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ORIGINAL RESEARCH REPORT

Separation at weaning from the family is stressful for naturally group-living, but not solitary-living, male African striped mice *Rhabdomys*

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Abstract

Early separation from a family is stressful for young mammals, but might be more stressful for group-living than solitary species. Using juvenile males of three African striped mice *Rhabdomys* taxa that are either group (*R. pumilio*) or solitary (*R. dilectus dilectus* and *R. d. chakae*) living, we predicted greater separation anxiety in *R. pumilio* than *R. dilectus* because group-living could reduce anxiety in *R. pumilio*. Three brothers from each of 10 litters per taxon were randomly assigned soon after natural weaning (25 days) to one of three treatments for 10 days: (1) remained with the family (philopatric); (2) separated from the family by a wire mesh barrier (separated); and (3) isolated from the family (isolated). Males were individually tested in a four-arm maze to assess their anxiety responses and sampled for corticosterone concentrations 20 mins and 10 days later. Compared to *R. dilectus* males, *R. pumilio* males showed a greater treatment response to separation: philopatric males used the light arms of the maze less and had higher corticosterone concentrations compared to isolated males, which spent the most time in the light arms and had the lowest corticosterone concentrations overall; separated males showed an intermediate behavioural response, but had similar corticosterone concentrations to philopatric males. Thus, separation from a family group is more stressful in group-living *Rhabdomys* and this stress response dissipates with time. Philopatry and group-living may be more important for young *R. pumilio*, whereas dispersal at weaning is an important life history event for solitary *R. dilectus*.

Keywords

Corticosterone, family group, isolation stress, separation anxiety, sociality, weaning

History

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Introduction

Isolation from a family group is stressful, particularly during the early developmental period (Mesquita et al., 2007). Stress is a natural response to adverse situations and heightens vigilance and awareness to potential threats and uncertainty (Zulkifli & Siegel, 1995). The family group therefore buffers against these stressors. Indeed, group-living mammals can manage stressful situations through interactions with conspecifics, which down-regulate the hypothalamic-pituitary-adenocortical (HPA) response (Taylor et al., 2000).

Group cohesion is maintained through strong bonds between individuals (Lukas & Clutton-Brock, 2013), regulated by neuropeptides, such as oxytocin and arginine vasopressin (AVP, Lim & Young, 2006). When social bonds break down (e.g. a mate dies) or are disrupted (e.g. separation from the family; Lidicker & Stenseth, 1992), individuals may experience separation anxiety, defined as an unpleasant emotional state or distress upon separation from a companion (Hock & Schirtzinger, 1992) or a caregiver (D'Amato et al., 2011).

Social isolation stress occurs in group-living species, such as laboratory rats and mice (Fone & Porkess, 2008) and common marmosets *Callithrix jacchus* (Dettling et al., 2002), and solitary species, such as golden-mantled ground squirrels *Callospermophilus lateralis* (Jesmer et al, 2011). Nonetheless, social organisation can influence social isolation because of differences in hormonal regulation of bond formation (Beery et al., 2008) and the age at which individuals disperse (Bennett & Jarvis, 1988). Therefore, arbitrarily removing individuals from a family group rather than natural separation post-weaning might mask the influences of social organisation on social isolation stress.

The African striped mouse *Rhabdomys* spp. is a small (ca. 80 g body weight, Schradin et al., 2012a) diurnal murid rodent with a wide distribution in South Africa (Skinner & Chimimba, 2005). Two species are recognised: *R. pumilio* in the western, xeric parts and *R. dilectus*, consisting of two subspecies *R. d. dilectus* and *R. d. chakae* in the moist eastern grasslands (Rambau et al., 2003). *Rhabdomys pumilio* in the arid Succulent Karoo is facultatively group-living, comprising 3–4 adult females, one adult male and their philopatric

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non-breeding adult offspring (Schradin & Pillay, 2004). Both *R. dilectus* subspecies are solitary, in which adult males and females maintain intrasexually exclusive territories (Schradin & Pillay, 2005) and weaning occurs at 16 days of age when young disperse (Willan & Meester, 1989).

We compared how group-living *R. pumilio* and solitary *R. dilectus* male juvenile striped mice respond to immediate separation and social isolation from the family soon after weaning using a four-arm maze and by measuring serum corticosterone concentrations. We also investigated whether individuals isolated from the family group or those prevented from making tactile contact with the family for 10 days prior to tests were as affected as those immediately removed from the family. *R. pumilio* is less stressed in an open arena, spending more time outside the nest and exploring novel objects and food more than *R. dilectus* (Rymer et al., 2008). Furthermore, *R. pumilio* shows lower levels of anxiety in the four-arm maze and open field than *R. dilectus* (Rymer & Pillay, 2012). Therefore, we hypothesized that group-living buffers young animals from social isolation stress, so that separation from a group at weaning is more stressful for males of naturally group-living than solitary living striped mice. We also hypothesized that social isolation stress would diminish over time as males habituate to being alone (Banerjee & Adkins-Regan, 2011). We made three predictions. (1) Male *R. pumilio* removed from the family and placed directly into the four-arm maze would be more anxious and have higher concentrations of corticosterone compared to male *R. dilectus*. (2) *R. pumilio* males separated from the family by a barrier would show similarly high levels of isolation stress to males remaining with the family, because of social contact across the barrier. (3) Males isolated from the family for 10 days would be the least anxious and have lower concentrations of corticosterone in the experiments.

