Reproductive isolation in four populations of the striped mouse *Rhabdomys*

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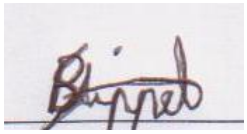
Declaration

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, South Africa. It has not been previously submitted for any degree or examination in any other university.



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Abstract

Reproductive isolation was investigated in four phylogenetically and/or geographically separated populations of *Rhabdomys* in South Africa. The four populations, Jonkershoek (*Rhabdomys pumilio*), Goegap (*R. pumilio*), Irene (*R. dilectus dilectus*) and Suikerbosrand (*R. d. chakae*), represent the two putative species, *R. pumilio* (Sparrman, 1784) and *R. dilectus* De Winton (1897), *as* well as the two sub-species, *R. d. dilectus* (Wroughton, 1905) and *R. d. chakae* (Wroughton, 1905), of *Rhabdomys*. The populations occur $\geq$ 900 km apart, except for the Irene and Suikerbosrand populations which are approximately 80 km apart. Inter- and intrapopulation breeding experiments and behavioural studies were used to test for pre- and/or postzygotic reproductive barriers. In breeding experiments, most intrapopulation pairs produced offspring. In the interpopulation breeding tests, except for one litter produced by an Irene-Jonkershoek pair, which did not survive, only the Jonkershoek-Goegap pairings produced offspring, which were fertile and had growth rates similar to those of offspring produced in intrapopulation pairings. However, the smaller litter size of the Jonkershoek-Goegap pairings compared to intrapopulation pairings, suggests post-zygotic failure between these two *R. pumilio* populations. In the behavioural experiments, I tested the responses of females to the soiled bedding of homotype (same population) and heterotype (different population) males. Two experiments were conducted: habituation-discrimination and habituation-generalization tests were used to investigate within- and between-taxon variations in male odour quality and female perception; and choice tests were used to test female preference. The results of the behavioural experiments indicate that there is variation within the *R. pumilio* (Jonkershoek and Goegap) taxon in odour quality, perception and preference. The Jonkershoek females could discriminate between their own population males and those of Goegap, and preferred their own males, while the Goegap females were unable to distinguish between their own population scent and the Jonkershoek population scent and therefore were unable to display a preference for their own population scent when a Jonkershoek/Goegap scent choice was presented. The two subspecies of *R. dilectus* perceived the scent of males from their own population as being more similar to each other than to that of *R. pumilio*, and Irene females perceived the two *R. pumilio* populations as different. All four populations displayed assortative mate preference and preferred their

own population's scent over all the others, with the exception of the Goegap population. My study indicates that phylogeny, and not geography, appears to be a more parsimonious explanation for the pattern of divergence in these four *Rhabdomys* populations, although ecological influences cannot be ruled out.

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Chapter 1: Introduction

1.1) Reproductive isolation

Although species are fundamental units of natural diversity and as such are of interest to biologists, from ecologists to systematists, there is still considerable debate within and among the biological disciplines about how a species should be defined (Harrison, 1998). The morphological species concept (MSC) dominated animal taxonomy during the 19th and 20th centuries but has since lost ground as morphological differences are now seen as playing a secondary role with reproductive isolation considered the primary criterion of species status (Mayr, 1963). There are two main problems with the MSC: firstly there can be great morphological variation among individuals of the same species and secondly there may not be morphological differences between some sympatric populations which otherwise have all the characteristics (genetic difference and reproductive isolation) of being separate species (Mayr, 1963). In contrast, the biological species concept (BSC), as defined by Mayr and Dobzhansky, uses reproductive isolation as its primary criterion and has become the default framework for the discourse about species and speciation (Harrison, 1998). According to the BSC, a species can be defined as a group of organisms that actually or potentially interbreed under natural conditions but which cannot or very rarely interbreed with animals of other groups (Dobzhansky, 1941; Schluter, 1998; Futuyma, 2005). Reproductive isolation is an important part in the process of speciation (Birkhead & Brillard, 2007) and is intimately related to the BSC. While the emergence of phylogenetic systematics has resulted in some controversy about the BSC, many evolutionary geneticists still use the BSC because of its heuristic value for identifying species (Harrison, 1998). Coyne (1994) stated that there is verification in the power of the BSC as virtually everyone studying the origin of species considers reproductive isolating mechanisms.

Reproductive isolation involves all the mechanisms that would keep individuals of different species from interbreeding, and represents the biological properties of individuals that prevent interbreeding between populations that are actually or potentially sympatric (Mayr, 1963; Harrison, 1998) or parapatric (Price, 2008). Historically, three major classes

of reproductive isolating mechanisms were distinguished: premating mechanisms (e.g. habitat isolation and seasonal isolation), mating-related mechanisms (e.g. sexual isolation and mechanical isolation) and postzygotic mechanisms (e.g. zygote mortality and hybrid inviability) (Dobzhansky, 1941). Reproductive isolating mechanisms can also be divided into two other categories, pre- and post-copulatory barriers (Birkhead & Brillard, 2007). Behavioural and geographic barriers are examples of pre-copulatory barriers while inviable (reduced growth or survival) and sterile hybrids are examples of post-copulatory barriers.

While it is easy to understand how reproductive isolation could work for populations that occurred in allopatry and then become sympatric subsequently, problems arise with the theory when populations have never been completely isolated. In these cases, isolation could be as a result of independent genetic change in isolation (Bickman & Baker, 1980). Although other factors, such as great diversity in available food types and habitats (Seger, 1985; Dieckmann & Doebeli, 1999), can also play a part in the level of separation between populations, with the retardation of gene flow being one of the most important of these factors. Gene flow can be defined as the movement of genes, gametes, individuals or groups of individuals within and between populations (Ferris *et al*., 1983). If there is a large amount of gene flow among local populations, they will be genetically similar (Slatkin, 1994) and thus reproductively isolating mechanisms are unlikely to occur. On the other hand, a low amount of or no gene flow among populations could result in genetic divergence and reproductive isolation.

The production of inviable hybrids can result in decreased fitness. Many authors (Liou & Price, 1994; Butlin, 1995; Noor, 1995; Noor *et al*., 2001) suggest that reinforcement, the process where selection favours the evolution of premating isolation mechanisms that would reduce hybridization (Dobzhansky, 1941; Howard, 1993; Butlin, 1995; Noor, 1999), which follows the production of unfit hybrids and leads to assortative mating (e.g. mating with compatible partners), can isolate sympatric populations, thus avoiding the production of unfit hybrids. While reinforcement has support as a process that can lead to reproductive isolation (Heth & Nevo, 1981), it has also been criticized for being not easily observed in nature and for lacking empirical evidence (Paterson, 1982; Howard, 1993).

As an alternative to the BSC, Paterson (1978) proposed the recognition concept (RC), which focused on the importance of mate recognition through species-specific signals and responses. In this definition of a species, organisms that share a common fertilisation system are considered to be the same species, and post-mating breakdown, such as hybrid infertility or sterility, are not as important as the behavioural recognition of conspecifics (Paterson, 1993). However, the RC and BSC can be seen as being two sides of the same coin (Pillay, 1994), as both maintain that behaviour promotes species cohesion or separation (Littlejohn, 1993). Behaviourally, the difference between the theories is that the RC focuses on recognition between individuals of two species while the BSC is concerned with discrimination between individuals.

Geneticists (e.g. Bush, 1982) have also criticized the concept of reproductive isolation as they feel it is a simplistic way of classifying species and does not take into account Mendelian, molecular and biochemical genetics. Some workers, such as Mallet (1995), argue in favour of looking at levels of gene flow and genetic structure to define species rather than using isolation. However, if reproductive isolation is tested using interfertility, or lack thereof, between populations, it can provide an indirect measure of gene flow and adaptations to local conditions (Pillay, 1994). I used this assumption in my study, where the lack of viable hybrids between populations was taken to indicate little gene flow and adaptation to local conditions whereas the production of viable hybrids indicated a greater level of gene flow. This assumption has been used previously in studies on reproductive isolation (e.g. Lancaster, 2001; Ahamed, 2006). Many studies, on a variety of animals (from insects to birds), have provided evidence for hybrid failure as a barrier to gene flow, and preference for the mating between same species, which would produce viable hybrids and no barrier to gene flow (e.g. Gerhardt, 1994; Pfennig, 2003; Noor, 1995, 1997; Hollocher *et al*., 1997; McMillan *et al*., 1997; Saetre *et al*., 1997; Rundle and Schluter, 1998; Nosil *et al*., 2003).

