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

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1 ABSTRACT

2

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

24 *Keywords:* cooperative breeding; endocrinology; estrogen; glucocorticoid; plural
25 breeding; single breeder; social environment; social flexibility, social organization;
26 sociality
27

28 **Introduction**

29

30 Alternative reproductive tactics (ARTs) are discrete reproductive phenotypes selected
31 to maximise fitness in two or more distinct ways in the same sex and population
32 (Gross, 1996). They can be plastic, whereby an individual is able to switch from one
33 ART to another, or they can be fixed for life (Taborsky, 1998). The differentiation and
34 maintenance of ARTs is mediated by changes in the secretion of steroid hormones
35 (reviewed in Oliveira et al., 2008). This idea was first conceptualised in the Relative
36 Plasticity Hypothesis (RPH), which predicts that fixed tactics are regulated by
37 organisational endocrine effects in early development, whereas switches between
38 plastic tactics are regulated by activational endocrine effects in sexually mature
39 individuals (Moore, 1991; Moore et al., 1998). Alternative adult phenotypes of
40 species with fixed ARTs should therefore have similar steroid profiles provided that
41 they experience the same social environment, while steroid levels are predicted to
42 differ between alternative adult phenotypes in species with plastic ARTs (Moore,
43 1991).

44

45 ARTs are expected to evolve when there is pronounced variance in reproductive
46 success within a sex, leading to reproductive competition (Taborsky et al., 2008).
47 Competition for mates is usually more intense in males than in females (Trivers,
48 1972), which probably explains why ARTs occur more frequently in males (Alonzo,
49 2008). Nevertheless, females of many species experience intense reproductive
50 competition (Stockley and Bro-Jørgensen, 2011), and an increasing number of female
51 ARTs has been described in recent years. Examples include brood parasitism versus
52 maternal care in ruddy ducks (*Oxyura jamaicensis*) (Reichart et al., 2010) and

53 monandry versus polyandry in horseshoe crabs (*Limulus polyphemus*) (Johnson and
54 Brockmann, 2012). Little, however, is known about the role of hormones in mediating
55 female ARTs (Oliveira et al., 2008).

56

57 Glucocorticoids (GCCs) regulate basal metabolism and facilitate appropriate
58 responses to stress (Reeder and Kramer, 2005; Sapolsky et al., 2000). In species with
59 plastic ARTs, bourgeois (dominant) males sometimes have higher GCC levels than
60 males of subdominant tactics (satellite, roamer, sneaker), while in other species the
61 pattern is reversed (Oliveira et al., 2008). This difference might depend on whether it
62 is more energetically demanding to occupy a dominant or a subordinate rank (Creel,
63 2001). Experimental manipulations of GCC levels in species with plastic ARTs can
64 induce males to switch tactics. For example, bourgeois male Great Plains toads (*Bufo*
65 *cognatus*) and Woodhouse's toads (*Bufo woodhousii*) with experimentally-elevated
66 corticosterone levels were more likely than controls to switch to a satellite tactic
67 (Leary et al., 2006). Given their role in mediating ARTs in males (Oliveira et al.,
68 2008) and transitions between life history stages in both sexes (Crespi et al., 2013;
69 Wada, 2008), GCCs are a promising candidate for regulating female ARTs.

70

71 In species with plastic ARTs, bourgeois males typically have higher androgen levels
72 than subordinates, and experimentally increasing androgen levels in subordinate
73 males can induce a switch to the bourgeois tactic (Oliveira et al., 2008). Marine
74 iguanas (*Amblyrhynchus cristatus*), for example, employ three plastic ARTs, with
75 satellite and sneaker males having lower androgen levels than territorial males
76 (Wikelski et al., 2005). Experimentally increasing androgen levels in satellites and
77 decreasing androgens in territorial males can bring about non-adaptive tactic switches

78 (Wikelski et al., 2005). Bourgeois males are more aggressive than subordinates in
79 many species (e.g. Corlatti et al., 2013; Schutz et al., 2010), and the role of androgens
80 in mediating male aggression is well-established (Wingfield et al., 1987). Fewer
81 studies have tested for an association between aggression and androgen levels in
82 females, but most work suggests that female testosterone levels vary in response to
83 intra-sexual competition and are under direct sex-specific selection (Rosvall, 2013).
84 This raises the possibility that testosterone could facilitate responses to intra-sexual
85 competition in females following different tactics.

