The behaviour of two sub-species of the striped mouse *Rhabdomys*: the role of phylogeny and the environment

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Declaration

I declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

Megan Mackay

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Abstract

The role of phylogeny and environmental influences on behaviour were investigated in two sub-species of *Rhabdomys dilectus*: *R. d. chakae* and *R. d. dilectus*. I compared populations of the two sub-species that occur about 70 km apart in superficially similar grasslands, south of Johannesburg (Walkers Fruit Farms) and at Irene in Gauteng Province, South Africa. The vegetation characteristics of the localities were assessed by measuring aerial cover, cover density, amount of dead material, and the maximum and minimum plant height. I studied the maintenance (non-social) and social behaviour of the sub-species in captivity. Three maintenance behaviours were studied: diel activity in an open arena, activity in an enclosed maze and in a modified plus maze. Social behaviour was studied by investigating the stress response of juvenile males after removal from their family groups and being placed in a plus maze, and the tolerance of unfamiliar same-sex consub-specifics in staged dyadic encounters. My results indicate that there were differences in the vegetation at the localities, most notably in the level of cover, which was greater at Irene (*R. d. dilectus*) than at Walkers Fruit Farms (*R. d. chakae*). Both sub-species displayed similar activity profiles (diurnal and crepuscular) and similar levels of activity in an enclosed maze. However, *R. d. dilectus* showed high levels of anxiety in an open arena and in a plus maze. The sub-species showed similar social behaviours: males of both sub-species that were removed from their family groups at the onset of weaning showed a lower stress response compared to the social sister species *R. pumilio.* Social interactions of same-sex intra-sub-specifics were mostly similar between the sub-species but there were slight discrepancies in behaviour associated with mating. Surprisingly, there were higher levels of amicability in all dyads than predicted by the solitary lifestyle of the two sub-species in nature. Behavioural differences (anxiety response) may be due to divergence in allopatry because of differences in vegetation characteristics. The similarities in activity profile and social behaviours could be explained by phylogenetic inertia or by similar selection pressures in similar environments.

Acknowledgements

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Thanks to Darryl Pepper for allowing me to trap and do my vegetation surveys on his farm – without his assistance, I would have had considerably more difficulty in doing the vegetation component of my project. Thank you also to the ARC in Irene, particularly Leon Kruger, who assisted me at the ARC grounds in Irene. Karin Coetzee from the department of Nature Conservation of the City of Tshwane and Riaan Marais from the Rietvlei Dam Nature Reserve were particularly helpful in allowing me to trap striped mice at Rietvlei dam, and I am indebted to them for allowing me prompt access to the reserve so I could trap enough animals for my project.

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I received two NRF bursaries (a grant-holder’s bursary in my first year and a scarce-skills bursary in my second year) for which I am grateful. I was allowed to trap and keep striped mice by the department of Agriculture, Conservation and Environment, Gauteng (permit number CPF6 – 0019). The study was approved by the Animal Ethics screening committee of the University of the Witwatersrand (clearance numbers 98/26/2A and 2009/25/2A).

Lastly, to my family, who also read numerous drafts of my work, assisted me in the field, and patiently listened while I explained my project to them.

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Chapter 1: General introduction

Behaviour is a product of heritable sources and environmental influences, both of which are important in shaping the behaviour of an animal (Chapman and Rothman, 2009). Genes are important in shaping the behaviour of an animal because they provide the potential for behaviour to be expressed through the production of tissues and hormones (Alcock, 1976). For example, male soil nematodes *Caenorhabditis elegans* require a specific gene in order to manufacture particular membrane proteins, which play a key role in locating females for mating (Barr and Sternberg, 1999). In rodents, specifically the house mouse *Mus musculus*, examples of genes influencing behaviour include aggression and the level of maternal care (Hood and Cairns, 1988; Chiang *et al.*, 2002; Frynta *et al.*, 2005), the development of anti-social behaviour (Raine, 2008) and the regulation of aggressive behaviour (Cases *et al.*, 1995). The environment is equally important, and can affect behaviour directly. For example, nutrition can affect the development of an animal and its behavioural phenotype, as brain functionality may be affected by malnutrition (Wainwright, 2001), competition, where a superior competitor (e.g. pacific rats, *Rattus exulans*) can induce behavioural flexibility in an inferior competitor (e.g. Duvaucel's geckos *Hoplodactylus duvaucelii*), whereby the geckos exploit less favourable niches in response to being outcompeted by the rats (Hoare *et al.*, 2007), and increased predation pressure can cause some species to choose safer nest sites, while causing others to increase their anti-predator response (Forstmeier and Weiss, 2004; Berger *et al.*, 2007).

Closely related species share several features as a consequence of their shared evolutionary history (i.e. their phylogeny). As an example, all species of deer mice (*Peromyscus*) have the ability to burrow (Weber and Hoekstra, 2009). Thus, for closely related species which live in different environments but have similar behaviours, phylogeny is the most parsimonious explanation for the similarity in behaviour, because the different environments are not exerting different selection pressures on the animals. Such phylogenetic inertia is exemplified by the remarkable similarities in competitive and social behaviour of macaque monkey (*Macaca* sp.) populations experiencing different ecological conditions (Chapman and Rothman, 2009). Alternatively, Losos (2008) proposes phylogenetic niche conservatism (PNC) as an explanation for phenotypic similarity between related species in different environments; the idea behind PNC maintains that species retain ancestral ecological characteristics, which are masked by phylogeny. In this case, closely related species will show similar behaviour in different environments because they are confined to niches occupied by their ancestors.

On the other hand, behavioural differences of closely related species that live in different environments are likely to be influenced more strongly by the environment. For example, two populations of cavies (*Microcavia australis*) located in different ecological conditions differed both in their sociality and aggressive behaviour, although they were very closely related (Taraborelli and Moreno, 2009). Similarly, several deer mice species (*Peromyscus)* which live in different ecological conditions have different burrowing habits, in that each species consistently creates distinct burrows (Weber and Hoekstra, 2009). However, for distantly related species that occupy similar niches, convergence is likely to be the basis of similar behaviour between the species. For example, spider monkeys *Ateles paniscus chamek* and chimpanzees *Pan troglodytes* are distantly related (Chapman and Rothman, 2009), occur in similar ecological niches and have similar association patterns, by remaining in subgroups of their own community and not mixing with other communities (Symington, 1990; Aureli *et al.*, 2008), suggesting convergence.

Environmental differences can cause divergence of behaviour in allopatric populations because of different selection pressures (e.g. different rearing environments and selection pressures lead to differences in anti-predator and fear-related responses in two populations of red jungle fowl *Gallus gallus* (Håkansson and Jensen, 2005; Håkansson *et al.*, 2007)). However, behavioural differences can also be caused by simple genetic drift (Foster, 1999). For example, different populations of the bumblebee *Bombus terrestris* have different flower colour preferences, possibly due to genetic drift (Raine *et al.*, 2006). A combination of genetic drift and different selection pressures could also influence behaviour, given that genetic drift could occur with different selection preferences. Finally, epigenetic influences of behaviour, or non-genetic influences (e.g. Avital and Jablonka, 2000; Sinha, 2005), can contribute to the differences between species. An example of this is the variation in the songs of white-crowned sparrows *Zonotrichialeucophrys nuttalli* in different regions of its geographical range, where song types are socially transmitted

(Baptista and King, 1980; Petrinovich, 1988). If different selection pressures and/or genetic drift are accompanied by non-genetic influences, the potential for a compounding effect exists, reinforcing the differences between species.

An important consideration of the behavioural phenotype is how genes and the environment (i.e. gene by environment) influence the behaviour of an animal. For similar species living in similar environments and which have similar behaviours, both phylogeny and the environment may have an effect on behaviour. In this case, phylogenetic inertia may constrain the expression of behaviour, and as such, the environment could have a limited effect on behaviour. However, the selection pressures (both biotic and abiotic) are also the same, which could also cause the behaviours to be similar, and thus the similarities could also be due to PNC (Losos, 2008). For example, dusky salamanders from the *Desmognathus* genus have maintained similar morphological characteristics despite having adaptively radiated into several similar niches (Kozak *et al.*, 2005). This could be the result of both ecological factors and phylogenetic constraints. Reaction norms, whereby a genotype could produce a range of phenotypes for different environmental conditions (Stearns, 1992), would by definition result in similar behaviour in similar environments. In the green frog *Rana clamitans*, differences in the climate affect growth rates, but when placed in the same environment, individuals from different climates have similar growth rates (Berven *et al.*, 1979).

The striped mouse *Rhabdomys* has a widespread distribution in southern Africa, occurring in all known biomes, but favouring grasslands in suitable habitats (Skinner and Chimimba, 2005). This small (approximately 40 g) rodent is a generalist omnivore, feeding on a wide range of plants (except grass; Perrin, 1980), seeds and some insects (De Graaff, 1981). *Rhabdomys* displays two chromosomal forms (2N=48 and 2N=46; Rambau *et al.*, 2003). In addition, the results of MtDNA analyses further split the genus into two groups (Rambau *et al.*, 2003): the xeric western form (clade 2), characterised by 2N=48, and designated *Rhabdomys pumilio*, the historical scientific name of the taxon; and a mesic eastern type (clade 1), designated *R. dilectus*. *Rhabdomys dilectus* was further divided into two sub-species: *R. d. chakae*, characterised by 2N=48 which occurs in the southern parts of South Africa, and *R. d. dilectus*, characterised by 2N=46, found in the northern parts of South Africa (Pillay, 2000; Rambau *et al.*, 2003). Both species and both sub-species are reproductively incompatible, since each prefers mates of their own taxon (Pillay, 2000) or of different populations of their taxon (Pillay *et al.*, 2006). The reproductive incompatibility is maintained through high levels of aggression (Pillay, 2000). Mate preference in the genus is based on divergence in the olfactory signals used in mate choice (Pillay *et al.*, 2006). Recent unpublished data indicate that the sub-species of *R. dilectus* have different molecular olfactory signatures (N. Pillay, G. Ganem, pers. com.).

At a regional level, studies on the Highveld region of Gauteng Province indicate that *R. d. chakae* occurs in grassland pockets in Johannesburg (Lancaster, 2001) and *R. d. dilectus* occurs north of Irene, Pretoria (Rambau *et al.*, 2003). More recently, striped mice with 2N=48 (*R. d. chakae*) have been found at Modderfontein, 15 km south of the *R. d. dilectus* population in Irene (Riyas Ahamed, 2006). This means that although the sub-species are characterised by different chromosomal and MtDNA profiles, they occupy superficially similar environments within the same biome. Moreover, while the sub-species are characterised by different olfactory cues used in mate choice (Pillay *et al.*, 2006), we do not know of other ways in which the sub-species differ behaviourally.

In the xeric, western parts of South Africa, which are inhabited by *R. pumilio,* striped mice mostly live in groups comprising of 3-4 breeding females, their offspring of varying ages, and 1 breeding male (Schradin and Pillay, 2004). *Rhabdomys pumilio* groups defend their territories against neighbouring groups (Schradin, 2004; Schradin and Pillay, 2005b), but are non-aggressive to members of their own group. Group-living in this species is facultative, however, and the social system can change (i.e. solitary living can occur) if the appropriate circumstances (e.g. low population density) are met (Schradin *et al.*, 2010). In the mesic grasslands in the eastern parts of southern Africa, which are inhabited by *R. dilectus*, striped mice are solitary, with both sexes maintaining intrasexually non-overlapping territories, and territories of males overlapping those of several females (Brooks, 1974; Schradin and Pillay, 2005b). The territoriality of the grassland striped mice, (like that of group living *R. pumilio*; Schradin and Pillay, 2005b) is maintained through aggression and possibly mutual avoidance, which may be related to the density of food (Schradin, 2004). In nature, there has been no evidence of group-living in *R. dilectus* (N. Pillay, pers. com.).

Interesting differences have been found in the behaviour of *R. pumilio* and *R. dilectus* in terms of exploratory behaviour, with *R. pumilio* displaying higher levels of exploration in an open arena than *R. dilectus* (Rymer *et al.*, in prep.). Rymer *et al.* (2008) maintain that this greater level of exploration may be to improve their encounters with food, but this would mean that these diurnal animals are exposed to raptors during foraging, which may explain why they remain in close proximity to bushes during foraging forays (Schradin, 2006). Surprisingly, suitable food (e.g. seeds and fruits) for *R. dilectus* is patchily distributed in the grasslands (Schradin, 2005), which results in larger home ranges for striped mice in the grasslands than in the semi-arid (open) habitats. In consequence, *R. dilectus* would need to cover large distances to locate food, so that aerial cover would be critical to reduce predation risk, particularly from birds of prey which feed on striped mice (De Graaff, 1981).

My project considers the maintenance (i.e. non social) and social behaviour of *R. d. dilectus* and *R. d. chakae*, to establish whether environmental or phylogenetic factors are more important in determining behaviour in these sub-species. Since both sub-species occur in the grassland biome, I expect that if behaviours of *R. d. dilectus* and *R. d. chakae* are different, phylogeny may have a more important role in affecting the behaviour of the sub-species. However, if the habitats are different and the behaviours are also different, the environment may be influencing behaviour. If phylogeny is found to be more important, the sub-species may have evolved along different trajectories. The lack of sympatry between the two sub-species (N. Pillay pers. com.) suggests that the potential exists for separate evolution by, for example, genetic drift or even population-specific selection pressures. To investigate differences in the behaviour between the *R. dilectus* sub-species, I selected two populations located 70 km apart on the Highveld in Gauteng Province South Africa. I first examined the microhabitats of the sites where the two populations were trapped. I next considered behaviours (activity, exploration, anxiety response) that are fundamentally important for survival and subsequently considered some aspects of the social behaviour that reflect social systems of the sub-species.

Taxonomic note

The genus *Rhabdomys* is now characterised by two species and two sub-species (as reported above) since the early 2000s. However, the historical name *R. pumilio* is used in all publications prior to the species name change, proposed by Rambau *et al.* (2003), and also in subsequent publications. To ensure that comparisons are made with the appropriate taxon in my dissertation, I used the geographic distribution of the taxon (after Rambau *et al.*, 2003) to infer the current taxonomic name of the population of striped mice in question. For example, striped mice in Irene in Gauteng Province, South Africa were regarded as *R. pumilio* by Pillay (2000) but are now referred to *R. dilectus dilectus*. Where there is uncertainty about the taxonomic status, I use just the genus name *Rhabdomys*.

Layout of the dissertation

Apart from the present chapter (introduction), the dissertation comprises 4 experimental chapters, and a general discussion (chapter 6). Chapter 2 focuses on the general habitat characteristics of the localities where I trapped the *R. dilectus* sub-species. The maintenance behaviour of the sub-species is described in chapter 3. The social behaviour of *R. dilectus* is considered in chapters 4 and 5, with chapter 4 concerning dispersal, stress and sociality in the two *R. dilectus* sub-species and *R. pumilio*, and chapter 5 considers the intra-specific social behaviour of the *R. dilectus* sub-species. Because of this format, there may be some repetition of introductory material, methodological details, or discussion. Figures and tables are numbered in sequence for each chapter, and not for the entire dissertation. One reference section is provided at the end of the dissertation, and pages are numbered sequentially.

Chapter 2: Vegetation characteristics of the study localities

Introduction

The striped mouse, genus *Rhabdomys*, is found in a wide variety of habitats, ranging from the western xeric succulent Karoo, to the eastern mesic grasslands (Schradin and Pillay, 2005b). The habitat shapes the biology of striped mice. For example, marked differences in the ecology of the areas result in group-living in the xeric-occurring *R. pumilio* and solitary-living in the mesic-occurring *R. dilectus* (Schradin, 2005; Schradin and Pillay, 2005b).

In the present study, I studied two populations of the *R. dilectus* sub-species (*R. d. chakae* and *R. d. dilectus*), which were located in a grassland biome (Mucina *et al.*, 2005) in South Africa. The localities from which the populations originated were approximately 70 km apart. Here, I compared the vegetation characteristics of these two localities, in order to place the findings reported elsewhere in my study in a broader ecological context. Due to the localities being within the same general habitat type, I expected the vegetation characteristics to be similar. However, ecosystem processes can have a marked effect on vegetation structure (Lauenroth *et al.*, 1993; Hejcmanovā-Nežerková and Hejcman, 2006). In turn, the ecosystem processes are affected by many factors including grazing by herbivores, water and soil type (Dantas and Batalha, 2010; Tessema *et al.*, 2011). These factors can result in unpredictable and stochastic patterns of variation between habitats, and as a consequence, I did not compare sites statistically, but rather my aim was to assess whether vegetation characteristics of the two localities differed qualitatively.

Materials and methods

I measured general vegetation characteristics at Walkers Fruit Farms (S 26°29’30’’ E 27°57’59’’) where *R. d. chakae* originated, and Irene (S 25°54’10” E 28°12’09”; Figure 1.1) where *R. d. dilectus* originated, during three winter months (June – August) and three summer months (November – January). Vegetation surveys were conducted at Irene between 2001 and 2002, and at Walkers Fruit Farms between 2009 and 2010. *Rhabdomys* *dilectus chakae* individuals are found at Walkers fruit farms and *R. d. dilectus* are found at Irene.

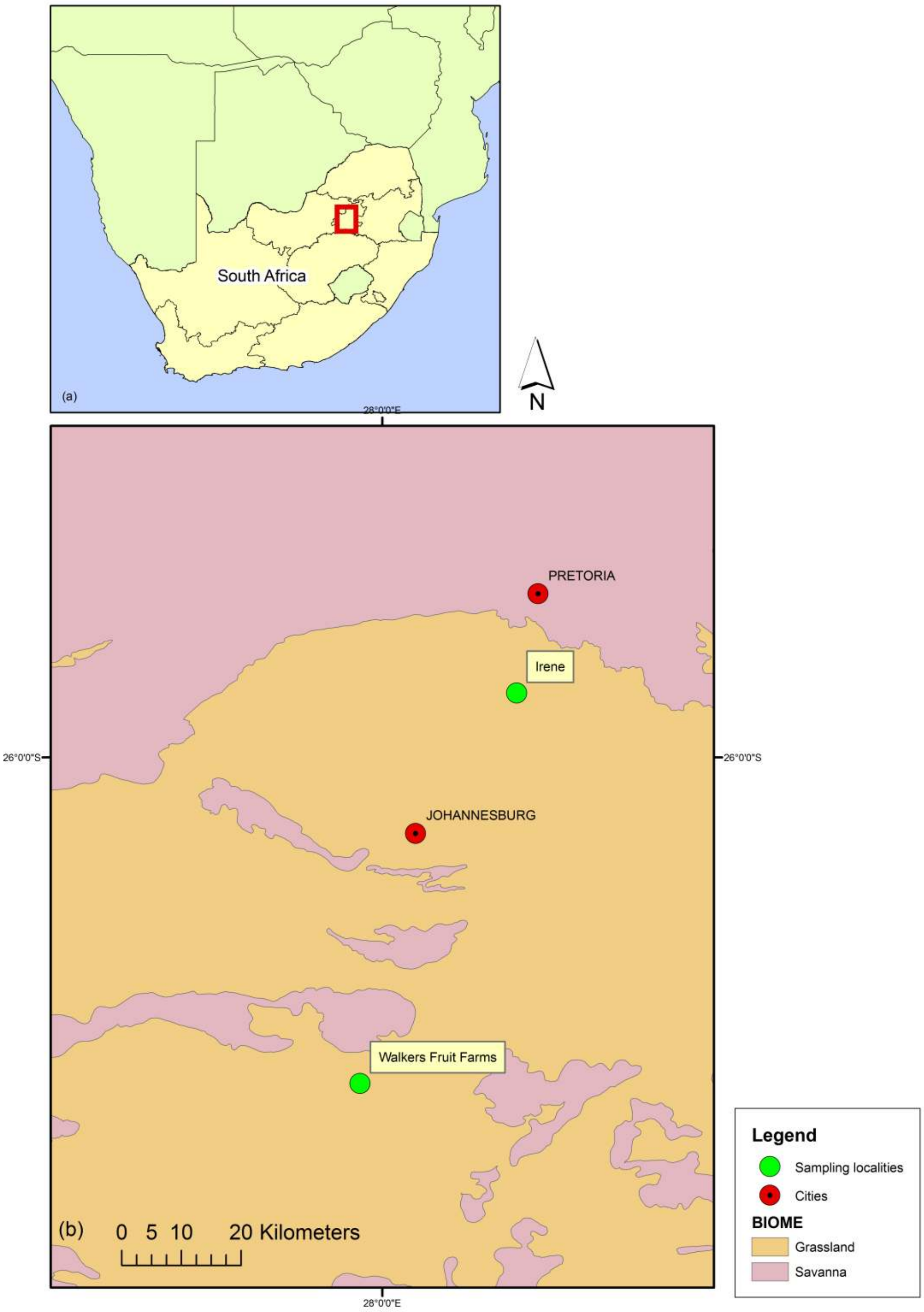

Figure 2.1. Map showing the locations of Walkers Fruit Farms and Irene in South Africa (inset) and the specific biomes in which they are located.