Methods

Animals

Captive populations (F3-F5 generation) of the three *Rhabdomys* taxa were used in this study. *Rhabdomys pumilio* originated from the arid Succulent Karoo (Goegap Nature Reserve, Northern Cape Province, South Africa, 29.41.56 S, 18.1.60 E) and both *R. dilectus* subspecies originated from grasslands in Gauteng Province, South Africa (*R. d. chakae* from Suikerbosrand Nature Reserve near Johannesburg, 26.31. S, 28.18 E; *R. d. dilectus* from Irene near Pretoria, 25.54.10 S, 28.12.09 E). Permits for their capture and maintenance in captivity were provided by the Northern Cape Department of Agriculture, Land Reform, Environment and Conservation (Fauna 689/2010) and Agriculture, Conservation and Environment, Gauteng (permit number CPF6 – 0019).

Each striped mouse taxon was housed in a separate room under partially controlled environmental conditions (14 h light: 10 h dark cycle, lights on at 05:00 h and off at 19:00 h; 22–24 °C; 30–60% relative humidity). All three taxa form stable male-female pairs in captivity (observations based on more than 15 years of data), and males from all taxa perform paternal care in captivity (Schradin & Pillay, 2003). We formed 45–54 breeding pairs ($n = 15$–18 per taxon), which were paired at sexual maturity (age at pairing (mean ± SEM): males 4 ± 0.12 months; females 5 ± 0.32 months). These animals were sourced from our breeding colony in which the group-living species were housed in same sex groups and the solitary species were housed alone. Pairs were housed in galvanized steel breeding tanks ($46 \times 31 \times 35$ cm) with a clear Perspex front. The floor of the tanks was covered with a layer of wood shavings for bedding. A plastic nesting box ($13 \times 9 \times 10$ cm) was provided. A handful of dry grass and approximately 5 g of paper towel was provided twice weekly for nesting material. Three cardboard rolls/paper cups were provided weekly per tank for environmental enrichment. The mice had access to water *ad libitum* and were fed approximately 5 g of mixed seed (sprinkled throughout the cage to stimulate foraging behaviour) and 10 g of fresh fruit/vegetables daily per mouse.

Experimental design

Like other species (e.g. rhesus monkeys *Macaca mulatta*; Mitchell & Stevens, 1968), most primiparous striped mouse mothers are often anxious and overcompensate maternal care when paired with inexperienced partners, which is known to influence offspring behavior (Rymer & Pillay, 2013). Therefore, to reduce the influence of parental experience and anxiety on offspring behavior, each pair was allowed to produce two litters (Figure 1). All offspring from first litters were removed from their parents at 25 days of age (inter-litter interval minimum 23 days, Dewsbury et al., 1984), prior to the birth of the second litter and coinciding with the natural period of dispersal at 16–20 days of age (Willan & Meester, 1989), and housed separately or in same-sex sibling pairs away from experimental pairs. Only pairs that produced a second litter of 5–6 pups, with at least 3 male offspring (30 litters in total, 10 litters per taxon) were used. Although *R. dilectus* is solitary in nature, it does form stable family groups in captivity (pers. obs.), allowing us to compare anxiety levels in the philopatric treatment (below).

At the birth of the second litter, a tank (identical to the breeding tank) was attached to one side of the breeding tank with a PVC pipe (±30 cm long, 4.5 cm diameter). A wire mesh grid was inserted into the pipe to prevent access to the adjoining tank. At 25 days of age, three male pups from each litter (10 litters per taxon) were randomly assigned to one of three treatments for a period of 10 days: (1) Isolated – a male was housed alone in a breeding tank in a separate room under the same conditions as its parents and siblings. (2) Separated – a male was housed in the tank adjoining the parental breeding tank. The male had visual, olfactory and auditory contact with its family (parents and siblings), but no physical contact. (3) Philopatric – a male remained with its family. All test males were uniquely marked with non-toxic hair dye (Inecto Rapid, Pinetown, South Africa; Schradin & Pillay, 2004).

Behavior responses in a four-arm maze

Males from all three treatments were tested individually in a modified four-arm maze 10 days after removal from the parents (35 days of age; Figure 1) to coincide with the natural dispersal stage of the solitary species. Tests took place

