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The development of exploratory behaviour in the African striped mouse *Rhabdomys*

reflects a gene x environment compromise

Development of exploratory behaviour. Rymer & Pillay

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Abstract

Behaviour results from the interaction of an individual's genotype with prevailing environmental conditions, resulting in local adaptation to specific habitats. We investigated the development of exploratory behaviour in two closely-related species of African striped mice from the semi-arid Succulent Karoo (*Rhabdomys pumilio*) and moist grassland (*R. dilectus chakae*) localities. Irrespective of sex, *R. pumilio* displayed greater exploratory behaviour (open-field) and greater use of the open arms of a modified plus maze, and thus were less anxious and bolder than *R. d. chakae*. When pups were cross-fostered between species, fostered individuals of both species showed an intermediate behavioural pattern between their foster and biological siblings: fostered *R. pumilio* explored more than their foster siblings but less than their biological siblings, whereas fostered *R. d. chakae* explored more than their biological siblings, but less than their foster siblings. Our study is one of the first to address how the underlying genotype and early postnatal experience interact to influence the expression of exploratory behaviour and personality. In particular, we showed that, in striped mice, the early postnatal environment shapes the anxiety responses and concomitant exploratory behaviour, but the genotype apparently modulates the phenotype and constrains the limit of behavioural flexibility.

Keywords: Anxiety, Behavioural development, Early rearing environment, Exploratory behaviour, Gene-environment interactions, *Rhabdomys*

Introduction

The phenotypic expression of behaviour results from a complex interaction between an individual's response to prevailing environmental (including social) conditions and an individual's genes (i.e. genotype; Sambandan et al. 2008). Consequently, populations of species may show environmentally-specific behaviours in different habitats (Christensen and Persson 1993) in order to ensure survival and enhance reproductive success, a concept known as local adaptation (Taylor 1991). For example, house mice *Mus musculus domesticus* inhabiting simulated human-built environments show a reduction in activity levels in habitats with little complexity and low vegetation cover, whereas mice in more complex environments with more cover show greater activity levels (Jensen et al. 2003). Changes in complex behaviours in response to ecological conditions can indicate that the phenotype may be under some genetic control. For example, Nachappa et al. (2010) showed that foraging behaviour in the predatory mite *Phytoseiulus persimilis* shows considerable phenotypic variation and the traits making up this behaviour (e.g. consumption and conversion efficiency) exhibit significant realized heritabilities. Changes in behaviour may itself expose animals to novel selection pressures in different environments, in the form of different social environments (e.g. *Marmota caligata*, Barash 1975), predation risk (Ghalambor and Martin 2002) or competitor density (Michel 2009), resulting in changes to the general phenotype (Duckworth 2009).

Animals use exploratory or investigatory behaviours (Tomlinson and Johnston 1991; Heyser and Chemero 2012) to determine the spatial and temporal relationships of resources (e.g. mole-rats *Spalax ehrenbergi*, *Cryptomys anselli* and *Heterocephalus glaber* and Chilean coruros *Spalacopus cyanus* used odour cues to locate palatable food resources underground, Heth et al. 2002), predators (e.g. grasshopper mice *Onychomys leucogaster* use visual cues to detect

predators, Langley 1989), travel routes (e.g. white-footed mice *Peromyscus leucopus* use trees to navigate during snowy conditions, Drickamer and Stuart 1984) and conspecifics (e.g. house mice *Mus domesticus* use conspecific olfactory cues to assess potential competitors and mates, Hurst 1990; Gosling et al. 1996) within their home range or territory (Shillito 1963). Furthermore, some studies have also suggested that animals may have episodic-like memory for objects and places. Dere et al. (2005) found that laboratory mice (strain C57BL/6) are able to recognize the type of object previously encountered, its spatial location and the relative recency of objects encountered.

Since habitats may be variable for a variety of factors, such as predator density or relative abundance of resources (Marín et al. 2003), species living in different habitats will show different exploratory phenotypes in order to minimize predation risk and maximise resource acquisition. For example, in a comprehensive study of 61 parrots species, Mettke-Hoffman et al. (2002) found that species that live in more complex habitats (e.g. forest edges) or habitats with low predation risk (e.g. islands) show greater exploratory behaviour than species living in less complex environments (e.g. grasslands). Furthermore, different exploratory behavioural phenotypes can occur in populations of species occurring in different habitats, because exploratory behaviour favoured in one particular environment may be less favoured in another. Three-spined sticklebacks *Gasterosteus aculeatus* from two populations in California show different behavioural responses after a predation threat (Bell 2005): sticklebacks from the Navarro River, which experience high predation levels due to dramatic seasonal changes in water flow, take longer to resume foraging and show lower activity levels than fish from Putah Creek, where predation levels are lower and there is abundant vegetation cover to avoid detection.

The African striped mouse (genus *Rhabdomys*) is a small (± 40 g) diurnal murid rodent, represented by two karyotypic forms ($2n = 46$ and $2n = 48$) in southern Africa (Ducroz et al. 1999). It provides a good model for investigating genetic and environmental influences of exploratory behaviour because at least two putative sister species of striped mice occur in different habitats (semi-arid Succulent Karoo and grassland) and show contrasting social systems. Striped mice living in the semi-arid Succulent Karoo of the Northern Cape Province of South Africa (*R. pumilio*), with patchy distribution of vegetation and increased vulnerability to aerial predation, are highly social (Schradin and Pillay 2004). In contrast, striped mice living in grassland regions (*R. dilectus chakae*), with high vegetation cover and comparatively decreased vulnerability to aerial predation, are solitary (Schradin and Pillay 2005). The species belong to different mtDNA clades (Rambau et al. 2003). They are morphologically similar, except for a paler coat colour and longer tails (Pillay 2000a), and larger testes and cauda epididymis (Schradin et al. 2009) in *R. pumilio*.

Our on-going research has suggested that there may also be geographic variation in other behaviours, notably exploratory behaviour. *R. pumilio* spends more time outside the nest box and investigating novel objects (i.e. greater exploration) compared to *R. d. chakae* (Rymer et al. 2008). This suggests that *R. pumilio* may have a bolder personality type than *R. d. chakae* in captivity, where boldness is defined as the animal's willingness to increase exploration in novel circumstances and engage in risky behaviours (Wilson and Stevens 2005; Wilson and Godin 2009; Couchoux and Cresswell 2012). Thus, we investigated species differences in exploratory behaviour and predicted that, in contrast to *R. d. chakae*, *R. pumilio* would show higher levels of exploratory behaviour in an open field, indicative of reduced anxiety (Prior et al. 2004) and a bolder personality.

It is apparent that variation in the behaviour, including sociality and exploratory behaviour, between the semi-arid and grassland species of *Rhabdomys* is likely to be a consequence of selection for different phenotypes in the different habitat types they occupy. Nonetheless, individuals in a population can change their behaviour in response to prevailing environmental conditions, demonstrating plasticity (Atwell et al. 2012). Thus, we also aimed to establish the norm of reaction of exploratory behaviour as a consequence of experiences during the early postnatal environment; our study is one of first to investigate how exploratory behaviour and personality is modulated by the interaction between the genotype and experience during the early life. For this, we cross-fostered young between the species during early life. Because young striped mice learn some behaviour from their parents (e.g. food preferences, Rymer et al. 2008; paternal care, Rymer and Pillay 2011), we predicted that cross-fostered young would show the same exploratory behaviour and anxiety responses as their foster parents, indicating that the environment shapes these behaviours (i.e. the behaviour is acquired by learning).

Methods

Subjects

Founder species of striped mice were live-trapped in a grassland (*R. d. chakae*; 20 males, 20 females; Alice, Eastern Cape Province, S 32 ∟ 48; E 26 ∟ 52) and Succulent Karoo (*R. pumilio*; 20 males, 20 females; Goegap Nature Reserve, Northern Cape Province, S 29°41.56; E 18°1.69) locality and housed in the Milner Park Animal Unit, University of the Witwatersrand, under partially controlled environmental conditions (14 L: 10 D cycle, lights on at 05 h 00; 20–24 °C; 30–60 % relative humidity).