Four 3000m^2 grids at least 140m apart were randomly selected and marked out using metal stakes at both Irene and Walkers Fruit Farms. The four grids were divided into 30 smaller plots of 100m^2 each, and within these plots, two 1m^2 steel quadrats were placed randomly in order to measure vegetation characteristics. I measured the maximum and minimum vegetation heights (cm), the aerial cover (%), and the amount of dead material (%). The maximum and minimum vegetation height measurements were taken from the tallest and shortest plants, regardless of species, in the 1m^2 quadrat. The aerial cover and amount of dead material was assessed visually using the Walker's scale (Walker, 1976), which is based on the proportion of the quadrat that was covered. In addition, I assessed the density of the cover randomly, four times within the 100m^2 plots, by taking measurements with a light meter (Yew, model number 3281) at the soil surface and directly above the vegetation layer, and dividing the bottom reading (soil surface) by the top reading (above vegetation layer), in order to obtain the proportion of light that passed through the vegetation. Cover density provided an estimate of light penetration to the zones of activity of the rodents, whereas aerial cover (see above) provided an assessment of vegetation cover over larger areas in a quadrat. Cover density measurements were taken as close to mid-day as possible, to minimise the effect of the angle of the sun on light measurements.

Data analyses

For each month, data were combined per grid to obtain the mean values of the measurements for all the variables recorded (see above). This was done because the variables measured in each plot may not have reflected the vegetation characteristics over the whole grid (Douglass *et al.*, 1992). I ran a Principle Components Analysis (PCA) for both winter and summer, in which locality (Walkers Fruit Farms and Irene) was the grouping variable, and cover density (arcsin transformed), maximum vegetation height, aerial cover and the dead material were factors (dependent variables) for analysis, and month was a supplementary variable. The PCA allowed me to describe patterns between localities in their vegetation characteristics. I excluded the minimum vegetation height from the analysis as it was strongly auto-correlated with maximum vegetation height. I also assessed the temporal variation in vegetation structure over the six sampling sessions, using repeated measures analysis of variance (ANOVA), which was run separately for each locality because of the random selection of grid positions within each locality. Fisher LSD tests were used as post-hoc tests. Where appropriate, all tests were two-tailed and the significance level was set at $p \leq 0.05$. All statistical analyses were run in Statistica v.8 (Statsoft, 2007).

Results

I compared the localities materially since I decided not to make statistical comparisons between them. On average, the percentage aerial cover was greater at Irene than Walkers Fruit Farms by 37% in winter and by 21% in summer (Figure 2.2). Walkers Fruit Farms had a greater amount of dead material than Irene by 63% in winter, but the localities did not differ in summer (Figure 2.2). The density of the cover was greater in Irene in winter (46%) but greater at Walkers Fruit Farms in summer (43%; Figure 2.2). The minimum height of the vegetation was not different between Walkers Fruit Farms and Irene in winter, but was lower by 90% at Walkers Fruit Farms than Irene in summer (Figure 2.3). Maximum vegetation height was similar between the localities in summer and winter (Figure 2.3).

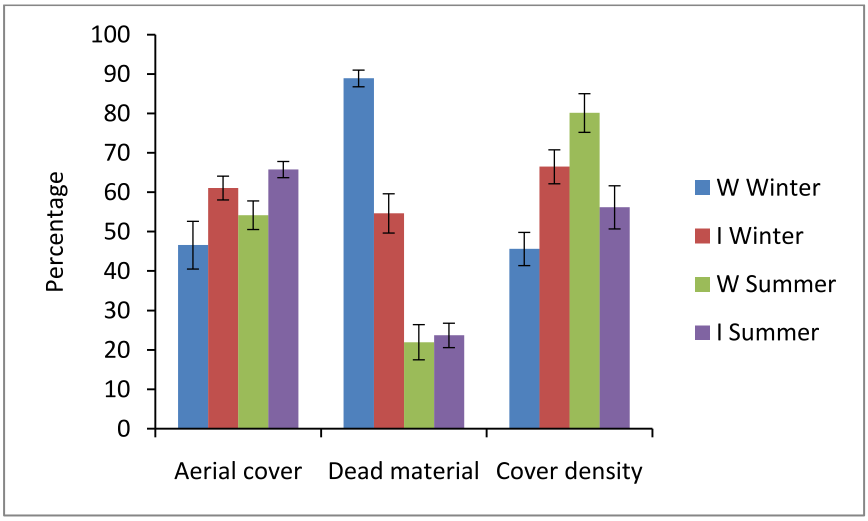

Figure 2.2. Mean (± SE) of aerial cover (%), dead material (%) and density of cover (%) at Walkers Fruit Farms (W) and Irene (I) during winter and summer months.

The principal components analysis for the winter months indicated that the first and second principal components accounted for 65 % and 35% of the variance, respectively. The

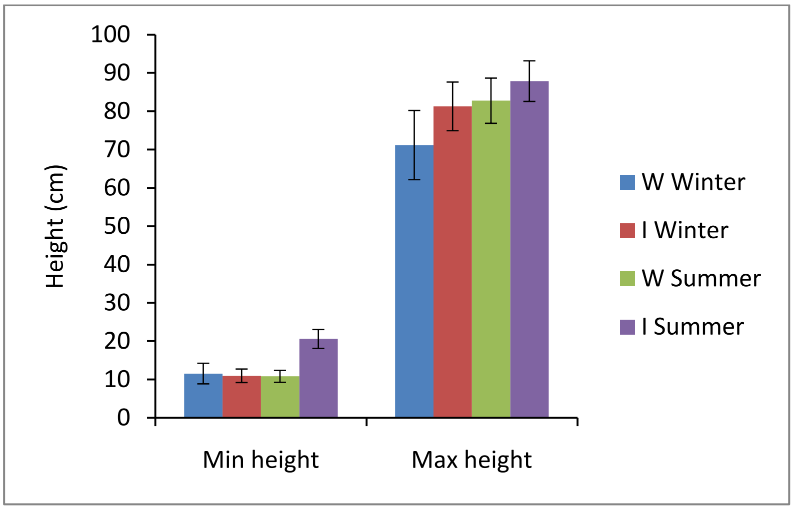

Figure 2.3. Mean (± SE) of minimum and maximum vegetation height (cm) at Walkers Fruit Farms (W) and Irene (I) during winter and summer months.

percentage dead material had the highest positive (0.814) and percentage cover had the highest negative (-0.878) influences on the first principal component. On the second principal component, cover density (measured using a light meter) had the highest positive (0.936) and maximum vegetation height the highest negative (-0.546) influences. By transposing the input matrix into the PCA, I was able to assess the data from the sampling grids (4 per locality) against the vegetation characteristics. During the winter months, Irene grids were mainly associated with higher cover density (Figure 2.4). Walkers Fruit Farms was mainly associated with lower cover and maximum height (Figure 2.4). Both localities were negatively associated with dead plant material (Figure 2.4).

The principle components analysis for the summer months showed that the first and second principle components accounted for 96% and 2% of the variance respectively. On the first principle component, percentage cover had the highest positive (0.951) and the cover density had the highest negative (-0.783) influences. The percentage of dead material had the highest positive (0.774) and percentage cover had the highest negative (-0.199) influences on the second principle component. In the summer months, the localities could not be distinguished as separate groups, and there were no positive associations between any of the variables and the localities, except for cover density (Figure 2.5).

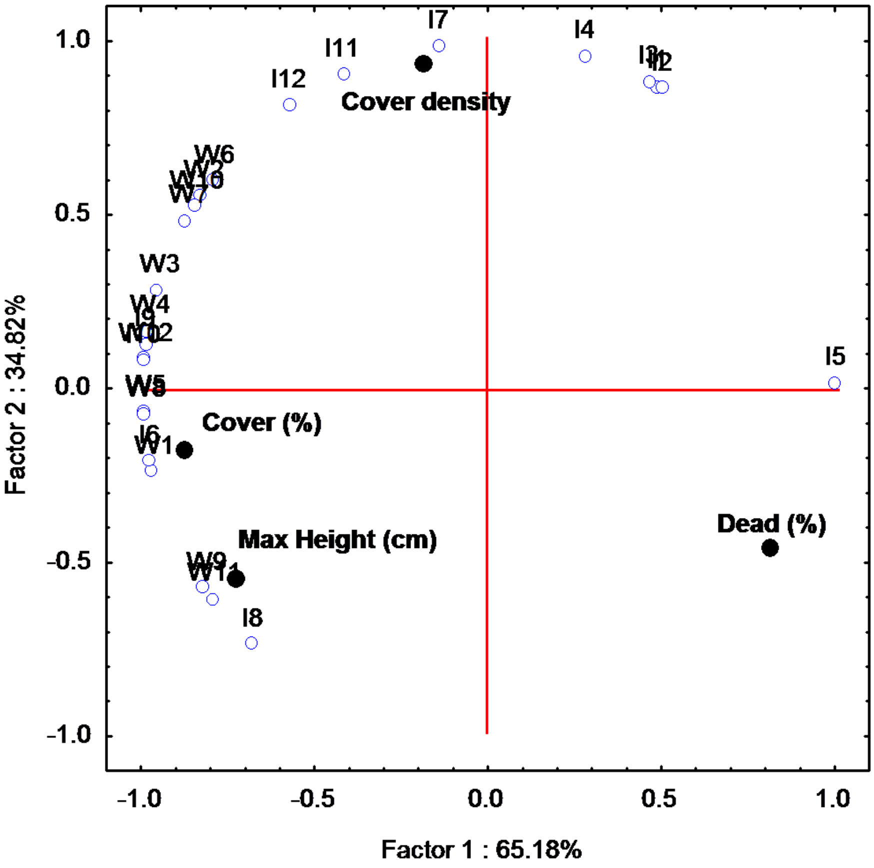

Figure 2.4. PCA of vegetation data for winter months, grouped according to locality (I = Irene; W = Walkers Fruit Farms), and overlaid by vegetation data collected in winter.

The month of sampling was a significant predictor of the cover density, maximum vegetation height, aerial cover and dead plant material at Walkers Fruit Farms, but was not a significant predictor of minimum vegetation height (Table 2.1). Cover density was greater in summer months than it was in winter months, and dead material was lower in summer than winter. There was no seasonal grouping for maximum vegetation height, and cover during late winter was the lowest compared to all other months (Figure 2.1, 2.2). At Irene, the month of sampling was a significant predictor of minimum and maximum vegetation height, aerial cover and the amount of dead material, but not the density of cover (Table 2.1). There was no clear seasonal grouping of minimum or maximum vegetation height, but on average, aerial cover was greater in summer months, and the amount of dead material was greater in winter months (Figure 2.1, 2.2).

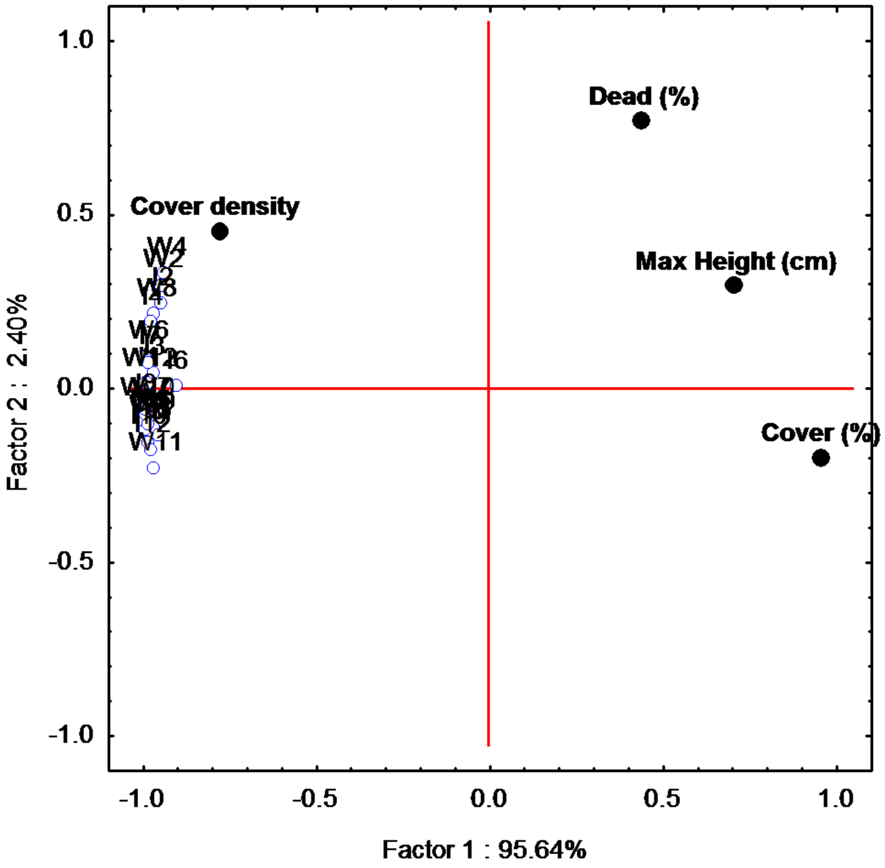

Figure 2.5. PCA of vegetation data for summer months grouped according to locality (I = Irene; W = Walkers Fruit Farms), and overlaid by vegetation data collected in summer.

Table 2.1. Results of Repeated measures ANOVA analysing the values for the vegetation per month at Walkers Fruit Farms and Irene. Bold numbers indicate statistically significant results.

	Walkers Fruit Farms	Irene
Cover density	**$F_{5,15}$=9.136, p < 0.001**	$F_{5,15}$=2.262, p = 0.101
Minimum vegetation height	$F_{5,15}$=0.679, p = 0.646	**$F_{5,15}$=10.570, p < 0.001**
Maximum vegetation height	**$F_{5,15}$=4.008, p = 0.016**	**$F_{5,15}$=6.293, p = 0.002**
Aerial cover	**$F_{5,15}$=3.887, p = 0.019**	**$F_{5,15}$=4.459, p = 0.001**
Dead material	**$F_{5,15}$=43.301, p < 0.001**	**$F_{5,15}$=28.891, p < 0.001**

In summary, Walkers Fruit Farms and Irene were associated with different vegetation characteristics, with Irene being associated with high cover density, and Walkers

Fruit Farms being associated with lower maximum heights and lower cover, and negatively associated with the amount of dead material, with more dead material than Irene. In summer, both sites were associated with higher cover density, although Walkers Fruit Farms had a higher cover density but lower amount of aerial cover than Irene. Interestingly, the monthly cover density did not change significantly at Irene.

Discussion

Rhabdomys associates with habitats that have high levels of cover (Monadjem, 1997; du Preez, 1998; Fuller and Perrin, 2001), most likely due to the protection from predation. I measured both the amount of aerial cover, as well as the density of the cover, since prey species might prefer areas that are more dense (e.g. barking deer, *Muntiacus muntjak* - Pokharel and Chalise, 2010). In winter, Irene (*R. d. dilectus*) was associated with both high amounts of aerial cover and high cover density compared to Walkers Fruit Farms (*R. d. chakae*). However, in summer, both localities were characterised by high cover density, although the density was higher at Walkers Fruit Farm than at Irene, and Irene had more aerial cover than Walkers Fruit Farms. This suggests that, although there might not be as much cover at Walkers Fruit Farms as at Irene, the plant material is denser at Walkers Fruit Farms, thus offering a greater degree of protection from aerial predation to small mammals. The negative relationship between the localities and the percentage of dead material suggests that both localities have high levels of live material, probably acting as a food source, since *R. dilectus* eats herbs, seeds and berries (Fuller and Perrin, 2001; Schradin and Pillay, 2005b).

Small mammals have been shown to have strong preferences for specific vegetation structures (see Ferreira and Van Aarde, 1999; Fuller and Perrin, 2001). Vegetative cover is important for protection against predation (Stokes *et al.*, 2004) and food resources, which affect territoriality (Schradin, 2004; Schradin and Pillay, 2005b). This small study, comparing the vegetation in the grids from where the two sub-species of *R. dilectus* were trapped indicates that, although the localities are situated within the same grassland biome (Mucina *et al.*, 2005), there are slight differences in the vegetation characteristics.

Chapter 3: Maintenance behaviour

Introduction

The environment in which an animal lives can influence its behaviour. In poor-quality or crowded soil, the earthworms *Aporrectodea icterica* and *Dendrobaena veneta* disperse faster than if conditions were uncrowded and favourable (Mathieu *et al.*, 2010). In southern elephant seals *Mirounga leonina* female foraging patterns depend on the condition of the pack ice, such that when the area of ice in the Antarctic expands, the females move to forage northwards (Bailleul *et al.*, 2007). Spotted sand lizards *Pedioplanis l. lineoocellata* switch from their natural 'sit-and-wait' foraging strategy to active foraging in overgrazed and more disturbed habitats (Wasiolka *et al.*, 2009).

For rodents, like all small prey animals, the availability of cover can influence their behaviour in fundamental ways, since a lack of cover increases predation risk. Consequently, predation risk is much higher in open habitats than in those that have greater cover (Kotler *et al.*, 1991). Therefore, the open habitats of two deer mice species, *Peromyscus polionotus* and *P. aztecus* expose individuals to high predation pressure, and they respond by burrowing long tunnels, with an escape tunnel in *P. polionotus* (Weber and Hoekstra, 2009). Another example is the dune hairy-footed gerbil *Gerbilliurus tytonis* which tends to forage closer to cover on full-moon nights when the risk of predation is higher (Hughes and Ward, 1993).

When rodents from areas of greater cover are exposed to areas with reduced cover, they alter their behaviour in order to reduce predation risk, such as avoiding open (Powell and Banks, 2004; Stokes *et al.*, 2004) and less structurally complex areas with less low-lying overhead cover (Baker and Brown, 2010). For example, house mice *Mus domesticus* prefer microhabitats that are complex, and have greater cover but when they are placed in situations that have little complexity and overhead cover, the mice concentrate their activity in and around their nest-boxes, or reduce their overall level of activity altogether (Jensen *et al.*, 2003). Thus when there are no perceived predators, animals from more open habitats should show greater levels of exploratory behaviour in open habitats (Rymer *et al.*, 2008).

The level of illumination and cover also play a role in the timing and level of activity in rodents. When the levels of illumination are high, Allenby's gerbil *Gerbillus allenbyi* and the greater Egyptian sand gerbil *G. pyramidum* respond by reducing their level of activity in open areas, foraging under cover instead (Kotler *et al.*, 1994). Kotler *et al*., (1994) suggested that the timing of activity did not appear to be affected by the amount of cover available to *G. allenbyi* and *G. pyramidum*, and was instead related to varying illumination during the lunar cycle. Two desert pocket mice species, *Perognathus amplus* and *P. baileyi*, respond similarly to cover (Brown *et al.*, 1988), and the presence of a predator caused *P. amplus*, *P. baileyi* and Merriam's kangaroo rat *Dipodomys merriami* to cease all activity in areas where there was low cover, and utilise habitats where the cover was higher (Brown *et al.*, 1988). Thus, although the amount of cover appears to affect the level of activity, it does not appear to affect the activity profiles (i.e. 24 hour activity) of rodents.

The striped mouse *Rhabdomys*, is widely distributed throughout South Africa, with *R. pumilio* inhabiting the arid western succulent Karoo, and *R. dilectus* inhabiting the moist eastern grasslands of South Africa (Schradin and Pillay, 2005b). More is known about the behaviour of free-living *R. pumilio* because of a research programme on these animals at the University of the Witwatersrand and because this species is easy to observe in its natural habitat (Schradin, 2006). The vegetation diversity of the succulent Karoo is high (Cowling *et al.*, 1999), although in general, there are large open patches with patchy shrub distribution (Schradin, 2005). *Rhabdomys pumilio* exhibits high levels of exploratory behaviour (Schradin, 2006), possibly to locate food (Rymer *et al.*, 2008). They also spend much time basking outside their nests in order to gain heat and save energy (Scantlebury *et al.*, 2006; Schradin *et al.*, 2007). Schradin *et al*. (2007) found that *R. pumilio* only start becoming active after their nests become sunlit in the mornings, cease their active period when the sun sets in the evening, and do not appear to be active during the night. During the heat of the day, striped mice rest in bushes (Schradin, 2006). Schumann *et al*. (2005) studied the activity profiles of *R. pumilio* from the Cape Flats under laboratory conditions using striped mice entrained on running wheels, and found a similar pattern of concentrated activity during the day, with peaks during morning and evenings.

In contrast to the arid regions, grasslands tend to have more cover, especially those in high rainfall areas with moderate fire and grazing regimes (O'Connor and Bredenkamp, 1997). The greater cover would provide small mammals with more protection against aerial predators, such as raptors. Therefore, it is predictable that, unlike striped mice from the succulent Karoo, those in the grasslands would be active under covered and not in open conditions. This expectation has been partially confirmed by anecdotal information available in Neville Pillay's laboratory at the University of the Witwatersrand. Another important difference between grassland and semi-arid areas is the differences in diel (i.e. 24 hour) temperature profiles, with the succulent Karoo being characterised by high diurnal and very low nocturnal temperatures (Schradin and Pillay, 2005a). The grasslands may also have similar temperature profiles (Schradin and Pillay, 2005b) but because vegetation may buffer extreme temperature fluctuation, as mentioned by Packer (1980), the temperature profiles under grassy cover may be more stable.

Perrin (1981) showed that captive *Rhabdomys* from the Eastern Cape (now referred to *R. d. chakae*; Rambau *et al.*, 2003), were also active during the day and had a strongly crepuscular activity profile under the artificial light regime. This diurnal activity profile is similar to *R. pumilio* but it is unclear whether the crepuscular activity is part of the diel activity profile of *R. d. chakae* or simply displaced behaviour in test subjects studied in an open arena in captivity and which originated from covered grasslands in nature.