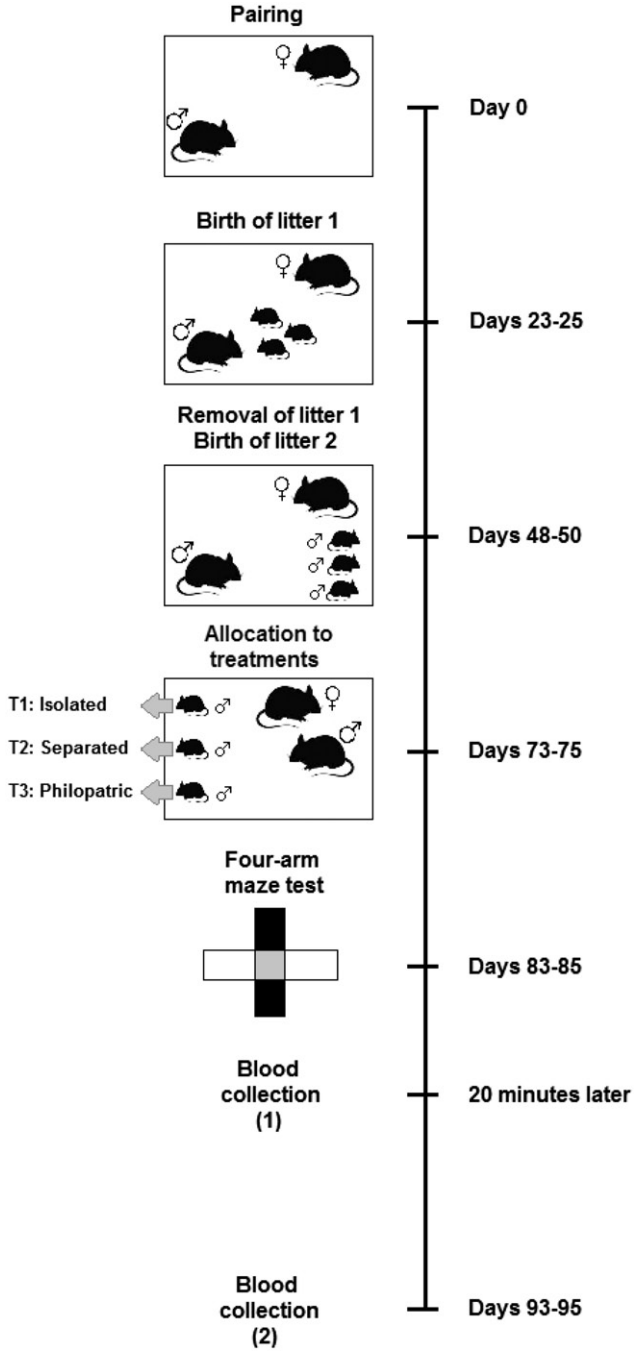

Figure 1. Time-line diagram of the experimental protocol. 30 breeding pairs (10 per taxon) were established (Day 0), with parturition of the first litter at Days 23–25. Offspring from the first litter were removed at 25 days of age (Days 48–50) just prior to the birth of the second litter. Pairs raised their second litter until juvenile males (30 per taxon) were allocated to one of three treatments at 25 days of age (Days 73–75). Juvenile males remained in their treatments for 10 days until 35 days of age and tested in a four-arm maze (Days 83–85). 20 min later, blood was taken from all males for corticosterone analysis. Blood for corticosterone analysis was collected again 10 days later at 45 days of age (Days 93–95) without exposure to the four-arm maze.

between 09:00 and 10:00 h, coinciding with the peak activity of striped mice (Rymer & Pillay, 2010). The four-arm maze comprised four enclosed arms (7.5×7.5 cm $\times$ 50 cm long) constructed from clear PVC connected to a central start box (11×11 cm $\times$ 15.5 cm high); the arms were enclosed because *Rhabdomys* regularly jumps off the maze (Jones et al., 2011). Two arms were painted black (dark) and two remained clear (light). A test mouse was placed in the start box and its behavior was video recorded (from directly above) under white light (room = 361 lux, light arms 279 lux) for 10 min from first entry into the maze. Following Espejo (1997), we recorded duration of time (seconds) spent in, and frequency of visits to, the light and dark arms of the maze. We also scored latency (seconds) to enter the dark and light arms (scored when the whole body and all limbs were inside the arm; Espejo, 1997) from the start box and the frequency of rearing against the maze walls, stretch attend postures (SAP), sniffing, freezing, grooming (in the light arms only as this was not possible in the dark arms) and returning to the dark arms of the maze. Mazes were thoroughly cleaned with soap and water and air dried between trials.

Corticosterone assays

To establish the physiological stress response of test subjects to social isolation, we measured serum corticosterone concentrations in all males per treatment per taxon ($n = 90$). Immediately after the four-arm maze tests, males were transferred to a small holding cage, furnished with woodshavings, food, water and hay. They were anaesthetized with Isoflurane 20 min later (09:30–10:30 h) and a blood sample of 100–200 µl was collected from the saphenous vein of one leg, a sampling procedure that does not result in elevated corticosterone concentrations in mice over and above the stress caused by the four-arm maze (ICR strain; Abatan et al., 2008). Males weighed 33–42 g at 35 days of age when blood was collected, and the volume of blood was sufficient for analysis without long-term impact (Schradin et al., 2009). We timed the blood collection to coincide with the peak of corticosterone release, which is estimated to be 30–40 min following a stress test in rats (Cavigelli & McClintock, 2003), and to control for circadian rhythm variation in hormone secretion. Striped mice were anaesthetized for 3–8 min and were returned to their designated housing conditions once awake and fully recovered. We collected blood samples from males 10 days later (45 days of age) by removing them from their housing treatment, anaesthetizing them and collecting blood within 5–8 min; this time was well before the peak corticosterone release in response to the sampling procedure (Cavigelli & McClintock, 2003). This sampling assessed corticosterone concentrations without exposure to the four-arm maze.

Blood samples were left at room temperature for one hour (11:30–12:30 h) and then centrifuged at 168 g twice for 10 min each (Raynaud & Schradin, 2013). The resulting serum was isolated and frozen in aliquots of 20 µl at −20 °C. Corticosterone analysis was performed using a commercial radioimmunoassay kit (MP Biomedical, Solon, OH). Serum samples were diluted (1:50) in buffer and assayed in duplicate (10 µl each); serial dilution followed the standard curve and slopes provided in the kits. The assay sensitivity was 0.57 ng/ml and the intra- and inter-assay variability was 12.1% and 4.3% respectively. All procedures were performed according to the manufacturer's instructions and final values were averaged for duplicated samples.

Individuals were returned to the colony for breeding later or euthanized with an Isoflurane overdose. This study

complied with the current laws and regulations in South Africa and experimental procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Screening number: 2009/25/2A).

Statistical analyses

We used Statistica 7.1 (Statsoft Inc, www.statsoft.com) for all analyses. The data met the assumptions of normality (Shapiro–Wilk's test) and homogeneity of variances (Levene's test), apart from the frequency of entries into the arms of the four-arm maze, which was square root transformed prior to analyses. The model-level significance was set at $\alpha = 0.05$.

For both experiments, we first ran a variance components analysis using the Expected Mean Squares method to assess the effects of two random factors, litter identity and litter size, on the behaviors. In all cases, neither random factor was significant ($p > 0.05$), and were not considered in further analyses. Thereafter, we ran General Linear Models (GLM) with multiple dependents to compare: (i) the duration of time spent in the light and dark arms; (ii) the frequency of visits to the light and dark arms; (iii) the frequency of rearing; (iv) the frequency of SAPs; (v) the frequency of sniffing, freezing and grooming; and (vi) the frequency of returns to the dark arms of the maze. Taxon and treatment were the categorical predictors. We ran GLMs to analyze the latency to enter the dark arms of the maze, as well as corticosterone concentrations in males per taxon and treatment. Finally, we analyzed corticosterone concentrations between two time periods (after the maze and 10 days later) using a GLM with a repeated measures design for males sampled in both time periods; 3 *R. d. dilectus* and 1 *R. d. chakae* males died before the second sampling and could not be considered in the analyses. In all cases, Tukey HSD *post hoc* and paired-t tests were used to identify the influences of the categorical predictors on behaviour or blood corticosterone concentrations.

