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Behavioural adaptive variation in the striped mouse *Rhabdomys*

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DECLARATION

I declare that this thesis, submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, is my own unaided work. It has not been submitted before for any degree or examination at any other University.

Megan Kirsten Mackay
30th day of May 2017

ABSTRACT

Under current and previous global climate change, environments are changing and have changed at a rapid rate. Species with the potential to undergo adaptive radiation are likely to survive environmental change. The genus *Rhabdomys* is widespread in southern Africa, occurring along the east-west rainfall gradient in South Africa. *Rhabdomys* may have undergone adaptive radiations in the past, which may have resulted in the current suite of species in various habitats of different aridity. Some *Rhabdomys* species also occur in sympatry in some locations in South Africa. The aim of my study was to investigate adaptive variation in *Rhabdomys* by studying the behaviour of 5 populations, representing 3 *Rhabdomys* species, across South Africa. Using selected taxa, my approach was, firstly, to describe variation in two traits, personality and spatial cogntion, well known for showing environmentally-linked (i.e. adaptive) variation. Secondly, I manipulated the development of exploratory and anxiety behaviour to assess the limits of the adaptive variation (i.e. test the nature of the reaction norm of the characters measured). I first established the taxon-level personality of 4 taxa (2 sympatric) in 5 standard behavioural tests. Generally, the semi-desert living *R. pumilio* was the boldest together, surprisingly, with *R. d. dilectus* occurring in grasslands of central South Africa, contradicting previously published results. Comparatively, *R. bechuanae* from central South Africa and *R. dilectus* from far north-eastern South Africa, also occurring in grasslands were less bold, even though *R. bechuanae* is sympatric with *R. dilectus* in central South Africa. My data indicate adaptive variation at the extreme populations and possibly character displacement in the sympatric populations. In the next chapter, I investigated whether early rearing environment shapes exploratory behaviour and anxiety responses of *R. pumilio* and *R. bechuanae*. I predicted that using an interspecies cross-fostering protocol would reveal a gene x environment interaction on behaviour, so that fostered offspring would display an intermediate behaviour phenotype compared to their non-fostered siblings. I showed that a novel rearing environment mostly did not influence the adult behaviour of cross-fostered inidividuals. This indicates genetic constraints on exploratory behaviour and anxiety responses. Next, I tested whether physical rearing environment shapes exploratory behaviour and anxiety responses. I reared semi-desert *R. pumilio*, sympatric *R. bechuanae* and *R. dilectus* and allopatric *R. bechuanae* under either no cover or high cover for 2 generations. The taxa were mostly similar and altering the phyical housing condition did not alter behaviour, but there were small differences between the taxa in exploratory behaviour. In the final experimental chapter, I established whether the environment predicts the spatial cognition in semi-desert *R. pumilio*, sympatric *R. bechuanae* and *R. dilectus* and an allopatric population of *R. dilectus* from far north-eastern South Africa. The populations showed very similar performance in a modified Barnes maze, indicating a possible phylogenetic constraint on spatial cognition. Overall, my study suggests that there is adaptive variation in personality but not spatial cognition. In contrast to previous studies in the genus, alterations to the social and physical environments failed to separate out genetic and environmental effects (i.e. reaction norm) that would potentially provide the mechanisms for adaptive variation within and between species. The similarity in spatial cognition between taxa and similar responses to environmental modification indicate phylogenetic constraints on traits that were predicted to vary geographically.

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To those who kept asking "When will you be finished?" or "How is it going?" You can stop now. It's finished.

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GENERAL INTRODUCTION

The phenotype of an animal is the result of complex interactions between its genes and the environment it occupies (Schlichting & Pigliucci 1998). Taylor (1991) proposed the term of local adaptation to describe intraspecific phenotypic variation depending on the different environmental conditions occupied by representative populations. For example, New Zealand trematodes *Microphallus* sp. are able to infect significantly more *Potamopyrgus antipodarum* snails from their own lake compared to *P. antipodarum* snails originating from different lakes (Lively 1989), indicating that trematodes have adapted to the snails from each lake, which also appear to be adapted to the conditions in the different lakes (Lively 1989).

Some genotypes are able to express multiple phenotypes in response to environmental variation, referred to as a reaction norm (Schlichting & Pigliucci 1998). For example, the squinting bush brown butterfly *Bicyclus anynana* lays different sized eggs, depending on the oviposition temperature (Fischer *et al.* 2003b) that result in better survival of offspring at the new oviposition temperature (Fischer *et al.* 2003a). The ability to respond to new environments has important consequences for species diversification (Losos 2010). For example, Hawaiian honeycreepers (subfamily Carduelinae) and Hawaiian thrushes (genus *Myadestes*) both colonized the Hawaiian islands at relatively the same time and were exposed to similar environments (Lovette *et al.* 2002). However, only the honeycreepers diversified in terms of their bill morphology and later speciated (Lovette *et al.* 2002). This suggests that the thrushes were not able to generate diverse phenotypes in response to an array of environmental conditions compared to the honeycreepers.

Adaptive radiation

The Hawaiian honeycreepers are one of the most well-known examples of adaptive radiation (Lovette *et al.* 2002), a process whereby a lineage diversifies into several different forms, each associated with particular ecological conditions (Schluter 1996; Givnish 1997; Schluter 2000; Glor 2010; Losos 2010; Losos & Mahler 2010). The diversification of descendants from a common ancestor is usually in response to some type of new ecological opportunity (niche), and the new ecological space acts as the selection pressure driving the diversification of descendant taxa (Schluter 1996; Glor 2010; Losos 2010; Losos & Mahler 2010). There are a number of ways that ecological space can become available, such as by the removal of a superior competitor for a new resource, the development of a trait that allows exploitation of a new resource, and/or the colonization of a new area (Losos 2010). Environmental change could also create new ecological space, but a species can only exploit such spaces if they have the ability to do so (Losos 2010), i.e. the species must have the potential to express multiple phenotypes under different environmental conditions (a broad reaction norm). With a change in ecological space, the species assemblage may change, where existing species may become inferior competitors for resources and may become locally extinct (Crombie 1947), whereas other, more competitive species, might thrive (Melville 2002).

Although many scientists have subtly different views on the exact definition of adaptive radiation (Losos 2010), most definitions have two common requirements (Glor 2010). 1) The number of descendent species with a common ancestor must increase. Givnish (1997) disagreed with this, arguing instead that adaptive radiation may not increase species number (e.g. pygopodid lizards; Webb & Shine 1994), and suggested that greater species richness should be tested rather than being an *a priori* condition for adaptive radiation. 2) The descendants must show an adaptation to the different environmental conditions (Glor 2010), which can be investigated using comparative population studies of different taxa, or through experimentation (Givnish 1997), where a trait is experimentally manipulated to show enhanced survival under particular ecological conditions (Givnish 1997). Both methods require extensive investigation, both in the laboratory and in nature. A comparative approach can reveal similar traits in similar environmental conditions of unrelated species (Arnold 1994) and, using phylogenetic analyses, can confirm species relatedness (Schluter 2000; Glor 2010; Weber & Agrawal 2012). However, Givnish (1997) suggested that a phylogenetic approach is not necessary – simply finding traits that are associated with the same ecological conditions in different species may be enough to suggest that the trait is adaptive.

Another widely debated criterion of adaptive radiation is that the descendant species must show extraordinary diversification (Glor 2010; Losos & Mahler 2010). This does not necessarily require a high species number, but rather that the descendant species have a greater degree of phenotypic diversity (disparity) than would be expected if populations had simply diverged over time (Losos & Mahler 2010) through, for example, genetic drift. For example, the group consisting of frogs and toads (order Anura) may not represent an exceptionally diverse group, since the species richness of the group is not significantly greater than would be expected given the age of the clade and the rate of diversification of other vertebrates (Alfaro *et al.* 2009).

Alternative theories of adaptive radiation

Glor (2010) provided an additional requirement for adaptive radiation – the descendant species or populations must have had access to the same opportunities as the parental species, without which, the descendant species would undergo species sorting or ecological speciation, which Glor (2010) argued are the other possibilities (alternatives) to adaptive radiation. The species sorting theory states that different species are adapted (i.e. have greater fitness) to different ecological niches (Leibold *et al.* 2004) and thus tend to be found in those niches. The idea of ecological speciation is similar to the species sorting theory – animals in different environments tend to develop adaptations associated with that environment (Schluter 2001; Losos & Mahler 2010). Arnold (1994) argued that the adaptive phenotype should only appear under new conditions. Similarly, according to Glor (2010), any pre-adaptations (or exaptations) would mean that simple species sorting had occurred, as species with a particular adaptation could be more likely to exploit the conditions for which they are adapted. However, many species have heterogeneous phenotypes (individuals have different phenotypes) in the same environment (e.g. male Verreaux's sifakas *Propithecus verreauxi verreauxi* either have brown stained fur around their sternal glands or clean fur; Lewis & van Schaik 2007) and without the ability to produce a variety of phenotypes, the potential for any radiation is limited or even unlikely (Glor 2010). Furthermore, diverse phenotypes may not necessarily result in an adaptation to a new environment (i.e. non-adaptive variation). Likewise, the process of ecological speciation in allopatric populations may be an adaptive radiation, though not all adaptive radiations occur through ecological speciation (for example, some radiations may happen through drift or character displacement; Schluter 2000; Losos & Mahler 2010).

The phenomenon of character displacement describes the situation of two closely related species occurring in sympatry and diverging in a manner that reduces competition, but when in allopatry, the species have similar character states (Brown & Wilson 1956). Examples of character displacement are relatively rare, but there are some documented examples, such as grebe (family Podicipedidae) beaks and prey types (Fjeldså 1983) and Appalachian salamander (genus *Plethodon*) head morphology (Adams 2004). In the *Plethodon* salamanders, competition interference drives character displacement in sympatric populations of *P. teyahalee* and *P. jordani*, since the head morphology of both species in allopatric populations is roughly similar (Adams 2004). Theoretically, character displacement has the potential to create an opportunity for adaptive radiation with the phenotypic divergence of closely related species in sympatry (Schluter 1996), but may also mask an adaptive divergence event between species that undergo multiple colonisations of one area from a different ancestral habitat.

Populations that have developed a variety of unique adaptations in response to environmental differences but that have not undergone speciation could be described as undergoing adaptive diversification (Glor 2010; Losos & Mahler 2010). For example, young brook charr *Salvelinus fontinalis* inhabiting different parts of streams have different foraging strategies within populations in response to environmental variation and different prey types (McLaughlin 2001). When individual brook charr that utilized different strategies occurred together, the strategies remained distinct, indicating that adaptive divergence had taken place (McLaughlin 2001). In many cases, adaptive diversification could be the precursor of adaptive radiations (Schluter 2000; Losos & Mahler 2010).

Behavioural adaptive radiation and personality

While morphological traits have been the focus of adaptive radiation (Johnson *et al.* 2009), there has been relatively little consideration of environmentally-linked behavioural adaptation (Rogers *et al.* 2002; Johnson *et al.* 2009). Behaviour may be one of the first traits to evolve under divergent selection pressure, compared to morphological traits, which tend to diversify relatively slowly (Rogers *et al.* 2002), since behaviour is generally more flexible than morphological traits (Price *et al.* 2003). Furthermore, many behaviours are closely linked to physiological or morphological traits (Martin 1972), and if physiological or morphological traits are under selection pressure, behaviour may also be altered in response to different selection pressures in a new environment. The behavioural phenotype is also under selection (Fox 1978; Dall *et al.* 2004; Dingemanse *et al.* 2007), which makes it surprising that comparatively few studies have focused on adaptive radiation or diversification of behaviour.

In order to study behaviour in the context of either adaptive radiation or adaptive diversification, one needs to show that the behaviour confers an advantage. While some behaviours clearly present advantages, such as flight-initiation distances in the Galápagos marine iguana *Amblyrhynchus cristatus* (Berger *et al.* 2007) or alarm calling and flocking behaviour in birds (reviewed in Lima & Dill 1990), personality traits may not present such clear advantages.

Personality, defined as consistent individual behavioural differences over different contexts (Gosling 2001; Sih *et al.* 2004) is a behavioural character that is usually associated non-randomly with particular ecological conditions (Dall *et al.* 2004). For example, individuals of a tropical poeciliid fish, the Panamanian bishop *Brachyraphis episcopi*, that were chased with nets daily (simulating high predation pressure), became bolder (i.e. took more risks and were less responsive to a high risk situation) than individuals that were left undisturbed (Brown *et al.* 2007). Studies of the fitness consequences of personality traits are not yet abundant (Brown *et al.* 2007, Smith & Blumstein 2008), and the findings are varied. Studies on poeciliid fish show that the bold-shy continuum (which considers an individual's responses to risky situations) is under direct selection pressure in the form of predation (Brown *et* *al.* 2005). In a meta-analysis on fitness consequences of personality, bolder individuals tended to have a higher reproductive output than shy individuals, but shy individuals lived longer (Smith & Blumstein 2008). Furthermore, the fitness of individuals with varying personality types strongly depended on the environmental context (Sih *et al.* 2004). Nevertheless, it may be possible to argue the advantage of intraspecific variation in personality between populations using the link between personality and physiology. Careau *et al.* (2009) and Biro and Stamps (2010) argued that the resting metabolic rate of an individual is tightly linked with personality. For example, more active deer mice *Peromyscus maniculatus* individuals had a higher metabolic rate (Chappell *et al.* 2004). In an environment with few or unpredictable resources, animals should explore more thoroughly (usually linked to a shy personality type) and have a lower basal metabolic rate to cope with these demands (Wolf *et al.* 2007; Careau *et al.* 2009), which ultimately is likely to affect fitness (Wolf & Weissing 2012).

Adaptive diversification and spatial cognition

Spatial cognition describes an array of mental representations of objects and the spatial relationship between the objects (Jacobs 2003). Spatial cognitive ability is under environmental selection pressure (Healy *et al.* 2009; Freas *et al.* 2012) and could therefore reflect adaptive diversification. The effect of environmental complexity and environmental demands on spatial cognition have been well documented in some animals. In the mole-rat *Cryptomys hottentotus natalensis*, wild-caught animals that originated from a spatially complex environment were better able to complete a navigation task than individuals reared in a spatially simple environment (du Toit *et al.* 2012b). Similarly, environments with different ecological demands (i.e. food and climate) also have an influence on spatial cognition. Black-capped chickadees *Poecile gambeli* living at high elevations (climatic extremes, lower food availability) in the Sierra Nevada Mountains performed better in spatial cognitive tasks, and had higher rates of hippocampal neurogenesis (which usually reflects better spatial working memory; Roth & Pravosudov 2009) than chickadees living at mid elevations (milder climate, higher food availability) approximately 10km away (Freas *et al.* 2012). Even in captivity, cognitive function is influenced by cage complexity (environmental enrichment; reviewed in Petrosini *et al.* 2009). Enriched housing with high spatial complexity improves spatial working memory in Wistar rats (Leggio *et al.* 2005). The relationship between spatial cognition and environmental conditions in nature and the malleability of spatial cognition shows that spatial cognition is a flexible trait depending on prevailing conditions, and possibly varies geographically.

Phenotypic plasticity and adaptive diversification

If environmental conditions change, organisms must either be able to shift their range to encompass areas where conditions are appropriate (Sol *et al.* 2005), or they require some degree of phenotypic plasticity (where one genotype has the potential to express multiple phenotypes in different environments; West-Eberhard 1989) in order to cope with different prevailing environmental conditions (Moran 1992; Mery & Burns 2010). According to Losos (2010), phenotypic plasticity is a requirement for adaptive radiation or diversification to occur, since it enables species to survive in different conditions. The selection pressure for particular phenotypes associated with the new environment may ultimately result in genetic assimilation (e.g. directional selection) of the new phenotype (Pigliucci *et al.* 2006).

In animals that are able to show plasticity, such plasticity can arise at different stages during an animal’s lifetime. Developmental plasticity describes the situation of an individual’s phenotype being set during its development (Fischer *et al.* 2003b) through organizational effects (Elekonich & Robinson 2000). In contrast, behavioural flexibility (i.e. a form of plasticity where individuals alter their behaviour in response to different environmental conditions; Gordon 1991) is reversible in an individual’s lifetime, since it is activated in response to different social or environmental conditions (Elekonich & Robinson 2000). Both behavioural flexibility and developmental plasticity could result in potential success in new environmental conditions and high variance of behaviour within and between populations.

The study species and motivation for the study

Rhabdomys

The African striped mouse, genus *Rhabdomys*, is a small, diurnal murid rodent that is distributed throughout southern Africa, occupying a variety of habitats (Skinner & Chimimba 2005). According to mitochondrial DNA (mtDNA) analyses, the genus was initially said to comprise two species, *R. pumilio* in the western and *R. dilectus* in the eastern parts of its geographic range. *R. dilectus* is further divided into two subspecies - *R. dilectus chakae* in the southern and *R. d. dilectus* in the northern parts of its range (Rambau *et al.* 2003). Following further genetic comparisons on *Rhabdomys* specimens from Namibia, Zimbabwe and South Africa, using mtDNA (Cytochrome Oxidase I) and nuclear interons Eef1a1, SPTBN1, MGF and Bfib7, du Toit (2012a) proposed that *R. pumilio* should be divided into three species. These were *R. pumilio* (southern and west coast regions of South Africa and Namibia), *R. intermedius* (central South Africa, mainly occurring in the Karoo) and *R. bechuanae* (central Namibia, central parts of the Northern Cape, North-West Province and Free State Province of South Africa; Figure 1.1A). The location from where the genus originated has been debated, with Rambau *et al*. (2003) proposing that the genus arose in the moist eastern grasslands and colonized the western semi-arid regions. More recently, du Toit *et al.* (2012a) proposed that *R. bechuanae* is the ancestral form of the genus, based on a phylogenetic tree and linkages (Figure 1.1B).

Genetic analyses of *Rhabdomys* populations occurring across South Africa have revealed that *R. bechuanae* also occurs in the grassland, syntopically with *R. dilectus*, in the Sandveld Nature Reserve in the Free State Province, South Africa (Ganem *et al.* 2012). The *R. bechuanae* form that occurs in the grassland should be more similar to *R. pumilio* based on genetic distances (du Toit *et al.* 2012a), but as yet, we do not know whether they differ phenotypically due to the different habitats that they occupy, the associated divergent selection pressures, and reduced gene flow over large distances (Figure 1.1A; approximately 800 km).

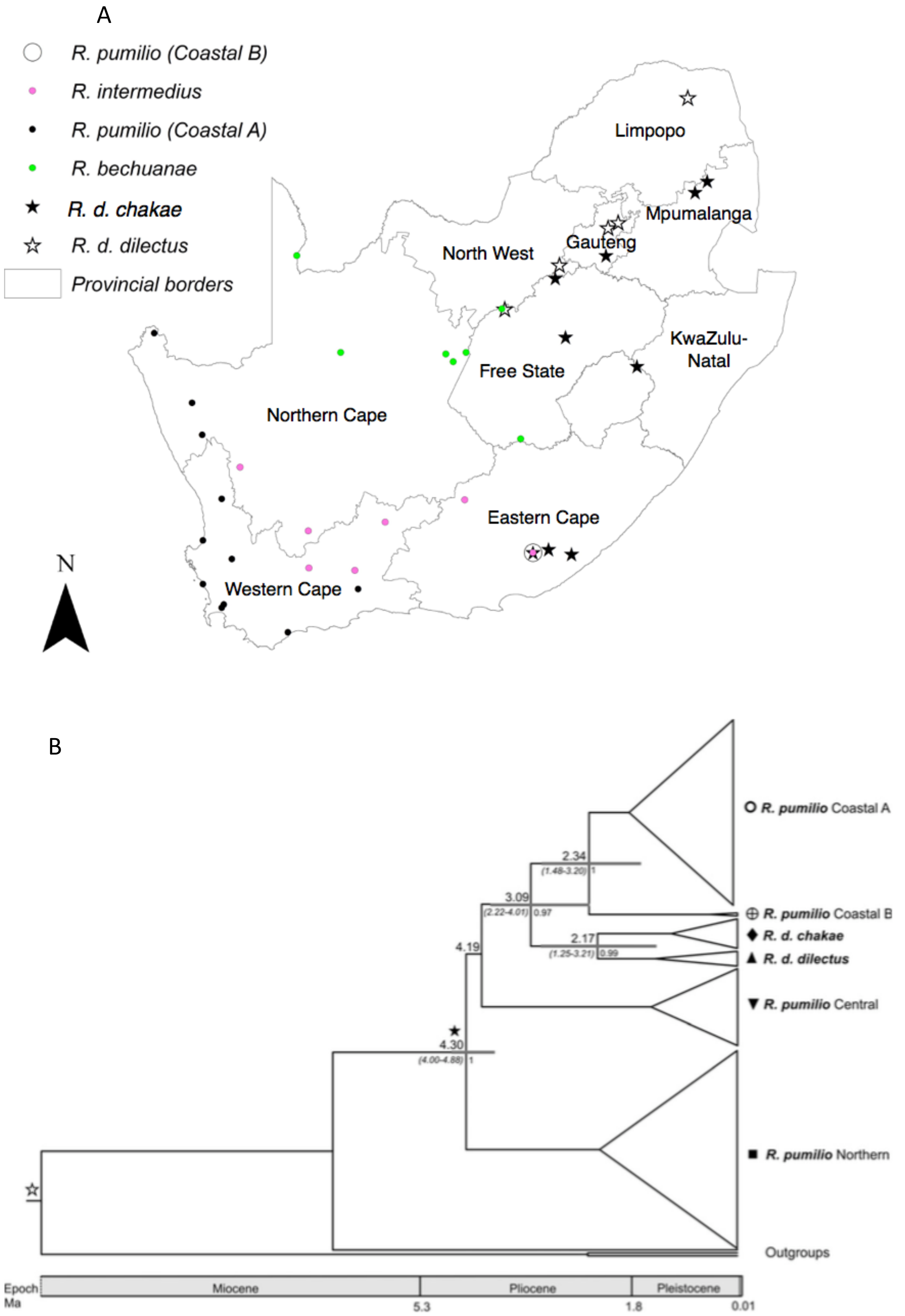

Figure 1.1A. Provincial map of South Africa, showing locations of genotyped *Rhabdomys* populations, courtesy of Candice Neves. Figure 1.1B. du Toit *et al*.'s (2012a) phylogeny of *Rhabdomys*, with permission from Professor Conrad Matthee. *R. pumilio* (Northern) corresponds to *R. bechuanae*, and *R. pumilio* (Coastal A) refers to the *R. pumilio* found at Goegap (see text).

My overall objective was to investigate adaptive variation and the mechanisms associated with behavioural and cognitive variation in a semi-arid population of *R. pumilio*, sympatric populations of *R. bechuanae* and *R. d. dilectus* and an allopatric population of *R. d. dilectus*. Only one population from each location was studied because only limited numbers of populations have been genotyped (Figure 1A) and therefore taxonomically known, and the situation of *R. bechuanae* living in sympatry with *R. dilectus* in the Sandveld Nature Reserve does not appear to occur elsewhere. However, the variation in characters among individuals within each population is assumed to be representative of the taxon in each locality.

Motivation for the study

The biology of *Rhabdomys* differs between the western, semi-arid-occurring *R. pumilio*, and the eastern, mesic-occurring *R. dilectus*. In the grasslands of South Africa, *R. dilectus* is solitary (Brooks 1974), and striped mice here maintain intra-sexually non-overlapping territories, although male territories overlap those of several females (Schradin & Pillay 2005b). In contrast, *R. pumilio* from the western semi-arid parts of South Africa (i.e. Goegap Nature Reserve; hereafter Goegap) is facultatively group-living, with groups comprising of 1-2 breeding males, 2-4 breeding females and several philopatric offspring (Schradin & Pillay 2004). However, males are more likely to become solitary when females nest alone (Schradin & Lindholm 2011), and females are more likely to nest alone when there is a greater availability of nesting sites (Schoepf *et al.* 2015) and when the females are heavier (Hill *et al.* 2015). In the regions where the genus has been studied, breeding starts in spring with the onset of the rainy season (Schradin & Pillay 2005a), but *R. dilectus* is able to continue breeding through the summer, while the breeding season for *R. pumilio* in Goegap only lasts 2-3 months due to the dry, hot summers in this region (Schradin & Pillay 2005b). Litter size ranges from 1-11 (mean of 6) are born in a single litter, and females have an inter-litter interval of approximately 30 days (Pillay 2000). Striped mice are short-lived, usually with a life-span of approximately 1 year in nature (Brooks 1974; Schradin & Pillay 2005a).

The behaviour of two *Rhabdomys* species has been relatively well studied. In a neutral laboratory environment, *R. pumilio* was less anxious and more explorative (i.e. bolder) than *R. dilectus*, which was more anxious and less exploratory (i.e. shy); the personality differences may reflect habitat differences of the species, such that the open spaces in the semi-desert selects for the bolder personality of *R. pumilio* (Rymer *et al.* 2008; Rymer & Pillay 2012). The differences are suggestive of adaptive variation (du Toit *et al.* 2012a; Ganem *et al.* 2012) or may even be the consequence of adaptive radiation, since different habitats can select for different personalities (Bókony *et al.* 2012).

That *R. bechuanae* occurs in the central grasslands of South Africa (Ganem *et al.* 2012) provides a unique opportunity to investigate adaptive variation in a genus with populations occurring under different environmental, and presumably, selection pressures. It would be important in this regard to establish the behaviour of putative ancestor *R. bechuanae*, and whether it behaves similarly to *R. dilectus* in sympatry (shy, high anxiety in open spaces; Rymer & Pillay 2012) or perhaps similar to that of *R. pumilio* from the semi-arid west. Likewise, environmental differences might also influence the spatial cognitive abilities of the species, because high spatial complexity has been shown to dramatically increase space use in house mice *Mus domesticus* (Jensen *et al.* 2003), and spatial working memory in Natal mole-rats *Cryptomys hottentotus natalensis* (du Toit *et al.* 2012b).

Dufour *et al.* (2015) found that *R. bechuanae* has larger home ranges than *R. d. dilectus* in sympatry compared to allopatric occurrences of these species, suggestive of character displacement. Differences in the behaviour between co-existing *R. bechuanae* and *R. dilectus* in the grasslands could be a consequence of character displacement, reducing competition in sympatry (Dufour 2014).

Social flexibility, a form of behavioural flexibility (plasticity) has been documented in *R. pumilio* in Goegap (Schradin *et al.* 2011). Overall, the environment determines social organisation in Goegap *R. pumilio* (Schradin *et al.* 2010a), and both female (Schradin *et al.* 2010a; Schradin *et al.* 2010b) and male striped mice (Schradin *et al.* 2009) change their reproductive tactic depending on prevailing environmental conditions, and male tactics are also dependent on female tactics (Schradin *et al.*

2012). When the population density is low (i.e. due to die off after a drought and insufficient food), heavier females (Hill *et al.* 2015) nest solitarily, exploiting the availability of nest sites and reducing reproductive competition present in communal nests (Schoepf & Schradin 2012). Males switch their reproductive tactic in response, such that when females are nesting solitarily, some males display a roaming tactic to solicit mating, but when females are nesting communally, males adopt a bourgeois (dominant) tactic and nest with a group of females (Schradin *et al.* 2010a). We do not know whether other *Rhabdomys* taxa show similar flexibility but such a potential might exist. Early development can result in changes in the behavioural phenotype through experience and/or learning. In support, Rymer and Pillay (2012) showed that the exploratory behaviour of *R. pumilio* pups that were fostered to *R. dilectus* mothers and *R. dilectus* pups that were fostered to *R. pumilio* mothers was altered by their rearing environment. The fostered pups did not behave like their non-fostered siblings but had a behaviour that was intermediate between *R. pumilio* and *R. dilectus*, indicating that *R. dilectus* and *R. pumilio* individuals develop flexible responses to prevailing conditions. The results of the fostering experiment also suggest that exploratory behaviour is organised in early life, and that the rearing environment modulates the genetic expression of exploratory behaviour in striped mice (Rymer & Pillay 2012).

Aims, objectives and hypotheses

In this thesis, I documented behavioural differences in personality and spatial cognition among 3 *Rhabdomys* species occurring in different biomes in South Africa, and then manipulated the early rearing environment to assess changes in behaviour. Goegap Nature Reserve (Figure 2), where *R. pumilio* occurs, is associated with open habitat, and there are wide spaces between bushes or clumps of grass in which *R. pumilio* nests (Schradin & Pillay 2004). Goegap has the lowest mean annual rainfall (160mm, mostly falling in winter; Schradin & Pillay 2005b). In contrast, the grasslands of Entabeni Forest Reserve (Figure 1.2) in northern South Africa, where *R. d. dilectus* occurs, have a high level of overhead grassy cover (Schradin & Pillay

2005b; Mostert *et al.* 2008) which *R. dilectus* prefers (Monadjem 1997; du Preez 1998; Fuller & Perrin 2001). Entabeni receives year round rainfall of approximately 1800mm (Mostert *et al.* 2008). The northern-central part of South Africa, where *R. bechuanae* and *R. d. dilectus* occur in sympatry, is classified as grassland/savanna (Mucina *et al.* 2006; Rutherford *et al.* 2006b), with overhead cover in the form of trees, shrubs and grass (Rutherford *et al.* 2006a), and intermediate rainfall (mainly in summer) of 500mm (Jankielsohn 2006). There is an overlap in the distributions of *R. bechuanae* and *R. d. dilectus* in the Sandveld Nature Reserve of the Free State Province, where the taxa occur in sympatry (Ganem *et al.* 2012). A second population (allopatric to the other taxa) of *R. bechuanae* from Tussen-die-Riviere Nature Reserve was studied in only one chapter, due to the small number of *R. bechuanae* individuals available for study in a specific locality.

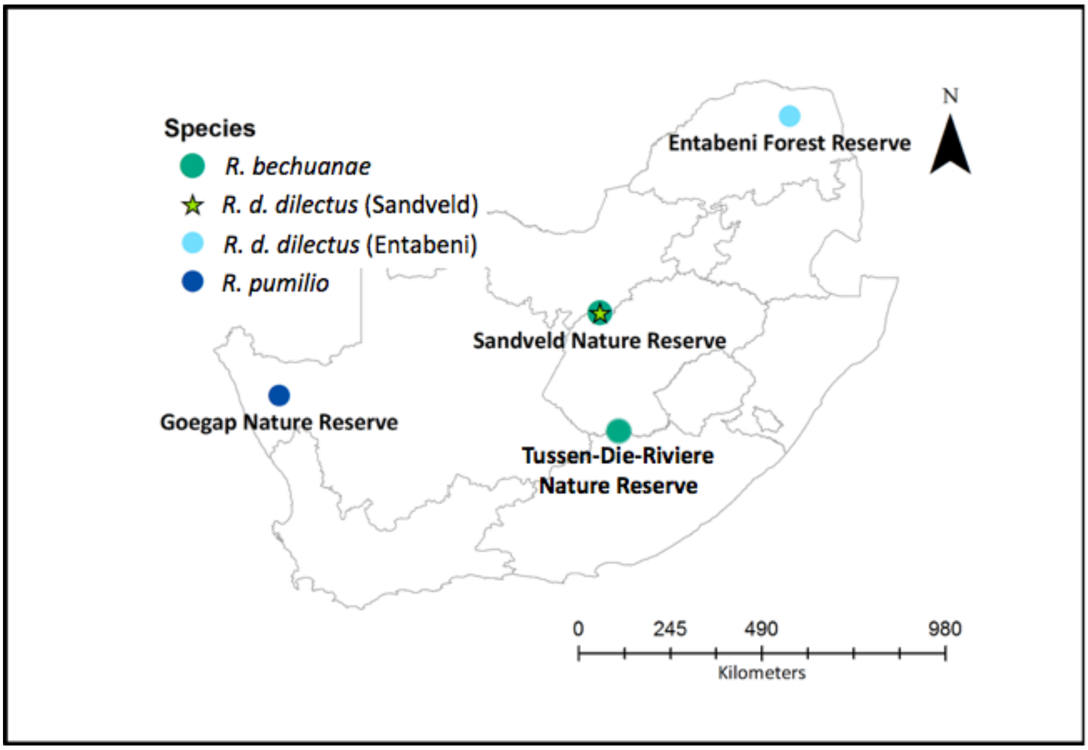

Figure 1.2. Map of South Africa, showing locations where the taxa selected for study originated. *Rhabdomys pumilio* originated from Goegap Nature Reserve (29° 41′ 33″ S, 18° 1′ 41″ E) in the Northern Cape Province, *R. bechuanae* and *R. d. dilectus* originated from the Sandveld Nature Reserve (27° 41′ 57″ S, 25° 44′ 13″ E) in the Free State Province. A second population of *R. bechuanae* originated from Tussen-Die-Riviere Nature Reserve (30° 28′ 4′′S 26° 9′ 31′′E), also in the Free State Province, and a second population of *R. d. dilectus* originated from the Entabeni Forest Reserve (22° 58′ 59″ S, 30° 16′ 56″ E) in the Limpopo Province.

I had two approaches to the study. I used a descriptive approach to record taxon-level differences in personality and spatial cognition. I also used a manipulative approach to assess whether adaptive variation in behaviour is fixed or if the behaviour can be modified by the environment. My first aim was to investigate whether and how environmental and geographic context influences the personality and the occurrence of behavioural syndromes in *R. pumilio*, sympatric *R. bechuanae* and *R. d. dilectus* from Sandveld, and an allopatric population of *R. d. dilectus*. I then compared the exploratory behaviour and anxiety response of *R. pumilio* and Sandveld *R. bechuanae* and tested whether altering their early social rearing environment can modulate these behaviours. Thirdly, I established whether different captive housing conditions influence the anxiety response and exploratory behaviour of *R. pumilio*, sympatric *R. bechuanae* and *R. d. dilectus*, and an allopatric population of *R. bechuanae*. Lastly, I investigated whether and how environmental and geographic context influences the spatial cognition ability of *R. pumilio*, sympatric *R. bechuanae* and *R. d. dilectus*, and an allopatric population of *R. d. dilectus*.

Outline of the thesis

All experimental chapters provide new information, test novel concepts and are intended for publication. Apart from the present chapter (main introduction), my thesis comprises 4 experimental chapters and a general discussion (Chapter 6). Chapter 2 (intended for Journal of Ethology) describes the personality and occurrence of behavioural syndromes in *R. pumilio*, sympatric *R. d. dilectus* and *R. bechuanae*, and an allopatric population of *R. d. dilectus*. Chapter 3 (intended for Behavior Genetics) considers the effect of the early social rearing environment on the development and later expression of anxiety response and exploratory behaviour in *R. pumilio* and *R. bechuanae*. Chapter 4 (intended for Developmental Neuroscience) is concerned with the effect of different captive housing conditions on the development and expression of exploratory behaviour and anxiety response in *R. pumilio*, sympatric *R. d. dilectus* and *R. bechuanae* and an allopatric population of *R. bechuanae*. Chapter 5 (intended for Animal Cognition) considers the spatial cognition ability of *R. pumilio*, sympatric *R. bechuanae* and *R. d. dilectus*, and an allopatric population of *R. d. dilectus.* Because of the format of the experimental chapters, there may be repetition of the introductory material, methodological details and/or discussion. Figures and tables are numbered in sequence for each chapter and not for the entire thesis. A reference section is provided at the end of each chapter. Pages are numbered sequentially.

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THE PERSONALITY OF *RHABDOMYS* TAXA INHABITING DIFFERENT ENVIRONMENTS

Abstract

Personality describes stable individual differences in behaviour, which arise in animal populations due to differences in genes, states, or imperfect matching of the environment. Behavioural syndromes describe personality traits that are correlated or co-vary across different contexts. Although syndromes are associated with limited plasticity, they may have benefits when the information about the environment is incomplete. Because personality and behavioural syndromes can be influenced by environmental selection pressure, different populations may have comparatively different personality types. I compared the personality of 4 populations, representing 3 taxa of the African striped mouse, *Rhabdomys*, in South Africa. The taxa differed in the environment from which they originated and their geographic context: *R. pumilio* originated from a locality with minimal natural cover in the western semi-arid region; *R. bechuanae* and *R. d. dilectus* occurred sympatrically in the central grasslands; and *R. d. dilectus* occurred allopatrically in the eastern grassland. From previous behaviour studies on *R. pumilio* and *R. dilectus*, I predicted that *R. pumilio* would be bolder than all of the other populations. I conducted light-dark, startle response, open field, novel object and plus maze tests to measure exploratory behaviour and anxiety responses in both sexes of each population. *R. pumilio* and the sympatric *R. d. dilectus* were bolder and more exploratory than *R. bechuanae* and the allopatric *R. d. dilectus*, and *R. pumilio*, *R. bechuanae* and the allopatric *R. d. dilectus* had fewer correlations between behaviours and between contexts than the sympatric *R. d. dilectus*. The bolder personality of *R. pumilio* could be a consequence of the open habitat that it occupies, where increased exploration and decreased anxiety may be adaptive, and the reduced exploration and increased anxiety of *R. bechuanae* and allopatric *R. d. dilectus* could be adaptive in areas with more cover. However, the similarity in personality between *R. pumilio* and the

sympatric *R. d. dilectus* cannot be explained by habitat, and might be a consequence of species coexistence (with *R. bechuanae*) in central South Africa.

Introduction

Personality describes consistent behavioural responses between individuals to different situations over time and in different contexts (Sih *et al.* 2004a; Dingemanse *et al.* 2009; Brown & Robinson 2016). The personalities of many animal species have been studied, ranging from invertebrates such as giant sea anemones *Condylactis gigantea* (Hensley *et al.* 2013) and European hermit crabs *Pagurus bernhardus* (Briffa *et al.* 2008) to wild guinea pigs *Cavia aperea* (Guenther *et al.* 2014). The most commonly studied personality traits are boldness-shyness (which considers responses to a risky situation), exploration-avoidance (which describes an animal's reaction to novelty), the general activity level of an animal, aggressiveness, and sociability (an individual's response to conspecifics; Réale *et al.* 2007). Other personality traits that have been studied include dominance and disposition (van Oers & Sinn 2013).

Individuals may differ in their behaviour due to genetic variation (reviewed in Wilson 1994; Bouchard & Loehlin 2001) which may produce multiple phenotypes, or differences in states (e.g. differences in size or nutrition) between individuals (reviewed in Wolf *et al.* 2013; Belgrad *et al.* 2017). Positive frequency-dependent selection describes the dominance of one phenotype in a population, whereas negative frequency-dependent selection acts by increasing the frequency of scarcer phenotypes (Maynard Smith 1982), and can result in the development of adaptive individual differences in behaviour by influencing density-dependent competition, predation or other selection pressures (Wolf *et al.* 2013). Moreover, when there is both negative frequency-dependent selection and positive frequency dependent selection acting on similar traits within a population, there may be multiple phenotypes coexisting at varying frequencies (Sinervo & Calsbeek 2006).

Genetic expression is modulated by prevailing environmental conditions, selecting for different phenotypes (Via & Lande 1985). Variation in environmental selection pressures may occur over time and space, creating novel phenotypes (Moran 1992). Sometimes, phenotypes may not match the environment perfectly, leading to multiple phenotypes (i.e. showing variation in matching with the environment) within a population (reviewed in Wolf *et al.* 2013). Male cichlids from multiple species are well known for showing differences in mating strategies, for example, satellite males, harem males, sneaker males or males that engaged in piracy (Martin & Taborsky 1997; Katoh *et al*. 2005; Ota & Kohda 2006). Such fixed individual phenotypic differences are evident in personalities. Thus, personality is maintained due to limited plasticity (the ability of a genotype to produce multiple phenotypes; West-Eberhard 1989), which may be advantageous when there is imperfect or incomplete information about the environment (Sih *et al.* 2004b). Limited plasticity could arise due to positive feedback loops (Sih *et al.* 2004b). This may reinforce a particular behaviour, and is often driven by experience and the costs or benefits derived from the behaviour (Wolf *et al.* 2013).