Although we know that there are slight differences in the activity profiles between *R. pumilio* and *R. dilectus*, *R. pumilio* tends to explore open arenas more readily than *R. dilectus* in captivity (Rymer *et al.*, in prep.), and thus *R. dilectus* may have a higher anxiety response than *R. pumilio*. We do not know whether the two subspecies, *R. d. chakae* and *R. d. dilectus*, have different maintenance behaviours (i.e. non-social behaviours). The aim of this study is to investigate three maintenance behaviours in the two populations of striped mice, representing each of the sub-species. I recorded the activity profile of individuals in open arenas, exploratory behaviour in an enclosed maze and their anxiety responses in a modified plus maze. I selected these behaviours for study because they represent fundamental aspects of the biology of animals, and are shaped by environmental (anxiety, reviewed in Clément *et al.*, 2002; activity, e.g. Pépin *et al.*, 2006; exploratory behaviour, e.g. Kazlauckas *et al.*, 2011) and phylogenetic factors (anxiety, reviewed in Clément *et al.*, 2002; activity patterns, reviewed in Roll *et al.*, 2006; exploratory behaviour; Vauti *et al.*, 2007).

I made two predictions. Firstly, from the Perrin (1981) study, I predicted that the sub-species would have crepuscular activity profiles. Secondly, Schradin and Pillay (2005b) showed that *R. dilectus* individuals have larger home-range sizes compared to *R. pumilio* individuals, and therefore I expected that representatives of both *R. dilectus* sub-species will explore large distances if placed under cover. However, they should show a high stress response if exposed to an open, novel environment (e.g. the plus maze), and thus should explore less when exposed to open environments.

Methods

General information

I trapped *Rhabdomys dilectus chakae* (2N=48) and *R. d. dilectus* (2N=46) using randomly placed plastic live-traps (290 x 60 x 80 mm). Trapping was done between April 2009 and June 2010. Traps were baited with a mixture of oats, sunflower seeds, salt, peanut butter and sunflower oil, and checked once daily. Traps contained cotton wool and were well covered with vegetation for insulation. Once animals were caught, they were transferred to plastic holding cages and transported by car to the University of the Witwatersrand. All *R. d. chakae* individuals (10 females and 13 males) were caught from Walkers Fruit farms (S 26°29’30’’ E 27°57’59’’). I trapped a total of 17 *R. d. dilectus* individuals (10 males and 7 females). Eight *R. d. dilectus* individuals (5 males and 3 females) were from the Agricultural Research Council (ARC) in Irene (S 25°54’10” E 28°12’09”), 2 individuals (1 male and 1 female) were from Vaal River (S 26°55’58” E 26°41’38”), and 5 male and 3 female striped mice were from Rietvlei Dam Nature Reserve (S 25°54’4’’ E 28°16’38’’), east of Irene. Where possible, all individuals were used in all the experiments described below, with the exception of the activity test or when some animals had died. All striped mice were adults at the time of testing, and had been in captivity for between 2 and 12 months prior to being tested in any experiment.

Subjects were housed singly in Lab-o-tec™ cages (150 x 420 mm x 150 mm high) with a standard 14 hr light, 10 hr dark cycle, temperature regulated between 22 and 24°C and 30 - 60% relative humidity, in the Milner Park animal unit, at the University of the Witwatersrand. Epol® mouse cubes and water were provided *ad libitum*, supplemented weekly with fresh fruit or vegetables and sunflower seeds. Cages were furnished with a plastic nesting box (130 x 90 mm x 100 mm high), a handful of dry grass, and a bedding of wood shavings. Cardboard toilet rolls and a handful of nesting material were provided bi-weekly for enrichment. Cages were cleaned every two weeks, and the cage contents replaced. The equipment used in all experiments was cleaned thoroughly with soap and water between uses. All experiments were recorded with a Sony Handycam (model: DCR – SR62E) connected to a Divis DVR security recording system.

Activity

I used 16 *R. d. chakae* (8 males; 8 females), and 14 *R. d. dilectus* striped mice (7 males; 7 females) to investigate taxon-specific activity patterns. The behaviour of subjects was studied individually in large galvanised steel tanks with a clear Perspex fronts (460 x 300 mm x 350 mm high) for 72 consecutive hours. The tanks had a layer of wood shavings, and were furnished with a plastic nesting box (130 x 90 mm x 100 mm high), a handful of grass, some paper towel as nesting material, and a toilet roll. Mouse cubes were placed in one corner of the tank, a handful of seeds was scattered throughout the tank to stimulate foraging, and a bottle of water was provided for the duration of the experiment.

Test subjects were placed individually in tanks 10-12 hours before the start of recording, in order for them to acclimatise to the tank. The behaviour of test subjects was video recorded via the Perspex window in the tank, and the recording started at midnight and ended 72 hours later. I scored active (feeding, grooming or walking around the tank) or inactive (sleeping or hiding in nesting material) behaviour using instantaneous sampling. During active periods (04h00-09h00; 17h00-21h00; Pillay *et al.*, 2006) samples were taken every 3 minutes, and were taken at 5 minute intervals for the remainder of the day.

Data analyses

I used mean substitution for 3 hours of data which were missing, and analysed the activity as a proportion of activity per hour (as the number of scoring periods differed between peak and non-peak periods; see above). I used a Variance Components Analysis with an expected mean squares model to investigate the role of presence of stereotypy on the expression of activity. Population and sex were fixed effects, stereotypy was a random effect and the behaviour (active or inactive) was the dependent factor. Sex was not a significant predictor of activity during all 72 hours of observations, and stereotypy was only a significant predictor in 2/72 (2.8%) of the hours. Therefore these two predictors were not considered further. I then used a repeated measures 2-way MANOVA to analyse whether taxon and sex predicted the hourly variation of active behaviour for each hour of the 72 hour experiment. The hour and day (every 24 hour period) were used as the repeated measures; the latter was included to ascertain whether the striped mice changed their activity patterns in their first introduction into the new tank and subsequent acclimation to the tank.

All statistics were done in Statistica v.8 (Statsoft, 2007), and were two-tailed and significance levels were set to 0.05. Where applicable, Fisher LSD *post hoc* tests were used to assess significant first and second order effects. Experiments were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Clearance number 2009/25/2A).

Plus maze

The behaviour of 20 *R. d. chakae* (12 males; 8 females) and 17 *R. d. dilectus* (10 males; 7 females) individuals was assessed in a modified plus maze. The maze consisted of 2 dark and 2 clear arms that were all enclosed (75 x 500 mm x 75 mm high) and a central chamber of 110 x 110 x 155 mm. A circular entry chamber (55 mm diameter x 100 mm height) was situated above one of the arms. I placed the subjects individually in the entry chamber and allowed them 5 minutes to enter the maze. The behaviour of test subjects was video recorded for 15 minutes. The duration spent in and the frequency of entries into the clear and the dark arms were recorded. Experiments were run between 06h00 and 09h00.

Data analyses

I assessed the duration (in seconds) spent in and the frequency of entries into both the clear and the dark arms of the plus maze, in order to determine whether the subjects experienced anxiety in the novel environment. I used a MANOVA to assess whether taxon and sex predicted differences in the duration spent in or frequency of visits into the clear and dark arms of the maze.

Exploratory behaviour

Exploratory behaviour is usually assessed using an open field test, which assumes that the subjects will display natural exploratory behaviour in an open arena that is representative of their species (Heth *et al.*, 1987). The localities where my study animals originated were quite dense with much cover (See chapter 2, page 7), and a closed arena would be more appropriate for testing exploratory behaviour in my test subjects. Therefore, I designed and constructed a closed, dark maze to test exploratory behaviour (Figure 3.1); the maze had small holes that allowed minimal light to enter. The maze was constructed from Perspex, and consisted of a box and lid with removable dividers to create passages and to facilitate cleaning. Before the start of a test, a start and end box were attached to the maze to enable subjects to turn around once in the maze. The start box also had an externally removable door which allowed me to keep the start box separate from the maze, so that test subjects could acclimate to the dark conditions before being allowed access into the maze itself.

The maze had 5 passages, which had infrared emitters and receivers placed at the start or end of each passage (Figure 3.1) and the emitters, receivers and wires placed inside the dividers. These were connected to a circuit board on top of the maze lid. Small LED lights that were connected to the receivers allowed me to establish whether the relevant beam had been broken. As the test subject crossed an infrared beam, an LED light corresponding to the receiver would go on for half a second after the animal had moved past the beam, which was achieved using 555 timers in each circuit. All circuit components were obtained from MANTECH, Johannesburg. The circuitry was connected to a rechargeable 12V battery.

The design of the maze allowed subjects to travel an infinite distance (up to the maximum time of 20 minutes in the maze) because of the start and end boxes which allowed the animals to turn around and also because the animals could turn around in the maze itself. The approximate distance that a mouse travelled was measured using the distances between emitter/receiver pairs (Figure 3.1).

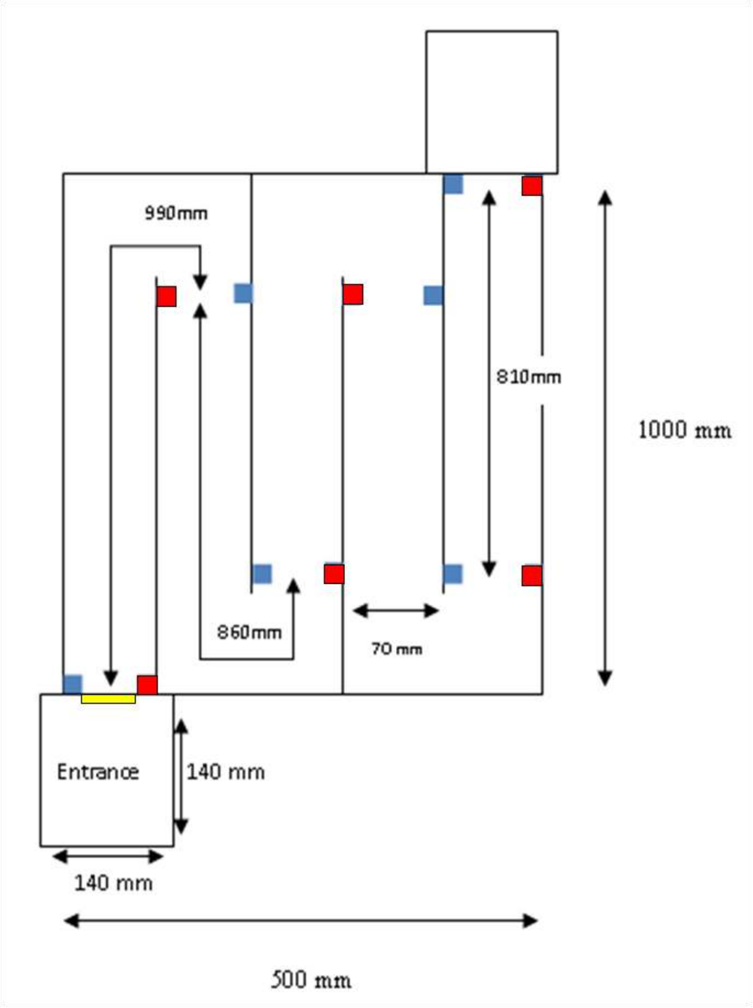

Figure 3.1. Diagrammatic representation of the floor plan of the maze used to test exploratory behaviour. Emitter (blue) and receiver (red) pairs and the externally removable door (yellow) are shown. The distances between emitter/receiver pairs are shown on the inside of the floor plan.

A total of 23 *R. d. chakae* individuals (13 males; 10 females) and 17 *R. d. dilectus* individuals (10 males; 7 females) were used in this experiment. Test subjects were placed individually in the entrance box for 5 minutes, after which they were allowed access into the maze, via the externally removable door in the start box. Individuals were allowed to explore the maze for 20 minutes. Subjects that had not left the entry box within five minutes of opening the maze were removed from the experiment and their data discarded from further analyses (2 of 23 *R. d. chakae* subjects only). Tests were run between 06h00 and 09h00, during the peak activity time for *Rhabdomys*.

The latency of test subjects to enter the maze, the number of emitter/receiver pairs crossed, the distance travelled, and the average speed were recorded. The number of emitter/receiver pairs crossed was established by counting the number of receiver activations. The distance travelled was measured using the receiver activations, as each emitter/receiver pair had a set distance between them, so I could calculate the distance each individual travelled by recording which emitter/receiver pair at point A and point B. The speed of the subjects was calculated using the distance between the emitter/receiver pairs and the time in which the subject travelled the distance. The body mass to the nearest gram of test subjects was recorded prior to experiments, to ascertain whether the mass of the animal affected their speed through the maze.

Data analyses

The data did not meet the assumptions of normality (Shapiro-Wilk test), so I used Generalized Linear Models (GLZ) with a normal distribution and log link function to investigate taxon and sex differences in exploratory behaviours of striped mice in a closed maze. The latency to enter the maze, the number of sensors crossed, the distance travelled, and the average speed were analysed separately as response variables, with body mass included as a covariate.

Results

Activity

I compared the hourly-level of activity of *R. d. chakae* and *R. d. dilectus* individuals over 72 hours. Taxon was a significant predictor of the level of activity ($F_{1,27}$=8.924, p = 0.006), with *R. d. chakae* significantly more active than *R. d. dilectus*. The day of testing was not a significant predictor of the level of activity ($F_{2,54}$=0.200, p = 0.819), nor was the statistical interaction between the day and taxon ($F_{2,54}$=2.317, p = 0.108). However, hour was a significant predictor of the level of activity ($F_{23, 621}$=19.444, p < 0.001), and the statistical interaction between hour and taxon also had a significant influence on the level of activity ($F_{23, 621}$=2.277, p = 0.001). Both taxa had peaks of activity between 04h00 and 06h00, and again between 19h00 and 21h00 (Figure 3.2). However, *R. d. chakae* had a significantly higher level of activity between 07h00 and 11h00, at 14h00, and between 19h00 and 23h00, compared to *R. d. dilectus* (Figure 3.2). The statistical interaction between day and hour ($F_{46, 1242}$=1.162, p = 0.215), and day, hour and taxon ($F_{46, 1242}$=1.312, p = 0.081) were not significant predictors of the level of activity.

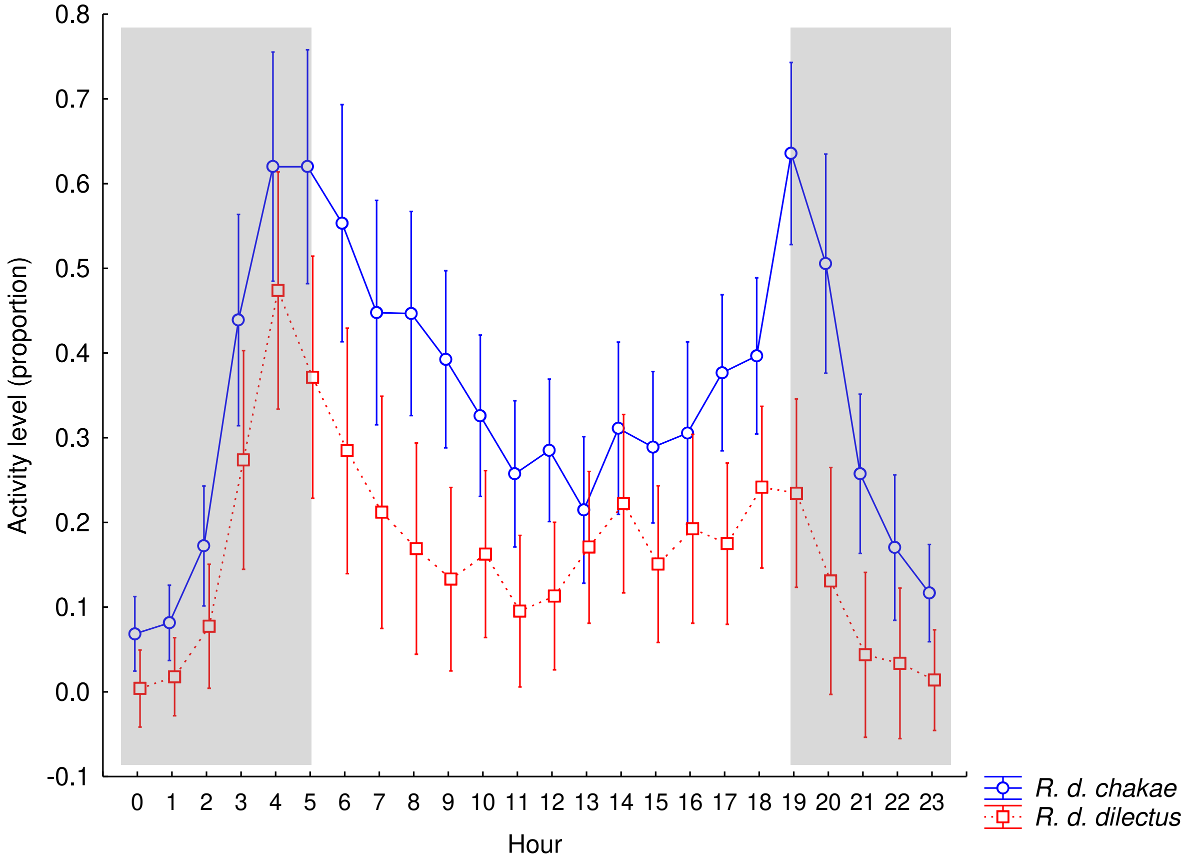

Figure 3.2. Mean ± SE activity level for 24 hours over 3 days for *R. d. chakae* and *R. d. dilectus*. The shaded grey areas indicate the dark phase of the light-dark cycle.

Plus maze

Sex did not influence the duration spent in the clear and dark arms of the plus maze or the frequency with which the arms were entered, and the data for the sexes were pooled in further analyses. Taxon was not a significant predictor of the duration spent in the clear or dark arms of the plus maze ($F_{2,34} = 3.127$, $p = 0.057$; Figure 3.3). In contrast, the frequency with which individuals entered clear or dark arms was significantly predicted by taxon ($F_{2,34}=4.628$, $p = 0.017$), with *R. d. dilectus* entering the clear and dark arms significantly more often than *R. d. chakae* (Figure 3.4). Taxon was also a significant predictor of the mean duration (i.e. total time spent per entry/number of entries) spent in the clear or dark arms ($F_{2,33}=6.704$, $p = 0.004$), with *R. d. chakae* spending significantly more time per entry in the clear arms of the plus maze than *R. d. dilectus*. There was no difference between *R. d. dilectus* and *R. d. chakae* for amount of time per entry in the dark arms of the plus maze (Figure 3.5).

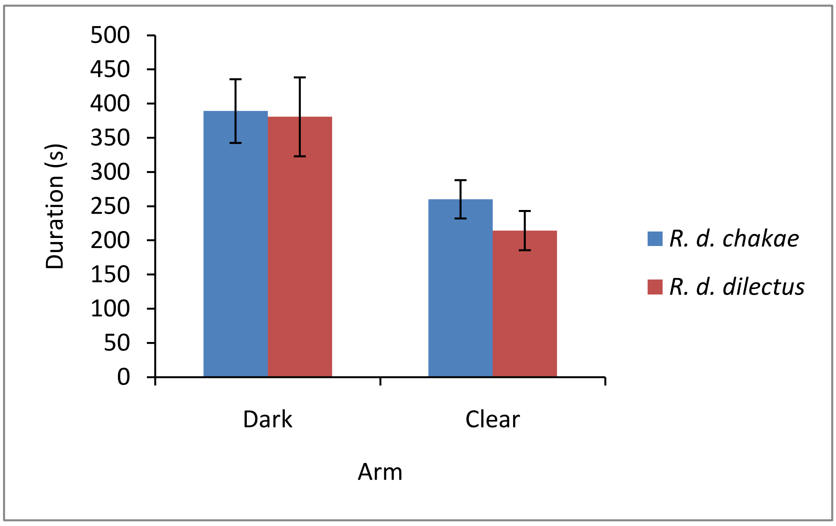

Figure 3.3. Mean duration (± SE) spent in the dark and clear arms of the plus maze by *R. d. chakae* and *R. d. dilectus.*

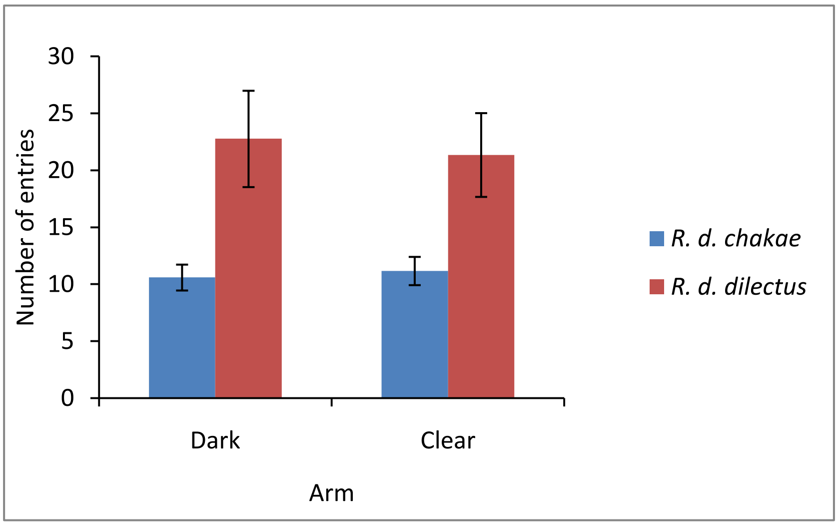

Figure 3.4. Mean (± SE) number of entries in the clear and dark arms of the plus maze by *R. d. chakae* and *R. d. dilectus*.

