



Corticosterone Levels Correlate With Alloparental Care in a Sex-Dependent Manner in African Striped Mice, *Rhabdomys pumilio*

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Abstract

Alloparental care of non-breeders is the main characteristic of cooperatively breeding species. While many studies have contributed to the understanding of the evolutionary reasons why individuals provide care to young that are not their own offspring, the variables influencing and causing alloparental care are less understood. We tested in African striped mice (*Rhabdomys pumilio*) whether age, sex, testosterone and corticosterone were correlated with alloparental care of non-breeding helpers. We studied 11 family groups under controlled conditions in the laboratory, each with two juvenile and two adult helpers, one being male and one being female in each age category. We predicted male helpers to show more alloparental care than female helpers, as males are the dispersing sex and might thus have to pay for staying. We also expected adult helpers to show more alloparental care than juvenile helpers and both corticosterone and testosterone to correlate negatively with alloparental care. We found high levels of alloparental care in non-breeding striped mice, which spent a significant amount of time in the nest, huddling and licking pups. There was neither a difference between the sexes nor between age categories (although both factors were significant in interaction terms), indicating either low costs and/or high benefits of alloparental care. Mothers showed significantly more care than helpers, and fathers showed similar levels of parental care as mothers but not significantly more than helpers. Although testosterone levels differed significantly between helpers of different age and sex, with adult male helpers showing the highest levels, we did not find any relationships between testosterone and the amount of alloparental care. Corticosterone levels were negatively correlated with alloparental care, and these effects were modulated by the sex and the age of helpers. In females, less alloparental care was shown with increasing corticosterone levels, while in males, the relationship was positive. Also, younger individuals with lower corticosterone levels showed more alloparental care than older individuals with low corticosterone levels. In sum, alloparental care is well developed in male and female non-breeding helpers of striped mice, both in adult and juvenile helpers, but independently of testosterone, with corticosterone showing an age- and sex-specific relationship with alloparental care.

Introduction

Cooperatively breeding species are characterised by non-reproducing helpers in their (often natal) group (Sherman et al. 1995). Helpers are often kin-related to the breeding individuals of their social group, and importantly, they provide caregiving (e.g. huddling pups, licking pups and feeding pups) to young which are not their own offspring – so-called alloparental care (Solomon & French 1997; Ligon & Burt 2004). Between and within cooperative breeding species, the amount of alloparental care can differ between helpers. While many studies investigated these interindividual differences in alloparental care to ultimately understand why helpers show alloparental care (Hamilton 1964; Trivers 1971; Riedman 1982; Balshine-Earn et al. 1998; Ligon & Burt 2004), less is known about the proximate mechanisms that cause differences in caregiving between helpers (Carter & Roberts 1997; Solomon & French 1997; Ligon & Burt 2004; Schoech et al. 2004).

Variation in the expression of alloparental might be due to variation in testosterone and corticosterone levels (Carter & Roberts 1997; Schoech et al. 2004). The hypothesis that elevated testosterone levels decrease parental care is generally accepted in birds (Wingfield et al. 1990). For example, in male helpers of *Aphelocoma* jays, *Aphelocoma* sp., testosterone levels decrease when chicks are present in the nest (Vleck & Brown 1999). In prairie voles, *Microtus ochrogaster*, male and female helpers show low testosterone levels and high corticosterone levels (Carter et al. 1986), and an experimental increase of testosterone reduces alloparental care in males but not in females, whereas an experimental increase of corticosterone reduces alloparental care in females but not in males (Roberts et al. 1996). These studies indicate that maintaining low testosterone levels might be important for the expression of alloparental care in male helpers. Interestingly, natural variation of testosterone and corticosterone levels in both male and female prairie vole helpers was not associated with differential responses in alloparental care (Roberts et al. 1998). In Mongolian gerbils, *Meriones unguiculatus*, high testosterone levels experienced *in utero* reduce the future expression of male alloparental care (Clark et al. 1998; Clark & Galef 2000). In male meerkat helpers, *Suricata suricatta*, testosterone levels are negatively correlated with pup feeding (Young et al. 2005). In sum, in mammals, effects of testosterone as well as corticosterone on alloparental care seem species and sex specific (Hirschenhauser & Oliveira 2006; Storey et al. 2006).