86

87 Progesterone and oestrogen control many aspects of female reproduction (Christensen
88 et al., 2012; Hewitt et al., 2005), and are associated with female-female competition in
89 some species (Goymann et al., 2008; Parn et al., 2008; Rubenstein and Wikelski,
90 2005) but not in others (Elekonich and Wingfield, 2000; Hay and Pankhurst, 2005;
91 Navara et al., 2006). In female house mice (*Mus musculus*), ovariectomy during
92 gestation brought forward the onset of maternal aggression (Ghiraldi et al., 1993),
93 while an experimental increase of oestrogen levels inhibited maternal aggression
94 (Svare and Gandelman, 1975). To our knowledge, no study has yet tested whether
95 females following alternative tactics differ in levels of progesterone and oestrogen,
96 and tests in males with plastic ARTs are limited to a few teleost species. Progesterone
97 levels are either higher in bourgeois than subdominant males (Cheek et al., 2000;
98 Oliveira et al., 1996) or do not differ (Hourigan et al., 1991; Ros et al., 2003).
99 Oestrogen levels are higher in subdominant than bourgeois male stoplight parrotfish
100 (*Sparisoma viride*) (Cardwell and Liley, 1991), but do not differ between ARTs in
101 saddleback wrasse (*Thalassoma duperrey*) (Hourigan et al., 1991). These studies

102 suggest that the role of progesterone and oestrogen in modulating female ARTs is
103 worth exploring.

104

105 Here, for the first time, we ask whether the RPH, which predicts differences in steroid
106 hormones in males that follow plastic ARTs (Moore et al., 1998), also applies to
107 females. The striped mouse (*Rhabdomys pumilio*) is an appropriate model in which to
108 test this because plastic ARTs occur in both sexes. Male striped mice have three
109 ARTs that differ in steroid hormone levels (Schradin et al., 2013; Schradin et al.,
110 2009b): 1) philopatric males have very high corticosterone and low testosterone
111 levels; 2) solitary-living roamers have low corticosterone and high testosterone levels;
112 and 3) dominant group-living breeding males have low corticosterone and
113 intermediate testosterone levels. Like males, female striped mice can breed in groups
114 or solitarily. Breeding groups usually comprise 2-4 closely related females and one
115 male (Schradin and Pillay, 2004). Communally-breeding females show alloparental
116 care, including allo-nursing (Schradin and Pillay, 2004; Schubert et al., 2009).
117 Nevertheless, reproductive competition between female nestmates is intense,
118 involving aggression and infanticide (Schradin et al., 2010). Females can avoid
119 reproductive competition by leaving the natal group to nest alone, and solitary and
120 communal females usually co-occur during the breeding season (Schoepf and
121 Schradin, 2012; Schradin et al., 2010). As an alternative to breeding solitarily or
122 communally, gestating females may adopt a third tactic termed 'returner' in which
123 they leave the group to give birth, but later return to it (Hill et al., revision under
124 review). Females can switch between the three phenotypes, which means that tactics
125 are flexible and likely to be regulated by activational endocrine effects.

126

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

152 tactic is indeed under hormonal control, we would therefore expect (v) solitary
153 breeders with living relatives to differ hormonally from solitary breeders without
154 living kin.

155

156

157 **Materials and Methods**

158

159 *Fieldwork*

160 We collected data every month during 2006-10 in Goegap Nature Reserve, South
161 Africa (S 29 41.56, E 18 1.60) using methods approved by the Animal Ethics
162 Committee at the University of the Witwatersrand (2004/87/2A, 2005/82/4 and
163 2007/10/01). The study site receives 180 mm precipitation *per annum*, mostly falling
164 between April and September (in austral winter and spring; C. Schradin, unpublished
165 data). It is an open habitat of shrubs, in which striped mice nest, and sandy areas.

166

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

177 sites (described in Schradin and Pillay, 2004). All adults trapped during the breeding
178 season were fitted with MD-2C radio-collars (Holohil, Canada). Radio-collars
179 weighed 2.5g, representing $5.4 \pm 0.07\%$ of the body mass of non-gestating adult
180 females ($N = 181$ records from 110 individuals). We assumed that juveniles (body
181 mass $< 30\text{g}$) were born at the nest where they were first trapped and observed
182 interacting with group members. This method was validated using microsatellite
183 markers for 2007 and 2008 (Schradin and Lindholm, 2011). We refer to females that
184 nested together or did so before becoming solitary as ‘relatives’ because genetic data
185 show that female group members are close kin (C. Schradin and A. K. Lindholm,
186 unpublished data).