1.1.1) The role of geographic distance

The mobility of an individual organism affects what modes of speciation seem likely for that organism (Palumbi, 1998). Different modes of speciation are more likely based on certain ranges of dispersal ability (Bush, 1975). For example, an organism with

low movement would be a more likely candidate for allopatric speciation than one with high movement (Palumbi, 1998). Gene flow due to dispersal can be tested using 'direct' and 'indirect' methods (Slatkin, 1994). 'Direct' methods depend on observations or experiments that measure the extent of dispersal, such as mark-release-capture studies, while 'indirect' methods use mathematical models, such as Wright's F_{ST} statistic (Wright, 1951), to predict how much gene flow must have been occurring for the patterns observed in the data (Slatkin, 1994). Studies using both 'direct' and 'indirect' methods suggest that species fall into roughly three categories: species with a high dispersal ability and high levels of gene flow, species with low dispersal and low gene flow and species with low dispersal but high levels of gene flow (Slatkin, 1994). Several species of birds (Price, 2008) and marine invertebrates (Slatkin, 1994) have high dispersal ability and high gene flow. In contrast, an example of a species with low dispersal and low gene flow is the salamander *Batracoseps campi* which is quite sedentary and individuals do not move even between adjacent populations that are in close geographic proximity (Yanef & Wake, 1981), with electrophoretic studies indicating that there is essentially no gene flow between different populations (Slatkin, 1994). The checkerspot butterfly *Euphydrias editha* also has limited dispersal potential, yet indirect studies have shown evidence of gene flow over long distances (Slatkin, 1985). One factor influencing the dispersal ability of an organism is whether it is a habitat specialist or a habitat generalist. Specialist species have a combination of morphological, behavioural or physiological adaptations to a particular habitat type and are less tolerant of disturbances (Henle *et al*., 2004) and thus more likely to have a lower dispersal ability. Generalist species are tolerant of a wider variety of habitat types and are likely to have a greater level of dispersal ability (Futuyma & Moreno, 1988).

Mayr (1942) postulated that a population that is geographically isolated from its parent species could acquire characteristics that would promote reproductive isolation when the external barriers break down. These external barriers can take many forms, from impassable mountains and rivers to the populations simply being too far from one another for individuals of the two populations to meet. The most convenient and common way to measure divergence between populations is to use physical distance (Edmands, 2002). The isolation by distance model developed by Wright (1943), predicts that the further away two populations are geographically, the more likely they are to be genetically different. Thus,

geographic distance would have an effect on gene flow, as individuals from one population would have to be able to reach another population for gene flow to be possible. Many studies have shown significant associations between gene flow and geographic distance in nearly all of the major groups of organisms, such as plants (e.g. Kaufman *et al*., 1998), insects (e.g. Britten *et al*., 1995), fish (e.g. Planes *et al*., 1996), birds (e.g. Martinez *et al*., 1999) and mammals (e.g. Burland *et al*., 1999).

However, distance alone is not the only barrier between populations that are geographically separated. Geographic distance can also include different habitats, some of which could be uninhabitable (and thus a barrier) for the animal. Environmental features making up a variety of habitats can either promote or restrict the movements of individuals in natural populations and therefore the extent of the flow of genes (Taylor., *et al*, 1993). Physical barriers that could impede animal dispersal include rivers, mountains and anthropogenic features, such as roads (Coulon., *et al* 2006). In a study on pumas *Puma concolor* in the southwestern USA, it was found that gene flow was strongly limited by distance, especially for those in areas of high habitat heterogeneity (McRae *et al*., 2005). Habitat barriers in the puma study included the ancient (historical grasslands and deserts) and the modern (metropolitan areas and interstate highways).

Behavioural traits can also exhibit geographic variation, and this variation is often a visible sign of underlying genetic variation (Foster, 1999). The best genotype in one environment will not always perform well in another as a result of either unfavourable environmental conditions (Mills *et al*., 2007) or environmentally induced biases in mate choice (Leal & Fleishman, 2004). Differentiation in courtship and mate choice between geographically separated populations are the most likely behavioural traits that contribute to speciation (Foster, 1999). In habitats where food and shelter are always available, females are expected to choose highly socially competitive males, whereas those that live in habitats where the availability of food and shelter fluctuates may select males that are less socially competitive but can survive environmental changes (Carere *et al*., 2005).

1.1.2) The role of behaviour in reproductive isolation

Behaviour plays an important role in maintaining species cohesion by affecting the level of gene flow between populations (Mayr, 1963; Littlejohn, 1993). Certain behavioural

differences can prevent between-population mating which is evolutionarily important as they decrease gene flow and therefore make further differentiation possible (Herring & Verrell, 1996). Reproductive compatibility in mate choice between individuals of different populations involves two processes: the ability to recognize each other as potential mates (Herring & Verrell, 1996) and assessment of mate quality (Ptacek, 2000). In mammals, inter-individual recognition, which consists of complex biocommunication systems, is important in mate choice (Alberts, 1992).

Organisms require a set of characteristics that function in bringing about mating. This set of characteristics makes up the specific-mate recognition system (SMRS), which forms the basis of the RC (Paterson, 1978). The SMRS defines recognition as the specific response of one mating partner to a specific signal from the other (Paterson, 1993). Signals and preferences are assumed to co-evolve, and divergence in one or both can lead to speciation by reducing the chances of mating between members of different populations of a taxon (Butlin & Ritchie, 1994). The evolution of mate recognition systems (MRSs) can be affected by factors such as phylogeny, ecology and geography (Pillay *et al*., 2006). Reproductive isolation can also occur when individuals of two populations behaviourally discriminate between same (homotype) and different (heterotype) population mates (Boyd & Blaustein, 1985).

Species recognition signals would mainly evolve as a result of intrasexual selection (sperm competition) for gamete recognition or possibly through natural selection against hybrid offspring of heterogametic pairings (Palumbi, 1994). An example of such a mating signal would be the species-specific recognition between sperm lysine proteins and conspecific egg membranes (Metz & Palumbi, 1996). Pre-mating reproductive isolation becomes possible among individuals of different populations if there is a breakdown in courtship behaviour (Boyd & Blaustein, 1985; Barnard & Fitzsimmons, 1989), leading to behavioural discrimination. This behavioural discrimination can be shown in various ways, including higher aggression and lower amicability towards different population mates, as seen in populations of the vlei rat *Otomys irroratus* (Pillay *et al*., 1995a ,b , c), the house mouse *Mus musculus* (Ganem *et al*., 1996), the striped mouse *Rhabdomys* (Pillay, 2000a) and gerbils, *Tatera* spp. (Dempster, 1996). This could promote behavioural divergence in mate recognition between populations (Ptacek, 2000).

Divergence in SMRS would lead to assortative mating (Butlin, 1994), which would ultimately lead to gene flow between similar populations and a reduction of gene flow between dissimilar populations (Hartfield & Schulter, 1996). Therefore, although divergence can occur either through recognition or discrimination, both can ultimately lead to assortative mating (Ganem & Searle, 1996) which may explain the non-mixing of individuals between different populations (Ganem, 1998).

1.1.2.1) Odour as a mate recognition signal

Mate recognition systems rely on potential mate partners sending and receiving signals that each can recognize. These signals can take many forms including visual, tactile, auditory and olfactory (Butlin & Ritchie, 1994). Visual (e.g. reptiles: Stamps & Barlow, 1973; mammals: Dempster & Perrin, 1991; birds: Sætre *et al*., 1997; fishes: Seehausen & van Alphen, 1998) and auditory (e.g. mammals: Dempster *et al*., 1992; birds: Grant & Grant, 1996; amphibians: Welch *et al*., 1998) cues are frequently tested. However, olfactory cues are rarely described, possibly due to the fact that they are difficult for human observers to distinguish and are complex to analyze (Pillay *et al*. 2006). Nonetheless, behavioural techniques, such as habituation-discrimination (Halpin, 1986) and habituation generalization (Todrank & Heth, 2003), make it possible to qualitatively assess odour signals and to compare the perception of these odours between individual organisms, populations and species (Todrank & Heth, 2003);the habituation-discrimination and habituation generalization techniques are described in the methods section (below). Scents play a major role in social communication in mammals (Ptacek, 2000). In rodents, while auditory, tactile and visual cues are important (Dempster *et al*., 1993), olfactory cues are the most important form of communication. Odours are used to detect food and predators, recognize individuals and to appraise sexual and social status of conspecifics (Berry, 1970).

Several studies have shown that female mate preference for conspecific male odours can play an important role in pre-mating reproductive isolation between closely related species that are not geographically separated (e.g. Ortells *et al*., 1989; Zambelli *et al*., 1994; Theiler & Blanco, 1996; Laukaitis *et al*., 1997).