Although personality describes within-population individual variation in behaviour, personality traits may vary between populations when the constituent individuals are subject to different selection pressures in different environments (Foster 1999). Because different habitats provide novel selection pressures, populations should differ in their behaviour to maximise fitness, although some overlap between populations can be expected due to the maintenance of multiple phenotypes within a population (discussed earlier). For example, Panamanian bishop fish *Brachyraphis episcopii* that had predator-experienced parents, were significantly bolder than fish that had predator-naïve parents (Brown *et al.* 2007), although there was overlap in boldness between the populations.

Different selection pressures in different environments may also influence exploration and anxiety. For example, European blackbirds *Turdus merula* that originate from urban areas are more neophobic (less exploratory, more anxious) than their rural counterparts, possibly due to the relative amount of danger for the birds in urban areas (Miranda *et al.* 2013). Predation risk is not the only important driver of such population-level personality differences. In great tits *Parus major*, when there was reduced competition for food during a beech masting year, selection favoured slow-exploring less aggressive females and fast-exploring aggressive males, while under reduced resource availability, fast-exploring aggressive females and slow-exploring less aggressive males predominated (Dingemanse *et al.* 2004).

While animal personality is a single trait that is consistent over contexts or time, behavioural syndromes are defined as personality traits that are correlated and co-vary across contexts or time in a group of individuals (Sih *et al.* 2004a; Bell 2007; Sih & Bell 2008). The presence of a behavioural syndrome implies limited plasticity (Sih *et al.* 2004a), and while it might be adaptive for an individual to constantly perceive its environment and respond in the most appropriate way to prevailing conditions (Dall *et al.* 2004), suites of correlated behaviours may reduce energy requirements due to imperfect information about the environment (Sih *et al.* 2004a). Behaviours that are genetically (Bell *et al.* 2013) or physiologically (Guenther *et al.* 2014) correlated may lead to behavioural syndromes. Like personality traits, environmental selection pressures (like predation) may influence the formation of behavioural syndromes (Bell & Stamps 2004). For example, the selection pressures associated with urbanisation drives the formation of behavioural syndromes, with urban populations of house sparrows *Passer domesticus* having fewer correlated behaviours than rural populations (Bókony *et al.* 2012).

The striped mouse *Rhabdomys* is a good model to study the relationship between habitat type and personality. *Rhabdomys* is a small diurnal murid rodent (± 80g), occurring in most biomes throughout southern Africa (Skinner & Chimimba 2005). The genus comprises at least 3 species, namely *R. bechuanae* (central south Africa), *R. pumilio* (western South Africa) and *R. dilectus* (eastern South Africa). *R. bechuanae* is proposed as the ancestral species (du Toit *et al.* 2012a). *R. bechuanae* (now in the central regions of South Africa) diverged first from a common ancestor with *R. pumilio* (du Toit *et al.* 2012a), which now occurs in the western, semi-arid parts of South Africa (du Toit *et al.* 2012a; Ganem *et al.* 2012). Two subspecies of *R. dilectus* (*R. d. chakae* and *R. d. dilectus*) occur from the northern grasslands of the central Free State Province of South Africa (*R. d. dilectus*) through to the east (*R. d. chakae*) of the country (Meynard *et al.* 2012). The Northern Cape Province, where *R.*

pumilio occurs, is associated with open habitat, and there are wide spaces between bushes or clumps of grass in which *R. pumilio* nests (Schradin & Pillay 2004). In contrast, the grasslands of northern South Africa, where *R. d. dilectus* occurs, have a high level of overhead grassy cover (Schradin & Pillay 2005b; Mostert *et al.* 2008) which *R. dilectus* prefers (Monadjem 1997; du Preez 1998; Fuller & Perrin 2001). The northern-central part of South Africa, where *R. bechuanae* occurs, is classified as grassland/savanna (Mucina *et al.* 2006; Rutherford *et al.* 2006b), with overhead cover in the form of trees, shrubs and grass (Rutherford *et al.* 2006a). There is an overlap in the distributions of *R. bechuanae* and *R. d. dilectus* in the northern parts of the Free State Province, where the taxa occur in sympatry (Ganem *et al.* 2012).

There are several behavioural and ecological differences between *R. pumilio* and a population of *R. d. chakae*, the better-studied subspecies of *R. dilectus*. *R. pumilio* is facultatively group-living, depending on environmental conditions (Schradin *et al.* 2012) with groups comprising of 2-4 adult females, along with a breeding male and several philopatric adult offspring (Schradin & Pillay 2004). Solitary living in adult males and females occurs at low population density (Schradin *et al.* 2012; Hill *et al.* 2015). *R. d. chakae*, on the other hand, is solitary (Brooks 1974), with individuals maintaining intra-sexually non-overlapping territories, and male territories overlapping those of several females (Schradin & Pillay 2005b). Previous studies have found that *R. pumilio* is more exploratory and less anxious (i.e. bolder) than *R. d. chakae* (Rymer & Pillay 2012) and *R. d. dilectus* (Mackay *et al.* 2014). However, to date, there is no information on the personality of *R. bechuanae*. In addition, the personalities of sympatric taxa of *Rhabdomys* have not been studied. A wider assessment of personalities of several *Rhabdomys* taxa would enable a better assessment of the environmental influences on personality in this rodent genus, with implications for other animal taxa too.

In this study, I investigated whether and how environmental (semi-arid vs grassland) and geographic context (sympatric vs allopatric) is associated with personality and the occurrence of behavioural syndromes in four populations, representing 3 taxa (*R. pumilio*, *R. bechuanae* and *R. d. dilectus*) of the genus *Rhabdomys*, in captivity. I investigated population-level differences to distinguish between the allopatric and sympatric populations of *R. d. dilectus*. For *Rhabdomys*, the availability of cover is likely to be an important selection pressure on behaviour (Mackay 2011) and possibly personality. *Rhabdomys* has been identified as an important prey item in barn owls (Skinner & Chimimba 2005), so cover may provide protection from aerial predators. I hypothesised that *R. pumilio*, which occurs in habitats with little cover, will be more exploratory and less anxious (i.e. bolder) than the other taxa. No *a priori* hypotheses were erected for geographic context, but existing niche differentiation (*R. bechuanae* has larger home ranges than *R. d. dilectus* in sympatry; Dufour *et al.* 2015) between *R. bechuanae* and *R. d. dilectus* in sympatry (Dufour 2014), suggests possible occurrence of different personality types.

Materials and methods

Study animals

Striped mice originating from four populations in three locations (Goegap Nature Reserve, Sandveld Nature Reserve, Entabeni Forest Reserve) in South Africa were used in experiments. *R. pumilio* originated from Goegap Nature Reserve in the Northern Cape Province (29°41’33”S, 18°1’41”E), which is situated in the semi-arid Succulent Karoo biome where vegetation cover is sparse, with large distances between clumps of bushes or grass (Schradin & Pillay 2005a). *R. bechuanae* originated from Sandveld Nature Reserve in the far north-western corner of Free State Province (27°41’57”S, 25°44’13”E). Sandveld Nature Reserve lies within the grassland/savanna biome, and vegetation here is characterised by grass interspersed with trees and shrubs (Rutherford *et al.* 2006a; Ganem *et al.* 2012). *R. d. dilectus* occurs in sympatry with *R. bechuanae* in this reserve (Ganem *et al.* 2012). A second population of *R. d. dilectus* originated from Entabeni Forest Reserve in the Limpopo Province (22°58’59”S, 30°16’56”E), which is classified as an afro-montane grassland/forest mosaic (Mostert *et al.* 2008). These three locations exist on an east-west rainfall gradient, with Goegap Nature Reserve receiving the lowest mean annual precipitation of 160mm (most of which falls in winter; Schradin & Pillay 2005b), Sandveld Nature Reserve receiving an intermediate amount of approximately 500mm (mainly in summer; Jankielsohn 2006) and Entabeni Forest Reserve receiving year-round rainfall of approximately 1800mm (Mostert *et al.* 2008).

Subjects

Subjects were 10 male and 10 female laboratory bred (F2 – F3) *R. pumilio*, 10 male and 10 female wild-caught *R. bechuanae* (tested after 5-6 months in captivity), 6 male and 12 female wild-caught *R. d. dilectus* (Sandveld; tested after 4-5 months in captivity) and 8 female and 10 male *R. d. dilectus* (Entabeni; tested after 2 months in captivity). *R. bechuanae* and sympatric and allopatric *R. d. dilectus* subjects were tested after at least 2 months in captivity in order to habituate the individuals to captivity. Yuen *et al.* (2016) and Yuen *et al.* (2015) showed that personality in *R. pumilio* is consistent, regardless of whether it is measured in the field or in the laboratory, and that personality traits are repeatable (Yuen *et al.* 2016). These studies indicate that the personality of *Rhabdomys* individuals is independent of housing condition, and that personality is independent of whether the individuals were lab-bred or free-living.

R. bechuanae and *R. d. dilectus* from Sandveld were genotyped before testing to confirm their identity (Dufour 2014), while the genotypes of the other populations were known from earlier publications (du Toit *et al.* 2012a). All individuals were adult at testing. All striped mice were housed in the Milner Park Animal Unit at the University of the Witwatersrand under partially controlled environmental conditions (22-26 °C; 20-50% RH; 14:10h light:dark cycle with lights on at 05h00). All study subjects were housed individually in lab-o-tec cages (15 x 42 x 15cm). Cages contained a layer of wood shavings, a handful of dry grass, paper towel for nesting material, a wood block for chewing and a cardboard toilet roll for enrichment. Subjects had free access to water, and were fed approximately 10g Epol© mouse cubes, along with 5ml of millet and 10g of fresh vegetables daily per individual.

Experimental design

All subjects were tested individually in each of five tests, with two pairs of sequential tests: 1) light-dark test immediately followed by; 2) startle-response test;

3) open field test immediately followed by; 4) novel object test; and 5) the plus maze. Individuals were exposed in random sequence to tests 1&2, 3&4 and 5. Individuals were tested once in each test, as Yuen *et al.* (2016) showed a high within-test repeatability of personality in *R. pumilio*.

Individuals went through one testing session (i.e. 1&2, or 3&4 or 5) per day (i.e. three days of testing) between 06h00 and 12h00, the peak activity period of striped mice (Schradin 2006). After testing on each day, subjects were returned to their home cage and rested for 24 hours prior to the next test. All testing apparatuses were thoroughly cleaned and air-dried after each use. All tests were video recorded from above the apparatus for later analysis using Observer 9 (Noldus 2009). The experimental protocol was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC 2012/27/2A).

1. Light-dark (LD) test

The light-dark test was conducted in a modified glass tank (46 x 30 x 35cm) that was divided into two equal sized chambers (each 23 x 30 x 35cm) by a black acrylic glass wall. The centre of the wall had a square opening (5 x 5cm) to allow the study subject to move between chambers. One chamber was painted black; including the lid on that side, while the other chamber and lid were left clear. I placed an individual into the centre of the light section of the tank and video-recorded its behaviour for 5 minutes, without any observers in the observation room. I later obtained measures of latency to enter the dark section (LD dark latency), the duration of time spent in the light section (LD light duration), and the number of entries into the light section (LD light transitions). The light-dark test was followed immediately by the startle-response test.

2. Startle response (SR) test

After the subject had spent 5 minutes in the light-dark chamber, I entered the observation room and clapped loudly next to the apparatus until the individual moved into the dark chamber of the tank, regardless of whether the individual was already in the dark chamber. I left the room, and the test arena was then filmed for a further 5 minutes. From the video footage, I later extracted the latency of the test subject to return to the light chamber (SR light latency), the duration of time spent in the light section (SR light duration) and the number of entries into the light section after the startle (SR light transitions).

3. Open field (OF) test

The open field test consisted of a glass tank (46 x 30 x 35cm) with opaque sides. I placed a single individual in the centre of the tank, measured by dividing the bottom of the tank into 9 equal squares (3 rows of 3) using electrical tape, which did not impede movement of a test mouse. I left the room and video-recorded the test for 10 minutes. From the video footage, I later extracted the duration of time spent in the centre of the tank (OF centre duration), the duration of exploration of the tank (moving around the tank; OF explore duration), and the number of times the individual moved from the periphery into the centre square (OF number centre entries).

4. Novel object (NO) test

The novel object test followed immediately after the open field test. After 10 minutes in the open field, I entered the room and placed a small table tennis ball (2cm diameter) in a corner of the tank directly opposite the study subject, leaving the room immediately afterwards. The ball was painted with purple, red and blue non-toxic paint and smeared with dilute lavender oil to create a novel scent. I video recorded the behaviour of the test subject for a further 10 minutes, later obtaining scores for the latency to approach within one mouse-length of the novel object (NO latency to approach) and the number of times the individual touched the novel object (NO interactions). I did not measure the duration of time that subjects spent interacting with the novel object, as subjects interacted with the novel object in very short durations, usually less than a second. I also measured the duration of time spent in the centre of the tank (NO duration centre), the number of times the individual moved from the periphery into the centre (NO number centre entries) and the duration of exploration of the tank (NO explore duration).

5. Plus maze (PM)

I used a modified plus maze constructed from acrylic glass to assess the anxiety of individuals (Jones *et al.* 2011; Mackay *et al.* 2014). The plus maze consisted of four enclosed arms, two of which were painted black and two left clear (all 7.5 x 50 x 7.5cm), arranged around a clear, square central chamber (11 x 11 x 15.5cm). The arms were enclosed to prevent test subjects from jumping out of the maze. I placed test subjects individually into a cylindrical entry chamber (10 cm long, 5.5 cm diameter) that was situated above one of the clear arms of the maze. This opened directly into the central chamber. I left the room and filmed the test for 10 minutes from above the maze. I later recorded the duration of time spent in the clear arms (PM clear duration), the number of times the individual entered the clear arms (PM number clear entries), and the latency to enter the centre chamber from the cylindrical entry chamber, as individuals may freeze in the entry cylinder (PM latency).

Data analysis

I analysed data using Statistica 12 (Statsoft 2013). Normality of the data was determined using Shapiro-Wilk's W tests. I used General Linear Models (GLMs) with multivariate design to analyse population and sex differences for each test. I used Fisher's LSD *post hoc* tests to identify the influences of the populations and sexes on behaviour. The model level significance was set at α = 0.05 and all tests were two-tailed.

I ran a Principle Components Analysis (PCA) to consider the combined influence of the various metrics obtained in the different personality tests on the similarities between individuals of the different populations. This analysis positions and compares individuals (rather than means and variances of populations, above), the basis of personality research. I excluded sex as it had no effect on behaviour in the GLMs (see Results). I analysed all 17 variables (listed in the descriptions of the tests above) in the PCA.

To investigate behavioural syndromes within each population separately, I analysed all of the variables from the dataset using Pearson product moment correlations. Correlations between variables are indicative of an association between them, and therefore can indicate a syndrome. In addition to $\alpha \leq 0.05$, I only considered responses with r-values greater than 0.5 to form syndromes.

Results

Population differences in personality

Light-dark test

Population was a significant predictor of the duration of time spent in the light chamber ($F_{3,63}$=8.93 p<0.001), with *R. pumilio* spending more time in the light than all other populations (Figure 2.1A). Sex ($F_{1,63}$=0.97 p=0.328, supplementary material S2) and population*sex ($F_{3,63}$=0.83 p=0.483, supplementary material S3) were not significant predictors of time spent in the light chamber. Population was also a significant predictor of the number of transitions into the light chamber ($F_{3,63}$=5.08 p=0.003), with *R. pumilio* and *R. d. dilectus* from Sandveld moving between chambers significantly more frequently than *R. d. dilectus* from Entabeni and *R. bechuanae* (Figure 2.1B). Sex ($F_{1,63}$=0.83 p=0.365, supplementary material S2) and population*sex ($F_{3,63}$=0.38 p=767, supplementary material S3) were not significant predictors of the number of entries into the light. Population ($F_{3,62}$=2.47 p=0.070; supplementary material S1), sex ($F_{1,63}$=0.26 p=0.610, supplementary material S2) and population*sex ($F_{3,63}$=0.87 p=0.463, supplementary material S3) were not significant predictors of the latency to enter the dark chamber after starting the test.

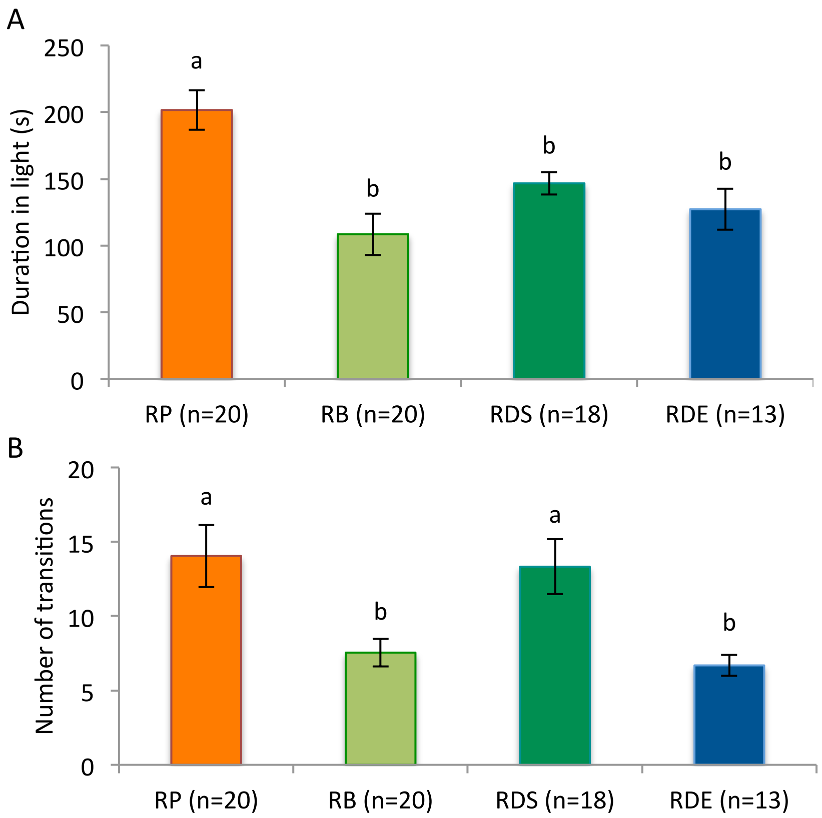

Figure 2.1. The duration of time spent in the light chamber in the light-dark test (Figure 2.1A) and the number of transitions into the light chamber (Figure 2.1B) by individuals of 4 populations of *Rhabdomys*. The populations were RP (*R. pumilio*), RB (*R. bechuanae*), sympatric with RDS (*R. d. dilectus* from Sandveld), and RDE (*R. d. dilectus* from Entabeni). Bars show means ± SE, and different letters indicate significant differences (Fisher's LSD *post-hoc* tests).

Startle response test

The latency to re-enter the light chamber after the startle was significantly predicted by population ($F_{3,63}$=9.04 p<0.001), with *R. pumilio* and *R. d. dilectus* from Sandveld re-entering the light chamber significantly sooner than *R. d. dilectus* from Entabeni and *R. bechuanae* (Figure 2.2A). Sex ($F_{1,63}$=0.31 p=0.581, supplementary material S2) and population*sex ($F_{3,63}$=0.71 p=0.550, supplementary material S3) were not significant predictors of the latency to re-enter the light chamber. The duration of time spent in the light chamber after the startle was also significantly affected by population ($F_{3,63}$=6.71 p<0.001), with *R. pumilio* spending significantly more time in the light chamber than all other populations (Figure 2.2B). Sex ($F_{1,63}$=1.71 p=0.196, supplementary material S2) and population*sex ($F_{3,63}$=0.48 p=0.697, supplementary material S3) were not significant predictors of the duration spent in the light chamber after the startle. Population was a significant predictor of the number of transitions into the light chamber ($F_{3,63}$=3.96 p=0.012) with *R. pumilio* making significantly more transitions than *R. d. dilectus* from Entabeni and *R. bechuanae* (Figure 2.2C). Sex ($F_{1,63}$=0.68 p=0.413, supplementary material S2) and population*sex ($F_{3,63}$=0.13 p=0.943, supplementary material S3) were not significant predictors of the number of transitions into the light chamber after the startle.

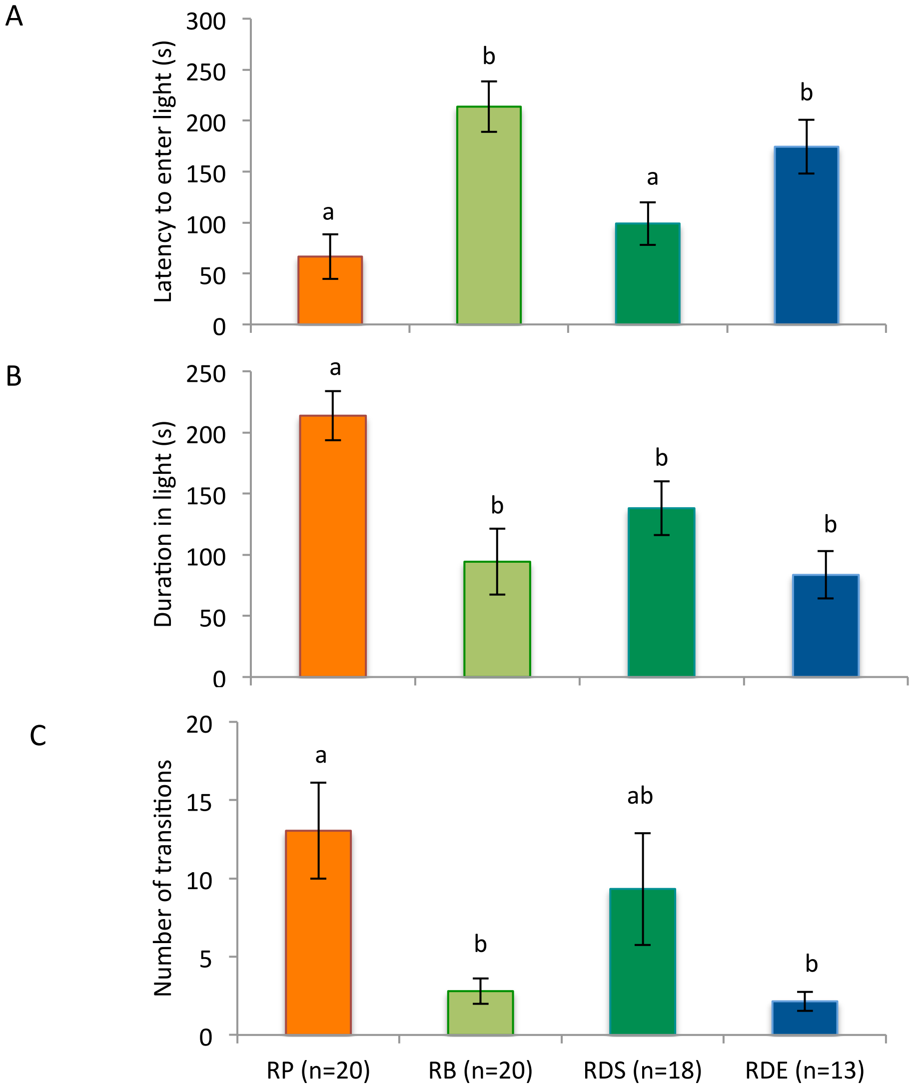

Figure 2.2. The latency to enter the light chamber during the startle response test (Figure 2.2A), the duration of time spent in the light chamber (Figure 2.2B) and the number of entries into the light chamber (Figure 2.2C) by individuals of 4 populations of *Rhabdomys*. Bars show means ± SE, and different letters indicate significant differences (Fisher's LSD *post-hoc* tests).

Open field test

Population ($F_{3,63}$=2.00 p=0.124, supplementary material S1), sex ($F_{1,63}$=0.53 p=0.471, supplementary material S2) and population*sex ($F_{3,63}$=1.10 p=0.356, supplementary material S3) were not significant predictors of the duration of time spent in the centre of the open field. The number of centre entries was also not significantly predicted by population ($F_{3,63}$=1.59 p=0.201, supplementary material S1), sex ($F_{1,63}$=0.28 p=0.598, supplementary material S2) or population*sex ($F_{3,63}$=1.07 p=0.369, supplementary material S3). However, the duration of exploration in the open field tank was significantly influenced by population ($F_{3,63}$=3.26 p=0.027), with *R. pumilio* and *R. d. dilectus* from Sandveld spending more time exploring than *R. d. dilectus* from Entabeni (Figure 2.3). Sex ($F_{1,63}$=0.11 p=0.738, supplementary material S2) and population*sex ($F_{3,63}$=0.98 p=0.408, supplementary material S3) were not significant predictors of the duration of exploration.

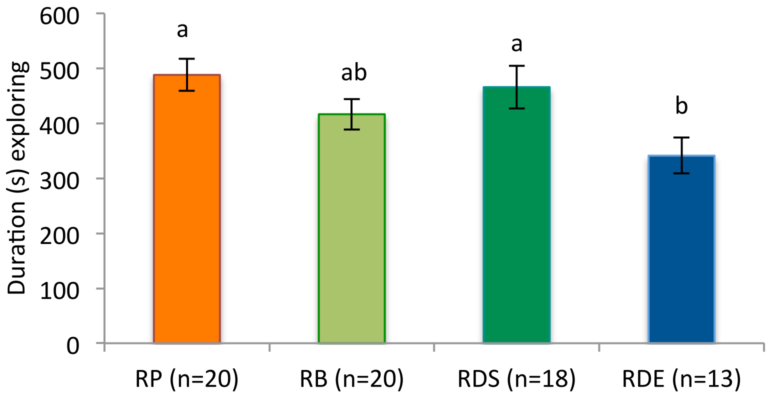

Figure 2.3. The duration of time spent exploring during the open field test by individuals of 4 populations of *Rhabdomys*. Bars show means ± SE, and different letters indicate significant differences (Fisher's LSD *post-hoc* tests).

Novel object test

The latency to touch the novel object was significantly influenced by population ($F_{3,63}$=2,93 p=0.041), with *R. pumilio* taking significantly less time to approach the novel object than *R. bechuanae* and *R. d. dilectus* from Entabeni (Figure 2.4A). Sex

($F_{1,63}$=0.94 p=0.337, supplementary material S2) and population*sex ($F_{3,63}$=1.94 p=0.132, supplementary material S3) were not significant predictors of the latency to touch the novel object. The number of interactions with the novel object was not significantly influenced by population ($F_{3,63}$=1.80 p=0.157, supplementary material S1), sex ($F_{1,63}$=0.07 p=0.787, supplementary material S2) or population*sex ($F_{3,63}$=0.02 p=0.997, supplementary material S3). Population ($F_{3,63}$=2.45 p=0.071, supplementary material S1), sex ($F_{1,63}$=059 p=0.443, supplementary material S2) and population*sex ($F_{3,63}$=1.47 p=0.231, supplementary material S3) also did not significantly predict the duration of time spent in the centre of the tank. The number of entries into the centre of the tank was also not significantly predicted by population ($F_{3,63}$=2.34 p=0.082, supplementary material S1), sex ($F_{1,63}$=2.90 p=0.093, supplementary material S2) or population*sex ($F_{3,63}$=0.73 p=0.540, supplementary material S3). However, population significantly predicted the duration of exploration in the tank ($F_{3,63}$=4.48 p=0.007) with *R. pumilio* and *R. d. dilectus* spending significantly more time exploring than *R. bechuanae* (Figure 2.4B). Sex ($F_{1,63}$=1.81 p=0.183, supplementary material S2) and population*sex ($F_{3,63}$=1.20 p=0.316, supplementary material S3) were not significant predictors of exploration.

Plus maze

The latency to enter the plus maze from the entry chamber was not significantly predicted by population ($F_{3,62}$=1.27 p=0.291, supplementary material S1), sex ($F_{1,63}$=0.09 p=0.767, supplementary material S2) or population*sex ($F_{3,63}$=0.15 p=0.932, supplementary material S3). Likewise, population ($F_{3,62}$=2.59 p=0.061, supplementary material S1), sex ($F_{1,63}$=0.38 p=0.542, supplementary material S2) and population*sex ($F_{3,63}$=2.45 p=0.072, supplementary material S3) did not significantly predict the duration of time spent in the clear arms of the plus maze. The number of entries into the light arms was not significantly predicted by population ($F_{3,62}$=2.15 p=0.103, supplementary material S1), sex ($F_{1,63}$=1.10 p=0.298, supplementary material S2) or population*sex ($F_{3,63}$=0.62 p=0.605, supplementary material S3).

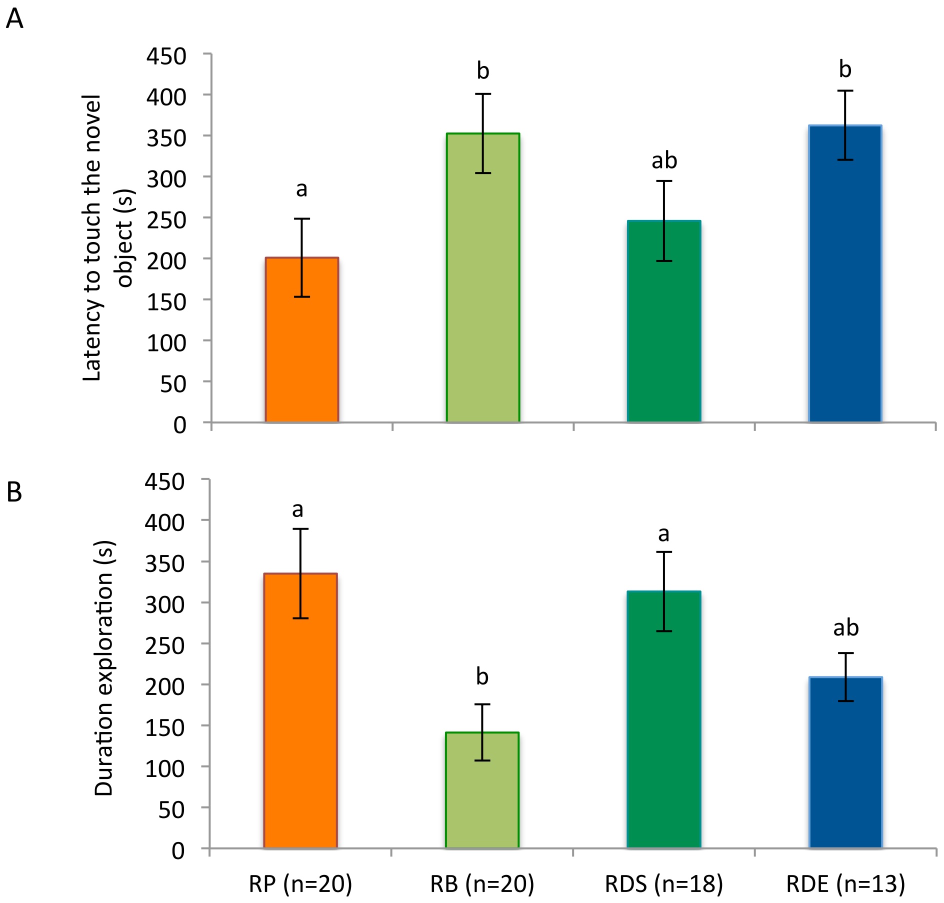

Figure 2.4. The latency to touch the novel object during the novel object test (Figure 2.4A) and the duration of exploration (Figure 2.4B) in the novel object test, by individuals of 4 populations of *Rhabdomys*. Bars show means ± SE, and different letters indicate significant differences (Fisher's LSD *post-hoc* tests).

Population similarities in personality

The principle components analysis (PCA) indicated that the first, second and third order principle components accounted for 35%, 11% and 10% of the variance, respectively. Such low explanation of variance is common in studies of personality (e.g. López *et al.* 2005; Lloyd *et al.* 2007; Carter & Feeney 2012). The latency to touch the novel object had the highest positive influences (factor loading) and the number of centre entries in the novel object test had the highest negative influences on PCA 1 respectively. The latency to enter the plus maze from the entry chamber and the duration of time spent in the light chamber in the startle response test had the highest positive and highest negative influences (factor loading) on PCA 2, respectively. The duration of time spent in the centre of the tank in the novel object test and the latency to enter the dark chamber during the light-dark test had the highest positive and highest negative influences (factor loading) on PCA 3 respectively (Table 2.1).

PCA 1 and 2 show that *R. bechuanae* and *R. d. dilectus* from Entabeni were mostly grouped together on the right half of the vertical axis, and mainly influenced by the high latency to approach the novel object (Figure 2.5A). In contrast, *R. pumilio* and *R. d. dilectus* from Sandveld were mainly grouped together on the left side of the vertical axis and influenced by a higher number of centre entries in the novel object test and longer duration in the light chamber in the startle response test (Figure 2.5A). PCA 1 and 3 showed that *R. bechuanae* and *R. d. dilectus* from Entabeni were mainly grouped on the right side of the vertical axis and influenced by a longer latency to touch the novel object (Figure 2.5B), while *R. pumilio* was mainly on the left side of the vertical axis, and below the horizontal axis, influenced by a higher frequency of centre entries in the novel object test (Figure 2.5B). PCA 2 and 3 showed that *R. pumilio* was mainly below the horizontal axis and also influenced by a longer latency to enter the dark chamber of the light-dark test, but there were no clear groupings for the other populations (Figure 2.5C).

Table 2.1. Factor loadings from a PCA of the influence of the combined metrics obtained in the light-dark, startle response, open field, novel object and plus maze tests on the groupings of individuals of 4 populations of *Rhabdomys*. Highest positive values are shown in orange and the highest negative values are highlighted in blue. LD refers to the light-dark test, SR refers to the startle response test, OF refers to the open field test, NO refers to the novel object test, and PM refers to the plus maze.

	PCA 1	PCA 2	PCA 3
LD light duration	-0.531	-0.469	-0.421
LD light transitions	-0.683	0.440	-0.373
LD dark latency	0.064	-0.236	-0.426
SR light duration	-0.632	-0.625	-0.300
SR light latency	0.703	0.349	0.276
SR light transitions	-0.804	0.401	-0.337
PM latency	0.086	-0.127	0.013
PM clear duration	-0.078	-0.129	-0.086
PM number clear entries	-0.738	0.555	-0.229
OF centre duration	-0.297	-0.468	0.261
OF number centre entries	-0.703	0.285	0.245
OF explore duration	-0.616	-0.193	0.268
NO latency to approach	0.725	0.204	-0.270
NO interactions	-0.513	0.014	0.395
NO duration centre	-0.176	-0.008	0.609
NO number centre entries	-0.843	0.229	0.182

	PCA 1	PCA 2	PCA 3
NO duration explore	-0.814	-0.188	0.256

Although there were clear groupings for each population, there was overlap between the populations, especially in PCA 2 and 3. In addition, *R. pumilio* and *R. d. dilectus* from Sandveld had the highest degree of overlap, while *R. d. dilectus* and *R. bechuanae* overlapped less with each other, and hardly with *R. pumilio* and *R. d. dilectus* from Sandveld (Figure 2.5A and 2.5B). However, all populations intersected near the centre (intersection of vertical and horizontal axes; Figure 2.5A and 2.5B), indicating that in all populations, there were one or two individuals that had similar personality.

Behavioural syndromes within populations

I used Pearson product moment correlations between the variables scored in the light-dark, startle response, open field, novel object and plus maze personality tests to investigate behavioural syndromes among individuals within each population. Out of a total of 136 possible correlations, *R. d. dilectus* from Entabeni and *R. pumilio* had 18 and 19 significant correlations respectively (with r > 0.5) between variables, while *R. bechuanae* and *R. d. dilectus* from Sandveld had 24 and 31 significant correlations respectively (Supplementary material S4 and S5). Within all four populations, all of the significant correlations mentioned above were either syndromic (any association between personality traits) or contextually correlated (a measure of personality that is consistent in different contexts), with relationships in all in the same direction (i.e. for tests measuring anxiety response, all positive associations or all negative associations).

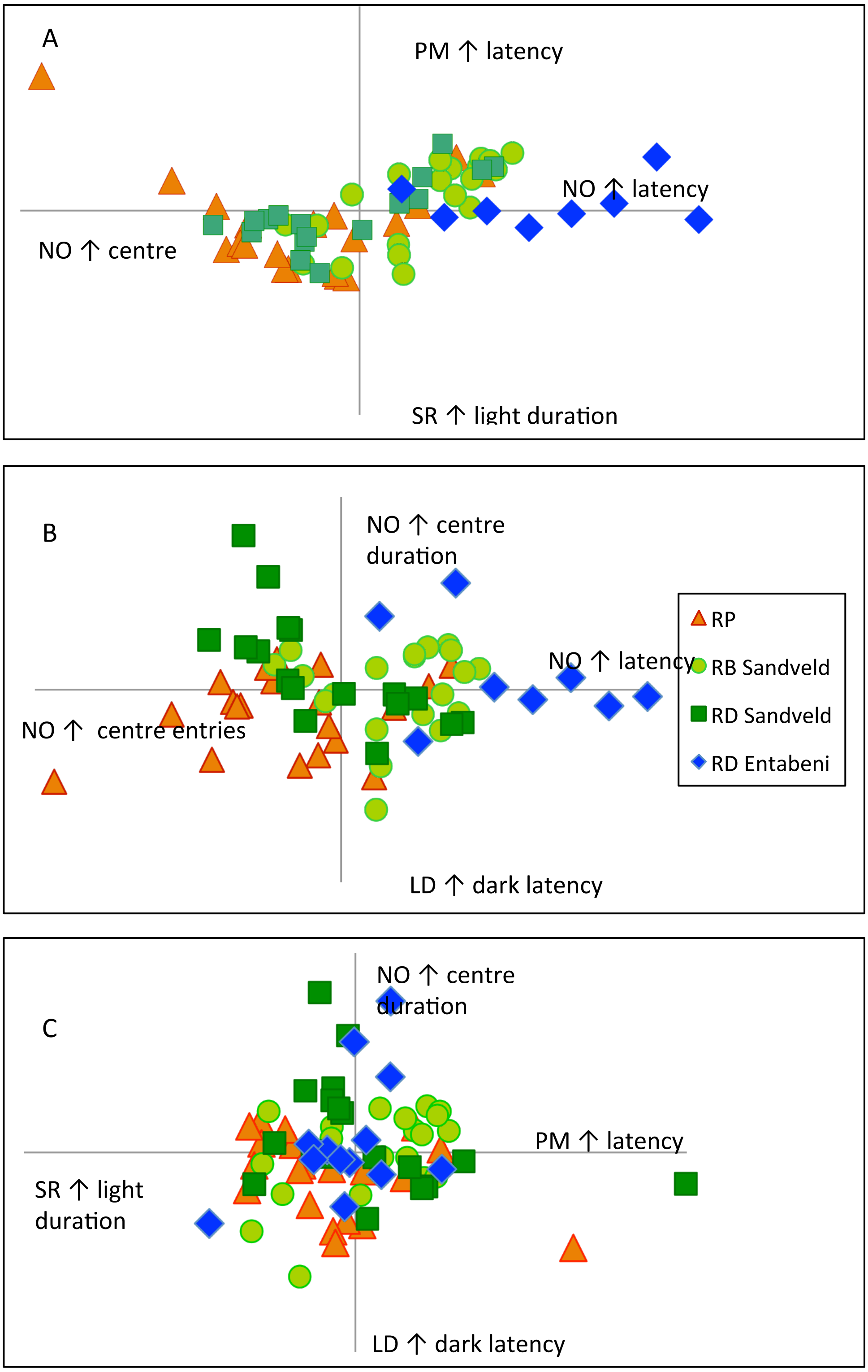

Figure 2.5. Principle components analysis of the combined metrics from the light-dark, startle response, open field, novel object and plus maze tests, to show the groupings of individuals in 4 populations of *Rhabdomys*. Figure 2.5A. PCA 1 and 2 account for 35% and 11% of the total variance respectively. Figure 2.5B. PCA 1 and 3 account for 35% and 10% of the total variance, respectively. Figure 2.5C. PCA 2 and 3 account for 11% and 10% of the total variance, respectively.