Results

Behavior responses in the four-arm maze

Taxon ($\lambda = 0.81$; $F_{4,160} = 4.41$; $p = 0.002$), treatment ($\lambda = 0.68$; $F_{4,160} = 8.60$; $p < 0.001$) and taxon × treatment ($\lambda = 0.60$; $F_{8,160} = 5.81$; $p < 0.001$) were all significant predictors of the duration of time spent in the light and dark arms of the four-arm maze. Within taxon comparisons revealed a graded response for *R. pumilio* males for time spent in the light (isolated > separated > philopatric) and dark arms (philopatric > separated > isolated) (Figures 2a, b). For the two *R. dilectus* subspecies, there was overlap among treatments in the times spent in the light and dark arms, with separated males spending the least time in the light arms and most time in the dark arms (Figures 2a, b). Between taxon comparisons showed that philopatric males spent the least and isolated *R. pumilio* males the most time in the light arms (Figure 2a). Separated male *R. pumilio* and all other taxa × treatment combinations spent an intermediate duration of time in the light arms. Philopatric *R. pumilio* and separated *R. d. dilectus* males spent the most time, whereas isolated

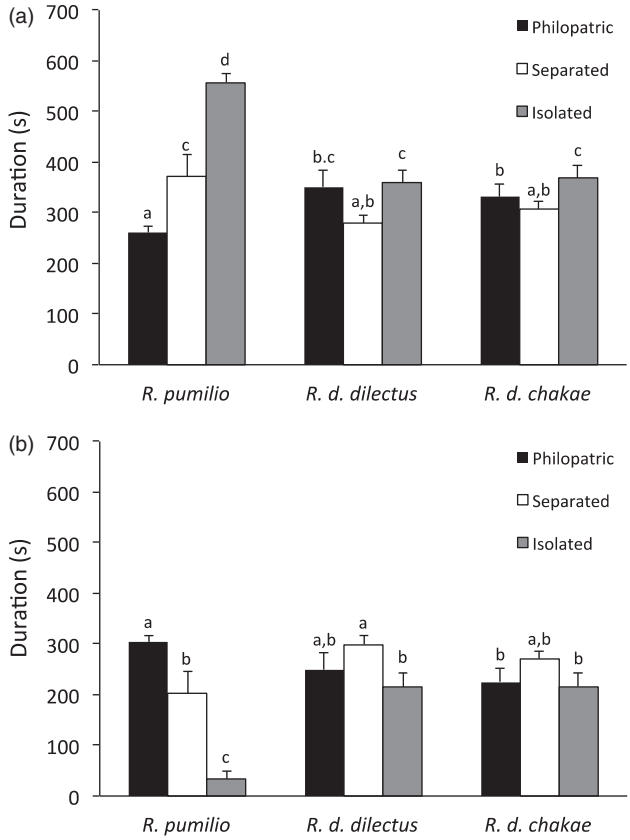

Figure 2. Time (s) spent by juvenile male striped mice *Rhabdomys* of three taxa, reared in three conditions, in the (a) light and (b) dark arms of a modified four-arm maze. $n = 30$ mice per taxon, 10 mice per treatment. Data are mean + SEM. Statistics: GLM with multiple dependents. Bars with different letters indicate significant differences (Tukey *HSD post hoc* tests; $p < 0.05$).

R. pumilio philopatric males spent the least in the dark arms (Figure 2b). The remaining taxa × treatment combinations occupied an intermediate position.

Taxon ($\lambda = 0.69$; $F_{4,160} = 8.24$; $p < 0.001$), treatment ($\lambda = 0.81$; $F_{4,160} = 4.53$; $p = 0.002$) and taxon × treatment ($\lambda = 0.73$; $F_{8,160} = 3.42$; $p = 0.001$) were all significant predictors of the number of entries into the light and dark arms of the four-arm maze. Within taxon comparisons for *R. pumilio* showed that isolated and separated males made more entries into the dark arms than the philopatric males. However, entries into the dark arms increased from philopatric to separated to isolated males (Figures 3a, b). All three treatments resulted in similar entries into the light and dark arms for both *R. d. dilectus* and *R. d. chakae* (Figures 3a, b). Comparisons between taxa showed that the frequency of entries into the light arms (Figure 3a) was greatest in separated and isolated *R. pumilio* males, whereas males of the remaining taxa and treatments showed an intermediate number of entries. The frequency of entries into the dark arms (Figure 3b) was similarly high in all taxa and treatments, apart from fewer entries by separated *R. pumilio* males and even fewer by isolated *R. pumilio* males.

