

Hill, Davina L., Pillay, Neville and Schradin, Carsten (2015)  
Alternative reproductive tactics in female striped mice: solitary  
breeders have lower corticosterone levels than communal  
breeders. *Hormones and Behavior*, 71 . pp. 1-9.

Downloaded from: <http://insight.cumbria.ac.uk/id/eprint/2544/>

*Usage of any items from the University of Cumbria's institutional repository 'Insight' must conform to the following fair usage guidelines.*

Any item and its associated metadata held in the University of Cumbria's institutional repository Insight (unless stated otherwise on the metadata record) may be copied, displayed or performed, and stored in line with the JISC fair dealing guidelines (available [here](#)) for educational and not-for-profit activities

**provided that**

- the authors, title and full bibliographic details of the item are cited clearly when any part of the work is referred to verbally or in the written form
- a hyperlink/URL to the original Insight record of that item is included in any citations of the work
- the content is not changed in any way
- all files required for usage of the item are kept together with the main item file.

**You may not**

- sell any part of an item
- refer to any part of an item without citation
- amend any item or contextualise it in a way that will impugn the creator's reputation
- remove or alter the copyright statement on an item.

The full policy can be found [here](#).

Alternatively contact the University of Cumbria Repository Editor by emailing [insight@cumbria.ac.uk](mailto:insight@cumbria.ac.uk).

Full text available from Elsevier:

<http://www.sciencedirect.com/science/article/pii/S0018506X15000379>

or (free until 29<sup>th</sup> May 2015):

<http://authors.elsevier.com/a/1Qq-Q,QxXF~qx>

Hill, D.L., Pillay, N. & Schradin, C. (2015) Alternative reproductive tactics in female striped mice: solitary breeders have lower corticosterone levels than communal breeders, *Hormones & Behavior*, **71**:1-9 doi:10.1016/j.yhbeh.2015.03.004

---

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

Davina L. Hill<sup>a\*</sup>, Neville Pillay<sup>a</sup> and Carsten Schradin<sup>a,b,c,d</sup>

<sup>a</sup>School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, Johannesburg, South Africa

<sup>b</sup>Université de Strasbourg, IPHC-DEPE, 23 rue Becquerel 67087 Strasbourg, France

<sup>c</sup>CNRS, UMR7178, 67087 Strasbourg, France

<sup>d</sup>Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

\* Author for correspondence and present address: Animal and Veterinary Sciences Research Group, Scotland's Rural College (SRUC), King's Buildings, West Mains Road, Edinburgh EH9 3JG, UK.

Email addresses: [davina.hill@sruc.ac.uk](mailto:davina.hill@sruc.ac.uk) (D. Hill), [neville.pillay@wits.ac.za](mailto:neville.pillay@wits.ac.za) (N. Pillay), [carsten.schradin@iphc.cnrs.fr](mailto:carsten.schradin@iphc.cnrs.fr) (C. Schradin).

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  $\mu$ 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  $-20^{\circ}\text{C}$ .

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  $\geq 1$  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  $\geq 1$  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

| <b>Breeding season</b> | <b>No. focal groups</b> | <b>No. focal females</b> | <b>Corticosterone</b> | <b>Testosterone</b>        | <b>Progesterone</b>       | <b>Oestrogen</b> |
|------------------------|-------------------------|--------------------------|-----------------------|----------------------------|---------------------------|------------------|
| 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                |
| <b>total</b>           | <b>27<sup>a</sup></b>   | <b>105<sup>a</sup></b>   | <b>179(90)</b>        | <b>229(95)<sup>a</sup></b> | <b>20(16)<sup>a</sup></b> | <b>30(25)</b>    |