187

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

201

202 Blood samples were collected between August and November of each year. Traps
203 were set close to nest sites in the morning and monitored from a distance of 5-10 m.
204 All blood sampling took place within 45 min of striped mice becoming active in the
205 morning to reduce the potential effects of circadian rhythms on hormone levels.
206 Trapped females were immediately anaesthetized with diethyl ether (validated in
207 Schradin (2008)), and a blood sample of 100-500 μ l (depending on body size) was
208 drawn from the sub-lingual vein as described in Heimann et al. (2009). We recorded
209 the time (s) taken to collect a blood sample from the moment an individual entered the
210 trap (sampling latency, see Measurement of hormone levels). Females were monitored
211 during recovery from anaesthesia and then weighed to the nearest gram. Blood was
212 left to clot at room temperature ($<20^{\circ}\text{C}$) for one hour, centrifuged to allow the serum
213 to be extracted and then stored at -20°C .

214

215 *Determination of parturition date and ART*

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

225

226 Females were classed as nesting ‘communally’ (sharing a nest with ≥ 1 adult female)
227 or not nesting communally on the night before they gave birth. Those that were not
228 nesting communally were further classified as: a) those that resumed nesting with
229 their original group ≥ 1 night after parturition (‘returners’); b) those that did not
230 resume nesting with the group although female relatives were still alive (‘solitary with
231 relatives’); and c) those whose female relatives had died (‘solitary without relatives’).

232 We use the term ‘reproductive phenotype’ (hereafter ‘RP’) to refer to the four
233 categories of breeding female (communal breeder, returner, solitary breeder with
234 relatives, solitary breeder without relatives), and ‘ART’ to describe the first three of
235 these categories, which are predicted to be under hormonal control. We ensured that
236 solitary females were not nesting with unmonitored females by observing the nests of
237 solitary females at dusk when striped mice were returning from foraging, and only
238 assigned a solitary or returner ART to a female if she and all her adult female relatives
239 were fitted with a radio-collar when she gave birth. The date of birth of each female
240 was estimated from the population-specific growth curve described in Schradin et al.
241 (2009c), and we used this to calculate the age of females at blood sampling. We
242 included in the study all females for which blood samples were available and for
243 which RP could be determined ($N = 105$ females from 27 groups; Table 1). Two
244 females provided blood samples and gave birth in two consecutive breeding seasons
245 (both in 2007-08); the remaining 103 individuals bred within a single season.

246

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

Breeding season	No. focal groups	No. 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)

251 ^a Some groups and individuals were sampled over multiple years; totals give the number of unique
 252 individuals and groups across all years

253

254 *Measurement of hormone levels*

255 Serum was analysed for total corticosterone, testosterone, progesterone and oestrogen
 256 levels using commercial Enzyme-Linked Immuno-sorbent Assay (ELISA) kits from
 257 IBL (Immuno Biological Laboratories, Hamburg). All measurements fell within the
 258 standard curves of the assays. Table 1 shows the number of serum samples assayed
 259 for the four hormones per breeding season (2006-10). The focus of our studies has
 260 always been on corticosterone and testosterone (e.g. Schradin, 2008; Schradin et al.,
 261 2009b), and this was also the case in the present study. Where additional aliquots
 262 were available, progesterone and oestrogen were analysed, resulting in a smaller
 263 sample size for those two hormones (Table 1). Progesterone and oestrogen assay kits
 264 were validated for the range of hormone levels found in females from the study
 265 population. Validation of corticosterone and testosterone kits for striped mouse serum
 266 is described in Schradin (2008). Serial dilution of two striped mouse sample pools

267 each for progesterone and oestrogen (this study) and for testosterone and
268 corticosterone (Schradin, 2008) closely followed the standard curves. Intra and inter-
269 assay variability was estimated using several pools from striped mice with low (L),
270 intermediate (I) and high (H) hormone values. Intra-assay variability was 4.0% (based
271 on 2 samples from a L corticosterone pool), 9.4% (2 samples, L), 9.9% (8 samples, L)
272 and 12.2% (10 samples, I) for corticosterone, 5.3% (10 samples, I), 8.8% (10 samples,
273 I) and 24.8% (7 samples, L) for testosterone, 3.7% (7 samples, H), 7.3% (8 samples,
274 H), 8.3% (2 samples, H) and 9.8% (9 samples, L) for progesterone, and 8.3% (6
275 samples, I) for oestrogen. Inter-assay variability was 8.1% (10 assays, I), 17.2% (3
276 assays, L) and 20.0% (4 assays, L) for corticosterone, 12.2% (13 assays, I), 13.7% (11
277 assays, I), 15.3% (4 assays, L) and 16.6% (4 assays, H) for testosterone, and 14.7% (2
278 assays, L) and 18.0% (3 assays, H) for progesterone. A single oestrogen assay was
279 carried out.

