capacity of females without relatives to meet the increased energetic demands of supplying milk and warmth to pups and responding to social challenges associated with territory defence without assistance from kin. In another study we found that solitary breeders with living relatives were heavier (measured shortly before gestation) than the other three female classes (Hill, D.L., Pillay, N. and Schradin, C., unpublished data). If greater body mass is advantageous to breeding females, this may enable solitary breeders with relatives to rear and defend young alone without having high corticosterone levels. Corticosterone levels might also be expected to decline in returners once they have left the group if group-living is associated with increased social stress, but we did not detect any differences in corticosterone with changes in social situation in returners. However, potential decreases in social stress after leaving the group could be offset by a different set of risks and challenges experienced away from the group, as observed in females without relatives. In summary, the social stress of group-living alone cannot explain the corticosterone levels we observed in female striped mice, especially the high levels in returners during their period away from the group and in females without living relatives. Instead, we expect that corticosterone modulates energy expenditure in response to different challenges, such as female–female competition and solitary breeding in females without relatives.

Oestrogen regulates many aspects of female reproduction (reviewed in Hewitt et al., 2005), including various sexual and maternal behaviours (Ghiraldi et al., 1993; Spiteri et al., 2012). We found that oestrogen levels were higher in returners than in communal and solitary breeders (with or without relatives). In returners, most (8/9) samples were taken from females before they left the group and gave birth, and the difference between reproductive phenotypes remained statistically significant ($\chi^2_3 = 18.56$, $P < 0.001$) when the single postpartum blood sample was excluded from the analysis. Breeding dispersal in the common vole, *Microtus arvalis*, occurs on the day before parturition, and was hypothesised to be triggered by a surge in oestrogen (Boyce and Boyce, 1988). Oestrogen levels peak around two days before parturition in house mice (which have a gestation of 19 days compared to 23 days in striped mice). In striped mice, returners leave the group around two days before giving birth (Hill, D.L., Pillay, N. and Schradin, C., unpublished data), which appears to correspond with the peak in oestrogen. Females (with living relatives) that became permanently solitary left the group at an earlier point in gestation than returners (Hill, D.L., Pillay, N. and Schradin, C., unpublished data). Accordingly, further studies should test whether solitary breeders have lower oestrogen levels than returners at the point of leaving the nest, and whether returners' oestrogen levels change before, during and after their period away from the group. In summary, our study points towards a modulatory role for oestrogen in inducing females to temporarily leave the group.

We did not detect an association between baseline progesterone levels and ARTs in non-gestating females. However, returners' progesterone levels were lower during their time away from the group (1–4 days postpartum) than after returning to it. Studies on the association between progesterone and the social environment have reported mixed findings: intra-sexual challenges have induced an increase (Rubenstein and Wikelski, 2005), a decrease (Davis and Marler, 2003; Goymann et al., 2008), or no change (Elekonich and Wingfield, 2000) in female progesterone levels. High levels of progesterone interfere with the onset of maternal behaviour in rats by reducing female responsiveness to oestrogen (Bridges and Feder, 1978; Numan, 1978; Sheehan and Numan, 2002). Therefore, as with testosterone, high baseline levels of progesterone might interfere with maternal and allo-parental care. Progesterone levels peak 2–4 days before parturition in house mice and fall sharply just before parturition (Barkley et al., 1979). Female striped mice most frequently become solitary (either on a temporary or permanent basis) during gestation than at other times (Hill, D.L., Pillay, N. and Schradin, C., unpublished data), so any modulatory action of progesterone is most likely to occur in gestating females, and may act in conjunction with oestrogen. Further studies should test whether progesterone or the ratio between oestrogen and progesterone levels differ between ARTs in gestating females.

Conclusions

Steroid hormones can follow physiological cycles and vary in response to changes in the social environment (Rubenstein and Wikelski, 2005; Wingfield et al., 1990). Changes in levels of these hormones in sexually mature individuals can induce them to switch from one ART to another, as predicted by the RPH (Moore, 1991; Moore et al., 1998). Female striped mice following different tactics differed in corticosterone and oestrogen levels, but not in testosterone or progesterone. Corticosterone levels were lower in solitary breeders with relatives than in communal breeders and returners, which suggests that group-living is more stressful and/or energetically demanding than following a solitary ART. Moreover, solitary breeders with living relatives had different corticosterone profiles from females that were constrained by mortality of their relatives to breed solitarily, even though the two female classes occupied a similar social environment. Oestrogen levels were higher in returners (mostly measured before leaving the group) than in communal and solitary breeders, which did not differ in oestrogen levels. This leads us to tentatively propose that the switchpoint between following a returner and an alternative tactic is controlled at a proximate level by variation in oestrogen levels. Moore et al. (1998) predicted that adults following alternative tactics will differ in hormone levels in species with plastic ARTs (the first prediction of the RPH sensu Oliveira et al., 2008). Although experimental manipulations of hormone levels and social situation are needed to confirm whether steroid hormones modulate female ARTs (the second prediction of the RPH: Moore et al., 1998; Oliveira et al., 2008), this correlative field study provides the first support for the RPH in females.

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