The influence of testosterone levels on alloparental care might be related to age-dependent developmental effects. For instance, in pied kingfishers, *Ceryle rudis*, older helpers show higher testosterone levels and provide less alloparental care than younger helpers (Reyer et al. 1986). In contrast, in callitrichid primates, older helpers typically show more alloparental care than younger helpers (Price 1992; Yamamoto & Box 1997; Achenbach & Snowdon 1998; Zahed et al. 2010). In common marmosets, *Callithrix jacchus*, fathers show a decrease in testosterone levels when exposed to infant stimuli (Prudom et al. 2008). However, to our knowledge, there is no study that has investigated the effects of age in relation to testosterone levels and alloparental care in any mammalian species.

The amount of alloparental care sometimes differs between the sexes. For instance, in callitrichids, males typically provide more alloparental care than females (Price 1992; Yamamoto & Box 1997; Achenbach & Snowdon 1998; Zahed et al. 2010), although age can interact with the sex effect. In cotton-top tamarins, *Saguinus oedipus*, young females show more alloparental care than young males (Price 1992). No sex differences in alloparental care has been found in Goeldi's monkeys, *Callimico goeldii* (Schradin & Anzenberger 2001a), nor in prairie voles (Roberts et al. 1998). In birds, females generally help less than males (Cockburn 1998), such as in Florida scrub jays, *Aphelocoma coerulescens* (Hailman et al. 1994). In cooperatively breeding fish species, no significant sex difference in alloparental care has been reported (Bruitjies & Taborsky 2008; Desjardins et al. 2008). In sum, the sex of helpers seems to influence alloparental care in a species-dependent manner. However, when sex differences in alloparental care exist, the proximate mechanisms underlying the sex-related alloparental care differences are poorly understood.

Here we studied variation in alloparental care as related to differences in steroid hormone levels (testosterone and corticosterone), age and sex in African striped mice (*Rhabdomys pumilio*). This diurnal rodent species shows social flexibility (Schradin et al. 2012) with individuals living either in extended family groups or solitarily, depending on environmental conditions (Schradin et al. 2010; Schoepf & Schradin 2012). Groups consist of one territorial dominant male, up to four breeding females, and sometimes more than 20 philopatric helpers of both sexes (Schradin & Pillay 2004). Philopatric helpers show lower testosterone and higher corticosterone levels than territorial dominant breeders and testosterone levels increase in male helpers with age (Schradin et al.

2009a,b). Interlitter intervals are 3–7 wk, and in the field, philopatric helpers can vary in age between 3 wk (juveniles) and 12 mo (fully adult) (Schradin & Pillay 2004). We first compared the amount of alloparental care with parental care by both parents and tested whether African striped mouse helpers show sex and age differences in providing alloparental care. We then compared steroid hormone levels between male and female helpers of two age classes (juvenile and adult) and breeding males (due to hormonal changes during pregnancy breeding females were not included). We finally tested whether interindividual variations of testosterone and corticosterone levels are correlated with alloparental care.

Materials and Methods

Animals and Breeding Conditions

The founder pairs of the striped mouse colony set up at the University of Zurich originated from individuals trapped in the Succulent Karoo in South Africa in 2002. Mice were housed under an 11:13 h light/dark regime with partly controlled temperature (approx. 23°C). Family groups were housed in two glass tanks (50 × 30 × 30 cm) which were connected to one another by a flexible plastic tube. Additionally, one plastic cage (20 × 13 × 15 cm) was provided with tissue as nesting material. All tanks and cages had 5 cm of wood shavings as bedding. Each group received a seed mixture in the morning (4 g/mouse), a fruit or lettuce at midday (1 g/mouse) and 2 mealworms/mouse in the afternoon. Water was provided *ad libitum*.

Protocol

Eleven family groups were studied, consisting of one breeding pair and their offspring from three litters. When the siblings of the first two litters were 21 d old, each litter was reduced in number to one female and one male. Thus, when the third litter was born, four helpers of different age and sex were present in each family unit: one male and one female adult helper (59.6 ± 3.3 d old) and one male and one female juvenile helper (29.2 ± 2.5 d old). Each mouse was marked with hair dye (Rapido, Pinetown, South Africa) for individual identification during observations.

We video-recorded the nests (which was always in the small plastic cage) from the day of the birth of the third litter for nine consecutive days (D0–D8), each day for 30 min. Recordings were carried out

alternatively during the morning between 9:00 am and 12:00 am or during the afternoon between 3:00 pm and 06:00 pm, when striped mice are most active. During recordings, no observer was inside the animal room, and the first 5 min of the recording were not analysed. We used the software ETHOLOG 2.25[©] (Otoni 2000) to record parental and alloparental care from the last 25 min of recording (after Schradin & Pillay 2003): huddling pups (seconds), licking pups (seconds), and presence in the nest with pups without licking or huddling pups (seconds).