1.1.3) The role of chromosomes in reproductive isolation

The frequency in occurrence of karyotypical differences among related species in several groups of organisms suggests that a change in karyotype may occur frequently in association with the speciation process (White, 1968). While some types of chromosomal changes can accompany but not be a cause of speciation (Spirito, 1998), the rearrangement of chromosomes could contribute to speciation (White, 1978), especially for major chromosomal rearrangements (e.g. Robertsonian rearrangements) involving a change in the position of the genes without a gain or loss of euchromatin (King, 1987). For speciation to occur, there must be a reproductive barrier (a biological factor which prevents successful interbreeding under natural conditions) formed between members of a species (Gibson, 1984). It is unlikely that chromosomal rearrangements themselves could be an effective pre-mating barrier, instead chromosomal differences (under particular conditions) are more likely to mainly cause post-mating reproductive isolation (Mayr, 1963). Chromosomal rearrangements are likely to inhibit interbreeding success mainly as a result of chromosomal heterozygosity which leads to a reduction in hybrid fertility and could even result in sterility owing to mal-segregation and germ cell death during gametogenesis (Meester, 1988). These changes in chromosomal structure could result in speciation occurring relatively rapidly (Gibson, 1984). Chromosomal evolution, by means of mutation events affecting the chromosomes, can therefore lead to divergence within a species. There are many ways in which mutations of the chromosomes can occur. Chromosomal rearrangements may alter the number of chromosomes, the number of chromosome arms, or both, with no apparent effect on the animal's appearance (Gibson, 1984). Some kinds of rearrangements produce obvious chromosomal changes, while others may be less obvious. Genes may be duplicated or depleted, or their sequence in the chromosome may be changed. Change in the position of a gene may affect its action (Wahl *et al*., 1984). Chromosomal rearrangements themselves could only be an effective pre-mating barrier, if the rearrangements change the genetic configuration and hence the phenotype of individuals (Spirito, 1998). Chromosomal evolution can be an efficient isolation mechanism, especially if chromosomal arrangements are complex (Britton-Davidian *et al*., 2005).

Robertsonian rearrangements are the result either of the fusion of two centromeres, or the fission of one centromere into two (Gibson, 1984). Although Robertsonian fusions are one of the more common types of chromosomal rearrangement (Fredga, 1977), Robertsonian fissions appear to be relatively rare in mammals (Gibson, 1984). Robertsonian rearrangements are capable of bringing about post-zygotic reproductive isolation (Ganem, 1998). In populations which differ by only a single Robertsonian rearrangement, hybrid fertility may not be significantly impaired, but hybrids between two chromosomal races which differ by multiple Robertsonian rearrangements are usually at least partially sterile (Gropp & Winking, 1981). The European house mice *Mus musculus* has populations with chromosome numbers ranging from 22 to 40 (Gropp & Winking, 1981), and these chromosomal races are reproductively isolated post-zygotically (Capanna & Corti, 1982).

Although it has been shown that populations that display chromosomal divergence can have problems interbreeding (e.g. Pillay *et al*., 1995c), the role of chromosomal rearrangements in promoting speciation is regularly debated and it is generally agreed that chromosomes are not directly linked to speciation (Meester, 1988). However, this does not mean that chromosomal changes have no affect on speciation, they merely play a more indirect role, as discussed below.

Hybrids that are formed between chromosomal groups that have structurally different chromosomes (which are mispaired during meiosis) have been shown to have a decrease in fertility, which would contribute to reproductive isolation between the parent populations (Nachman & Searle, 1995). The role of chromosomal rearrangements as a post-mating reproductive isolation mechanism has been well studied in the mouse *Mus musculus domesticus* (Redi & Capanna, 1988; Wallace *et al.*, 1992; Hauffe & Searle, 1998; Castiglia & Capanna, 2000). Post-zygotic reproductive isolation because of chromosomal variation has been shown in populations of several other rodent species, such as the phyllotine rodent *Graomys griseoflavus* (Theiler & Blanco, 1996), the vlei rat *Otomys irroratus* (Pillay *et al*. 1992, 1995c), and the mole rat *Spalax ehrenbergi* (Nevo, 1991).

1.2) Examples of reproductive isolation in rodents

Reproductive isolation has been demonstrated in a number of rodent species. Due to the fact that rodents exhibit chromosomal and genetic differences even in closely related species or populations means they are ideal to test hypotheses in speciation.

The South American murid rodent *Graomys griseoflavus* has a high degree of chromosomal polymorphism which has led to the formation of distinct chromosomal races (Zambelli *et al*., 1994). It was shown that receptive female *G. griseoflavus* individuals discriminate between the odour of males from a similar chromosome race from the odour cues of males with which she could not have offspring or the hybrids would be sterile (Theiler & Blanco, 1996). Similarly *Mus musculus musculus* females discriminate between homotype and heterotype males based on a fixed allele difference at the salivary androgen binding protein (ABP) locus, and females showed a strong preference for males of their own ABP type (Laukaitis *et al*., 1997).

Some of the most extensive work on reproductive isolation in chromosomally different populations has been done on the house mouse. In Scotland, *M. musculus musculus* is found to discriminate between individuals from their own and different populations using behavioural displays, leading to assortative mating even though the populations were closely related genetically and morphologically (Ganem & Searle, 1996; Ganem, 1998). Cox (1984) showed that reproductive isolation occurs between two different populations of *M. musculus* because of behavioural differences. In a study on *M. m. musculus* from Northern Italy, mating pairs from different populations experienced reduced reproductive success (Capanna *et al*., 1985). It has also been shown that two different subspecies of house mice (*M. m. musculus* and *M. m. domesticus*) can have different patterns of mate preference, with *M. m. musculus* females showing a stronger preference for homosubspecific individuals, whereas *M. m. domesticus* had no preference between homosubspecific and heterosubspecific males (Smadja & Ganem, 2005). This study also found that preference was assortative in populations that are in contact but nondirectional for populations in allopatry. *Mus m. musculus* individuals also showed strong assortative preference particularly for urinary signals of consubspecifics, while *M. m. domesticus* did not (Smadja *et al*., 2004).

In Israel, there are four chromosomal races of the mole rat *Spalax ehrenbergi* (Heth & Nevo, 1981). However, there are narrow areas of hybridization between the races, which suggests that the differences in chromosomal structure are insufficient for reproductive isolation to occur (Nevo & Heth, 1976; Heth & Nevo, 1981). There are still pre-mating barriers between these mole rats of different populations, which is probably why there is only a small area where hybrids are found. Effective pre-mating barriers in *S. ehrenbergi* include aggression and differences in olfactory and vocalization cues (Nevo & Heth, 1976; Heth & Nevo, 1981).

Two rodent groups have received attention in southern Africa, gerbils *Tatera spp*. and *Gerbillurus spp*. and the vlei rat *Otomys irroratus*. Gerbils are able to recognize conspecifics using species-specific behaviour (pre-mating isolation) and hybrids have high mortality rates (post-mating isolation: Dempster *et al*., 1992, 1993; Dempster, 1996). The vlei rat also displays both pre- and post-mating isolation (Pillay *et al*., 1995a, b, c). Populations of *O. irroratus* that were geographically close together but chromosomally different had reduced interpopulation breeding success, with high pre-weaning mortality of hybrids (Pillay *et al*., 1995b). Pre-mating isolation comprised of high aggression, olfactory preferences for the homotype mates and population-specific courtship behaviour (Pillay *et al*., 1995a, c)

1.3) The study animal *Rhabdomys*

The African four striped mouse, *Rhabdomys* (Sparrman, 1784) is a murid rodent with a wide distribution south of the Sahara (Skinner & Chimimba, 2005). Adult males and females are similar in size, with the males weighing a mean of 43g and the females 41g (Pillay, 2000b). Their large numbers and ability to exploit varied habitats means that *Rhabdomys* is an ideal model for testing hypotheses and theoretical concepts in behavioural ecology, reproduction, evolution and phylogeny. In addition there is a plethora of research on this African rodent species by Pillay and colleagues.