280

281 To reduce variation in progesterone levels as a result of the stage of gestation, we
282 assayed progesterone from females whose body mass and reproductive records
283 suggested that they were not gestating at the time of sampling. We did not assay
284 progesterone in females without living relatives due to the small sample size. For
285 corticosterone, only blood samples collected with a sampling latency ≤ 180 s were
286 assayed to avoid a potential stress response, and there was no effect of sampling
287 latency on log-transformed corticosterone levels (ng/ml) within this range (Linear
288 Mixed effects Model: $\beta = -0.002 \pm 0.002$ (mean slope \pm standard error), $t_{174.4} = 1.17$, P
289 $= 0.245$, controlling for random intercepts of individual identity, group identity and
290 year; see Table 1 for N). Sampling latency did not influence log-transformed levels of
291 testosterone ($\beta = -0.0005 \pm 0.002$, $t_{225.5} = 0.31$, $P = 0.760$; sampling latency range: 78-

292 260s, 80.1% of samples collected within 180s), oestrogen ($\beta = 0.002 \pm 0.002$, $t_{20.7} =$
293 1.10, $P = 0.286$; sampling latency range: 104-180s, 86.7% of samples <180s) or
294 progesterone ($\beta = -0.001 \pm 0.006$, $t_{12.8} = 0.09$, $P = 0.928$; sampling latency range: 115-
295 225s, 90.0% of samples <180s).

296

297 *Statistical analysis*

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

309

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

312

$$y_j = \mu + RP + PL + PL^2 + RP \times PL + RP \times PL^2 + mass_j + age_j \\ + id + group + year + \varepsilon$$

313

(1)

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

325

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

333

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

337

$$y_j = \mu + SS_j + mass_j + age_j + id + group + year + \varepsilon$$

338 (2)

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

344

345 Returners experience a similar change in social situation from living in a group to
346 giving birth alone and returning to the group. To test whether these changes are
347 accompanied by changes in hormone levels, we compared log-transformed
348 corticosterone, testosterone and progesterone levels between returners that had been
349 sampled before the temporary solitary stage, while nesting alone and after re-joining
350 the group. The LMM used was the same as Eq. (2) except that SS was a three-level
351 fixed factor (before, during time alone, after) for corticosterone and testosterone, and
352 a two-level factor (during, after) for progesterone; samples from gestating females
353 (before) were not assayed for progesterone. We did not control for body mass and age
354 on progesterone levels in Eq. (2) because of small sample size, and did not consider
355 the effects of changes in social situation in returners on oestrogen levels because 8 of
356 9 samples were collected before females left the group. Where paired samples were
357 available (2008-10), we ran a paired t-test to compare hormone levels in returners
358 before they became temporarily solitary and after they re-joined the group.

359

360 We found no significant heterogeneity of variance across the four female RPs for
361 parturition latency or body mass. We report parameter estimates and degrees of
362 freedom from Type II ANOVA Wald chi-square tests, assuming significance where P

363 < 0.05. Multiple comparisons were carried out using Tukey contrasts with *P*-values
364 adjusted using a single-step method from the multcomp package (Hothorn et al.,
365 2014). Statistical tests are two-tailed. Means are least-squares means ± SE expressed
366 on the original response scale using the lsmeans package (Lenth, 2014).