Breeding pairs were established using wild caught adults that were randomly paired using individuals trapped at least 200 m apart to reduce the chances of previous encounters and inbreeding (see Kinahan and Pillay 2008). Breeding pairs were housed in glass tanks (46 x 30 x 32 cm) and the floor of each tank was covered with coarse wood shavings for bedding. A plastic nest box (13 x 9 x 10 cm) and hay were provided for nesting. Each pair received Epol^{TM} mouse cubes and water ad libitum, as well as approximately 5 g of mixed seeds (parrot food) twice a week, spread around the cage to stimulate foraging behaviour. Cages were cleaned weekly.

Experiment 1: Species differences

Offspring from the second litters (F1) of 40 wild caught breeding pairs (20 per species) were used to assess baseline levels of behaviour for each species (offspring from first litters were used in cross-fostering experiments - Experiment 2 below). We used the second litter of pairs to account for mating and parental care experience. At 5 days of age, all young were marked at the base of the tail with permanent non-toxic black hair dye for later identification. Adults were similarly marked on the back to remove the potential confounding effects of marking on behaviour and to reduce the risk of pup rejection. In all experiments, young were raised by both parents since male striped mice also care for their offspring (Schradin and Pillay 2003). After weaning at 20 days of age, two randomly selected offspring (one male and one female) were housed individually in plastic holding cages (25 x 12 x 20 cm) until use in experiments. The remaining offspring from each litter were returned to the breeding colony for use in other experiments.

Test protocols

We used two protocols - open field and plus maze tests - that we have used on striped mice previously (see Jones et al. 2011). These complementary protocols both test unconditioned, and hence generalized, behavioural expression, and can detect species-specific behavioural responses (Ohl 2003). We measured exploratory behaviour in both tests (as described below), which was used to infer anxiety: more exploratory equals less anxious (Prior et al. 2004).

Test 1: Open-field

The open-field test is a standard laboratory test used to measure exploratory behaviour and anxiety in laboratory rodents (Walsh and Cummins 1976), where increased time spent exploring the centre of the open-field is interpreted as reduced anxiety (Carola et al. 2002), whereas thigmotaxis (movement hugging the arena walls; Matynia et al. 2010) and inactivity (including freezing, sitting or lying with no visible body movements; Walsh and Cummins 1976) indicate high anxiety. Measuring exploratory behaviour in an open-field can then be used to assess personality type. For example, ''fast'' great tits *Parus major* are bold and proactive, actively exploring their environment, in contrast to ''slow'' great tits that are shy and reactive (Verbeek et al. 1994). Using siblings (1 male, 1 female) from 10 litters ($n = 20$ per species), behavioural observations were made twice per individual to account for differences due to behavioural development: first when they reached 30–35 days old (juveniles) and again when they reached 55–60 days (sub-adult stage; Brooks 1982). Between 07 h 00 and 12 h 00, which is the peak period of striped mice activity, one individual was placed in a clean glass tank (open-field) with a ±2 cm layer of coarse wood shavings and allowed to acclimate for 5 min. Thereafter, its behaviour was video-recorded for 15 min under white light. Four behaviours were measured and

generally categorised as either exploratory (walking or digging the wood shavings in the centre of the arena) or inactive (sitting or lying with no visible body movements, which always occurred in a corner of the arena); thigmotaxis was rare (<2 %) of observations and was not considered in analyses. Scoring of behaviour was done by NP who was blind to the species of test subjects. After each test, the open-field was thoroughly cleaned with soapy water and 70 % ethanol and left to air dry.

Test 2: Plus maze

The plus maze, like the open-field test, is a standard laboratory test used to measure exploratory behaviour and activity, which are then used to assess anxiety in rodents, where greater activity/exploration of open arms of the maze is interpreted as reduced anxiety (Carola et al. 2002). We studied the behaviour of male and female striped mouse siblings ($n = 20$ per species) in a modified plus maze made from transparent Perspex (see below); these individuals were not used in the open-field tests and were tested as sub-adults (60–65 days old); we did not test juveniles because of the absence of an age effect in open-field tests (see "Results" section).

Unlike standard plus mazes, the modified plus maze is completely enclosed, as striped mice readily jump out of the apparatus. The maze comprised of four arms: two dark and two light arms, each measuring 50 x 8 x 9 cm, which radiated from a central introduction chamber (12 x 12 x 12 cm) with an exterior cylindrical holding tube (10 x 7 cm). Between 07 h 00 and 12 h 00, one individual was placed into the central introduction chamber of the maze (using the holding tube) 5 min prior to testing to reduce the effects of novelty. Thereafter, duration of time (s) spent in, and number of visits to (frequency), the light arms was video recorded for 15 min under white

light. Scoring was done by NP who was blind to the species of test subjects. After each test, the maze was thoroughly cleaned with soapy water and 70 % ethanol and left to air dry.

Experiment 2: Cross-fostering

The first litter of wild caught parents (described above) were outbred at 100 days of age with other same-species F1 individuals. They raised two litters under the same conditions described previously and their second (F2) litters (to control for prior mating and caring experience) were used in a cross-fostering experiment. Cross-fostering in *Rhabdomys* is possible because they have synchronous births; cross-fostering must occur before 10 days of age for pups to be accepted by foster parents and fostering does not impair growth and development in the taxon (Pillay 2000b; Schwaibold and Pillay 2001). We used 40 litters (20 per species) that produced 5 or more pups (mean ± SE: 6.7 ± 2.1) and which had 2 males and 2 females per litter.

Fostering occurred when pups were at least 2–4 days of age and between litters that were born no more than 2 days apart. When two breeding pairs (one from each species) produced litters during this time frame, two offspring (one male and one female) from each litter were randomly selected, marked between the ears with permanent non-toxic black hair dye and cross-fostered to the other species litter (i.e. litters donated and received 2 pups in the inter-species fostering). The remainder of the offspring remained with their biological parents. Both adults and non-fostered young were also marked (adults on the back, young above the base of the tail) with dye. After weaning at 20 days of age, both fostered offspring and two randomly selected biological offspring (one male and one female) were housed individually in plastic holding cages under similar conditions to their parents. Their behaviour in the open-field and modified plus maze was analysed later when they were juveniles (open-field) and sub-adults (open-field and

plus maze), as described above. Again, siblings were tested in each test. We tested 10 individuals of each sex per species per treatment (fostered and non-fostered).

Experiment 3: Control-fostering

To control for the effects of the cross-fostering procedure, we cross-fostered pups between breeding pairs of the same species (i.e. intra-species fostering). Twenty litters (10 per species; mean litter size 6.2 ± 1.2) produced by F1 parents were used. We followed the same protocol as for the cross-fostering experiment (i.e. second litters, marking of individuals, and selection of test subjects). We scored the behaviour of 10 individuals per species, sex and treatment combination in the open-field and modified plus maze of control-fostered and two randomly selected biological offspring (one male and one female) at the juvenile (open-field) and sub-adult stages (open-field and plus maze), as described above.

Ethical Note

The experimental procedures used here have been used in numerous studies on other species and had no obvious negative effects on the welfare of striped mice. After tests, all animals were returned to the captive striped mouse colony and used in other breeding experiments. This study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Animal ethics clearance no. 99/26/1) and complied with the current laws and regulations in South Africa.

Statistical analysis

We used Statistica 7.1 (Statsoft Inc, www.statsoft.com) for all analyses. All data sets met the assumptions of normality (Shapiro-Wilk's test) and homogeneity of variances (Levene's test), apart from the number of entries into the open arms of the modified plus maze (frequency), which was square root transformed prior to analyses. For all three experiments, we first analysed the data with the variance components analysis using the Restricted Maximum Likelihood method to assess the effects of breeding pair identity (random factor: considered because pairs donated or received pups in the cross-fostering experiments), litter identity (random factor: considered because two biological and/or two fostered subjects per litter were used), age at fostering and litter size (both covariates) on behaviours in the open-field (explore, inactive) and modified plus maze (time spent in, and number of entries into, light arms). For all tests, breeding pair identity, litter identity and litter size were not significant predictors of behaviour ($P > 0.05$). Therefore, they were not considered in further analyses.