There are two karyotypic forms of *Rhabdomys* in South Africa (Mahida *et al*., 1999). Several populations show a 2N = 48 form whereas a 2N = 46 form, which is the result of a Robertsonian fusion, is found in some populations in the northern parts, particularly in the highveld region of South Africa (Ducroz *et al*., 1999; Rambau *et al*.,

2003). This Robertsonian fusion in *Rhabdomys* is unlikely to influence interfertility (Pillay, 2000b). *Rhabdomys* was historically considered as a monospecific genus (De Graaff, 1981), but a recent phylogeographical study based on studies of mtDNA revealed two major lineages which supports the existence of two putative species: *R. pumilio* (clade 2) which occurs in the western (xeric) parts of South Africa and displays the ancestral karyotype of the genus (2n = 48) and *Rhabdomys dilectus* (De Winton (1897)**;** clade 1) which is found in mesic areas (Rambau *et al*., 2003). Clade 1, based on diploid number dichotomy and mtDNA sequence divergence, can be further divided into two subspecies: *Rhabdomys dilectus chakae* (2n = 48) in the east and south-east of South Africa and *R. dilectus dilectus* (2n = 46; (Wroughton, 1905)) in the north (Rambau *et al*., 2003).

The three groups differ in their sociality with *R. pumilio* (clade 2), at least those found in the arid succulent karoo, forming social groups made up of multiple adults of both sexes which share a nest and territory (Schradin, 2004; Schradin & Pillay, 2005). In contrast, *R. d. chakae* (clade 1), found in the moist grasslands of South Africa, is solitary (Schradin & Pillay, 2005). *Rhabdomys dilectus dilectus* (clade 1) appears to have a similar social organization to *R. d. chakae* (Brooks, 1974).

In studies of two *R. pumilio* (clade 2; Alice and Goegap) and one *R.d. dilectus* (clade 1; Irene) allopatric populations that represented the extremes in the distribution of *Rhabdomys* (> 900 km apart), it has been shown that females in two of the populations (Alice and Irene) selected males from their own population over males of a different population in choice tests. The Goegap population was the exception as females showed equal preference for its own population males and males from Alice (both populations being *R. pumilio*; Pillay, 2000a). Interpopulation hybrids were rare, indicating that pre-mating rather than post-zygotic barriers were responsible for the reproductive isolation between the populations (Pillay, 2000b). In another study, Lancaster (2001) showed that for two closely-occurring populations (< 100 km), from the same clade (clade 1) but representing the two sub-species, *Rhabdomys dilectus dilectus* (Irene) and *R. d. chakae* (Suikerbosrand Nature Reserve), neither sex showed a choice for the same or different population mates, and interbreeding was successful, but there was F2 generation hybrid failure, due to hybrid sterility. Lancaster's study therefore indicates that striped mice in these two closely-situated populations were reproductively isolated because of post-zygotic

(hybrid) breakdown which contradicted the studies by Pillay (2000a, b). This suggests that patterns of divergence across the distributional range of the taxon is unpredictable. Next, Ahamed (2006) studied an *R. d. chakae* population (Midrand) and an *R .d. dilectus* population (Irene) that were 15 km apart which were behaviourally incompatible (i.e. highly aggressive) and therefore produced few hybrids. Finally, Pillay *et al*. (2006) tested the divergence in mate recognition, using habituation-discrimination/generalization tests and two-way choice tests, between the two putative species *R. pumilio* and *R. dilectus*. Two geographically distinct populations per taxon were tested and it was found that there was no within taxon variation but there was a difference in the odour characteristic between *R. pumilio* and *R. dilectus*, indicating that phylogeny, rather than geographic distance, was a key predictor of divergence (Pillay *et al*., 2006). However, it was not possible to rule out ecology as a driver of this difference, as *R. pumilio* occurs in arid regions while *R. dilectus* occurs in mesic areas (Pillay *et al*., 2006). The previous studies on *Rhabdomys* have thus shown varied patterns of divergence (either pre- or post-mating), with phylogeny (populations from different taxa are more likely to have diverged from one another) being more important than geography (little within taxon variation) in explaining divergence and reproductive compatibility.

To investigate the effects of phylogeny and geography on reproductive isolation through divergence of behaviour, odour and reproduction between different *Rhabdomys* populations, I studied the interfertility and scent perception between four populations, representing three taxonomic groups: Jonkershoek (*R. pumilio* 2n = 48, clade 2), Goegap (*R. pumilio* 2n = 48, clade 2), Irene (*R. d. dilectus* 2n = 46, clade 1) and Suikerbosrand (*R. d. chakae* 2n = 48, clade 1). The two *R. pumilio* populations, Jonkershoek and Goegap, are situated far apart (> 900 km), whereas the two *R. dilectus* subspecies, Irene and Suikerbosrand, are situated < 100 km apart. The two *R. pumilio* populations and the two *R. dilectus* subspecies are situated > 1000 km apart. Therefore, my study was designed to consider the importance of geographic distance (Jonkershoek and Goegap; *R. pumilio* and *R. dilectus*) and phylogeny (all four populations). While studies of reproductive isolation have been done on the Goegap, Irene and Suikerbosrand striped mouse populations, the Jonkershoek population has not been studied. The Jonkershoek population was chosen for this study, since it is situated far away from the other three populations and it occurs in a

different habitat (shrubland, rather than desert or grassland) from the other three populations and apparently displays an intermediate sociality between the other populations (N. Pillay, pers. comm.).

1.4) Aims

This study involved breeding studies, habituation-discrimination/generalization tests of the odour based mate recognition system, and mate choice using olfactory cues. My study tested for the existence of pre-mating and post-mating reproductive barriers between different populations. Aims and appropriate predictions are provided below. For the two-way choice and habituation-discrimination/generalization tests, only female choice was considered, as mate choice is largely made by female striped mice (Bennett, 1999) and is more easily detected in female *Rhabdomys* (Bennett & Pillay, 2001).

Aim 1. To determine the interfertility between the four *Rhabdomys* populations.

Prediction 1. Assuming that the putative species are correct and that phylogeny is a predictor of reproductive isolation, interpopulation pairings between two populations of the same species (i.e. Jonkershoek and Goegap, *R. pumilio*) are expected to have greater reproductive success than pairings between populations that involve different species (Jonkershoek and Irene; Jonkershoek and Suikerbosrand; Goegap and Irene; Goegap and Suikerbosrand) or different sub-species (Suikerbosrand, *R. d. chakae*, and Irene, *R. d. dilectus*).

Prediction 2. The populations of *R. pumilio* (Jonkershoek and Goegap) are situated > 900 km apart. If geographic distance limits gene flow between these populations rather than phylogeny, as assumed above, I expected that these populations are reproductively isolated.

Aim 2. To evaluate the similarities or differences in male odour signal quality and to assess the female's ability to detect these similarities and differences, using habituation-discrimination/generalization tests.

Prediction 1. Females from Jonkershoek and Goegap would perceive signals of males of both these populations as being more similar to signals of Irene and Suikerbosrand males. Similarly, females from Irene and Suikerbosrand would perceive signals of males of both these populations as being more similar than signals of Jonkershoek and Goegap males. This will show a phylogenetic pattern of signal divergence.

Prediction 2. In contrast to the previous prediction, for both Jonkershoek and Goegap females, the olfactory cues of Goegap and Jonkershoek males should be perceived as different from each other due to divergence in geographically distant populations.

Aim 3. To determine the mate choice decisions made by females in two-way choice tests.

Prediction 1. Females will prefer males of their own population to that of a different population

Prediction 2. Females will prefer phylogenetically more similar males than more distant males.

Chapter 2: Materials and Methods

2.1) Study subjects and odour samples

The study subjects used in this experiment were either wild caught or captive F2-F5 born progeny of striped mice derived from four different South African localities (Jonkershoek, Goegap, Suikerbosrand and Irene; Fig 1). The Jonkershoek and Goegap populations represent the *Rhabdomys pumilio* taxon, clade 2, while the Irene population represents the *R. dilectus dilectus* taxon, clade 1, and the Suikerbosrand population represents the *R. dilectus chakae* taxon, clade 1 (Rambau *et al*., 2003).

Figure 1. The geographic location of the *Rhabdomys* populations used in this study (figure not drawn to scale).

Both species of *Rhabdomys* and the two subspecies of *R. dilectus* have wide geographic distributions (Rambau *et al*., 2003), which may include different habitat types

(Pillay *et al*., 2006). Table 1 provides the habitat characteristics of the localities from which the four populations used in this study originated.

Table 1. Grid position, habitat type, range in rainfall (average in brackets), and vegetation type (after Low and Rebelo, 1998) of the four locations from which striped mice of the four populations of *Rhabdomys* originated.