367

368

369 **Results**

370

371 *Were corticosterone levels associated with reproductive phenotype?*

372 Breeding season corticosterone levels in female striped mice were lower in solitary
373 breeders with relatives than in communal breeders, returners and solitary breeders
374 without relatives, but there was no difference in corticosterone between any of the
375 other reproductive phenotypes (Fig. 1A, Table 2, overall effect of RP: $\chi^2_3 = 18.53$, $P <$
376 0.001). Corticosterone levels increased with body mass ($\chi^2_1 = 16.19$, $P < 0.001$) but
377 did not vary with age ($\chi^2_1 = 0.23$, $P = 0.629$). Corticosterone did not increase in the
378 days leading up to parturition or decrease after it (linear term of PL: $\chi^2_1 = 0.76$, $P =$
379 0.383 ; quadratic term: $\chi^2_1 = 0.05$, $P = 0.816$; sampling range: 99 days before
380 parturition to 97 days after). The relationship between corticosterone levels and
381 parturition latency did not vary with RP (RP × PL linear term: $\chi^2_3 = 0.357$, $P = 0.949$,
382 quadratic term: $\chi^2_3 = 1.22$, $P = 0.748$). A second ANOVA examining females only
383 after they became solitary showed that corticosterone levels were lower in solitary
384 breeders with living relatives (881 ± 192 ng/ml; $N = 17$ samples from 11 females) than
385 in females that were solitary because their relatives had died (2006 ± 391 ng/ml, $N = 19$
386 samples from 10 females; $\chi^2_1 = 13.91$, $P < 0.001$, controlling for body mass) in spite
387 of the similar social environments.

389 **Table 2** Linear Mixed effects Models testing for associations between females' reproductive phenotypes and circulating hormone levels (ng/ml, log-transformed). All models
 390 controlled for random intercepts of individual identity, group identity and year. Estimates were calculated using Tukey contrasts with *P*-values adjusted for multiple testing
 391 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
 392 bold

	Corticosterone			Testosterone			Progesterone			Oestrogen		
	$\beta \pm SE$	<i>Z</i>	<i>P</i>	$\beta \pm SE$	<i>Z</i>	<i>P</i>	$\beta \pm SE$	<i>Z</i>	<i>P</i>	$\beta \pm SE$	<i>Z</i>	<i>P</i>
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

393 *Did corticosterone levels change with females' social situation?*

394 In solitary females with living relatives, there was no difference in corticosterone
395 levels before (816 ± 364 ng/ml, $N = 4$ samples from 4 females, 7.1 ± 2.05 days before
396 becoming solitary) and after (1044 ± 290 ng/ml, $N = 17$ samples from 11 females,
397 27.8 ± 5.44 days after) females became solitary ($\chi^2_1 = 0.66$, $P = 0.416$). A separate
398 ANOVA revealed that corticosterone levels were not associated with social situation
399 in returners ($\chi^2_2 = 0.44$, $P = 0.801$, controlling for body mass). Pairwise Tukey
400 comparisons based on the latter model did not detect a difference in corticosterone
401 levels before (1478 ± 175 ng/ml, $N = 35$ samples from 26 females) and during
402 returners' solitary period (1666 ± 293 ng/ml, $N = 13$ samples from 13 females sampled
403 1.5 ± 1.32 days postpartum; $\beta = 0.12 \pm 0.18$, $Z = 0.65$, $P = 0.788$), during females' time
404 away from the group and after returning to the group (1494 ± 217 ng/ml, $N = 23$
405 samples from 19 females; $\beta = 0.11 \pm 0.20$, $Z = 0.54$, $P = 0.849$), nor before returners
406 became solitary and after they returned to the group ($\beta = 0.01 \pm 0.15$, $Z = 0.07$, $P =$
407 0.997).

408

409 *Were testosterone levels associated with reproductive phenotype?*

410 Testosterone levels were not associated with RP ($\chi^2_3 = 1.77$, $P = 0.621$; Table 2, Fig.
411 1B), body mass ($\chi^2_1 = 1.86$, $P = 0.173$), age ($\chi^2_1 = 2.41$, $P = 0.120$) or parturition
412 latency (linear term: $\chi^2_1 = 0.57$, $P = 0.452$; quadratic term: $\chi^2_1 = 1.00$, $P = 0.318$;
413 sampling range: 99 days before parturition to 97 days after). There was no interaction
414 between RP and parturition latency (RP \times PL linear term: $\chi^2_3 = 2.03$, $P = 0.566$; RP \times
415 PL quadratic term χ^2_3 : 1.25 , $P = 0.740$).