For the open-field tests, data were analysed using General Linear Models (GLM) with repeated measures, multivariate design. Species, sex and whether or not offspring were fostered (if applicable) were the categorical predictors, the two behaviours (explore and inactive) were the dependent variables, and the age categories (juvenile and sub-adult) were the repeated measures variables (to assess changes in behaviour over time). For the plus maze tests, data were analysed using GLM, where species, sex and whether or not offspring were fostered (if applicable) were the categorical predictors, and duration (time spent in the open arms) or frequency (entries into the open arms) was the dependent variable. For all analyses, specific differences were identified using Fisher's LSD post hoc tests. All tests were two-tailed and the model-level significance was determined at $\alpha = 0.05$.

Results

Experiment 1: Species differences

Species was a significant predictor of time spent exploring and time spent inactive in the open-field (Table 1; Fig. 1). *R. pumilio* spent more time exploring the centre of the open-field than *R. d. chakae* (Fig. 1). Sex and age at testing (juvenile and sub-adult) were not significant predictors of behaviour in the open-field (Table 1). Furthermore, none of the interactions (species x sex; species x age; age x sex) were significant predictors of behaviour in the open-field (Table 1).

The behavioural responses seen in the modified plus maze were similar to those of the open-field. *R. pumilio* spent significantly more time in (Table 1; Fig. 2), and made significantly more entries into (Table 2), the open arms of the plus maze than *R. d. chakae*. We found no sex or species x sex interaction (Tables 1 and 2).

Experiment 2: Cross-fostering

Species significantly predicted the behaviour of test subjects in the open-field when offspring were cross-fostered from one species to another (Table 1). As in Experiment 1, *R. d. chakae* showed less exploratory behaviour and a higher level of inactivity than *R. pumilio* (Fig. 3). In contrast, fostering (i.e. whether test subjects were raised by their biological mother or a foster mother), did not predict behaviour of striped mice (Table 1). However, the interaction between species and fostering did significantly influence behaviour (Table 1), with fostered offspring occupying a position intermediate (fostered *R. pumilio* = lower explore, higher inactivity; fostered *R. d. chakae* = higher explore, lower inactivity) between non-fostered offspring of both

taxa (Fig. 3). Sex, age at testing and the interactions (species x sex, species x age, age x sex, age x fostering) did not influence behaviour (Table 1).

Similar to Experiment 1, *R. pumilio* spent more time in, and made more visits to, the open arms of the modified plus maze than *R. d. chakae* (Tables 1, 2; Fig. 4). However, species x fostering significantly influenced the duration of time spent in the open arms, with the fostered offspring from both species occupying an intermediate position between their respective non-fostered siblings (Fig. 4). We found no effect of sex, fostering or the interaction between species and sex for either time spent in, or visits made to, the open arms (Tables 1, 2).

Experiment 3: Control-fostering

Unlike cross-fostered striped mice, individuals fostered within the same species were not affected by the fostering procedure. As in Experiment 1, there was a species effect with *R. pumilio* showing greater levels of exploratory behaviour and *R. d. chakae* showing higher levels of inactive behaviour (Table 1; Fig. 5). Sex, fostering, age at testing, species x sex, species x fostering, species x age, age x sex and age x fostering all did not influence behaviour in the open-field (Table 1).

Similarly, fostering within the same species did not alter use of the modified plus maze (Tables 1, 2; Fig. 6). Again, there was a species effect, with *R. pumilio* spending more time in, and making more entries into, the open arms of the plus maze than *R. d. chakae*. There were no sex, foster, species x sex or species x foster effects (Tables 1 and 2).

Discussion

Striped mice show geographical differences in sociality (Schradin and Pillay 2005). Here, we

showed geographic variation in exploratory behaviour, reflecting underlying differences in

anxiety and indicating that these sister species are locally adapted to their respective habitats.

Our study corroborates preliminary observations (Rymer et al. 2008) that semi-arid occurring *R.*

pumilio has a bolder personality type (defined as an showing the same behavioural

response/willingness to take a risk in response to different situations or over a period of time;

Wilson and Godin 2009); Couchoux and Cresswell (2012), in captivity than *R. d. chakae*,

displaying higher levels of exploratory behaviour in the centre of the arena (open field) or greater

use of the open arms (plus maze), both indicating lower levels of anxiety.

Differences in exploratory behaviour between the striped mouse species are possibly related

to the spatial and temporal availability of resources and the level of exposure during foraging.

The Succulent Karoo has a stable annual food supply, although nutrient-rich food resources (i.e.

wild flowers, newly-emerged plant material and insects) are transient and unpredictable

(Schradin 2005). Concomitantly, cover, in the form bushes of the dominant plant species

Zygophyllum retrofractum, is also patchy and there are large open sandy patches in between

bushes (Schradin and Pillay 2004). Thus, while rodents occurring in areas with low vegetation

cover are expected to reduce activity to minimize predation risk (e.g. house mice, Jensen et al.

2003), *R. pumilio* forages alone and under reduced cover to increase its encounter rate with

patchily distributed protein-rich foods (Schradin 2007). This appears to have selected for a

bolder personality phenotype that is less anxious in open spaces. In contrast, the ground in the

grasslands is generally completely covered by vegetation (grasses and herbs, Schradin 2005).

However, although *R. d. chakae* has access to cover when foraging, it does not eat grass, and the

primary source of its diet (i.e. seeds, berries and herbs; Curtis and Perrin 1979; Perrin 1980) is not stable but is scarce and patchily distributed (Schradin 2005). *R. d. chakae* shows greater anxiety in open areas, perhaps reflecting an evolutionary response to greater predation risk or environmental complexity, as suggested for parrots (Mettke-Hoffman et al. 2002).

Fostered young did not retain the behavioural phenotype of their biological parents. This indicates that the levels of the anxiety, as revealed by exploratory behaviour in the test apparatuses, are not genetically fixed, however, and are influenced by environmental factors during early development, as also seen in collared *Dicrstonyx groenlandicus* and brown lemmings *Lemmus trimucronatus* (Huck and Banks 1980a, b). Numerous studies have shown that behavioural development, particularly the development of the anxiety response and investigation of novelty, is mediated during the early rearing period by the quality and quantity of maternal care received (Liu et al. 1997; Meaney 2001; Curley et al. 2011), and our results indicate the importance of the early environment in shaping these behaviours. Contrary to expectations, however, the behaviour of offspring cross-fostered between the species did not precisely match the behaviour of their foster siblings, instead showing an intermediate response between that of their foster and biological siblings. The process of cross-fostering did not appear to influence the development of exploratory behaviour because within species fostering had no influence on the behaviour of fostered offspring. Moreover, the expression of exploratory behaviour in striped mice does not appear to be eroded over time because both juveniles and sub-adults retained the behaviour acquired in the fostered nest. However, it is possible that a rapid shift in environmental conditions in adulthood could trigger associated changes in hormonal response and, consequently, exploratory behaviour (e.g. dark-eyed juncos *Junco hyemalis*; Atwell et al. 2012). This is in contrast to studies of other complex behaviours, such as song

learning in birds, where individuals may learn the song type of their foster parents, but revert to the song type of their biological parents later (e.g. song *Melospiza melodia* and swamp *M. georgiana* sparrows, Nowicki and Marler 1988).

Cross-fostering is a valuable experimental technique to assess whether behaviour has a strong underlying genetic basis (Drickamer and Vessey 1986) or if there is a degree of flexibility or plasticity modulated by the social and physical environment. If genetically related animals are raised under different circumstances and environments, yet still show similar a behaviour pattern, the assumption is that the behaviour is primarily genetically determined and will remain relatively fixed (i.e. less plastic) regardless of environmental fluctuations (Huck and Banks 1980a; Drickamer and Vessey 1986; Bize et al. 2012). However, the significant changes in behaviour following fostering indicate that the behaviour pattern is plastic, and less under genetic control, and can change in response to the social and/or physical environment (i.e. the individual learns the behaviour and/or the behaviour is influenced by parental effects, as seen in house mice *M. musculus*; Penn and Potts 1998). In this context, the intermediate expression of exploratory behaviour (anxiety) in our study indicates, firstly, that the expression of these behaviours are under the combined influences of the early rearing environment in the foster nest and genes (species differences), and secondly, both species display a degree of phenotypic plasticity.