Taxon	**Location**	**Grid position**	**Habitat**	**Rainfall (mm)**	**Vegetation**
R. pumilio	Goegap	29º 37'S 17º 59'E	Desert, semidesert	20-290 (160)	Succulent Karoo
R. pumilio	Jonkershoek	33º 59'S 18º 57'E	Shrubland	250-400 (300)	Central mountain renosterveld
R. d. dilectus	Irene	25º 53'S 28º 18'E	Grassland	650-750 (720)	Rocky highveld grassland
R.d. chakae	Suikerbosrand	26º 31'S 28º 15'E	Grassland	650-750 (710)	Rocky highveld grassland

Striped mice were housed under partially controlled laboratory conditions (23-26°C, 30-50% rH, light regime 14L: 10D, lights on at 05h00). They were housed either singly or in same sex groups (two or three individuals) in Labotec cages (25 x 25 x 12 cm). The cages were provided with a 2 cm layer of wood shavings for bedding, hay for nesting material and a plastic nest box. Striped mice were fed a mixture of sunflower seeds, millet and Capstone Lifetime Balancer pellets (containing protein, essential amino acids and vitamins) every day. Either fresh fruit or vegetables were also provided daily while mouse cubes were given once a week. The striped mice had constant access to water. Individuals of the four populations were housed in separate rooms, so as to eliminate exposure to the scent of individuals of different populations prior to experiments.

2.2) Experimental procedures

2.2.1) Breeding studies

For the breeding study, 57 intrapopulation breeding pairs were established, involving striped mice from Jonkershoek, Goegap, Irene and Suikerbosrand. A total of 152 interpopulation breeding pairs were also established. These were bidirectional (males of one population paired with females of another population and vice versa), involving males and females from all interpopulation combinations. Individuals used in interpopulation pairings were mostly virgins but a few non-virgins were also used to achieve the required sample sizes. The treatments used were: Jonkershoek – Goegap, Jonkershoek – Irene, Goegap – Irene, Jonkershoek – Suikerbosrand, Goegap – Suikerbosrand and Irene – Suikerbosrand. Breeding pairs were maintained as described above, but were fed more mouse cubes to support pregnancy. In addition, a second nest box was included to ensure that the pairs would not be forced to share the same nest box, thereby reducing the potential for high levels of aggression.

Breeding pairs were monitored closely after pairing and those pairs engaging in damaging fights were separated immediately (after Pillay *et al.*, 1995a). The number of pairs that had to be separated due to damaging fights was recorded. Otherwise, pairs were allowed to produce a litter within 50 days. Pairs that did not produce offspring after 50 days were separated. Starting from 20 days after pairing, the cages were inspected daily for litters, as gestation is approximately 23 days (Brooks, 1982). Females from pairs that did not produce offspring were not immediately paired again. Instead, they were house alone to determine whether fertilization had occurred (for successful females, for the most part, produced offspring within 30 days after pairing). Males from pairs that were not successful were used again with some producing offspring with their second partner and some not. I recorded the reproductive success of treatments. For successful pairs (those producing offspring), I recorded the latency from pairing to parturition. Litter size was noted on the day of parturition, when possible, or otherwise on the next day. The sex ratio of the offspring was recorded at weaning (i.e. 20 days of age), whereafter offspring were housed either singly or in same sex groupings (of no more than two individuals) as described above. The mass (in grams) of individual offspring in a litter was determined on days 0, 20,

30, 40, 50 and 60. Growth rate of litters was calculated for pre- (0-20 days) and post-weaning (20-60 days) intervals. Pre-weaning growth rates were calculated using the equation: (Ln (birth mass) – Ln (day 20 mass))/20. Post-weaning growth rates were calculated using the equation: (Ln (day 20 mass) – Ln (day 60 mass))/40. The survival rate of individuals in the litters was also recorded at weaning and at day 60 which was the end of the experiment.

The fertility of hybrid young produced was tested by backcrossing 10 of the hybrids per interpopulation pairing with individuals of the parent stock (after Pillay *et al.*, 1995a; Pillay, 2000b). The reproductive variables mentioned above for intra- and interpopulation treatments were also measured for the hybrid pairings.

2.2.2) Mate choice experiments

Only oestrous females were tested, which was confirmed by vaginal smears taken two hours before the start of every experiment.

Four weeks before mate choice experiments were conducted, all test females and scent donor males were kept singly. The stimulus used in these experiments was the soiled bedding of adult males. I used soiled bedding as the odour source as it is known to be an effective carrier of mate choice signals (Pillay, 2000b). It has been shown that female *Rhabdomys* show virtually identical strength and direction of preference when using wood shavings soiled with faeces and urine (soiled bedding) as they do when presented with a male *Rhabdomys* (Pillay, 2000b). Variation in odours was controlled for by housing the donor males singly and under the same standardized environmental conditions and diet (Pillay *et al*., 2006). To control for individual differences in the volatile and non-volatile components of the bedding between donors, I pooled the bedding from at least four different males to produce a population odour signal (Pillay *et al*., 2006). The soiled bedding was stored at -20ºC before the experiments and was thawed at room temperature immediately before the tests. The soiled bedding was placed in a petri dish (diameter 8.5 cm) during the experiments.

All experiments took place between 08:00 and 12:00 and between 15:00 and 17:00, coinciding with the peak activity period of *Rhabdomys* (Schradin, 2006). The behaviour of all the test females during the experiments was video-recorded.

2.2.2.1) Odour discrimination/generalization tests

One way to assess perception of differences in odour signals is the habituation-discrimination procedure (Halpin, 1986). Two variations of this procedure were used for these experiments. Both of the procedures started with a habituation phase where the test subject was presented with a "habituation" odour. Habituation would occur when the subject's interest in the odour decreased (Todrank & Heth, 2003). For the first procedure, the habituation phase was followed by a discrimination phase during which the test subject was presented with the habituation odour again (but a different sample) and a different test odour at the same time. If the subject spent more time investigating the test odour, it was determined that it perceives the second test odour as different from the habituation odour. The second procedure is known as habituation-generalization (Todrank & Heth, 2003). In this procedure, the two odours presented to the test subject in the test phase were both different to the habituation odour. Therefore, this procedure allowed for the assessment of the similarities between the two test odours and the habituation odour. A statistically significant difference in the time spent investigating one or the other test odour would indicate that they are perceived differently and therefore the odour that is investigated less is regarded as more similar to the habituation odour.

A total of 10-30 females per population were used in the odour discrimination tests. Both virgin and non-virgin females were used. Females were not used more than twice; those that were used twice were exposed to different combination of stimuli and had a rest period of 10 days between tests.

Tests took place in a plexiglas apparatus (Figure 2), consisting of a start box and a test box (both: length = 36 cm, width = 20 cm, height = 16 cm) connected by a short tunnel (internal diameter 4.6 x 18 cm). Between each experiment, the entire apparatus was thoroughly washed with soap, water and a 20% alcohol solution to remove the odour of previous occupants.

The habituation phase lasted for 10 min. This time was chosen as a previous study showed that female *Rhabdomys* had a comparatively slow response time to the test apparatus (Pillay *et al*., 2006). In the habituation phase, the female test subject was placed in the start box, at the wall directly opposite from the entry into the box (Figure 2). There

was first a short familiarization period when the female was prevented from entering the test box as the tunnel entrance was blocked by a piece of cardboard (length = 6 cm, width = 4.5 cm). The cardboard was then removed and the female was allowed to enter the test box and the time she spent sniffing the stimulus was recorded. A 9 min discrimination phase, as suggested by Pillay *et al*. (2006), immediately followed the habituation phase when the test box was replaced by a new test box containing the two other stimuli, which were placed on the floor at the left and right extremities of the new box (Figure 2). Laterality was controlled for by alternating the left and right position of each type of stimulus presented to test females within each population. The time the female spent sniffing each stimulus was recorded. If the test subject was in contact with the stimulus or was within a 1 cm radius of the stimulus and had its nose pointed towards the stimulus, it was recorded as sniffing.