416

417 *Did testosterone levels change with females' social situation?*

418 Testosterone levels did not differ in females with living relatives before (0.51 ± 0.27
419 ng/ml, $N = 6$ samples from 6 females taken 6.4 ± 1.71 days before becoming solitary)
420 and after (0.46 ± 0.17 ng/ml, $N = 24$ samples from 15 females, 35.2 ± 4.93 days after)
421 they became solitary ($\chi^2_1 = 0.08$, $P = 0.781$). Testosterone levels in returners showed a
422 trend towards an association with social situation ($\chi^2_2 = 5.15$, $P = 0.076$). Pairwise
423 comparisons based on this model suggested that returners had higher testosterone
424 levels before (0.70 ± 0.20 ng/ml, $N = 44$ samples from 31 females) leaving the group
425 than after returning to it (0.45 ± 0.15 ng/ml, $N = 29$ samples from 22 females), but this
426 was not statistically significant after adjusting for multiple testing ($\beta = 0.37 \pm 0.18$, $Z =$
427 2.10 , $P = 0.088$). There was no difference in testosterone levels before and during
428 (0.48 ± 0.17 ng/ml, $N = 18$ samples from 16 females, 1.3 ± 1.16 days postpartum)
429 returners' time away from the group ($\beta = 0.32 \pm 0.21$, $Z = 1.49$, $P = 0.293$), nor during
430 their time away from the group and after returning to it ($\beta = 0.05 \pm 0.23$, $Z = 0.23$, $P =$
431 0.970). In returners for which paired samples were available, females had higher
432 testosterone levels before leaving the group (1.46 ± 0.23 ng/ml, sampled 13.9 ± 3.02
433 days antepartum) than after returning to it (0.78 ± 0.21 ng/ml, sampled 10.4 ± 1.09 days
434 postpartum; $t_6 = 3.37$, $P = 0.015$).

435

436 *Were progesterone levels associated with alternative reproductive tactic?*

437 Circulating progesterone levels were not associated with ART ($\chi^2_2 = 0.890$, $P = 0.641$,

438 Table 2; Fig. 1C) nor the number of days since parturition ($\chi^2_1 = 2.01$, $P = 0.156$;

439 range: blood sampled 1-35 days after breeding) in non-gestating female striped mice.

440

441 *Did progesterone levels change when returners temporarily became solitary?*

442 Returners had lower progesterone levels during their time away from the group
443 (11.8 ± 10.49 ng/ml, $N = 2$ samples from 2 females, sampled 2.5 ± 1.50 days
444 postpartum) than after they had returned to it (51.0 ± 36.50 ng/ml, $N = 4$ samples from
445 3 females, 18.0 ± 3.51 days postpartum; $\chi^2_1 = 8.98$, $P = 0.003$).

446

447 *Were oestrogen levels associated with reproductive phenotype?*

448 Circulating oestrogen levels in female striped mice were associated with RP ($\chi^2_3 =$
449 18.48 , $P < 0.001$, Table 2, Fig. 1D) but were not influenced by body mass ($\chi^2_1 = 1.76$,
450 $P = 0.184$), age ($\chi^2_1 = 0.22$, $P = 0.637$), or latency to parturition (linear term: $\chi^2_1 =$
451 2.21 , $P = 0.137$; quadratic term: $\chi^2_1 = 1.63$, $P = 0.202$; range: blood sampled 48 days
452 before parturition to 39 days postpartum). Oestrogen levels were higher in returners
453 than in all other reproductive phenotypes, which did not differ from each other (Table
454 2, Fig. 1D).

455

456 *Did oestrogen levels change with solitary breeders' social situation?*

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

461

462

463 **Discussion**

464

465 We found that alternative reproductive tactics were associated with differences in
466 baseline levels of steroid hormones in female striped mice, as reported previously in

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

478

479 In male striped mice, baseline levels of testosterone are higher in solitary than in
480 group-living individuals (Schoepf and Schradin, 2013; Schradin et al., 2009b;
481 Schradin and Yuen, 2011), but no difference in testosterone levels has been observed
482 between ARTs (this study) or social tactics (Schoepf and Schradin, 2013) in female
483 striped mice. The influence of testosterone on female phenotypes is not well
484 understood (Staub and DeBeer, 1997), but levels of testosterone within females are
485 usually higher in species and situations where reproductive competition is more
486 pronounced (Chapman et al., 1998; Ketterson et al., 2005; Langmore et al., 2002;
487 Møller et al., 2005). Reproductive competition in female striped mice occurs
488 primarily when females are caring for young (Schradin et al., 2009a; Schradin et al.,
489 2010). High levels of testosterone suppress parental care in males (Wingfield et al.,
490 2001 but see Trainor and Marler, 2001), and decrease the expression of certain
491 maternal behaviours (Gandelman, 1973; O'Neal et al., 2008), including pup defence