Discussion

The aim of this study was to compare the personalities and existence of behavioural syndromes in four populations, representing 3 taxa, of *Rhabdomys*. The populations differed in their environmental characteristics (with Sandveld and Entabeni having greater vegetative cover compared to Goegap) and geographic context (allopatric vs sympatric). In general, *R. pumilio* was the most exploratory and least anxious (and therefore significantly bolder), and *R. d. dilectus* from Sandveld (sympatric with *R. bechuanae*) had intermediate levels of exploratory behaviour and anxiety, while *R. bechuanae* and allopatric *R. d. dilectus* from Entabeni were the least exploratory and most anxious (and therefore significantly less bold) in the light-dark, startle response, open field and novel object tests, but there were no significant population differences in the plus maze. I also found that *R. pumilio* and the Sandveld *R. d. dilectus* population were grouped together, and the *R. bechuanae* and the Entabeni *R. d. dilectus* population were grouped together in an analysis of the variables combined in PCA, demonstrating similar personality. Therefore, although *R. pumilio* was as bold as sympatric *R. d. dilectus*, the difference in boldness between *R. pumilio* and *R. bechuanae* and allopatric *R. d. dilectus* leads me to accept my hypothesis that *R. pumilio* is bolder than all other taxa. Lastly, there were low numbers of significant correlations between behaviours, and populations had different significantly correlated variables, indicating that there were syndromes and similar behaviour in different contexts, but that personality was less fixed than if there were high numbers of significant correlations.

Bold individuals are defined as having lower levels of anti-predator behaviour (Bell *et al.* 2013) along with higher levels of risky behaviour (Sneddon 2003), more activity in a novel environment and lower latencies to emerge into a novel environment (Kortet & Hedrick 2007), lower levels of neophobia (Martins *et al.* 2012), and higher exploration of novelty (Frost *et al.* 2007). In contrast, less bold (or shy) individuals are defined as having behaviour that is at the opposite end on a bold-shy continuum (Wilson *et al.* 1993). Carter *et al.* (2012) raised concerns that different tests of boldness may be measuring different traits, so I measured exploration and anxiety in several different tests, which can then be used to infer boldness. I found population-level differences in behaviour in the light-dark test, the startle response, open field test and novel object test, with all of the tests being indicative of exploration and anxiety (Walsh & Cummins 1976; Bourin & Hascoët 2003; Burns 2008; Maximino *et al.* 2012; Beckmann & Biro 2013; Perals *et al.* 2017).

R. pumilio and *R. d. dilectus* from Entabeni (allopatric) had overall similar behaviour to that reported in previous studies for both species. Rymer *et al.* (2008) showed that *R. pumilio* individuals were less neophobic (and bolder) in comparison to *R. d. dilectus* individuals, in concordance with my results. The behaviour of the sympatric populations (Sandveld *R. bechuanae* and *R. d. dilectus*), however, only partly agrees with predictions, because only *R. bechuanae* had similar behaviour to *R. d. dilectus* from Entabeni. In contrast, *R. d. dilectus* in Sandveld (sympatric with *R. bechuanae*) resembled *R. pumilio.*

In agreement with the analyses of the separate tests of personality, a PCA showed that *R. pumilio* and *R. d. dilectus* from Sandveld were grouped together and *R. bechuanae* and allopatric *R. d. dilectus* from Entabeni were grouped together. The groupings are surprising, given the similar environments of Sandveld and Entabeni (high levels of cover). This also contradicts previous comparative *Rhabdomys* studies, where other populations of *R. dilectus* were less exploratory and more anxious than *R. pumilio* (Rymer & Pillay 2012; Mackay *et al.* 2014). From the PCA, individuals of both *R. pumilio* and *R. d. dilectus* from Sandveld had higher numbers of entries into the centre of the tank during the novel object test, and spent a longer duration of time in the light chamber during the startle response test. In contrast, individuals of *R. bechuanae* and Entabeni *R. d. dilectus* both had a high latency to approach within one mouse length of the novel object.

The PCAs may provide important information on the spread of personalities within populations, which is rarely considered in the literature. This might be because populations generally have multiple phenotypes (Via & Lande 1985; Moran 1992). However, the spread of phenotypes within a population may be indicative of the selection pressure acting on the population. For example, stabilising selection pressure reduces the phenotypic variation of a population (Hohenboken 1985) while disruptive (Lande 1980) or directional (Rieseberg *et al.* 2002) selection pressure could allow for a greater spread of phenotypes (i.e. greater variation within a population). For example, stabilising selection in years without beech masting (less food), led to great tit *Parus major* offspring having an intermediate level of exploratory behaviour, while disruptive selection in good years favoured both fast and slow exploratory behaviour (Dingemanse & Réale 2005). However, directional and stabilising selection pressures together may result in tighter clustering of phenotypes (Arnold 1992). In the personality tests, individuals of the different populations overlapped in some behaviours of exploration and anxiety (variance and principal components), rather showing distinct population clusters. The variation within populations, and the overlap between the populations (from the PCA) is indicative of disruptive selection pressure. However, the patterns of grouping, where *R. pumilio* and *R. d. dilectus* from Sandveld were grouped and different to *R. d. dilectus* from Entabeni, may be indicative of directional selection pressure. Moreover, the existence of multiple phenotypes in the populations could also occur because of simultaneous negative and positive frequency-dependent selection (Sinervo & Calsbeek 2006).

Correlations between behaviours in different contexts, and correlations between behaviours themselves, are used to assess behavioural syndromes (Valladares *et al.* 2014). Behavioural syndromes occur because of selection for correlated behaviours (Sih *et al.* 2004b) or if behaviours are genetically linked (Bell *et al.* 2013), where there are behaviours that are adaptive in different contexts, or different behaviours that are adaptive together (reviewed in Dingemanse *et al.* 2007). Studies on behavioural syndromes typically select between two and ten behaviours or behaviours between contexts to investigate correlations (e.g. Duckworth 2006; Bell & Sih 2007; Dingemanse *et al.* 2007; Dochtermann & Jenkins 2007; Johnson & Sih 2007; Moretz *et al.* 2007; Garamszegi *et al.* 2009; Evans *et al.* 2010; Pruitt *et al.* 2010; Wisenden *et al.* 2011 and others) by choosing a few behaviours or measures to be representative of a personality trait (i.e. cherry picking), but see Wilson and Godin (2009); Dingemanse *et al.* (2010); Wilson *et al.* (2010); and Chapman *et al.* (2011). Because most studies on syndromes are so selective, comparisons between studies are difficult (if not impossible) and it is therefore challenging to make statements about the meaning of many or few correlations. *R. d. dilectus* from Sandveld (sympatric with *R. bechuanae*) had the highest number of significant correlations between variables compared to the other populations. However, lower numbers of correlations are suggestive of plasticity in behaviour (reviewed in Sih *et al.* 2004b), where an individual can behave optimally under different conditions (Briffa *et al.* 2008). *R. pumilio* and allopatric *R. d. dilectus* from Entabeni had the lowest numbers of significant correlations followed by *R. bechuanae*, implying that these populations have the ability to behave flexibly under different conditions.

R. pumilio displays remarkable behavioural plasticity (Schradin *et al.* 2012; Rymer *et al.* 2013), which might explain the comparatively low numbers of correlations for this species. The low numbers of correlations in *R. bechuanae* and *R. d. dilectus* from Entabeni are therefore also suggestive of plasticity in these populations. The disparity in the number of correlations in Sandveld *R. d. dilectus* compared to *R. bechuanae* (which occur in sympatry) suggests that these populations might be under different selection pressures, despite them occurring syntopically. It is also interesting that, although they are the same species, Sandveld *R. d. dilectus* (sympatric) had more correlations and consistency between contexts than *R. d. dilectus* from Entabeni (allopatric). This in turn suggests that Entabeni *R. d. dilectus* individuals had the ability to behave differently in different situations, while Sandveld *R. d. dilectus* individuals are likely to have set behaviour across situations, which suggests that there may be an advantage (Sih *et al.* 2004a) in having behavioural syndromes in Sandveld, and may be a potential adaptive response to competition in Sandveld.

The differences between *R. pumilio* and *R. d. dilectus* from Entabeni, along with the similarity of *R. bechuanae* to Entabeni *R. d. dilectus* support an environmental influence on behaviour. du Toit *et al.* (2012a) indicated that *R. bechuanae* is a basal clade in the genus, and that *R. pumilio* and *R. dilectus* are derived from a common ancestor with *R. bechuanae*. This would in turn suggest that the differences between *R. pumilio* and *R. d. dilectus* from Entabeni are driven by environmental conditions, particularly levels of vegetation cover influencing exploratory behaviour and anxiety. Similarly, the concordance of behaviour between *R. d. dilectus* from Entabeni and *R. dilectus* from other studies (e.g. Rymer *et al.* 2008; Mackay 2011; Mackay *et al.* 2014), indicates that the behaviour of Sandveld *R. d. dilectus* is anomalous and that there may be some factor within Sandveld that may explain this.

Where two similar or closely related species have overlapping distributions, interspecific competition can act to reduce conflict by promoting phenotypic divergence (character displacement; Brown & Wilson 1956; Grether *et al.* 2009). Given that *R. d. dilectus* from Sandveld and *R. d. dilectus* from Entabeni are most closely related, and their habitats were more similar to each other than with that of *R. pumilio*, this raises the possibility that co-existence of the species might be resulting in different selection pressures in allopatry compared to sympatry. Although character displacement is usually demonstrated through morphological changes (Brown & Wilson 1956; Grether *et al.* 2009; Pfennig & Pfennig 2009), the presence of different personalities within populations could be maintained though character displacement (Dayan & Simberloff 2005). Dufour (2014) found that there was competition between *R. bechuanae* and *R. d. dilectus* in sympatry, and showed that the home ranges of *R. bechuanae* were larger in sympatry. This supports the character displacement idea, and suggests that *R. d. dilectus* may have different behaviour in sympatry as a result of competition with *R. bechuanae*.

I found no significant differences between the populations in their behaviour in the plus maze. The plus maze is designed to measure an individual's anxiety response (Carobrez & Bertoglio 2005), with more anxious individuals spending less time in, and fewer entries into the light arms compared to the dark arms (Carola *et al.* 2002). In previous studies, *R. pumilio* individuals were consistently less anxious in a plus maze than *R. d. chakae* individuals (Rymer & Pillay 2012). In comparison, in my study, all populations spent similar durations of time in the clear arms, and comparatively, *R. pumilio* and *R. dilectus* spent 36% and 27% less time in the clear arms compared to the Rymer and Pillay (2012) study. Given the similarity in anxiety of *R. d. chakae* and *R. d. dilectus* individuals in Mackay *et al.* (2014), as well as the consistent low anxiety shown by *R. pumilio* individuals compared to *R. d. chakae* individuals in both Rymer and Pillay (2012) and Mackay *et al.* (2014), it is surprising that I found no differences in anxiety between the four populations. However, the relative increase in anxiety of *Rhabdomys* individuals between studies is suggestive of sensitisation to captivity, but further studies would be needed to test this idea.

Interestingly, neither sex nor population*sex significantly influenced behaviour. There is mixed support for sex-based personality differences (support sex-based differences, Schuett & Dall 2009; no sex differences, Guenther *et al.* 2014). Sex influences personality when there is sexual selection on behaviour (Schuett *et al.* 2010), and where a behaviour influences the likelihood of mating success (e.g. Godin & Dugatkin 1996). Likewise, different populations should only have sex-based behavioural differences where the populations are under different sexual selection pressures (West-Eberhard 1983), or when the sexes have different spatial ecology (Lucon-Xiccato & Bisazza 2017). In *R. pumilio*, males and females have different reproductive tactics with males either remaining philopatric (non-breeder), roaming (breeding), or dominant breeder males (Schradin *et al.* 2009), while females may choose to nest in groups or alone (Schoepf *et al.* 2015). Likewise, male *R. d. chakae* territories are bigger than female territories in the central-eastern grasslands of South Africa (Schradin & Pillay 2005b). The similarity in personalities between the sexes does not mirror the tactics, both in my study and in Yuen *et al.* (2015).

In conclusion, I established that there were differences and similarities in personality in 3 species (4 populations) of *Rhabdomys.* The personality of the *R. pumilio* and *R. d. dilectus* from Entabeni (allopatric) were in agreement with previous studies, and together with the behaviour of *R. bechuanae*, support an environmental influence (cover levels) of behaviour on these populations. The disparity of the personality type of Sandveld *R. d. dilectus* is suggestive of character displacement in competition with *R. bechuanae*, but further studies are required to test this hypothesis. Finally, the low numbers of correlations between measures of behaviour are indicative of behavioural plasticity in *R. pumilio*, *R. bechuanae* and *R. d. dilectus* from Entabeni, while the comparatively high number of correlations in Sandveld *R. d. dilectus* is indicative of reduced plasticity, and a possible advantage of having reduced plasticity in Sandveld for *R. d. dilectus*. Finally, my data indicate that population differences in personality are a matter of degree (stabilising selection) and direction (combination of both positive and negative frequency-dependant selection) of selection rather than absolute differences between taxa. However, further studies should investigate the influence of housing condition (i.e. free-living or captive-bred) on personality in all *Rhabdomys* taxa to assess whether captivity modulates personality.

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Supplementary Material

S1. Model outcomes from ANOVAs, and data summary for non-significant variables influencing behaviour of individuals from four populations of *Rhabdomys*. Data are presented as means ± SE.

Tests	Statistics	*R. pumilio*	*R. bechuanae*	*R. d. dilectus* Sandveld	*R. d. dilectus* Entabeni
LD dark latency	$F_{3,62}$=2.47 p=0.070	16.38 ± 5.70	29.58 ± 9.47	2.99 ± 0.70	19.15 ± 8.47
OF centre duration	$F_{3,63}$=2.00 p=0.124	27.57 ± 3.84	21.60 ± 4.16	37.28 ± 6.47	39.47 ± 7.91
OF centre number	$F_{3,63}$=1.59 p=0.201	16.60 ± 1.83	14.45 ± 1.97	21.28 ± 4.87	9.62 ± 2.00
NO interactions	$F_{3,63}$=1.80 p=0.157	29.60 ± 8.80	12.80 ± 4.67	40.72 ± 17.38	7.08 ± 1.86
NO centre duration	$F_{3,63}$=2.45 p=0.071	17.97 ± 6.55	4.83 ± 1.85	30.33 ± 12.21	36.27 ± 17.49
NO centre number	$F_{3,63}$=2.34 p=0.082	8.40 ± 1.86	2.65 ± 1.00	8.61 ± 2.70	4.77 ± 1.17
PM latency	$F_{3,62}$=1.27 p=0.291	20.82 ± 16.27	59.91 ± 37.91	2.93 ± 0.56	2.51 ± 0.66
PM clear duration	$F_{3,62}$=2.59 p=0.061	288.79 ± 31.38	177.25 ± 36.25	220.16 ± 20.72	295.05 ± 63.07
PM clear number	$F_{3,62}$=2.15 p=0.103	31.75 ± 11.41	8.25 ± 1.65	28.94 ± 10.60	6.92 ±1.76

S2. Model outcomes from ANOVAs, and data summary for non-significant variables influencing behaviour of females and males. Data are presented as means ± SE.

Tests	Statistics	Female	Male
LD light duration	$F_{1,63}$=0.97 p=0.328	143.23 ± 10.58	153.17 ± 12.70
LD light transitions	$F_{1,63}$=0.83 p=0.365	11.79 ± 1.38	9.42 ± 1.01
LD dark latency	$F_{1,63}$=0.26 p=0.610	14.93 ± 4.42	19.83 ± 5.89
SR light duration	$F_{1,63}$=1.71 p=0.196	123.23 ± 16.84	153.03 ± 19.82
SR light latency	$F_{1,63}$=0.31 p=0.581	136.99 ±19.32	134.92 ± 19.34
SR light transitions	$F_{1,63}$=0.68 p=0.413	8.53 ± 2.35	5.73 ± 1.14
OF centre duration	$F_{1,63}$=0.53 p=0.471	28.80 ± 3.84	32.52 ± 4.06
OF centre number	$F_{1,63}$=0.28 p=0.598	16.92 ± 2.53	14.73 ± 1.57
OF explore duration	$F_{1,63}$=0.11 p=0.738	432.41 ± 25.48	438.99 ± 22.27
NO latency	$F_{1,63}$=0.94 p=0.337	258.72 ± 33.97	314.57 ± 36.82
NO interactions	$F_{1,63}$=0.07 p=0.787	26.50 ± 7.40	20.18 ± 7.84
NO centre duration	$F_{1,63}$=059 p=0.443	18.75 ± 4.35	23.06 ± 9.44
NO centre number	$F_{1,63}$=2.90 p=0.093	7.92 ± 1.57	4.15 ± 0.93
NO explore duration	$F_{1,63}$=1.81 p=0.183	288.68 ± 33.76	209.08 ± 33.41
PM latency	$F_{1,63}$=0.09 p=0.767	18.79 ± 15.71	29.87 ± 17.88

Tests	Statistics	Female	Male
PM clear duration	$F_{1,63}$=0.38 p=0.542	242.20 ± 26.16	239.87 ± 28.14
PM clear number	$F_{1,63}$=1.10 p=0.298	25.34 ± 7.68	13.58 ± 2.95

S3. Model outcomes from ANOVAS, and data summary for non-significant variables influencing behaviour of females (F) and males (M) from four populations of *Rhabdomys*. Data are presented as means ± SE.

Tests	Statistics	*R. pumilio* F	*R. pumilio* M	*R. bechuanae* F	*R. bechuanae* M	*R. d. dilec* Sandveld
LD light duration	$F_{3,63}$=0.83 p=0.483	199.17 ± 22.87	204.13 ± 19.99	117.10 ± 20.49	99.89 ± 24.28	138.96 ±
LD light transitions	$F_{3,63}$=0.38 p=767	15.80 ± 3.12	12.30 ± 2.82	8.80 ± 1.54	6.30 ± 0.99	13.92 ± 2
LD dark latency	$F_{3,63}$=0.87 p=0.463	11.38 ± 5.82	21.38 ± 9.89	35.45 ± 14.15	23.70 ± 13.07	3.81 ± 0.
SR light duration	$F_{3,63}$=0.48 p=0.697	198.40 ± 34.75	229.14 ± 21.05	84.29 ± 34.14	104.36 ± 43.79	111.69 ± 24.10
SR light latency	$F_{3,63}$=0.71 p=0.550	65.22 ± 35.54	67.99 ± 27.68	198.44 ± 40.44	229.07 ± 29.88	120.51 ± 28.92
SR light transitions	$F_{3,63}$=0.13 p=0.943	15.50 ± 5.62	10.60 ± 2.62	3.30 ± 1.18	2.30 ± 1.15	9.92 ± 5.
OF centre duration	$F_{3,63}$=1.10 p=0.356	25.19 ± 5.52	29.96 ± 5.53	13.30 ± 1.52	29.89 ± 7.45	40.98 ± 8
OF centre number	$F_{3,63}$=1.07 p=0.369	16.90 ± 2.90	16.30 ± 2.40	11.60 ± 2.00	17.30 ± 3.26	24.42 ± 6
OF explore duration	$F_{3,63}$=0.98 p=0.408	476.94 ± 56.14	499.37 ± 19.95	379.43 ± 41.56	453.81 ± 35.18	492.85 ± 42.61
NO latency	$F_{3,63}$=1.94 p=0.132	104.53 ± 44.68	297.62 ± 75.05	365.95 ± 66.22	339.20 ± 73.52	277.76 ± 69.79
NO interactions	$F_{3,63}$=0.02 p=0.997	30.30 ± 7.02	28.90 ± 16.66	15.70 ± 7.99	9.90 ± 5.13	40.75 ± 2
NO centre duration	$F_{3,63}$=1.47 p=0.231	27.87 ± 12.42	8.07 ± 2.32	3.43 ± 2.30	6.24 ± 2.96	18.09 ± 5
NO centre number	$F_{3,63}$=0.73 p=0.540	12.10 ± 2.82	4.70 ± 1.91	2.90 ± 1.52	2.40 ± 1.37	9.75 ± 3.
NO explore duration	$F_{3,63}$=1.20 p=0.316	435.21 ± 66.03	234.54 ± 76.83	157.71 ± 49.74	124.61 ± 48.76	308.21 ± 61.82
PM latency	$F_{3,63}$=0.15 p=0.932	4.33 ± 0.89	37.31 ± 32.50	63.01 ± 59.67	56.81 ± 50.05	2.40 ± 0.
PM clear duration	$F_{3,63}$=2.45 p=0.072	290.89 ± 39.76	286.68 ± 50.77	135.60 ± 40.52	218.89 ± 59.35	209.61 ± 26.61
PM clear number	$F_{3,63}$=0.62 p=0.605	44.00 ± 20.92	19.50 ± 8.87	7.90 ± 2.31	8.60 ± 2.49	34.50 ± 1

S4. Pearson correlations of all the variables, to investigate associations between behaviours in *R. pumilio* (top) and *R. bechuanae* (bottom). Correlated behaviours are indicative of behavioural syndromes or contextual consistency. Cells highlighted in yellow show a significant relationship, and significant relationships with r > 0.5 are highlighted in bold light blue.

		LD L duration	LD L number	LD D latency	SR L duration	SR L latency	SR L number	OF centre duration	OF centre number	OF explore duration	NO latency	NO inter tions
										R. pumilio		
LD L duration	***R. bechuan***		0.23 p=.330	-0.23 p=.321	**0.62** **p=.004**	-0.26 p=.271	0.27 p=.248	0.37 p=.113	**0.62** **p=.004**	0.37 p=.104	-0.30 p=.197	0. p=.4

		LD L duration	LD L number	LD D latency	SR L duration	SR L latency	SR L number	OF centre duration	OF centre number	OF explore duration	NO latency	NO interactions
		R. pumilio										
LD L number		**0.52** **p=.019**		0.05 p=.843	0.05 p=.846	-0.50 p=.025	**0.79** **p=.000**	-0.03 p=.896	0.14 p=.555	0.14 p=.565	-0.16 p=.509	0. p=.3
LD D latency		**0.60** **p=.005**	0.02 p=.928		0.13 p=.591	-0.20 p=.390	0.22 p=.357	-0.39 p=.088	-0.29 p=.215	0.21 p=.371	-0.03 p=.895	0. p=.6
SR L duration		**0.72** **p=.000**	0.21 p=.371	0.30 p=.193		-0.44 p=.052	0.06 p=.817	0.46 p=.041	0.44 p=.053	**0.71** **p=.000**	-0.36 p=.118	0. p=.3
SR L latency		-0.44 p=.051	-0.40 p=.080	0.03 p=.887	**-0.75** **p=.000**		-0.48 p=.032	-0.36 p=.122	-0.32 p=.176	**-0.58** **p=.007**	0.22 p=.343	-0. p=.3
SR L number		**0.55** **p=.012**	0.44 p=.053	0.04 p=.867	**0.75** **p=.000**	**-0.93** **p=.000**		-0.04 p=.878	0.43 p=.060	0.35 p=.134	-0.37 p=.104	0. p=.1
OF centre duration		0.12 p=.607	-0.15 p=.536	-0.27 p=.246	0.49 p=.029	-0.41 p=.075	0.41 p=.071		0.46 p=.041	0.27 p=.243	-0.05 p=.831	-0. p=.4
OF centre number		-0.33 p=.159	-0.04 p=.882	-0.39 p=.087	0.14 p=.557	-0.40 p=.079	0.23 p=.325	**0.52** **p=.019**		**0.59** **p=.006**	-0.41 p=.071	0. p=.4
OF explore duration		0.02 p=.928	-0.08 p=.740	-0.22 p=.360	0.21 p=.365	-0.26 p=.270	0.18 p=.455	0.37 p=.113	**0.52** **p=.018**		-0.46 p=.043	0. p=.3
NO latency		-0.30 p=.198	-0.18 p=.449	-0.16 p=.505	-0.46 p=.042	0.31 p=.184	-0.34 p=.148	-0.22 p=.346	-0.08 p=.728	-0.17 p=.472		**-0.** **p=.0**
NO interactions		0.18 p=.454	0.49 p=.028	-0.16 p=.489	0.39 p=.092	**-0.57** **p=.009**	0.48 p=.031	0.10 p=.666	0.32 p=.171	0.30 p=.206	**-0.63** **p=.003**	
NO centre duration		0.32 p=.167	0.05 p=.825	0.28 p=.235	0.32 p=.169	0.04 p=.877	-0.10 p=.676	0.09 p=.709	-0.15 p=.524	0.17 p=.471	**-0.54** **p=.015**	0. p=.1
NO centre number		0.18 p=.451	0.23 p=.321	-0.03 p=.914	0.33 p=.149	-0.33 p=.157	0.19 p=.419	0.23 p=.335	0.17 p=.476	0.29 p=.219	**-0.59** **p=.006**	**0.** **p=.0**
NO explore duration		0.32 p=.173	0.38 p=.097	-0.05 p=.843	**0.52** **p=.018**	**-0.63** **p=.003**	**0.54** **p=.013**	0.25 p=.293	0.23 p=.320	0.34 p=.147	**-0.70** **p=.001**	**0.** **p=.0**
PM latency		0.10 p=.671	-0.08 p=.737	-0.18 p=.438	0.12 p=.626	-0.19 p=.424	0.15 p=.528	-0.07 p=.767	-0.16 p=.498	0.22 p=.361	0.09 p=.693	-0. p=.4
PM Clear duration		0.32 p=.172	0.12 p=.609	0.21 p=.374	0.34 p=.143	-0.06 p=.816	0.11 p=.632	0.08 p=.745	-0.18 p=.458	0.04 p=.869	-0.04 p=.865	0. p=.4
PM Clear number		0.36 p=.116	0.47 p=.034	-0.01 p=.955	0.36 p=.122	-0.42 p=.066	**0.54** **p=.014**	0.43 p=.061	0.11 p=.653	-0.08 p=.725	-0.34 p=.143	0. p=.0

S5. Pearson correlations of all the variables, to investigate associations between behaviours in *R. d. dilectus* (Sandveld, top) and *R. d. dilectus* (Entabeni, bottom). Correlated behaviours are indicative of behavioural syndromes or contextual consistency. Cells highlighted in yellow show a significant relationship, and significant relationships with r > 0.5 are highlighted in bold light blue.

		LD L duration	LD L number	LD D latency	SR L duration	SR L latency	SR L number	OF centre duration	OF centre number	OF explore duration	NO latency	NO interactions

		***R. d. dilectus* (Sandveld)**										
LD L duration	***R. d. dilectus* (Entabeni)**	1.0000 p= ---	0.04 p=.865	-0.29 p=.238	0.59 p=.010	-0.36 p=.144	0.08 p=.750	0.41 p=.087	0.22 p=.389	0.22 p=.378	-0.23 p=.353	-0.07 p=.769
LD L number		0.30 p=.317	1.00 p= ---	0.27 p=.276	0.06 p=.816	-0.09 p=.728	0.88 p=.000	-0.09 p=.734	0.80 p=.000	0.22 p=.383	-0.18 p=.482	-0.02 p=.948
LD D latency		0.65 p=.015	-0.06 p=.856	1.00 p= ---	-0.27 p=.287	0.35 p=.151	0.25 p=.314	-0.12 p=.622	0.36 p=.143	0.16 p=.517	-0.02 p=.931	0.59 p=.010
SR L duration		0.66 p=.015	0.27 p=.365	0.54 p=.059	1.00 p= ---	-0.77 p=.000	0.27 p=.276	0.50 p=.035	0.27 p=.276	0.43 p=.076	-0.69 p=.002	0.05 p=.832
SR L latency		-0.55 p=.053	-0.32 p=.281	-0.40 p=.174	-0.91 p=.000	1.00 p= ---	-0.40 p=.098	-0.47 p=.049	-0.32 p=.197	-0.38 p=.122	0.71 p=.001	-0.10 p=.701
SR L number		0.20 p=.523	0.42 p=.159	0.05 p=.865	0.77 p=.002	-0.76 p=.003	1.00 p= ---	-0.02 p=.924	0.83 p=.000	0.22 p=.378	-0.40 p=.103	0.11 p=.663
OF centre duration		0.29 p=.333	-0.08 p=.803	0.60 p=.029	0.21 p=.494	-0.16 p=.593	0.05 p=.872	1.00 p= ---	0.29 p=.248	0.65 p=.003	-0.47 p=.051	0.07 p=.772
OF centre number		-0.35 p=.248	-0.14 p=.659	-0.08 p=.794	-0.33 p=.272	0.32 p=.279	-0.01 p=.983	0.49 p=.087	1.00 p= ---	0.60 p=.009	-0.52 p=.026	0.26 p=.296
OF explore duration		-0.16 p=.611	-0.09 p=.782	-0.15 p=.616	-0.49 p=.088	0.52 p=.069	-0.28 p=.353	0.34 p=.261	0.73 p=.005	1.00 p= ---	-0.48 p=.044	0.26 p=.292
NO latency		0.17 p=.575	0.24 p=.421	0.30 p=.321	0.10 p=.752	-0.22 p=.467	-0.03 p=.926	0.08 p=.792	-0.53 p=.063	-0.31 p=.301	1.00 p= ---	-0.37 p=.135
NO interactions		-0.09 p=.766	-0.15 p=.635	-0.33 p=.264	-0.02 p=.937	0.08 p=.791	0.10 p=.757	0.02 p=.942	0.45 p=.122	0.46 p=.114	-0.75 p=.003	1.00 p= ---
NO centre duration		-0.19 p=.530	-0.17 p=.580	-0.17 p=.569	-0.42 p=.154	0.47 p=.109	-0.25 p=.404	-0.01 p=.984	0.65 p=.017	0.57 p=.041	-0.66 p=.015	0.24 p=.435
NO centre number		-0.33 p=.264	-0.20 p=.508	-0.26 p=.394	-0.36 p=.222	0.32 p=.286	-0.10 p=.736	0.10 p=.747	0.82 p=.001	0.48 p=.096	-0.75 p=.003	0.59 p=.036
NO explore duration		0.01 p=.984	-0.48 p=.099	0.31 p=.296	0.01 p=.984	0.00 p=.997	-0.04 p=.899	0.54 p=.058	0.63 p=.020	0.40 p=.173	-0.41 p=.162	0.50 p=.079
PM latency		0.40 p=.174	0.18 p=.551	0.24 p=.436	0.45 p=.122	-0.36 p=.225	0.29 p=.333	0.12 p=.703	0.07 p=.819	0.22 p=.480	-0.39 p=.190	0.48 p=.100
PM Clear duration		-0.28 p=.348	-0.47 p=.102	-0.47 p=.104	-0.17 p=.579	0.24 p=.430	-0.05 p=.883	-0.33 p=.275	-0.24 p=.423	-0.09 p=.778	-0.06 p=.854	0.14 p=.638
PM Clear number		-0.17 p=.586	0.77 p=.002	-0.27 p=.370	-0.12 p=.686	-0.02 p=.948	0.14 p=.651	-0.32 p=.289	-0.11 p=.728	0.04 p=.899	0.33 p=.266	-0.17 p=.569

THE DEVELOPMENT OF ANXIETY AND EXPLORATION IN TWO SPECIES OF THE AFRICAN STRIPED MOUSE *RHABDOMYS*

Abstract

Genes and the environment interact to produce complex, environmentally relevant behaviours. Most habitats are variable, and behaviour may be modulated by the environment. What is not often known is whether behaviours are set during gestation or modified during early rearing. I tested whether the exploratory behaviour and anxiety of two species of the striped mouse (*Rhabdomys pumilio* and *R. bechuanae*) are modulated by the alteration of their early social rearing environment. The species originated from different habitat types: *R. pumilio* from a semi-arid area and *R. bechuanae* from a grassland. I studied the exploratory behaviour and anxiety responses of adults of both species in an open field and plus maze respectively. I used a cross-fostering technique to establish whether a novel rearing environment influences the behavioural development in each species. I expected that *R. pumilio* would be more exploratory and less anxious in open spaces than *R. bechuanae* and that fostering would modify both behaviours in both taxa. Individuals were cross-fostered between species between 3-5 days, weaned at 20 days and tested as adults (>60 days). My results showed that the species were very similar in their response, but that *R. bechuanae* was slightly more exploratory and less anxious than *R. pumilio* in the open field and novel object tests. Furthermore, fostering between species did not alter the species-typical patterns of exploratory behaviour and anxiety responses. In contrast to findings from earlier studies of striped mice, my study shows that habitat of origin does not influence exploration and anxiety in predictable ways. Moreover, these behaviours were not disrupted by early rearing environments, indicating a strong genetic influence on behavioural development. This could also suggest that the early rearing environments of *R. pumilio* and *R. bechuanae* are not sufficiently different to cause a change in behaviour, especially in *R. pumilio*.

Introduction

The development of behaviour is influenced by the interactions of the genotype and environment of an animal, with the environment often modulating the genetic expression of the behaviour (e.g. norm of reaction; Fuller *et al.* 2005). Therefore, populations of the same species are likely to have behaviours that are specific to different environments they occupy, which maximises fitness (a process called local adaptation; Taylor 1991). For example, in low-density populations of Merriam's kangaroo rats *Dipodomys merriami*, individuals mainly used a larder-hoarding technique to cache their seeds (Murray *et al.* 2006). In comparison, in high-density populations, individuals mainly scatter-hoarded their seeds (Murray *et al.* 2006), with the scatter-hoarding reducing the risk of cache-pilfering by other rodents. Likewise, poeciliid fish *Brachyraphis episcopi* which originated from a population which experienced low predation pressure had better spatial cognition than fish from a high predation population (Brown & Braithwaite 2005).

Because habitats are variable, for example in their predator density (Ferrari *et al.* 2009) or food resources (Lurz *et al.* 2000; Renton 2001), populations of species should differ in their exploratory and/or anxiety related phenotypes, in order to maximise their fitness under the conditions in their habitat. Animals may explore their environment to determine the location of resources (Hughes 1997). For example, snail kites *Rostrhamus sociabilis* explore more when their prey are widely available, but unevenly distributed through the environment (Bennetts & Kitchens 2000). Exploratory behaviour may also assist in territory maintenance and defense. For example, male deer mice *Peromyscus maniculatus bairdii* have greater levels of exploratory behaviour in order to expand their home ranges, compared to male California mice *Peromyscus californicus insignis* (Jašarević *et al.* 2012). Likewise, red-backed salamanders *Plethodon cinereus* use glandular secretions to mark territories and elicit higher exploration in males that smell the secretions (Simons & Felgenhauer 1992). Animals may use exploratory behaviour to determine the location of predators (Archard & Braithwaite 2011). For example, Iberian wall lizards

Podarcis hispanica that were more exploratory were able to assess risk better than lizards that explored less (Rodríguez-Prieto *et al.* 2011).

Animals become anxious in unpredictable situations. For example, C57BL/6 mice that were reared with unpredictable food supply were more anxious than mice that had stable food supply (Coutellier *et al.* 2009). Similarly, environments with high or unpredictable predation regimes, combined with open habitats that offer little cover from predation, may be associated with high anxiety. For example, a high, unpredictable risk of predation increases glucocorticoid levels (associated with anxiety) and decreases reproductive output in snowshoe hares *Lepus americanus* (Sheriff *et al.* 2009). Likewise, house mice *Mus domesticus* had a much higher giving up density (GUD) in open environments that were associated with the risk of predation, compared to areas that were densely covered (Powell & Banks 2004), indicating a higher anxiety in the open areas.

The African striped mouse (genus *Rhabdomys*) has a widespread distribution throughout southern Africa, from the western semi-arid parts of South Africa through to the moist eastern parts of the country (Skinner & Chimimba 2005). The genus is divided into several putative species (du Toit *et al.* 2012a), with *R. pumilio* occupying the western coastal parts of South Africa, two subspecies of *R. dilectus* (*R. d. dilectus* and *R. d. chakae*) inhabiting the northeast and eastern parts, and *R. bechuanae* occupying the central parts of South Africa (du Toit *et al.* 2012a; Meynard *et al.* 2012). *R. bechuanae* is proposed to be ancestral, speciating into *R. pumilio* (and others) approximately 4.3 MYA (du Toit *et al.* 2012a). A more recent (3.09 MYA) radiation of *R. pumilio* into the central grasslands (Ganem *et al.* 2012) resulted in the formation of *R. dilectus* (du Toit *et al.* 2012a). *R. pumilio* from the Northern Cape Province of South Africa occurs in a relatively open habitat with wide spaces between shrubs and other cover (Schradin & Pillay 2004). In contrast, both *R. dilectus* (Schradin & Pillay 2005) and *R. bechuanae* (Dufour *et al.* 2015) occur in areas with much higher levels of continuous grass cover.

Differences between the social and non-social behaviours of *R. pumilio* and *R. d. chakae* have been extensively described previously. *R. pumilio* is facultatively group-living (Schradin & Pillay 2005), with groups consisting of 2-4 breeding females, a breeding male and several philopatric offspring (Schradin & Pillay 2004), and males displaying paternal care in nature (Schradin & Pillay 2003). In contrast, *R. d. chakae* is solitary living, with males and females maintaining intra-sexually non-overlapping territories, but a male's territory may overlap those of several females (Schradin & Pillay 2005). *R. pumilio* is more exploratory and less anxious than *R. d. chakae* in laboratory experiments (Rymer *et al.* 2008; Rymer & Pillay 2012a). The development of exploratory behaviour and anxiety responses in these two species appears to be influenced by an interaction between their genes and their social rearing environment (Rymer & Pillay 2012a): offspring cross-fostered between species showed an intermediate exploratory phenotype to their biological parents. In a previous study, *R. pumilio* individuals were more exploratory and less neophobic than *R. bechuanae* individuals (Chapter 2), but in contrast to *R. pumilio* and *R. dilectus*, little is known about the development of behaviour (especially non-social behaviour) in *R. bechuanae.*

I compared the exploratory behaviour and anxiety of two species of the striped mouse (*R. pumilio* and *R. bechuanae*) and tested whether these behaviours can be modulated by altering their early social rearing environment. Using a cross-fostering technique, I established whether a novel rearing environment influences the behavioural development in each species. I constructed two hypotheses. 1) Because *Rhabdomys* shows variation in behaviour that is linked to environmental variation (Rymer & Pillay 2012a), I hypothesised that there is an environmental component to behaviour, such that *R. pumilio* is more exploratory and less anxious than *R. bechuanae* (similar to *R. d. chakae* from Rymer & Pillay 2012a). 2) I hypothesised that cross-fostering would reveal a gene x environment interaction on behaviour, so that fostered offspring would display an intermediate behaviour phenotype compared to their non-fostered siblings.