251 <sup>a</sup> 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  $\leq 180$ 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  $j$ ;  $\mu$  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  $\varepsilon$  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  $\leq 2.37$  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±192ng/ml;  $N = 17$  samples from 11 females) than  
385 in females that were solitary because their relatives had died (2006±391ng/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     | <b>&lt;0.001</b> |
| solitary with relatives vs communal                   | -0.53±0.14     | 3.72     | <b>0.001</b>     | -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     | <b>&lt;0.001</b> | -0.11±0.19     | 0.57     | 0.938    | -0.19±0.66     | -0.95    | 0.954    | -0.36±0.14     | 2.57     | <b>0.049</b>     |
| 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     | <b>0.012</b>     |
| solitary without relatives vs solitary with relatives | 0.66±0.18      | 3.67     | <b>0.001</b>     | 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 ( $816 \pm 364$  ng/ml,  $N = 4$  samples from 4 females,  $7.1 \pm 2.05$  days before  
396 becoming solitary) and after ( $1044 \pm 290$  ng/ml,  $N = 17$  samples from 11 females,  
397  $27.8 \pm 5.44$  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 ( $1478 \pm 175$  ng/ml,  $N = 35$  samples from 26 females) and during  
402 returners' solitary period ( $1666 \pm 293$  ng/ml,  $N = 13$  samples from 13 females sampled  
403  $1.5 \pm 1.32$  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 ( $1494 \pm 217$  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  $\chi^2_3$ :  $1.25$ ,  $P = 0.740$ ).

416

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

418 Testosterone levels did not differ in females with living relatives before ( $0.51 \pm 0.27$   
419 ng/ml,  $N = 6$  samples from 6 females taken  $6.4 \pm 1.71$  days before becoming solitary)  
420 and after ( $0.46 \pm 0.17$  ng/ml,  $N = 24$  samples from 15 females,  $35.2 \pm 4.93$  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 ( $0.70 \pm 0.20$  ng/ml,  $N = 44$  samples from 31 females) leaving the group  
425 than after returning to it ( $0.45 \pm 0.15$  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 ( $0.48 \pm 0.17$  ng/ml,  $N = 18$  samples from 16 females,  $1.3 \pm 1.16$  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 ( $1.46 \pm 0.23$  ng/ml, sampled  $13.9 \pm 3.02$   
433 days antepartum) than after returning to it ( $0.78 \pm 0.21$  ng/ml, sampled  $10.4 \pm 1.09$  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



645 **Acknowledgements**

646

647 We are grateful to the manager and staff of the Goegap Nature Reserve for support  
648 and the Northern Cape Nature Conservation Service for research permits. We would  
649 like to thank South Africa's National Research Foundation (Grant Number 75057 to  
650 DLH), the Swiss National Science Foundation (31003A-135770/1 to CS), the Fonds  
651 zur Förderung des akademischen Nachwuchses des Zürcher Universitätsvereins (to  
652 CS), the Baugarten Stiftung (to CS), the Swiss South African Joint Research  
653 Programme (to DLH), the University of Zurich and the University of the  
654 Witwatersrand (University Research Committee Fellowship to DLH) for funding.  
655 This study was made possible through the administrative and technical support of the  
656 Succulent Karoo Research Station (registered South African NPO 122-134), where  
657 fieldwork took place. We thank Ed Yuen and Ivana Schoepf for collecting many of  
658 the analysed blood samples, Laura Hastie, Megan MacKay and Ivana Schoepf for  
659 assisting with the hormone assays, and numerous field assistants for help with data  
660 collection. We are also grateful to two anonymous referees for their helpful comments  
661 on the manuscript.

662

663

664 **References**

665

666 Alonzo, S.H., 2008. Conflict between the sexes and alternative reproductive tactics  
667 within a sex, in: Oliveira, R.F., Taborsky, M., Brockmann, H.J. (Eds.),  
668 Alternative Reproductive Tactics: An Integrative Approach. Cambridge  
669 University Press, Cambridge, pp. 435-450.

670 Barkley, M.S., Geschwind, H., Bradford, G.E., 1979. Gestational pattern of estradiol,  
671 testosterone and progesterone secretion in selected strains of mice. Biol.  
672 Reprod. 20, 733-738.

673 Bates, D., Maechler, M., Bolker, B.M. & Walker, S. (2014) Linear mixed-effects  
674 models using Eigen and S4. R package version 1.1-7. Retrieved July 20,  
675 2014, from <http://lme4.r-forge.r-project.org/>

676 Bridges, R.S., Feder, H.H., 1978. Inhibitory effects of various progestins and  
677 deoxycorticosterone on rapid onset of maternal-behavior induced by  
678 ovariectomy-hysterectomy during late pregnancy in rats. Horm. Behav. 10,  
679 30-39.

680 Cardwell, J.R., Liley, N.R., 1991. Hormonal control of sex and color change in the  
681 stoplight parrotfish, *Sparisoma viride*. Gen. Comp. Endocrinol. 81, 7-20.