Blood Sampling

When the offspring of the second litter were 21 d old, a first blood sample was collected from each helper and each breeding male as basal level before birth of the third litter (minimum birth interval in striped mice is 23 d; Dewsbury et al. 1984). As such, this first blood sample was taken on a median of 5 d (1st and 3rd quartile: 4 and 6 d; $\bar{x} + SD$: 8.2 ± 2.5 d) before the birth of the third litter, called D–5. We took a second blood sample from each helper and each breeding male at the end of the experiment, which means 9 d after the birth of the third litter, called D+9. Blood samples were collected in the morning within 1 h after the lights went on to control for a possible circadian rhythm of hormone secretions. Mice were anaesthetized with ether, and a blood sample of 200 µl was collected from the sublingual vein (Heimann et al. 2009) within <3 min. Blood samples were left at room temperature for 1 h to clot and then centrifuged two successive times for 10 min. The resulting serum was frozen in aliquots of 50 µl for testosterone and 10 µl for corticosterone assays.

Hormone Assays

We ran three testosterone and three corticosterone assays with commercial kits (IBL Hamburg, Germany), previously validated for striped mice serum (Schradin 2008). For corticosterone, we used the kit RE52211 with a sensitivity of 0.47 ng/ml, and for testosterone, we used the kit RE52151 with a sensitivity of 0.083 ng/ml. As basal corticosterone levels are very high in striped mice (Schradin 2008), samples for the corticosterone assay were diluted 2:48 with the zero standards. For three samples of testosterone, the amount of available serum was too low and was thus diluted 1:1 with the zero standards. The intra-assay coefficients of variation were 1.6% for testosterone and 3.7% for corticosterone. The interassay coefficients

of variation were 1.3% for testosterone and 8.7% for corticosterone.

Ethical Note

Animal ethical clearance was provided by the Kantonale Veterinärämte of the Kanton Zürich in Switzerland (ethical clearance number 91/2006). The research adhered to the ASAB/ABS Guidelines for the Use of Animals in Research. We provided animals with environmental enrichment. The welfare of the animals was monitored by checking them three times a day visually during feeding, and during behavioural observations. Three adult male helpers were euthanised because of injury caused by the breeding males and females – aggression had started after the birth of the third litter. These three males were removed and euthanised to avoid any suffering as these individuals could not have been used in the breeding stock.

Data Analyses

Statistical analyses were carried out with R 3.0.2 and GRAPHPAD INSTAT 3.05. Results are presented as $\bar{x} \pm SE$, and significance was accepted at $\alpha < 0.05$. As data did not differ significantly from a normal distribution, we tested for behavioural and hormonal differences using repeated measures analysis of variance followed by Tukey–Kramer multiple comparison test. Data from the three adult male helpers that were evicted from their group were not included into analysis. For behavioural data, paired comparisons were first carried out with the remaining eight pairs and all social categories to test whether adult male helpers differed from any other social category. Comparisons between all other categories were made with the full dataset from 11 families. ANOVA statistics are only reported for the dataset of 11 families. Correlations between hormone levels taken at D–5 and D+9 were calculated using the Spearman rank correlation.

We ran three linear mixed effect models (LMMs), for which the response variable was square-root-transformed to reach normality of residuals, as tested with the Shapiro–Wilk test. We used the packages LME4 and LMERTTEST, performing type 3 modelling and backwards stepwise deletion of non-significant effects. We tested whether sex, age, testosterone and corticosterone levels (measured at day 9) and their interactions were correlated with (1) the time spent in the nest with pups, that is, response variable of LMM1; (2) the time spent huddling the pups, that is, response variable of LMM2; and (3) the time spent

licking pups, that is, response variable of LMM2. Family affiliation was added as a random factor for each LMM. For figures illustrating significant interactions, regression lines based on back-transformed parameter estimates of the multivariate model were included. R-scripts for the LMM and the figures were provided by Sven Krackow (Zurich, Switzerland: <http://www.yourstat.ch>).

Results

Alloparental and Parental Care

There was no significant difference in the time spent in the nest with pups between mothers, fathers and helpers of the different categories ($N = 11$, $F_{5,4} = 1.218$, $p = 0.32$; Fig. 1). The time pups were huddled differed significantly between social categories ($N = 11$, $F_{5,4} = 6.590$, $p = 0.004$; Fig. 1), and mothers spent more time huddling than helpers of any category, but not more than fathers (Table 1). The time spent licking pups differed significantly between social categories ($N = 11$, $F_{5,4} = 7.757$, $p = 0.0001$, Fig. 1), and mothers spent more time licking pups than helpers of any category and more than fathers (Table 1). Fathers did not show more care than helpers, and helpers of different age and different sex did not differ in alloparental care (Table 1).