Materials and methods

Adult striped mice used in this study originated from the Sandveld Nature Reserve (*R. bechuanae*, Free State Province of South Africa, 27°41'57"S, 25°44'13"E, wild-caught and 1st generation in captivity) and Goegap Nature Reserve (*R. pumilio*, Northern Cape Province, 29°41’33”S, 18°1’41”E, 2nd-3rd generation in captivity). Individuals were housed in the Milner Park Animal Unit at the University of the Witwatersrand, under partially controlled conditions with a 14:10h L:D cycle, lights on at 05h00, at a temperature of 22-25°C, and 30-60%RH. I established breeding pairs which were housed in standard clear lab-o-tec cages (15 x 42 x 15cm). Cages were furnished with wood shavings, a handful of dry grass, tissue paper for nesting material, and a toilet roll and wood block for enrichment. Pairs were fed a handful of Epol™ mouse cubes, 10g of fresh vegetables and 10g of sunflower seeds daily. Water was available *ad libitum*. The study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (2012/27/2A).

Pairs consisted of randomly chosen unrelated individuals. I used 20 litters (10 per species) that were born 2 days apart or less (to prevent rejection by the foster parents; Pillay 2000). I also only used litters that had at least 4 pups, with at least 1 male and 1 female. When litters met the above criteria, all individuals including the parents were marked with non-toxic hair dye (Inecto™), with *R. bechuanae* individuals marked on the head and *R. pumilio* individuals marked at the base of the tail to facilitate species recognition and to reduce the risk of rejection (as all individuals smelled of dye). Litter sizes were not altered in any way. When pups were between 3 and 5 days old, 1 male and 1 female pup were removed from their own litter and placed with their age-matched litter of the opposite species, so that litters donated and received 1 male and 1 female of the opposite species.

The duration of fostering was 15-17 days. At weaning (20 days of age), juveniles were placed individually into lab-o-tec cages with the same furnishings as the breeding pairs. Individuals were fed 10g of Epol™ mouse cubes, 5g of fresh vegetables and 5g of millet daily, with water available *ad libitum*. When subjects were between 60 and 90 days of age, their anxiety and exploratory behaviour were tested in a modified plus maze and the open field/novel object test. Both fostered individuals and two non-fostered individuals (1 male and 1 female if possible) were tested from each litter. Individuals were tested in the plus maze and open field/novel object apparatus on different days, and returned to their home cage immediately after testing. All testing apparatus was cleaned with an odourless disinfectant and water after each use, to remove the odour of the previous occupant.

Plus maze

I used a modified plus maze made from acrylic glass to assess the anxiety of individuals. The plus maze consisted of four enclosed arms, two of which were painted black and two left clear (all 7.5 x 50 x 7.5cm), arranged around a clear, square central chamber (11 x 11 x 15.5cm). The arms were enclosed to prevent test subjects from jumping out of the maze (following Jones *et al.* 2011). I placed test subjects into a cylindrical entry chamber (10cm long, 5.5cm diameter) that was situated above one of the clear arms of the maze. This opened directly into the central chamber. I then left the room and filmed the apparatus from above for 10 minutes, and I later recorded the duration of time that the test subject spent in the clear and dark arms and the number of times the individual entered the clear and dark arms. The duration of time that an individual spent in the centre compartment was not considered in the analysis.

Open field test

The open field test was conducted in an arena (a 46 x 30 x 35cm glass tank) with opaque sides, and the bottom was marked into 9 equal squares with electrical tape. I placed an individual mouse in the centre square (on the bottom of the arena), left the room and video recorded the test for 10 minutes. I later recorded the duration of time that the mouse spent in the centre of the arena and the number of times the individual moved from the periphery into the centre of the arena.

Novel object test

The novel object test followed immediately after the open field test. After 10 minutes in the open field arena, I placed a small table tennis ball (2cm diameter) in the corner of the tank directly opposite to the study subject. The ball was painted with purple, red and blue non-toxic paint and dilute lavender oil to create a novel scent. I then left the room and video recorded the behaviour of the test subject for a further 10 minutes. I later recorded the frequency and duration of interactions with the novel object. Interactions with the novel object were defined as the individual being within one mouse-length of the novel object.

Data analysis

All video recordings were scored using Observer XT software (Noldus 2009), and the statistical analysis done in R software (version 3.3.0; R Core Team 2016). I included both the litter of origin and the litter that an individual was housed with until weaning as random factors, because 2 individuals were used from each litter and also to control for the effect of litter size. Likewise, both the litter of origin and recipient litter may have influenced the offspring behaviour. I checked the model fit for each variable (described below), and used the most appropriate model, based on the plot of the residuals against the fitted values from each model (Crawley 2007). For all variables, I used treatment, sex, species and two-way interactions (treatment*sex, species*sex and treatment*species) as independent factors, but excluded species*treatment*sex which was not relevant to the aims of the study as I was not interested in investigating the differences between sexes among species, between treatments. I generated p values using likelihood ratio tests (Bates *et al.* 2015). Data are presented as notched boxplots with the median and 1st and 3rd interquartiles, with the means shown by a diamond within the boxplot. The notches represent 95% confidence limits (Krzywinski & Altman 2014), and where the statistics indicate significant differences, the notches can be used to indicate where groups are significantly different. A full description of the boxplots and notches is provided in Supplementary Material S1. For non-significant predictors, I constructed tables and provided medians and 1st and 3rd quartiles and presented these as supplementary material.

Plus maze

I analysed the duration of time spent in the clear arms with the *cpglmm* function (compound poisson linear mixed model, from the cplm package; Zhang 2013), the duration of time spent in the dark arms with the *lmer* function and gaussian distribution (lme4 package; Bates *et al.* 2015), the frequency of visits to the clear arms with the *glmer* function and poisson distribution (lme4 package; Bates *et* *al.* 2015), and the frequency of visits to the dark arms with the *glmer* function and a poisson distribution (lme4 package; Bates *et al.* 2015).

Open field test

I analysed the duration of time spent in the centre of the open field with the *lmer* function, and the number of times an individual entered the centre of the open field with the *glmer* function and poisson distribution (both from the lme4 package; Bates *et al.* 2015).

Novel object test

I analysed the duration of time spent interacting with, or in close contact with the novel object with square-root transformed data (in order for the data to approach normality), using the *lmer* function. I analysed the number of times that an individual touched the novel object with the *glmer* function and poisson distribution (both from the lme4 package; Bates *et al.* 2015).

Results

Plus maze

Duration in clear and dark arms

The duration of time spent in the clear arms of the plus maze was not significantly influenced by treatment, species or sex (Supplementary Material 2). The duration of time in the clear arms was also not significantly influenced by treatment*species, or treatment*sex (Supplementary Material 2). Species*sex was a significant predictor of time spent in the clear arms (χ^2_1=6.58, p=0.010, Figure 3.1), with *R. bechuanae* females spending significantly less time in the clear arms than *R. bechuanae* males, but *R. bechuanae* males and females were not significantly different to *R. pumilio* males and females. The duration of time spent in the dark arms of the plus maze was not significantly influenced by treatment, species, sex, treatment*species, treatment*sex, or species*sex (Supplementary Material 3).

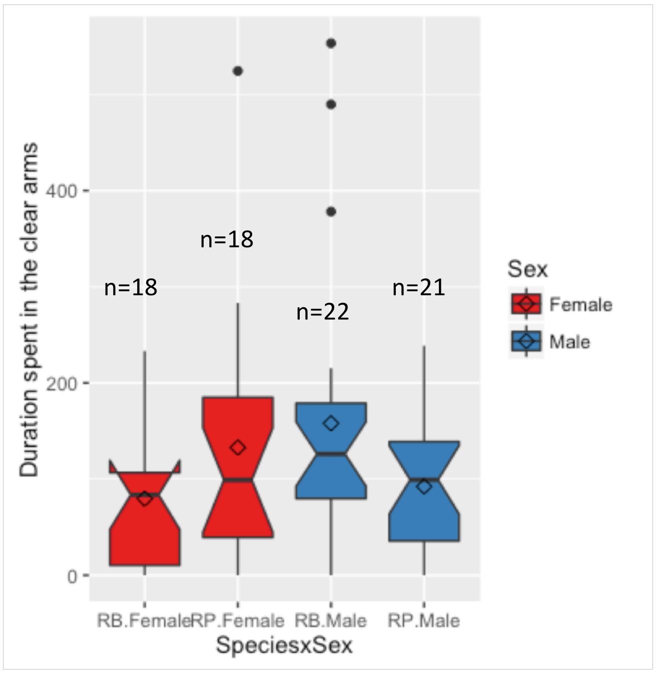

Figure 3.1. The duration spent in the clear arms of the plus maze by both sexes of *R. bechuanae* (RB) and *R. pumilio* (RP), combining fostered and non-fostered individuals. A full description of the boxplots is provided in the supplementary material.

Number of entries into clear and dark arms

Treatment, species, treatment*species, treatment*sex and species*sex (Supplementary Material 4) were not significant predictors of the number of entries into the clear arms of the plus maze, but the number of entries into the clear arms of the plus maze was significantly influenced by sex (χ^2_1=5.10, p=0.024, Figure 3.2), with females entering the clear arms more often than males. The number of entries into the dark arms of the plus maze was not significantly influenced by treatment, species, treatment*species, and treatment*sex (Supplementary Material 5). However, both sex (χ^2_1=22.13, p<0.001, Figure 3.3) and species*sex (χ^2_1=7.35, p=0.007, Figure 3.3) were significant predictors of the number of dark arm entries, with females entering the dark arms significantly more frequently than males, and *R. pumilio* females entering the dark arms significantly more often than *R. bechuanae* males, but not significantly more often than *R. bechuanae* females or *R. pumilio* males.

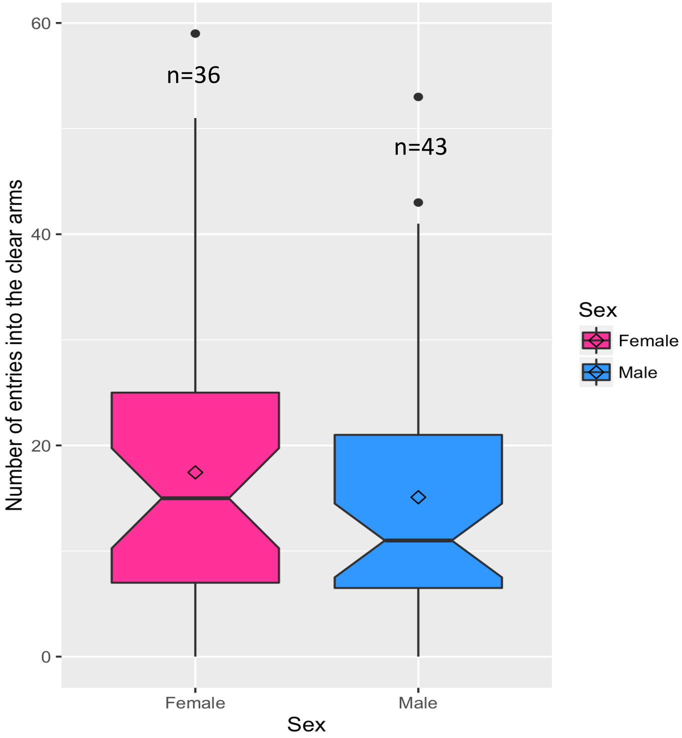

Figure 3.2. The number of entries into the clear arms of the plus maze by *Rhabdomys* males and females. A full description of the boxplots is provided in the supplementary material.

Open field test

Duration in the centre

The duration of time spent in the centre of the open field arena was not significantly influenced by treatment, species, sex, treatment*species, treatment*sex, or species*sex (Supplementary Material 6).

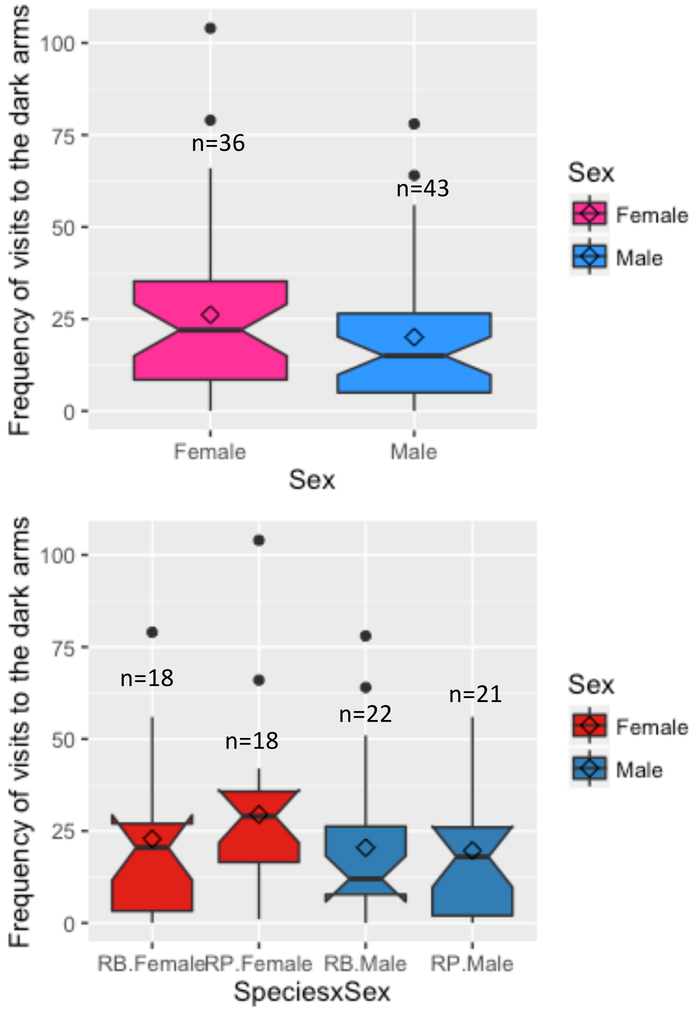

Figure 3.3. The frequency of entries into the dark arms of the plus maze by *Rhabdomys* males and females (top) and by male and female *R. pumilio* and *R. bechuanae* (bottom). A full description of the boxplots is provided in the supplementary material.

Frequency of centre entries

In contrast to the duration spent in the centre, treatment (χ^2_1=10.99, p<0.001), species (χ^2_1=6.14, p=0.014), sex (χ^2_1=19.23, p<0.001), and treatment*sex (χ^2_1=42.94, p<0.001) were significant predictors of the number of entries into the centre of the open field arena (Figure 3.4). Fostered individuals entered the centre significantly more than non- fostered individuals, *R. bechuanae* individuals entered the centre more than *R. pumilio* individuals, males entered the centre significantly more than females, and fostered males entered the centre significantly more frequently than fostered females and non-fostered males, but not significantly more than non-fostered females. Treatment*species and species*sex (Supplementary

Material 7) were not significant predictors of the number of times an individual entered the centre of the arena.

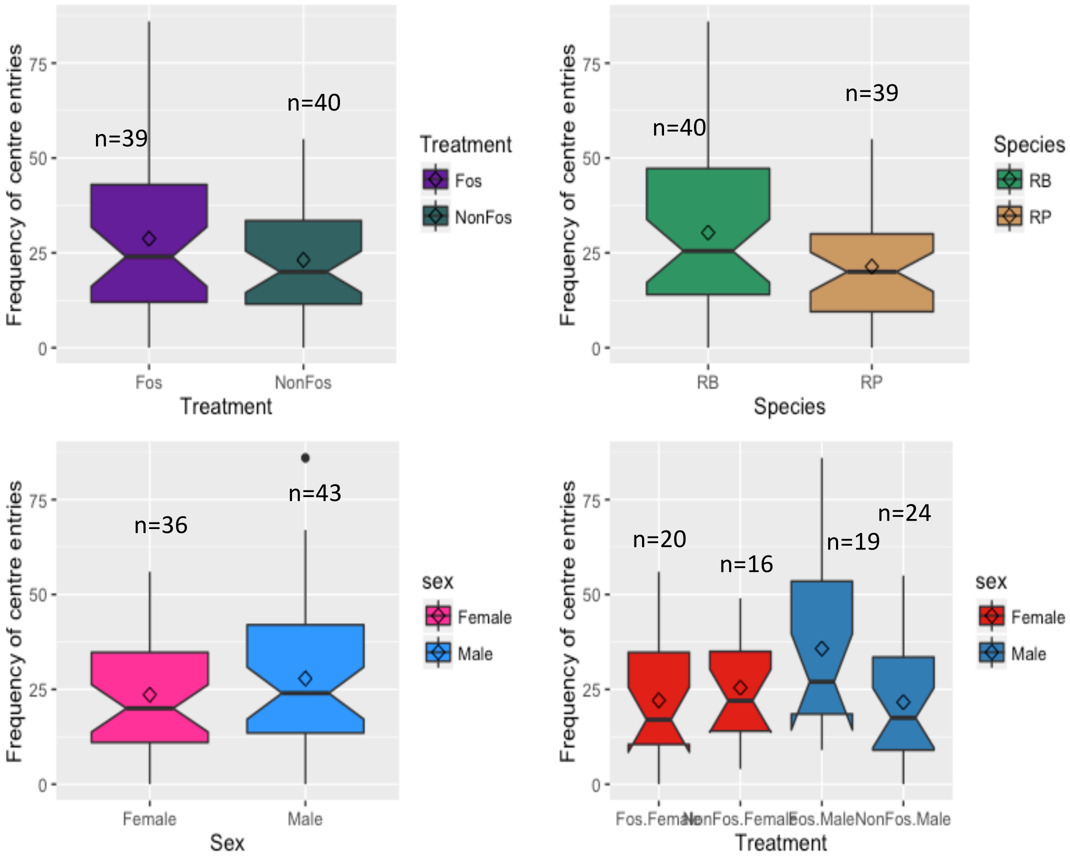

Figure 3.4. The number of entries into the centre of the open field, by fostered (Fos) and non-fostered (NonFos) individuals (top left), *R. bechuanae* and *R. pumilio* individuals (top right), males and females (bottom left) and by fostered and non-fostered males and females (bottom right). A full description of the boxplots is provided in the supplementary material.

Novel object

Duration spent in contact with the novel object

The duration of time spent with the novel object was not significantly influenced by treatment, species, sex, treatment*species, treatment*sex or species*sex (Supplementary Material 8).

Number of interactions with the novel object

The number of interactions with the novel object was significantly influenced by treatment (χ^2_1=32.88, p<0.001, Figure 3.5), with non-fostered individuals interacting with the novel object significantly more than fostered individuals. Species was a significant predictor of the number of interactions (χ^2_1=8.03, p=0.005, Figure 3.5), with *R. bechuanae* individuals interacting with the novel object significantly more than *R. pumilio* individuals. The number of object interactions was also significantly predicted by species*sex (χ^2_1=28.99, p<0.001, Figure 3.5), with *R. bechuanae* females interacting with the novel object significantly more than *R. pumilio* females, but not significantly more than males of both species. Sex, treatment*species, and treatment*sex (Supplementary Material 9) were not significant predictors of the number of interactions with the novel object.

Discussion

I investigated species differences and the development of exploratory behaviour and anxiety responses in two species of *Rhabdomys*. In the plus maze, increased time spent in, and higher numbers of entries into the clear arms usually indicates lower levels of anxiety (Carola *et al.* 2002). Similarly, increased numbers of entries into the centre of the open field arena reflects higher exploration, and a longer duration of time spent in the centre of the open field arena indicates lower anxiety (Prut & Belzung 2003). Likewise, increased duration of time spent with a novel object, as well as higher numbers of interactions with the novel object usually indicate decreased neophobia (Martins *et al.* 2012) and anxiety (Belzung & Le Pape 1994). There were no significant differences between the species or treatments in

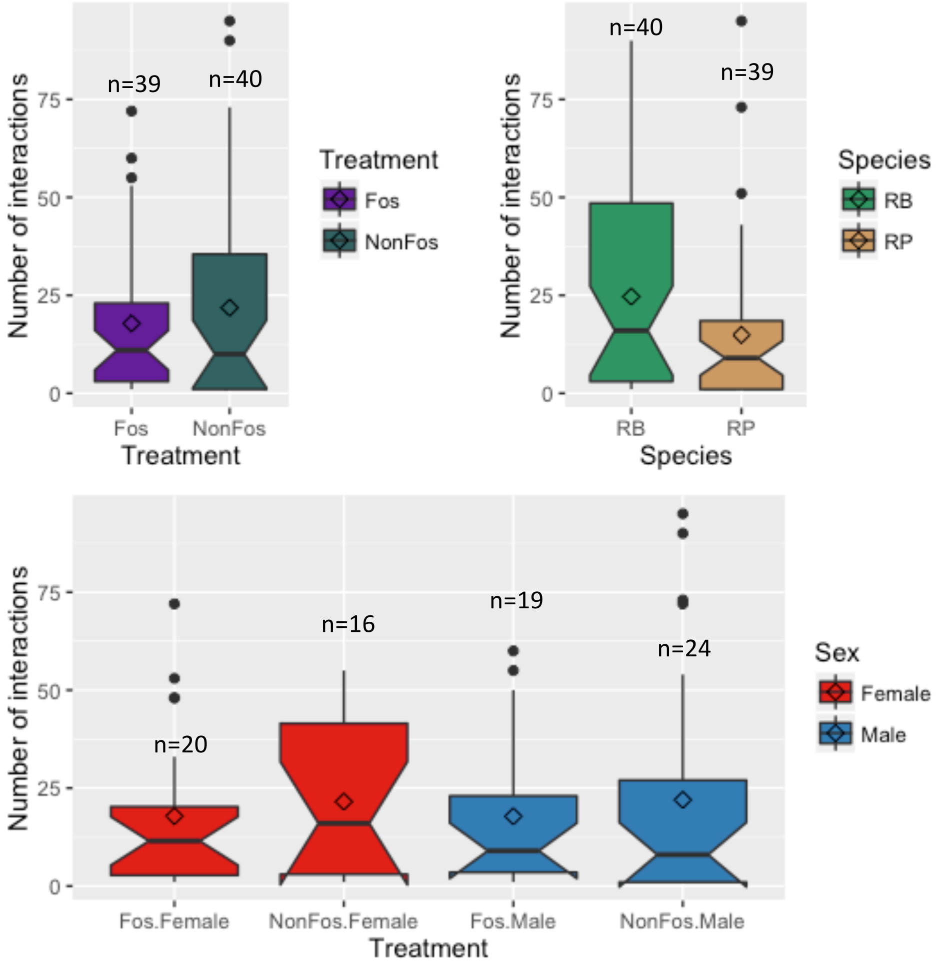

Figure 3.5. The number of interactions with the novel object by fostered (Fos) and non-fostered (NonFos) individuals (top left), *R. bechuanae* and *R. pumilio* (top right), and by males and females from fostered and non-fostered treatments (bottom). A full description of the boxplots is provided in the supplementary material.

the plus maze, indicating no significant differences in anxiety, causing me to reject my hypothesis that there is an environmental component to behaviour. In the open field and novel object test, *R. bechuanae* was significantly less anxious and more exploratory than *R. pumilio* by showing greater number of entries into the centre of the open field arena and a higher frequency of interactions with the novel object. Fostering did not influence behaviour in the plus maze and produced inconsistent results in the open field and novel object tests: fostered individuals made more entries into the centre of the arena but interacted with the novel object less than non-fostered individuals. This also causes me to reject my second hypothesis that cross-fostering would reveal a gene x environment interaction. There were also inconsistent sex effects, where females were significantly less anxious (but more active overall) in the plus maze, but significantly more anxious by entering the centre of the open field more often than males. There were also inconsistent differences between sexes within species, and within sexes between treatments.

Rymer and Pillay (2012a) found that the *R. pumilio*, which originates from an open habitat, was more exploratory and less anxious in open spaces than *R. d. chakae* which originates from a closed habitat (Rymer & Pillay 2012a). In that study, *R. pumilio* spent as much as 50% of the time exploring and 25% more time in the clear arms of a plus maze (Rymer & Pillay 2012a). Striped mice were maintained under the same conditions as Rymer and Pillay (2012a), and therefore the differences in behaviour between the studies remains to be explored in future.

In my study, the species were largely similar for most of measurements recorded, with only two incidences of lower anxiety in *R*. *bechuanae* compared to *R. pumilio*. My data do not support my prediction that *R. pumilio* would be more exploratory and less anxious than *R*. *bechuanae*, in concordance with the environment-related differences in exploratory behaviour and anxiety suggested by Rymer and Pillay (2012a). There are two explanations for the similarities in responses. Firstly, it is possible that the tests were not able to detect species level differences. This is unlikely given the findings in several *Rhabdomys* studies (e.g. Rymer & Pillay 2012a; Yuen *et al.* 2015; Joshi & Pillay 2016a; Joshi & Pillay 2016b). Secondly, the species I studied are closely related (du toit *et al*. 2012a; Ganem *et al.* 2012; Meynard *et al.* 2012) and it is possible that the exploratory behaviour and anxiety responses are largely phylogenetically constrained.

The slight differences between species were contrary to my predictions: *R. bechuanae* was less anxious than *R. pumilio*. This was unexpected given that *R. d. chakae* (the grassland counterpart to *R. bechuanae*) is more anxious than *R. pumilio* (Rymer & Pillay 2012a). It is possible that *R. bechuanae* developed reduced anxiety in response to specific selection pressures in its habitat. For example, the grassland habitats of *R. bechuanae* are patchy, interspersed with open areas (Dufour *et al.* 2015), whereas grassland habitats inhabited by *R. d. chakae* provide continuous cover (Ganem *et al.* 2012). If so, *R. bechuanae* occupies an intermediate habitat type where a less anxious behavioural phenotype might be selected for.

The different results from the plus maze and the open field and novel object tests, where the plus maze showed no difference between species, in contrast to the open field and novel object test, were also unexpected, because both tests apparently measure anxiety (Ramos & Mormède 1997; Carola *et al.* 2002; Murphy *et al.* 2014). However, there is some evidence that these tests do not measure exactly the same trait (Ramos *et al.* 2008), with some studies suggesting that situations in which the individual is forced to confront novelty (i.e. plus maze and open field test) measures state anxiety (i.e. the anxiety that an individual shows at a particular point; Goes *et al.* 2015), while free exploration of novelty measures trait anxiety (i.e. the inherent anxiety of an individual; Belzung & Le Pape 1994; Goes *et al.* 2015). However, this does not explain the similarity of results in the open field and novel object tests, and thus it remains unclear why the results of the plus maze are different to the results of the open field test.

Like the species level differences, fostering, which considers the influence of early rearing environments, had small and inconsistent influences on the development of exploratory behaviour and anxiety responses. This is in contrast to Rymer and Pillay's (2012a) findings, which showed that fostered striped mice had an intermediate phenotype between the biological and fostered siblings. Other studies have also shown that the behaviour of cross-fostered offspring differed from both their biological parents and siblings, and their behaviour was different to that of their foster parents and foster siblings (e.g. Zebra finch *Taeniopygia guttata* song learning; Woodgate *et al.* 2014). Although there is evidence that the early rearing environment influences behaviour, there is also evidence in other taxa that the postnatal environment influences behaviour to a far lesser degree. For example, cross-fostered Japanese (*Macaca fuscata*) and rhesus macaques (*M. mulatta*) both retained their species-specific call types in comparison to their non-fostered counterparts (Owren *et al.* 1993), indicating a strong genetic component to the development of calls. The similar behaviour between fostered and non-fostered individuals within *R. bechuanae* and *R. pumilio* indicates that exploratory behaviour and anxiety are not altered in a fostered environment and thereby indicate a stronger genetic influence on development (Kruuk & Hadfield 2007).

While learning may be possible in a fostered environment, there was no phenotypic change between fostered and non-fostered individuals, implying that there was no learning. Furthermore, the lack of significant differences resulting from fostering also suggests that the rearing environments are sufficiently similar to not influence behavioural development, at least of the behaviours recorded here. In the earlier study of *R. pumilio* and *R. d. chakae*, differences in the parental rearing environment between species may have accounted for some of the environmental effects on the behaviour of *R. pumilio* and *R. d. chakae* (Rymer & Pillay 2012a). However, there are no obvious differences in the quality of maternal care between *R. pumilio* and *R. d. chakae* (N. Pillay, *pers. comm.*), which possibly means that the maternal environments are similar between species. Nonetheless, the fostering process can be stressful, which might explain the inconsistent findings in the open field and novel object tests. For example, male Swiss CD1 laboratory mice fostered to unrelated Swiss CD1 mothers were more stressed in adulthood compared to individuals that were removed from their nests and replaced with their own mothers (Bartolomucci *et al.* 2004). However, Rymer and Pillay (2012a) did not find a fostering effect when they fostered individuals to other litters of the same species, possibly indicating that the fostering procedure itself does not influence *Rhabdomys* behaviour.

In light of the inconsistent results on fostering, sex may play a large role in determining the individual response to the tests, similar to the results in Chapter 5. The significant treatment*sex effect in open field test, where fostered males were more explorative in the open field than fostered females and non-fostered males, possibly indicates that fostered males have a similar exploratory phenotype to females, regardless of treatment. In *R. pumilio*, parental care displayed later on by males (i.e. sons) depends on the level of maternal care they received themselves postnatally, while maternal care received appears to have little effect on later female (i.e. daughters) maternal care behaviour (Rymer & Pillay 2011; Rymer & Pillay 2012b), indicating a sex determined response to the postnatal environment.

Therefore, males appear to be more influenced by the treatment (fostering vs non-fostering), while the species seems to predict female response in the behaviour tests (see below).

Females were significantly less anxious in the plus maze but also significantly less exploratory in the open field/novel object arena than males, with males entering the centre of the open field more than females, but spending a shorter duration in the centre, indicating that overall, females were less anxious than males. When I considered the species*sex effect, *R. bechuanae* females were significantly less neophobic and significantly more exploratory in the open field/novel object arena than *R. pumilio* females and all males. However, when sex was not taken into consideration, the behaviour of *R. pumilio* and *R. bechuanae* in the open field and plus maze were not significantly different. Taken together, these results could possibly indicate that *R. bechuanae* females were driving the sex effect, as they were much less anxious than all other groups.

In conclusion, the general similarities in the behaviour between *R. bechuanae* and *R. pumilio* suggest that the development of exploratory behaviour and anxiety responses are possibly phylogenetically constrained in *Rhabdomys*. Moreover, my study indicates that fostering does not create a sufficiently novel environmental influence on the development of behaviours in the two species. I found a strong sex-dependent influence, whereby fostering treatment was a significant predictor of male responses, while species was a significant predictor of female responses, suggesting a complex sex-determined phenotypic expression of behaviour. Nonetheless, my study cannot discount potential convergence or potential adaptive variation (i.e. differences in the open field and novel object tests) in these behaviours in allopatry, which should be considered in future studies of several populations of these species.

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Supplementary Material 1

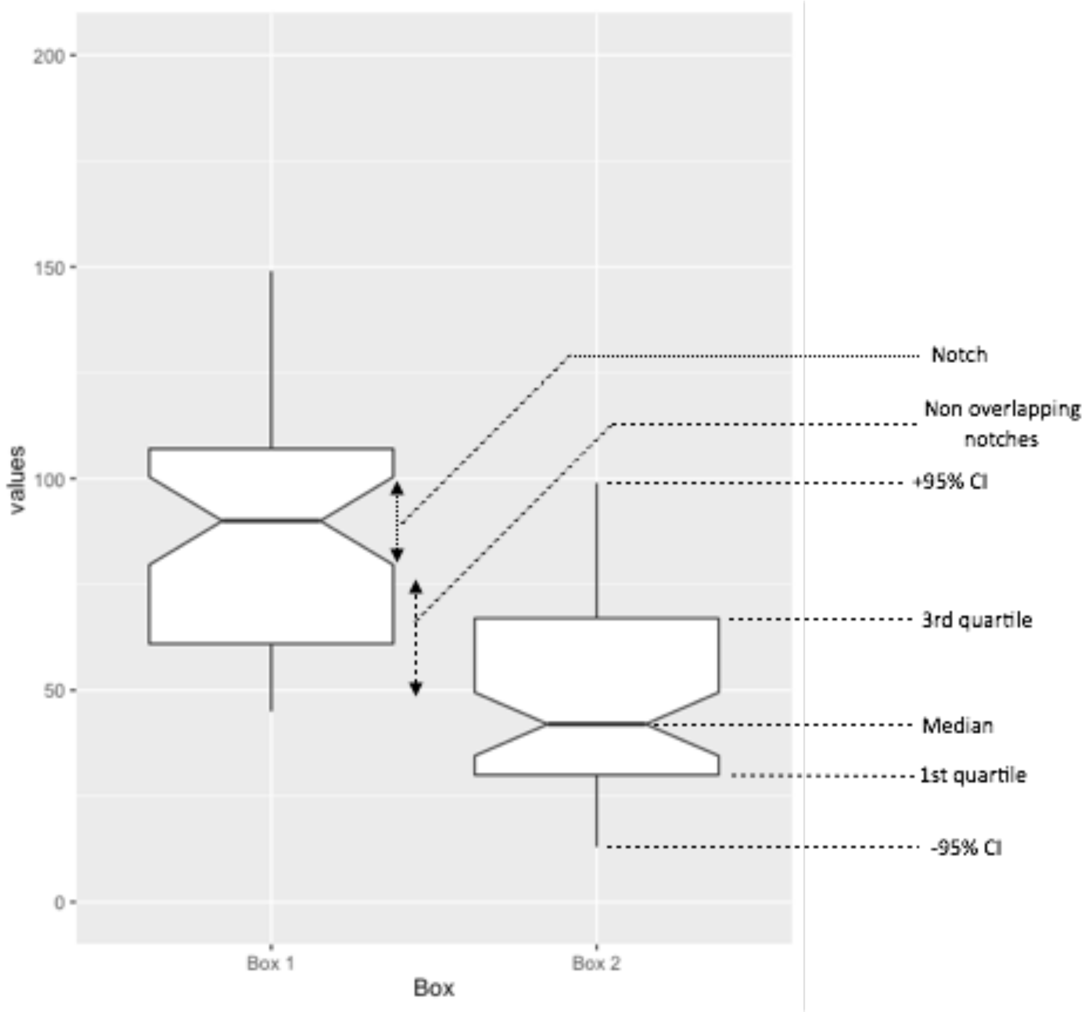

Figure S1. Hypothetical boxplot. Boxes show medians (middle of boxes) and 1st (top box) and 3rd (bottom box) quartiles. Whiskers show confidence limits, and dots outside of boxes indicate outliers. Non-overlapping notches indicate differences between groups.

Supplementary Material 2

Model outcomes (Table S1A) and data summary (Table S1B) for non-significant factors influencing the duration spent in the clear arms of the plus maze, by male and female *R. bechuanae* (RB) and *R. pumilio* (RP) that were fostered or non-fostered (i.e. remained with their litter of origin).

Table S1A. Model outcomes for non-significant factors influencing the duration spent in the clear arms of the plus maze.

Predictor	**Statistics**
Treatment	χ^2_1=0.03, p=0.865
Species	χ^2_1=0.14, p=0.709
Sex	χ^2_1=0.676, p=0.411
Treatment*species	χ^2_1=0.01, p=0.984
Treatment*sex	χ^2_1=0.11, p=0.739

Table S1B. Data summary for the non-significant factors influencing the duration spent in the clear arms of the plus maze.

Level	**Duration in the clear arms**		
	Median	**1st quartile**	**3rd quartile**
Fostered	96.76	57.05	172.57
Non-fostered	105.42	17.30	139.71
RB	99.37	57.31	144.12
RP	99.22	36.73	159.14
Female	91.21	36.71	155.24
Male	110.31	57.05	144.12
Fostered RB	96.49	60.54	169.87
Fostered RP	99.22	49.85	174.35
Non-fostered RB	105.42	36.82	142.04
Non-fostered RP	101.02	0.00	138.95
Fostered Female	93.20	41.42	167.34
Fostered Male	101.97	60.99	175.72
Non-fostered Female	80.68	28.33	145.99
Non-fostered Male	116.69	17.30	138.95

Supplementary Material 3

Model outcomes (Table S2A) and data summary (Table S2B) for non-significant factors influencing the duration spent in the dark arms of the plus maze by male and female fostered and non-fostered *R. pumilio* (RP) and *R. bechuanae* (RB).

Table S2A. Model outcomes for non-significant factors influencing the duration spent in the dark arms of the plus maze.

Predictor	**Statistics**
Treatment	χ^2_1=2.08, p=0.149
Species	χ^2_1=0.14, p=0.708
Sex	χ^2_1=0.16, p=0.687
Treatment*species	χ^2_1=0.01, p=0.956
Treatment*sex	χ^2_1=3.24, p=0.072
Species*sex	χ^2_1=3.25, p=0.071

Table S2B. Data summary for non-significant factors influencing the duration spent in the dark arms of the plus maze.

Level	**Duration in the dark arms**		
	Median	**1st quartile**	**3rd quartile**
Fostered	228.66	173.01	282.72
Non-fostered	262.57	191.48	407.81
RB	237.14	30.50	308.32
RP	235.81	194.10	338.99
Female	228.99	181.93	337.87
Male	240.87	189.67	307.87
Fostered RB	208.00	163.22	280.29
Fostered RP	236.75	199.62	292.59
Non-fostered RB	268.33	185.20	354.64
Non-fostered RP	233.64	194.75	452.51
Fostered Female	228.99	181.73	357.62
Fostered Male	222.73	171.10	252.20
Non-fostered Female	218.06	183.55	302.21
Non-fostered Male	268.33	208.03	462.70
RB Female	237.14	184.78	531.48
RB Male	231.80	170.75	170.75
RP Female	200.93	178.12	318.08
RP Male	247.18	222.33	404.34

Supplementary Material 4

Model outcomes (Table S3A) and data summary (Table S3B) for non-significant factors influencing the number of entries into the clear arms of the plus maze by male and female fostered and non-fostered *R. pumilio* (RP) and *R. bechuanae* (RB).

Table S3A. Model outcomes for non-significant factors influencing the number of entries into the clear arms of the plus maze**.**

Predictor	**Statistics**
Treatment	χ^2_1=0.01, p=0.963
Species	χ^2_1=3.72, p=0.054
Treatment*species	χ^2_1=0.69, p=0.407
Treatment*sex	χ^2_1=2.13, p=0.144
Species*sex	χ^2_1=1.45, p=0.229

Table S3B. Data summary for non-significant factors influencing the number of entries into the clear arms of the plus maze.

Level	**Number of entries into the clear arms**		
	Median	**1st quartile**	**3rd quartile**
Fostered	13.00	9.00	21.50
Non-fostered	13.00	4.25	21.75
RB	11.00	6.75	19.25
RP	17.00	7.50	28.00
Fostered RB	12.5.0	8.50	20.25
Fostered RP	17.00	10.00	26.00
Non-fostered RB	10.50	5.00	16.00
Non-fostered RP	18.00	0.75	31.25
Fostered Female	15.50	8.75	25.00
Fostered Male	12.00	11.00	20.50
Non-fostered Female	14.50	5.75	22.00
Non-fostered Male	11.00	4.00	21.75
RB Female	13.00	3.25	19.75
RB Male	11.00	7.00	13.00
RP Female	20.00	8.25	29.50
RP Male	16.00	3.00	24.00

Supplementary Material 5

Model outcomes (Table S4A) and data summary (Table S4B) for non-significant factors influencing the number of entries into the dark arms of the plus maze by male and female fostered and non-fostered *R. pumilio* (RP) and *R. bechuanae* (RB).

Table S4A. Model outcomes for non-significant factors influencing the number of entries into the dark arms of the plus maze.