682 Chapman, J.C., Christian, J.J., Pawlikowski, M.A., Michael, S.D., 1998. Analysis of  
683 steroid hormone levels in female mice at high population density. Physiol.  
684 Behav. 64, 529-533.

685 Cheek, A.O., Thomas, P., Sullivan, C.V., 2000. Sex steroids relative to alternative  
686 mating behaviors in the simultaneous hermaphrodite *Serranus subligarius*  
687 (Perciformes: Serranidae). Horm. Behav. 37, 198-211.

688 Christensen, A., Bentley, G.E., Cabrera, R., Ortega, H.H., Perfito, N., Wu, T.J.,  
689 Micevych, P., 2012. Hormonal Regulation of Female Reproduction. *Horm.*  
690 *Metab. Res.* 44, 587-591.

691 Corlatti, L., Caroli, M., Pietrocini, V., Lovari, S., 2013. Rutting behaviour of  
692 territorial and nonterritorial male chamois: Is there a home advantage? *Behav.*  
693 *Process.* 92, 118-124.

694 Creel, S., 2001. Social dominance and stress hormones. *Trends Ecol. Evol.* 16, 491-  
695 497.

696 Crespi, E.J., Williams, T.D., Jessop, T.S., Delehanty, B., 2013. Life history and the  
697 ecology of stress: how do glucocorticoid hormones influence life-history  
698 variation in animals? *Funct. Ecol.* 27, 93-106.

699 Cummings, M.E., Gelineau-Kattner, R., 2009. The energetic costs of alternative male  
700 reproductive strategies in *Xiphophorus nigrensis*. *J. Comp. Physiol. A.* 195,  
701 935-946.

702 Davis, E.S., Marler, C.A., 2003. The progesterone challenge: steroid hormone  
703 changes following a simulated territorial intrusion in female *Peromyscus*  
704 *californicus*. *Horm. Behav.* 44, 185-198.

705 Elekonich, M.M., Wingfield, J.C., 2000. Seasonality and hormonal control of  
706 territorial aggression in female song sparrows (Passeriformes: Emberizidae:  
707 *Melospiza melodia*). *Ethol.* 106, 493-510.

708 Fox, J. and Weisberg, S. (2014). *car*: Companion to Applied Regression. R package  
709 version 2.0-18. Retrieved July 20, 2014, from  
710 <http://cran.rproject.org/web/packages/car/index.html>

711 Gandelman, R., 1973. Reduction of maternal nest building in female mice by  
712 testosterone propionate treatment. *Dev. Psychobiol.* 6, 539-546.

713 Ghiraldi, L.L., Plonsky, M., Svare, B.B., 1993. Postpartum aggression in mice - The  
714 role of ovarian hormones. *Horm. Behav.* 27, 251-268.

715 Goymann, W., Wittenzellner, A., Schwabl, I., Makomba, M., 2008. Progesterone  
716 modulates aggression in sex-role reversed female African black coucals. *P.*  
717 *Roy. Soc. Lond. B. Biol.* 275, 1053-1060.

718 Gross, M.R., 1996. Alternative reproductive strategies and tactics: Diversity within  
719 sexes. *Trends Ecol. Evol.* 11, 92-98.

720 Hay, A.C., Pankhurst, N.W., 2005. Effect of paired encounters on plasma androgens  
721 and behaviour in males and females of the spiny damselfish *Acanthochromis*  
722 *polyacanthus*. *Mar. Freshw. Behav. Physiol.* 38, 127-138.

723 Heimann, M., Kaesermann, H.P., Pfister, R., Roth, D.R., Buerki, K., 2009. Blood  
724 collection from the sublingual vein in mice and hamsters: a suitable  
725 alternative to retrobulbar technique that provides large volumes and  
726 minimizes tissue damage. *Lab. Anim.* 43, 255-260.

727 Hewitt, S.C., Harrell, J.C., Korach, K.S., 2005. Lessons in estrogen biology from  
728 knockout and transgenic animals, *Annu. Rev. Physiol.*, pp. 285-308.

729 Hill, D.L., Pillay, N., Schradin, C., revision under review. A single strategy with  
730 three alternative reproductive tactics in female striped mice (*Rhabdomys*  
731 *pumilio*).