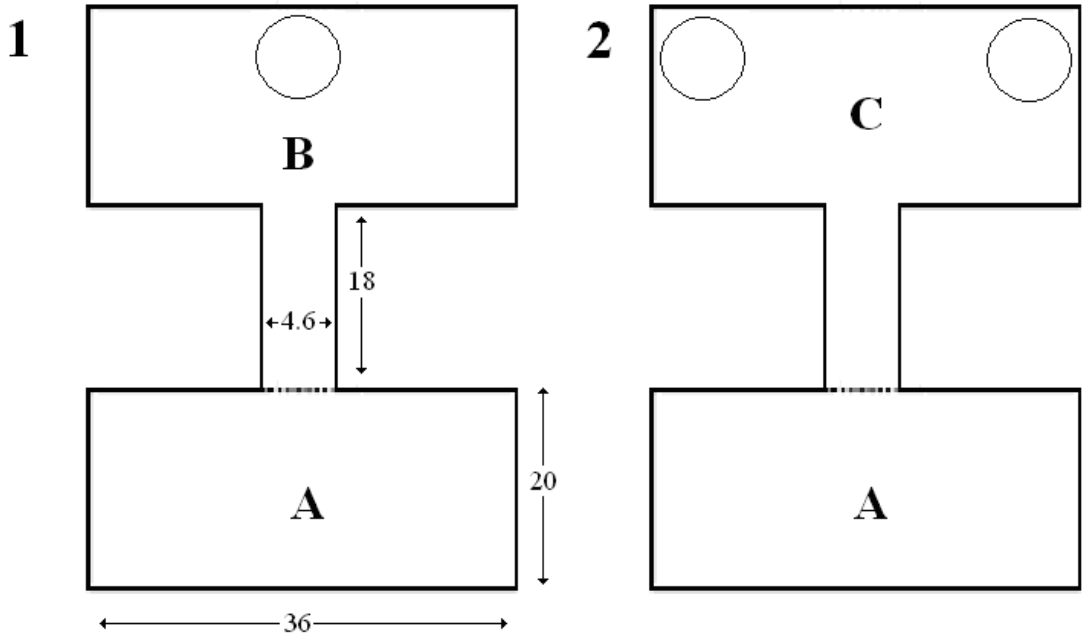

Figure 2. Plexiglas habituation apparatus. 1 is the habituation phase and 2 is the discrimination/generalization phase. A is the start chamber, B is the habituation chamber and C is the discrimination/generalization chamber. The circles represent the position of the petri dishes containing the odiferous bedding. Stippled lines represent the position of the cardboard pieces. Measurements are in centimetres.

2.2.2.2) Preference tests

The preference tests consisted of two-way choice tests, comprising of two treatments. The first was used to assess the preference of striped mice females for male odours of the same population against one of the other three different populations while the second treatment was used to assess the preference between male odours of two different populations. Ten females per treatment were tested (60 females per population). Due to a limited number of striped mice and ethical considerations of using too many individuals, some of the females used in the habituation tests were also used here. There was however a three-month gap between the beginning of the habituation tests and the end of the preference tests. Females once again were not used more than twice and those that were used twice were exposed to different combination of stimuli and had a rest period of 10 days between tests.

Each choice test lasted 18 minutes (after Pillay *et al*., 2006). Experiments were conducted in a choice apparatus built of transparent plexiglas material (Figure 3), consisting of a start box (length = 36 cm, width = 20 cm, height = 16 cm) connected by a Y maze (internal diameter 4.6 cm; main branch: 32 cm long; secondary branches/arms: 22 cm long) to two choice chambers (length = 36 cm, width = 20 cm, height = 16 cm). Between each experiment, the entire apparatus was thoroughly washed with soap, water and a 20% alcohol solution to remove odours from previous occupants.

At the beginning of each test, a female test subject was placed in the start box, with each choice chamber containing a petri dish with 25 g of soiled bedding (collected as described above). The entrance to the Y maze was blocked off by a piece of cardboard (length = 6 cm, width = 4.5 cm) while the subject was given 5 min of familiarization before being allowed to enter the Y maze. Recording was started once the female entered the maze. Laterality was controlled for by alternating the position of the two stimuli (left and right) between tests. The time the female spent in contact with, sniffing or licking each stimulus was recorded.

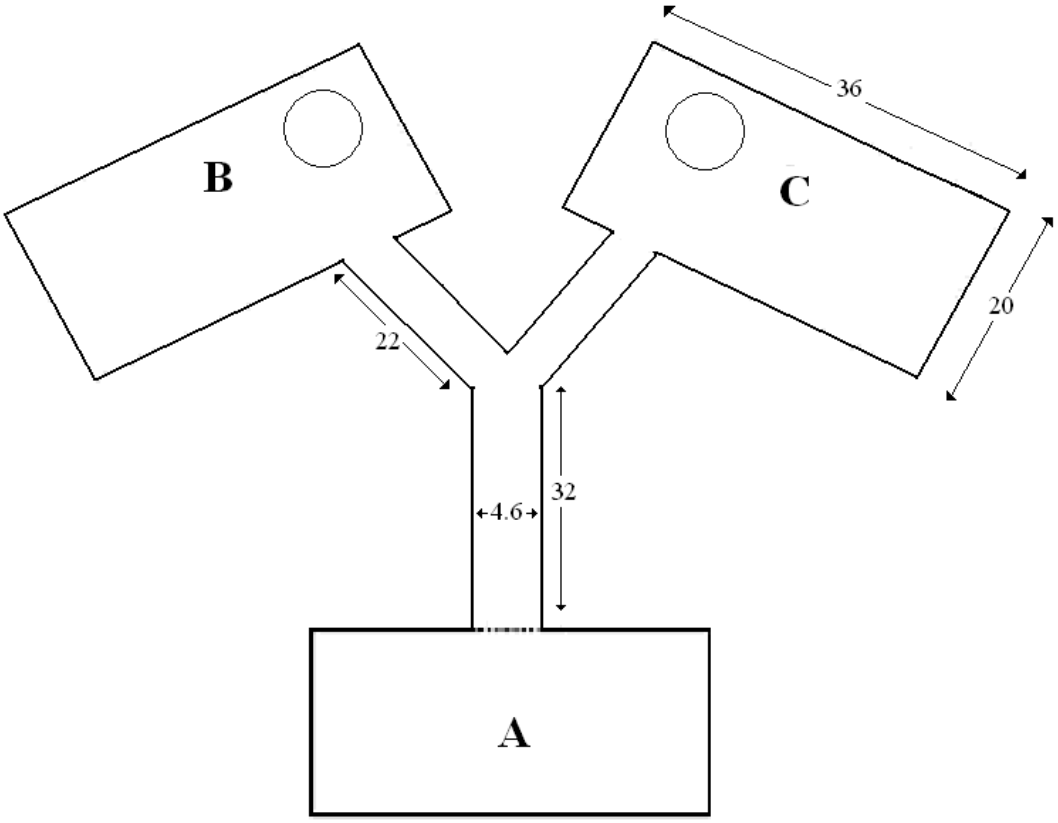

Figure 3. Plexiglas choice apparatus. A is the start chamber and B and C are choice chambers. The circles represent the position of the petri dishes containing the odiferous bedding. Stippled lines represent the position of the cardboard piece. Measurements are in centimetres.

2.3) Data analysis

All data sets were tested for normality and appropriate statistical tests were used. STATISTICA 6 (2001) was used to run all statistical tests. For the breeding experiments, ANCOVA tests were used to assess differences in litter size among treatments; maternal mass at weaning was the covariate. Logistic regression analyses were used to compare reproductive success (the proportion of pairs within a treatment that had offspring), the proportion of pairs that engaged in damaging fights, sex ratio, and the survival of offspring to day 60. For the logistic analyses, 95% confidence intervals (CI) were plotted to identify treatment effects. A General linear model (GLM) was used to compare the latency from

pairing to parturition. A repeated measures ANOVA was used to compare pre- and post-weaning growth rates between treatments. Tukey post hoc tests were used to identify specific differences among the treatments when ANCOVA and GLM analyses were significant at $\propto \leq 0.05$.

For the odour discrimination/generalisation tests, to assess whether habituation had occurred, the habituation phase was separated into two five minute segments and the time that females spent with the stimulus during each of these two segments was compared using a Wilcoxon matched pairs test. Habituation occurred if females showed a significant decline of interest in the habituation odour from the first five minutes onwards. The comparison of the time spent sniffing the scent during the discrimination/generalisation phase was analyzed using a Wilcoxon matched pairs test.

For the preference tests, paired t-tests were used to compare the time spent sniffing the two odours in the Y maze.