Predictor	**Statistics**
Treatment	χ^2_1=0.44, p=0.509
Species	χ^2_1=0.55, p=0.460
Treatment*species	χ^2_1=0.30, p=0.585
Treatment*sex	χ^2_1=2.39, p=0.122

Table S4B. Data summary for non-significant factors influencing the number of entries into the dark arms of the plus maze.

Level	**Number of entries into the dark arms**		
	Median	**1st quartile**	**3rd quartile**
Fostered	22.00	11.00	32.50
Non-fostered	15.50	1.75	31.00
RB	15.00	6.00	27.00
RP	22.00	8.50	34.50
Fostered RB	16.00	10.75	34.75
Fostered RP	22.00	17.00	32.00
Non-fostered RB	13.50	2.50	27.00
Non-fostered RP	19.00	1.75	36.75
Fostered Female	22.00	14.50	33.50
Fostered Male	18.00	11.00	28.50
Non-fostered Female	24.00	3.50	36.75
Non-fostered Male	14.00	1.00	26.25

Supplementary Material 6

Model outcomes (Table S5A) and data summary (Table S5B) for non-significant factors influencing the duration of time spent in the centre of the open field by male and female fostered and non-fostered *R. pumilio* (RP) and *R. bechuanae* (RB).

Table S5A. Model outcomes for non-significant factors influencing the duration spent in the centre of the open field.

Predictor	**Statistics**
Treatment	χ^2_1=0.07, p=0.794
Species	χ^2_1=0.07, p=0.790
Sex	χ^2_1=0.01, p=0.963
Treatment*species	χ^2_1=2.92, p=0.087
Treatment*sex	χ^2_1=3.19, p=0.074
Species*sex	χ^2_1=0.94, p=0.334

Table S5B. Data summary for non-significant factors influencing the duration spent in the centre of the open field.

Level	**Duration in the centre**		
	Median	**1st quartile**	**3rd quartile**
Fostered	36.16	23.15	43.24
Non-fostered	29.54	15.86	51.81
RB	36.19	17.82	47.50
RP	32.94	16.29	42.94
Female	33.14	15.31	45.01
Male	34.40	19.13	43.12
Fostered RB	39.23	30.92	47.50
Fostered RP	31.95	16.03	39.84
Non-fostered RB	19.89	14.61	44.82
Non-fostered RP	34.08	17.55	51.81
Fostered Female	35.74	15.05	43.33
Fostered Male	36.22	31.58	43.12
Non-fostered Female	30.63	16.88	60.98
Non-fostered Male	25.69	15.71	42.83
RB Female	37.85	15.37	57.37
RB Male	31.01	18.32	41.14
RP Female	26.59	15.60	35.02
RP Male	36.72	26.81	45.22

Supplementary Material 7

Model outcomes (Table S6A) and data summary (Table S6B) for non-significant factors influencing the number of entries into the centre of the open field by male and female fostered and non-fostered *R. pumilio* (RP) and *R. bechuanae* (RB).

Table S6A. Model outcomes for non-significant factors influencing the number of entries into the centre of the open field.

Predictor	**Statistics**
Treatment*species	χ^2_1=3.71, p=0.054
Species*sex	χ^2_1=3.56, p=0.059

Table S6B. Data summary for non-significant factors influencing the number of entries into the centre of the open field.

Level	**Number of entries into the centre**		
	Median	**1st quartile**	**3rd quartile**
Fostered RB	36.00	17.75	52.75
Fostered RP	18.00	10.00	28.00
Non-fostered RB	20.50	13.50	36.50
Non-fostered RP	20.00	9.75	32.00
RB Female	28.50	14.75	41.50
RB Male	23.50	14.00	51.00
RP Female	13.00	9.25	27.75
RP Male	24.00	11.00	34.00

Supplementary Material 8

Model outcomes (Table S7A) and data summary (Table S7B) for non-significant factors influencing the duration of time spent in contact with the novel object by male and female fostered and non-fostered *R. pumilio* (RP) and *R. bechuanae* (RB).

Table S7A. Model outcomes for non-significant factors influencing the duration spent with the novel object.

Predictor	**Statistics**
Treatment	χ^2_1=0.08, p=0.777
Species	χ^2_1=1.94, p=0.164
Sex	χ^2_1=1.65, p=0.197
Treatment*species	χ^2_1=0.52, p=0.470
Treatment*sex	χ^2_1=0.52, p=0.472
Species*sex	χ^2_1=2.32, p=0.128

Table S7B. Data summary for non-significant factors influencing the duration spent with the novel object.

Level	**Duration spent with the novel object**		
	Median	**1st quartile**	**3rd quartile**
Fostered	36.16	9.63	64.73
Non-fostered	29.54	0.00	56.07
RB	36.19	6.75	71.22
RP	32.94	0.00	48.09
Female	33.14	9.70	71.22
Male	34.40	0.00	56.45
Fostered RB	39.23	12.00	65.89
Fostered RP	31.95	8.12	56.85
Non-fostered RB	19.89	9.14	78.68
Non-fostered RP	34.08	0.00	45.14
Fostered Female	35.74	8.72	67.56
Fostered Male	36.22	11.99	62.60
Non-fostered Female	30.63	13.67	85.65
Non-fostered Male	25.69	0.00	41.21
RB Female	37.85	21.93	115.05
RB Male	31.01	2.36	2.36
RP Female	26.59	4.25	48.37
RP Male	36.72	0.00	44.35

Supplementary Material 9

Model outcomes (Table S8A) and data summary (Table S8B) for non-significant factors influencing the number of interactions with the novel object by male and female fostered and non-fostered *R. pumilio* (RP) and *R. bechuanae* (RB).

Table S8A. Model outcomes for non-significant factors influencing the number of interactions with the novel object.

Predictor	**Statistics**
Sex	χ^2_1=0.03, p=0.874
Treatment*species	χ^2_1=1.47, p=0.225
Treatment*sex	χ^2_1=0.02, p=0.899

Table S8B. Data summary for non-significant factors influencing the number of interactions with the novel object.

Level	**Number of interactions with the novel object**		
	Median	**1st quartile**	**3rd quartile**
Female	11.00	2.00	33.75
Male	7.00	0.00	25.00
Fostered RB	16.00	2.00	47.50
Fostered RP	5.00	1.50	11.00
Non-fostered RB	11.00	1.50	41.50
Non-fostered RP	9.00	0.00	27.75
Fostered Female	10.50	1.75	19.25
Fostered Male	8.00	2.50	22.00
Non-fostered Female	15.50	2.00	39.75
Non-fostered Male	6.00	0.00	27.75

ANXIETY AND EXPLORATORY BEHAVIOUR IN THE AFRICAN STRIPED MOUSE *RHABDOMYS* ARE NOT MODIFIED BY THE PHYSICAL REARING ENVIRONMENT

Abstract

Genes and the environment usually interact to produce behaviours that are environmentally appropriate, with the environment modulating genetic influences of behavioural expression. Behaviour may be set either during early development or modified by the rearing environment. I tested whether the physical rearing environment (without changing complexity) modulates the anxiety and exploratory behaviour of four populations, representing three taxa, of the striped mouse *Rhabdomys*. The populations originated from different habitat types: *R. pumilio* from a semi-arid, open habitat, and two *R. bechuanae* populations and a *R. d. dilectus* population (sympatric with one of the *R. bechuanae* populations) all originating from habitats with continuous grass cover. I raised individuals from all four populations in captivity under either cover or no cover for two generations. Then, using open field, light-dark and startle response tests, I assessed the anxiety responses and exploratory behaviour of adults from both treatments. I expected that *R. pumilio* would be the least anxious and most exploratory compared to all grassland populations when raised under no cover, and that all populations would have increased anxiety and decreased exploration when raised under high cover. Contrary to my predictions, all individuals reared under high cover were both significantly more anxious and more exploratory, regardless of population and generation. In contrast to previous studies, *R. pumilio* was significantly less exploratory than all of the grassland populations. Furthermore, the treatment (both cover and no cover) appeared to reduce the differences in behaviour previously reported between populations, suggesting that the physical environment has a small influence (positive gene x environment influence), but that phylogeny may constrain the behaviour of *Rhabdomys.*

Introduction

Interactions between an individual's genotype and the environment influence the behaviour of animals (Thoday 1965), often in unpredictable ways (reviewed in Hershberger 1990). The environment may modulate the genetic expression of a behaviour in a norm of reaction (genotypes producing different phenotypes in different environments; Fuller *et al.* 2005), but the degree (and direction) to which a behaviour is influenced depends on the extent of genetic and environmental influences (Plomin *et al.* 1980). For example, two genotypes may produce similar phenotypes in one environment and, in a different environment, one genotype could produce a similar phenotype to the first environment (i.e. phenotypically stable; Zewdie & Bosland 2000), suggesting this genotype is under limited environmental control. The second genotype could produce a different phenotype to the first environment, suggesting that the environment has an important influence on the phenotype (Plomin *et al.* 1980; Fuller *et al.* 2005). In addition, the environment may interact with genotype to produce a phenotype that is modulated (i.e. decrease in the level of a behaviour) or produce an intensified phenotype (Plomin *et al.* 1980).

Because of gene-environment interactions, different populations of the same species may have behaviours that are specific to their environments, which may maximise fitness (known as local adaptation; Taylor 1991). For example, populations of killifish *Fundulus heteroclitus* are locally adapted to either fresh or brackish water, measured by assessing swimming performance (time and distance until an individual is exhausted) in both salty water and fresh water (Brennan *et al.* 2016). Fish from brackish-water populations performed significantly better than fresh-water populations in high salinity conditions (Brennan *et al.* 2016). Another example is that of mole rats *Spalax ehrenbergi* in which individuals that originated from a more humid environment had higher levels of exploratory behaviour (greater distance explored, lower latency to enter an unfamiliar area) compared to populations that originated from drier regions, possibly as an adaptation to conserve water and energy in the drier regions (Heth *et al.* 1987).

Finding food (e.g. Vonshak *et al.* 2009) and evading predators (e.g. Brown *et al.* 1988) present two major daily challenges for small mammals. Habitats are usually variable in their available resources and predatory risks (Kotler *et al.* 1991; Kotler *et al.* 1994; Abu Baker & Brown 2011; Perea *et al.* 2011), and the variability of these factors results in population specific differences in exploratory behaviour and anxiety responses. Animals explore their environment in order to determine the location of resources (Hughes 1997), and food resources that are unevenly or sparsely distributed increase exploration (Bennetts & Kitchens 2000). For example, great tits *Parus major* that experienced food restriction explored more than birds that did not experience food restriction (Carere *et al.* 2005). Predation risk influences behaviour by increasing vigilance, which reduces the time available to forage (Lima & Dill 1990; Lima & Bednekoff 1999; Ferrari *et al.* 2009). Therefore, high predation pressure should select for individuals that show increased risky behaviour (increased boldness and decreased anxiety) in order to forage (e.g. Brydges *et al.* 2008).

The availability of cover influences the behaviour of small mammals because it reduces predation risk by reducing visibility to predators or provides a means of escape (Jensen *et al.* 2003). For example, dune hairy-footed gerbils *Gerbillurus tytonis* prefer to forage closer to covered areas than further away (Hughes & Ward 1993), indicating that they perceive areas further from cover as more risky. In the greater Egyptian sand gerbil *Gerbillus pyramidum* and Allenby's gerbil *G. allenbyi*, individuals had greater giving up densities (GUDs) in patches without cover, when there was increased illumination (i.e. greater predation risk), or when there were predators in the vicinity (Kotler *et al.* 1991). Likewise, midday gerbils *Meriones meridianus* had significantly higher GUDs in uncovered seed trays that were situated far from burrows (high predation risk) compared to seed trays close to burrows (Shuai & Song 2011).

Increased predation risk is also associated with greater boldness (Niemelä *et al.* 2012). Bolder animals tend to take more risks (Sneddon 2003), as well as have lower levels of anti-predator behaviour (Bell *et al.* 2013). Predation risk presents a selection pressure that drives boldness: Panamanian bishop fish *Brachyraphis episcopii* which had parents that experienced predators, were bolder than fish whose parents were predator-naïve (Brown *et al.* 2007). On the other hand, due to danger (from novel situations or anthropogenic objects) in urban areas, European blackbirds *Turdus merula* were less neophobic, more exploratory and less anxious (i.e. bolder) in rural areas than in urban areas (Miranda *et al.* 2013). Thus, because predation risk is associated with increased boldness, and predation risk is higher in open areas (Perea *et al.* 2011), open habitats should also select for increased boldness.

The African striped mouse (genus *Rhabdomys*) is widespread throughout southern Africa, occurring from the semi-arid western parts of South Africa to the wetter eastern parts of the country (Skinner & Chimimba 2005). The genus is comprised of several putative species (du Toit *et al.* 2012a), with *R. pumilio* occupying the western coastal parts, *R. bechuanae* occupying the central grasslands, and two subspecies of *R. dilectus* occupying the northern and eastern grasslands of South Africa (du Toit *et al.* 2012a; Meynard *et al.* 2012). *R. pumilio* in the Northern Cape Province occurs in an open habitat, characterised by wide sandy spaces between small bushes (Schradin & Pillay 2004). In contrast, the central and eastern grasslands (where both *R. dilectus* and *R. bechuanae* occur) are associated with much higher levels of continuous grass cover (Schradin & Pillay 2005; Dufour *et al.* 2015).

Previous studies have demonstrated behavioural differences between *R. pumilio* and *R. dilectus chakae*. *R. pumilio* is facultatively group living (Schradin & Pillay 2005), with groups consisting of 2-4 breeding females, a breeding male and several philopatric offspring (Schradin & Pillay 2004). The offspring may disperse when vacant nesting sites become available (Schoepf & Schradin 2012). In contrast, *R. d. chakae* lives solitarily, where females maintain non-overlapping territories, but male territories may overlap those of several females (Schradin & Pillay 2005). In the laboratory, *R. pumilio* is more exploratory and less anxious than *R. d. chakae* (Rymer *et al.* 2008; Rymer & Pillay 2012). Furthermore, the development of exploratory behaviour and anxiety in *R. pumilio* and *R. d. chakae* appears to be influenced by an interaction of their genes and their social rearing environment, with between-species cross-fostered offspring showing an intermediate anxiety response and exploratory behaviour to the biological parents and siblings (Rymer & Pillay 2012). However, in contrast to *R. pumilio* and *R. dilectus*, little is known about the development of behaviour in *R. bechuanae*. In a previous experiment studying the personality profiles of *R. pumilio*, *R. bechuanae* sympatric with *R. d. dilectus*, and allopatric *R. d. dilectus*; *R. pumilio* and sympatric *R. d. dilectus* were bolder and more exploratory than *R. bechuanae* (Chapter 2). However, in another experiment on the effects of cross-fostering on behavioural development, the behaviour of *R. pumilio* and *R. bechuanae* were not significantly different, and altering the early social environment by cross-fostering individuals did not significantly disrupt exploratory behaviour and anxiety responses (Chapter 3).

The aim of the current study was to establish whether the behaviour of four populations (representing three taxa) of *Rhabdomys* is influenced by different captive housing conditions, over two generations. I raised individuals under two treatments (high cover and no cover), and tested the anxiety response and exploratory behaviour of adult individuals from both generations and cover levels. *Rhabdomys* shows variation in behaviour that may be linked to variation in the environment (Rymer & Pillay 2012), such that, in standardised laboratory tests, semi-arid-occurring *R. pumilio* is not anxious and highly exploratory, and grassland-occurring *R. d. chakae* is anxious and shows low levels of exploratory behaviour. I made three hypotheses about exploratory behaviour and anxiety response in individuals reared in different housing treatments. 1) Because the grassland populations were more anxious and less exploratory in comparison to semi-arid-occurring *R. pumilio* (from the results of Chapter 2), when individuals are raised under no cover, *R. pumilio* individuals should be less anxious and more exploratory than all other populations. 2) If they are raised under high cover, individuals of all populations will be more anxious and less exploratory. 3) I predicted similar responses to treatment by F1 (first generation) and F2 (second generation) individuals.

Materials and methods

Striped mice from four populations were used in this experiment. The populations were *R. pumilio* from Goegap Nature Reserve (29°41’33’’S, 18°1’41’’E), *R. bechuanae* sympatric with *R. d. dilectus* from Sandveld Nature Reserve (27°41’57’’S, 25°44’23’’E), and *R. bechuanae* from Tussen-die-Riviere Nature Reserve (TDR; 30°28’4’’S, 26°9’31’’E). All of the striped mice originated from laboratory populations, except those from TDR which were wild-caught. The study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (2013/18/2A).

Five breeding pairs from each population were established in tanks (46 x 30 x 35cm) that had 3 metal sides, a clear acrylic glass front, and a wire mesh lid. Each tank was equipped with a acrylic glass platform (33 x 30cm) placed 16cm above the floor of each tank. The platform was clear or spray painted dark colours (Figure 4.1). Platforms were the width of the tank and were placed against one of the short sides of the tanks. The tanks contained a 2cm layer of wood shavings, approximately 5g of dry grass and 2g of paper towel for nesting material, a wooden block for chewing and a cardboard toilet roll for enrichment. An opaque PVC box (7 x 7 x 10cm) was also provided for nesting. Water and food were available *ad libitum*.

Each pair was allowed to produce 2 litters. The first litter (F1-no cover) was reared in tanks that had clear acrylic glass platforms (Figure 4.1A,C). At weaning (20 days), 2 pups (1 male and 1 female, where possible) were placed individually in identical tanks to the breeding tanks (Figure 4.1A,C) with clear acrylic glass platforms. For the second litter, the parents’ tank was modified by adding the spray-painted platform (Figure 4.1B), and part of the tank covered with dark cardboard (Figure 4.1D) to simulate dense cover. Thereafter, the second litter (F1-cover) was born and reared in cover (Figure 4.2). At weaning, 2 pups (1 male and 1 female, where possible) from each litter were placed individually into tanks with cover identical to the tanks in which they were reared (Figure 4.2).

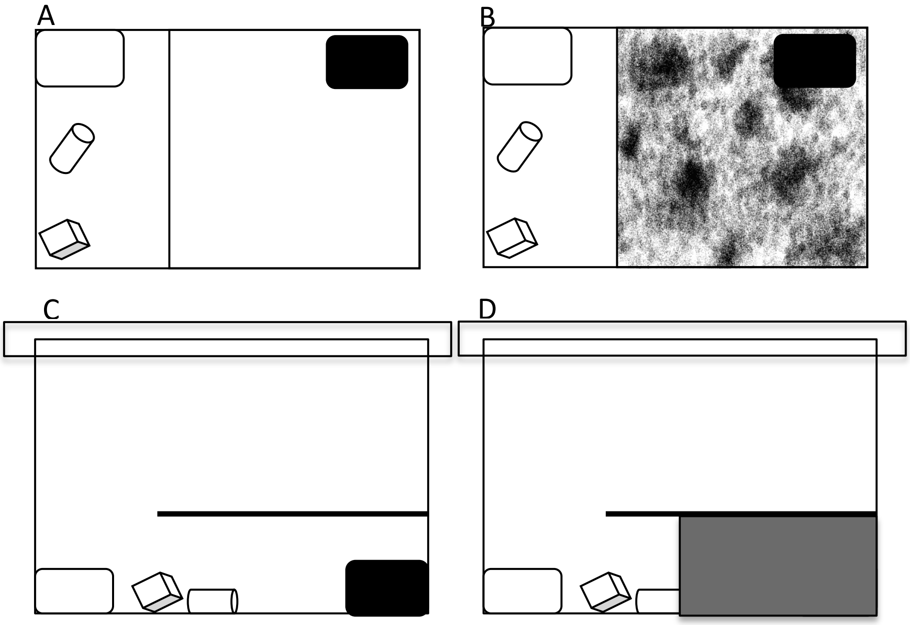

Figure 4.1. Configuration of the tanks used to breed and house individuals. View from above a tank with a clear platform (Figure 4.1A) and a tank with a spray painted platform (Figure 4.1B). Each tank had a brick, a toilet roll, a wooden block, an opaque nesting box which was placed under the platform, as well as grass and paper towel for nesting material. The view from the side (Figure 4.1C), shows the placement of the platform (held in place with brackets bolted to the sides of the tank), and tanks that simulated cover (Figure 4.1D), had an additional piece of dark cardboard covering part of the front of the tank, to provide additional cover.

At 60 days of age (early adulthood), the exploratory behaviour and anxiety response of all F1 mice were tested in two experiments (see below). At 90 days, they were paired with other F1 individuals from the same treatment and population (e.g. an F1-no cover male was paired with an F1-no cover female of the same population and an F1-cover male was paired with an F1-cover female of the same population). Pairs were kept in the same treatment conditions under which they were bred and raised. Both F1-cover and F1-no cover pairs were allowed to produce and raise one litter. At weaning, 2 pups (1 male and 1 female, where possible) from each F2 litter from both treatments were placed individually into tanks identical to those in which they were reared. Once the individuals reached 60 days of age, their exploratory behaviour and anxiety responses were tested (Figure 4.2).

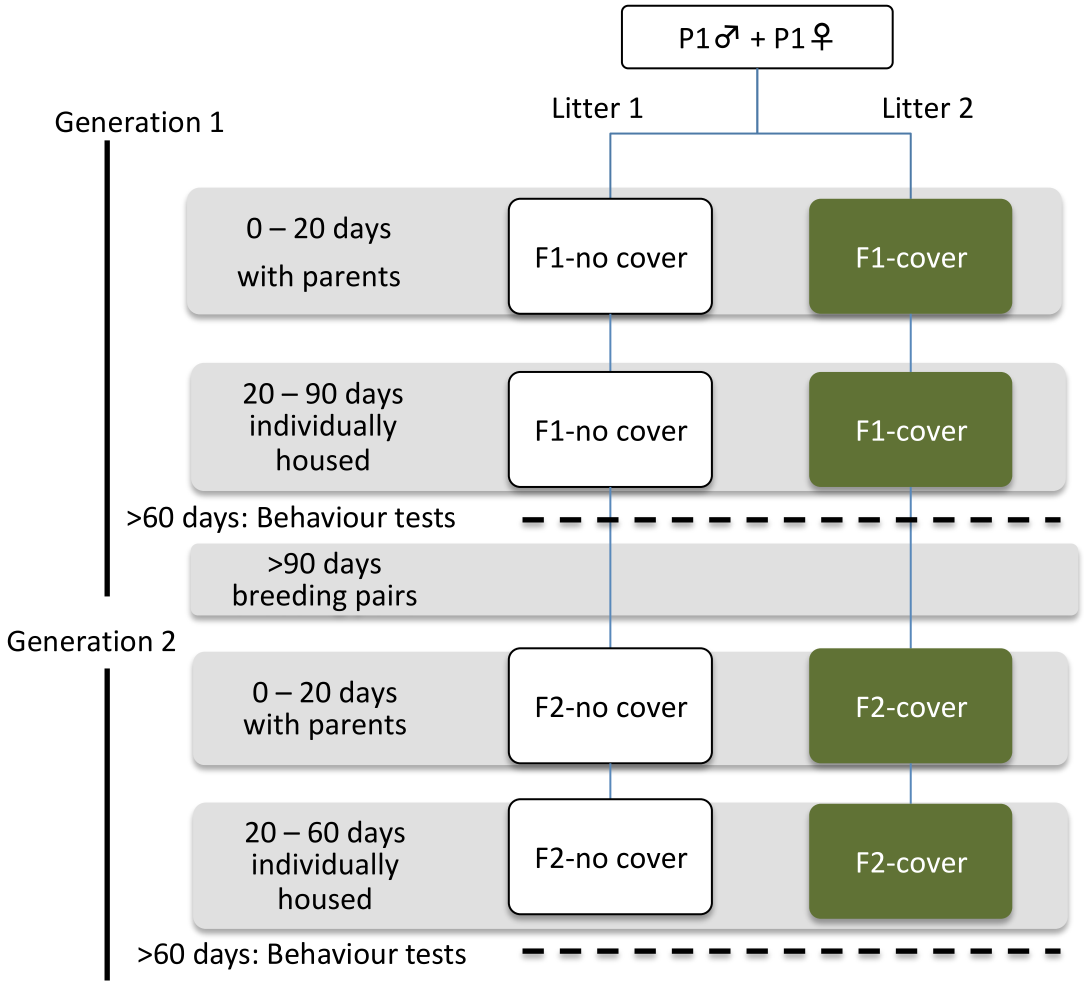

Figure 4.2. Timeline of cover experiment, showing the timing of behaviour tests as well as the timing of pairing and weaning for no cover and cover litters.

Behavioural tests

F1 and F2 individuals of both treatments were tested individually in an open field (exploratory behaviour), and light-dark (anxiety), and startle response (anxiety) tests in two apparatuses, described below. These tests have been used previously to measure behaviour in striped mice (Rymer & Pillay 2012; Joshi & Pillay 2016b). Between each use, all apparatuses were thoroughly washed and air-dried to remove the odour of the previous test subject.

Open field test

The open field test was conducted in a glass tank (46 x 30 x 35cm) with opaque sides and a clear lid. A test subject was placed in the centre of the tank, and its behaviour video recorded for 10 minutes by a camera positioned directly above the tank. The bottom of the tank was divided into 9 equally sized rectangles with electrical tape, to demarcate the centre and periphery of the arena. I recorded the duration of time that the test individual spent in the centre of the tank and the number of entries into the centre of the tank.

Light-dark test

The light-dark test and startle test were conducted in a modified glass tank (46 x 30 x 35cm), which was divided into two equally sized chambers (each 23 x 30 x 35cm) by an acrylic glass wall that was painted black. The centre of the wall had a square opening (5 x 5cm) to allow a test subject to move between the compartments. One chamber was painted black, while the other chamber was left clear to allow as much light as possible into the chamber. In addition, half of the lid of the test apparatus was painted black, while the other half was left clear to facilitate light entering the apparatus from above. A test subject was placed into the clear half of the chamber and its behaviour video recorded from above for 5 minutes. I recorded the latency to move into the dark chamber, the latency to return to the clear chamber after the subject first moved to the dark chamber, and the number of times the animal moved between the chambers.

Startle response test

At the end of the light-dark test, I entered the room and startled the test subject by clapping my hands near the apparatus, prompting the mouse to move into the dark chamber. The subject was video recorded for a further 5 minutes, and the latency to return to the clear chamber after being startled was recorded.

Data analysis

All video recordings were scored using Ethovision™ (Noldus 2013) video tracking software. I used R (version 3.3.0; R Core Team 2016), and the *lmer* function in the Lme4 package (Bates *et al.* 2015) to analyse all variables, as the models fit each variable adequately (based on the plot of the residuals and the fitted values from each model; a straight line indicates an adequate fit). Mother ID and father ID were the random factors for each individual, since some individuals were used in different pairings. Fixed factors included population, treatment, generation (F1 and F2), sex and their interactions. Sample sizes are shown in Table 4.1. I generated p-values using likelihood ratio tests (Bates *et al.* 2015). I plotted significant predictors using notched box plots with medians (middle of boxes) and 1st and 3rd quartiles. I compared notch (showing 95% confidence intervals) overlap to show significant differences between groups, as described by Krzywinski and Altman (2014). A full description along with a hypothetical boxplot is provided in Supplementary Material 1. For non-significant predictors, I constructed tables and provided medians and 1st and 3rd quartiles and presented these as supplementary material.

Table 4.1. Sample sizes of number of individuals tested for each of the species, for each treatment and sex.

Species	Sex	Treatment				Total sample size
		F2-cover	F1-cover	F1-no cover	F2-no cover	
R. pumilio	Female	3	5	5	5	45
	Male	2	5	6	5	
TDR *R. bechuanae*	Female	5	7	7	5	62
	Male	5	7	7	5	
Sandveld *R. bechuanae*	Female	2	3	2	2	34
	Male	4	3	6	4	
Sandveld *R. d. dilectus*	Female	9	9	11	9	96
	Male	9	10	10	9	

Results

Open field test

Duration in the centre of the open field

The duration of time spent in the centre of the open field arena was significantly influenced by treatment (χ^2_1=10.22, p=0.001), with cover individuals spending more time in the centre than no cover individuals, as indicated by Figure 4.3. The duration of time spent in the centre of the open field was also significantly predicted by treatment*population*generation (χ^2_6=13.25, p=0.039). *R. pumilio* (F1-cover) spent the most time in the centre, and *R. pumilio* F2-cover, F1-

no cover and F2-no cover spent the least time in the centre, followed by *R. bechuanae*. All other population*treatment*generation groups occupied intermediate positions (Figure 4.4).

The duration spent in the centre of the arena was not significantly influenced by population, generation, sex, population*treatment, population*sex, treatment*generation, population*treatment*sex, and population*treatment*generation*sex (Supplementary Material 2).

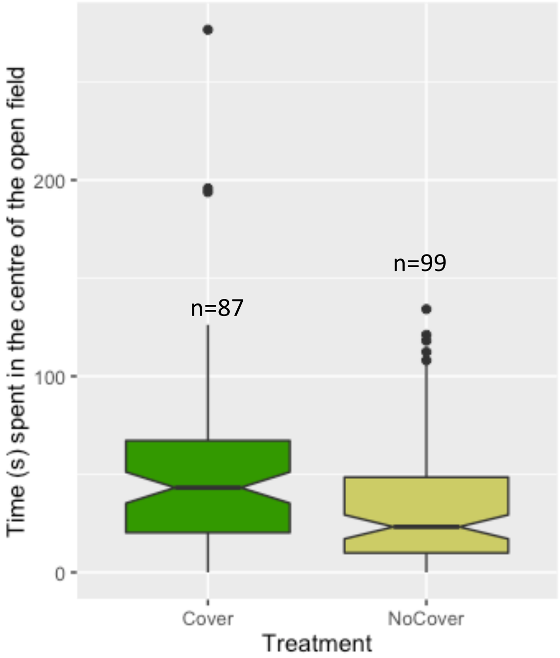

Figure 4.3. The duration spent in the centre of the open field arena by individuals that were raised under cover (Cover), or individuals raised under no cover (NoCover). Descriptions of the boxes and notches are available in Supplementary Material 1.

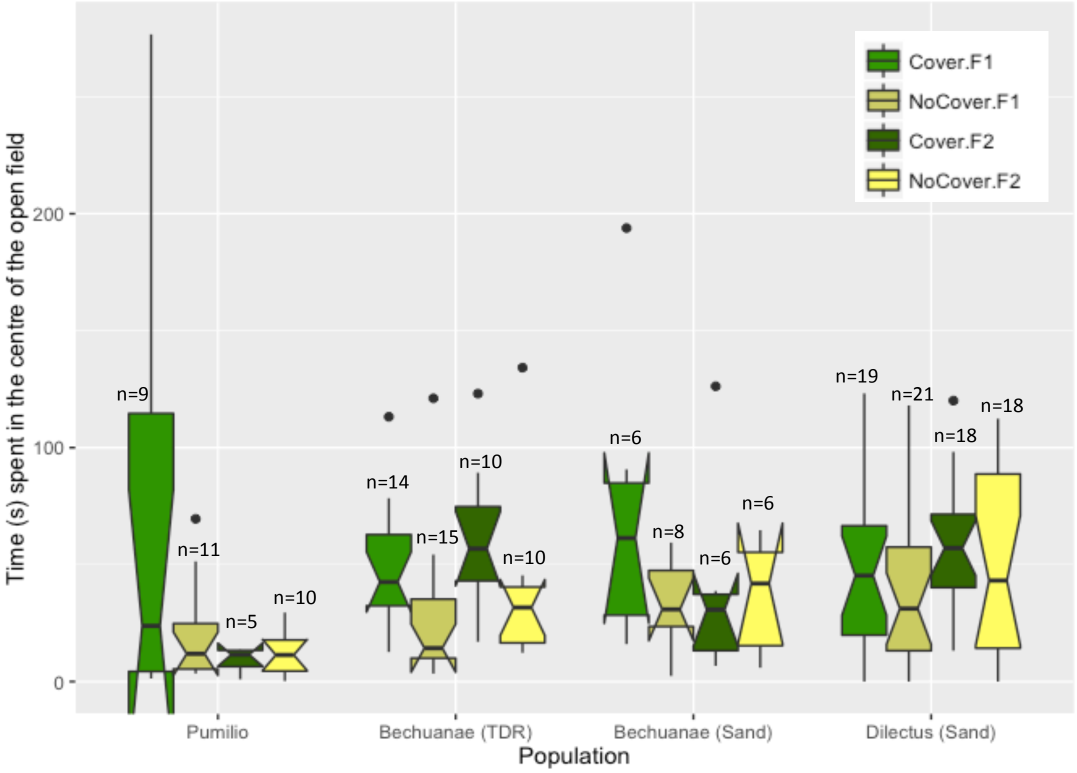

Figure 4.4. The duration spent in the centre of the open field by F1 and F2 individuals from both the cover and no cover treatments, from each population. The populations were Pumilio (*Rhabdomys pumilio*), Bechuanae (TDR - *R. bechuanae* from TDR), Bechuanae (Sand - *R. bechuanae* from Sandveld) and Dilectus (Sand - *R. d. dilectus* from Sandveld). Descriptions of the boxes and notches are available in Supplementary Material 1.

Number of entries into the centre of the open field

The number of entries into the centre of the open field arena was significantly influenced by population (χ^2_3=12.57, p=0.006), with *R. pumilio* entering the centre of the arena significantly less than Sandveld *bechuanae*, but not significantly less than TDR *R. bechuanae* and Sandveld *R. d. dilectus* (Figure 4.5). Treatment, generation, sex, population*treatment, population*sex, treatment*generation, population*treatment*sex, population*treatment*generation and population*treatment*generation*sex were not significant predictors of the number of entries into the centre of the open field arena (Supplementary Material 3).

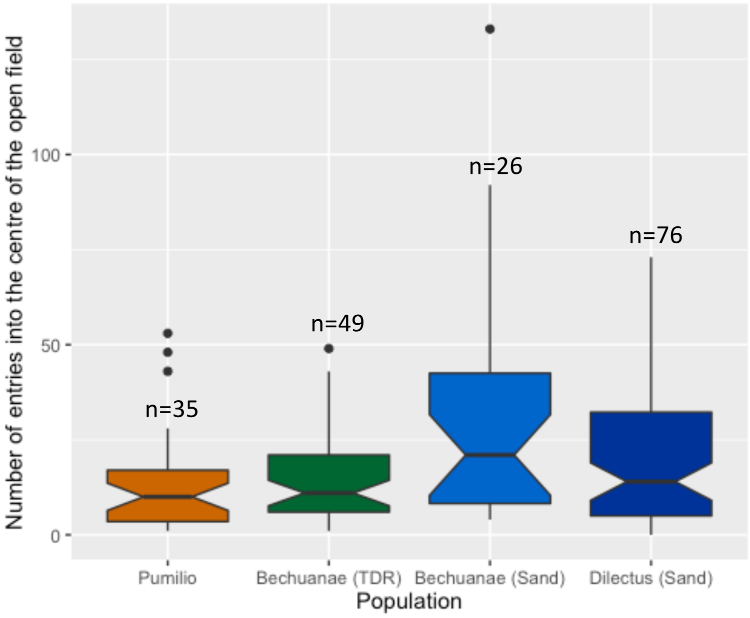

Figure 4.5. The number of entries into the centre of the open field by Pumilio (*Rhabdomys pumilio*), Bechuanae (TDR - *R. bechuanae* from TDR), Bechuanae (Sand - *R. bechuanae* from Sandveld) and Dilectus (Sand - *R. d. dilectus* from Sandveld) individuals. Descriptions of the boxes and notches are available in Supplementary Material 1.

Light-dark test

Duration of time in light compartment

Treatment had a significant influence on the duration of time spent in the light compartment of the light-dark chamber (χ^2_1=10.29, p=0.001). No cover individuals spent more time in the light compartment compared to cover individuals (Figure 4.6). The duration spent in the light compartment was also significantly predicted by population*treatment*sex (χ^2_4=9.58, p=0.048), where *R. pumilio* males spent less time in the light compartment than males from all other populations and treatments. In all populations, no cover males and females spent a similar amount of time in the light, and generally more time in the light than cover males and females, apart from *R. pumilio* cover females (Figure 4.7). Population, generation, sex, population*treatment, population*sex, treatment*generation, population*treatment*generation, and population*treatment*generation*sex were not significant predictors of the duration of time spent in the light compartment (Supplementary Material 4).

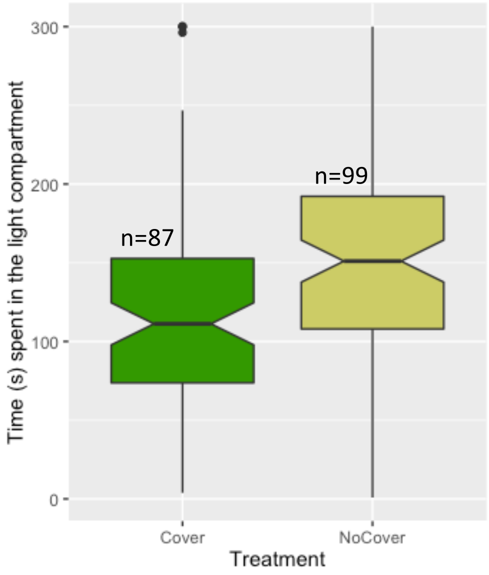

Figure 4.6. The duration of time spent in the light compartment of the light-dark test, by individuals that were raised under cover, and individuals that were raised under no cover. Descriptions of the boxes and notches are available in Supplementary Material 1.

Number of entries into the light compartment

The number of entries into the light compartment was not significantly influenced by population, treatment, generation, sex, population*treatment, population*sex, treatment*generation, population*treatment*sex, population*treatment*generation and population*treatment*generation*sex (Supplementary Material 5).

Startle response test

Latency to re-enter the light chamber after the startle

The latency to re-enter the light compartment was not significantly influenced by population, treatment, generation, sex, population*treatment, population*sex, treatment*generation, population*treatment*sex, population*treatment*generation and population*treatment*generation*sex (Supplementary Material 6).

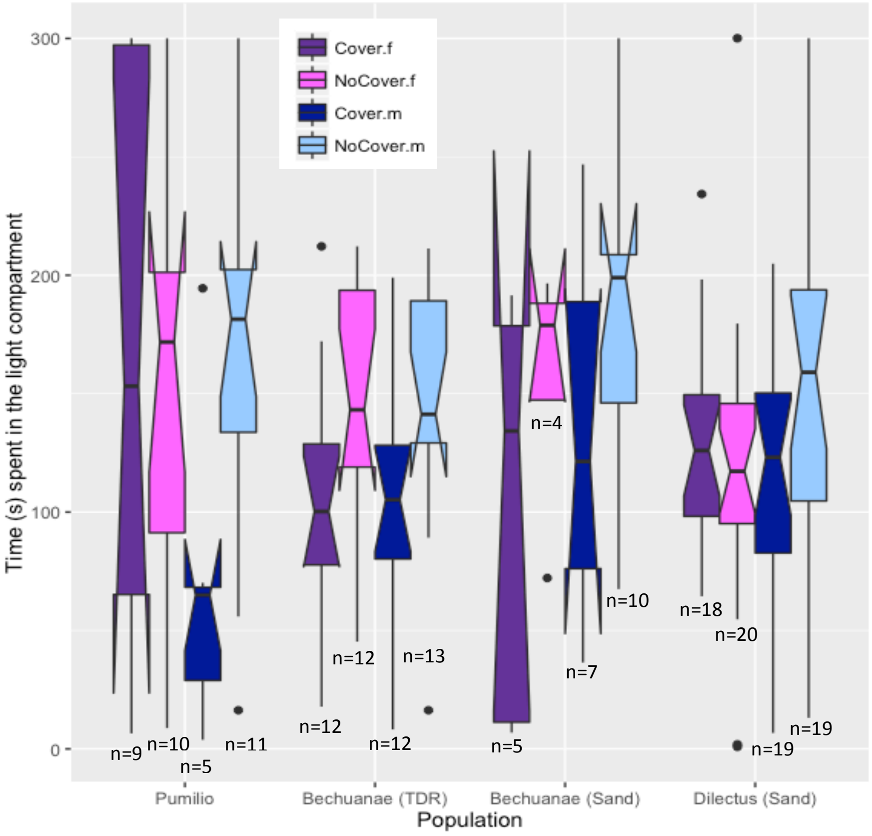

Figure 4.7. The duration of time spent in the light compartment of the light-dark test, by males and females from the cover and no cover treatments, from the Pumilio (*Rhabdomys pumilio*), Bechuanae (TDR - *R. bechuanae* from TDR), Bechuanae (Sand - *R. bechuanae* from Sandveld) and Dilectus (Sand - *R. d. dilectus* from Sandveld) populations. Descriptions of the boxes and notches are available in Supplementary Material 1.