Testosterone

At D–5, testosterone levels differed significantly between social classes ($N = 11$; $F_{5,4} = 7.871$;

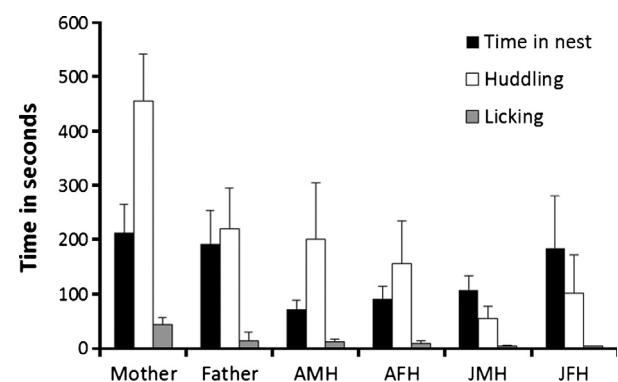


Fig. 1: Parental and alloparental care shown by parents and helpers of African striped mice. Both male and female helpers showed high amounts of alloparental care independent of age, comparable to values shown by fathers. Mothers showed significantly more huddling and licking than helpers, but did not spend more time in the nest (without licking or huddling pups). $\bar{x} \pm SE$ are shown. AMH, adult male helper; AFH, adult female helpers; JMH, juvenile male helpers; JFH, juvenile female helpers.

Table 1: Repeated measures analysis of variance followed by Tukey–Kramer multiple comparison test for three kinds of parental/alloparental care (top: time spent in nest with pups; middle: time spent huddling pups; bottom: time spent licking pups)

Category	Time in the nest pups	Adj. p-values				
		Mothers	Fathers	AMH	AFH	JMH
Mothers	211.6 ± 51.7					
	455.1 ± 87.3					
	44.2 ± 12.5					
Fathers	192.4 ± 61.8	n.s.				
	219.5 ± 75.3	n.s.				
	13.7 ± 16.3	<0.05				
AMH	71.9 ± 16.3	n.s.	n.s.			
	199.6 ± 105.2	<0.05	n.s.			
	11.4 ± 5.3	<0.01	n.s.			
AFH	90.6 ± 23.1	n.s.	n.s.	n.s.		
	156.4 ± 78.6	<0.05	n.s.	n.s.		
	8.7 ± 4.1	<0.01	n.s.	n.s.		
JMH	106.6 ± 27.3	n.s.	n.s.	n.s.	n.s.	
	55.3 ± 23.0	<0.001	n.s.	n.s.	n.s.	
	2.9 ± 2.4	<0.001	n.s.	n.s.	n.s.	
JFH	184.4 ± 97.3	n.s.	n.s.	n.s.	n.s.	n.s.
	102.1 ± 69.5	<0.01	n.s.	n.s.	n.s.	n.s.
	2.5 ± 1.2	<0.001	n.s.	n.s.	n.s.	n.s.

Data are presented as $\bar{x} \pm SE$. AMH, adult male helpers; AFH, adult female helpers; JMH, juvenile male helpers; JFH, juvenile female helper. Significant differences are marked in bold.

$p < 0.0001$; Fig. 2a). Fathers and adult male helpers had similar testosterone levels that were significantly higher than those of adult female helpers ($p < 0.01$ and $p < 0.05$) and juvenile female helpers ($p < 0.01$ and $p < 0.05$). Fathers also had significantly higher testosterone levels than juvenile male helpers ($p < 0.01$), but adult and juvenile male helpers did not differ from each other. No other significant differences in testosterone levels were found.

At D+9, testosterone levels did not differ significantly between social classes ($N = 8$; $F_{3,1} = 0.8337$; $p = 0.063$) when only the eight pairs were considered in which data for all social categories were available. In a second analysis with all 11 pairs, but excluding the male helpers (for which data were only available for eight pairs), we found an overall significant difference ($N = 11$, $F_{4,3} = 6.246$, $p = 0.002$). Fathers had higher testosterone levels than adult and juvenile female helpers (both $p < 0.01$), while no other significant differences were found.