732 Hothorn, T., Bretz, F., Westfal, P., 2014. multcomp: Simultaneous inference in  
733 General Parametric Models. version 1.3-6, [http://multcomp.R-forge.R-](http://multcomp.R-forge.R-project.org)  
734 [project.org](http://multcomp.R-forge.R-project.org).

735 Hourigan, T.F., Nakamura, M., Nagahama, Y., Yamauchi, K., Grau, E.G., 1991.  
736 Histology, ultrastructure, and in vitro steroidogenesis of the testes of 2 male

737 phenotypes of the protogynous fish, *Thalassoma duperrey* (Labridae). Gen.  
738 Comp. Endocrinol. 83, 193-217.

739 Johnson, S.L., Brockmann, H.J., 2012. Alternative reproductive tactics in female  
740 horseshoe crabs. Behav. Ecol. 23, 999-1008.

741 Ketterson, E.D., Nolan, V., Sandell, M., 2005. Testosterone in females: Mediator of  
742 adaptive traits, constraint on sexual dimorphism, or both? Am. Nat. 166, S85-  
743 S98.

744 Kitaysky, A.S., Piatt, J.F., Wingfield, J.C., Romano, M., 1999. The adrenocortical  
745 stress-response of Black-legged Kittiwake chicks in relation to dietary  
746 restrictions. J. Comp. Physiol. B. 169, 303-310.

747 Langmore, N.E., Cockrem, J.F., Candy, E.J., 2002. Competition for male  
748 reproductive investment elevates testosterone levels in female dunnocks,  
749 *Prunella modularis*. P. Roy. Soc. Lond. B. Biol 269, 2473-2478.

750 Leary, C.J., Garcia, A.M., Knapp, R., 2006. Elevated corticosterone levels elicit non-  
751 calling mating tactics in male toads independently of changes in circulating  
752 androgens. Horm. Behav. 49, 425-432.

753 Lenth, R.V., 2014. lsmeans: Least-Squares Means. R package version 2.00-5.  
754 Retrieved June 28, 2014, from <http://CRAN.R-project.org/package=lsmeans>.

755 Lewanzik, D., Kelm, D.H., Greiner, S., Dehnhard, M., Voigt, C.C., 2012. Ecological  
756 correlates of cortisol levels in two bat species with contrasting feeding habits.  
757 Gen. Comp. Endocrinol. 177, 104-112.

758 Møller, A.P., Garamszegi, L.Z., Gil, D., Hurtrez-Bousses, S., Eens, M., 2005.  
759 Correlated evolution of male and female testosterone profiles in birds and its  
760 consequences. Behav. Ecol. Sociobiol. 58, 534-544.

761 Moore, M.C., 1991. Application of organization activation theory to alternative male  
762 reproductive strategies - A review. *Horm. Behav.* 25, 154-179.

763 Moore, M.C., Hews, D.K., Knapp, R., 1998. Hormonal control and evolution of  
764 alternative male phenotypes: Generalizations of models for sexual  
765 differentiation. *Am. Zool.* 38, 133-151.

766 Navara, K.J., Siefferman, L.M., Hill, G.E., Mendonca, M.T., 2006. Yolk androgens  
767 vary inversely to maternal androgens in eastern bluebirds: An experimental  
768 study. *Funct. Ecol.* 20, 449-456.

769 Numan, M., 1978. Progesterone inhibition of maternal behaviour in the rat. *Horm.*  
770 *Behav.* 11, 209-231.

771 O'Neal, D.M., Reichard, D.G., Pavilis, K., Ketterson, E.D., 2008. Experimentally-  
772 elevated testosterone, female parental care, and reproductive success in a  
773 songbird, the Dark-eyed Junco (*Junco hyemalis*). *Horm. Behav.* 54, 571-578.

774 Oliveira, R., Canário, A.V.M., Ros, A.F.H., 2008. Hormones and alternative  
775 reproductive tactics in vertebrates, in: R. Oliveira, Taborsky, M., Brockmann,  
776 H.J. (Eds.), *Alternative Reproductive Tactics: An Integrative Approach*.  
777 Cambridge University Press, Cambridge, U.K.

778 Oliveira, R.F., Almada, V.C., Canario, A.V.M., 1996. Social modulation of sex  
779 steroid concentrations in the urine of male cichlid fish *Oreochromis*  
780 *mossambicus*. *Horm. Behav.* 30, 2-12.