During the early postnatal period, offspring are subjected to numerous novel stimuli (e.g. the mother, Mousseau and Fox 1998; litter mates, Laviola and Alleva 1995; ambient conditions, Drickamer and Vessey 1986) that combine to modify their behavioural development. For example, mothers primarily influence the development and later expression of paternal care behaviour in striped mice during early development (Rymer and Pillay 2011). Furthermore, within a litter, transmission of behaviour (e.g. learning about novel foods from parents in young

striped mice, Rymer et al. 2008) is a major contributor to the early development of individual

behaviour (Coussi-Korbel and Fragaszy 1995; Previde and Poli 1996). Therefore, social

dynamics within a litter should lead to behavioural homogeneity because nest mates engage in

activities simultaneously, Coussi-Korbel & Fragaszy 1995). The physical or environmental

stimuli experienced by cross fostered *Rhabdomys* was different to that experienced by their non

fostered biological siblings, which would explain why they differed behaviourally from their

siblings raised by their biological parents.

The interaction between an organism's genotype and the environment drives the strength of

natural selection (Odling-Smee et al. 2003). Since no single genotype is optimal in all

environments (Gillespie and Turelli 1989), and an individual's behaviour can be influenced by its

physical and social environment (Coussi-Korbel and Fragaszy 1995), the expression of

behaviour ultimately results from selection for the phenotype most suited to that environment.

Our data indicate that fostered individuals altered their responses in the direction of their foster

parents, partially overriding the behaviour patterns inherited from their biological parents.

Thomson et al. (2011) suggested that boldness (categorised as a complex behavioural

phenotype), which is associated with a particular coping style (Koolhaas et al. 2007), should be

correlated with an individual's physiological response to stress (i.e. its hormonal levels, in

particular, plasma cortisol). Personality differences can be explained partly by additive genetic

variation (van Oers et al. 2005). Moreover, personality can also be influenced by previous

experience and social interactions (Oosten et al. 2010). This interplay between intrinsic (genetic)

and extrinsic (environmental) factors may organise the development of personality. For example,

the prenatal environment can drive adult behaviour and anxiety in laboratory mice, indicating an

organisational effect on personality (Van den Hove et al. 2011). Furthermore, laboratory mice

displayed an intermediate form of anxiety-related behaviours between cross fostered mouse strains, suggesting than an additional ''trigger'' might be required to shift the behavioural phenotype (i.e. an activational effect; Van den Hove et al. 2011). Therefore, it is possible that the intermediate form of personality type, as revealed by exploratory behaviour, displayed by cross fostered *Rhabdomys* could be the result of an interaction between intrinsic factors, such as divergence in particular genes associated with the stress response (Yao and Denver 2007; Thomson et al. 2011) interacting with extrinsic pressures, such as rearing environment (Sundström et al. 2004). This corroborates well with previous findings in this taxon that a combination of organizational and activational effects influence the expression of behaviour (e.g. parental care behaviour; Rymer and Pillay 2011; Rymer and Pillay 2012).

Our study shows an underlying genetic basis selecting for different behavioural phenotypes for exploratory behaviour in open environments (i.e. a measure anxiety) in the two species of *Rhabdomys* (originating in semi-arid and grassland habitats). Nonetheless, exploratory behaviour, a measure of anxiety and personality, like other behavioural phenotypes (e.g. social, Brooks 1982; Schradin and Pillay 2004; parental care, Schradin and Pillay 2004; food preferences, Rymer et al. 2008) can be altered during early development by the parental environment. Such behavioural flexibility favours the establishment of environmentally-specific phenotypes that could have significant consequences for the successful exploitation of the diverse habitats occupied by *Rhabdomys*. However, the underlying genotype modulates the phenotype and constrains the limit of behavioural flexibility within this taxon.

Acknowledgments

References

Atwell JW, Cardoso GC, Whittaker DJ, Campbell-Nelson S, Robertson KW, Ketterson ED (2012) Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation. Behav Ecol 23: 237-241

Barash DP (1975) Ecology of paternal behavior in the hoary marmot (*Marmota caligata*): an evolutionary interpretation. J Mammal 56: 613-618

Bell AM (2005) Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). J Evol Biol 18: 464-473

Bize P, Diaz C, Lindström J (2012) Experimental evidence that adult antipredator behaviour is heritable and not influenced by behavioural copying in a wild bird. Proc Roy Soc Lond B 279: 1380-1388

Brooks PM (1982) Aspects of the reproduction, growth and development of the four-striped field mouse, *Rhabdomys pumilio* (Sparrman, 1784). Mammalia 46: 53-64

Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P (2002) Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav Brain Res 134: 49-57

Christensen B, Persson L (1993) Species-specific antipredatory behaviours: effects on prey choice in different habitats. Behav Ecol Sociobiol 32: 1-9

Couchoux C, Cresswell W (2012) Personality constraints versus flexible antipredator behaviors: how important is boldness in risk management of redshanks (*Tringa totanus*) foraging in a natural system. Behav Ecol 23: 290-301

Coussi-Korbel S, Fragaszy DM (1995). On the relation between social dynamics and social learning. Anim Behav 50: 1441-1453

Curley JP, Jensen CL, Mashoodh R, Champagne FA (2011) Social influences on neurobiology and behavior: epigenetic effects during development. Psychoneuroendocrinology 36: 352-371

Curtis BA, Perrin MR (1979) Food preferences of the vlei rat (*Otomys irroratus*) and the four-striped mouse (*Rhabdomys pumilio*). SA J Zool 14: 224-229

Dere E, Huston JP, De Souza Silva, MA (2005) Episodic-like memory in mice: simultaneous assessment of object, place and temporal order memory. Brain Res Prot 16: 10-19

Drickamer LC, Stuart J, (1984) Peromyscus: snow tracking and possible cues used for navigation. Am Midl Nat 111: 202-204

Drickamer LC, Vessey SH (1986) Control of behaviour. In: Drickamer LC, Vessey SH (ed) Animal Behaviour. Concepts, Processes and Methods. PWS Publishers, Boston. pp 89-110

Duckworth RA (2009) The role of behavior in evolution: a search for mechanism. Evol Ecol 23: 513-531

Ducroz J–F, Granjon L, Lombard M, Volobouev V (1999) Comparative chromosome analysis (R- and C-bands) of two South African murid species, *Lemniscomys rosalia* and *Rhabdomys pumilio* (Rodentia: Murinae). Cytogen Cell Gen 87: 69-84

Ghalambor CK, Martin TE (2002) Comparative manipulation of predation risk in incubating birds reveals variability in the plasticity of responses. Behav Ecol 13: 101-108

Gillespie JH, Turelli M (1989) Genotype-environment interactions and the maintenance of polygenic variation. Genetics 121: 129-138

Gosling LM, Atkinson NW, Collins SA, Roberts RJ, Walters RL (1996) Avoidance of scent-marked areas depends on the intruder's body size. Behaviour 133: 491-502

Heth G, Todrank J, Begall S, Koch R, Zilbiger Y, Nevo E, Braude SH, Burda H (2002) Odours underground: subterranean rodents may not forage "blindly". Behav Ecol Sociobiol 52: 53-58

Heyser CJ, Chemero A (2012) Novel object exploration in mice: not all objects are created equal. Behav Proc 89: 232-238

Huck UW, Banks EM (1980a) The effects of cross-fostering on the behaviour of two species of North American Lemmings, *Dicrostonyx groenlandicus* and *Lemmus trimucronatus*: I. Olfactory Preferences. Anim Behav 28: 1046-1052