Testosterone levels did not differ significantly between D–5 and D+9 for fathers ($N = 11$; paired $t_{10} = 1.2115$; $p = 0.25$), adult male helpers ($N = 8$; paired $t_7 = 0.278$; $p = 0.79$), adult female helpers

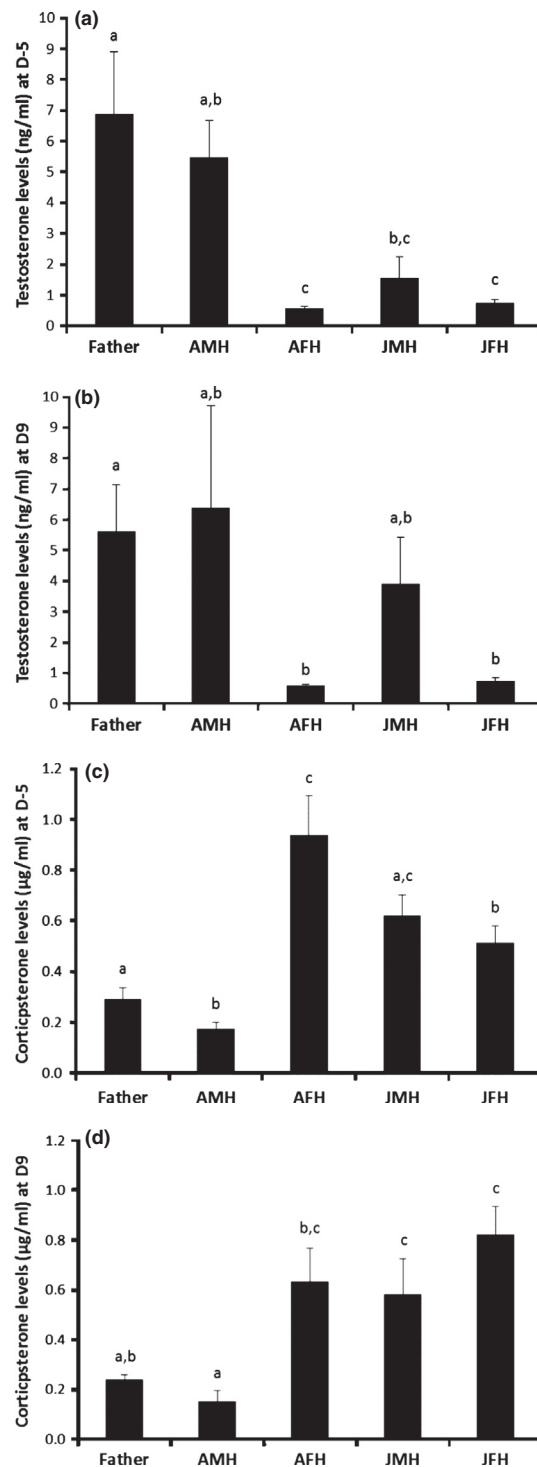


Fig. 2: Testosterone levels (a) 8.2 ± 2.5 before the birth of a litter (D–5), and (b) 9 d after the birth of a litter (D+9) and corticosterone levels (bottom) at D–5 (c) and at D+9 (d). AMH, adult male helper; AFH, adult female helpers; JMH, juvenile male helpers; JFH, juvenile female helpers. Differences in letters above the boxes indicate statistically significant differences. Statistics are based on a paired data design, using 11 family groups for D–5 and eight family groups for D+9.

($N = 11$; paired $t_{10} = 0.9904$; $p = 0.35$), juvenile male helpers ($N = 11$; paired $t_{10} = 1.293$; $p = 0.23$) and juvenile female helpers ($N = 11$; paired $t_{10} = 0.084$; $p = 0.94$; Fig. 2b). Testosterone levels of the blood sample on D-5 correlated significantly with testosterone levels on D+9 ($r_s = 0.61$, $N = 52$, $p < 0.0001$), indicating individual consistency in hormone levels.

Corticosterone

At D-5, corticosterone levels differed significantly between social classes ($N = 11$; $F_{5,4} = 11.636$; $p < 0.0001$; Fig. 2c). Adult female helpers had significantly higher corticosterone levels than fathers ($p < 0.001$), adult male helpers ($p < 0.001$) and juvenile female helpers ($p < 0.05$), but did not differ from juvenile male helpers. Adult male helpers had significantly lower levels than juvenile male helpers ($p < 0.01$). All other comparisons were non-significant.

At D+9, corticosterone levels differed significantly between social classes ($N = 8$; $F_{3,9} = 11.997$; $p < 0.0001$; Fig. 2d). Fathers had significantly lower levels than both juvenile female ($p < 0.001$) and male helpers ($p < 0.01$), but did not differ from adult helpers. Adult male helpers had significantly lower levels than helpers of any other category (adult female helpers, $p < 0.05$; juvenile female helpers, $p < 0.001$; juvenile male helpers, $p < 0.01$). No other comparisons were significant.