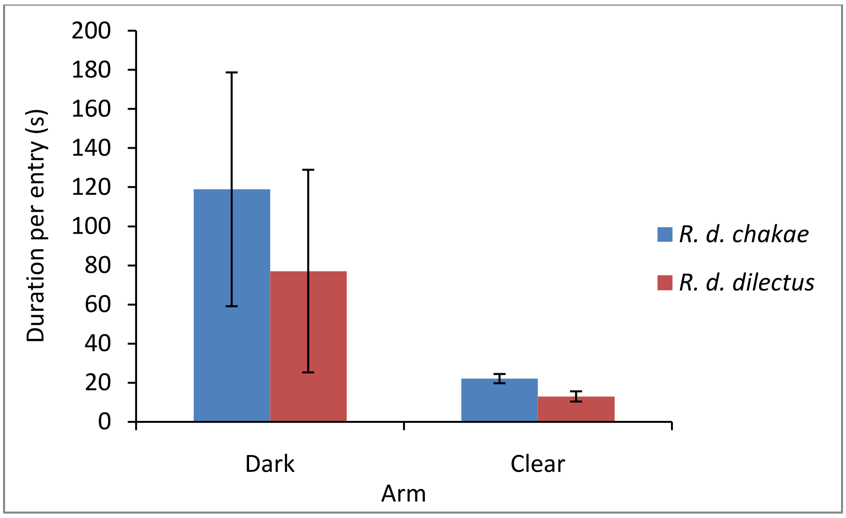

Figure 3.5. Mean (± SE) duration per entry in the clear and dark arms of the plus maze for *R. d. chakae* and *R. d. dilectus*.

Exploratory behaviour

The latency to enter the enclosed maze was not significantly influenced by the taxon, or the statistical interaction between taxon and sex, but sex and weight were significant predictors of the latency of an individual to enter the closed maze (Table 3.1). Females entered the maze significantly faster than males (Figure 3.6), and striped mice that were heavier took less time to enter the maze (Figure 3.7). *Rhabdomys dilectus chakae* striped mice weighed between 55 g and 105 g, and *R. d. dilectus* individuals weighed between 40 g and 96 g. Taxon, sex, sex x taxon, and weight were not significant predictors for the number of sensors that the test subjects crossed, the total distance that the subjects travelled, or the average speed of the subjects through the maze (Table 3.1, Table 3.2).

Table 3.1. Results of the GLZ analyses for taxon, sex, taxon x sex and weight (as a covariate) effects on the latency to enter the closed maze, the number of sensors, the total distance travelled, and the average speed through the maze. All statistics reported are Wald χ^2 statistics. β-coefficients (in bold) are shown for significant statistics.

	Latency to enter	Sensors crossed	Total distance travelled	Average speed
Taxon	χ^2_1=1.091, p = 0.296	χ^2_1=0.003, p = 0.955	χ^2_1=0.188, p = 0.665	χ^2_1=0.222, p = 0.637
Sex	**χ^2_1=6.455, p = 0.011; β = 32.439; p=0.011**	χ^2_1=0.112, p = 0.738	χ^2_1=0.089, p = 0.766	χ^2_1=0.114, p = 0.735
Taxon x sex	χ^2_1=1.073, p = 0.300	χ^2_1=0.002, p = 0.965	χ^2_1=1.372, p = 0.241	χ^2_1=0.620, p = 0.431
Weight	**χ^2_1=5.406, p = 0.020; β = -2.165; p=0.020**	χ^2_1=0.017, p = 0.896	χ^2_1=0.024, p = 0.877	χ^2_1=0.279, p = 0.597

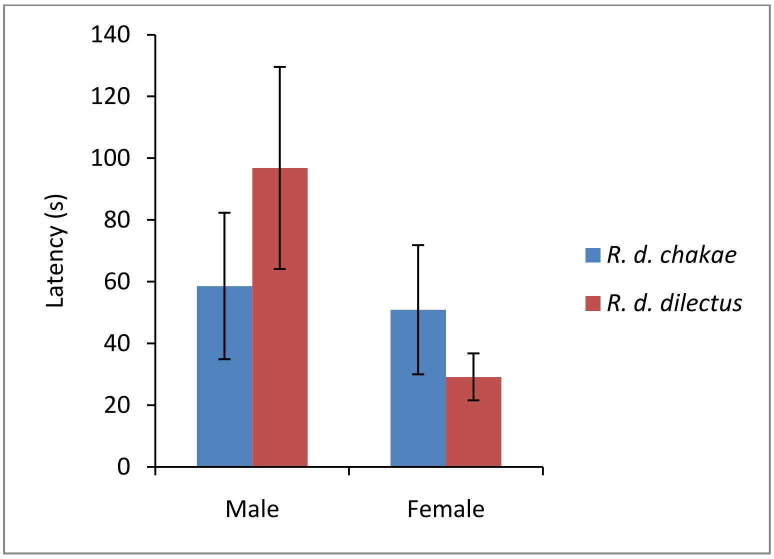

Figure 3.6. Mean (± SE) latency of males and female by *R. d. chakae* and *R. d. dilectus* to enter the closed maze.

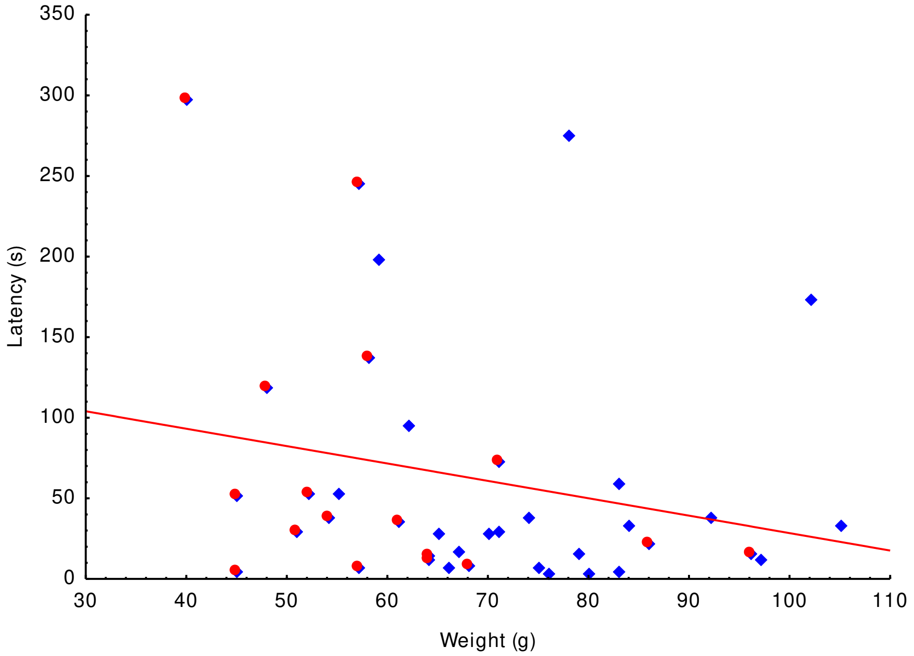

Figure 3.7. Scatterplot of the influence of weight on the latency to enter the closed maze by *R. d. chakae* (blue) and *R. d. dilectus* (red). The regression line: y=136.401-1.0796*x.

Table 3.2. Mean (± SE) sensors crossed, total distance and average speed in the exploratory maze for *R. d. chakae* and *R. d. dilectus*.

		Sensors crossed	Total distance (m)	Average speed (m/s)
R. d. chakae	Male	63.417 ± 7.309	29.85 ± 3.708	0.105 ± 0.014
	Female	65.5 ± 19.443	24.904 ± 9.666	0.0880 ± 0.0318
R. d. dilectus	Male	62.3 ± 15.876	19.029 ± 5.302	0.087 ± 0.017
	Female	67.286 ± 12.634	27.589 ± 4.847	0.099 ± 0.008

Discussion

Contrary to expectations, I found differences in the level of activity between *R. d. chakae* and *R. d. dilectus*, as well as differences in their anxiety responses in the plus maze. However, I found no differences in the intra-sub-specific activity profiles, or the intra-sub-specific exploratory behaviour, consistent with my predictions.

Rhabdomys dilectus chakae and *R. d. dilectus* had similar diel activity profiles, both with a diurnal activity profile, and weak peaks during the dark phase of the light-dark cycle, just before and just after the lights went on in the morning and off in the evenings respectively. This crepuscular activity is consistent with the findings of Perrin (1981) for *R. d. chakae*. Lima and Bednekoff (1999) maintain that animals should forage and devote periods of high activity (e.g. foraging) to times when the risk of predation is low. Crepuscular activity is associated with predator avoidance, since the level of illumination is low, and striped mice may escape detection from predators like jackals or diurnal raptors, which prey on striped mice (De Graaff, 1981). Mornings and evenings are also associated with colder temperatures, so predators which hunt below cover, such as snakes, may also be less active (e.g. Aleksiuk, 1976; DeNardo *et al.*, 2002).

The marked differences in the levels of activity of *R. d. chakae* and *R. d. dilectus* are not so easily explainable. Differences in the energy budgets may account for distinct activity patterns, since animals may mediate activity according to their energetic constraints (Biro and Stamps, 2010). Williams *et al*. (2007) showed that the activity levels of California sea lions *Zalophus californianus* are strongly dependent on metabolic rate and food intake. However, as I did not measure energy in any way, any conclusions drawn on the basis of metabolic constraints would be purely speculative. Food was also available *ad libitum* throughout the testing period, so that test subjects would not have suffered an energy deficiency throughout the 72 hours over which they were tested, and thus differences in activity levels due to energetic constraints seems unlikely. Another possibility is that the difference in the level of activity is in response to the testing arena itself. To facilitate video-taping, the tanks in which the activity study was undertaken had minimal cover, apart from a nest box and a handful of dry grass. Jensen *et al*. (2003) maintain that cover and habitat complexity is essential for house mice *Mus domesticus*, and that a lack of cover will cause a decrease in the level of activity. Similarly, the *R. d. dilectus* striped mice may be responding to decreased cover by decreasing their activity level compared to *R. d. chakae*.

If the lack of cover explains the comparatively low activity of *R. d. dilectus*, their behaviour in an enclosed maze should be similar between the sub-species, and the plus maze would reveal whether the activity of *R. d. dilectus* is a stress response in the open arena. In support of my expectation, the exploratory behaviour in a closed arena was not different between the sub-species. The maze tested the behaviour under cover for a shorter period of 20 minutes, and showed that the long distances and short latency of most test subjects to enter the maze suggests that both *R. d. chakae* and *R. d. dilectus* prefer areas that offer high amounts of cover. Interestingly, females entered the maze faster than males, and heavier individuals entered the maze faster than lighter individuals. While I cannot explain the quicker entry of heavier individuals, studies have revealed that female rodents have a shorter latency to leave their home cages, and spend more time in the novel chambers in choice-tests than males (reviewed in Corey, 1978). This may be because males have a heightened neophobic response, as occurs in deer mice *Peromyscus maniculatus* (Choleris *et al.*, 2000).

In contrast to the activity test (open environment) and the exploratory test (closed environment), the plus maze consisted of both dark and clear arms and could test the preference of the sub-species to cover. The modified plus maze that I used was a combination of two tests for anxiety: the elevated plus maze, and the light/dark test. The elevated plus maze is generally used to assess anxiety, whereby individuals that are more stressed usually spend more time in the arms with high walls (Martínez *et al.*, 2002). The light/dark box test is a test of anxiety, where anxious individuals spend more time in the dark compartment (Bourin and Hascoët, 2003). Thus, striped mice that were more anxious should spend more time in the dark arms of the plus maze, and should enter the dark arms more frequently than the clear arms. *Rhabdomys dilectus dilectus* entered both clear and dark arms significantly more frequently that *R. d. chakae*, which suggests that *R. d. dilectus* may experience exposure to an open environment as more stressful than *R. d. chakae*. For example, male Long-Evans hooded rats that were raised by mothers who were repeatedly exposed to predator odours (stressed), tended to be more anxious, and spent large amounts of time in contact with walls in an open field test (Mashoodh *et al.*, 2009). However, the time that *R. d. dilectus* spent per entry in the dark and clear arms showed that they spent a significantly shorter duration per entry in the clear arms than *R. d. chakae*, while there was no difference between the subspecies in the amount of time per entry into the dark arms of the plus maze. Thus, *R. d. dilectus* may minimise the amount of time spent exposed, which may explain the differences in the level of activity in a relatively open tank (activity profiles).

The low latencies of male striped mice to enter the covered maze should have been related to a stress response in the plus maze, but there was no sex difference in the anxiety response of the sexes. Therefore, the neophobic response of the males to the closed maze may be a response to the closed environment itself, since the plus maze had both clear and dark elements.

The results of the present study suggest that *R. d. chakae* might have a more flexible response to the level of cover present, while *R. d. dilectus* might have a less flexible response, and may not be able to respond to changing conditions. The anxiety response of *R. d. dilectus* in open areas may be caused by altered fore-brain structure and function (Berntson *et al.*, 1998; Schulkin *et al.*, 1998), or the stimulus of high levels of light may cause changes in the hormonal patterns, particularly glucocorticoids (Korte, 2001). If the anxiety was inherent (i.e. brain function may be different to that of *R. d. chakae*), *R. d. dilectus* should have shown anxiety-related behaviour in the closed maze as well as the plus maze. However, the lack of differences in the responses in the closed maze between *R. d. dilectus* and *R. d. chakae* suggest that the anxiety is related to open spaces.

In conclusion, the similarity in diel activity profile between the two sub-species and *R. d. chakae* in another study (Perrin, 1981) indicates that activity is conservative over a wide geographic range of the *R. dilectus* sub-species. It is possible that the crepuscular activity in striped mice in both studies may be a response to the open test arena, but activity needs to be studied in free-living *Rhabdomys* from the grasslands to confirm the findings made here. However, the differences in the levels of activity between the sub-species appear to be related to a stress response in *R. d. dilectus*. If so, the populations appear to have diverged in their response to an open habitat. Future studies should focus on whether the population differences are representative of the sub-specific differences, which would require testing of several populations of each sub-species.

Chapter 4: Social stress and dispersal

Introduction

Animal social systems range along a continuum (Banks, 1977) from solitary to group living, characterised by increasingly complex social interactions (de Waal and Tyack, 2003). Group-living confers particular benefits to animals, including reduced predation risk (Alexander, 1974), foraging benefits (e.g. Clode, 1993), increased breeding success with helpers (Jennions and Macdonald, 1994) and thermoregulatory advantages (Scantlebury *et al.*, 2006). However, living in a group has its disadvantages, such as competition for food (Schradin *et al.*, 2010), reproductive suppression (e.g. Dalerum *et al.*, 2006) and increased reproductive competition (Schradin *et al.*, 2010). Therefore, groups will form when the benefits of group-living outweigh the costs (Baker *et al.*, 1998; Lett *et al.*, 2004; Solomon and Crist, 2008). Group-living can be facultative or obligate, which is determined by evolutionary and ecological constraints such as fluctuating food resources which may lead to facultative group-living (Randall *et al.*, 2005) or high costs of reproducing, which could lead to obligate group-living (Emlen, 1994; Jennions and Macdonald, 1994).

An important component of group-living animals is delayed dispersal and natal philopatry which leads to group formation (reviewed in Koenig *et al.*, 1992; and Emlen, 1994). In mammals which form obligate social groups, dispersal is unlikely to occur due to the costs of dispersing (e.g. social mole rats; Faulkes and Bennett, 2001) whereas in facultative species dispersal is likely when the benefits of group-living no longer outweigh the costs (Randall *et al.*, 2005). In contrast to group-living species, dispersal in solitary mammal species usually occurs soon after weaning. Young can be tolerated for a time within the maternal territory but may be eventually evicted when the next generation of offspring are born or approach weaning. The timing and the social interactions surrounding dispersal can vary greatly between group-living and solitary mammals, but it is possible that dispersal is a stressor for the dispersing animal, regardless of its social system. The costs of attaining a new territory, combined with the risk of dispersal itself (Solomon, 2003) is universal to both solitary and social animals.

My study is concerned with understanding isolation anxiety (see below), potentially associated with dispersal in the striped mouse. The genus *Rhabdomys*, a diurnal murid rodent, inhabits large parts of southern Africa (Skinner and Chimimba, 2005). There are two species of *Rhabdomys: R. pumilio* inhabits the western, xeric parts of South Africa, while *R. dilectus*, comprising of two sub-species *R. d. dilectus* and *R. d. chakae*, occurs in the moist eastern grasslands (Schradin and Pillay, 2005b). The species differ in their social organisation. *Rhabdomys pumilio* lives in groups comprised of up to four breeding females and one breeding male, as well as their philopatric offspring of various ages (Schradin and Pillay, 2004). However, *R. pumilio* has been shown to switch between group and solitary living during periods when population density is low, suggesting that reproductive competition may favour solitary-living (Schradin *et al.*, 2010). Under low population density, adult females switch from group nesting to solitary breeding (Schradin *et al.*, in review). In response, adult males adopt one of three different reproductive tactics based on their age and mass: large dominant breeding males live with a group of females, displaying paternal care; roaming males are smaller and display a solitary habit, mating with solitary and group-living females but do not display paternal care; and younger, lighter philopatric males remain within a group, sometimes mate with neighbouring females and display alloparental care to young in their own group (Schradin *et al.*, 2009). In contrast, the grassland dwelling *R. dilectus* species is solitary, with males and females maintaining intrasexually exclusive territories (Schradin and Pillay, 2005b). In the grasslands, young are weaned at 16 days of age (Brooks, 1982) and dispersal is biphasic, in that young remain in the outer parts of their mother’s territory until the birth of the next litter (Willan, 1982).

The differences in the social systems of *R. pumilio* and *R. dilectus* provide an ideal opportunity to study the effects of separation from a family group in *Rhabdomys*. Specifically, my aim was to investigate whether juvenile males removed from a family group display a stress response in a novel environment in the group-living *R. pumilio* and two sub-species of the solitary *R. dilectus* (*R. d. dilectus* and *R. d. chakae*). Since male *R. pumilio* are philopatric post-weaning and exposure to a novel environment could be potentially stressful, I hypothesised that following weaning, a period of isolation in a novel environment would result in a greater stress response in the group-living *R. pumilio* than the two *R. dilectus* taxa. I recorded the behaviour of males in a plus maze (novel environment) and measured the levels of faecal corticosterone in males in the three taxa that were exposed to one of three treatments: i) males that were removed from their family groups and housed singly in another cage for a week before being tested in the plus maze and their faecal corticosterone measured a day after exposure to the maze (to control for the effects of a novel environment); ii) males separated with a barrier from their family group for a week before being tested in the maze (to control for the effects separation from the group); and iii) males were removed from their family group and immediately placed in plus maze and their faecal corticosterone measured twice: first, a day after exposure to the maze, and again, a week after their exposure to the maze. I made 4 predictions. 1) Males of group-living *R. pumilio* that are removed from their family groups and immediately isolated, would show greater avoidance of the light arms of the plus maze, and show greater levels of corticosterone after exposure to plus maze but lower levels a week after the experience. In contrast, males of the solitary *R. dilectus* sub-species would display more use of the open arms of the maze and have lower levels of corticosterone. 2) Sexually mature philopatric male *R. pumilio* have the highest corticosterone of males adopting the three different mating strategies in this species, indicating that living in a group may lead to anxiety for philopatric males (Schradin *et al.*, 2009). Although it is unknown whether juvenile males have comparatively high corticosterone levels, I nonetheless alternatively predicted that removal from a social group would not alter corticosterone levels if they display high levels of corticosterone already as a consequence of being in a social group, but I did expect that they would display stress behaviour in the plus maze. 3) For the males separated from their family group with a barrier, I expected the responses to be similar to that of the males that were removed from their family groups. 4) Males of all 3 taxa that were isolated from their groups for a week prior to tests would show a lesser degree of stress response in the plus maze.

Materials and methods

Subjects

Three *Rhabdomys* taxa from captive populations were used in this study, all occurring in South Africa. *Rhabdomys pumilio* originated from the arid succulent Karoo

(Goegap Nature Reserve, Northern Cape Province, S 29°41’56” E 18°1’60”), while both *R. d. chakae* (Suikerbosrand Nature Reserve near Johannesburg, 26°31'S, 28°18'E) and *R. d. dilectus* (Irene near Pretoria, S 25°54’10” E 28°12’09”) originated from grasslands in Gauteng Province. Each striped mouse taxon was housed in a separate room at the Milner Park animal unit, at University of the Witwatersrand, under partially controlled environmental conditions (with a standard 14 hr light, 10 hr dark cycle, temperature regulated between 22 and 24°C and 30 - 60% relative humidity). Breeding pairs per taxon were established with randomly chosen individuals and housed in galvanised steel tanks (465 x 310 mm x 350 mm high) with a clear Perspex front. Within each tank, bedding (wood shavings), a plastic nesting box (130 x 90 mm x 100 mm high) and a handful of dry grass was provided. Approximately 5 g of paper towel was provided twice weekly and one cardboard roll⁄ paper cup was provided weekly per tank for environmental enrichment. Subjects had access to water *ad libitum* and were fed approximately 5 g of mixed seed (sprinkled throughout the cage to stimulate foraging behaviour) and 10 g of fresh fruit/ vegetables daily per striped mouse.