781 Parn, H., Lindstrom, K.M., Sandell, M., Amundsen, T., 2008. Female aggressive  
782 response and hormonal correlates - an intrusion experiment in a free-living  
783 passerine. *Behav. Ecol. Sociobiol.* 62, 1665-1677.

784 R Development Core Team, 2014. *R: A Language and Environment for Statistical*  
785 *Computing*. R Foundation for Statistical Computing, Vienna, Austria.

786 Reeder, D., Kramer, K.M., 2005. Stress in free-ranging mammals: integrating  
787 physiology, ecology, and natural history. *J. Mammal.* 86, 225-235.

788 Reichart, L.M., Anderholm, S., Munoz-Fuentes, V., Webster, M.S., 2010. Molecular  
789 identification of brood-parasitic females reveals an opportunistic reproductive  
790 tactic in ruddy ducks. *Mol. Ecol.* 19, 401-413.

791 Ros, A.F.H., Canario, A.V.M., Couto, E., Zeilstra, I., Oliveira, R.F., 2003. Endocrine  
792 correlates of intra-specific variation in the mating system of the St. Peter's fish  
793 (*Sarotherodon galilaeus*). *Horm. Behav.* 44, 365-373.

794 Rosvall, K.A., 2013. Proximate perspectives on the evolution of female aggression:  
795 good for the gander, good for the goose? *Philos. T. Roy. Soc. B.* 368,  
796 20130083.

797 Rubenstein, D.R., Wikelski, M., 2005. Steroid hormones and aggression in female  
798 Galapagos marine iguanas. *Horm. Behav.* 48, 329-341.

799 Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids  
800 influence stress responses? Integrating permissive, suppressive, stimulatory,  
801 and preparative actions. *Endocr. Rev.* 21, 55-89.

802 Scantlebury, M., Waterman, J.M., Bennett, N.C., 2008. Alternative reproductive  
803 tactics in male Cape ground squirrels *Xerus inauris*. *Physiol. Behav.* 94, 359-  
804 367.

805 Schoepf, I., Schradin, C., 2012. Better off alone! Reproductive competition and  
806 ecological constraints determine sociality in the African striped mouse  
807 (*Rhabdomys pumilio*). *J. Anim. Ecol.* 81, 649-656.

808 Schoepf, I., Schradin, C., 2013. Endocrinology of sociality: Comparisons between  
809 sociable and solitary individuals within the same population of African striped  
810 mice. *Horm. Behav.* 64, 89-94.

811 Schradin, C., 2008. Seasonal changes in testosterone and corticosterone levels in four  
812 social classes of a desert dwelling sociable rodent. *Horm. Behav.* 53, 573-579.

813 Schradin, C., Kenkel, W., Krackow, S., Carter, C.S., 2013. Staying put or leaving  
814 home: endocrine, neuroendocrine and behavioral consequences in male  
815 African striped mice. *Horm. Behav.* 63, 136-143.

816 Schradin, C., Kinahan, A.A., Pillay, N., 2009a. Cooperative breeding in groups of  
817 synchronously mating females and evolution of large testes to avoid sperm  
818 depletion in African Striped Mice. *Biol. Reprod.* 81, 111-117.

819 Schradin, C., König, B., Pillay, N., 2010. Reproductive competition favours solitary  
820 living while ecological constraints impose group-living in African striped  
821 mice. *J. Anim. Ecol.* 79, 515-521.

822 Schradin, C., Lindholm, A.K., 2011. Relative fitness of alternative male reproductive  
823 tactics in a mammal varies between years. *J Anim. Ecol.* 80, 908-917.

824 Schradin, C., Pillay, N., 2004. The striped mouse (*Rhabdomys pumilio*) from the  
825 succulent karoo, South Africa: A territorial group-living solitary forager with  
826 communal breeding and helpers at the nest. *J. Comp. Psychol.* 118, 37-47.

827 Schradin, C., Scantlebury, M., Pillay, N., König, B., 2009b. Testosterone levels in  
828 dominant sociable males are lower than in solitary roamers: physiological  
829 differences between three male reproductive tactics in a sociably flexible  
830 mammal. *Am. Nat.* 173, 376-388.

831 Schradin, C., Schneider, C., Yuen, C.H., 2009c. Age at puberty in male African  
832 striped mice: the impact of food, population density and the presence of the  
833 father. *Funct. Ecol.* 23, 1004-1013.