Corticosterone levels of juvenile female helpers increased significantly from D-5 to D+9 ($N = 11$; paired $t_{10} = 2.514$; $p = 0.03$). Corticosterone levels did not differ significantly between D-5 and D+9 for fathers ($N = 11$; paired $t_{10} = 1.302$; $p = 0.22$), adult male helpers ($N = 8$; paired $t_7 = 0.744$; $p = 0.48$), adult female helpers ($N = 11$; paired $t_{10} = 2.048$; $p = 0.07$) and juvenile male helpers ($N = 11$; paired

$t_{10} = 0.24$; $p = 0.82$). Corticosterone levels of the blood sample on D-5 correlated significantly with corticosterone levels on D9 ($r_s = 0.49$, $N = 52$, $p = 0.0002$), indicating individual consistency in hormone levels.

Age, Sex, Testosterone, Corticosterone and Alloparental Care

In the LMM1, no independent variable was significantly associated with the time philopatrics spent in the nest with pups (statistics for the main effects: testosterone: $F_{1,30} = 0.1258$; $p = 0.73$; age: $F_{1,31} = 0.0491$; $p = 0.83$; corticosterone: $F_{1,32} = 1.9662$; $p = 0.17$; sex: $F_{1,28} = 0.6712$; $p = 0.42$, Table 2), and all interactions were non-significant ($p > 0.13$). The time spent huddling pups was significantly associated with sex, age, corticosterone levels, and the interactions between sex and age (Fig. 3), and sex and corticosterone levels (Fig. 4) as well as the interaction between age and corticosterone levels (Fig. 5). Testosterone was the only fixed effect that was eliminated from the model. In the LMM3, no independent variable was significantly associated with the time philopatrics spent licking pups (statistics for the main effects: testosterone: $F_{1,32} = 0.118$; $p = 0.73$; age: $F_{1,36} = 0.19$; $p = 0.67$; corticosterone: $F_{1,36} = 0.216$; $p = 0.65$; sex: $F_{1,30} = 3.727$; $p = 0.06$) and all interactions were non-significant ($p > 0.06$).

Discussion

Both male and female non-reproducing helpers showed high amount of alloparental care in our study, and this was the case both for adult and juvenile helpers. While the existence of alloparental care by non-reproducing helpers has been reported for

Table 2: Results from linear mixed models with huddling as the response variable. Non-significant interaction terms were stepwise removed and models were re-calculated after each step

Original model	Huddling = sex × age × corticosterone × testosterone, family as random factor				
Final model	Huddling = sex + age + corticosterone + sex × age + sex × corticosterone + age × corticosterone, family as random factor				
Fixed effect	Mean sq	NumDF	DenDF	F	p
Testosterone	54.12	1	31.56	0.1684	0.68
Sex	66.28	1	28.93	7.2097	0.01
Age	68.89	1	31.15	6.9169	0.01
Corticosterone	51.60	1	33.40	5.8291	0.02
Sex × age	7.68	1	29.67	4.4943	0.04
Sex × cort	103.83	1	28.50	7.2856	0.01
Age × cort	201.94	1	33.95	5.2538	0.03

Significant differences are marked in bold.

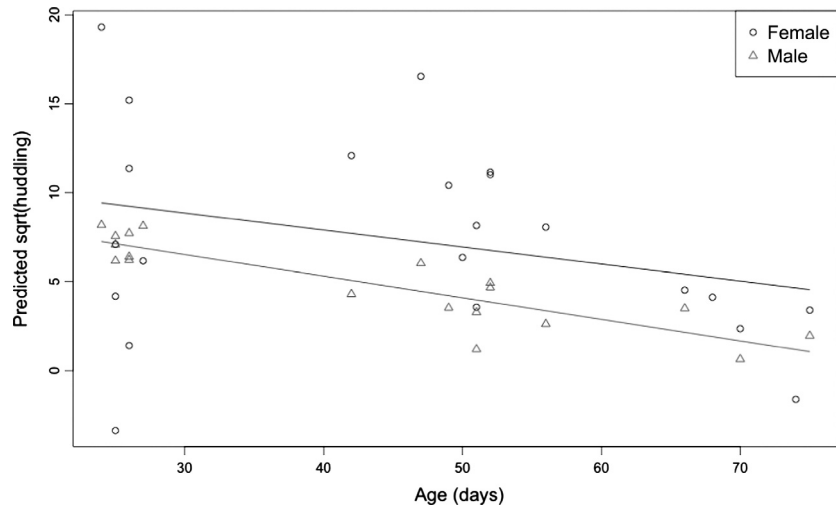


Fig. 3: The correlation between age (in days) and the time spent huddling (log-transformed) depending on the sex of helpers. While this interaction term was significant, the regression lines (based on back-transformed parameter estimates of the multivariate model) do not cross within the studied age range.