Behaviors in the four-arm maze are summarized in Table 1. Latency to enter the dark arms was significantly influenced by taxon ($F_{2,81} = 5.06$; $p = 0.009$), treatment ($F_{2,81} = 3.56$; $p = 0.033$) and taxa × treatment ($F_{4,81} = 8.43$; $p < 0.001$). Within taxa, philopatric *R. pumilio* males had the shortest

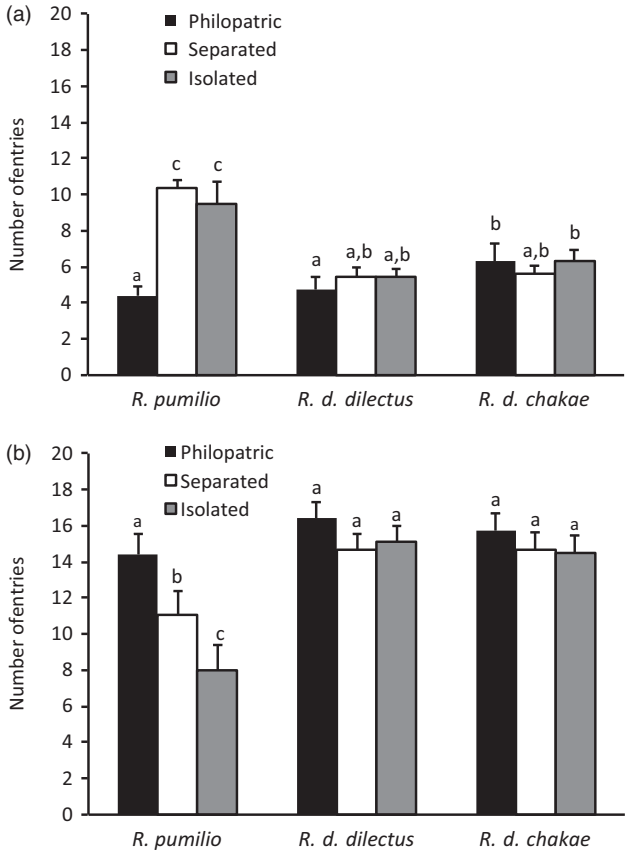

Figure 3. Number of visits to (a) light and (b) dark arms of a modified four-arm maze by juvenile male striped mice *Rhabdomys* of three taxa, reared in three conditions. $n = 30$ mice per taxon, 10 mice per treatment. Data are mean + SEM. Statistics: GLM with multiple dependents. Bars with different letters indicate significant differences (Tukey *HSD post hoc* tests; $p < 0.01$).

latency to enter the dark arms, followed by separated and isolated males. Treatment effects did not differ between the two *R. dilectus* subspecies (Table 1). Among taxa, philopatric *R. pumilio* and isolated *R. d. dilectus* males showed the shortest latencies, followed by separated *R. d. chakae* males and then all other taxa × treatment combinations, apart from isolated *R. pumilio* males, which had the longest latency (Table 1).

The frequencies of behaviors in the light arms were affected by taxon ($\lambda = 0.63$; $F_{12,152} = 3.32$; $p < 0.001$), treatment ($\lambda = 0.58$; $F_{12,152} = 3.95$; $p < 0.001$) and taxon × treatment ($\lambda = 0.38$; $F_{24,266} = 2.91$; $p < 0.001$). Three behaviors (SAP, freeze and return to dark arms) differed significantly between taxa and treatments. Within taxa comparisons showed that the treatment effects between the *R. dilectus* subspecies did not differ for the three behaviors. In contrast, in *R. pumilio*, frequencies of SAP, freezing and returning to the dark arms were greater in the philopatric treatment group and lowest in the isolated treatment group, while the separated treatment group occupied an intermediate position. Between taxa, isolated *R. pumilio* males had the lowest frequencies of SAP, freezing and returns to the dark arms, whereas philopatric *R. pumilio* males had the highest frequencies, and separated *R. pumilio* males and the other taxa × treatment males occupied intermediate positions (Table 1). The frequency of rearing, sniffing

Table 1. The behavioral responses of male striped mice of three taxa in a modified four-arm maze.

	R. pumilio			*R. d. dilectus*			*R. d. chakae*		
Parameters	Philopatric	Separated	Isolated	Philopatric	Separated	Isolated	Philopatric	Separated	Isolated
Latency into dark (s)	17.8 (5.06)b	31.8 (2.14)a,b	84.7 (14.67)a	26.6 (11.36)b	23.9 (4.85)b	21.1 (3.86)b	25.8 (8.53)b	48.2 (7.86)b	27.7 (5.20)b
Frequency of rearing	4.2 (1.19)a	6.9 (2.14)a	7.9 (0.72)a	4.9 (1.14)a	4.1 (0.91)a	7.1 (1.39)a	6.2 (1.21)a	7.2 (1.16)a	7.4 (1.88)a
Frequency SAP	17.2 (2.38)a	9.5 (1.17)a,b	5.1 (1.40)b	6.7 (1.30)b	6.0 (1.15)b	6.7 (1.40)b	5.9 (1.66)b	7.5 (1.50)b	6.7 (1.54)b
Frequency sniff	12.4 (1.96)a	8.7 (0.90)a	9.1 (1.70)a	12.4 (1.40)a	11.9 (1.09)a	13.4 (1.19)a	12.5 (2.29)a	10.0 (1.52)a	12.9 (0.96)a
Frequency freeze	12.9 (1.26)a	6.7 (1.52)b	0.8 (0.29)c	6.2 (1.16)b	9.2 (0.55)b	7.8 (0.77)b	7.2 (1.65)b	8.0 (0.45)b	8.8 (1.39)b
Frequency groom	0.6 (0.22)a	0.5 (0.17)a	0.7 (0.21)a	0.8 (0.29)a	0.7 (0.26)a	0.6 (0.15)a	1.0 (0.32)a	0.8 (0.25)a	1.0 (0.26)a
Frequency of return to dark arms	7.7 (1.16)a	1.3 (0.37)b, c	0.3 (0.21)c	2.1 (0.35)b,c	2.4 (0.27)b,c	2.0 (0.52)b,c	3.6 (0.83)b	3.1 (0.84)b	3.2 (0.53)b

The latency to return into the dark arms was analyzed using a GLM. The frequencies of rearing, SAP (stretch attend posture), sniffing, freezing, grooming and returns to the closed arms, were analyzed using a GLM with a multivariate design. Data are shown as mean (SEM), $n = 10$ striped mice per group. Cells in rows with same letter are not significantly different (Tukey *post hoc* tests): Latency into the dark – a versus b $p < 0.001$; SAP – a versus b, $p < 0.01$; freeze and return to dark arms – a versus b, c, $p < 0.001$, b versus c, $p < 0.05$.

and grooming did not differ among taxa and treatments (Table 1).