834 Schradin, C., Yuen, C.-H., 2011. Hormone levels of male African striped mice  
835 change as they switch between alternative reproductive tactics. *Horm. Behav.*  
836 60, 676-680.

837 Schubert, M., Pillay, N., Schradin, C., 2009. Parental and alloparental care in a  
838 polygynous mammal. *J. Mammal.* 90, 724-731.

839 Schutz, D., Pachler, G., Ripmeester, E., Goffinet, O., Taborsky, M., 2010.  
840 Reproductive investment of giants and dwarfs: specialized tactics in a cichlid  
841 fish with alternative male morphs. *Funct. Ecol.* 24, 131-140.

842 Sheehan, T., Numan, M., 2002. Estrogen, progesterone, and pregnancy termination  
843 alter neural activity in brain regions that control maternal behavior in rats.  
844 *Neuroendocrinology* 75, 12-23.

845 Spiteri, T., Ogawa, S., Musatov, S., Pfaff, D.W., Agmo, A., 2012. The role of the  
846 estrogen receptor  $\alpha$  in the medial preoptic area in sexual incentive motivation,  
847 proceptivity and receptivity, anxiety, and wheel running in female rats.  
848 *Behav. Brain Res.* 230, 11-20.

849 Staub, N.L., DeBeer, M., 1997. The role of androgens in female vertebrates. *Gen.*  
850 *Comp. Endocrinol.* 108, 1-24.

851 Stockley, P., Bro-Jørgensen, J., 2011. Female competition and its evolutionary  
852 consequences in mammals. *Biol. Rev.* 86, 341-366.

853 Svare, B., 1980. Testosterone propionate inhibits maternal aggression in mice.  
854 *Physiol. Behav.* 24, 435-439.

855 Svare, B., Gandelman, R., 1975. Postpartum aggression in mice - inhibitory effect of  
856 estrogen. *Physiol. Behav.* 14, 31-35.

857 Taborsky, M., 1998. Sperm competition in fish: 'bourgeois' males and parasitic  
858 spawning. *Trends Ecol. Evol.* 13, 222-227.

859 Taborsky, M., Oliveira, R.F., Brockmann, H.J., 2008. The evolution of alternative  
860 reproductive tactics: concepts and questions, in: Oliveira, R.F., Taborsky, M.,  
861 Brockmann, H.J. (Eds.), *Alternative Reproductive Tactics: An Integrative*  
862 *Approach*. Cambridge University Press, Cambridge, pp. 1-21.

863 Trainor, B.C., Marler, C.A., 2001. Testosterone, paternal behavior, and aggression in  
864 the monogamous California mouse (*Peromyscus californicus*). *Horm. Behav.*  
865 40, 32-42.

866 Trivers, R.L., 1972. Parental investment and sexual selection, in: Campbell, B. (Ed.),  
867 *Sexual selection and the descent of man*. Aldine Atherton, Chicago, pp. 136-  
868 179.

869 Wada, H., 2008. Glucocorticoids: Mediators of vertebrate ontogenetic transitions.  
870 *Gen. Comp. Endocrinol.* 156, 441-453.

871 Wikelski, M., Steiger, S.S., Gall, B., Nelson, K.N., 2005. Sex, drugs and mating role:  
872 testosterone-induced phenotype-switching in Galapagos marine iguanas.  
873 *Behav. Ecol.* 16, 260-268.

874 Wingfield, J.C., Ball, G.F., Dufty, A.M., Hegner, R.E., Ramenofsky, M., 1987.  
875 Testosterone and aggression in birds. *Am. Sci.* 75, 602-608.

876 Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The Challenge  
877 Hypothesis - Theoretical implications for patterns of testosterone secretion,  
878 mating systems, and breeding strategies. *Am. Nat.* 136, 829-846.

879 Wingfield, J.C., Lynn, S.E., Soma, K.K., 2001. Avoiding the 'costs' of testosterone:  
880 Ecological bases of hormone-behavior interactions. *Brain Behav. Evolut.* 57,  
881 239-251.

882 Woodruff, J.A., Lacey, E.A., Bentley, G.E., Kriegsfeld, L.J., 2013. Effects of social  
883 environment on baseline glucocorticoid levels in a communally breeding

884 rodent, the colonial tuco-tuco (*Ctenomys sociabilis*). Horm. Behav. 64, 566-

885 572.

886

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.