Each pair was allowed to produce two litters. However, I used only those pairs that produced a second litter of 5-6 pups, with at least 3 male offspring (30 litters in total). Offspring from the first litter were weaned at 25 days of age and housed elsewhere.

Social anxiety: behavioural responses

At the birth of the second litter, two tanks similar to the breeding tank were attached on either side of the breeding tank with PVC pipes (± 300 mm long, 45 mm diameter). A wire mesh grid was inserted in the PVC pipes to prevent access to the adjoining tanks by the striped mice. At 25 days of age, three male pups from each litter were randomly assigned to one of three treatments: (1) Isolated – a male was housed alone in a tank identical to the breeding tank, under the same conditions as its parents and siblings. The tank with the solitary male was placed at the opposite side of the room. (2) Separated – a male was housed in the other adjoining tank. It was physically separated from its parents and siblings but remained in visual and olfactory contact with its parents and siblings through the wire mesh barrier. (3) Removed – a male stayed with its parents and remaining siblings and was later used in experiments (below). To enable identification of the housed male, all striped mice were uniquely marked with non-toxic hair dye (Inecto Rapid – Pinetown, South Africa; see Schradin and Pillay, 2003) prior to assignment to treatments. All individuals had access to food and water, as described above.

Ten days thereafter (at 35 days of age), the behaviour of test subjects was assessed in a modified plus maze. The maze comprised of two dark and two clear arms that were all enclosed (dimensions: 75 x 500 mm x 75 mm high) to prevent individuals jumping out of the maze during experiments. The maze had a central start box (110 x 110 mm x 155 mm high). A test subject was placed in the start box and its behaviour was video recorded for 10 minutes from the moment it first entered the maze. Since the aim of the study was to assess the anxiety response of the test subjects, I recorded the duration of time spent in, and frequency of visits to the clear arms only. Mazes were thoroughly cleaned with soap and water and air dried between trials. Test subjects were returned to their respective housing conditions immediately after trials.

Social anxiety: corticosterone levels

In order to measure the physiological stress response of test subjects, I used non-invasive faecal corticosterone sampling. Corticosterone levels are an accepted measure of hypothalamic-pituitary-adrenal (HPA) axis activity, with high levels reflecting increased stress (Touma *et al.*, 2003). Corticosterone is released into the blood in response to a stressful event, is then reabsorbed and later partially eliminated in the faeces. Faecal samples were collected approximately 24 hours after the anxiety response experiment (designated as Time 1), between 08:00 and 10:00, to minimise variation due to fluctuation in circadian corticosterone production (Touma *et al.*, 2003). In order to collect samples, the subjects were individually transferred from their tank into clean holding cages (150 x 420 mm x 150 mm high) where they remained until five faecal pellets had been collected or one hour had elapsed (following Jones *et al.*, in review). The subjects were then returned to their respective tanks. Faecal samples were again collected from group housed test subjects (Removed treatment) one week after they were returned to their social groups (designated as Time 7) in order to measure the effect of social group on modulating the stress response of the test subject. All faecal samples were frozen immediately after collection and later sent for analysis to Gordon Laboratories (Sedgefield, Stockton-on-Tees, UK). Each sample was dried, weighed, reconstituted with a small amount of water and homogenised. Corticosterone was extracted using a solvent and the resulting eluate was allowed to evaporate. The residue was resolved in a dilution buffer and the concentration measured using a commercially available ELISA (Enzyme-linking immunosorbent assay) kit.

Data analyses

I ran General Linear Models (GLM) using Statistica version 7.1 (Statsoft, 2007). Taxon and treatment were the fixed effects, litter identity and litter size were random effects (as the origin of the test subjects might have influenced their behaviour), corticosterone level was the covariate and the duration of time spent in, or the frequency of visits to the clear arms were the dependent variables. As corticosterone levels had a significant effect on the duration of time spent in the clear arms, I compared corticosterone levels between taxa and treatments using an Analysis of Variance (ANOVA). Finally, for the Removed treatment, I ran a Repeated Measures ANOVA to compare corticosterone levels between Time 1 and Time 7; at separation and after time spent with the social group.

Results

Behavioural responses

Taxon was a significant predictor of the duration of time spent in the clear arms of the plus maze. *Post hoc* tests showed that *R. pumilio* (group-living) individuals spent more time in the clear arms of the plus maze than *R. dilectus chakae* and *R. d. dilectus* individuals (both solitary) ($F_{2,18.22} = 9.05$, p=0.002; Figure 4.1). There was also a significant treatment effect for the duration of time spent in the clear arms, with subjects from the Removed and Separated treatments spending less time in the clear arms than individuals from the Isolated treatment ($F_{2,52.0} = 28.07$, p<0.001; Figure 4.1). The statistical interaction between taxon and treatment was a significant predictor of the duration of behaviour, with the group-living *R.*

pumilio individuals which were isolated from their family groups spending more time in the clear arms than individuals from any of the other taxa and treatments ($F_{4,52.0} = 15.08$, $P<0.001$; Figure 4.1). Interestingly, *R. pumilio* showed a significant graded response (i.e. Isolated>Separated>Removed) whereas there was no significant treatment effect for *R. d. chakae* and *R. d. dilectus* (Figure 4.1). The random factors included into the GLM analyses did not influence the duration of entry into the clear arms of the plus maze: litter identity ($F_{27,52.25} = 1.57$, $p=0.081$) and litter size ($F_{1,52.0} = 0.08$, $p=0.781$). Since the level of corticosterone was a significant covariate predictor of the responses of test subjects ($F_{1,52.0} = 23.22$, $p<0.001$), I ran a GLM analysis to compare corticosterone levels among the taxa and treatments. Taxon was a significant predictor of corticosterone levels ($F_{2,81} = 17.69$, $p<0.001$), where *R. pumilio* individuals had higher levels of corticosterone than *R. d. chakae* and *R. d. dilectus* individuals (Figure 4.2). Treatment was also a significant predictor of corticosterone levels ($F_{2,81} = 3.69$, $p=0.029$), with group housed (Removed) individuals showing the highest levels of the hormone compared to those in the Isolated and Separated treatments (Figure 4.2). Taxon x treatment had a significant effect on corticosterone levels ($F_{4,81} = 3.58$, $p=0.010$). The Removed and Separated treatment individuals for *R. pumilio* had the highest levels of corticosterone compared to all other taxon-treatments, and the *R. pumilio* Separated treatment had similar corticosterone levels to all treatments for *R. d. chakae* and *R. d. dilectus* (Figure 4.2).

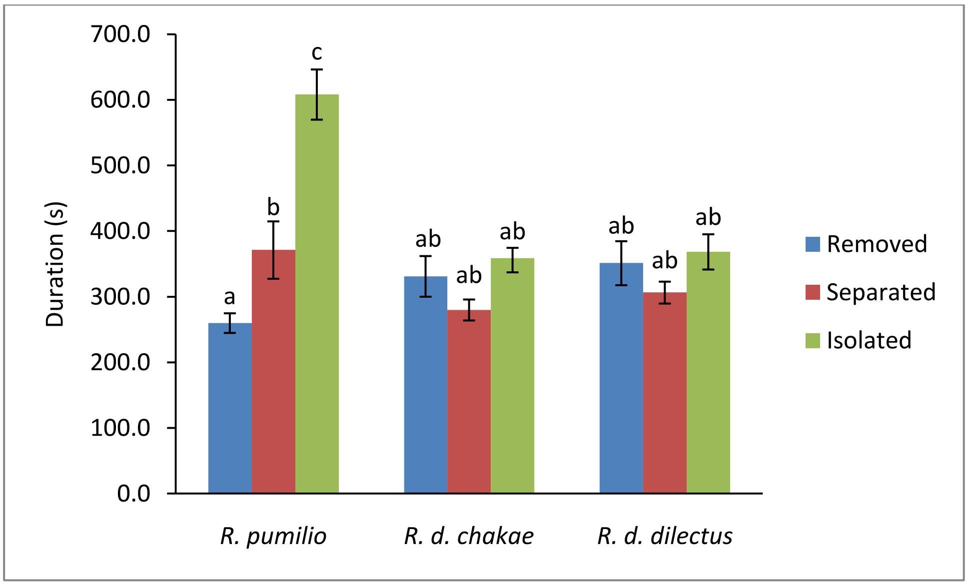

Figure 4.1. Mean (± SE) of the duration (s) spent in the clear arms of a modified plus maze. Bars with the same letters denote significant groups (post hoc tests).

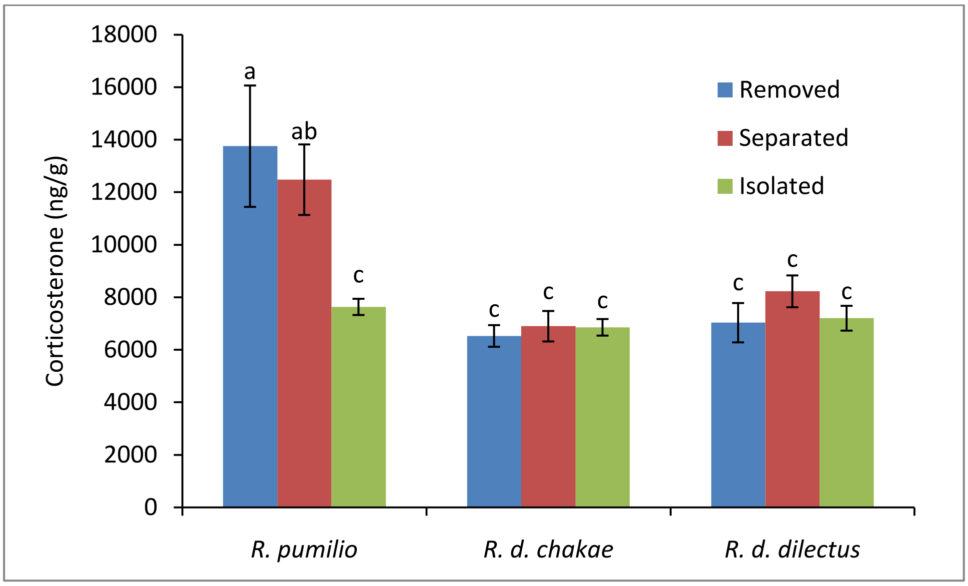

Figure 4.2. Mean (± SE) corticosterone levels, one day after tests in the modified plus maze. Bars with the same letters denote significant groups (post hoc tests).

R. pumilio individuals entered the clear arms of the plus maze more frequently than *R. d. chakae* and *R. d. dilectus* individuals ($F_{2,4.42}$ = 6.8, p=0.045; Figure 4.3). There was also a significant treatment effect ($F_{2,52.0}$ = 4.09, p=0.022). *Post hoc* analyses showed that Removed treatment subjects entered the clear arms significantly fewer times than Separated or Isolated treatment subjects (Figure 4.3). There was a significant statistical interaction between population and treatment ($F_{4,52.0}$ = 3.54, p=0.013), with all *R. d. chakae*, *R. d. dilectus* treatments and Removed (group housed) *R. pumilio* individuals entering the clear arms of the plus maze less frequently than Separated and Isolated treatment *R. pumilio* individuals (Figure 4.3). Neither the litter identity ($F_{27,32.4}$ = 1.56, p=0.114), litter size ($F_{1,52.0}$ = 2.97, p=0.091) nor corticosterone level ($F_{1,52.0}$ = 1.2, p = 0.279), significantly affected the number of clear-arm entries in the plus maze.

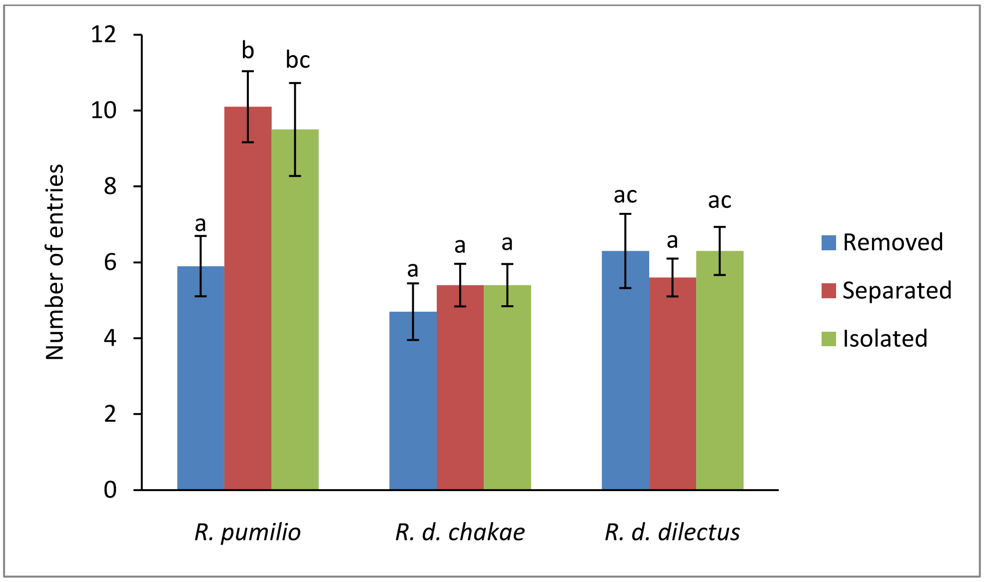

Figure 4.3. Mean (± SE) frequency of open arm entries. Letters denote significant groups (*post hoc* tests).

Corticosterone levels

I compared the corticosterone levels of test subjects in the Removed treatment when they were exposed to the plus maze (Time 1) and one week later when they had been in their family groups (Time 7). Taxon was not a significant predictor of corticosterone levels ($F_{2,27}$ = 2.42, p=0.108). However, test subjects showed elevated levels of corticosterone at Time 1 compared to Time 7 ($F_{1,27}$ = 15.19, p=0.001). There was also a significant effect of taxon x time ($F_{2,27}$ = 25.37, p<0.001). The important finding was the elevated corticosterone levels at Time 1 in *R. pumilio* compared to all other taxon-time combinations, which were grouped together. The difference in corticosterone between Time 1 and Time 7 was exaggerated in *R. pumilio* and negligible in *R. d. chakae* and *R. d. dilectus* (Figure 4.4).

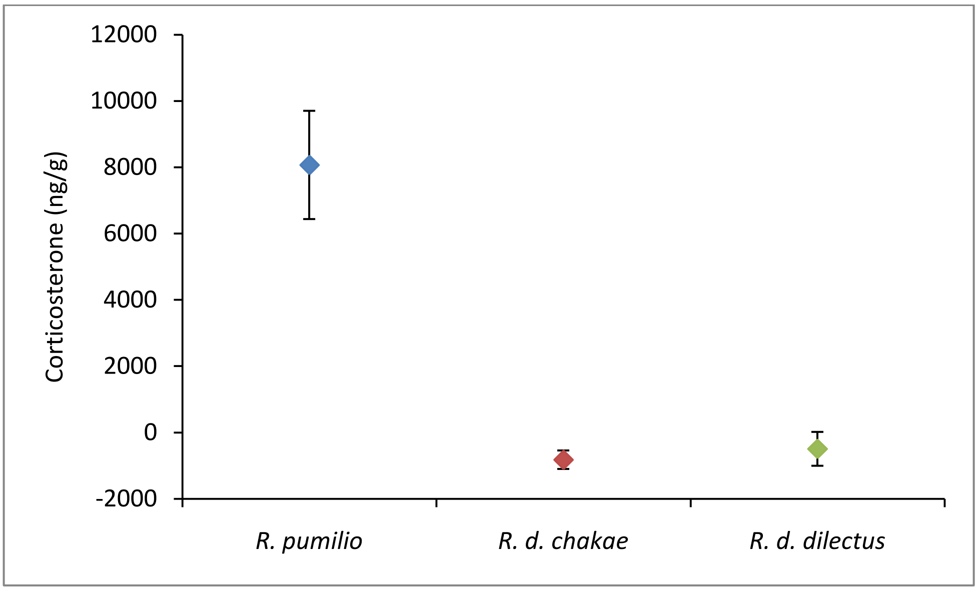

Figure 4.4. Mean (± SE) difference in corticosterone levels between Time 1 and Time 7 (Time 1-Time 7) in three *Rhabdomys* taxa.

Discussion

The aim of the present study was to establish whether young males removed from their family groups show a stress response in a novel environment in *Rhabdomys* species that display different social organisations in nature. Compared to males of the solitary-living *R. d. dilectus* and *R. d. chakae*, males of the group-living *R. pumilio* that were removed from their family groups (Removed treatment) showed a heightened anxiety response as evidenced by the minimal time in the clear arms of the plus maze, the fewer entries into the clear arms and elevated corticosterone levels. This finding supports my first prediction. However, there was no support for my second prediction that philopatric *R. pumilio* males are anxious since these males had lower corticosterone levels a week after exposure to the maze when they were living with the family group. In support of my third prediction, male *R. pumilio* that were separated from their family group with a barrier (Separated) had a similar response to Removed males, but there was no difference between Removed and Separated males in both *R. dilectus* sub-species. Finally, *R. pumilio* and *R. dilectus* males isolated from their family groups for 10 days prior to testing showed a much lower stress response in the plus maze and low levels of corticosterone which supports my fourth predictions.

I used two independent methods of establishing the stress response of the individuals: behaviour in a plus maze and corticosterone levels. The plus maze has been shown to assess the level of anxiety in a subject, where individuals that are more anxious tend to avoid open areas (Martínez *et al.*, 2002; Bourin and Hascoët, 2003). Corticosterone has been shown to be released from the cerebral cortex in response to a stressor (Charmandari *et al.*, 2005), and thus animals that are more anxious should have higher levels of corticosterone. The behaviour of test subjects in the plus maze concurred with the data obtained in the analysis of corticosterone. Concordance between behavioural and physiological measures of stress is well known in the literature (reviewed in Kim and Haller, 2007), but is not universal (see Creel *et al.*, 2009).

For *R. pumilio*, my study revealed contradictory outcomes. In the plus maze, males of *R. pumilio*, a group-living species, spent more time in, and entered the clear arms of the plus maze more frequently when they were isolated from their family group for 10 days. Yet, when males were separated from the family group and immediately placed in the plus maze, they avoided the open arms. This paradox was not apparent in *R. d. chakae* and *R. d. dilectus*. These apparently contradictory results can be explained by the biology of the species. Rymer *et al*. (2008) found that *R. pumilio* tends to explore open areas more readily than *R. dilectus*, which may be related to the habitats occupied by the two species. Both *R. dilectus* sub-species live in the grassland, a naturally dense environment with high levels of over-head cover (Schradin and Pillay, 2005b), and thus may naturally avoid open areas. In support, both individuals of both sub-species showed high levels of activity in an enclosed maze (Chapter 3, page 15). *Rhabdomys pumilio* on the other hand, lives in a much more open habitat (Schradin, 2005), and appears to cope with open spaces without apparent stress (M. Jones, pers. com). Thus in general, it is expected that *R. pumilio* has a lower stress response in the plus maze compared to *R. dilectus* individuals.

The corticosterone results showed a clear stress response of *R. pumilio* after exposure to the novel environment in the Removed and Separated treatments but not in the other taxa-treatment combinations. Interestingly, males of both *R. dilectus* sub-species in all three treatments did not use the clear arms of the maze as much as *R. pumilio* in the Isolated treatment, indicating that the *R. dilectus* sub-species mostly occupied the dark arms. Moreover, males of the sub-species did not have elevated levels of corticosterone compared to *R. pumilio* (Removed and Separated treatments). These findings indicate that the dark arms provided an opportunity for *R. dilectus* to avoid exposure in an open area of the plus maze and potential stress. If one follows this idea to its logical conclusion, the stress response of *R. pumilio* in the Removed and Separated treatments, in which they avoided the clear arms of the maze and had higher corticosterone levels, indicates that they were most likely stressed in a novel environment (i.e. a neophobic response) even when they had a choice between dark and clear arms.

Upon return to the family group, the two solitary subspecies, *R. d. chakae* and *R. d. dilectus* showed a negligible change in corticosterone, which could be due to the comparatively low level of stress experienced in the plus maze, but also points towards a negligible level of stress experienced during the period leading to dispersal. In contrast, the group-living *R. pumilio* had a large reduction in corticosterone when they time spent with the family group following their experience in the plus maze.

Groups form because the benefits of remaining philopatric outweigh the costs of dispersing (Koenig *et al.*, 1992; Solomon, 2003). The costs of dispersing include limited availability of food and territories (Emlen, 1982) and the risk of predation (McGuire *et al.*, 2002; Solomon, 2003). Thus in a social species, being removed from the group is a stressful experience, because the animal faces a novel environment alone (Morgan and Tromborg, 2007). In solitary species, while dispersal may be a stressful event, the benefits of dispersing outweigh the costs (Ostfeld, 1985), and thus may be less stressful.