Corticosterone assays

Taxon ($F_{2,81} = 5.18$; $p = 0.008$), treatment ($F_{2,81} = 11.91$; $p < 0.001$) and the taxon × treatment interaction ($F_{4,81} = 3.50$; $p = 0.011$) were significant predictors of corticosterone concentrations. Within taxa, *R. pumilio* males showed graded differences: philopatric > separated > isolated (Figure 4). For both *R. d. dilectus* and *R. d. chakae* corticosterone concentrations were slightly elevated in the philopatric treatments but there was overlap among treatments. Among taxa, philopatric and separated *R. pumilio* males had the highest corticosterone concentrations, followed by the philopatric *R. d. chakae* and *R. d. dilectus* males. All other males from the remaining treatments of both *R. dilectus* subspecies had lower corticosterone concentrations and the lowest concentrations were recorded in isolated *R. pumilio* males (Figure 4).

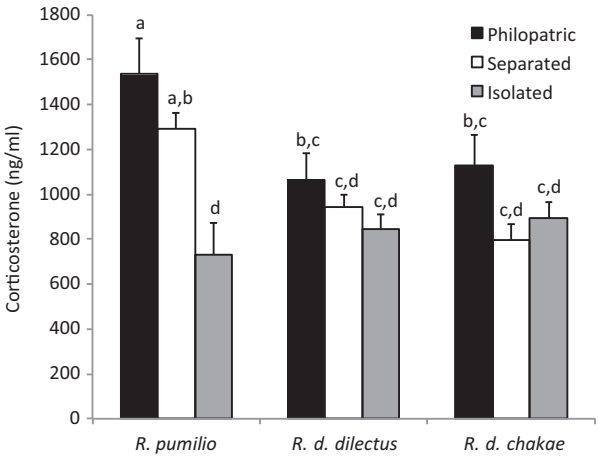

Figure 4. Serum corticosterone concentrations (ng/ml) of juvenile male striped mice *Rhabdomys* of three taxa reared in three conditions, after a 10 min period in a modified four-arm maze. Data are mean + SEM. $n = 30$ mice per taxon, 10 mice per treatment. Statistics: GLM design. Bars with different letters indicate significant differences (Tukey *HSD post hoc* tests; $p < 0.05$).

Figure 5 presents the changes in corticosterone concentrations from 20 min after the maze experiments to 10 days later, with mice kept in their original housing treatment. Overall, corticosterone concentrations were significantly lower 10 days after the maze exposure ($F_{1,77} = 45.66$; $p < 0.001$) (Figure 5). Among taxa comparisons indicated temporal variation ($F_{2,77} = 5.23$; $p = 0.007$), with corticosterone concentrations of *R. pumilio* philopatric males in the first sampling session (20 min after the maze) being significantly greater than all other taxa × treatment combinations (Figure 5); there were no significant differences between groups 10 days later, regardless of treatment. Comparisons between sampling times within taxa (Figure 5) were analyzed using paired-t tests. For *R. pumilio*, corticosterone concentrations decreased significantly in the philopatric ($t_9 = 3.96$,