Discussion

I aimed to establish whether the physical rearing and housing environment (high cover or no cover) over two generations affected the exploratory behaviour and anxiety responses in adult striped mice from 4 populations, representing 3 *Rhabdomys* taxa. I tested the behaviour of individuals using the open field, light-dark and startle response tests. A higher duration of time spent in the centre of the open field, as well as a greater number of entries into the centre is usually indicative of higher exploration (Prut & Belzung 2003). A longer duration spent in the light compartment of the light-dark test, as well as a higher number of entries into the light compartment (reviewed in Bourin & Hascoët 2003; Maximino *et al.* 2012) and a shorter duration to emerge after a startle reflects lower anxiety (de Jongh *et al.* 2002; Ardayfio & Kim 2006; Mineur *et al.* 2006).

I hypothesised that when striped mice were raised under high cover, all populations should be more anxious and less exploratory, and if striped mice are raised under no cover, *R. pumilio* should be less anxious and more exploratory because it is adapted to habitats with sparse cover. However, *R. pumilio* was significantly the least exploratory of all the populations while Sandveld *R. bechuanae* was the most exploratory, although there were no significant population level differences in anxiety, leading me to reject my first hypothesis. Nevertheless, generally, among all populations, individuals that were raised under cover were both significantly more exploratory and more anxious than individuals raised under no cover, leading me to partially reject my second hypothesis. There were a few minor differences in the responses of individuals in the four populations to the treatments, and *R. pumilio* had the fewest significant differences between the treatments and generations. However, this was only in the duration spent in the centre of the open field, suggesting that population and treatment differences were negligible. The similar responses of F1 and F2 generations confirmed my prediction that there would not be a generation effect due to similar social rearing environments within treatments.

Since the environment may interact with an individual's genotype to modify the phenotype (gene x environment interaction), a protocol to study the influences of genes and the environment is to alter the environmental conditions during development. The phenotype that is altered by environmental changes indicates phenotypic plasticity (i.e. norm of reaction; Schlichting & Pigliucci 1998). Phenotypic plasticity describes the expression of multiple phenotypes in response to prevailing environmental conditions during development (West-Eberhard 1989), which is non-reversible (Moczek *et al.* 2011) due to organisational processes which make permanent changes to the neural systems that underlie behaviour (West-Eberhard 1989). The effects of altering the housing conditions during ontogeny in my study tested developmental plasticity (i.e. non-reversible changes to the phenotype due to changes in the developmental environment; Fischer *et al.* 2003). In my study, regardless of species, individuals that were reared under cover tended to be significantly more exploratory as well as more anxious than individuals reared without cover, indicating that the genus *Rhabdomys* may be sensitive to changes in the developmental environment.

The significant differences in exploration between individuals from the two treatments is unlikely to be an enrichment effect, because the cages did not differ in complexity (Leggio *et al.* 2005). However, anxiety is likely due to the level of cover available to animals. Lack of cover is associated with anxiety because open areas are associated with increased predation risk (Brown *et al.* 1988; Lima & Dill 1990) and rodents may use structurally complex and covered areas in preference to barren areas (Jensen *et al.* 2003). This suggests that *Rhabdomys* may be less anxious when raised with higher levels of cover. This also suggests that exploratory behaviour and anxiety are decoupled, and may not form a behavioural syndrome (behaviours which are coupled together; Sih *et al.* 2004). For example, under natural conditions, male Siamese fighting fish *Betta splendens* have a boldness-aggression syndrome (males that are bolder are also more aggressive), but when exposed to 17 α-ethinyloestradiol (a pollutant often found in sewage), the syndrome becomes decoupled and males behave less consistently (Hebert *et al.* 2014). This also suggests that exploratory behaviour and anxiety have different developmental pathways in *Rhabdomys* (i.e. not pleiotropic).

The effects of the physical rearing environment on behaviour have been relatively well studied, but altering the levels of cover available has rarely been done, and the results are equivocal. Spotted sand lizards *Pedioplanis lineoocellata* that occurred in areas with low vegetation cover were active foragers (lizards were more active and travelled further), than lizards in areas with high vegetation cover (typically sit-and wait foragers; Wasiolka *et al.* 2009), indicative of lower anxiety in the low cover lizards. In contrast, there are very few studies that experimentally manipulated rearing environments by increasing or decreasing the amount of cover available to animals. Most of the studies on altering physical environment focus on enrichment (Newberry 1995). In general, enrichment tends to improve animal welfare, but the effects on behaviours are inconsistent and depend on the species concerned. One study reported that Mongolian gerbils *Meriones unguiculatus* that were reared in tunnels were bolder and less anxious than gerbils that were reared in open tanks (Clark & Galef 1977). However, BALB/c laboratory mice that were reared in enriched conditions (more objects in standard cages) were more anxious than un-enriched mice, the opposite reaction to C57BL enriched and un-enriched mice (Van de Weerd *et al.* 1994).

Three of the four populations originated from grassland habitats and only *R. pumilio* originated from an open semi-arid habitat. The similarity in behaviour and anxiety between the semi-arid *R. pumilio* and all of the grassland populations implies that the environment of origin might have a lesser influence on behaviour. Instead, the similar responses of the populations to available cover appear to show an underlying phylogenetically conserved response, as the environment of origin does not appear to have a large effect on behaviour. *R. bechuanae*, *R. pumilio* and *R. dilectus* have a common ancestor which appears to have originated in the central parts of South Africa (du Toit *et al.* 2012a). The dissimilar habitats that the species now occupy and the similar response of the species to the provision of cover would then support a phylogenetically conserved response to cover. There are many examples of phylogenetic constraints on behaviour. For example, closely related Madagascar lemur (*Eulemur*) species had similar social organisation, with similar group sizes, numbers of males and females per group and the sex ratios in each group, than species that were more distantly related (Ossi & Kamilar 2006). Likewise, a phylogenetic analysis of macaque *Macaques* spp. behaviour showed that the social organisation, patterns of male dispersion, and female rank acquisition are similar in closely related taxa (Thierry *et al.* 2000). Since the Goegap habitat of *R. pumilio* is so different to the grassland localities of the other populations (Schradin & Pillay 2005; Rutherford *et al.* 2006), it is parsimonious to assume a phylogenetic conservatism in its responses to the cover treatments.

Although the semi-desert and grassland habitats are different, environmental selection pressures on exploratory behaviour and anxiety responses are putative rather than known. Therefore, differences in selection pressures may potentially explain the small differences between the four populations. However, although phylogenetic constraint and potentially different selection pressures combined provide reasonable explanations for the overall similarity and subtle differences in behaviour of the four populations, it does not explain why *R. pumilio* was as anxious, but less exploratory than *R. d. dilectus* and both populations of *R. bechuanae*. This is opposite to what has been reported in another study, which reported higher levels of exploratory behaviour and anxiety in *R. pumilio* compared to a population of *R. d. chakae* (Rymer & Pillay 2012). It is possible that the tests (open field, light-dark and startle), were not able to detect population-level differences in anxiety, but this is unlikely given the similar testing protocols and findings in other studies of *Rhabdomys* (Rymer & Pillay 2012; Yuen *et al.* 2015; Joshi & Pillay 2016a; Joshi & Pillay 2016b), as well as the small but significant difference in exploratory behaviour between the populations. It is also possible that the treatment (adding a platform, either simulating cover or no cover) itself might have increased the anxiety of *R. pumilio* (i.e. positive genotype-environment effect), with the result of overall similarity in behaviour between populations.

Individuals of the two generations did not have different anxiety and exploratory behaviour, confirming my third prediction. Since there was no difference in the treatment between the first and second generations, it would have been unlikely that rearing individuals in a particular treatment (cover or no cover) for a subsequent generation would have altered behaviour. However, *Rhabdomys* is associated with remarkable behavioural flexibility, where individuals are able to change their reproductive phenotype within a lifetime (Schradin *et al.* 2009; Schradin *et al.* 2010). Nevertheless, there was no selection pressure or differential fitness associated with my treatments and therefore there was unlikely to be a dramatic shift in behaviour over one generation.

Unlike my previous studies (Chapter 3 & 5), I found few significant sex effects. This is surprising given the large overall sample size, and sex-based differences I found in the previous experiment (Chapter 3) where I altered social environment, and may reflect different developmental neural pathways that are influenced by the social environment. The literature has mixed reports of sex effects on exploration and anxiety, with some studies finding that sex has an important effect on behaviour, while others report no effect of sex. In great tits *P. major*, there was an important effect of both sex and personality on learning, with fast-exploring (bolder) males performing a learning test better than less bold males, while slow-exploring (less bold) females performed the learning test better than bolder females (Titulaer *et al.* 2012). In contrast, there was no sex effect on the exploration behaviour found in a colonial cichlid *Neolamprologus caudopunctatus*, with no differences in exploration between males and females (Martins *et al.* 2012). Generally, sex effects are expected when males and females have different spatial ecology (Lucon-Xiccato & Bisazza 2017), but in grassland *R. d. chakae*, home range sizes of males are bigger than females (Schradin & Pillay 2005), which should promote sex differences, especially in exploration. Therefore, this remains to be explored in future studies.

In conclusion, individuals that were raised under high levels of cover were significantly less anxious and more exploratory than individuals that were raised under no cover, suggesting firstly that these behaviours are developmentally plastic (because there was a treatment effect) and secondly that anxiety and exploratory behaviour may have different developmental neural pathways in *Rhabdomys*. However, there were limited responses to the treatments, suggesting that the housing environment plays a small role in influencing behaviour. The overall similarity of behaviour between *R. pumilio*, allopatric *R. bechuanae* from TDR Nature Reserve and sympatric *R. bechuanae* and *R. d. dilectus* from Sandveld Nature Reserve, suggests that exploratory behaviour and anxiety response may be phylogenetically constrained in this genus. The small population differences in exploratory behaviour may potentially be due to different selection pressures in different environments. Also, there appears to have been a modulating effect of the treatment (i.e. positive genotype-environment effect; Plomin *et al.* 1980), which may have resulted in the similarities of the behavioural phenotypes in the four populations (three taxa).

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Supplementary Material 1

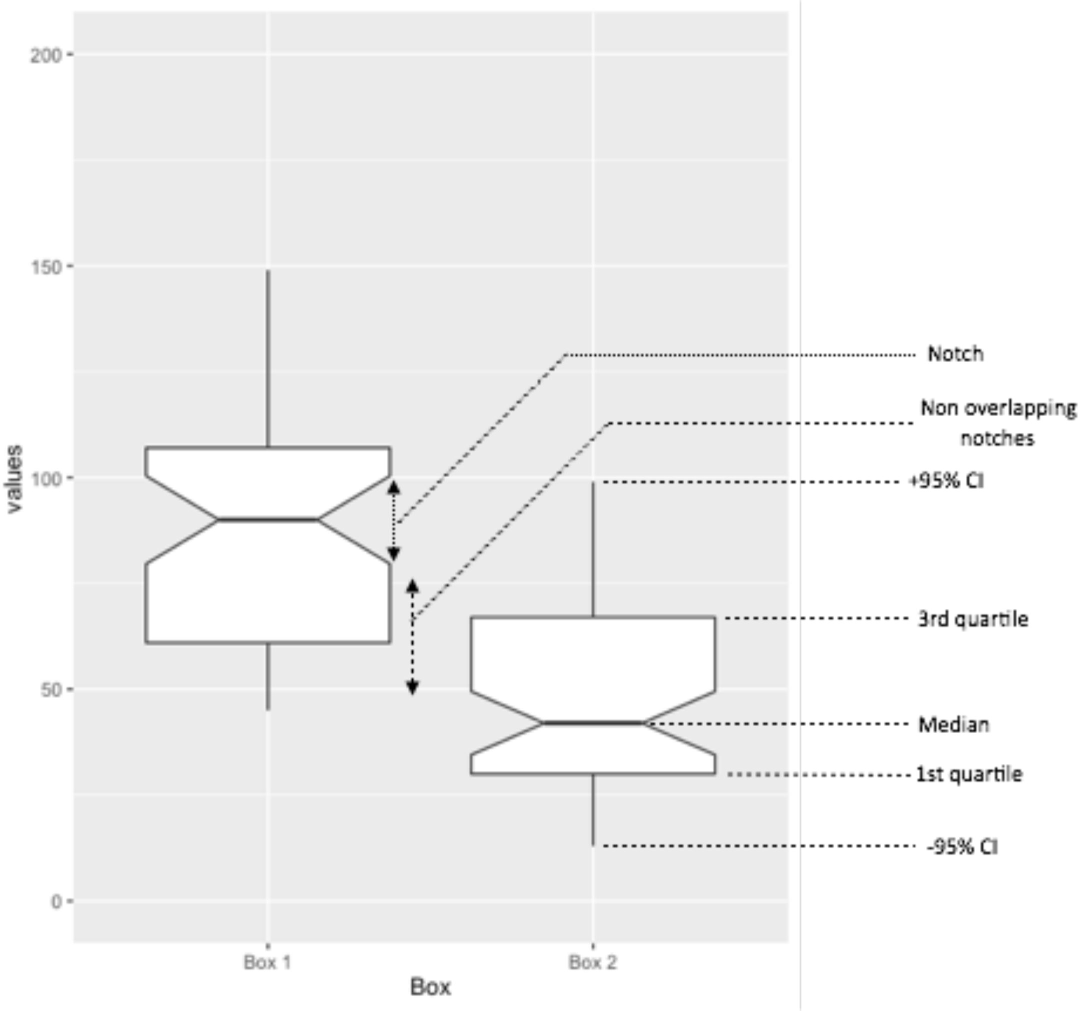

Figure S1. Hypothetical boxplot. Boxes show medians (middle of boxes) and 1st (top box) and 3rd (bottom box) quartiles. Whiskers show confidence limits, and dots outside of boxes indicate outliers. Non-overlapping notches indicate differences between groups.

Supplementary Material 2

Model outcomes (Table S1A) and data summary (Table S1B) for non-significant factors influencing the duration spent in the centre of the open field arena by male and female individuals, from four populations, that were raised under cover and no cover for two generations. The populations were Pumilio (*Rhabdomys pumilio*), Bechuanae (TDR - *R. bechuanae* from TDR), Bechuanae (Sand - *R. bechuanae* from Sandveld) and Dilectus (Sand - *R. d. dilectus* from Sandveld). Treatments were first generation (F1) and second generation (F2) individuals raised under cover or no cover.

Table S1A. Model outcomes for non-significant factors influencing the duration spent in the centre of the open field.

Predictor	**Statistics**
Population	χ^2_3=5.81, p=0.121
Generation	χ^2_1=0.01, p=0.930
Sex	χ^2_1=0.29, p=0.593
Population*treatment	χ^2_3=3.28, p=0.350
Population*sex	χ^2_3=7.37, p=0.061
Treatment*generation	χ^2_1=0.82, p=0.364
Population*treatment*sex	χ^2_4=7.58, p=0.108
Population*treatment*generation*sex	χ^2_8=3.97, p=0.859

Table S1B. Data summary for the non-significant factors influencing the duration spent in the centre of the open field.

Level	**Duration in the centre**		
	Median	**1st quartile**	**3rd quartile**
Pumilio Cover F1 Female	23.80	4.32	195.80
Pumilio Cover F1 Male	17.86	6.25	50.11
Pumilio Cover F2 Female	11.52	8.96	12.44
Pumilio Cover F2 Male	7.28	4.12	10.44
Pumilio No Cover F1 Female	9.52	5.04	12.32
Pumilio No Cover F1 Male	12.72	7.05	30.45
Pumilio No Cover F2 Female	16.24	2.36	17.76
Pumilio No Cover F2 Male	6.48	5.60	17.76
Bechuanae (TDR) Cover F1 Female	34.20	27.05	48.12
Bechuanae (TDR) Cover F1 Male	54.30	38.75	71.63
Bechuanae (TDR) Cover F2 Female	59.30	34.20	64.30
Bechuanae (TDR) Cover F2 Male	54.30	43.20	78.30

Bechuanae (TDR) No Cover F1 Female	14.30	11.17	35.07
Bechuanae (TDR) No Cover F1 Male	17.65	10.50	35.66
Bechuanae (TDR) No Cover F2 Female	32.10	23.30	43.20
Bechuanae (TDR) No Cover F2 Male	31.20	14.30	32.10
Bechuanae (Sand) Cover F1 Female	55.32	37.36	124.60
Bechuanae (Sand) Cover F1 Male	67.36	41.70	79.04
Bechuanae (Sand) Cover F2 Female	82.48	60.60	104.36
Bechuanae (Sand) Cover F2 Male	18.30	7.80	29.58
Bechuanae (Sand) No Cover F1 Female	44.80	37.50	52.10
Bechuanae (Sand) No Cover F1 Male	30.86	10.25	42.86
Bechuanae (Sand) No Cover F2 Female	19.68	12.80	26.56
Bechuanae (Sand) No Cover F2 Male	53.64	40.13	58.84
Dilectus (Sand) Cover F1 Female	34.30	21.40	47.40
Dilectus (Sand) Cover F1 Male	53.31	24.53	66.81
Dilectus (Sand) Cover F2 Female	59.76	73.20	73.20
Dilectus (Sand) Cover F2 Male	54.30	48.80	66.40
Dilectus (Sand) No Cover F1 Female	31.20	11.53	43.50
Dilectus (Sand) No Cover F1 Male	49.70	14.91	91.57
Dilectus (Sand) No Cover F2 Female	14.30	11.20	87.30
Dilectus (Sand) No Cover F2 Male	67.20	32.10	93.84

Supplementary Material 3

Model outcomes (Table S2A) and data summary (Table S2B) for non-significant factors influencing the number of entries into the centre of the open field arena by male and female individuals, from four populations, that were raised under cover and no cover for two generations. The populations were Pumilio (*Rhabdomys pumilio*), Bechuanae (TDR - *R. bechuanae* from TDR), Bechuanae (Sand - *R. bechuanae* from Sandveld) and Dilectus (Sand - *R. d. dilectus* from Sandveld). Treatments were first generation (F1) and second generation (F2) individuals raised under cover or no cover.

Table S2A. Model outcomes for the non significant factors influencing the number of entries into the centre of the open field

Predictor	Statistics
Treatment	χ^2_1=0.99, p=0.319
Generation	χ^2_1=0.04, p=0.844
Sex	χ^2_1=2.62, p=0.105
Population*treatment	χ^2_3=6.27, p=0.099
Population*sex	χ^2_3=1.99, p=0.575
Treatment*generation	χ^2_1=0.64, p=0.424
Population*treatment*sex	χ^2_4=3.02, p=0.554
Population*treatment*generation	χ^2_6=7.97, p=0.240
Population*treatment*generation*sex	χ^2_8=13.25, p=0.104

Table S2B. Data summary for the non-significant factors influencing the number of entries into the centre of the open field.

Level	Frequency of centre entries		
	Median	1st quartile	3rd quartile
Pumilio Cover F1 Female	6.00	4.00	12.00
Pumilio Cover F1 Male	14.50	7.50	22.00
Pumilio Cover F2 Female	13.00	10.00	14.50
Pumilio Cover F2 Male	2.00	1.50	2.50
Pumilio No Cover F1 Female	10.00	7.00	12.00
Pumilio No Cover F1 Male	14.50	8.00	19.50
Pumilio No Cover F2 Female	10.00	3.00	17.00
Pumilio No Cover F2 Male	9.00	6.00	14.00
Bechuanae (TDR) Cover F1 Female	14.00	5.50	22.50
Bechuanae (TDR) Cover F1 Male	21.00	17.00	27.00
Bechuanae (TDR) Cover F2 Female	11.00	6.00	13.00

Level	Frequency of centre entries		
	Median	1st quartile	3rd quartile
Pumilio Cover F1 Female	6.00	4.00	12.00
Bechuanae (TDR) Cover F2 Male	23.00	17.00	23.00
Bechuanae (TDR) No Cover F1 Female	9.00	4.00	19.00
Bechuanae (TDR) No Cover F1 Male	10.50	5.25	12.25
Bechuanae (TDR) No Cover F2 Female	9.00	6.00	11.00
Bechuanae (TDR) No Cover F2 Male	7.00	5.00	8.00
Bechuanae (Sand) Cover F1 Female	12.00	10.50	35.50
Bechuanae (Sand) Cover F1 Male	45.00	27.50	47.50
Bechuanae (Sand) Cover F2 Female	29.00	24.50	33.50
Bechuanae (Sand) Cover F2 Male	9.50	6.25	13.25
Bechuanae (Sand) No Cover F1 Female	58.00	41.00	75.00
Bechuanae (Sand) No Cover F1 Male	15.00	7.25	26.50
Bechuanae (Sand) No Cover F2 Female	17.50	12.25	22.75
Bechuanae (Sand) No Cover F2 Male	40.00	28.50	66.25
Dilectus (Sand) Cover F1 Female	15.00	6.00	23.00
Dilectus (Sand) Cover F1 Male	17.50	12.00	32.75
Dilectus (Sand) Cover F2 Female	31.00	38.00	38.00
Dilectus (Sand) Cover F2 Male	31.00	17.00	34.00
Dilectus (Sand) No Cover F1 Female	12.00	3.50	15.00
Dilectus (Sand) No Cover F1 Male	22.00	6.00	36.75
Dilectus (Sand) No Cover F2 Female	7.00	2.00	8.00
Dilectus (Sand) No Cover F2 Male	12.00	4.00	22.00

Supplementary Material 4

Model outcomes (Table S3A) and data summary (Table S3B) for non-significant factors influencing the duration spent in the light chamber of the LD test by male and female individuals, from four populations, that were raised under cover and no cover for two generations. The populations were Pumilio (*Rhabdomys pumilio*), Bechuanae (TDR - *R. bechuanae* from TDR), Bechuanae (Sand - *R. bechuanae* from Sandveld) and Dilectus (Sand - *R. d. dilectus* from Sandveld). Treatments were first generation (F1) and second generation (F2) individuals raised under cover or no cover.

Table S3A. Model outcomes for the non-significant factors influencing the duration of time spent in the light chamber of the LD test.

Predictor	**Statistics**
Population	χ^2_3=2.73, p=0.435
Generation	χ^2_1=2.84, p=0.092
Sex	χ^2_1=0.09, p=0.759
Population*treatment	χ^2_3=2.202, p=0.532
Population*sex	χ^2_3=4.04, p=0.257
Treatment*generation	χ^2_1=2.43, p=0.119
Population*treatment*generation	χ^2_6=6.31, p=0.389
Population*treatment*generation*sex	χ^2_8=3.47, p=0.902

Table S3B. Data summary for the non-significant factors influencing the duration of time spent in the light chamber of the LD test.

Level	Duration spent in light chamber		
	Median	1st quartile	3rd quartile
Pumilio Cover F1 Female	157.84	148.56	296.24
Pumilio Cover F1 Male	66.32	64.96	70.16
Pumilio Cover F2 Female	83.12	44.84	191.60
Pumilio Cover F2 Male	28.92	19.38	38.46
Pumilio No Cover F1 Female	174.40	40.80	189.36
Pumilio No Cover F1 Male	192.52	88.12	203.44
Pumilio No Cover F2 Female	169.20	119.12	205.20
Pumilio No Cover F2 Male	158.16	151.04	181.44
Bechuanae (TDR) Cover F1 Female	78.20	56.86	116.15
Bechuanae (TDR) Cover F1 Male	89.20	74.04	108.70
Bechuanae (TDR) Cover F2 Female	124.30	89.32	142.20
Bechuanae (TDR) Cover F2 Male	126.20	109.20	134.30
Bechuanae (TDR) No Cover F1 Female	132.10	115.96	199.47
Bechuanae (TDR) No Cover F1 Male	137.74	121.45	155.42
Bechuanae (TDR) No Cover F2 Female	154.30	123.10	163.20
Bechuanae (TDR) No Cover F2 Male	154.20	132.10	189.20
Bechuanae (Sand) Cover F1 Female	134.24	70.56	162.88
Bechuanae (Sand) Cover F1 Male	110.32	87.52	158.96
Bechuanae (Sand) Cover F2 Female	94.96	53.12	136.80
Bechuanae (Sand) Cover F2 Male	132.48	84.48	189.64
Bechuanae (Sand) No Cover F1 Female	122.28	97.22	147.34
Bechuanae (Sand) No Cover F1 Male	149.88	129.72	196.74
Bechuanae (Sand) No Cover F2 Female	190.96	188.16	193.76
Bechuanae (Sand) No Cover F2 Male	203.40	200.28	228.92
Dilectus (Sand) Cover F1 Female	121.90	101.20	132.20
Dilectus (Sand) Cover F1 Male	134.87	103.62	172.23
Dilectus (Sand) Cover F2 Female	143.20	86.24	175.68
Dilectus (Sand) Cover F2 Male	109.20	71.20	134.30
Dilectus (Sand) No Cover F1 Female	109.20	81.93	122.25
Dilectus (Sand) No Cover F1 Male	150.47	101.45	191.91
Dilectus (Sand) No Cover F2 Female	127.36	113.20	153.76
Dilectus (Sand) No Cover F2 Male	159.04	123.20	191.20

Supplementary Material 5

Model outcomes (Table S4A) and data summary (Table S4B) for non-significant factors influencing the frequency of entries into the light chamber of the LD test by male and female individuals, from four populations, that were raised under cover and no cover for two generations. The populations were Pumilio (*Rhabdomys pumilio*), Bechuanae (TDR - *R. bechuanae* from TDR), Bechuanae (Sand - *R. bechuanae* from Sandveld) and Dilectus (Sand - *R. d. dilectus* from Sandveld). Treatments were first generation (F1) and second generation (F2) individuals raised under cover or no cover.

Table S4A. Model outcomes for the non-significant predictors influencing the number of entries into the light chamber in the LD test.

Predictor	**Statistics**
Treatment	χ^2_1=1.32, p=0.251
Population	χ^2_3=2.36, p=0.501
Generation	χ^2_1=0.78, p=0.376
Sex	χ^2_1=0.24, p=0.627
Population*treatment	χ^2_3=2.43, p=0.488
Population*sex	χ^2_3=2.51, p=0.474
Treatment*generation	χ^2_1=2.24, p=0.134
Population*treatment*sex	χ^2_4=3.22, p=0.521
Population*treatment* generation	χ^2_6=7.21, p=0.302
Population*treatment*generation*sex	χ^2_8=1.94, p=0.983

Table S4B. Data summary for the non-significant predictors influencing the number of entries into the light chamber of the LD test.

Level	**Number of entries into light chamber**		
	Median	**1st quartile**	**3rd quartile**
Pumilio Cover F1 Female	1.00	1.00	2.00
Pumilio Cover F1 Male	4.00	3.00	6.00
Pumilio Cover F2 Female	1.00	1.00	6.00
Pumilio Cover F2 Male	6.00	3.50	8.50
Pumilio No Cover F1 Female	5.00	2.00	5.00
Pumilio No Cover F1 Male	5.50	2.00	7.50
Pumilio No Cover F2 Female	6.00	1.00	13.00
Pumilio No Cover F2 Male	5.00	5.00	6.00
Bechuanae (TDR) Cover F1 Female	6.00	5.00	8.00

Level	Number of entries into light chamber		
	Median	1st quartile	3rd quartile
Bechuanae (TDR) Cover F1 Male	6.00	3.00	9.00
Bechuanae (TDR) Cover F2 Female	7.00	6.00	9.00
Bechuanae (TDR) Cover F2 Male	8.00	8.00	9.00
Bechuanae (TDR) No Cover F1 Female	8.00	4.50	11.00
Bechuanae (TDR) No Cover F1 Male	4.00	3.75	5.50
Bechuanae (TDR) No Cover F2 Female	3.00	3.00	7.00
Bechuanae (TDR) No Cover F2 Male	4.00	4.00	7.00
Bechuanae (Sand) Cover F1 Female	6.00	4.50	7.00
Bechuanae (Sand) Cover F1 Male	7.00	6.00	9.00
Bechuanae (Sand) Cover F2 Female	3.00	2.00	4.00
Bechuanae (Sand) Cover F2 Male	3.00	2.50	4.50
Bechuanae (Sand) No Cover F1 Female	8.00	7.50	8.50
Bechuanae (Sand) No Cover F1 Male	6.00	6.00	6.75
Bechuanae (Sand) No Cover F2 Female	7.50	7.25	7.75
Bechuanae (Sand) No Cover F2 Male	4.50	3.25	6.50
Dilectus (Sand) Cover F1 Female	5.00	3.00	6.00
Dilectus (Sand) Cover F1 Male	6.50	6.00	9.75
Dilectus (Sand) Cover F2 Female	9.00	6.00	11.00
Dilectus (Sand) Cover F2 Male	9.00	5.00	11.00
Dilectus (Sand) No Cover F1 Female	6.00	3.50	9.50
Dilectus (Sand) No Cover F1 Male	4.00	2.25	8.75
Dilectus (Sand) No Cover F2 Female	5.00	4.00	7.00
Dilectus (Sand) No Cover F2 Male	7.00	3.00	8.00

Supplementary Material 6

Model outcomes (Table S5A) and data summary (Table S5B) for non-significant factors influencing the latency to re-enter the light compartment after a startle by male and female individuals, from four populations, that were raised under cover and no cover for two generations. The populations were Pumilio (*Rhabdomys pumilio*), Bechuanae (TDR - *R. bechuanae* from TDR), Bechuanae (Sand - *R. bechuanae* from Sandveld) and Dilectus (Sand - *R. d. dilectus* from Sandveld). Treatments were first generation (F1) and second generation (F2) individuals raised under cover or no cover.

Table S5A. Model outcomes for the non-significant predictors influencing the latency to re-enter the light compartment after a startle.

Predictor	Statistics
Treatment	χ^2_1=0.36, p=0.546
Population	χ^2_3=3.93, p=0.269
Generation	χ^2_1=0.09, p=0.760
Sex	χ^2_1=1.41, p=0.234
Population*treatment	χ^2_3=5.25, p=0.154
Population*sex	χ^2_3=0.96, p=0.811
Treatment*generation	χ^2_1=0.01, p=0.963
Population*treatment*sex	χ^2_4=1.08, p=0.898
Population*treatment*generation	χ^2_6=9.31, p=0.157
Population*treatment*generation*sex	χ^2_8=10.40, p=0.238

Table S5B. Data summary of factors influencing the non-significant factors influencing the latency to re-enter the light compartment after a startle.

Level	Latency to re-enter light chamber		
	Median	1st quartile	3rd quartile
Pumilio Cover F1 Female	300.00	120.00	300.00
Pumilio Cover F1 Male	222.00	75.00	223.00
Pumilio Cover F2 Female	234.00	136.00	267.00
Pumilio Cover F2 Male	300.00	300.00	300.00
Pumilio No Cover F1 Female	23.00	4.00	85.00
Pumilio No Cover F1 Male	300.00	94.50	300.00
Pumilio No Cover F2 Female	126.00	85.00	161.00
Pumilio No Cover F2 Male	157.00	76.00	187.00
Bechuanae (TDR) Cover F1 Female	105.40	36.70	230.50

Level	Latency to re-enter light chamber		
	Median	1st quartile	3rd quartile
Pumilio Cover F1 Female	300.00	120.00	300.00
Pumilio Cover F1 Male	222.00	75.00	223.00
Pumilio Cover F2 Female	234.00	136.00	267.00
Pumilio Cover F2 Male	300.00	300.00	300.00
Pumilio No Cover F1 Female	23.00	4.00	85.00
Pumilio No Cover F1 Male	300.00	94.50	300.00
Pumilio No Cover F2 Female	126.00	85.00	161.00
Pumilio No Cover F2 Male	157.00	76.00	187.00
Bechuanae (TDR) Cover F1 Male	89.20	38.75	213.05
Bechuanae (TDR) Cover F2 Female	101.20	89.20	198.20
Bechuanae (TDR) Cover F2 Male	190.20	101.20	213.20
Bechuanae (TDR) No Cover F1 Female	178.20	70.70	259.60
Bechuanae (TDR) No Cover F1 Male	190.10	33.20	300.00
Bechuanae (TDR) No Cover F2 Female	65.40	35.20	109.20
Bechuanae (TDR) No Cover F2 Male	59.30	39.20	231.10
Bechuanae (Sand) Cover F1 Female	185.00	110.50	242.50
Bechuanae (Sand) Cover F1 Male	182.00	109.50	241.00
Bechuanae (Sand) Cover F2 Female	229.50	194.25	264.75
Bechuanae (Sand) Cover F2 Male	300.00	269.00	300.00
Bechuanae (Sand) No Cover F1 Female	171.00	106.50	235.50
Bechuanae (Sand) No Cover F1 Male	282.50	98.50	300.00
Bechuanae (Sand) No Cover F2 Female	211.00	207.50	214.50
Bechuanae (Sand) No Cover F2 Male	223.00	111.50	300.00
Dilectus (Sand) Cover F1 Female	143.00	123.90	161.00
Dilectus (Sand) Cover F1 Male	169.10	67.35	300.00
Dilectus (Sand) Cover F2 Female	43.20	34.20	96.00
Dilectus (Sand) Cover F2 Male	60.00	17.00	98.20
Dilectus (Sand) No Cover F1 Female	104.00	89.70	163.50
Dilectus (Sand) No Cover F1 Male	265.55	98.95	300.00
Dilectus (Sand) No Cover F2 Female	243.00	190.20	300.00
Dilectus (Sand) No Cover F2 Male	123.20	34.20	300.00

SPATIAL COGNITION IN *RHABDOMYS* TAXA ORIGINATING FROM DIFFERENT ECOLOGICAL CONTEXTS

Abstract

Variation in spatial cognition is correlated with differences in environments, such that different environments may select for different levels of cognitive ability. The aim of my study was to establish whether four populations, representing 3 taxa, of the striped mouse genus *Rhabdomys*, have different spatial cognition. I studied two sympatric and two allopatric populations from three locations across a rainfall gradient in southern Africa, which vary in habitat complexity. Using a Barnes maze, I tested individuals for their learning ability, their use of external and internal cues for navigation, and their spatial memory. In three variables (errors in finding the escape hole, latency to find the escape hole and distance travelled), populations showed similarities in learning, the use of internal cues in the maze and general spatial memory. Populations differed only slightly in their accuracy when internal cues were removed and in their spatial memory, with *R. bechuanae* initially performing significantly better without external cues, and both *R. pumilio* and *R. bechuanae* initially performing significantly better in the spatial memory test. However, the taxa performed similarly in all other aspects of testing. The similarities suggest that the environment and context (sympatric or allopatric) of origin do not influence spatial cognition of the taxa in a Barnes maze. I suggest that spatial cognition is phylogenetically constrained in the genus or there might be similar selection pressures, such as a similar need to forage over large distances, in this omnivorous genus.

Introduction

Environmental variation can influence the phenotypes of species that are geographically widespread. Selection pressure in different environments can increase the prevalence of certain phenotypes, such as behavioural phenotypes

(Foster 1999), a process known as local adaptation (Taylor 1991). For example, white-crowned sparrows *Zonotrichia leucophrys oriantha* show geographically-mediated population-specific differences in their song dialects (Baptista & King 1980). Mean population-level differences in behaviour can also occur due to random stochastic processes, like genetic drift (Lande 1976). However, populations could be similar, due to gene flow between populations (Alleaume-Benharira *et al.* 2006; Edelaar *et al.* 2008), similar ecological selection pressures (Futuyma 2009), or to phylogenetic constraints (e.g. Roll *et al.* 2006).

Selection pressures within an organism's environment may influence brain evolution (Clutton-Brock & Harvey 1980; Mace *et al.* 1981; Safi & Dechmann 2005) and cognitive ability (reviewed in Healy *et al.* 2009) in different species and populations (Roth *et al.* 2012). For example, Atlantic cod *Gadus morhua* individuals raised in physically highly complex hatcheries, learn how to handle novel prey quicker than those raised in hatchery tanks without complexity (Strand *et al.* 2010). In the tropical poeciliid fish *Brachyraphis episcopi*, individuals from low predation areas solved a maze faster and more accurately than fish from the same stream but in an area with more predators (Brown & Braithwaite 2005; Beri *et al.* 2014). In contrast, Burns and Rodd (2008) found that free-living guppies *Poecilia reticulata* from high predation sites made slow but accurate decisions when solving a maze, compared to guppies from low predation sites. In these examples, variation in cognition is linked to variation in fitness (Dukas 2004), which may be driven by predation pressure (Kelley & Brown 2011; Beri *et al.* 2014).

Spatial cognition is defined as an array of mental representations of objects and the spatial relationships between them (Jacobs 2003). It includes the navigation of an animal in its environment, as well as mental mapping and spatial memory of points, and the ability of an animal to re-orientate itself successfully when it becomes disorientated (Jacobs 2003; Lee *et al.* 2012). There is evidence that different selection pressures in different geographic locations (allopatric populations) influence spatial cognition within species (Freas *et al.* 2012; White & Brown 2015). For example, mountain chickadees *Poecile gambeli* living at higher elevations (associated with harsher climate) have better spatial memory than chickadees living at lower elevations (associated with a milder climate), with the climate acting as selection pressure on spatial cognition (Croston *et al.* 2016).

Food caching may also be under environmental selection pressure. The ability to cache food and reliably remember the cache locations increases fitness (reviewed in Healy & Jones 2002). Food storing is a complex task that requires spatial memory to recall the location of the cache (Healy *et al.* 2009). Remembering the locations of good foraging sites may require good spatial memory, even for non-caching species. Likewise, an animal's home range size and resource distribution may also be associated with spatial ability (Gaulin & FitzGerald 1986), because the animal will encounter more spatial features in large, compared to small, home ranges. For example, male deer mice *Peromyscus maniculatus bairdii*, which expand their home ranges during the breeding season, have better spatial memory than male California mice *Peromyscus californicus insignis*, which do not show any range expansion (Jašarević *et al.* 2012).

Many studies previously considered individual variation as noise around a population average (Chittka *et al.* 2012; Thornton & Lukas 2012), but individual variation has important fitness consequences (Chittka *et al.* 2012). Populations consist of genetically different individuals, so there are usually multiple phenotypes across a heterogeneous environment (Via & Lande 1985). Like many behavioural phenotypes, individuals show inter-individual variability in their cognitive ability (Cole *et al.* 2011). This comes about through genetic differences, environmental variation and the interaction of both (reviewed in Thornton & Lukas 2012), as well as stochastic events during ontogeny (Dall *et al.* 2012). Nonetheless, there may be environmental selection pressures or phylogenetic constraints on cognitive phenotypes in the population, leading to low variation within the population. Therefore, the degree of variation within a population can be indicative of the evolutionary pressures on the phenotypes within the population (Valladares *et al.* 2014).