Chapter 3: Results

3.1) Breeding studies

Pairing was a significant predictor of reproductive success (Figure 4) between all the pairings (logistic regression: Wald $\chi^2_{17} = 30.19$, $P = 0.025$). The confidence intervals indicate that this difference is mainly due to the high success of the intrapopulation and the Jonkershoek (*R. pumilio*)-Goegap (*R. pumilio*; bi-directional) pairings and a lack of success of the Jonkershoek (*R. pumilio*)-Irene (*R. d. dilectus*), Jonkershoek (*R. pumilio*)-Suikerbosrand (*R. d. chakae*), Goegap (*R. pumilio*)-Irene (*R. d. dilectus*), Goegap (*R. pumilio*)-Suikerbosrand (*R. d. chakae*) and Irene (*R. d. dilectus*)- Suikerbosrand (*R. d. chakae*) pairings. As can be seen in Figure 4, none of the pairings between the *R. pumilio* populations (Jonkershoek or Goegap) and the *R. dilectus* populations (Suikerbosrand or Irene) or between the two sub-species populations (Irene, *R. d. dilectus*, and Suikerbosrand, *R.d. chakae*) produced hybrid offspring, apart from one hybrid pup from a Jonkershoek-Irene pair that died a few days after birth. The only successful interpopulation pairing were the two *R. pumilio* populations, Jonkershoek and Goegap. There were a similar proportion of reproductively successful pairs whether the male was from the Jonkershoek population and the female from the Goegap population or vice versa. This was not the case for the backcross hybrids. Hybrids produced from a Goegap father and a Jonkershoek mother had a marked decrease in reproductive success (three pairs out of 10 were successful) when compared to hybrids from a Jonkershoek father and a Goegap mother (nine out of 10 pairs were successful). The intrapopulation and the backcross Jonkershoek-Goegap pairings had the greatest reproductive success (Figure 4).

Pairing was not a significant predictor of the percentage of pairs fighting (Wald $\chi^2_{17} = 3.45$, $P = 0.999$; Table 2). Pairs were sometimes separated before they had the chance to fight, but even when those pairs that were separated early were not considered in the analyses, there is still no significant difference (Wald $\chi^2_{17} = 7.35$, $P = 0.979$). However, while there was no statistically significant difference, none of the intrapopulation pairs were separated for fighting while the Jonkershoek (*R. pumilio*)-Goegap (*R. pumilio*) pairings had only a small

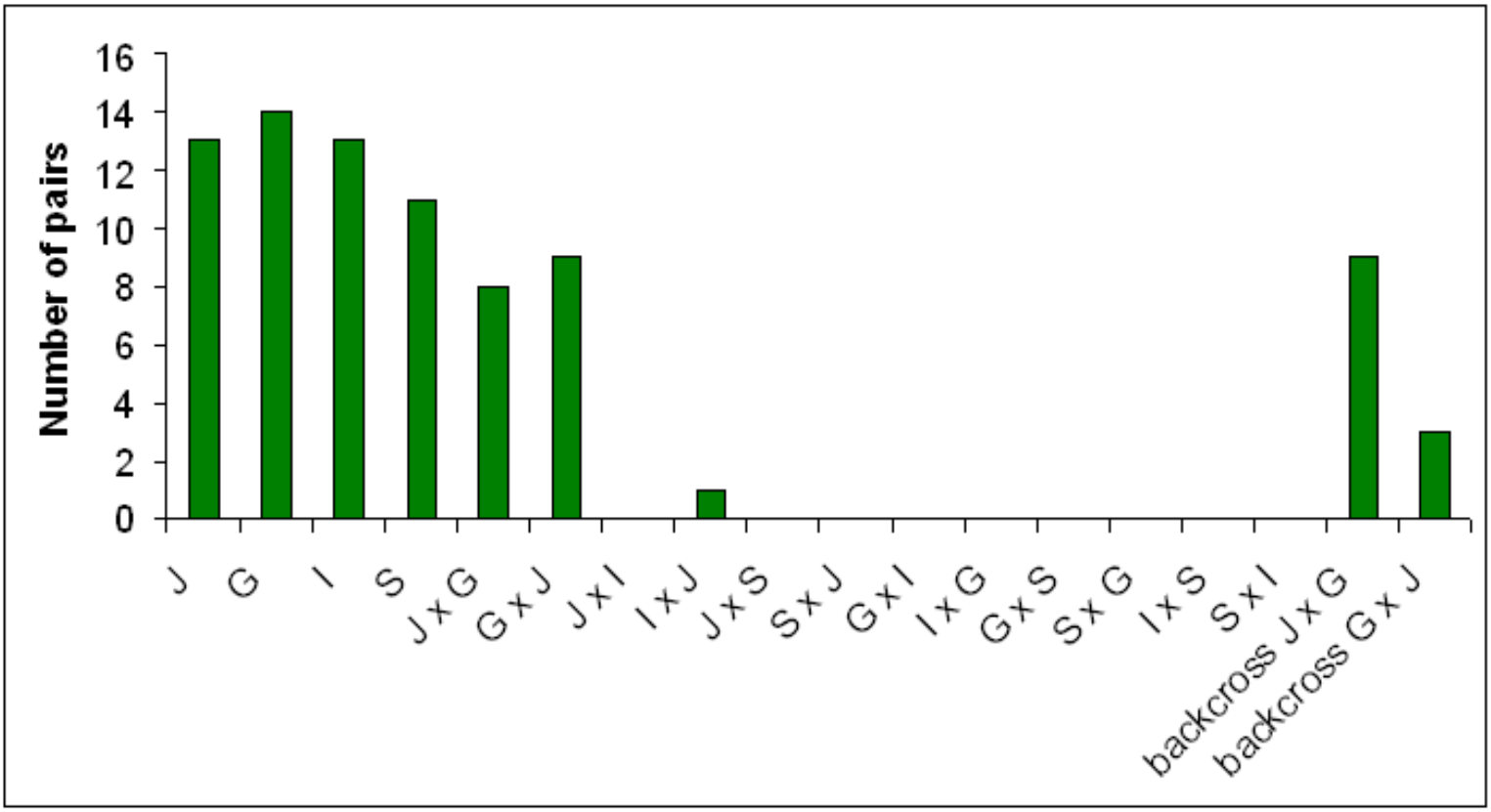

Figure 4. The number of successful pairs in intra- and interpopulation *Rhabdomys* pairs and their hybrids. Interpopulation and backcross pairings indicated as male x female. J = Jonkershoek, G = Goegap, I = Irene and S = Suikerbosrand.

percentage of fighting. The highest percentages (between 20-30%) for fighting were found in the Goegap (*R. pumilio*)-Irene (*R. d. dilectus*) and Goegap (*R. pumilio*)-Suikerbosrand (*R. d. chakae*) pairings. The Jonkershoek (*R. pumilio*)-Irene (*R. d. dilectus*) pairing was the only pairing that showed a large difference in the percentage of pairs fighting, depending on the male-female combination: when a Jonkershoek male was paired with an Irene female, there were no incidents of fighting, but when an Irene male was paired with a Jonkershoek female, up to 50% of the pairs (discounting the pairs that were separated before they could fight) had to be separated due to fighting (Table 2).

Table 2. Incidence of damaging fights, litter sex ratio and interval between pairing and production of the first litter for striped mice used in intra- and interpopulation pairs and their hybrids. Interpopulation and backcross pairings indicated as male x female. *n* = sample size; dashes indicate no data available.

Pairings	% Fighting	Interval between pairing and first litter		Sex ratio F : M
		n	$\bar{X}$ (SE)	
Same population:				
Jonkershoek (*R. pumilio*)	0	13	26.2(0.8)	48 : 49
Goegap (*R. pumilio*)	0	14	28 (1.2)	55 : 52
Irene (*R. d. dilectus*)	0	13	29.8 (3)	51 : 43
Suikerbosrand (*R. d. chakae*)	0	11	28 (1.3)	41 : 40
Different population:				
Jonkershoek (*R. pumilio*) x Goegap (*R. pumilio*)	7.7	8	29.5 (1)	25 : 12
Goegap (*R. pumilio*) x Jonkershoek (*R. pumilio*)	7.1	9	28.1 (1)	28 : 19
Jonkershoek (*R. pumilio)* x Irene (*R. d. dilectus*)	0	-	-	-
Irene (*R. d. dilectus*) x Jonkershoek (*R. pumilio*)	21.1	1	33	-
Jonkershoek (*R. pumilio*) x Suikerbosrand (*R. d. chakae*)	0	-	-	-
Suikerbosrand (*R. d. chakae*) x Jonkershoek (*R. pumilio*)	0	-	-	-
Goegap (*R. pumilio*) x Irene (*R. d. dilectus*)	20	-	-	-
Irene (*R. d. dilectus*) x Goegap (*R. pumilio*)	30	-	-	-
Goegap (*R. pumilio*) x Suikerbosrand (*R. d. chakae*)	20	-	-	-
Suikerbosrand (*R. d. chakae*) x Goegap (*R. pumilio*)	20	-	-	-
Irene (*R. d. dilectus*) x Suikerbosrand (*R. d. chakae*)	0	-	-	-
Suikerbosrand (*R. d. chakae*) x Irene (*R. d. dilectus*)	0	-	-	-
Backcrosses with offspring of:				
Jonkershoek (*R. pumilio*) x Goegap (*R. pumilio*)	0	9	33.6(2.1)	18 : 15
Goegap (*R. pumilio*) x Jonkershoek (*R. pumilio*)	10	3	28 (0.7)	3 : 5

Pairing did not influence the time from pairing to first litter (Table 2) for any of the pairs that produced offspring (GLM: $F_{7, 74} = 1.12$, $P = 0.359$). Most of the litters for all the pairings were born between day 26 and day 33 after pairing. Additionally, pairing did not affect the sex ratio (Wald $\chi^2_7 = 5.46$, $P = 0.604$), although intrapopulation and hybrid pairings produced sex ratios close to parity while the interpopulation pairings did not (Table 2). In interpopulation pairings, there were more females than males produced.