492 (Svare, 1980). This suggests that female tactics associated with higher testosterone
493 levels would potentially incur a net fitness cost owing to reduced maternal care if
494 testosterone were to modulate female ARTs. This may explain why no association
495 was found. Consistent with this, dominant breeding male striped mice, which must
496 balance paternal care with defending a territory and harem, had lower testosterone
497 levels than solitary-living roamer males, which invade dominant breeders' territories
498 to seek matings, and provide no paternal care (Schradin et al., 2009b). In our study,
499 returners' testosterone levels did, however, decrease between leaving the group and
500 returning to it postpartum. This cannot be explained by a change in returners'
501 reproductive state because testosterone levels did not vary with the number of days
502 before or after parturition. Instead, this might reflect differences in the social
503 environment: perhaps returners experienced greater aggression before leaving the
504 group than after returning to it. Our findings suggest that baseline levels of
505 testosterone do not differ between female ARTs in this species but that testosterone
506 levels within a tactic might be influenced by aspects of the social environment.

507

508 Among female striped mice with living relatives, solitary breeders had lower baseline
509 levels of corticosterone than group-living females (communal breeders and returners).
510 Corticosterone levels did not differ before and after females became solitary, which
511 raises the possibility that hormonal differences were present in these females even
512 before they left the nest. Interestingly, an experimental field study showed a trend
513 towards lower corticosterone levels in group-living male striped mice that later
514 became solitary (i.e. sampled before leaving the group) than in males that remained
515 permanently group-living (Schoepf and Schradin, 2013). Schoepf and Schradin (2013)
516 did not detect a difference in corticosterone levels between females sampled before

517 leaving the group and those that were permanently group-living, although
518 corticosterone levels were significantly lower after leaving the group than ~9 days
519 before leaving it in both sexes. Whether the switch to a solitary ART might be elicited
520 by a decrease in corticosterone while individuals are still group-living is a promising
521 area for future research.

522

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

537

538 A further possibility is that high levels of corticosterone in group-living females are a
539 consequence of social stress arising from reproductive competition or other
540 interactions within the natal group. Indeed, female aggression and infanticide,
541 indicators of reproductive competition in this species, occurred more frequently in

542 communally-breeding groups of striped mice than in male-female pairs (Schradin et
543 al., 2010). However, in tuco-tucos, *Ctenomys sociabilis*, a plurally-breeding rodent,
544 corticosterone levels were higher in solitary than in group-living females (Woodruff et
545 al., 2013). This might reflect differences in the physical and social environments
546 occupied by the two species. Similarly, corticosterone levels can be higher in
547 bourgeois than in subdominant males in some species, while in other species,
548 including male striped mice (Schradin et al., 2009b), the inverse is true (Oliveira et
549 al., 2008). In summary, studies in female striped mice suggest that living in a group
550 and breeding communally is stressful and potentially more energetically demanding
551 than solitary-living and breeding.

552

553 Nevertheless, if social stress from reproductive competition in group-living females
554 were the only explanation for high corticosterone levels, then we would expect to find
555 low corticosterone level in all classes of solitary-breeding female striped mice. By
556 contrast, we found that solitary breeders whose female relatives had died did not
557 differ in corticosterone levels from group-living females. Moreover, corticosterone
558 levels were lower in solitary breeders with living relatives than in those without
559 relatives even though they experienced similar social environments. This may reflect
560 differences in their coping abilities. By regulating energy availability, elevated GCC
561 levels are likely to increase the capacity of females without relatives to meet the
562 increased energetic demands of supplying milk and warmth to pups and responding to
563 social challenges associated with territory defence without assistance from kin. In
564 another study we found that solitary breeders with living relatives were heavier
565 (measured shortly before gestation) than the other three female classes (Hill et al.,
566 revision under review). If greater body mass is advantageous to breeding females, this

567 may enable solitary breeders with relatives to rear and defend young alone without
568 having high corticosterone levels. Corticosterone levels might also be expected to
569 decline in returners once they have left the group if group-living is associated with
570 increased social stress, but we did not detect any differences in corticosterone with
571 changes in social situation in returners. However, potential decreases in social stress
572 after leaving the group could be offset by a different set of risks and challenges
573 experienced away from the group, as observed in females without relatives. In
574 summary, the social stress of group-living alone cannot explain the corticosterone
575 levels we observed in female striped mice, especially the high levels in returners
576 during their period away from the group and in females without living relatives.
577 Instead, we expect that corticosterone modulates energy expenditure in response to
578 different challenges, such as female-female competition and the solitary breeding in
579 females without relatives.