A good study model to demonstrate the influence of environmental variation on spatial cognition is the striped mouse *Rhabdomys*, a small (±80 g) diurnal murid rodent, occurring in most biomes throughout southern Africa (Skinner & Chimimba

2005). The genus consists of at least four species (du Toit *et al.* 2012a). In South Africa, *R. bechuanae* occurs in the central parts of the country, and is proposed as being derived first from a *Rhabdomys* ancestor (du Toit *et al.* 2012a). *R. pumilio* arose during a radiation into the arid western parts (Meynard *et al.* 2012). *R. dilectus* occurs in the central Free State Province grasslands and eastwards, and diverged from *R. pumilio* during a second adaptive radiation during the Pliocene (Meynard *et al.* 2012). *R. dilectus* is further subdivided, with *R. d. dilectus* occurring from the northern Free State Province through to the moist northern parts of South Africa, while *R. d. chakae* occurs from the south-eastern parts of the Free State Province, eastwards (Ganem *et al.* 2012). I studied four populations of *Rhabdomys* in three geographic locations along the east-west rainfall gradient in South Africa, including one *R. pumilio* population from the semi-arid west, a population each of *R. bechuanae* and *R. d. dilectus* occurring in sympatry in the northern parts of the Free State Province and an allopatric population of *R. d. dilectus* originating from the northern parts of Limpopo Province.

There are interesting biological and ecological differences between a population of *R. pumilio* from Goegap Nature Reserve in the west and *R. d. chakae*, the better studied sub-species of *R. dilectus*. *R. pumilio* lives in small colonies of 2-4 adult females, a breeding male and several philopatric adult offspring (Schradin & Pillay 2004), although group-living is facultative, depending on environmental conditions (Schradin *et al.* 2012) and the mass of females (Hill *et al.* 2015). In contrast, *R. dilectus* in the grasslands is solitary-living (Brooks 1974), and is characterized by intra-sexually non-overlapping territories, where male territories overlap those of several females (Schradin & Pillay 2005). Although *R. dilectus* lives solitarily, its territories in one population were 6-10 times larger than *R. pumilio* (4662m^2-14466m^2 vs 819m^2-1530m^2 respectively; Schradin & Pillay, 2005). It has been proposed that the territory size is related to the availability of food, which is patchily distributed in grasslands (Schradin & Pillay 2005). Accordingly, *R. d. dilectus* individuals must travel further than *R. pumilio* individuals on a daily basis in order to forage.

While spatial cognition has been tested in *R. pumilio* in the semi-arid Succulent Karoo (Maille *et al.* 2015), nothing is known about the spatial cognition of other *Rhabdomys* taxa. In addition, little is known about how striped mice navigate, apart from Maille *et al*.'s 2015 study, which demonstrated that *R. pumilio* is able to navigate through a Barnes maze with external maze cues. The Barnes maze is designed to test spatial cognition ability by requiring study subjects to learn, discriminate and recall a specific location within a round enclosure (Barnes 1979; Paul *et al.* 2009). Training to establish the target exit is conducted for a set number of sessions using internal maze cues, external maze cues or no cues, followed by a testing phase in order to assess spatial learning (Harrison *et al.* 2006).

To date, few studies have investigated differences in cognition, among populations and closely related species across an environmental gradient (principally rainfall) or between populations of different, closely related species living in sympatry. The aim of this study was to establish whether there were differences in the spatial cognition ability of four *Rhabdomys* populations representing three taxa, in three geographic locations that are distributed across a rainfall gradient. I asked two questions about the learning and memory of the *Rhabdomys* taxa in a Barnes maze. Question 1a: Can study subjects from all populations learn to navigate in the Barnes maze? Question 1b: If learning does occur, do individuals from all populations respond equally to the removal of external cues from the Barnes maze (i.e. can they navigate using internal maze cues)? Question 2: What are the similarities and differences in the spatial memory of the 4 populations, and are these related to the geographic location or context (sympatry or allopatry)? No *a priori* hypotheses were constructed due to the lack of information about spatial cognition in *Rhabdomys* taxa.

Materials and methods

Study subjects and husbandry

I tested 14 male and 14 female study subjects each from Goegap Nature Reserve (*R. pumilio*), Sandveld Nature Reserve (*R. bechuanae*) and Entabeni Forest

Reserve (allopatric *R. d. dilectus*) and 14 male and 14 female sympatric *R. d. dilectus* from Sandveld Nature Reserve (Figure 5.1). Goegap is characterised by low scrubby bushes with large open spaces between bushes (Schradin & Pillay 2005). In contrast, both Sandveld and Entabeni have high levels of grassy cover, with Sandveld having more trees (Ganem *et al.* 2012) and bushes than Entabeni, which consists of an Afro-montane mosaic of forest and grasslands (Mostert *et al.* 2008). Goegap is the most arid of the three sites with a mean annual rainfall of approximately 160mm, most of which falls in winter (Schradin & Pillay 2005). Sandveld Nature reserve is characterised by summer rainfall of approximately 500mm (Jankielsohn 2006), and Entabeni Forest reserve receives year-round rainfall of approximately 1800mm per annum (Mostert *et al.* 2008).

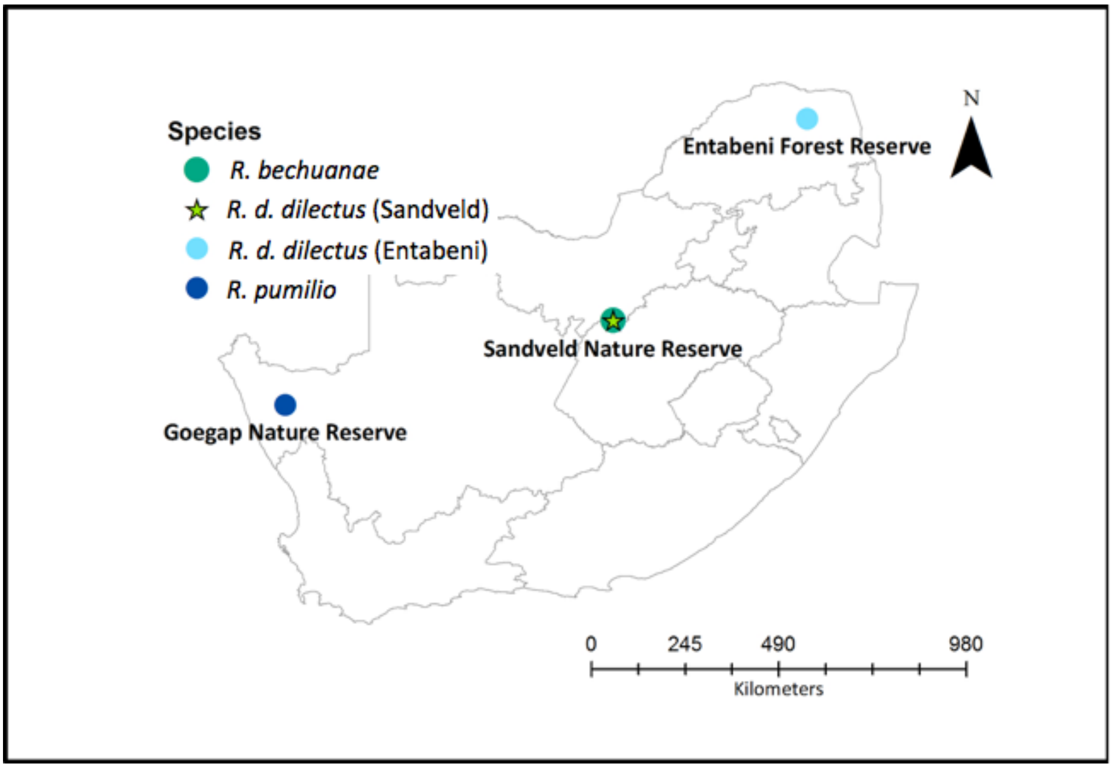

Figure 5.1. Map of South Africa showing locations where the taxa originated. *Rhabdomys pumilio* originated from Goegap Nature Reserve (29°41’33”S, 18°1’41”E) in the Northern Cape Province, *R. bechuanae* and *Rhabdomys dilectus dilectus* originated from the Sandveld Nature Reserve (27°41’57”S, 25°44’13”E) in the Free State Province, and *R. d. dilectus* originated from the Entabeni Forest Reserve (22°58’59”S, 30°16’56”E) in the Limpopo Province.

Study subjects of *R. bechuanae*, sympatric *R. d. dilectus* and allopatric *R. d. dilectus* were wild caught and kept in captivity for at least 6 months prior to testing. *R. pumilio* study subjects were captive-bred (3-5th generation). Captive *R. pumilio* individuals have been shown to have similar behaviour to individuals tested in nature (Maille et al. 2015; Yuen *et al.* 2015). Study subjects were housed individually in standardised tanks (46 x 30 x 35cm) with mesh lids for approximately 1 week prior to the start of the experiment. Tanks contained a layer of wood shavings, a handful of dry grass, a plastic nesting box (10 x 10 x 10cm), paper towel for nesting material, a cardboard roll for enrichment and a wooden block to chew on. Individuals were fed daily with Epol® mouse cubes, approximately 5g of fresh vegetables and a teaspoon of millet per day. Water was available *ad libitum*. Study subjects were kept in a room with partially controlled environmental conditions (22-24 °C, RH 30-50%, 14:10 L:D cycle, with lights on at 05h00). Training and testing took place between 06h00 and 12h00 since striped mice are diurnal (Schradin 2006). The experimental protocol was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC 2013/18/2A).

Equipment and design

I used a modified Barnes maze, following Jašarević *et al*. (2012), which consisted of a circular base (150cm diameter) surrounded by a 50cm high opaque wall with five 5cm diameter holes spaced equally along the wall, placed 1cm above the base. Clear curved tunnels were attached to the outside of the maze, one of which was connected to a test subject's home tank via made-to-fit circular openings in the wall of the home tanks. In the Barnes maze, the remaining four tunnels were blocked off at the tunnel entrance using clear acrylic glass sheets. Tunnels were closed off when a different subject was trained using another tunnel (which was opened). The location of the subject's home tunnel remained constant relative to the maze for the entire experiment. Five objects (a rock, ceramic pot, stack of wooden dowel sticks, a round pebble and a plastic aquarium plant) were placed inside the arena in marked positions. External (room) cues consisted of two doors at the opposite ends of the room, a large table against an adjacent wall and a metal sink in the opposite corner to the table. There were no adornments on the walls.

Study subjects were transferred into the maze using a clear starting box with a removable platform placed in the centre of the arena. Since a subject could turn in the starting box, the box was not placed facing any particular direction, and the test subject could start the session facing in any direction. Sunflower seeds were placed in the home tank of the individual as a positive stimulus to entice the mouse back into their home tank.

I trained striped mice subjects 3 times per day for 7 days (i.e. 21 training sessions in total), with at least 5 minutes between each of the 3 training sessions per day. During training trials (days 1 to 4), the start box was lifted off the platform and the subject was allowed a maximum of 5 minutes to explore the maze and find its home tank. The trial ended immediately after the subject found its home tunnel, if the subject exited the maze within the allotted time. If it did not find the home tunnel, I gently guided the individual there. On day 5, I hung opaque mosquito-net draping around the maze to remove all extra-maze cues for navigation (Paul *et al.* 2009). During all further training and testing, the maze remained in the same configuration. Training continued with the curtain in place for a further two days (days 6 and 7). I measured latency to find the home tunnel, the distance that each study subject travelled as well as the number of errors (defined as the number of times a study subject went within 1 mouse body-length of an incorrect tunnel). On day 8 and 9, each individual underwent one 90 second probe test each day (probe test 1 and 2), with the curtain around the maze, in order to test for memory for the exit hole without external cues available. All tunnels were blocked by clear acrylic glass sheets, which meant that there was no exit from the Barnes maze.

Study subjects were trained using different tunnels, and at the end of each day, the arena and all the objects were washed with laboratory disinfectant and water to remove the smell of the occupants from that day. Since the test subjects were not trained using the same exit tunnel, if the smell from a previous occupant affected the training, subsequent occupants would not have been trained to their specific exit tunnel. Instead, all subjects found their own exit tunnels, regardless of the location of the exit tunnel of the previous test subject. This was shown by the short latency times on day 4 (see Results). During the probe test phase, I cleaned the maze thoroughly between tests of each subject in order to remove all scent cues. All training testing sessions and probe tests were filmed using a camera mounted above the maze and footage was analysed later using Ethovision™ software (Noldus 2013).

Data analysis

I checked for normality of the data using Shapiro-Wilks W tests and used repeated measures ANOVAs to analyse the number of errors made, distance travelled, and the latency to find the correct tunnel in Statistica 12 (Statsoft 2013). Population and sex were fixed effects and day of testing (e.g. day 1 or day 4) was the repeated measures. Post-hoc comparisons were done using Fisher's LSD *post hoc* tests for significant first and second order effects. For significant three-way interactions, I assessed the influence of predictors using linear and polynomial contrasts. I plotted graphs using means and standard errors. Non-significant results are presented in tables in Supplementary material. All tests were two-tailed and alpha set at 0.05.

To answer question 1a, I established whether study subjects could learn the location of their home tunnel by comparing their performance on day 1 to day 4. For question 1b, I compared performance in the Barnes maze on day 4 to day 5, with the curtain placed around the maze to establish whether external cues were important for navigation. To answer question 2, I established whether the populations had different spatial memory by comparing performance in the Barnes maze in probe tests 1 and 2.

Results

Q1a. Did individuals from all 4 populations learn the location of their home tunnels in the training phase?

Errors

Day and sex were significant predictors of the number of errors (day: $F_{1,85}$=5.581, p=0.020; sex: $F_{1,85}$=4.785, p=0.031), with individuals of all populations making fewer errors on day 4 than day 1 and females making fewer errors than males (Figure 5.2). Population, population*sex, day*population, day*sex and day*population*sex were not significant predictors of errors (Supplementary Material 1).

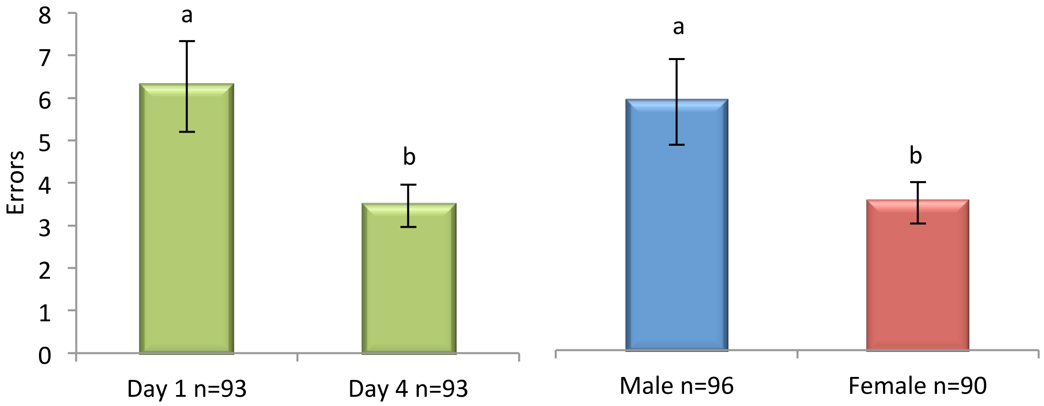

Figure 5.2. Mean (± SE) number of errors made in the Barnes maze. Day and sex were significant predictors of errors. Fisher's post hoc tests showed differences between groups, indicated by different letters.

Distance

Sex was a significant predictor of distance travelled ($F_{1,85}$=6.589, p=0.012) with females travelling less than males (Figure 5.3). Population, day, population*sex, day*population, day*sex and day*population*sex were not significant predictors of distance travelled (Supplementary Material 2).

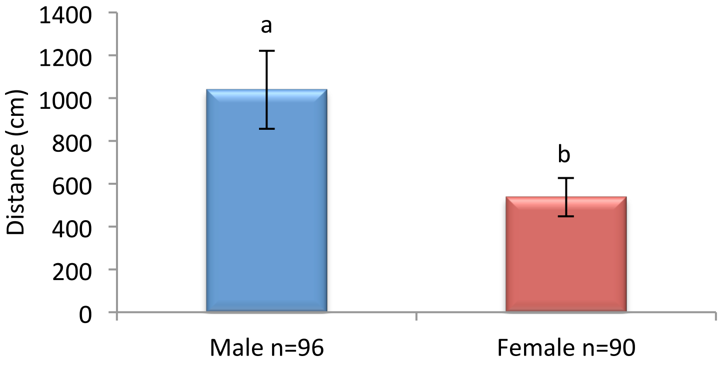

Figure 5.3. Mean (± SE) distance travelled in the Barnes maze over days 1 and 4. Sex was a significant predictor of distance travelled. Fisher's post hoc tests showed differences between groups, indicated by different letters.

Latency

Day was a significant predictor of latency to find the correct tunnel ($F_{1,85}$=14.936, p=0.001), with individuals from all populations taking longer to find their home tunnel on day 1 than day 4 (Figure 5.4). Day*population*sex was also a significant predictor of latency ($F_{3,85}$=3.289, p=0.025). Linear polynomial contrasts were not significant (t=-1.413, p=0.161), but quadratic polynomial contrasts showed a significant temporally fluctuating relationship between the predictors and latency (t=2.347, p=0.021). Population, sex, population*sex, day*population and day*sex were not significant predictors of latency to find the correct tunnel (Supplementary Material 3).

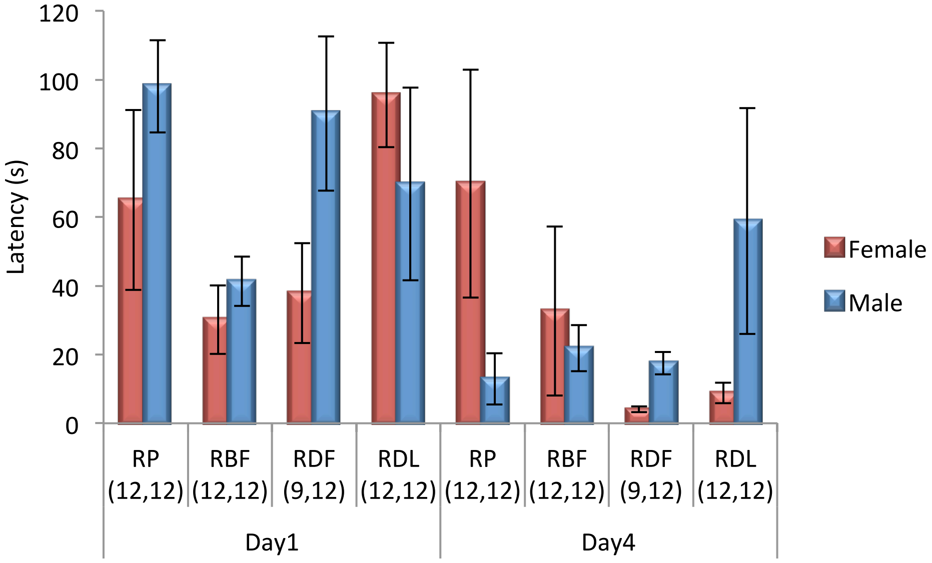

Figure 5.4. Mean (±SE) latency to find the correct tunnel in the Barnes maze. Day and day*population*sex were significant predictors of latency to find the correct tunnel. Fisher's post hoc tests showed that individuals found the correct tunnel quicker on day 4. Quadratic polynomial contrasts showed a significant temporally fluctuating relationship between the predictors and latency. Sample sizes are indicated in brackets.

Q1b. Do individuals from all populations respond equally to the removal of external cues from the Barnes maze?

I compared the number of errors, distance travelled and latency to find the correct home tunnels on day 4 (external cues present) and day 5 (external cues absent).

Errors

Day*population was a significant predictor of the number of errors made ($F_{3,85}$=2.762, p=0.047), with *R. bechuanae* making more errors on day 4 than day 5, and more errors than sympatric *R. d. dilectus* (day 5) and allopatric *R. d. dilectus* (day 4), and *R. pumilio* making more errors on day 5 than allopatric *R. d. dilectus* on day 4, and more errors than *R. bechuanae* and sympatric *R. d. dilectus* on day 5 (Figure 5.5). Population, sex, day, population*sex, day*sex and day*population*sex were not significant predictors of errors (Supplementary Material 4).

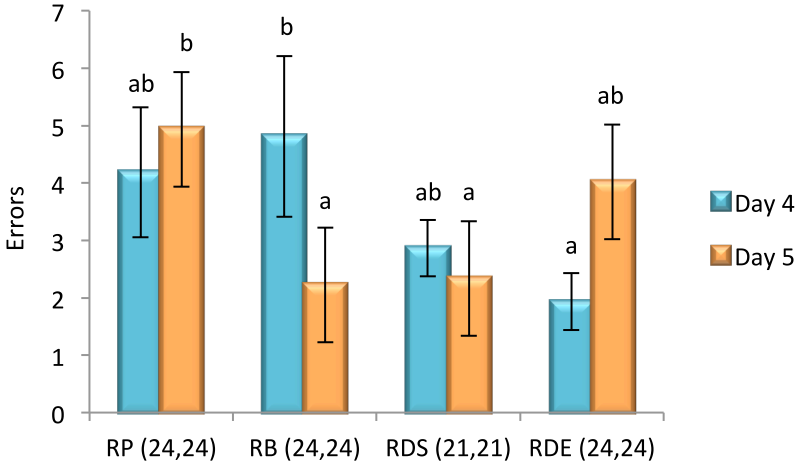

Figure 5.5. Mean (±SE) errors made during day 4 (regular training) and day 5 (external cues removed). Population*day was a significant predictor of errors made. Fisher's post hoc tests showed differences between groups, indicated by different letters.

Distance

Sex was a significant predictor of distance travelled ($F_{1,85}$=4.544, p=0.036) with females travelling less than males (Figure 5.6). Day*population was also a significant predictor of distance travelled ($F_{3,85}$=3.147, p=0.029), with *R. bechuanae* travelling further on day 4 than on day 5, and more than sympatric *R. d. dilectus* and allopatric *R. d. dilectus* on both day 4 and 5 (Figure 5.6). Population, day, population*sex, day*sex and day*population*sex were not significant predictors of distance travelled (Supplementary Material 5).

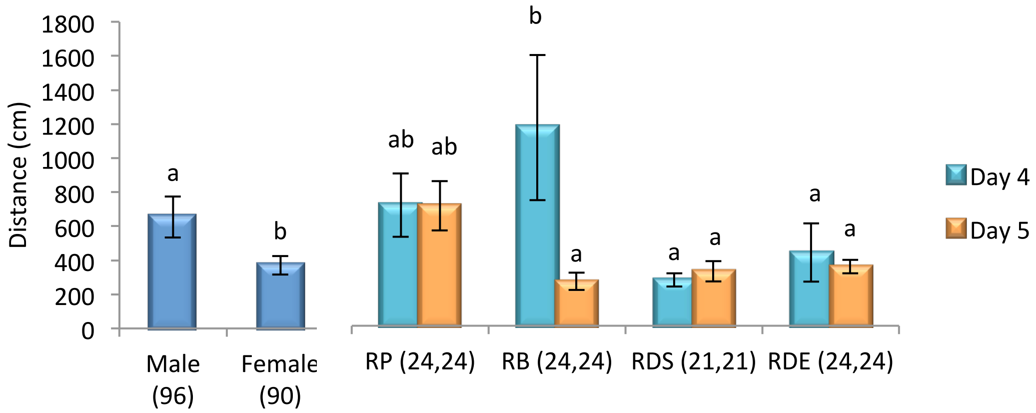

Figure 5.6. Mean (±SE) distance travelled during day 4 (regular training) and day 5 (external cues removed and maze rotated). Sex and Day*Population were significant predictors of distance travelled. Fisher's post hoc tests showed differences between groups, indicated by different letters. Sample sizes are indicated in brackets.

Latency

None of the predictors (population, sex, day, or any of the interactions) influenced the latency to find the correct tunnel (Supplementary Material 6).

Q2. Do the populations differ in their spatial memory in the probe tests?

I compared the spatial memory of the populations by analysing the number of errors, distance travelled and latency to find their correct tunnel during two probe tests, 24 and 48 hours after the last training session.

Errors

Population was a significant predictor of errors made ($F_{3,23}$=4.925, p=0.009), with *R. bechuanae* and *R. pumilio* making significantly fewer errors than sympatric *R. d. dilectus* (Figure 5.7). Test was a significant predictor of errors ($F_{1,23}$=27.159, p=0.000), with individuals making fewer errors during probe test 2 (Figure 5.7). Test*population was also a significant predictor of the number of errors ($F_{3,23}$=5.775, p=0.004), with sympatric *R. d. dilectus* making more errors during probe test 1 than probe test 2, and more errors than *R. bechuanae* and *R. pumilio* in both probe tests. Allopatric *R. d. dilectus* also made significantly more errors during probe test 1 than probe test 2 (Figure 5.7). Sex, population*sex, test*sex and test*population*sex were not significant predictors of errors (Supplementary Material 7).

Distance

Test was a significant predictor of distance travelled ($F_{1,23}$=15.274, p=0.001), with individuals travelling further during probe test 1 than probe test 2 (Figure 5.8). Population, sex, population*sex, test*population, test*sex and test*population*sex were not significant predictors of distance travelled (Supplementary Material 8).

Latency

None of the predictors (population, sex, test, or any of the interaction terms) significantly predicted latency (Supplementary Material 9).

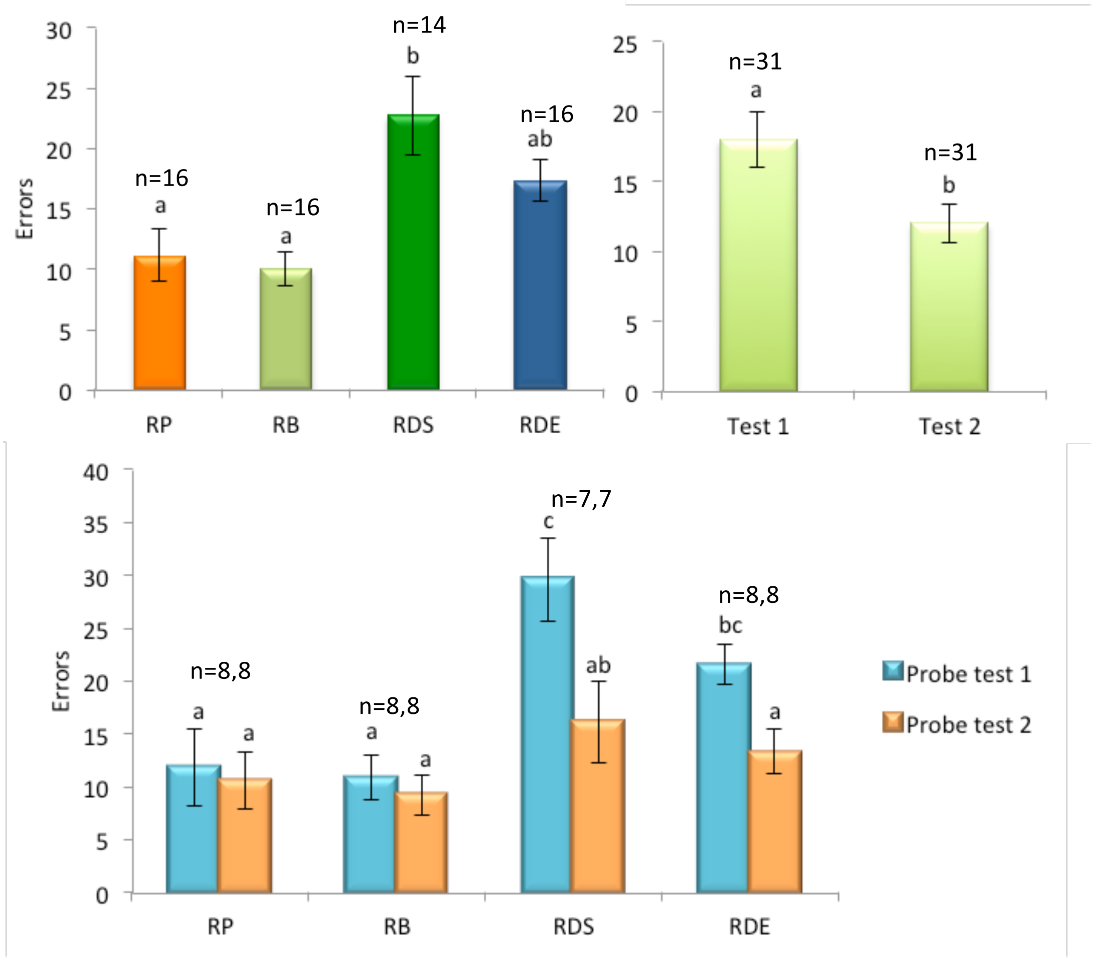

Figure 5.7. Mean (±SE) errors made during probe test 1 and probe test 2, two 90 second tests that are run 24 and 48 hours after the last training session. Population (top left), test (top right) and population*test (bottom) were significant predictors of errors made. Fisher's post hoc tests showed differences between groups, indicated by different letters.

Discussion

The aim of this study was to establish the spatial cognitive ability of 4 *Rhabdomys* populations (with two populations occurring in sympatry), representing three taxa that are distributed across the southern African rainfall gradient. These comparisons of closely related species can potentially provide information on cognitive adaptations within the genus. To investigate spatial navigation and memory among the four populations, I asked two questions, which are discussed below.

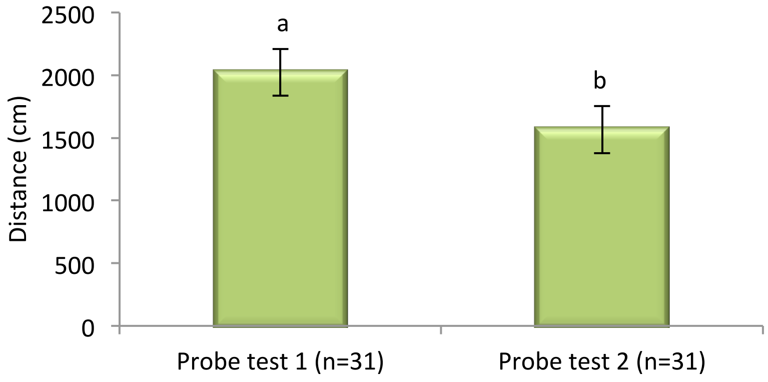

Figure 5.8. Mean (±SE) distance travelled during the two probe tests conducted 24 and 48 hours after the last training session respectively. Test was a significant predictor of distance travelled. Fisher's post hoc tests showed differences between groups, indicated by different letters.

Q1a. Did individuals of all 4 populations learn the location of their home tunnels in the training phase?

In the Barnes maze, the widely-used measures of errors, distance travelled and latency to find the correct tunnel provide an interdependent measure of cognitive performance and learning (Harrison *et al.* 2009). Decreases over time in latency, number of errors, and distance travelled, are all indicative that an animal has learnt the maze. Individuals performed significantly better on day 4 compared to day 1, regardless of population, indicating that individuals from all populations have the ability to learn the location of their home tunnel in the Barnes maze. This is unsurprising given that this was an associative learning task, which most animals are able to accomplish (Dickinson 2012; Heyes 2012).

There were some significant sex differences. Males travelled further than females, a behaviour typical of male rodents, which generally tend to be more explorative than females (e.g. flying squirrels *Pteromys*, Selonen & Hanski 2006; deer mice *Peromyscus*, Jašarević *et al.* 2012). Because the likelihood of making errors increases with distance travelled and activity in the Barnes maze (Gaulin *et al.* 1990), males also made significantly more errors. Regardless, males and females did not significantly differ in latency to find the correct tunnel, indicating a similar ability to learn over time. Female superiority in accuracy during the learning phase was surprising, as studies have shown a significant male advantage in spatial learning tasks for laboratory rats and mice (Jonasson 2005; Heyes 2012), as well as other taxa (reviewed in Jones *et al.* 2003). In contrast, Popović *et al.* (2010) argued that male and female degus *Octodon degu* used different searching strategies while learning, with males motivated to explore and thus make more errors than females (which made fewer errors but also explored less). This is in line with males being more explorative than females, and therefore in *Rhabdomys*, while the sexes differ in their exploration and errors, there may not be any sex advantage associated with learning as the sexes found the tunnel in the same amount of time.

The quadratic polynomial contrast for population*sex*day was significant, indicating that these predictors influence latency in a non-linear fluctuating manner, making for complex interpretation of the relationships as emergent patterns may not be consistent. Although there were some significant population-level differences in latency, when all measures are taken together, learning ability appears to be relatively similar across populations.

Q1b. Do the populations respond differently to the removal of external cues?

Previous studies suggest that other rodents preferentially use external or room cues over internal maze cues (Harrison *et al.* 2006) as would be shown by a decline in performance once external cues were removed. This was not the case in my study, as the performance of the striped mice was largely unaffected by the removal of external cues from the Barnes maze on day 4 and day 5.

All populations showed similar latency to find the correct tunnel. However, *R. bechuanae* individuals had significantly decreased numbers of errors and shorter distances when external cues were initially removed, suggesting that external cues were not necessary for navigation in *R. bechuanae*. This is similar to male deer mice *Peromyscus maniculatus bairdii*, which use intra-maze cues for navigation (Jašarević *et al.* 2012). By probe test 1, all populations had similar performances, suggesting that *R. pumilio*, and both sympatric and allopatric *R. d. dilectus* were able to compensate for the absence of external cues by learning to use intra-maze cues to navigate to their tunnel.

Q2: Do the populations differ in their spatial memory?

Latency to find the correct tunnel may be the most important measure of memory, while the number of errors made and distance travelled provide information on searching and accuracy during the 90 second probe tests. Declines in performance during the probe test phase may indicate memory decay (Ziegler & Wehner 1997; Kleen *et al.* 2006). In my study, latency to find the correct tunnel was unchanged from probe test 1 to probe test 2 for both sexes in all populations, but improved error rate and shorter distances travelled may reflect improved accuracy during the second probe test. Regardless, the similar performance of all populations during probe test 2 suggests a lack of memory decay (i.e. high recall), which may be due to the short inter-test interval (Hilton & Krebs 1990). In contrast to the previous results from questions 1a&b, the lack of significant sex differences in performance showed that the sexes have similar spatial memory.

I found significant population-level differences in the number of errors made, with *R. pumilio* and *R. bechuanae* making fewer errors than sympatric *R. d. dilectus*, while allopatric *R. d. dilectus* was not different to all populations, in both probe tests. The accuracy of sympatric and allopatric *R. d. dilectus* improved significantly during the second test, in comparison to *R. pumilio* and *R. bechuanae*, which remained relatively consistent over both tests. The significant decrease in errors made during the second probe test for both *R. dilectus* populations could be indicative of decreased stress or habituation to the testing conditions. Cognitive ability is diminished at both high and low stress levels, but enhanced at intermediate levels

(Kim & Haller 2007; Lupien *et al.* 2007), which may explain the decrease in errors for *R. dilectus* during the second probe test, as habituation can reduce stress levels (reviewed in Barnum *et al.* 2007). *R. pumilio* and both populations of *R. dilectus* do not respond in the same way to anxiogenic tests like the four-arm plus maze or open field tests (Rymer & Pillay 2012) or isolation stress (Mackay *et al.* 2014), where *R. dilectus* showed a high stress response (at least, initially) in comparison to *R. pumilio*. Although there were population and population*test differences in the accuracy of spatial memory, there were no differences in distance travelled or latency to find the correct tunnel, indicating that the populations have similar spatial memory with variation in accuracy only initially.

Animals that use their spatial cognition potential may tend to have better ability than animals in environments where good spatial cognition is not required (e.g. Maguire *et al.* 2003; Freas *et al.* 2012). For example, du Toit *et al*. (2012b) compared the spatial cognition of Natal mole-rats *Cryptomys hottentotus natalensis* which were raised in sparsely furnished laboratory cages to that of wild-caught mole-rats. Wild-caught mole-rats, which maintain highly complex tunnels in nature, had better spatial cognitive ability compared to their laboratory counterparts which did not maintain any burrow systems (du Toit *et al.* 2012b). Like complex environments, large home ranges are related to better spatial cognition, possibly because individuals need to travel over greater distances (Börger *et al.* 2008), especially for foraging (Hills 2006; Janson & Byrne 2007), thereby encountering more objects in their home ranges. In sympatry, *R. d. dilectus* has a smaller home range size than *R. bechuanae* (sympatric *R. d. dilectus*: 2000m^2 - 4000m^2 vs sympatric *R. bechuanae*: 2500m^2 - 10000m^2; Dufour *et al*. 2015), which might explain why *R. bechuanae* performs slightly better in the Barnes maze. However, the reported home range size of *R. bechuanae* (ca 2500m^2-10000m^2; Dufour *et al.* 2015) is larger than home range sizes of *R. pumilio* (819m^2-1530m^2; Schradin & Pillay 2005). Therefore, home range size alone does not provide a satisfactory explanation for the similarity in spatial ability between *R. pumilio* and *R. bechuanae*, but may provide a potential explanation for the superior performance of *R. bechuanae* when external cues were removed.

Evolutionary considerations

Low levels of individual variation within a population are suggestive of stabilising selection pressure on a trait (Brodie *et al.* 1995), or tight phylogenetic constraint (Roll *et al.* 2006). Conversely, high levels of variation between individuals within a population could be indicative of relaxed phylogenetic effects or different selection pressures in different environments. However, if different populations occurring in different environments have similar behaviour with high levels of variation, this could be indicative of an ancestral trait (phylogenetic constraint; McKitrick 1993), along with, or relaxed selection pressures (Lahti *et al.* 2009) even in different environments.

Environmental influences on spatial cognition have been widely studied, but in my study, the locality of origin was not a significant predictor of spatial cognition. It is possible that phylogeny or similar selection pressures are responsible for the maintenance of spatial cognition ability in this genus. This was indicated by the ability of all striped mice to learn, and by the similar spatial memory of all populations. There were small variations in spatial memory, with both *R. d. dilectus* populations showing similar, potentially anxiety-related, responses to the spatial memory test, also indicating a phylogenetic signal, or similar selection pressures in different environments.

In conclusion, I studied *Rhabdomys* taxa distributed across an environmental gradient, but I did not find a similar gradient in the spatial cognition ability of the populations studied. I also found limited environmental influences on the spatial learning and the use of different cues (local/internal or global/external maze cues). Therefore spatial cognition could be strongly constrained by phylogeny or similar selection pressures, such as a need to forage over large distances, in this genus. Future studies using a Barnes maze with more escape tunnels may detect differences at a finer scale, as this would place more demand on the cognitive abilities of the test subjects.

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Supplementary Material 1

Model outcomes (Table S1A) and data summary (Table S1B) for non-significant factors influencing the number of errors made during day 1 and day 4 of training, in the Barnes maze by male and female *Rhabdomys* individuals. RP (*R. pumilio*) originated from the semi-arid western parts of South Africa, RB and RDS (sympatric *R. bechuanae* and *R. d. dilectus*) originated from the central grasslands, and RDE (allopatric *R. d. dilectus*) originated from the moist eastern parts of South Africa.

Table S1A. Model outcomes for non-significant factors influencing the number of errors made in the Barnes maze.

Predictor	**Statistics**
Population	$F_{3,85}$=1.750, p=0.163
Population*sex	$F_{3,85}$=2.006, p=0.119
Day*population	$F_{3,85}$=1.016, p=0.390
Day*sex	$F_{1,85}$=1.602, p=0.209
Day*population*sex	$F_{3,85}$=1.786, p=0.156

Table S1B. Data summary for the non-significant factors influencing the number of errors made in the Barnes maze.