Huck UW, Banks EM (1980b) The effects of cross-fostering on the behaviour of two species of North American Lemmings, *Dicrostonyx groenlandicus* and *Lemmus trimucronatus*: II. Sexual Behaviour. Anim Behav 28: 1053-1062

Hurst JL (1990) Urine marking in populations of wild house mice *Mus domesticus* Rutty. I. Communication between males. Anim Behav 40: 209-222

Jensen SP, Gray SJ, Hurst JL (2003) How does habitat structure affect activity and use of space among house mice? Anim Behav 66: 239-250

Jones M, Mason G, Pillay N (2011) Correlates of birth-origin effects on the development of stereotypic behaviour in striped mice, *Rhabdomys*. Anim Behav 82: 149-159

Kinahan AA, Pillay N (2008) Dominance status influences female reproductive strategy in a territorial African rodent *Rhabdomys pumilio*. Behav Ecol Sociobiol 62: 579-587

Koolhaas JM, De Boer SF, Buwalda B, Van Reenen CG (2007) Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. Brain Behav Evol 70: 218-226

Langley WM (1989) Grasshopper mouse's use of visual cues during a predatory attack. Behav Proc 19: 115-125

Laviola G, Alleva E (1995) Sibling effects on the behavior of infant mouse litters (*Mus domesticus*). J Comp Psychol 109: 68-75

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277: 1659-1662

Marín AI, Hernández L, Laundré JW (2003) Predation risk and food quantity in the selection of habitat by black-tailed jackrabbit (*Lepus californicus*): an optimal foraging approach. J Arid Environ 55: 101-110

Matynia A, Ng CH, Dansithong W, Chiang A, Silva AJ, Reddy S (2010) Muscleblind1, but Not Dmpk or Six5, contributes to a complex phenotype of muscular and motivational deficits in mouse models of myotonic dystrophy. PLoS One 5: e9857

Meaney MJ (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Ann Rev Neurosci 24: 1161-1192

Mettke-Hoffman C, Winkler H, Leisler B (2002) The significance of ecological factors for exploration and neophobia in parrots. Ethology 108: 249-272

Michel MJ (2009) Influence of multiple environments on tadpole phenotypes: plasticity, adaptation, and conservation implications. PhD dissertation, University of Notre Dame, Indiana, USA

Mousseau TA, Fox CW (1998) The adaptive significance of maternal effects. Trends Ecol Evol 13 403-407

Nachappa P, Margolies DC, Nechols JR, Morgan TJ (2010) Response of a complex foraging phenotype to artificial selection on its component traits. Evol Ecol 24: 631-655

Nowicki S, Marler P (1988) How do birds sing? Mus Percep 5: 391-426

Odling-Smee FJ, Laland KN, Feldman MW (2003) Niche construction: the neglected process in evolution. Princeton University Press, Princeton

Ohl F (2003) Testing for anxiety. Clin Neurosci Res 3: 233-238

Oosten JE, Magnhagen C, Hemelrijk CK (2010) Boldness by habituation and social interactions: a model. Behav Ecol Sociobiol 64: 793-802

Penn D, Potts W (1998) MHC-disassortative mating preferences reversed by cross-fostering. Proc Roy Soc Lond B 265: 1299-1306

Perrin MR (1980) The feeding habits of two co-existing rodents, *Rhabdomys pumilio* (Sparman, 1784) and *Otomoys irroratus* (Brants, 1827), in relation to rainfall and reproduction. Acta Oecol 1: 71-89

Pillay N (2000a) Female mate preference and reproductive isolation in populations of the striped mouse *Rhabdomys pumilio*. Behaviour 137: 1431-1441

Pillay N (2000b) Fostering in the African striped mouse: implications for kin recognition and dominance. Acta Theriol 45: 193-200

Previde EP, Poli MD (1996) Social learning in the golden hamster (*Mesocricetus auratus*). J Comp Psychol 110: 203-208

Prior H, Schwegler H, Marashi V, Sachser N (2004) Exploration, emotionality, and hippocampal mossy fibers in nonaggressive AB/Gat and congenic highly aggressive mice. Hippocampus 4: 135–140

Rambau RV, Robinson TJ, Stanyon R (2003). Molecular genetics of *Rhabdomys pumilio* subspecies boundaries: mtDNA phylogeography and karyotypic analysis by fluorescence in situ hybridization. Mol Phylogenet Evol 28: 564-575

Rymer TL, Pillay N (2011) The influence of the early rearing environment on the development of paternal care in African striped mice. Ethology 117: 284-293

Rymer TL, Pillay N (In press) Maternal care in the African striped mouse *Rhabdomys pumilio*: a behaviourally flexible phenotype that is modified by experience. Dev Psychobiol doi:10.1002/dev.21020

Rymer T, Schradin C, Pillay N (2008) Social transmission of information about novel food in two populations of the African striped mouse, *Rhabdomys pumilio*. Anim Behav 76: 1297-1304

Sambandan D, Carbobne MA, Anholt RRH, Mackay TFC (2008) Phenotypic plasticity and genotype by environment interaction for olfactory behavior in *Drosophila melanogaster*. Genetics 179: 1079-1088

Schradin C (2005) When to live alone and when to live in groups: ecological determinants of sociality in the African striped mouse (*Rhabdomys pumilio*, Sparrman, 1784). Belg J Zool 135: 77-82

Schradin C (2007) Information transfer about food locations is not a benefit of group-living in the solitary foraging striped mouse (*Rhabdomys pumilio*). J Ethol 25: 83-86

Schradin C, Pillay N (2003) Paternal care in the social and diurnal striped mouse (*Rhabdomys pumilio*): Laboratory and field evidence. J Comp Psychol 117: 317-324

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

Schradin C, Pillay N (2005) Intraspecific variation in the spatial and social organization of the African striped mouse. J Mammal 86:99-107

Schradin C, Kinahan AA, Pillay N (2009) Cooperative breeding in groups of synchronously mating females and evolution of large testes to avoid sperm depletion in African striped mice. Biol Repro 81: 111-117

Schwaibold U, Pillay N (2001) Stereotypic behaviour is genetically transmitted in the African striped mouse *Rhabdomys pumilio*. Appl Anim Behav Sci 74:273-280

Shillito EE (1963) Exploratory behaviour in the short-tailed vole, *Microtus agrestis*. Behaviour 21:145-154

Sundström LF, Petersson E, Höjesjö J, Johnsson JI, Järvi T (2004) Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance. Behav Ecol 15: 192-198

Taylor EB (1991) A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture 98: 185-207

Thomson JS, Watts PC, Pottinger TG, Sneddon LU (2011) Physiological and genetic correlates of boldness: characterising the mechanisms of behavioural variation in rainbow trout, *Oncorhynchus mykiss*. Horm Behav 59: 67-74

Tomlinson WT, Johnston TD (1991) Hamsters remember spatial information derived from olfactory cues. Anim Learn Behav 19: 185-190

Van den Hove DLA, Jakob SB, Schraut KG, Kennis G, Schmitt AG, Kneitz S, Scholz C-J, Wiescholleck V, Ortega G, Prickaerts J, Steinbusch H, Lesch K-P (2011) Differential effects of prenatal stress in 5-Htt deficient mice: towards molecular mechanisms of gene x environment interactions. PLoS One 6: e22715

van Oers K, de Jong G, van Noordwijk AJ, Kempenaers B, Drent PJ (2005) Contribution of genetics to the study of animal personalities: a review of case studies. Behaviour 142: 1191-1212

Verbeek MEM, Drent PJ, Wiepkema PR (1994). Consistent individual differences in early exploratory behaviour of male great tits. Anim Behav 48: 1113-1121

Walsh RN, Cummins RA (1976) The open field test: a critical review. Psychol Bull 83: 482-504

Wilson ADM, Godin JGJ (2009) Boldness and behavioral syndromes in the bluegill sunfish, *Lepomis macrochirus*. Behav Ecol 20: 231-237

Wilson ADM, Stevens ED (2005) Consistency in context-specific measures of shyness and boldness in rainbow trout, *Oncorhynchus mykiss*. Ethology 111: 849-862