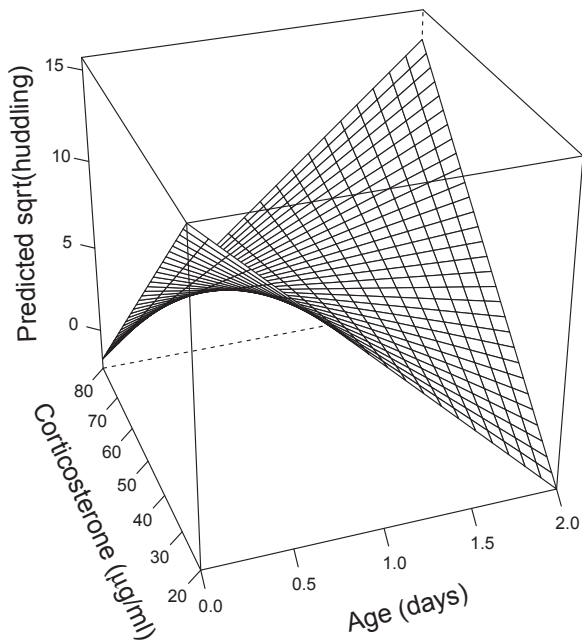


Fig. 4: The correlation between corticosterone levels (in $\mu\text{g/ml}$) and the time spent huddling (log-transformed) depending on the age of helpers (in days).

striped mice before (Schradin & Pillay 2004) and studied separately in females (Rymer & Pillay 2014) and in males (Raynaud & Schradin 2014), this is the most detailed study on it. Mothers provided more care to the offspring (time spent in the nest with pups, huddling pups and licking pups) than any categories of helpers, which is in agreement with the general pattern observed in mammals (Kleiman & Malcom 1981). We also confirmed the important role of fathers (Schradin & Pillay 2003; Schubert et al. 2009),

who on average showed more care than helpers, but did not differ significantly from them. We showed that hormonal profiles differ between helpers of different sex and age and that sex, age and their interactions with corticosterone levels but not testosterone correlated with the expression of alloparental care. In sum, our study indicates that alloparental care is well developed in this species.

In cooperatively breeding callitrichid primates, older helper shows more alloparental care than younger ones (Price 1992; Yamamoto & Box 1997; Achenbach & Snowdon 1998; Zahed et al. 2010). Alloparental care in callitrichids consists mainly of carrying the offspring on the back, which induces higher costs on juvenile helpers, which are physically less strong than older helpers (Schradin & Anzenberger 2001b). In cooperatively breeding rodents, younger helpers do not experience higher costs of huddling and licking pups than older helpers, which could explain why we found no effect when directly comparing older with younger helpers (Fig. 1). However, Rymer & Pillay (2014) found that juvenile striped mouse females show less alloparental care than subadults, when the mother was removed from the cage. This could either indicate that the amount of alloparental care shown is depending on the interaction between age and social condition, or – alternatively – that juveniles are more stress responsive than subadults to the removal of the mother. For huddling, we found a significant effect of age and significant interactions between age and corticosterone, indicating that younger individuals with lower corticosterone levels showed more alloparental care than older individuals with low corticosterone levels. The significant interaction between age and sex is difficult to interpret, as the two regression lines do not cross

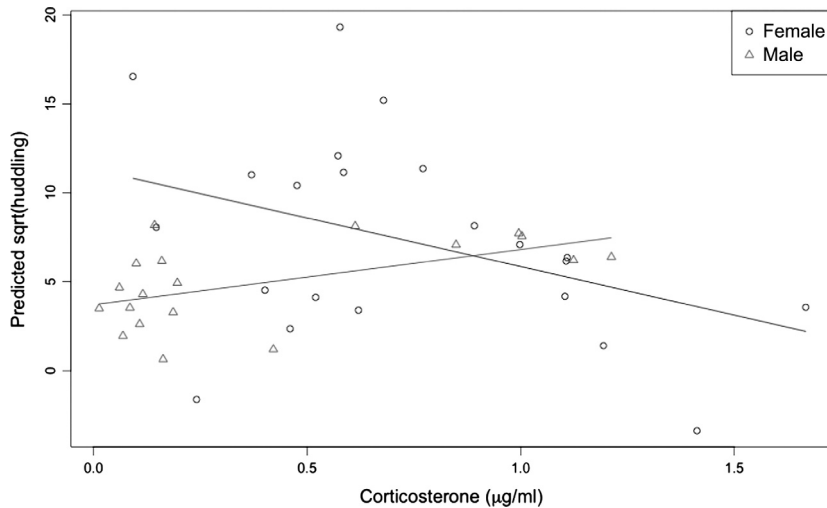


Fig. 5: The correlation between corticosterone levels (in $\mu\text{g/ml}$) and the time spent huddling (log-transformed) depending on the sex of helpers. Regression lines are based on back-transformed parameter estimates of the multivariate model.