In conclusion, in social species, the levels of stress are high in animals that disperse compared to naturally solitary species. I artificially imposed separation of test subjects from their families, which may itself cause high levels of stress. However, the differences in the stress associated with immediate isolation between the solitary *R. dilectus* sub-species and the social *R. pumilio* suggests that the dispersal is a natural occurrence in juvenile male *R. dilectus* but philopatry is a natural occurrence in juvenile male *R. pumilio*. Nevertheless, after a period of separation, *R. pumilio* from a family group does not show an apparent stress response in a novel environment, as revealed by the Isolated treatment in the present study and in another study on social learning (Rymer *et al.*, 2008). Finally, the similarity in the responses of the two sub-species suggests that the social system concerning juvenile dispersal (Willan, 1982) is similar in *R. d. dilectus* and *R. d. chakae*.

Chapter 5: Social behaviour

Introduction

The social interactions between individuals determine the social organisation of a species (e.g. Karelina *et al.*, 2010). Solitary animals display high levels of intra-specific aggression and/or mutual avoidance (i.e. less tolerance of conspecifics) and territoriality (e.g. Yahner, 1978; Pillay *et al.*, 1995), whereas group-living species are primarily amicable, which promotes mutual attraction and tolerance of conspecifics (Taraborelli and Moreno, 2009). For example, the northern hopping-mouse *Notomys alexis* engages in attracting behaviour between multiple individuals of both sexes, resulting in the formation of groups, whereas the brown desert mouse *Pseudomys desertor* is solitary and all adults are strongly repelled by each other, apart from male and female pairs during mating (Happold, 1976). Nonetheless, group-living social systems can be either despotic or egalitarian (Vehrencamp, 1983), depending on the level of aggression and amicability (Hemelrijk, 1999), with egalitarianism being characterised by high levels of amicability (e.g. samango monkeys, *Cercopithecus mitis erythrarchus*; Payne *et al.*, 2003), and despotism characterised by both aggression and amicability, with high levels of within-group competition (e.g. chacma baboons, *Papio cynocephalus ursinus*; reviewed in Payne *et al.*, 2003). Moreover, since social organisation ranges along a continuum from solitary to social (Banks, 1977), it is expected that the comparative levels of amicability and aggression also vary along a continuum.

Social systems are constrained by intrinsic (i.e. mating and rearing strategies) and extrinsic (i.e. predation, refugia and resource abundance and distribution) factors (Crook *et al.*, 1976). Of the extrinsic factors, the distribution and abundance of resources is a primary determinant of social systems (Carr and Macdonald, 1986; Johnson *et al.*, 2002; Akre *et al.*, 2010). When resources, especially those of high quality, are patchily distributed, the size of a group that is able to exploit the resource is dependent on the extent of the patch (Symington, 1990), such that group-living occurs when there are large areas of high quality resources (Johnson *et al.*, 2002; Campbell *et al.*, 2006) and solitary living occurs when the resource quality may be too poor to support more than one animal, or the resources are randomly spaced (Kleiman, 1972; Johnson *et al.*, 2002).

The striped mouse genus *Rhabdomys* inhabits a wide variety of habitats, from the arid succulent Karoo (*R. pumilio*), to the mesic grasslands of South Africa (*R. dilectus*; Schradin and Pillay, 2005b). *Rhabdomys pumilio* forms egalitarian groups, comprising of up to 30 adults: 2-4 breeding females, a breeding male and their philopatric offspring (Schradin and Pillay, 2004) and groups defend their territories against other groups through aggression (Schradin, 2004). In contrast, *R. dilectus* is solitary, with males and females occupying intra-sexually non-overlapping territories (Schradin and Pillay, 2005b), and offspring leave the maternal territory soon after weaning (Willan, 1982). The main ecological determinants of the social organisation of the two species appear to be population density, the availability of nesting sites and food abundance (Schradin, 2005; Schradin and Pillay, 2005b). For the group-living *R. pumilio*, a high population density, large quantities of protein-rich food in small patches and limited nest sites constrain dispersal and promotes group-living, although huddling and communal sun-basking are additional advantages for *R. pumilio* in arid habitats (Schradin *et al.*, 2007; Scantlebury *et al.*, 2010). In contrast, *R. dilectus* occurs at low population density because protein-rich food is sparsely distributed and juvenile mortality is high, which together with the abundance of suitable nest sites, contributes to a dispersed social structure and solitary living (reviewed in Schradin, 2005). The grassland localities occupied by *R. dilectus* are relatively homogenous, such that the social system of both sub-species (*R. d. dilectus* and *R. d. chakae*) may be similar. In support, studies by Brooks (1974) on *R. d. dilectus* and Willan (1982) on *R. d. chakae* (both sub-species previously recognised as *R. pumilio*) indicate that the sub-species have similar social systems. However, comparative social behaviour for the two sub-species is not available. The aim of this study is to investigate the intra-taxon, same-sex social behaviour of *R. d. chakae* and *R. d. dilectus*. Since social behaviour reflects social organisation, I expected that the intra-sub-specific social behaviour of the taxa would be similar, characterised by high levels of aggression and a lack of intra-sexual tolerance.

Materials and methods

I trapped 23 (13 male and 10 female) *R. d. chakae* (2N=48) at Walkers Fruit Farms (S 26°29'30'' E 27°57'59''), and 17 (10 male and 17 female) *R. d. dilectus* (2N=46) at Irene (S 25°54' E 28°12'), Rietvlei dam (S 25°54'4'' E 28°16'38''), and Vaal River (S 26°55'58" E 26°41'38"). I used randomly placed plastic live-traps (290 x 60 x 80 mm) to capture striped mice. Trapping was done between April 2009 and June 2010. Traps were baited with a mixture of oats, sunflower seeds, salt, peanut butter and sunflower oil, and checked once daily. Traps contained cotton wool and were well covered with vegetation for insulation. Once animals were caught, they were transferred to plastic holding cages and transported by car to the University of the Witwatersrand. All *R. d.* chakae individuals had been housed in captivity for at least 12 months prior to testing, and *R. d.* dilectus individuals had been in housed captivity for between 2 and 9 months. All striped mice were adult at the time of testing.

Subjects were housed singly in Lab-o-tec ™ cages (150 x 420 mm x 150 mm high) with a standard 14 hr light, 10 hr dark cycle, temperature regulated between 22 and 24°C and 30 - 60% relative humidity, in the Milner Park animal unit, at the University of the Witwatersrand. Epol® mouse cubes and water were provided *ad libitum*, supplemented weekly with fresh fruit or vegetables and sunflower seeds. Cages were furnished with a plastic nesting box (130 x 90 mm x 100 mm high), a handful of dry grass, and a bedding of wood shavings. Cardboard toilet rolls and a handful of nesting material were provided bi-weekly for enrichment. Cages were cleaned every two weeks, and the cage contents replaced.

I assessed the social interactions of 34 dyads of same-sex consub-specific striped mice. I weighed all individuals prior to pairing to the nearest 1 g. I pseudo-randomly established dyads by assigning a letter to each individual and drawing pairs. Striped mice were only paired when the difference in the body masses was within 20% (to reduce the influence of body mass on social behaviour during encounters; see Verbeek *et al.*, 1996). To ensure that dyads were unrelated, dyads were established with individuals trapped at least 50 m away from each other or from different localities (*R. d. dilectus*). Individuals were used in a maximum of two dyads, and the minimum interval between testing for an individual was two days. All subjects were individually marked with non-toxic dye (Inecto Rapid – Pinetown, South Africa; see Schradin and Pillay, 2003) one week prior to testing.

I studied 20 *R. d. chakae* dyads (11 male-male; 9 female-female) and 14 *R. d. dilectus* dyads (8 male-male; 6 female-female). Dyadic encounters took place in large, rectangular glass tanks (300 x 450 mm, 320 mm high). The floor of the tank was covered with a layer of wood shavings and had three sides covered with white paper to reduce the amount of external light during video-recording. Tanks were cleaned with soapy water between tests.

Testing took place between 06h00 and 09h00, the peak activity time of striped mice (Pillay *et al.*, 2006). Prior to testing, the tank was divided in half by a cardboard barrier, secured in the corners of the tank, and test subjects were placed on either side. The barrier remained in place for 5 minutes to allow individuals to become accustomed to the novel environment. Thereafter, the barrier was removed and the interactions of the dyad was recorded for 10 minutes using a Sony Handycam (model: DCR – SR62E) connected to a Divis DVR security recording system. I remained in the room to watch for aggression between subjects, but my presence did not appear to affect the behaviour of the animals. An encounter was terminated whenever heightened aggression compromised the welfare of the animals, and the pair removed from further analyses; this occurred in 1 male *R. d. chakae* dyad of 35 dyads in total. I recorded the duration and frequency of the behaviour of dyads using the categories described in Table 5.1 and the data were combined for the dyad. Test subjects were returned to their separate home-cages at the end of the test. I did not consider oestrus in females, since oestrus stage does not influence female-female interactions (Pillay, 2000). Experiments were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance number 98/26/2A).

Data analyses

The data did not meet the assumptions of normality (Shapiro-Wilk test). Therefore, I analysed the frequency and duration of each behaviour per taxon using the Generalized Linear Model (GLZ) in Statistica v.8 (Statsoft, 2007). I ran separate GLZs (multinomial distribution and logit link function) for the duration and frequency of behaviour, which were the response variables; behaviour (Table 5.1) was the dependent (multivariate) variable and sex and taxon were the categorical predictors. For each individual, frequency was assessed as a proportion of the total frequency of all behaviours combined. The incidence of mounting was rare, so I only assessed the frequency with which it was performed. β estimates were used to analyse which behaviours had significant first and second order effects. The model level significance was set at 0.05 and all tests were two-tailed.

Table 5.1. Definitions of the behaviours scored during dyadic encounters.

Behaviour categories	Descriptions
Sniff	One or both animals sniffing each other naso-nasally
Follow	One individual following the other at low speed
Amicable	Sitting in bodily contact, and/or allo-grooming
Aggressive	Tail-rattling, chasing at high speed, biting and/or aggressive vocalising
Mount	One individual attempting to mount the other
Other	Behaviours which did not fit into the categories above, including non social behaviour

Results

Taxon was a significant predictor for the duration of behaviour (Table 5.2). Sniffing and following were the only two behaviours that differed between taxa (Figure 5.1): *R. d. chakae* individuals spent less time sniffing and more time following than *R. d. dilectus* individuals (Figure 5.1). Sex was also a significant predictor for the duration of all behaviour, apart from sniffing (Table 5.2). Male dyads spent more time being aggressive and less time following one another compared to female dyads, but spent similar amounts of time doing all other behaviours (Figure 5.1). The interaction between taxon and sex was a significant predictor of the duration of sniffing, amicable and aggressive behaviour, and following (Table 5.2). *Rhabdomys dilectus chakae* males spent the least amount of time sniffing compared to other taxa-dyad combinations, spent less time engaged in other (non-social) behaviour and were more amicable than *R. d. dilectus* males (Figure 5.1). *Rhabdomys dilectus dilectus* females were more amicable than *R. d. dilectus* males and *R. d. chakae* females (Figure 5.1).

Regardless of taxon, males were more aggressive than females (Figure 5.1). Male *R. d. dilectus* spent less time following than *R. d. chakae* individuals of either sex, while *R. d. chakae* females spent more time following than all other taxa-dyad combinations (Figure 5.1).

Taxon, sex, and taxon x sex did not have a significant influence on the frequency of behaviours (Table 5.2, Figure 5.2).

Table 5.2.Results of the GLZ analyses for taxon, sex and taxon x sex effects on the durations and frequencies of behaviour recorded in male-male and female-female dyads in 2 *Rhabdomys* taxa.

Parameter	Wald statistic	Sniff	Amicable	Aggressive	Follow	Other
Duration						
Taxon	**χ^2_4 = 502.23; p<0.001**	**β = -1.03; p<0.001**	**β = -0.53; p<0.001**	**β = -0.49; p<0.001**	**β = 0.47; p<0.001**	**β = -0.61; p<0.001**
Sex	**χ^2_4 = 611.52; p<0.001**	β = 0.03; p=0.51	**β = 0.08; p<0.01**	**β = 1.54; p<0.001**	**β = -1.52; p<0.001**	**β = 0.03; p<0.001**
Taxon x sex	**χ^2_4 = 969.04; p<0.001**	**β = -0.13; p<0.01**	**β = 0.43; p<0.01**	**β = -0.49; p<0.001**	**β = 0.40; p<0.001**	β = -0.04; p=0.17
Frequency						
Taxon	χ^2_5 = 1.76; p = 0.88					
Sex	χ^2_5 = 1.44; p = 0.92					
Taxon x sex	χ^2_5 = 0.66; p = 0.99					

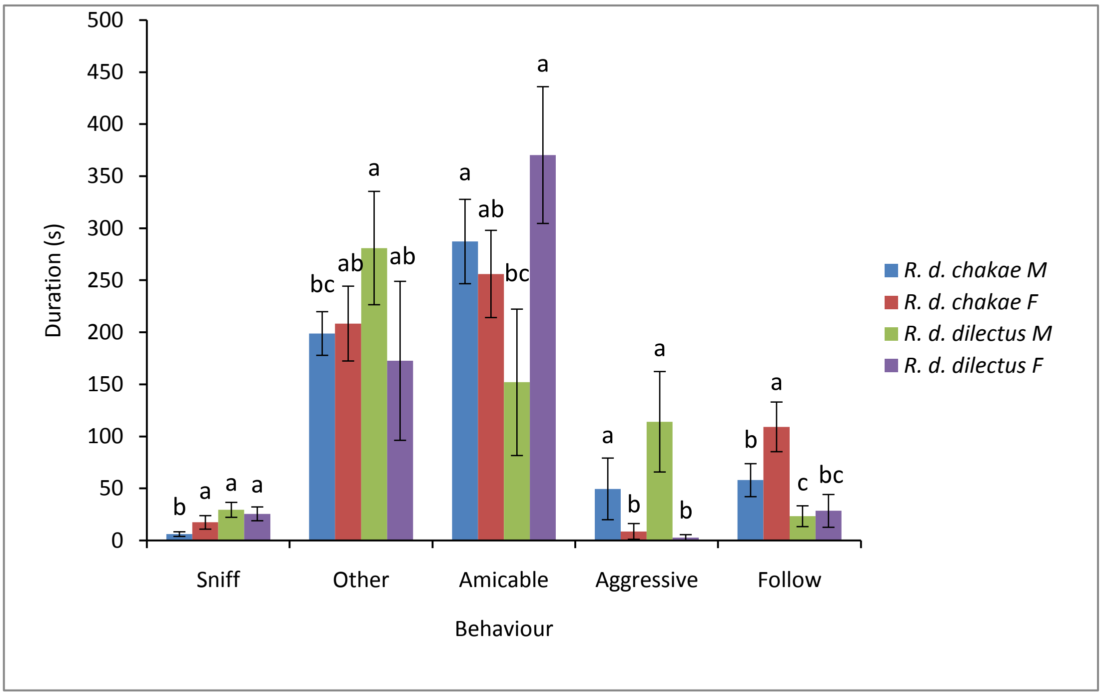

Figure 5.1.Duration of time (mean ± SE) of the behaviour during the male-male and female-female dyads in 2 *Rhabdomys* taxa. Letters denote non-significant differences for each behaviour.

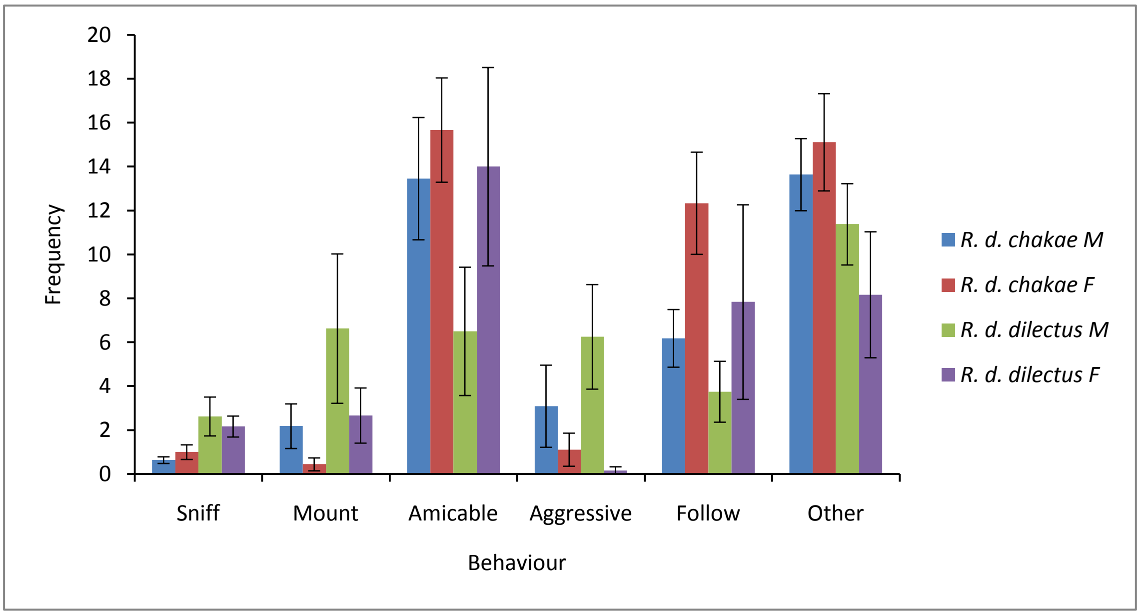

Figure 5.2. Frequency (mean ± SE) of the behaviour during the male-male and female-female dyads in 2 *Rhabdomys* taxa.

Discussion

My study reveals two main patterns. Firstly, contrary to expectations, dyads were characterised by higher levels of amicability and tolerance of conspecifics than I predicted. Secondly, and again contrary to expectations, there were subtle differences in social behaviour between the two sub-species.

I expected high levels of aggressive behaviour in both *R. d. chakae* and *R. d. dilectus*, but I found that both taxa exhibited high levels of amicable behaviour instead. Agonistic behaviour is important for maintaining territories in solitary animals (Madison, 1980; Ostfeld, 1985), and thus territorial species are expected to be highly aggressive toward conspecifics (Pillay *et al.*, 1995). Perrin *et al*. (2001) found that wild-caught grassland *Rhabdomys* showed high levels of aggressive behaviour in dyads that were apparently tested on the day of capture, but this could simply be a stress response to capture. In contrast, Willan (1982) found that striped mice that had been kept under laboratory conditions for a number of months displayed high levels of amicable behaviour similar to what I observed with subjects housed for 2 to 12 months in captivity. Willan (1982) suggested that the amicability is an artefact of the laboratory conditions under which the striped mice used in his study were housed (separate cages in close proximity in one room). This is suggestive of the ‘dear enemy’ phenomenon which explains the reduction in aggression between territorial neighbours, either because it is energetically expensive to fight, or because familiarity with neighbours can reduce the probability of a damaging fight (Ydenberg *et al.*, 1988; Temeles, 1994). Temeles (1994) argued that the ‘dear enemy’ phenomenon should be strongest when individuals are defending multi-purpose territories. Since I tested animals in a neutral arena, I had no method of assessing whether the elevated levels of amicable behaviour can be attributed to the ‘dear enemy’ phenomenon.

In contrast to Willan’s (1982) conclusion, territorial vlei rats (*Otomys irroratus*; Pillay *et al.*, 1995) and blind mole-rats (*Spalax ehrenbergi*; Zuri *et al.*, 1998) were always aggressive to conspecifics despite spending many months in captivity, indicating that other factors may account for the amicable behaviour in striped mice than prior familiarity in captivity. One possible explanation for the higher amicable behaviour is social flexibility, for which striped mice are notorious (e.g. see Kinahan and Pillay, 2008; Schradin *et al.*, 2009).

Group-living *R. pumilio* can switch from group-living to solitary living depending on prevailing ecological and social circumstances (Schradin *et al.*, 2010). Since *R. pumilio* represents the ancestral form of the genus (Rambau *et al.*, 2003), it is possible that *R. dilectus* has become secondarily solitary, and may have retained some ancestral characteristics, such as amicability during social interactions under particular circumstances. Ecologically, mutual avoidance in *R. dilectus* is apparently related to environmental constraints of protein rich food on the one hand and an abundance of nest sites on the other hand (Schradin, 2005). Ecological factors could have either provided the selection pressures in the past that committed *R. dilectus* to territoriality (i.e. phylogeny) or caused changes in behavioural responses under specific circumstances (i.e. environment). In other words, although *R. dilectus* is territorial in nature, this may be because of mutual avoidance rather than aggression. Thus, when they meet under captive conditions without limiting resources, they may be more amicable. In support, Dalerum *et al*. (2006) found that in the absence of ecological constraints, naturally solitary wolverines (*Gulo gulo*) will adopt a group-living strategy, with increased socio-positive behaviour in captive environments.

At the taxon-level, I found differences in the duration of sniffing and following behaviour, which is usually associated with mating behaviour in rodents (see Fleming *et al.*, 1981). Therefore, there are subtle differences in the social behaviours between the sub-species, which are possibly unrelated to territoriality. The sexes differed in the level of aggression and following behaviour, with males being more aggressive and spending less time following than females. Male *Rhabdomys* have been found to be more aggressive than females in other studies, and this has been attributed to kin-selection by females (Perrin *et al.*, 2001).