Yao M, Denver RJ (2007) Regulation of vertebrate corticotropin-releasing factor genes. Gen Comp Endocrinol 153: 200-216

**Table 1** Results of GLM analyses for open-field and modified plus maze experiments

Experiment	Test	Species	Sex	Age	Foster	Spp x Sex	Spp x Age	Spp x Fos	Age x Sex	Age x Fos
Species differences	Open-field	$F_{2,35} = 23.71$; $P < 0.001$	$F_{2,35} = 0.22$; $P = 0.803$	$F_{2,35} = 0.19$; $P = 0.829$	-	$F_{2,35} = 0.44$; $P = 0.646$	$F_{2,35} = 1.87$; $P = 0.169$	-	$F_{2,35} = 0.78$; $P = 0.465$	-
	Plus maze	$F_{1,36} = 25.35$; $P < 0.001$	$F_{1,36} = 0.01$; $P = 0.911$	-	-	$F_{1,36} = 3.46$; $P = 0.071$	-	-	-	-
Cross-fostering	Open-field	$F_{2,73} = 53.43$; $P < 0.001$	$F_{2,73} = 2.09$; $P = 0.131$	$F_{2,73} = 0.48$; $P = 0.619$	$F_{2,73} = 2.87$; $P = 0.063$	$F_{2,73} = 1.95$; $P = 0.150$	$F_{2,73} = 1.74$; $P = 0.183$	$F_{2,73} = 42.26$; $P < 0.001$	$F_{2,73} = 0.23$; $P = 0.794$	$F_{2,73} = 2.56$; $P = 0.084$
	Plus maze	$F_{1,73} = 6.92$; $P = 0.010$	$F_{1,73} = 0.70$; $P = 0.407$	-	$F_{1,73} = 0.01$; $P = 0.931$	$F_{1,74} = 0.63$; $P = 0.429$	-	$F_{1,73} = 6.02$; $P = 0.02$	-	
Control-fostering	Open-field	$F_{2,73} = 67.70$; $P < 0.001$	$F_{2,73} = 0.01$; $P = 0.991$	$F_{2,73} = 1.05$; $P = 0.354$	$F_{2,73} = 1.71$; $P = 0.182$	$F_{2,73} = 0.02$; $P = 0.984$	$F_{2,73} = 1.27$; $P = 0.288$	$F_{2,73} = 2.27$; $P = 0.110$	$F_{2,73} = 0.33$; $P = 0.718$	$F_{2,73} = 1.03$; $P = 0.361$
	Plus maze	$F_{1,73} = 47.80$; $P < 0.001$	$F_{1,73} = 2.08$; $P = 0.154$	-	$F_{1,73} = 0.00$; $P = 0.982$	$F_{1,73} = 0.68$; $P = 0.413$	-	$F_{1,73} = 0.25$; $P = 0.620$	-	-

Results are presented for *first order* and *second order* effects where appropriate. Significant values are presented *in bold*. *Post hoc* analyses are reported in *text and figures*

Table 2 Mean (± SE) number of entries into the open arms of the modified plus maze

Experiment	Species	Sex/Foster	Number of entries	Statistics
Species differences	Grassland	Female	6.30 (0.98)a	**Spp: $F_{1,36} = 20.66; P < 0.001$**
		Male	5.10 (0.82)a	Sex: $F_{1,36} = 1.63; P = 0.210$
	Desert	Female	10.80 (0.87)b	Spp x Sex: $F_{1,36} = 0.00; P = 0.960$
		Male	9.50 (1.20)b	
Cross-foster	Grassland	Own	5.35 (0.45)a	**Spp: $F_{1,73} = 39.55; P < 0.001$**
		Foster	6.20 (0.85)a	Sex: $F_{1,73} = 0.00; P = 1.000$
	Desert	Own	11.15 (0.98)b	Fos: $F_{1,73} = 0.07; P = 0.792$
		Foster	9.90 (0.58)b	Spp x Sex: $F_{1,73} = 0.53; P = 0.469$
				Spp x Fos: $F_{1,73} = 1.93; P = 0.169$
Control-foster	Grassland	Own	5.35 (0.92)a	**Spp: $F_{1,73} = 18.78; P < 0.001$**
		Foster	4.30 (0.82)a	Sex: $F_{1,73} = 0.01; P = 0.944$
	Desert	Own	8.95 (1.23)b	Fos: $F_{1,73} = 0.01; P = 0.944$
		Foster	9.85 (1.14)b	Spp x Sex: $F_{1,73} = 0.85; P = 0.359$
				Spp x Fos: $F_{1,73} = 0.85; P = 0.359$