within the age range in which the study was conducted (Fig. 2). Alternatively, as the results are correlative, this could be due to older individuals being more willing to show huddling and thus experiencing an increase in corticosterone levels due to alloparental investment. In sum, the relationship between alloparental care and age is less obvious than in other cooperatively breeding taxa, especially callitrichids, and while older helpers tend to show more care, also juvenile helpers invest heavily in alloparental care.

In callitrichid primates, male helpers often show more alloparental care than female helpers (Price 1992; Yamamoto & Box 1997; Achenbach & Snowdon 1998; Zahed et al. 2010), although this has not been reported in all studies (Box 1977; Locke-Haydon & Chalmers 1981; Tardif et al. 1992). The sex difference found in helpers might be explained by sex differences in parental care, as in callitrichids, fathers show more care than mothers (Locke-Haydon & Chalmers 1981; Goldizen 1987). In striped mice, no sex difference exists between maternal and paternal care in single family groups (Schradin & Pillay 2003; current study), which can explain why we found no differences between male and female helpers in our direct comparison (Fig. 1). However, we found that male helpers show more huddling than female helpers. On the other hand, the significant interaction between age and sex indicates that younger males might show less care than other helpers. Our results are thus best interpreted as there being much less pronounced differences in alloparental care between males and females than having been described in callitrichids. Similarly, in family-living prairie voles, where fathers also show high amounts of paternal care, no sex difference in alloparental care was found (Roberts et al.

1998). Rymer and Pillay (2014) found indication that the presence of helpers reduces maternal care, and Schubert et al. (2009) found indication that fathers reduced paternal care when helpers are present (in this study, helpers were other breeding females of the communal group), indicating that helper really help, reducing the investment needed by parents. In sum, whether or not female and male helpers of cooperatively breeding mammals differ in the amount of alloparental care might depend on whether differences exist between the sexes in the amount of parental care shown.

We observed significant variation in testosterone levels (Fig. 2) that were in agreement with the expectation that adult males have higher testosterone levels than females. We did not find any evidence that testosterone plays a role in the regulation of alloparental care in African striped mice under the tested conditions. Natural variation of testosterone levels in helpers of different sex and age did not correlate with alloparental care. Our present results are in agreement with an experimental study, where an increase of testosterone levels via implants in male philopatric helpers did not decrease the amount of alloparental care (Raynaud & Schradin 2014). In sum, our testosterone data revealed expected sex and age differences, but without any evidence of a role of testosterone in the regulation of alloparental care.

As for corticosterone, we observed significant variation in corticosterone levels (Fig. 2) that was in agreement with the expectation that adult males have low corticosterone levels (Schradin 2008; Schradin et al. 2013) and the fact that testosterone is suppressing corticosterone secretion (Raynaud et al. 2012). In prairie voles, a post-natally experimental increase of

corticosterone reduced the time spent in contact with pups in female but not in male helpers (Roberts et al. 1996). We found a negative relationship between corticosterone levels and the time spent huddling pups, and this was especially the case for male and for younger helpers. This could either indicate that high corticosterone levels are negatively influencing huddling or that huddling has a calming effect, reducing corticosterone secretion. Our result suggests age-dependent corticosterone-related mechanisms in the regulation of alloparental care that vary between the sexes.

We found that both male and female striped mice show extensive amounts of alloparental care independent of their testosterone levels, and alloparental care was already well developed in juvenile helpers. In combination with other studies on striped mice (Schradin et al. 2009b; Raynaud et al. 2012; Schradin et al. 2013), this suggests that testosterone does not have a negative effect on caregiving in striped mice. The relationship with corticosterone is more difficult as the effect differed between ages and sex, and due to the correlative nature of the data. It remains to be tested whether an experimental increase of corticosterone levels would negatively influence alloparental care in female helpers but not in males and whether the effects of corticosterone manipulation on the time spent huddling pups change with the age of helpers. In sum, our study demonstrated well-developed alloparental care in striped mice, a cooperatively breeding species with helpers at the nest.

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