The differences in the social behaviour between the two sub-species of *R. dilectus* are not easily explainable. On the one hand, the taxa occupy slightly different habitats (e.g. differences in cover; Chapter 2) and it is tempting to suggest that differences in habitats selected for differences in social behaviour. On the other hand, high levels of amicability could be due to an ancestral character shared with *R. pumilio* and any differences in social behaviour (i.e. following and sniffing behaviour) could simply be due to divergence in the allopatric sub-species. An example to illustrate this point: all vlei rats (*Otomys* sp.) use nipple-clinging behaviour to retrieve and transport offspring, but one species, the bush

Karoo rat *O. unisulcatus* also retrieve pups by mouth-carrying behaviour, even though they have the necessary adaptations for nipple-clinging behaviour (i.e. females have inguinal nipples and young are born with notched incisors), indicating an alteration of the ancestral behaviour in arid habitats where mouth-carrying could be advantageous (Pillay *et al.*, 1993).

In conclusion, I found higher levels of amicable behaviour than I expected, which was possibly due to phylogenetic effects, while the slight differences in the social behaviour could be due to undetected selection pressures, stochastic processes such as genetic drift or non-genetic differences. The tolerance between unfamiliar conspecifics could be an ancestral characteristic shared with *R. pumilio*, particularly for females. Male-male dyads were more aggressive than female-female dyads but males also displayed more amicability than is expected in typically solitary rodents (e.g. vlei rats *Otomys irroratus*; Pillay *et al.*, 1995), and even in *R. pumilio,* unfamiliar males are aggressive (Schradin, 2004). Such ambiguities in the apparent tolerance for unrelated males require further testing.

Chapter 6: General discussion and conclusion

Behaviour is shaped by the phylogenetic history of a species as well as evolutionary selective forces, often as consequence of the environment (Chapman and Rothman, 2009). My study is concerned with assessing how the twin forces of phylogeny and the environment influence the behaviour of two sub-species of *Rhabdomys dilectus* (*R. d. chakae* and *R. d. dilectus*) on the Highveld region of South Africa. The distance between the localities from where study specimens were obtained is approximately 70 km and the localities occur within the grassland biome. Specifically, the objectives of my study were to obtain an impression of the broad vegetation profile of the localities from where the majority of the specimens were collected, and assess maintenance (non-social) and social behaviour of the sub-species, which would provide insight into the evolutionary divergence between them.

Table 6.1 provides a comparison of the behaviour and vegetation characteristics of the localities for each sub-species. Generally, the behaviour of the sub-species was similar, but *R. d. dilectus* had a greater stress response in open environments, and both *R. d. chakae* and *R. d. dilectus* differed slightly in some of the components of their social behaviour which are usually associated with mating (Table 6.1). I also found differences in the aerial cover, cover density, dead material and maximum heights between the localities where I trapped *R. d. chakae* and *R. d. dilectus* (Table 6.1). In particular, the locality occupied by *R. d. dilectus* (Irene) was characterised by greater levels of cover than the locality occupied by *R. d. chakae* (Walkers Fruit Farms; Table 6.1).

I studied three maintenance behaviours (activity patterns, exploratory behaviour, and anxiety) that are fundamental to the biology of most small rodents (Champagne *et al.*, 2010), since these behaviours would influence the fitness, and variation in these behavioural phenotypes is likely to be under strong selection pressure from the environment (Clément *et al.*, 2002; Pépin *et al.*, 2006; Kazlauckas *et al.*, 2011). The sub-species generally displayed a genus-specific activity profile (i.e. diurnal; Schradin, 2006), and interestingly also displayed a species-specific profile (crepuscular - *R. d. chakae*; Perrin, 1981). Such conservatism is expected since major morphological, physiological and behavioural adaptations often accompany the specialisation in nocturnal and diurnal ways of life, so that diel activity patterns are usually phylogenetically constrained in rodents (Roll *et al.*, 2006). The similarities of intra-sub-specific behaviour in the enclosed maze, but not the plus maze, and the reduced levels of activity in the open arena indicate that *R. d. dilectus* displays a stress response that does not match with a common phylogenetic signal between the sub-species. Divergence in anxiety is expected if animals are exposed to different environments (Jones *et al.*, in review), which is considered below.

Table 6.1. Summary of comparative differences in maintenance and social behaviour of *R. d. chakae* and *R. d. dilectus* and differences in the vegetation from representative localities of the sub-species. The symbols ↓ and ↑ denote higher and lower levels of behaviour respectively, and ≈ denotes a similar level of behaviour.

	R. d. chakae	*R. d. dilectus*
Maintenance behaviour		
Activity patterns (open tank)	≈ patterns	≈ patterns
	↑ level	↓level
Exploratory behaviour (closed maze)	≈	≈
Anxiety	↓	↑
Social behaviour		
Dispersal stress	≈	≈
Social tolerance	↑ intra-sex tolerance	↑ intra-sex tolerance
	↑following	↑sniffing
Environment		
Aerial cover	↓	↑
Cover density	↑	↓
Dead material	↑	↓
Maximum height	↓	↑

The social behaviour of a species usually reflects its social system (Lott, 1991; Pillay, 1993). Since *R. dilectus* is solitary across its distributional range (Brooks, 1974; Willan, 1982; Schradin and Pillay, 2005b), I expected that (i) dispersal in the two sub-species would occur soon after weaning, such that males removed from their family groups would be comparatively less stressed, and (ii) social interactions would be characterised by mutual avoidance, high aggression and lower levels of intra-specific tolerance, as occurs in some other rodents (*Otomys irroratus* - Pillay *et al.*, 1995; *Spalax ehrenbergi* - Zuri *et al.*, 1998). I studied male removal to understand the events, especially stress, associated with potential dispersal and I also studied social interactions between same-sex dyads to assess whether social behaviour indeed reflects the territorial social system of both sub-species. Firstly, the events surrounding dispersal of *R. d. chakae* and *R. d. dilectus* appear to be driven by similar mechanisms, since juvenile males had comparatively low levels of stress when they were removed from their family group. In contrast, *R. pumilio* juvenile males experienced high levels of stress upon separation from their family group. Thus, dispersal behaviour appears to be phylogenetically constrained within the grassland *R. dilectus*. Secondly, although the social behaviour did not vary much between the sub-species, the high level of amicable behaviour in same-sex dyads was unexpected. High levels of amicability occur in group-living species (Taraborelli and Moreno, 2009) and notably, in the facultatively group-living *R. pumilio*. However, *R. pumilio* can switch from group-living to solitary living depending on the prevailing ecological and social conditions (Schradin *et al.*, 2010), and appear to have flexible tolerance levels, depending on whether they are group-living or solitary living (Schradin *et al.*, in review). Therefore, my results indicate a mismatch between social behaviour and social organisation. However, it is also possible that during opportune conditions, such as high resource abundance, both *R. d. chakae* and *R. d. dilectus* could similarly show tolerance to conspecifics. *Rhabdomys pumilio* is apparently closely related to the *Rhabdomys* ancestor (Rambau *et al.*, 2003), suggesting that tolerance of conspecifics may be an ancestral character, which could be retained in both *R. dilectus* sub-species.

I found differences in the vegetation characteristics that are important for small mammals, such as aerial cover and cover density (Jensen *et al.*, 2003; Stokes *et al.*, 2004) for the two sub-species. *Rhabdomys* prefers a high levels of cover in grasslands, but both du Preez (1998) and Fuller and Perrin (2001) argued that striped mice are opportunistic when conditions become unfavourable, and can be associated with many different vegetation types. I found that *Rhabdomys* is found in areas with differing vegetation characteristics, which supports the findings of both du Preez (1998) and Fuller and Perrin (2001), and has important implications for the success of *Rhabdomys dilectus* in changing habitats and climate regimes. Although both *R. d. chakae* and *R. d. dilectus* were trapped in areas that had high levels of cover, the high level of anxiety shown by *R. d. dilectus* in response to an open arena in captivity suggests that *R. d. dilectus* may be less successful if conditions change in the habitat where it lives.

However, the relatively small scale at which I sampled the vegetation could possibly mean that the differences that I found may not be representative of the wider areas in which the sub-species are found. Vegetation is affected by ecosystem processes (Lauenroth *et al.*, 1993; Hejcmanovā-Nežerková and Hejcman, 2006), which in turn, are affected by grazing, soil types and water (Dantas and Batalha, 2010; Tessema *et al.*, 2011), and which can differ markedly between areas in close proximity. Nevertheless, the high level of anxiety of *R. d. dilectus* compared to that of *R. d. chakae* appears to be coincidentally related to the greater levels of cover found at Irene. Thus, future studies should examine multiple populations of both sub-species as well as their associated vegetation to ascertain how much of an effect the vegetation has on the behaviour of the sub-species. If the anxiety of a sub-species is solely dependent on environment (vegetation characteristics), which can change so distinctly between areas, one would expect there to be differences between populations of both sub-species of *R. dilectus* across its range. In contrast to this expectation, a study by Pillay *et al*. (2006) on the variation in olfactory cues used in mate choice between multiple geographically distinct populations of *R. pumilio*, *R. d. chakae* and *R. d. dilectus* showed that olfactory signatures were similar in populations within a taxon but the cues were distinct for each taxon. This study showed that the olfactory signals used in mate choice are taxon- but not population-specific, regardless of habitat type (Pillay *et al.*, 2006).

Another interpretation for the anxiety response is that both *R. d. dilectus* and *R. d. chakae* have a similar genotype which expresses multiple phenotypes in different environments (e.g. reaction norms; Stearns, 1992), similar to the alternative male mating tactics in *R. pumilio* (Schradin *et al.*, 2010). Thus, different environments could select for different stress responses. In order to test for this possibility, one could raise members of each taxon in the environment of the other one; in other words, raise *R. d. chakae* individuals under the conditions found at Irene, where *R. d. dilectus* is found, and raise *R. d. dilectus* individuals under the conditions found at Walkers Fruit Farms, where *R. d. chakae* is found.

Finally, it is possible that the anxiety response is simply a product of variation in a population independent of direct selection (e.g. genetic drift) or as consequence of non-genetic inheritance, which is currently being investigated in *Rhabdomys* (Rymer *et al.*, in prep.).

Conclusion

The reasons of the similarities and differences in the behaviour of *R. d. chakae* and *R. d. dilectus* are not easily explained. On the one hand, the relative similarity of the environments in which the sub-species occur, the lack of co-existence between the sub-species in nature (N. Pillay pers. com.) and reproductive isolation in captivity (Lancaster, 2001; Riyas Ahamed, 2006), suggest that any behavioural differences between the sub-species occurred in allopatry over the past 600 000 years when the sub-species shared a common ancestor (Rambau *et al.*, 2003). On the other hand, the similarities in the behaviour (activity patterns, social behaviour) between *R. d. dilectus* and *R. d. chakae* could be due to one or more of three processes. 1) The similarities could also be explained by convergence, but this would require that the sub-species had diverged over time, and then due to inhabiting similar environments, converged through natural selection (Ackerly, 2004). However, due to the definition of convergence, which explains the similarities of independent lineages and species that are not closely related (Ackerly, 2004), and since the *R. dilectus* sub-species are closely related, convergence is unlikely to explain the similarities between the sub-species. 2) A more parsimonious explanation for the behavioural similarities is phylogenetic inertia, which maintains that more closely related species will have similar characteristics (following Blomberg and Garland, 2002). Phylogeny appears to be a driving factor in the social behaviour of *R. d. dilectus* and *R. d. chakae*, especially with regard to the unexpected amicability of the sub-species. 3) Another consideration is that of phylogenetic niche conservatism (PNC), where species are more similar than can be expected, after accounting for genetic drift (Losos, 2008). Under PNC, species that are similar may select for a particular niche, which conserves the phenotypes. Thus, even if the environment changes over time, if the niches are available in allopatry, the species may remain similar, due to similar selective forces. I cannot definitively conclude whether the cause of the similarities of the behaviour of *R. d. chakae* and *R. d. dilectus* is due to phylogeny or PNC, since this would require a detailed knowledge of the ancestral niches which the sub-species inhabited, and it would also require that I calculate the expected similarity of the sub-species taking into account genetic drift. Whether the similarities in the behaviour of *R. d. chakae* and *R. d. dilectus* can be ascribed to phylogeny or PNC is unclear. The relatively small observed differences in behaviour that may be associated with mating (sniffing and following; Fleming *et al.*, 1981), and the differences in mate-preference through olfactory cues (Pillay, 2000; Pillay *et al.*, 2006) indicate that the sub-species may be diverging in allopatry either through natural selection, genetic drift or non-genetic factors. The differences in the anxiety response of the sub-species have potential implications under changing environmental conditions, particularly if the behaviour is selected for in *R. d. dilectus*. Nevertheless*, Rhabdomys* is an opportunistic omnivore that displays a wide niche breadth in terms of the biomes and altitudes in which it occurs (Skinner and Chimimba, 2005) and is capable behavioural flexibility (Kinahan and Pillay, 2008; Schradin *et al.*, 2009). The persistence of *R. dilectus* under changing conditions may ultimately depend on such social flexibility.

References

Ackerly, D. D. (2004). Adaptation, Niche Conservatism, and Convergence: Comparative Studies of Leaf Evolution in the California Chaparral. *The American Naturalist*. 163**,** 654 - 672.

Akre, A. K., Hovland, A. L. and Bakken, M. (2010). The effects of resource distribution on behaviour in pair housed silver fox vixens (*Vulpes vulpes*) subsequent to mixing. *Applied Animal Behaviour Science*. 126**,** 67-74.

Alcock, J. (1976). Animal Behaviour: An evolutionary approach. Sinauer Associates, Inc., Sunderland.

Aleksiuk, M. (1976). Metabolic and behavioural adjustments to temperature change in the red-sided garter snake (*Thamnophis sirtalis parietalis*): An integrated approach. *Journal of Thermal Biology*. 1**,** 153-156.

Alexander, R. D. (1974). The Evolution of Social Behavior. *Annual Review of Ecology and Systematics*. 5**,** 325-383.

Aureli, F., Schaffner, C. M., Boesch, C., Bearder, S. K., Call, J., Chapman, C. A., Connor, R., Di Fiore, A., Dunbar, R. I. M., Henzi, S. P., Holekamp, K., Korstjens, A. H., Layton, R., Lee, P., Lehmann, J., Manson, J. H., Ramos-Fernandez, G., Strier, K. B. and van Schaik, C. P. (2008). Fission-Fusion Dynamics: New Research Frameworks. *Current Anthropology*. 49**,** 627-654.

Avital, E. and Jablonka, E. (2000). Animal Traditions: Behavioural Inheritance in Evolution. Cambridge University Press, Cambridge.

Bailleul, F., Charrassin, J.-B., Ezraty, R., Girard-Ardhuin, F., McMahon, C. R., Field, I. C. and Guinet, C. (2007). Southern elephant seals from Kerguelen Islands confronted by Antarctic Sea ice. Changes in movements and in diving behaviour. *Deep Sea Research Part II: Topical Studies in Oceanography*. 54**,** 343-355.

Baker, M. A. A. and Brown, J. S. (2010). Islands of fear: effects of wooded patches on habitat suitability of the striped mouse in a South African grassland. *Functional Ecology*. 24**,** 1313-1322.

Baker, P. J., Robertson, C. P. J., Funk, S. M. and Harris, S. (1998). Potential fitness benefits of group living in the red fox, *Vulpes vulpes*. *Animal Behaviour*. 56**,** 1411-1424.

Banks, E. M. (1977), Introduction. In: E. M. Banks, (Ed.), Vertebrate social organisation. Dowden, Hutchinson & Ross, Inc., Stroudsburg, pp. 1-10.

Baptista, L. F. and King, J. R. (1980). Geographical Variation in Song and Song Dialects of Montane White-Crowned Sparrows. *The Condor*. 82**,** 267-284.

Barr, M. M. and Sternberg, P. W. (1999). A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans*. *Nature*. 401**,** 386-389.

Berger, S., Wikelski, M., Romero, M., Kalko, E. K. V. and Rödl, T. (2007). Behavioral and physiological adjustments to new predators in an endemic island species, the Galápagos marine iguana. *Hormones and Behavior*. 52**,** 653–663.

Berntson, G. G., Sarter, M. and Cacioppo, J. T. (1998). Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link. *Behavioural Brain Research*. 94**,** 225-248.

Berven, K. A., Gill, D. E. and Smith-Gill, S. J. (1979). Countergradient Selection in the Green Frog, *Rana clamitans*. *Evolution*. 33**,** 609-623.

Biro, P. A. and Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution*. 25**,** 653-659.

Blomberg, S. P. and Garland, T. J. (2002). Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *Journal of evolutionary biology*. 15**,** 899-910.

Bourin, M. and Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology*. 463**,** 55-65.

Brooks, P. M. (1974) The ecology of the four-striped field mouse, *Rhabdomys pumilio* (Sparrman, 1784), with particular reference to a population on the Van Riebbeeck Nature Reserve, Pretoria. Unpubl. doctoral dissertation, University of Pretoria, Pretoria, South Africa.

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

Brown, J. S., Kotler, B. P., Smith, R. J. and Wirtz, W. O. (1988). The effects of owl predation on the foraging behavior of heteromyid rodents. *Oecologia*. 76**,** 408-415.

Campbell, P., Akbar, Z., Adnan, A. M. and Kunz, T. H. (2006). Resource distribution and social structure in harem-forming Old World fruit bats: variations on a polygynous theme. *Animal Behaviour*. 72**,** 687-698.

Carr, G. M. and Macdonald, D. W. (1986). The sociality of solitary foragers: a model based on resource dispersion. *Animal Behaviour*. 34**,** 1540-1549.

Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Pournin, S., Müller, U., Aguet, M., Babinet, C., Chen shih, J. and De Maeyer, E. (1995). Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. *Science*. 268**,** 1763-1766.

Champagne, D. L., Hoefnagels, C. C. M., de Kloet, R. E. and Richardson, M. K. (2010). Translating rodent behavioral repertoire to zebrafish (*Danio rerio*): Relevance for stress research. *Behavioural Brain Research*. 214**,** 332-342.

Chapman, C. A. and Rothman, J. M. (2009). Within-species differences in primate social structure: evolution of plasticity and phylogenetic constraints. *Primates*. 50**,** 12-22.

Charmandari, E., Tsigos, C. and Chrousos, G. (2005). Endocrinology of the Stress Response. *Annual Review of Physiology*. 67**,** 259-284.

Chiang, C., Johnson, R. K. and Nielsen, M. K. (2002). Selection for maternal behavior in mice - direct and correlated responses. *Applied Animal Behaviour Science*. 79**,** 311-323.

Choleris, E., Thomas, A. W., Ossenkopp, K.-P., Kavaliers, M., Valsecchi, P. and Prato, F. S. (2000). Sex differences in conditioned taste aversion and in the effects of exposure to a specific pulsed magnetic field in deer mice *Peromyscus maniculatus*. *Physiology & Behavior*. 71**,** 237-249.

Clément, Y., Calatayud, F. and Belzung, C. (2002). Genetic basis of anxiety-like behaviour: a critical review. *Brain Research Bulletin*. 57**,** 57-71.

Clode, D. (1993). Colonially breeding seabirds: Predators or prey? *Trends in Ecology & Evolution*. 8**,** 336-338.

Corey, D. T. (1978). The determinants of exploration and neophobia. *Neuroscience & Biobehavioral Reviews*. 2**,** 235-253.

Cowling, R. M., Esler, K. J. and Rundel, P. W. (1999). Namaqualand, South Africa – an overview of a unique winter-rainfall desert ecosystem. *Plant Ecology*. 142**,** 3-21.

Creel, S., Winnie, J. A. and Christianson, D. (2009). Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. *Proceedings of the National Academy of Sciences*. 106**,** 12388-12393.

Crook, J. H., Ellis, J. E. and Goss-Custard, J. D. (1976). Mammalian social systems: Structure and function. *Animal Behaviour*. 24**,** 261-274.

Dalerum, F., Creel, S. and Hall, S. B. (2006). Behavioral and endocrine correlates of reproductive failure in social aggregations of captive wolverines (*Gulo gulo*). *Journal of Zoology*. 269**,** 527-536.

Dantas, V. d. L. and Batalha, M. A. (2010). Vegetation structure: Fine scale relationships with soil in a cerrado site. *Flora - Morphology, Distribution, Functional Ecology of Plants*. In Press, Corrected Proof.

De Graaff, G. (1981). The rodents of Southern Africa. Butterworths, Durban.

de Waal, F. B. M. and Tyack, P. L. (2003), Evolution of Cooperative Strategies. In: F. B. M. de Waal, P. L. Tyack, (Eds.), Animal Social Complexity. Harvard University Press, Cambridge, Massachusetts.

DeNardo, D. F., Luna, J. V. and Hwang, M. (2002). Ambient Temperature Activity of Horned Adders, *Bitis caudalis*: How Cold Is Too Cold? *Journal of Herpetology*. 36**,** 688-691.

Douglass, R. J., Douglass, K. S. and Rossi, L. (1992). Ecological distribution of bank voles and wood mice in disturbed habitats : preliminary results. *Acta Theriologica*. 37**,** 359 - 370.

du Preez, H. (1998) Small mammal ecology in an agricultural land-use system. Unpubl. MSc. dissertation, University of the Witwatersrand, Johannesburg.

Emlen, S. T. (1982). The Evolution of Helping. I. An Ecological Constraints Model. *The American Naturalist*. 119**,** 29-39.

Emlen, S. T. (1994). Benefits, constrainsts and the evolution of the family. *Trends in Ecology & Evolution*. 9**,** 282-285.