Data are presented for female/male and own/foster (Fos) for *R. dilectus chakae* and *R. pumilio* striped mice. Data for the sexes were pooled for the Cross-foster and Control-foster experiments because there was no sex effect. Statistics = GLM analyses; significant predictors are shown in *bold* and values in a row with *same alphabets* are not significantly different (*Post hoc* tests)
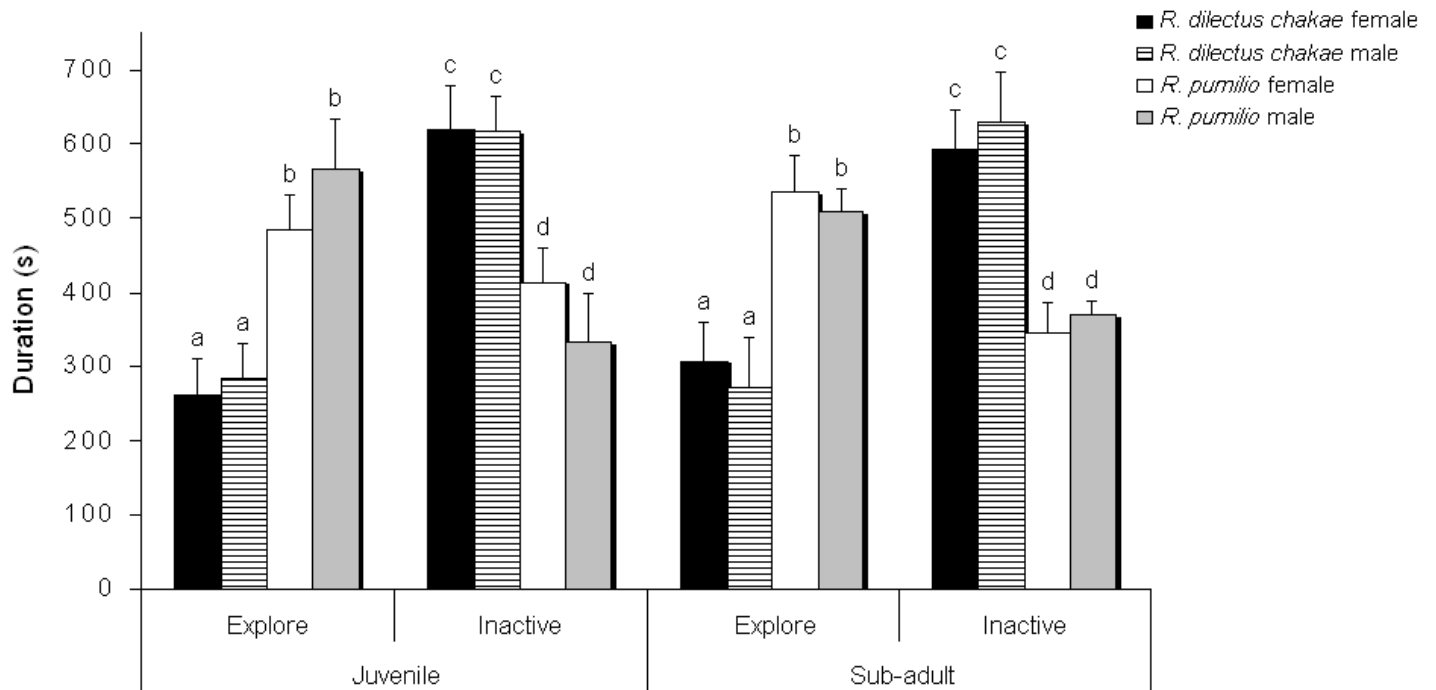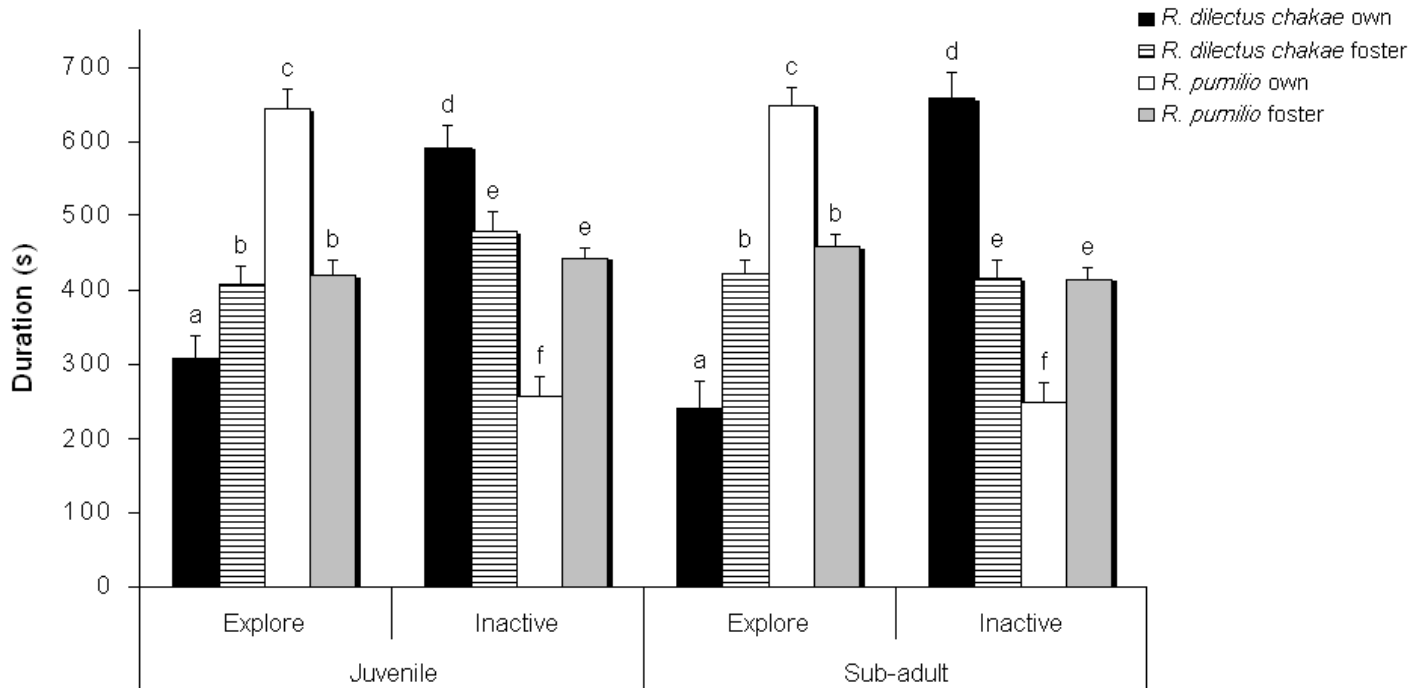
Fig 1. Mean (± SE) duration (s) of exploratory and inactive behaviour by *R. d. chakae* and *R. pumilio* female and male striped mice. Test subjects were tested as juveniles (30-35 days of age) and again as sub-adults (55-60 days of age). *Bars* with the *same alphabets* are not significantly different.
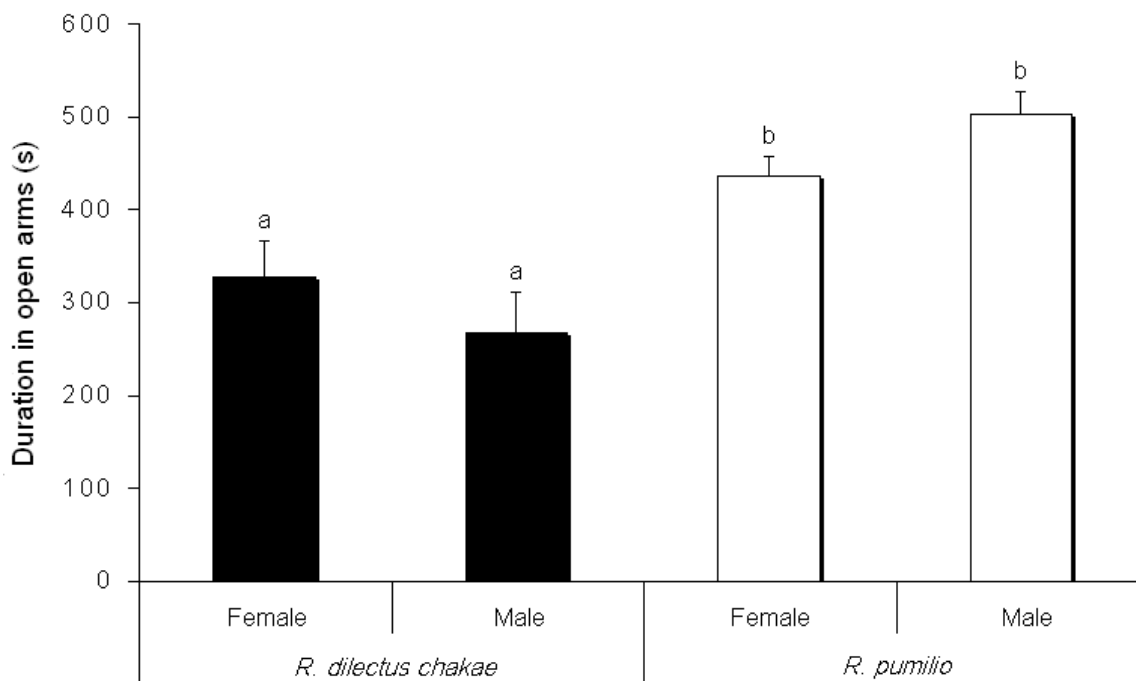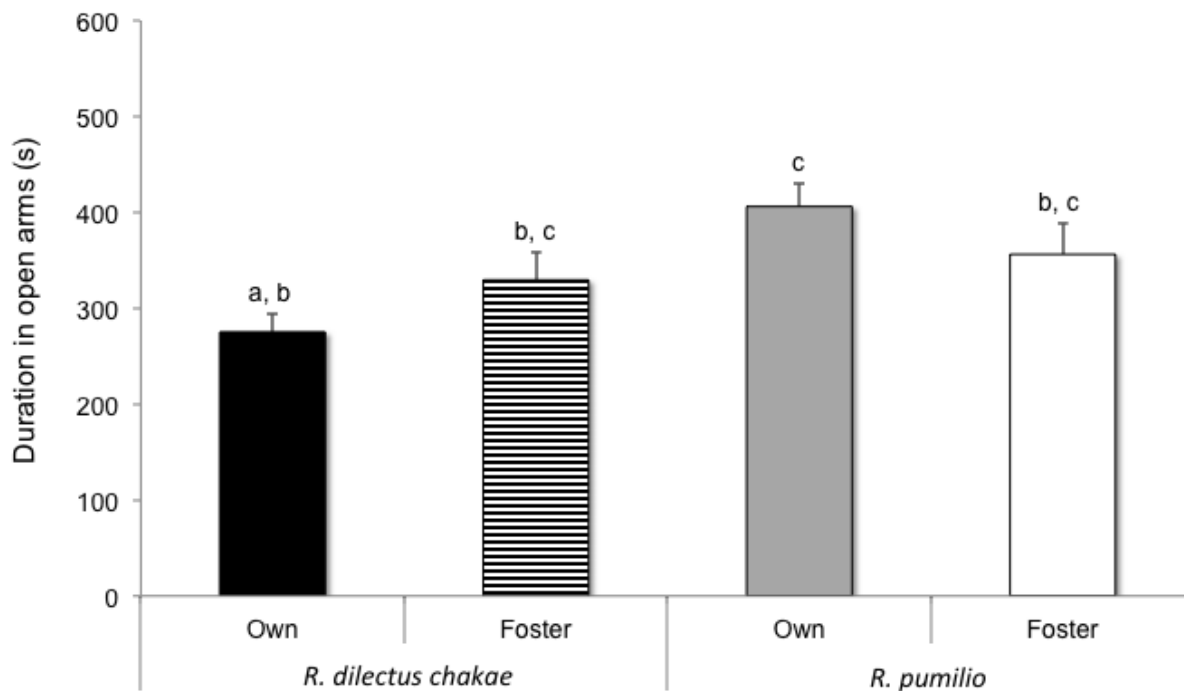
Fig 2. Mean (± SE) duration of time (s) spent in the open arms of the modified plus maze by *R. d. chakae* and *R. pumilio* female and male striped mice. *Bars* with the *same alphabets* are not significantly different.
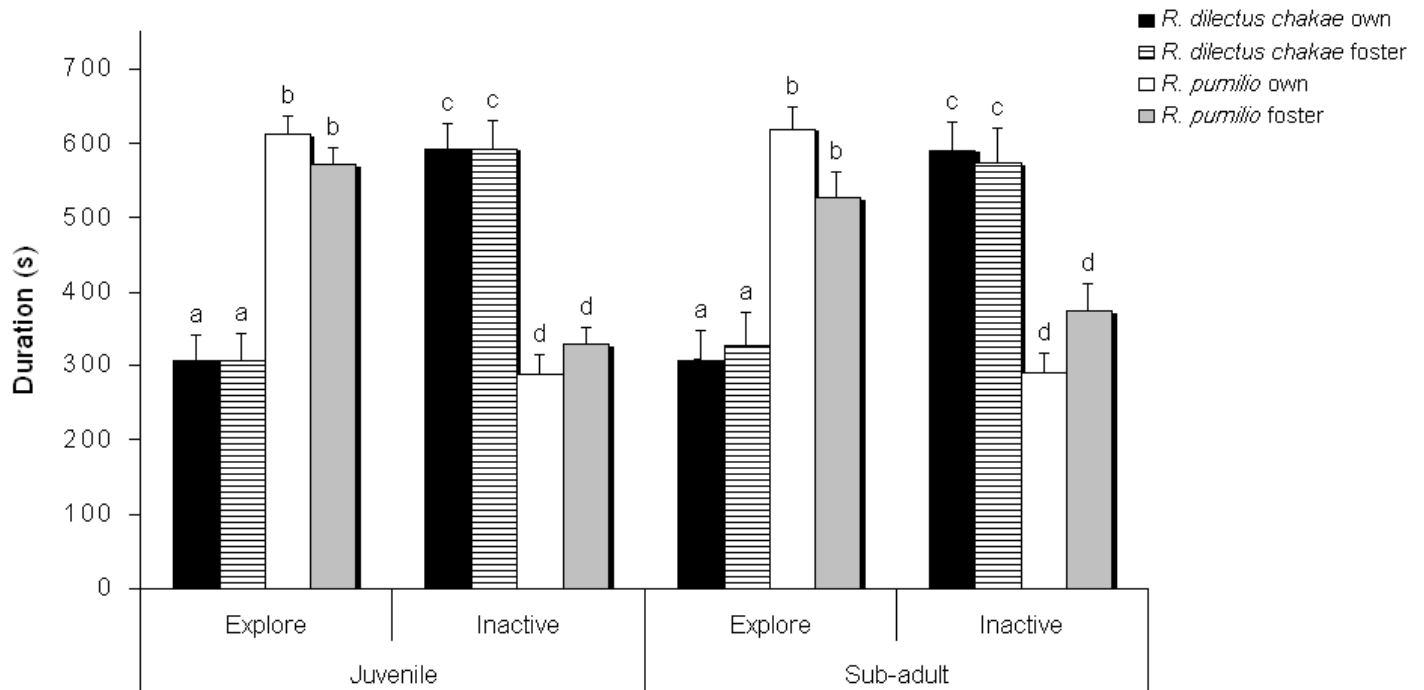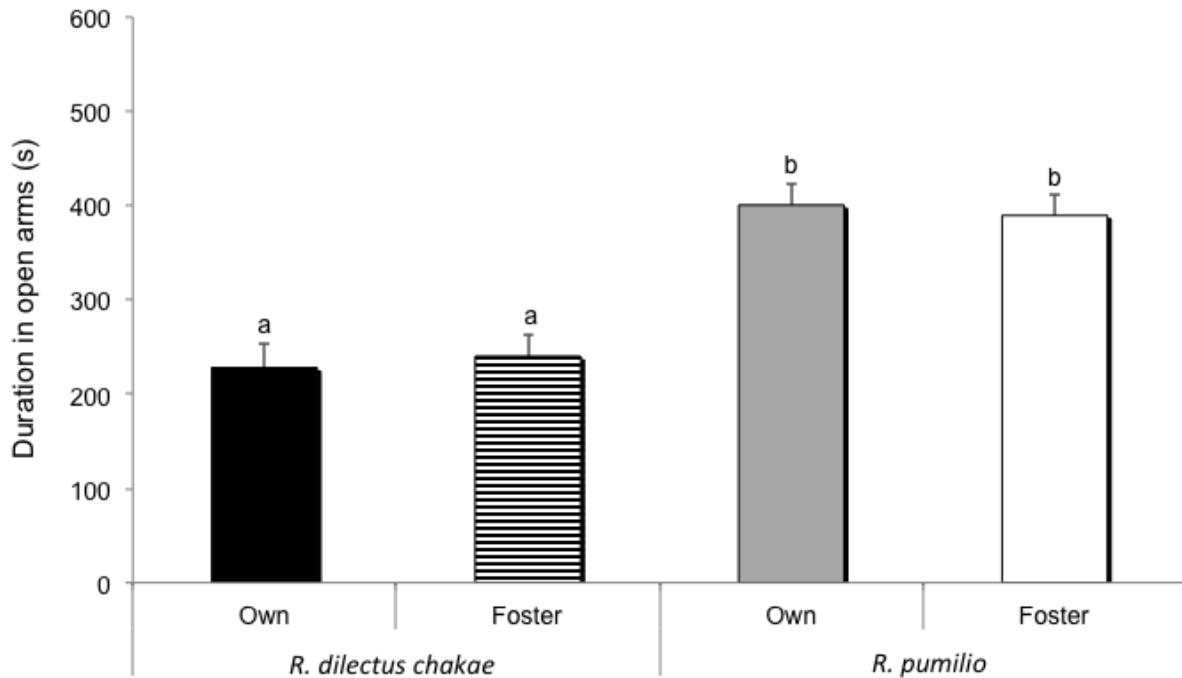
Fig 3. Mean (± SE) duration (s) of exploratory and inactive behaviour by *R. d. chakae* and *R. pumilio* striped mice that were raised by the biological mother (own) or different species foster mother (foster). Test subjects were tested as juveniles (30-35 days of age) and again as sub-adults (55-60 days of age). *Bars* with the *same alphabets* are not significantly different.

Fig 4. Mean (± SE) duration of time (s) spent in the open arms of the modified plus maze by *R. d. chakae* and *R. pumilio* striped mice that were raised by the biological mother (own) or different species foster mother (foster). *Bars* with the *same alphabets* are not-significantly different.

Fig 5. Mean (± SE) duration (s) of exploratory and inactive behaviour by *R. d. chakae* and *R. pumilio* striped mice that were raised by the biological mother (own) and same species foster mother (foster) – control fostering. Test subjects were tested as juveniles (30-35 days of age) and again as sub-adults (55-60 days of age). *Bars* with the *same alphabets* are not significantly different.

Fig 6. Mean (± SE) duration of time (s) spent in the open arms of the modified plus maze by *R. d. chakae* and *R. pumilio* striped mice that were raised by the biological mother (own) and same species foster mother (foster) – control fostering. *Bars* with the *same alphabets* are not-significantly different.