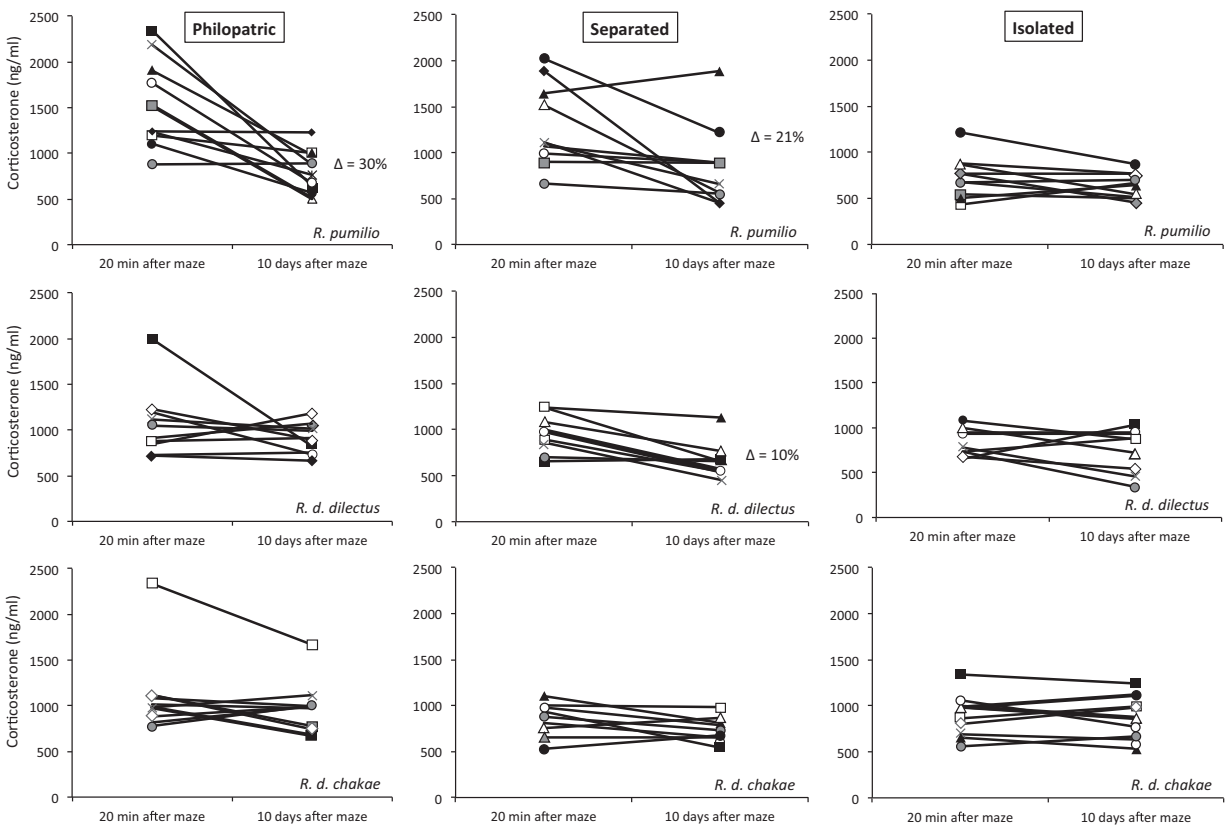

Figure 5. Change in corticosterone concentrations in individuals in philopatric (column 1), separated (column 2) and isolated (column 3) juvenile male striped mice 20 min after exposure to a four-arm maze and 10 days later. Row 1 is the group-living *R. pumilio* and rows 2 and 3 are the solitary *R. d. dilectus* and *R. d. chakae*, respectively ($n = 10$ for each taxon and treatment, except *R. d. dilectus* separated ($n = 9$) and isolated ($n = 8$), and *R. d. chakae* isolated ($n = 9$). The mean percentage change in concentrations (Δ) is presented inside each figure for significant within taxon differences: *R. pumilio* philopatric, $p = 0.003$, separated, $p = 0.023$; *R. d. dilectus*, separated, $p = 0.003$ (paired-*t* test).

$p = 0.003$) and separated ($t_9 = 2.73$, $p = 0.023$) treatments but there was no difference in the isolated treatment group ($t_9 = 1.41$, $p = 0.193$). For *R. d. dilectus*, separated males showed a significant reduction in corticosterone concentrations ($t_8 = 4.29$, $p = 0.003$) but there were no significant changes for the philopatric ($t_7 = 1.07$, $p = 0.321$) and isolated ($t_8 = 1.01$, $p = 0.344$) treatments. *Rhabdomys chakae* males did not show significant differences in corticosterone levels, regardless of treatment: philopatric ($t_9 = 1.28$, $p = 0.232$); separated ($t_8 = 1.73$, $p = 0.123$) and isolated ($t_9 = 0.55$, $p = 0.598$). The interaction between taxon, treatment and time did not influence the change in corticosterone concentration ($F_{4,77} = 2.29$; $p = 0.068$).

Discussion

We used two different methods (four-arm maze and corticosterone concentrations) to assess how early separation and social isolation from the family influences anxiety behavior and HPA activity of captive juvenile males of two species of *Rhabdomys* with different social organizations in nature. In support of our first prediction, philopatric males of the group-living *R. pumilio* were more anxious in the four-arm maze, spending more time in the dark arms and showing higher frequencies of stress-related behaviors (SAP, freezing and returning to the dark arms) compared to the other taxa/treatment combinations. In addition, circulating corticosterone concentrations in philopatric *R. pumilio* males were greater compared to *R. dilectus* males (both subspecies) after exposure to the maze. Comparing the two sampling times (after the maze and 10 days later), corticosterone concentrations were elevated by ca. 30% in philopatric *R. pumilio* males, 3–5 times greater compared to philopatric *R. dilectus* males, yet males of all taxa showed similar concentrations 10 days after exposure to the maze. Our data indicate that social isolation is more stressful for group-living *R. pumilio* species. Stress stimulates the HPA axis, which regulates the secretion of adrenocorticotrophic hormone (ACTH), stimulating an increase in glucocorticoid synthesis and release, resulting in increased circulating corticosterone concentrations (Charmandari et al., 2005). This, in turn, stimulates mobilization of glucose, which is diverted to skeletal muscles, supporting an appropriate behavioral response, either escape or fight (Sapolsky et al., 2000). Our results indicate concordance between behavioral and physiological measures of stress in the striped mouse, as also occurs in Sprague-Dawley rats (Lee et al., 1994).

In support of our second prediction, *R. pumilio* males separated from the family group by a barrier showed similar high circulating corticosterone concentrations to philopatric *R. pumilio* after exposure to the maze, which was significantly higher by ca. 21%, compared to serum concentrations 10 days after the maze experiment. These data indicate that separated *R. pumilio* males were as stressed in the maze as philopatric males and more so than isolated males, which did not show a significant difference in corticosterone concentrations. However, separated *R. pumilio* males did not show greater behavioral anxiety in the four-arm maze compared to philopatric *R. pumilio*, but rather displayed intermediate levels of behavioral anxiety between philopatric (highest anxiety) and isolated (lowest anxiety) males. Separated males spent less time in the light arms, and had a shorter latency to enter the dark arms than isolated males, but spent more time in the light arms, and had a longer latency to enter the dark arms, than philopatric males. Furthermore, separated males showed fewer SAPs, less freezing and fewer returns to the dark arms compared to philopatric males, but more SAPs, freezing and returns to the dark arms than isolated males. Thus, the behavioral and the corticosterone stress responses do not seem to follow a similar pattern (i.e. high physiological stress and high behavioral stress) and appear contradictory (see below) in *R. pumilio* males, although separated males were still more behaviorally anxious than isolated males.