580

581 Oestrogen regulates many aspects of female reproduction (reviewed in Hewitt et al.,
582 2005), including various sexual and maternal behaviours (Ghiraldi et al., 1993; Spiteri
583 et al., 2012). We found that oestrogen levels were higher in returners than in
584 communal and solitary breeders (with or without relatives). In returners, most (8/9)
585 samples were taken from females before they left the group and gave birth, and the
586 difference between reproductive phenotypes remained statistically significant ($\chi^2_3 =$
587 18.56, $P < 0.001$) when the single postpartum blood sample was excluded from the
588 analysis. Breeding dispersal in the common vole, *Microtus arvalis*, occurs on the day
589 before parturition, and was hypothesised to be triggered by a surge in oestrogen
590 (Boyce & Boyce 1988). Oestrogen levels peak around two days before parturition in
591 house mice (which have a gestation of 19 days compared to 23 days in striped mice).

592 In striped mice, returners leave the group around two days before giving birth (Hill et
593 al., revision under review), which appears to correspond with the peak in oestrogen.
594 Females (with living relatives) that became permanently solitary left the group at an
595 earlier point in gestation than returners (Hill et al., revision under review).
596 Accordingly, further studies should test whether solitary breeders have lower
597 oestrogen levels than returners at the point of leaving the nest, and whether returners'
598 oestrogen levels change before, during and after their period away from the group. In
599 summary, our study points towards a modulatory role for oestrogen in inducing
600 females to temporarily leave the group.

601

602 We did not detect an association between baseline progesterone levels and ARTs in
603 non-gestating females. However, returners' progesterone levels were lower during
604 their time away from the group (1-4 days postpartum) than after returning to it.
605 Studies on the association between progesterone and the social environment have
606 reported mixed findings: intra-sexual challenges have induced an increase
607 (Rubenstein and Wikelski, 2005), a decrease (Davis and Marler, 2003; Goymann et
608 al., 2008), or no change (Elekonich and Wingfield, 2000) in female progesterone
609 levels. High levels of progesterone interfere with the onset of maternal behaviour in
610 rats by reducing female responsiveness to oestrogen (Bridges and Feder, 1978;
611 Numan, 1978; Sheehan and Numan, 2002). Therefore, as with testosterone, high
612 baseline levels of progesterone might interfere with maternal and allo-parental care.
613 Progesterone levels peak 2-4 days before parturition in house mice and fall sharply
614 just before parturition (Barkley et al., 1979). Female striped mice most frequently
615 become solitary (either on a temporary or permanent basis) during gestation than at
616 other times (Hill et al., revision under review), so any modulatory action of

617 progesterone is most likely to occur in gestating females, and may act in conjunction
618 with oestrogen. Further studies should test whether progesterone or the ratio between
619 oestrogen and progesterone levels differ between ARTs in gestating females.

620

621 *Conclusions*

622 Steroid hormones can follow physiological cycles and vary in response to changes in
623 the social environment (Rubenstein and Wikelski, 2005; Wingfield et al., 1990).

624 Changes in levels of these hormones in sexually mature individuals can induce them
625 to switch from one ART to another, as predicted by the RPH (Moore, 1991; Moore et
626 al., 1998). Female striped mice following different tactics differed in corticosterone
627 and oestrogen levels, but not in testosterone or progesterone. Corticosterone levels
628 were lower in solitary breeders with relatives than in communal breeders and
629 returners, which suggests that group-living is more stressful and/or energetically
630 demanding than following a solitary ART. Moreover, solitary breeders with living
631 relatives had different corticosterone profiles from females that were constrained by
632 mortality of their relatives to breed solitarily, even though the two female classes
633 occupied a similar social environment. Oestrogen levels were higher in returners
634 (mostly measured before leaving the group) than in communal and solitary breeders,
635 which did not differ in oestrogen levels. This leads us to tentatively propose that the
636 switchpoint between following a returner and an alternative tactic is controlled at a
637 proximate level by variation in oestrogen levels. Moore et al. (1998) predicted that
638 adults following alternative tactics will differ in hormone levels in species with plastic
639 ARTs (the first prediction of the RPH sensu Oliveira et al., 2008). Although
640 experimental manipulations of hormone levels and social situation are needed to
641 confirm whether steroid hormones modulate female ARTs (the second prediction of

642 the RPH: Moore et al., 1998; Oliveira et al., 2008), this correlative field study

643 provides the first support for the RPH in females.

644

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646

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662

663

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665

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