Level	**Number of errors**	
	Mean	**SE**
RP	5.94	1.49
RB	4.77	0.92
RDS	5.86	1.62
RDE	2.65	0.49
RP Female	3.71	1.14
RP Male	8.17	2.71
RB Female	3.08	1.12
RB Male	6.46	1.40
RDS Female	3.44	0.93
RDS Male	7.67	2.71
RDE Female	3.75	0.85
RDE Male	1.54	0.40
Day 1 RP	7.79	2.74
Day 1 RB	4.83	1.23
Day 1 RDS	8.95	3.09
Day 1 RDE	3.46	0.83
Day 4 RP	4.08	1.13
Day 4 RB	4.71	1.40
Day 4 RDS	2.76	0.49
Day 4 RDE	1.83	0.50
Day 1 Female	4.13	0.76
Day 1 Male	8.08	1.92

Level	Number of errors	
	Mean	**SE**
Day 4 Female	2.87	0.68
Day 4 Male	3.83	0.74
Day 1 RP Female	3.42	1.43
Day 1 RP Male	12.17	5.07
Day 1 RB Female	3.91	1.80
Day 1 RB Male	6.08	1.80
Day 1 RDS Female	4.22	1.73
Day 1 RDS Male	12.50	5.11
Day 1 RDE Female	5.33	1.38
Day 1 RDE Male	1.58	0.57
Day 4 RP Female	4.00	1.83
Day 4 RP Male	4.17	1.42
Day 4 RB Female	2.58	1.54
Day 4 RB Male	6.83	2.23
Day 4 RDS Female	2.67	0.75
Day 4 RDS Male	2.83	0.68
Day 4 RDE Female	2.17	0.81
Day 4 RDE Male	1.50	0.60

Supplementary Material 2

Model outcomes (Table S2A) and data summary (Table S2B) for non-significant factors influencing the distance travelled during day 1 and day 4 of training, in the Barnes maze by male and female *Rhabdomys* individuals. RP (*R. pumilio*) originated from the semi-arid western parts of South Africa, RB and RDS (sympatric *R. bechuanae* and *R. d. dilectus*) originated from the central grasslands, and RDE (allopatric *R. d. dilectus*) originated from the moist eastern parts of South Africa.

Table S2A. Model outcomes for non-significant factors influencing the number of errors made in the Barnes maze.

Predictor	Statistics
Population	$F_{3,85}$=1.226, p=0.305
Day	$F_{1,85}$=2.526, p=0.116
Population*sex	$F_{3,85}$=1.716, p=0.170
Day*population	$F_{3,85}$=2.525, p=0.063
Day*sex	$F_{1,85}$=0.051, p=0.823
Day*population*sex	$F_{3,85}$=2.491, p=0.066

Table S2B. Data summary for the non-significant factors influencing the distance travelled in the Barnes maze.

Level	Distance (cm)	
	Mean	**SE**
RP	1000.33	239.72
RB	870.68	220.09
RDS	817.70	236.06
RDE	500.90	136.13
Day 1	958.70	167.56
Day 4	634.80	127.02
RP Female	488.65	139.30
RP Male	1512.02	438.97
RB Female	534.90	145.48
RB Male	1206.46	408.66
RDS Female	544.47	227.87
RDS Male	1022.63	375.22
RDE Female	585.88	219.24
RDE Male	415.92	164.44
Day 1 RP	1313.46	440.15
Day 1 RB	612.76	146.17
Day 1 RDS	1374.86	442.77
Day 1 RDE	585.73	218.25
Day 4 RP	687.21	180.59
Day 4 RB	1128.60	413.30
Day 4 RDS	260.54	37.10
Day 4 RDE	416.07	165.76

Level	Distance (cm)	
	Mean	SE
Day 1 Female	670.84	149.70
Day 1 Male	1228.56	289.16
Day 4 Female	405.30	97.88
Day 4 Male	849.95	225.27
Day 1 RP Female	438.79	120.15
Day 1 RP Male	2188.13	810.31
Day 1 RB Female	504.59	175.51
Day 1 RB Male	749.63	243.81
Day 1 RDS Female	831.36	442.46
Day 1 RDS Male	1782.49	694.09
Day 1 RDE Female	977.46	412.06
Day 1 RDE Male	194.00	38.65
Day 4 RP Female	538.50	257.40
Day 4 RP Male	835.91	257.10
Day 4 RB Female	593.91	247.75
Day 4 RB Male	1663.29	775.23
Day 4 RDS Female	257.57	65.83
Day 4 RDS Male	262.77	44.90
Day 4 RDE Female	194.30	57.76
Day 4 RDE Male	637.84	320.36

Supplementary Material 3

Model outcomes (Table S3A) and data summary (Table S3B) for non-significant factors influencing the latency to find the correct tunnel during day 1 and day 4 of training, in the Barnes maze by male and female *Rhabdomys* individuals. RP (*R. pumilio*) originated from the semi-arid western parts of South Africa, RB and RDS (sympatric *R. bechuanae* and *R. d. dilectus*) originated from the central grasslands, and RDE (allopatric *R. d. dilectus*) originated from the moist eastern parts of South Africa.

Table S3A. Model outcomes for non-significant factors influencing the latency to find the correct tunnel in the Barnes maze.

Predictor	**Statistics**
Population	$F_{3,85}$=2.449, p=0.069
Sex	$F_{1,85}$=0.751, p=0.389
Population*sex	$F_{3,85}$=0.942, p=0.424
Day*population	$F_{3,85}$=1.082, p=0.361
Day*sex	$F_{1,85}$=0.922, p=0.340

Table S3B. Data summary for the non-significant factors influencing the latency to find the correct tunnel in the Barnes maze.

Level	**Latency (s)**	
	Mean	**SE**
RP	60.41	11.77
RB	30.49	6.93
RDS	38.73	8.73
RDE	57.19	12.00
Female	43.42	7.53
Male	50.28	7.03
Day 1	65.85	6.92
Day 4	28.07	7.09
RP Female	66.35	20.67
RP Male	54.47	11.64
RB Female	30.39	12.98
RB Male	30.60	5.26
RDS Female	19.93	8.16
RDS Male	52.83	13.44
RDE Female	51.15	11.78
RDE Male	63.22	21.13
Day 1 RP	80.50	14.80
Day 1 RB	34.75	6.15
Day 1 RDS	66.72	15.11
Day 1 RDE	81.55	15.80
Day 4 RP	40.32	17.66
Day 4 RB	26.23	12.52

Level	Latency (s)	
	Mean	SE
Day 4 RDS	10.75	2.39
Day 4 RDE	32.82	16.95
Day 1 Female	57.39	9.52
Day 1 Male	73.79	9.96
Day 4 Female	29.45	11.39
Day 4 Male	26.78	8.76

Supplementary Material 4

Model outcomes (Table S4A) and data summary (Table S4B) for non-significant factors influencing the number of errors made during day 4 and day 5 of training, in the Barnes maze by male and female *Rhabdomys* individuals. RP (*R. pumilio*) originated from the semi-arid western parts of South Africa, RB and RDS (sympatric *R. bechuanae* and *R. d. dilectus*) originated from the central grasslands, and RDE (allopatric *R. d. dilectus*) originated from the moist eastern parts of South Africa.

Table S4A. Model outcomes for non-significant factors influencing the number of errors made in the Barnes maze.

Predictor	**Statistics**
Population	$F_{3,85}$=1.960, p=0.126
Sex	$F_{1,85}$=1.621, p=0.206
Day	$F_{1,85}$=0.012, p=0.914
Population*sex	$F_{3,85}$=0.561, p=0.642
Day*sex	$F_{1,85}$=0.113, p=0.737
Day*population*sex	$F_{3,85}$=1.537, p=0.211

Table S4B. Data summary for the non-significant factors influencing the number of errors made in the Barnes maze.

Level	**Errors**	
	Mean	**SE**
RP	4.46	0.73
RB	3.42	0.77
RDS	2.50	0.32
RDE	2.88	0.46
Female	2.96	0.41
Male	3.70	0.46
Day 4	3.37	0.50
Day 5	3.31	0.36
RP Female	3.92	1.04
RP Male	5.00	1.03
RB Female	2.46	0.91
RB Male	4.38	1.23
RDS Female	2.44	0.49
RDS Male	2.54	0.44
RDE Female	2.88	0.58
RDE Male	2.88	0.73
Day 4 Female	2.87	0.68
Day 4 Male	3.83	0.74
Day 5 Female	3.04	0.47
Day 5 Male	3.56	0.55
Day 4 RP Female	4.00	1.83
Day 4 RP Male	4.17	1.42

Level	Errors	
	Mean	SE
Day 4 RB Female	2.36	1.67
Day 4 RB Male	6.83	2.23
Day 4 RDS Female	2.67	0.75
Day 4 RDS Male	2.83	0.68
Day 4 RDE Female	2.17	0.81
Day 4 RDE Male	1.50	0.60
Day 5 RP Female	3.83	1.06
Day 5 RP Male	5.83	1.50
Day 5 RB Female	2.33	1.03
Day 5 RB Male	1.92	0.47
Day 5 RDS Female	2.22	0.68
Day 5 RDS Male	2.25	0.57
Day 5 RDE Female	3.58	0.83
Day 5 RDE Male	4.25	1.24

Supplementary Material 5

Model outcomes (Table S5A) and data summary (Table S5B) for non-significant factors influencing the distance travelled during day 4 and day 5 of training, in the Barnes maze by male and female *Rhabdomys* individuals. RP (*R. pumilio*) originated from the semi-arid western parts of South Africa, RB and RDS (sympatric *R. bechuanae* and *R. d. dilectus*) originated from the central grasslands, and RDE (allopatric *R. d. dilectus*) originated from the moist eastern parts of South Africa.

Table S5A. Model outcomes for non-significant factors influencing the distance travelled in the Barnes maze.

Predictor	**Statistics**
Population	$F_{3,85}$=2.350, p=0.078
Day	$F_{1,85}$=3.448, p=0.067
Population*sex	$F_{3,85}$=0.474, p=0.701
Day*sex	$F_{1,85}$=1.749, p=0.190
Day*population*sex	$F_{3,85}$=1.521, p=0.215

Table S5B. Data summary for the non-significant factors influencing the distance travelled in the Barnes maze.

Level	**Distance (m)**	
	Mean	**SE**
RP	684.68	113.18
RB	690.11	215.60
RDS	285.78	34.44
RDE	376.11	84.33
Day 4	634.80	127.02
Day 5	397.95	44.81
RP Female	495.57	142.43
RP Male	873.79	170.15
RB Female	440.65	133.66
RB Male	939.57	408.30
RDS Female	252.33	41.54
RDS Male	310.86	51.76
RDE Female	263.49	38.93
RDE Male	488.72	162.62
Day 4 Female	405.30	97.88
Day 4 Male	849.95	225.27
Day 5 Female	335.48	46.94
Day 5 Male	456.52	74.37
Day 4 RP Female	538.50	257.40
Day 4 RP Male	835.91	257.10
Day 4 RB Female	598.39	271.35
Day 4 RB Male	1663.29	775.23
Day 4 RDS Female	257.57	65.83

Level	**Distance (m)**	
	Mean	**SE**
Day 4 RDS Male	262.77	44.90
Day 4 RDE Female	194.30	57.76
Day 4 RDE Male	637.84	320.36
Day 5 RP Female	452.65	135.07
Day 5 RP Male	911.67	233.90
Day 5 RB Female	287.39	95.15
Day 5 RB Male	215.84	30.58
Day 5 RDS Female	247.08	54.70
Day 5 RDS Male	358.95	93.64
Day 5 RDE Female	332.68	46.18
Day 5 RDE Male	339.60	62.55

Supplementary Material 6

Model outcomes (Table S6A) and data summary (Table S6B) for non-significant factors influencing the latency to find the correct tunnel during day 4 and day 5 of training, in the Barnes maze by male and female *Rhabdomys* individuals. RP (*R. pumilio*) originated from the semi-arid western parts of South Africa, RB and RDS (sympatric *R. bechuanae* and *R. d. dilectus*) originated from the central grasslands, and RDE (allopatric *R. d. dilectus*) originated from the moist eastern parts of South Africa.

Table S6A. Model outcomes for non-significant factors influencing the latency to find the correct tunnel in the Barnes maze.

Predictor	Statistics
Population	$F_{3,85}$=0.784, p=0.506
Sex	$F_{1,85}$=0.001, p=0.979
Day	$F_{1,85}$=2.759, p=0.100
Population*sex	$F_{3,85}$=0.844, p=0.473
Day*population	$F_{3,85}$=1.947, p=0.128
Day*sex	$F_{1,85}$=0.039, p=0.844
Day*population*sex	$F_{3,85}$=1.072, p=0.365

Table S6B. Data summary for the non-significant factors influencing the latency to find the correct tunnel in the Barnes maze.

Level	Latency (s)	
	Mean	**SE**
RP	47.53	12.59
RB	38.16	12.13
RDS	21.02	9.80
RDE	41.41	13.02
Female	38.32	9.42
Male	36.82	7.72
Day 4	48.03	10.29
Day 5	27.06	6.19
RP Female	64.03	22.81
RP Male	31.03	10.18
RB Female	29.49	17.04
RB Male	46.84	17.44
RDS Female	19.40	16.51
RDS Male	22.24	12.18
RDE Female	35.65	16.80
RDE Male	47.16	20.19
Day 4 RP	40.32	17.66
Day 4 RB	57.11	22.73
Day 4 RDS	23.13	13.97
Day 4 RDE	68.45	24.83

Level	Latency (s)	
	Mean	SE
Day 5 RP	54.75	18.20
Day 5 RB	19.21	7.30
Day 5 RDS	18.91	14.08
Day 5 RDE	14.37	3.55
Day 4 Female	48.47	15.22
Day 4 Male	47.61	14.09
Day 5 Female	28.17	11.09
Day 5 Male	26.03	6.11
Day 4 RP Female	68.74	33.18
Day 4 RP Male	11.89	7.50
Day 4 RB Female	57.58	36.14
Day 4 RB Male	60.87	32.43
Day 4 RDS Female	3.07	0.87
Day 4 RDS Male	38.18	23.93
Day 4 RDE Female	57.39	32.84
Day 4 RDE Male	79.50	38.44
Day 5 RP Female	59.32	32.72
Day 5 RP Male	50.17	17.62
Day 5 RB Female	5.63	2.09
Day 5 RB Male	32.80	13.60
Day 5 RDS Female	35.72	33.04
Day 5 RDS Male	6.31	1.12
Day 5 RDE Female	13.92	3.99
Day 5 RDE Male	14.82	6.07

Supplementary Material 7

Model outcomes (Table S7A) and data summary (Table S7B) for non-significant factors influencing the number of errors made during probe test 1 and 2, in the Barnes maze by male and female *Rhabdomys* individuals. RP (*R. pumilio*) originated from the semi-arid western parts of South Africa, RB and RDS (sympatric *R. bechuanae* and *R. d. dilectus*) originated from the central grasslands, and RDE (allopatric *R. d. dilectus*) originated from the moist eastern parts of South Africa.

Table S7A. Model outcomes for non-significant factors influencing the number of errors in the Barnes maze.

Predictor	Statistics
Sex	$F_{1,23}$=0.780, p=0.386
Population*sex	$F_{3,23}$=0.534, p=0.663
Test*sex	$F_{1,23}$=1.732, p=0.201
Test*population*sex	$F_{3,23}$=0.876, p=0.468

Table S7B. Data summary for the non-significant factors influencing the number of errors in the Barnes maze.

Level	Errors	
	Mean	SE
Female	16.00	1.64
Male	14.06	1.83
RP Female	14.75	3.22
RP Male	7.38	2.42
RB Female	11.13	1.52
RB Male	8.75	2.27
RDS Female	21.67	5.14
RDS Male	23.50	4.40
RDE Female	17.88	2.70
RDE Male	16.63	2.26
Test 1 Female	19.67	2.49
Test 1 Male	16.38	2.98
Test 2 Female	12.33	1.73
Test 2 Male	11.75	2.07
Test 1 RP Female	17.00	5.12
Test 1 RP Male	6.50	3.88
Test 1 RB Female	13.75	2.17
Test 1 RB Male	7.75	3.07
Test 1 RDS Female	28.67	8.41
Test 1 RDS Male	30.00	4.18
Test 1 RDE Female	21.50	2.63
Test 1 RDE Male	21.25	2.95
Test 2 RP Female	12.50	4.35
Test 2 RP Male	8.25	3.42

Level	**Errors**	
	Mean	**SE**
Test 2 RB Female	8.50	1.19
Test 2 RB Male	9.75	3.75
Test 2 RDS Female	14.67	3.48
Test 2 RDS Male	17.00	6.67
Test 2 RDE Female	14.25	4.27
Test 2 RDE Male	12.00	0.91

Supplementary Material 8

Model outcomes (Table S8A) and data summary (Table S8B) for non-significant factors influencing the distance travelled during probe test 1 and 2, in the Barnes maze by male and female *Rhabdomys* individuals. RP (*R. pumilio*) originated from the semi-arid western parts of South Africa, RB and RDS (sympatric *R. bechuanae* and *R. d. dilectus*) originated from the central grasslands, and RDE (allopatric *R. d. dilectus*) originated from the moist eastern parts of South Africa.

Table S8A. Model outcomes for non-significant factors influencing the distance travelled in the Barnes maze.

Predictor	**Statistics**
Population	$F_{3,23}$=2.147, p=0.122
Sex	$F_{1,23}$=0.721, p=0.405
Population*sex	$F_{3,23}$=0.049, p=0.985
Test*population	$F_{3,23}$=0.924, p=0.445
Test*sex	$F_{1,23}$=0.143, p=0.709
Test*population*sex	$F_{3,23}$=2.025, p=0.138

Table S8B. Data summary for the non-significant factors influencing the distance travelled in the Barnes maze.

Level	**Distance (cm)**	
	Mean	**SE**
RP	1599.50	196.13
RB	1223.75	176.09
RDS	2435.93	249.66
RDE	1997.63	340.14
Female	1929.57	217.09
Male	1667.19	161.77
RP Female	1718.50	365.08
RP Male	1480.50	165.93
RB Female	1363.75	198.56
RB Male	1083.75	296.43
RDS Female	2527.33	311.48
RDS Male	2367.38	386.04
RDE Female	2258.13	633.77
RDE Male	1737.13	273.44
Test 1 RP	1686.75	312.85
Test 1 RB	1424.38	267.50
Test 1 RDS	2810.43	307.35
Test 1 RDE	2267.50	422.61
Test 2 RP	1512.25	254.59
Test 2 RB	1023.13	223.25
Test 2 RDS	2061.43	359.00
Test 2 RDE	1727.75	544.45

Level	Distance (cm)	
	Mean	SE
Test 1 Female	2181.60	275.82
Test 1 Male	1873.63	252.79
Test 2 Female	1677.53	331.80
Test 2 Male	1460.75	196.41
Test 1 RP Female	2040.50	584.83
Test 1 RP Male	1333.00	176.92
Test 1 RB Female	1637.25	306.97
Test 1 RB Male	1211.50	457.69
Test 1 RDS Female	2757.33	451.36
Test 1 RDS Male	2850.25	476.99
Test 1 RDE Female	2435.25	763.87
Test 1 RDE Male	2099.75	480.82
Test 2 RP Female	1396.50	459.19
Test 2 RP Male	1628.00	287.55
Test 2 RB Female	1090.25	199.70
Test 2 RB Male	956.00	435.56
Test 2 RDS Female	2297.33	477.99
Test 2 RDS Male	1884.50	559.01
Test 2 RDE Female	2081.00	1126.96
Test 2 RDE Male	1374.50	173.43

Supplementary Material 9

Model outcomes (Table S9A) and data summary (Table S9B) for non-significant factors influencing the latency to find the correct tunnel during probe test 1 and 2, in the Barnes maze by male and female *Rhabdomys* individuals. RP (*R. pumilio*) originated from the semi-arid western parts of South Africa, RB and RDS (sympatric *R. bechuanae* and *R. d. dilectus*) originated from the central grasslands, and RDE (allopatric *R. d. dilectus*) originated from the moist eastern parts of South Africa.

Table S9A. Model outcomes for non-significant factors influencing the latency to find the correct tunnel in the Barnes maze.

Predictor	Statistics
Population	$F_{3,23}$=1.053, p=0.388
Sex	$F_{1,23}$=0.199, p=0.659
Test	$F_{1,23}$=0.257, p=0.617
Population*sex	$F_{3,23}$=1.658, p=0.204
Test*population	$F_{3,23}$=0.801, p=0.506
Test*sex	$F_{1,23}$=0.669, p=0.422
Test*population*sex	$F_{3,23}$=0.729, p=0.545

Table S9B. Data summary for the non-significant factors influencing the latency to find the correct tunnel in the Barnes maze.

Level	Latency (s)	
	Mean	SE
RP	6.62	2.44
RB	17.89	4.33
RDS	11.37	5.49
RDE	9.29	4.48
Female	12.70	3.32
Male	9.97	2.77
Test 1	10.35	2.29
Test 2	12.23	3.65
RP Female	5.89	3.40
RP Male	7.36	3.71
RB Female	23.60	7.14
RB Male	12.19	4.46
RDS Female	3.26	1.36
RDS Male	17.46	9.19
RDE Female	15.70	8.59
RDE Male	2.87	0.64
Test 1 RP	10.55	4.46
Test 1 RB	14.88	4.44
Test 1 RDS	8.05	5.57
Test 1 RDE	7.65	4.43
Test 2 RP	2.70	1.09

Level	Latency (s)	
	Mean	SE
Test 2 RB	20.91	7.61
Test 2 RDS	14.70	9.79
Test 2 RDE	10.93	8.09
Test 1 Female	10.14	3.43
Test 1 Male	10.55	3.17
Test 2 Female	15.26	5.75
Test 2 Male	9.39	4.64
Test 1 RP Female	8.79	6.65
Test 1 RP Male	12.31	6.81
Test 1 RB Female	14.66	7.94
Test 1 RB Male	15.09	5.40
Test 1 RDS Female	2.03	0.15
Test 1 RDS Male	12.56	9.62
Test 1 RDE Female	13.05	8.46
Test 1 RDE Male	2.24	0.87
Test 2 RP Female	2.99	1.99
Test 2 RP Male	2.40	1.24
Test 2 RB Female	32.53	11.03
Test 2 RB Male	9.28	7.63
Test 2 RDS Female	4.49	2.76
Test 2 RDS Male	22.36	16.91
Test 2 RDE Female	18.35	16.37
Test 2 RDE Male	3.50	0.95

GENERAL DISCUSSION

The behaviour of an animal results from interactions between its genotype and the environment (Schlichting & Pigliucci 1998). Because different environments impose different selection pressures on animals, they are likely to be locally adapted to the environments they occupy (Taylor 1991). Populations or closely related species that develop adaptations in response to differences in their environments may have undergone an adaptive diversification (Glor 2010; Losos & Mahler 2010) or adaptive radiation (Schluter 2000). The genus *Rhabdomys* is a good study model to investigate behavioural adaptive radiation because several species occupy different habitats across South Africa. Previous studies on two taxa (*R. pumilio* and *R. dilectus*) have indicated differences in behaviour that may be linked to environmental variation (Schradin & Pillay 2005; Rymer *et al.* 2008; Rymer & Pillay 2012; Mackay *et al.* 2014). In addition, the phylogeographic history of the genus indicates that *Rhabdomys* taxa radiated from a common ancestor (Ganem *et al.* 2012), possibly in central South Africa (du Toit *et al.* 2012a), with later radiations into the eastern grasslands (Ganem *et al.* 2012). At least three species occupy different habitats with potentially different selection pressures, and at least one location in central South Africa has co-existing taxa, which provides an additional opportunity to study the influence of co-existence on the behavioural phenotypes of similar taxa.

My specific objectives were to establish the personality and the presence of behavioural syndromes, and describe the spatial cognition ability of *R. pumilio*, sympatric *R. bechuanae* and *R. d. dilectus*, and an allopatric population of *R. d. dilectus*. I studied personality and spatial cognition because other studies have shown environmentally linked adaptive variation (e.g. personality: Brown *et al.* 2005; spatial cognition: Freas *et al.* 2012). I then established developmental plasticity (a requirement for adaptive radiation or diversification; Losos 2010) by altering the social rearing environment in *R. pumilio* and *R. bechuanae*, and the physical rearing environment in *R. pumilio*, sympatric *R. d. dilectus* and *R. bechuanae*, an allopatric population of *R. bechuanae*. The first approach was descriptive, and the second was manipulative. I used both approaches as I first needed to document taxon-level differences, and then used the experimental approach to consider genotype-environment interactions. The manipulative approach further tested whether any taxon-level differences are genetically fixed (i.e. changes to the environment do not alter the phenotype; Zewdie & Bosland 2000), or whether the environment largely influences the phenotype. For example, all species of deer mice (genus *Peromyscus*) retain the ability to burrow, even after several generations in captivity and kept in different environmental conditions (Weber & Hoekstra 2009), indicating a strong genetic component to this behaviour. In contrast, the social organisation of different populations of cavies *Microcavia australis* varies according to the environmental conditions. At night, in locations with extreme climatic conditions, burrows are shared by larger groups of cavies, compared to groups occurring at milder climates (Taraborelli & Moreno 2009). This indicates that the environment modifies the social organisation in cavies.

Phenotypic variation

When I assessed the personality and behavioural syndromes of *R. pumilio*, sympatric *R. bechuanae* and *R. d. dilectus*, and an allopatric population of *R. d. dilectus*, I found that there was some predictable variation in personality types. *R. pumilio* and allopatric *R. d. dilectus* conformed to the results of other studies (Rymer & Pillay 2012; Mackay *et al.* 2014), whereby *R. pumilio* is significantly bolder and the allopatric *R. d. dilectus* is significantly less bold. A bolder personality type may be advantageous in open areas (because predation risk is higher in open areas; Kotler *et al.* 1991; Perea *et al.* 2011) and animals need to traverse open areas (thereby risking predation) in order to forage (Hughes & Ward 1993). In support, midday gerbils *Meriones meridianus* had a significantly greater giving-up density (GUD) in uncovered seed trays, compared to seed trays that were under cover or close to burrows (Shuai & Song 2011). Areas further away from cover were associated with increased predation risk (Brown *et al.* 1988), and increased predation risk is associated with increased boldness (Niemelä *et al.* 2012), so habitats that are more open may be associated with bolder individuals. The significantly less bold phenotype of sympatric *R. bechuanae* was not unexpected because the habitat where it occurs is more similar to the grassland habitat of allopatric *R. d. dilectus* than to the semi-arid habitat of *R. pumilio*. The surprising result was that sympatric *R. d. dilectus* was more similar to *R. pumilio* (i.e. bolder), which I suggest may be due to inter-specific competition between *R. bechuanae* and *R. d. dilectus* in sympatry. In addition, the sympatric *R. d. dilectus* had more indications of behavioural syndromes than any of the other populations, possibly related to competition in sympatry. Co-existence has been studied previously amongst *R. d. dilectus* and *R. bechuanae* in central South Africa (Dufour 2014). In sympatry, the home ranges of *R. bechuanae* and *R. d. dilectus* were larger than allopatric populations, indicating that competition between the species may be influencing home range sizes (Dufour *et al.* 2015) and possibly other traits.

Although there were taxon-level personality differences, there was some degree of overlap in personality among and within taxa, which indicates that there is both directional and stabilising selection (Arnold 1992) on personality among the taxa. Directional selection is evident in the overall degree of differences in personality between the taxa (Rieseberg *et al.* 2002), while stabilising selection is evident in the level of overlap between the taxa (Hohenboken 1985). Thus, the clear patterns of boldness in my study are suggestive of some form of environmental selection pressure on personality. Previously, Mackay (2011) suggested that cover is an important selection pressure driving behaviour in *Rhabdomys*, but environmental selection pressure may not be the only driver of taxon-level differences, since genetic drift may also account for behavioural differences between taxa.

There were small but significant differences in spatial cognition, with *R. bechuanae* significantly better able to navigate without external cues, and *R. bechuanae* and *R. pumilio* having marginally better spatial memory than both sympatric and allopatric *R. d. dilectus*, although both *R. d. dilectus* populations improved over time. Thus, there was variation in spatial cognition, but this was not consistent and not predictable across all taxa. This suggests that spatial cognition may be phylogenetically constrained and immutable, or there might be similar pressures driving spatial cognition even in the different habitats.

Often, there is an association between personality and cognition (Carere & Locurto 2011), where bolder, more exploratory individuals make less accurate decisions, but may be faster decision makers than less bold, less exploratory individuals (Sih & Del Giudice 2012). For example, rainbow trout *Oncorhynchus mykiss* individuals that spent more time and were more active in open areas (i.e. bolder), learnt faster in a simple associative learning task than less bold trout (Sneddon 2003). The differences between the taxa in personality but not spatial cognition suggest that personality and spatial cognition may not be linked in *Rhabdomys*. I propose that spatial cognition may be phylogenetically constrained and that divergence in personality is associated with adaptive variation or possibly genetic drift in the genus.

Genetic drift is a process where the frequencies of genotypes change randomly between generations (Lande 1976; Ridley 2004), and could account for differences in behaviour between taxa. For example, in the bumblebee *Bombus terrestris*, different populations have different preferences for flower colour, which can be attributed to genetic drift (Raine *et al.* 2006). The influences of genetic drift may be more pronounced in small (Lande 1976) and founder (Templeton 1980; Ridley 2004) populations, and changes in the frequencies of genotypes could lead to speciation in allopatry (Lande 1976; but see Turelli *et al.* 2001). In *Rhabdomys*, range contractions during the Last Glacial Maximum could have resulted in population bottlenecks (du Toit *et al.* 2012a), with the implication that genetic drift could be a plausible explanation for phenotypic differences between the taxa.

Gene-environment interactions

I assessed the influences of social (early social rearing environment) and physical (housing) environments on exploratory behaviour and anxiety. Exploratory behaviour and anxiety are associated with different loci (Gershenfeld *et al.* 1997; Clément *et al.* 2002), and are subject to independent evolution unless they form a behavioural syndrome (Bell *et al.* 2013). Although I found evidence of syndromes when I described the personality and behavioural syndromes (Chapter 2), the syndromes appeared to become decoupled when I manipulated the rearing environment, because significant taxon differences in exploratory behaviour were not mirrored by taxon differences in anxiety when I manipulated both the physical and social early rearing environment. This suggests that *Rhabdomys* is able to show more plasticity than would be expected if individuals showed an exploration-anxiety syndrome (Sih *et al.* 2004; Bell & Sih 2007).

Phenotypes that are influenced by genotype-environment interactions are considered to be more flexible than phenotypes that are mostly influenced by the genotype alone (Plomin *et al.* 1980; Schlichting & Pigliucci 1998). This is because when the environment modulates phenotypes, the organism is able to respond flexibly to the prevailing environmental conditions (West-Eberhard 1989). *R. pumilio* is known to be phenotypically flexible (Schradin *et al.* 2012). For example, male *R. pumilio* have alternate breeding tactics (philopatric, roaming or breeding), depending on the distribution of females (Schradin & Lindholm 2011). Females choose to nest solitarily when population density is low, and communally when density is high (Schoepf *et al.* 2015). The gene-environment interaction that Rymer and Pillay (2012) found in both *R. pumilio* and *R. d. chakae* suggests that phenotypic flexibility may be widespread in the genus. However, the lack of phenotypic modulation when I altered both the social and physical environments, suggests the opposite.

Another issue to explore is the overall similarity between the taxa in both of the manipulative studies. Environmental interactions with a genotype may either increase (intensify) or decrease (modulate) the levels of a phenotype (Plomin *et al.* 1980). In Chapter 2, *R. pumilio* and *R. d. dilectus* (sympatric with *R. bechuanae*) were bold, and displayed high levels of exploratory behaviour and low anxiety responses, while *R. bechuanae* (sympatric with *R. d. dilectus*) was significantly less bold, displayed low levels of exploratory behaviour and a high anxiety response. In contrast, when the early social and physical environments were manipulated (Chapters 3 and 4), the differences between the taxa were reduced (such that the taxa only had minor differences between them). Gene-environment interactions may have the effect of reducing differences between taxa when the selection pressure acting on the populations is removed or altered (Schlichting & Pigliucci 1998). Although this is a possibility, neither of the manipulative experiments included an un-manipulated control, so this remains to be tested in future.

The overall similarities of the taxa make it difficult to assess the results of manipulating the environment. The lack of response to cross-fostering could also be due to taxon similarities in the social rearing environments, and the lack of response to altering cover in the housing conditions could suggest that cover does not significantly influence the developmental neural pathways to modify the exploratory behaviour or anxiety of striped mice. Another possibility is that the response to altering the early social environment (*R. pumilio* and *R. bechuanae*) and physical housing condition (*R. pumilio*, *R. bechuanae* and *R. d. dilectus*) is phylogenetically constrained in the genus.

The influence of phylogeny

Rhabdomys is thought to have arisen in the central parts of South Africa, with *R. bechuanae* and then the rest of the *Rhabdomys* taxa diverging from a common ancestor approximately 4.3 MYA (Figure 1.1B; du Toit *et al.* 2012a). *R. pumilio* and *R. dilectus* then diverged from a common ancestor approximately 3.09 MYA (du Toit *et al.* 2012a), probably following a radiation from the semi-arid west into the central and eastern grasslands (Ganem *et al.* 2012). Since *R. bechuanae* is basal in the clade (Figure 1.1B), if all taxa behave similarly to *R. bechuanae*, they may be showing a phylogenetically conserved response, which appeared to be the case in the cover experiment. Other studies on derived *Rhabdomys* taxa have shown similar patterns. Paternal care behaviour, which is expressed both in nature and in the laboratory in *R. pumilio*, but only in the laboratory in *R. dilectus* (Schradin & Pillay 2003), suggests that paternal care is plesiomorphic in *R. dilectus* and that a common ancestor of *R. pumilio* and *R. dilectus* arose in the semi-arid western parts of southern Africa where paternal care is adaptive (Rymer *et al.* 2013).

Ganem *et al.* (2012) suggested that *Rhabdomys* may have undergone multiple radiations, with speciation following adaptive radiations into different habitats. From previous studies, *R. pumilio* has different social ecology and behaviour to both subspecies of *R. dilectus* (Schradin & Pillay 2005; Pillay *et al.* 2006; Rymer *et al.* 2008; Rymer & Pillay 2012; Mackay *et al.* 2014), supporting the adaptive radiation theory in derived *Rhabdomys* taxa (i.e. *R. pumilio* and *R. dilectus*). In order to demonstrate that adaptive radiation in *Rhabdomys*, with *R. bechuanae* as the common ancestor of *R. pumilio* and *R. dilectus*, the behaviour of *R. dilectus* should match other typical grassland species, and be similar to *R. bechuanae, as R. dilectus* re-colonised the grasslands secondarily (du Toit *et al.* 2012a). In my studies, *R. bechuanae* had similar behaviour to allopatric *R. d. dilectus* and similar behaviour to other *R. d. dilectus* populations studied previously (Mackay 2011; Mackay *et al.* 2014). In contrast, the similarity in spatial cognition of *R. pumilio* and *R. d. dilectus* to *R. bechuanae* suggests that spatial cognition may be phylogenetically constrained. Nonetheless, there is also the possibility that there are similar selection pressures acting on spatial cognition in the different environments.

Sympatric and allopatric populations

Although phylogenetic conservatism is a plausible explanation for the similarities between taxa in spatial cognition and genetic constraint is a good explanation for the lack of gene-environment interactions, significant taxon differences in behaviour in sympatry and allopatry need further discussion. In Chapter 2, allopatric *R. d. dilectus* were less bold (shy), and their behaviour agreed with previous studies (Mackay *et al.* 2014). In contrast, *R. d. dilectus* (sympatric with *R. bechuanae*) were bold, similar to *R. pumilio*, while *R. bechuanae* behaved similarly to allopatric *R. d. dilectus*. These results taken in isolation appear to suggest character displacement (Brown & Wilson 1956) in the sympatric *R. d. dilectus*. In addition, allopatric *R. bechuanae* also had similar personality (Chapter 4) to *R. pumilio* and different personality to sympatric *R. bechuanae* (Chapter 2), suggesting that the behaviour of *R. bechuanae* could also reflect character displacement. In support, Dufour (2014) found that there was competition between *R. bechuanae* and *R. d. dilectus* in sympatry, leading to larger home ranges of *R. bechuanae* in sympatry compared to allopatric populations (Dufour *et al.* 2015).

Conclusions and future directions

The genus *Rhabdomys* is widespread, occurring throughout southern Africa. Recent genetic analyses have revealed the existence of several *Rhabdomys* species occupying different biomes across southern Africa, arising from an ancestor in central South Africa (du Toit *et al.* 2012a). In addition, recent surveys found the sympatric occurrence of at least two *Rhabdomys* (ancestral *R. bechuanae* and derived *R. d. dilectus*) in the central Free State grasslands (du Toit *et al.* 2012a; Ganem *et al.* 2012). I postulated that speciation in the genus is related to adaptive variation in contrasting environments, which is also suggested by theoretical models (Rymer *et al.* 2013).

The overall objective of my study was to investigate adaptive variation and the mechanisms associated with behavioural and cognitive variation in the semi-arid *R. pumilio*, sympatric *R. bechuanae* and *R. d. dilectus*, and allopatric populations of *R. d. dilectus* and *R. bechuanae*. My study is one of few that investigate variation, linked to adaptation, in personality and spatial cognition in different but closely related taxa. In addition, few other studies have considered taxa across such a wide environmental range (i.e. rainfall). My study is also unique in that few others modify the developmental environment to investigate whether inter-specific variation is fixed, or if it can be modified by altering the environment. Furthermore, there are no other studies that investigate the influence of cover on personality traits, by altering cover levels without changing environmental complexity. I found some support for adaptive variation in personality, and additional influences possibly indicating character displacement in sympatry. Yet, geographic variation did not influence spatial cognition in the taxa, indicating that spatial cognition and personality might be not be functionally related in *Rhabdomys*. Changes in the social and physical environments failed to influence exploration and anxiety, contrasting with previous studies in *Rhabdomys*, and indicating a genetic influence on these behaviours, suggesting phylogenetic constraint and/or similar selection pressures.

I adopted a common approach to previous studies on personality in *Rhabdomys* (Rymer & Pillay 2012; Mackay *et al.* 2014; Yuen *et al.* 2015; Joshi & Pillay 2016a; Joshi & Pillay 2016b; Yuen *et al.* 2016). The topic of personality is constantly evolving, and multiple approaches to test personality traits and behavioural syndromes have been proposed. For example, there is concern that tests for a certain trait may be measuring a different trait (Carter *et al.* 2012; Carter *et al.* 2013), and many studies assessing behavioural syndromes typically assess correlations between one or two measures of behaviour (see Chapter 2). Therefore, future studies should consider other tests of personality in *Rhabdomys*, in order to confirm the correct usage of tests for personality traits. Secondly, multiple populations from different taxa should be studied, in order to establish whether differences are population-specific, whether any differences are a result of genetic drift, and whether character displacement affects behaviour in sympatric populations. Thirdly, future studies should consider the same taxa/populations for both descriptive and manipulative studies, as this would confirm the mechanisms of adaptive variation. Lastly, since *R. bechuanae* appears to respond to different contexts and treatments inconsistently, there is a need for in-depth studies on this taxon in both laboratory contexts and in nature.

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