Regardless of treatment, the solitary-living *R. dilectus* males (both subspecies) showed a similar intermediate pattern of response to that of separated *R. pumilio* males (i.e. length of time in the light arms, latency to enter the dark arms, SAP, freezing and returns to the dark arms were between those of philopatric and isolated *R. dilectus* males), although philopatric *R. dilectus* males (both taxa) were less stressed (based on changes in corticosterone concentrations between exposure to the maze and 10 days later) than philopatric and separated *R. pumilio* males. It is possible that separated *R. pumilio* males showed intermediate behavioral anxiety compared to philopatric and isolated *R. pumilio* males because of the nature of the physical barrier separating males from the family. Although separated males were in auditory, olfactory and visual contact with the family group, the lack of tactile contact is apparently stressful for *R. pumilio* males, which are naturally group-living, remaining with the family group into adulthood (Schradin & Pillay, 2004). Similarly, although *R. dilectus* is solitary after weaning at 16 days of age (Brooks, 1982), offspring sometimes associate with the mother for another 20 days until the birth of the next litter. Wilson (2001) found that lack of tactile stimulation, even in the presence of other sensory stimuli, was sufficient to enhance stress of Long-Evans hooded rats in a novel open field. Similarly, exposure to olfactory cues of familiar conspecifics did not decrease anxiety of male Wistar rats in an elevated plus maze (Nakayasu & Kato, 2011). This suggests that the barrier may have been sufficient for maintaining social bonds, to some degree, but the lack of physical contact resulted in stress in these *Rhabdomys* males when placed in a novel environment.

In support of our third prediction, 10 days of isolation from the birth group resulted in males of all taxa showing lower levels of behavioral anxiety and lower concentrations of corticosterone than philopatric males. Isolated *R. pumilio* males spent more time in, and entered the light arms of the four-arm maze proportionately more frequently than any other taxon/treatment combination. These males also had the lowest corticosterone concentrations immediately after the maze exposure. Such habituation to living alone occurs in zebra finch, *Taeniopygia guttata*, males, in which isolation for 30 min caused no measurable increase in faecal corticosterone metabolites (Banerjee & Akins-Regan, 2011).

The contrast between the behavioral (i.e. use of the dark and light arms) and hormonal stress responses in our study is interesting. Numerous other studies demonstrated a correlation between behavioral anxiety and hormone (corticosterone or equivalent) secretion in response to stress

(Rodgers et al., 1999), but Dickens & Romero (2013) provided evidence that the relationship between endocrine profile and stress exposure can be equivocal. Although we did not measure initial baseline corticosterone, using the data for anxiety behavior in the four-arm maze and the corticosterone concentration immediately after the maze and 10 days later, we could discern four patterns of behavioral/hormonal response to the maze in our study. (1) Philopatric and separated *R. pumilio* males showed increased use of the darks arms and elevated concentrations of corticosterone. (2) Separated *R. pumilio* showed increased entries into the light arms and had high concentrations of corticosterone. (3) *R. dilectus* males showed increased use of the dark arms and associated low concentrations of corticosterone. (4) Isolated *R. pumilio* males showed increased use of the light arms and associated low concentrations of corticosterone. Patterns 1 and 2 reflect a treatment effect discussed earlier. We suggest that the apparent contradiction between the behavioral and hormonal stress response in patterns 3 and 4 reflect differences in ''personality'' (Pervin & John, 1999; Rymer & Pillay, 2012; Sih et al., 2004), social organization and natural dispersal tendencies of the different *Rhabdomys* species.

Rhabdomy pumilio is socially flexible (Schradin et al., 2012b) and, although mainly group-living, can live solitarily depending on prevailing environmental conditions (Schradin et al., 2009). Dispersal is generally delayed for several months (Schradin et al., 2010). Furthermore, *R. pumilio* in the arid Succulent Karoo has little or no vegetation cover (Schradin, 2005), has a bolder personality than *R. dilectus* and generally shows lower levels of anxiety in the four-arm maze and open field than *R. dilectus* (Rymer & Pillay, 2012). In contrast, both *R. dilectus* sub-species are solitary (Brooks, 1982), live in naturally dense grasslands with high levels of over-head cover (Schradin & Pillay, 2005) and have a less bold personality than *R. pumilio* (Rymer & Pillay, 2012). Furthermore, *R. dilectus* disperse soon after weaning (Brooks, 1982). While we did not test whether dispersal *per se* was stressful, the differences between species and treatments indicate that social isolation, which we imposed on test subjects as a form of involuntary dispersal, is more stressful for group-living *R. pumilio* males. However, the stress response is reduced when males are housed alone for 10 days, as also reported for other species (Gunnar et al., 1981; Vecsey et al., 2013).

While social isolation stress may result in behavioral, cognitive and endocrine abnormalities in animals (Weltman et al., 1962), stress is a natural response to adverse situations of short duration (Charmandari et al., 2005). Social groups confer multiple benefits to philopatrics (Scantlebury et al., 2006; Silk, 2007) that outweigh the costs associated with dispersing, such as limited availability of food and territories (Emlen, 1982) and the risk of predation (Solomon, 2003). Furthermore, tactile and olfactory interactions with conspecifics down-regulate the HPA response to stress (Taylor et al., 2000), at least for rodents, through a process known as social buffering (Kikusui et al., 2006). Therefore, involuntary removal from the group is stressful and individuals experience separation anxiety when encountering a novel environment. For solitary species, dispersal is also stressful but the benefits of dispersing most likely outweigh the costs (Ostfeld, 1985). Isolated *R. dilectus* had lower corticosterone concentrations than philopatric *R. pumilio* after exposure to the maze, indicating that solitary animals may be more able to tolerate isolation than group-living animals.

In conclusion, our study highlights the importance of considering social organization, ecology and concomitant timing of dispersal as predictors of social isolation stress. Male group-living *R. pumilio* display an acute stress response when separated from their families and placed in a novel environment, favoring the dark arms of the four-arm maze and showing elevated corticosterone. However, group-living *R. pumilio* males become habituated to separation when isolated from the breeding pair for a period of time, and the general stress response to isolation dissipates over time. Males of the solitary *R. dilectus* are less stressed in a novel environment and avoid open areas, potentially related to their closed, covered habitat and solitary life. The responses of the solitary species suggest an adaptive response to early post-weaning dispersal, whereas the anxiety displayed by the group-living species in response to disruption of social bonds reflects a constraint of living with kin.

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