Faulkes, C. G. and Bennett, N. C. (2001). Family values: group dynamics and social control of reproduction in African mole-rats. *Trends in Ecology & Evolution*. 16**,** 184-190.

Ferreira, S. M. and Van Aarde, R. J. (1999). Habitat associations and competition in Mastomys-Saccostomus-Aethomys assemblages on coastal dune forests. *African Journal of Ecology*. 37**,** 121-136.

Fleming, A. S., Chee, P. and Vaccarino, F. (1981). Sexual behaviour and its olfactory control in the desert woodrat (*Neotoma lepida lepida*). *Animal Behaviour*. 29**,** 727-745.

Forstmeier, W. and Weiss, I. (2004). Adaptive plasticity in nest-site selection in response to changing predation risk. *Oikos*. 104**,** 487-499.

Foster, S. A. (1999). The geography of behaviour: an evolutionary perspective. *Trends in Ecology & Evolution*. 14**,** 190-195.

Frynta, D., Slábová, M., Váchová, H., Volfová, R. and Munclinger, P. (2005). Aggression and Commensalism in House Mouse: a Comparative Study Across Europe and the Near East. *Aggressive Behaviour*. 31**,** 283-293.

Fuller, J. A. and Perrin, M. R. (2001). Habitat assessment of small mammals in the Umvoti Vlei Conservancy, KwaZulu-Natal, South Africa. *South African Journal of Wildlife Research*. 31**,** 1.

Håkansson, J., Bratt, C. and Jensen, P. (2007). Behavioural differences between two captive populations of red jungle fowl (Gallus gallus) with different genetic background, raised under identical conditions. *Applied Animal Behaviour Science*. 102**,** 24-38.

Håkansson, J. and Jensen, P. (2005). Behavioural and morphological variation between captive populations of red junglefowl (Gallus gallus) - possible implications for conservation. *Biological conservation*. 122**,** 431-439.

Happold, M. (1976). Social behavior of the conilurine rodents (Muridae) of Australia. *Zeitschrift für Tierpsychologie*. 40**,** 113-182.

Hejcmanovā-Nežerková, P. and Hejcman, M. (2006). A canonical correspondence analysis (CCA) of the vegetation-environment relationships in Sudanese savannah, Senegal. *South African Journal of Botany*. 72**,** 256-262.

Hemelrijk, C. K. (1999). An individual-orientated model of the emergence of despotic and egalitarian societies. *Proceedings of the Royal Society of London. Series B: Biological Sciences*. 266**,** 361-369.

Heth, G., Nevo, E. and Beiles, A. (1987). Adaptive exploratory behaviour: Differential patterns in species and sexes of subterranean mole rats. *Mammalia*. 51**,** 27-37.

Hoare, J. M., Pledger, S., Nelson, N. J. and Daugherty, C. H. (2007). Avoiding aliens: Behavioural plasticity in habitat use enables large, nocturnal geckos to survive Pacific rat invasions. *Biological conservation*. 136**,** 510 - 519.

Hood, K. E. and Cairns, R. B. (1988). A Developmental-Genetic Analysis of Aggressive Behavior in Mice. II. Cross-Sex Inheritance. *Behavior genetics*. 18**,** 605-619.

Hughes, J. J. and Ward, D. (1993). Predation risk and distance to cover affect foraging behaviour in Namib Desert gerbils. *Animal Behaviour*. 46**,** 1243-1245.

Jennions, M. D. and Macdonald, D. W. (1994). Cooperative breeding in mammals. *Trends in Ecology & Evolution*. 9**,** 89-93.

Jensen, S. P., Gray, S. J. and Hurst, J. L. (2003). How does habitat structure affect activity and use of space among house mice? *Animal Behaviour*. 66**,** 239-250.

Johnson, D. D. P., Kays, R., Blackwell, P. G. and Macdonald, D. W. (2002). Does the resource dispersion hypothesis explain group living? *Trends in Ecology & Evolution*. 17**,** 563-570.

Jones, M. A., Mason, G. J. and Pillay, N. (in review). Correlates of birth-origin effects on the development of stereotypic behaviour in striped mice, *Rhabdomys*. *Animal Behaviour*.

Karelina, K., Walton, J. C., Weil, Z. M., Norman, G. J., Nelson, R. J. and DeVries, A. C. (2010). Estrous phase alters social behavior in a polygynous but not a monogamous *Peromyscus* species. *Hormones and Behavior*. 58**,** 193-199.

Kazlauckas, V., Pagnussat, N., Mioranzza, S., Kalinine, E., Nunes, F., Pettenuzzo, L., O.Souza, D., Portela, L. V., Porciúncula, L. O. and Lara, D. R. (2011). Enriched environment effects on behavior, memory and BDNF in low and high exploratory mice. *Physiology & Behavior*. 102**,** 475-480.

Kim, J. J. and Haller, J. (2007). Glucocorticoid hyper- and hypofunction: stress effects on cognition and aggression. *Annals of the New York Academy of Sciences*. 1113**,** 291-303.

Kinahan, A. A. and Pillay, N. (2008). Dominance status influences female reproductive strategy in a territorial African rodent *Rhabdomys pumilio*. *Behavioral Ecology and Sociobiology*. 62**,** 579-587.

Kleiman, D. G. (1972). Social Behavior of the Maned Wolf (*Chrysocyon brachyurus*) and Bush Dog (*Speothos venaticus*): A Study in Contrast. *Journal of Mammalogy*. 53**,** 791-806.

Koenig, W. D., Pitelka, F. A., Carmen, W. J., Mumme, R. L. and Stanback, M. T. (1992). The Evolution of Delayed Dispersal in Cooperative Breeders. *The Quarterly Review of Biology*. 67**,** 111-150.

Korte, S. M. (2001). Corticosteroids in relation to fear, anxiety and psychopathology. *Neuroscience & Biobehavioral Reviews*. 25**,** 117-142.

Kotler, B. P., Ayal, Y. and Subach, A. (1994). Effects of predatory risk and resource renewal on the timing of foraging activity in a gerbil community. *Oecologia*. 100**,** 391-396.

Kotler, B. P., Brown, J. S. and Hasson, O. (1991). Factors Affecting Gerbil Foraging Behavior and Rates of Owl Predation. *Ecology*. 72**,** 2249-2260.

Kozak, K. H., Larson, A., Bonett, R. M. and Harmon, L. J. (2005). Phylogenetic analysis of ecomorphological divergence, community structure, and diversification rates in dusky salamanders (Plethodontidae: Desmognathus). *Evolution*. 59**,** 2000-2016.

Lancaster, J. L. (2001) Reproductive isolation in two populations of the striped mouse *Rhabdomys pumilio*. Unpubl. MSc dissertation, University of the Witwatersrand, Johannesburg.

Lauenroth, W. K., Urban, D. L., Coffin, D. P., Parton, W. J., Shugart, H. H., Kirchner, T. B. and Smith, T. M. (1993). Modeling vegetation structure-ecosystem process interactions across sites and ecosystems. *Ecological Modelling*. 67**,** 49-80.

Lett, C., Auger, P. and Gaillard, J.-M. (2004). Continuous cycling of grouped vs. solitary strategy frequencies in a predator-prey model. *Theoretical Population Biology*. 65**,** 263-270.

Lima, S. L. and Bednekoff, P. A. (1999). Temporal Variation in Danger Drives Antipredator Behavior: The Predation Risk Allocation Hypothesis. *The American Naturalist*. 153**,** 649-659.

Losos, J. B. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*. 11**,** 995-1007.

Lott, D. F. (1991). Intraspecific Variation in the Social Systems of Wild Vertebrates. Cambridge University Press, New York.

Madison, D. M. (1980). Space use and social structure in meadow voles, *Microtus pennsylvanicus*. *Behavioral Ecology and Sociobiology*. 7**,** 65-71.

Martínez, J. C., Cardenas, F., Lamprea, M. and Morato, S. (2002). The role of vision and proprioception in the aversion of rats to the open arms of an elevated plus-maze. *Behavioural Processes*. 60**,** 15-26.

Mashoodh, R., Sinal, C. J. and Perrot-Sinal, T. S. (2009). Predation threat exerts specific effects on rat maternal behaviour and anxiety-related behaviour of male and female offspring. *Physiology & Behavior*. 96**,** 693-702.

Mathieu, J., Barot, S., Blouin, M., Caro, G., Decaëns, T., Dubs, F., Dupont, L., Jouquet, P. and Nai, P. (2010). Habitat quality, conspecific density, and habitat pre-use affect the dispersal behaviour of two earthworm species, *Aporrectodea icterica* and

Dendrobaena veneta, in a mesocosm experiment. *Soil Biology and Biochemistry*. 42, 203-209.

McGuire, B., Getz, L. L. and Oli, M. K. (2002). Fitness consequences of sociality in prairie voles, *Microtus ochrogaster*: influence of group size and composition. *Animal Behaviour*. 64, 645-654.

Monadjem, A. (1997). Habitat preferences and biomasses of small mammals in Swaziland. *African Journal of Ecology*. 35, 64-72.

Morgan, K. N. and Tromborg, C. T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science*. 102, 262-302.

Mucina, L., Rutherford, M. C. and Powrie, L. W. (Eds.). (2005). Vegetation map of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria.

O'Connor, T. G. and Bredenkamp, G. J. (1997), Grassland. In: R. M. Cowling, et al., (Eds.), Vegetation of Southern Africa. Cambridge University Press, Cambridge.

Ostfeld, R. S. (1985). Limiting Resources and Territoriality in Microtine Rodents. *The American Naturalist*. 126, 1-15.

Packer, W. C. (1980). Nest-building and activity patterns in four sympatric rodent species. *South African Journal of Zoology*. 15, 50-55.

Payne, H. F. P., Lawes, M. J. and Henzi, S. P. (2003). Competition and the Exchange of Grooming among Female Samango Monkeys (*Cercopithecus mitis erythrarchus*). *Behaviour*. 140, 453-471.

Pépin, D., Renaud, P.-C., Dumont, B. and Decuq, F. (2006). Time budget and 24-h temporal rest-activity patterns of captive red deer hinds. *Applied Animal Behaviour Science*. 101, 339-354.

Perrin, M. R. (1980). The feeding habits of two co-existing rodents, *Rhabdomys pumilio* (Sparrman, 1784) and *Otomys irroratus* (Brants, 1827), in relation to rainfall and reproduction. *Acta Oecologica*. 1, 71-89.

Perrin, M. R. (1981). Notes on the activity of 12 species of southern African rodents and a new design of activity monitor. *South African Journal of Zoology*. 16, 248-258.

Perrin, M. R., Ercoli, C. and Dempster, E. R. (2001). The role of agonistic behaviour in the population regulation of two syntopic African grassland rodents, the striped mouse *Rhabdomys pumilio* (Sparrman 1784) and the multimammate mouse *Mastomys natalensis* (A. Smith 1834) (Mammalia Rodentia). *Tropical Zoology*. 14, 7 - 29.

Petrinovich, L. (1988). Individual Stability, Local Variability and the Cultural Transmission of Song in White-Crowned Sparrows (Zonotrichia leucophrys nuttalli). *Behaviour*. 107**,** 208-240.

Pillay, N. (1993) The evolution and socio-ecology of two populations of the vlei rat *Otomys irroratus*. Unpbl. PhD thesis, University of Natal, South Africa.

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

Pillay, N., Eborall, J. and Ganem, G. (2006). Divergence of mate recognition in the African striped mouse (*Rhabdomys*). *Behavioural Ecology*. 17**,** 757-764.

Pillay, N., Willan, K. and Cooke, J. (1995). Evidence of pre-mating reproductive isolation in two populations of the Vlei rat *Otomys irroratus* : experiments of intra- and interpopulation male-female encounters. *Zeitschrift für Säugetierkunde*. 60**,** 352-360.

Pillay, N., Wolhuter, W. and Willan, K. (1993). Pup retrieval in the bush Karoo rat. *Acta Theriologica*. 38**,** 339-343.

Pokharel, K. and Chalise, M. K. (2010). Status and Distribution Pattern of Barking Deer (*Muntiacus muntjak* Zimmermann) in Hemja VDC, Kaski. *Nepal Journal of Science and Technology*. 11**,** 223 - 228.

Powell, F. and Banks, P. B. (2004). Do house mice modify their foraging behaviour in response to predator odours and habitat? *Animal Behaviour*. 67**,** 753-759.

Raine, A. (2008). From Genes to Brain to Antisocial Behavior. *Current Directions in Psychological Science*. 17**,** 323-328.

Raine, N. E., Ings, T. C., Dornhaus, A., Saleh, N. and Chittka, L. (2006). Adaptation, Genetic Drift, Pleiotropy, and History in the Evolution of Bee Foraging Behavior. *Advances in the study of behaviour*. 36**,** 305-354.

Rambau, R. V., Robinson, T. J. and Stanyon, R. (2003). Molecular genetics of *Rhabdomys pumilio* subspecies boundaries: mtDNA phylogeography and karyotypic analysis by fluorescence in situ hybridization. *Molecular Phylogenetics and Evolution*. 28**,** 564-575.

Randall, J. A., Rogovin, K., Parker, P. G. and Eimes, J. A. (2005). Flexible social structure of a desert rodent, *Rhombomys opimus*: philopatry, kinship, and ecological constraints. *Behavioral Ecology*. 16**,** 961-973.

Riyas Ahamed, A. M. (2006) Reproductive isolation in the striped mouse *Rhabdomys*: a case for reinforcement? Unpubl.MSc thesis, University of the Witwatersrand., Johannesburg.

Roll, U., Dayan, T. and Kronfeld-Schor, N. (2006). On the role of phylogeny in determining activity patterns of rodents. *Evolutionary Ecology*. 20**,** 479-490.

Rymer, T., Hinze, A. and Pillay, N. (in prep.). Behavioural development in two populations of the african striped mouse, *Rhabdomys*: genetic and environmental influences.

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

Scantlebury, M., Bennett, N. C., Speakman, J. R., Pillay, N. and Schradin, C. (2006). Huddling in groups leads to daily energy savings in free-living African Four-Striped Grass Mice, *Rhabdomys pumilio*. *Functional Ecology*. 20**,** 166-173.

Scantlebury, M., Krackow, S., Pillay, N., Bennett, N. and Schradin, C. (2010). Basking is affected by season and influences oxygen consumption in desert-living striped mice. *Journal of Zoology*. 281**,** 132-139.

Schradin, C. (2004). Territorial Defense in a Group-Living Solitary Forager: Who, Where, against Whom? *Behavioral Ecology and Sociobiology*. 55**,** 439-446.

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

Schradin, C. (2006). Whole-day follows of striped mice (*Rhabdomys pumilio*), a diurnal murid rodent. *Journal of Ethology*. 24**,** 37-43.

Schradin, C., König, B. and Pillay, N. (2010). Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology*. 79**,** 515-521.

Schradin, C., Krackow, S., Schubert, M., Keller, C., Schradin, B. and Pillay, N. (2007). Regulation of Activity in Desert-Living Striped Mice: The Importance of Basking. *Ethology*. 113**,** 606-614.

Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C.-H., König, B. and Pillay, N. (in review). Social flexibility as a tool to understand social behaviour. *Molecular Ecology*.

Schradin, C. and Pillay, N. (2003). Paternal Care in the Social and Diurnal Striped Mouse (*Rhabdomys pumilio*): Laboratory and Field Evidence. *Jounal of Comparative Psychology*. 117**,** 317–324.

Schradin, C. and Pillay, N. (2004). The Striped Mouse (*Rhabdomys pumilio*) from the Succulent Karoo, South Africa: A territorial group-living solitary forager with communal breeding and helpers at the nest. *Journal of Comparative Psychology*. 118**,** 37-47.

Schradin, C. and Pillay, N. (2005a). Demography of the striped mouse (*Rhabdomys pumilio*) in the succulent karoo. *Mammalian Biology*. 70**,** 84-92.

Schradin, C. and Pillay, N. (2005b). Intraspecific variation in the spatial and social organization of the african striped mouse. *Journal of Mammalogy*. 86**,** 99-107.

Schradin, C., Scantlebury, M., Pillay, N. and Konig, B. (2009). Testosterone levels in dominant sociable males are lower than in solitary roamers: physiological differences between three male reproductive tactics in a sociably flexible mammal. *The American Naturalist*. 173**,** 376-388.

Schulkin, J., Gold, P. W. and McEwen, B. S. (1998). Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology*. 23**,** 219-243.

Schumann, D. M., Cooper, H. M., Hofmeyr, M. D. and Bennett, N. C. (2005). Circadian rhythm of locomotor activity in the four-striped field mouse, *Rhabdomys pumilio*: A diurnal African rodent. *Physiology & Behavior*. 85**,** 231-239.

Sinha, A. (2005). Not in their genes: Phenotypic flexibility, behavioural traditions and cultural evolution in wild bonnet macaques. *Journal of Biosciences*. 30**,** 51-64.

Skinner, J. D. and Chimimba, C. T. (2005). The Mammals of the Southern African Subregion. Cambridge University Press, Cape Town.

Solomon, N. G. (2003). A reexamination of factors influencing philopatry in rodents. *Journal of Mammalogy*. 84**,** 1182-1197.

Solomon, N. G. and Crist, T. O. (2008). Estimates of reproductive success for group-living prairie voles, *Microtus ochrogaster*, in high-density populations. *Animal Behaviour*. 76**,** 881-892.

Statsoft, I. (2007) STATSOFT (Data analysis software system).

Stearns, S. C. (1992). The Evolution of Life Histories. Oxford University Press, New York.

Stokes, V. L., Pech, R. P., Banks, P. B. and Arthur, A. D. (2004). Foraging behaviour and habitat use by *Antechinus flavipes* and *Sminthopsis murina* (Marsupialia: Dasyuridae) in response to predation risk in eucalypt woodland. *Biological conservation*. 117**,** 331-342.

Symington, M. (1990). Fission-fusion social organization in *Ateles* and *Pan*. *International Journal of Primatology*. 11**,** 47-61.

Taraborelli, P. and Moreno, P. (2009). Comparing composition of social groups, mating system and social behaviour in two populations of *Microcavia australis*. *Mammalian Biology - Zeitschrift fur Saugetierkunde*. 74**,** 15-24.

Temeles, E. J. (1994). The role of neighbours in territorial systems: when are they 'dear enemies'? *Animal Behaviour*. 47**,** 339-350.

Tessema, Z. K., de Boer, W. F., Baars, R. M. T. and Prins, H. H. T. (2011). Changes in soil nutrients, vegetation structure and herbaceous biomass in response to grazing in a semi-arid savanna of Ethiopia. *Journal of Arid Environments*. In Press, Corrected Proof.

Touma, C., Sachser, N., Möstl, E. and Palme, R. (2003). Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *General and Comparative Endocrinology*. 130**,** 267-278.

Vauti, F., Goller, T., Beine, R., Becker, L., Klopstock, T., Hölter, S. M., Wurst, W., Fuchs, H., Gailus-Durner, V., de Angelis, M. H. and Arnold, H.-H. (2007). The mouse Trm1-like gene is expressed in neural tissues and plays a role in motor coordination and exploratory behaviour. *Gene*. 389**,** 174-185.

Vehrencamp, S. L. (1983). A model for the evolution of despotic versus egalitarian societies. *Animal Behaviour*. 31**,** 667-682.

Verbeek, M. E. M., Boon, A. and Drent, P. J. (1996). Exploration, Aggressive Behaviour and Dominance in Pair-Wise Confrontations of Juvenile Male Great Tits. *Behaviour*. 133**,** 945-963.

Wainwright, P. E. (2001). The role of nutritional factors in behavioural development in laboratory mice. *Behavioural Brain Research*. 125**,** 75-80.

Walker, B. H. (1976). An approach to the monitoring of changes in the composition and utilization of woodland and savanna vegetation. *South African Journal of Wildlife Research*. 6**,** 1 - 32.

Wasiolka, B., Blaum, N., Jeltsch, F. and Henschel, J. (2009). Behavioural responses of the lizard *Pedioplanis l. lineoocellata* to overgrazing. *Acta Oecologica*. 35**,** 157-162.

Weber, J. N. and Hoekstra, H. E. (2009). The evolution of burrowing behaviour in deer mice (genus *Peromyscus*). *Animal Behaviour*. 77**,** 603-609.

Willan, K. (1982) Social ecology of *Otomys irroratus*, *Rhabdomys pumilio* and *Praomys natalensis*. Unpubl. Ph.D. Thesis, University of Natal Pietermaritzburg.

Williams, T. M., Rutishauser, M., Long, B., Fink, T., Gafney, J., Mostman-Liwanag, H. and Casper, D. (2007). Seasonal Variability in Otariid Energetics: Implications for the Effects of Predators on Localized Prey Resources. *Physiological and Biochemical Zoology*. 80**,** 433-443.

Yahner, R. H. (1978). The adaptive nature of the social system and behavior in the eastern chipmunk, *Tamias striatus*. *Behavioral Ecology and Sociobiology*. 3**,** 397-427.

Ydenberg, R. C., Giraldeau, L. A. and Falls, J. B. (1988). Neighbours, strangers, and the asymmetric war of attrition. *Animal Behaviour*. 36**,** 343-347.

Zuri, I., Gottreich, A. and Terkel, J. (1998). Social stress in neighbouring and encountering blind mole-rats (*Spalax ehrenbergi*). *Physiology & Behavior*. 64**,** 611- 620.