There was an indication that there could be post-zygotic barriers to reproductive success between the Jonkershoek and Goegap populations. Pairing was a significant predictor of litter size (ANCOVA: $F_{7, 70} = 6.76$, $P < 0.001$; Figure 5); maternal mass did not significantly influence litter size ($F_{1, 70} = 0.47$, $P = 0.494$). Post hoc tests showed that intrapopulation pairings had a greater litter size than the interpopulation pairs and their hybrids. The hybrids that had a Jonkershoek (*R. pumilio*) father and a Goegap (*R. pumilio*) mother had the lowest number of offspring of all the pairings.

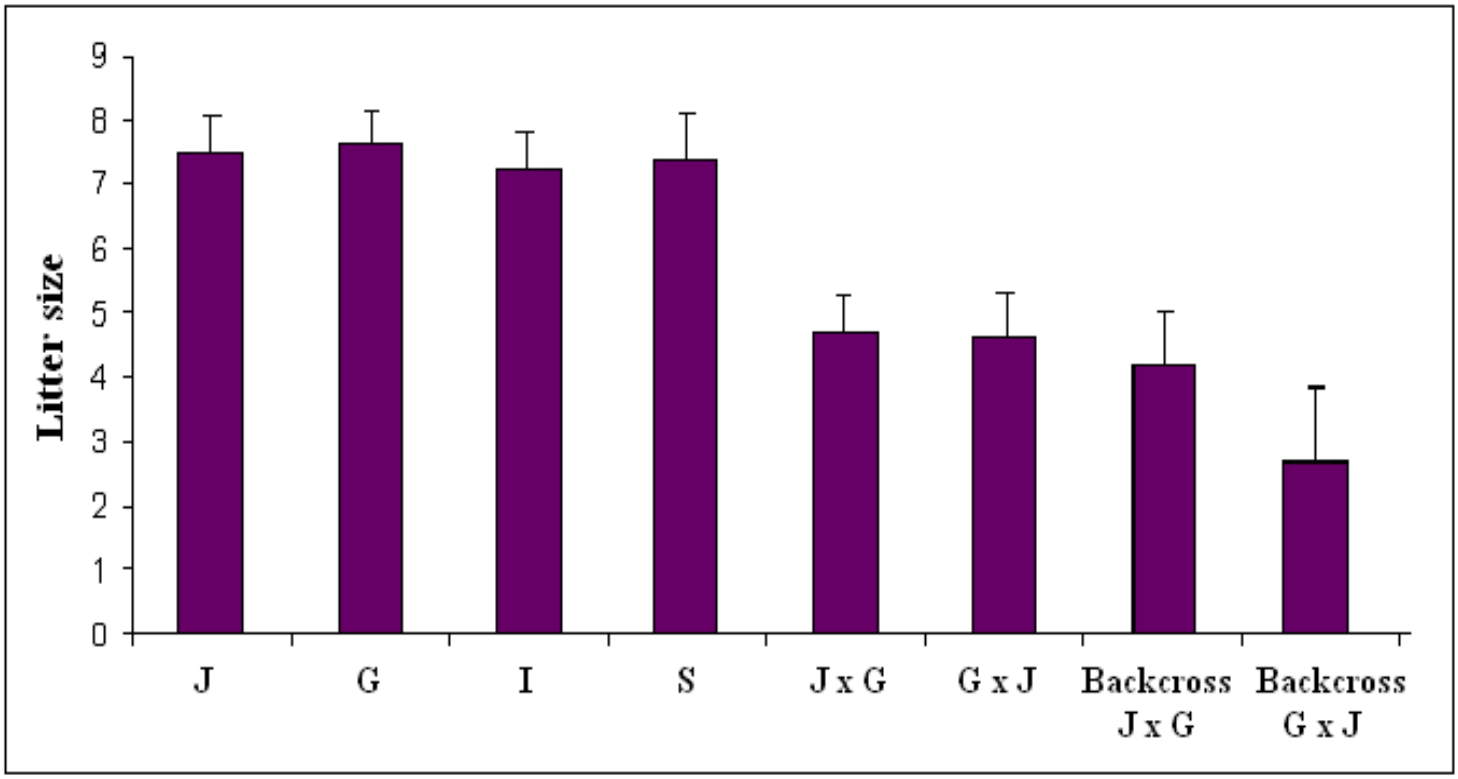

Figure 5. Mean (+SE) litter size of successful intra- and interpopulation *Rhabdomys* pairs and their hybrids. Interpopulation and backcross pairings indicated as male x female. J = Jonkershoek, G = Goegap, I = Irene and S = Suikerbosrand.

Treatment was not a significant predictor of growth rate of offspring of the intrapopulation pairings and the interpopulation hybrids before weaning or after weaning ($F_{5, 63} = 1.20$, $P = 0.318$; $F_{5, 63} = 0.52$, $P = 0.760$; Table 3). Moreover, pairing was not a significant predictor of the survival of offspring from the intrapopulation pairs, the

interpopulation hybrids and the backcross hybrids (Wald $\chi^2_7 = 0.19$, $P = 0.999$; Table 3). For all the intrapopulation pairings, there were a few offspring deaths, generally one or two.

Table 3. Survival of striped mice offspring (±SE) of the intrapopulation pairs, hybrids of the Jonkershoek-Goegap pairings and their backcrosses, from birth to day 60. Interpopulation and backcross pairings indicated as male x female. *n* = sample size; dashes indicate no data available.

Pairing	*n*	Birth		Weaning			Day 60		
		$\bar{X}$ survived	$\bar{X}$ deaths	$\bar{X}$ survived	$\bar{X}$ deaths	$\bar{X}$ growth rate	$\bar{X}$ survived	$\bar{X}$ deaths	$\bar{X}$ growth rate
Same population:									
Jonkershoek (*R. pumilio*)	13	7.46 (0.60)	0	7.15 (0.56)	1.33 (0.33)	0.085 (0.002)	7.08 (0.59)	1	0.013 (0.001)
Goegap (*R. pumilio*)	14	7.64 (0.58)	0	7.64 (0.58)	0	0.091 (0.003)	7.64 (0.58)	0	0.015 (0.001)
Irene (*R. d. dilectus*)	13	7.23 (0.51)	0	6.92 (0.37)	2	0.090 (0.003)	6.84 (0.34)	1	0.014 (0.001)
Suikerbosrand (*R. d. chakae*)	11	7.36 (0.66)	0	6.91 (0.59)	1.67 (0.67)	0.091 (0.002)	6.91 (0.59)	0	0.014 (0.001)
Different population:									
Jonkershoek (*R. pumilio*) x Goegap (*R. pumilio*)	8	4.63 (0.63)	0	4.63 (0.63)	0	0.092 (0.003)	4.63 (0.63)	0	0.015 (0.001)
Goegap (*R. pumilio*) x Jonkershoek (*R. pumilio*)	10	4.70 (0.50)	0	4.70 (0.50)	0	0.088 (0.003)	4.70 (0.50)	0	0.013 (0.001)
Backcrosses with offspring of:									
Jonkershoek (*R. pumilio*) x Goegap (*R. pumilio*)	9	4.57 (0.78)	3 (2.00)	4.57 (0.78)	0	-	4.57 (0.78)	0	-
Goegap (*R. pumilio*) x Jonkershoek (*R. pumilio*)	3	2.67 (1.20)	0	2.67 (1.20)	0	-	2.67 (1.20)	0	-

3.2) Odour discrimination/generalization tests

To establish whether habituation occurred during the habituation-discrimination tests, I compared the time spent sniffing the habituation odour in two 5 min segments (Figure 6). In all tests, females showed a significant decline of interest in the habituation odour from the first five minutes onwards ($T = 18.01$, $n = 150$, $P < 0.001$), indicating that they had become habituated to the stimulus during the habituation phase.

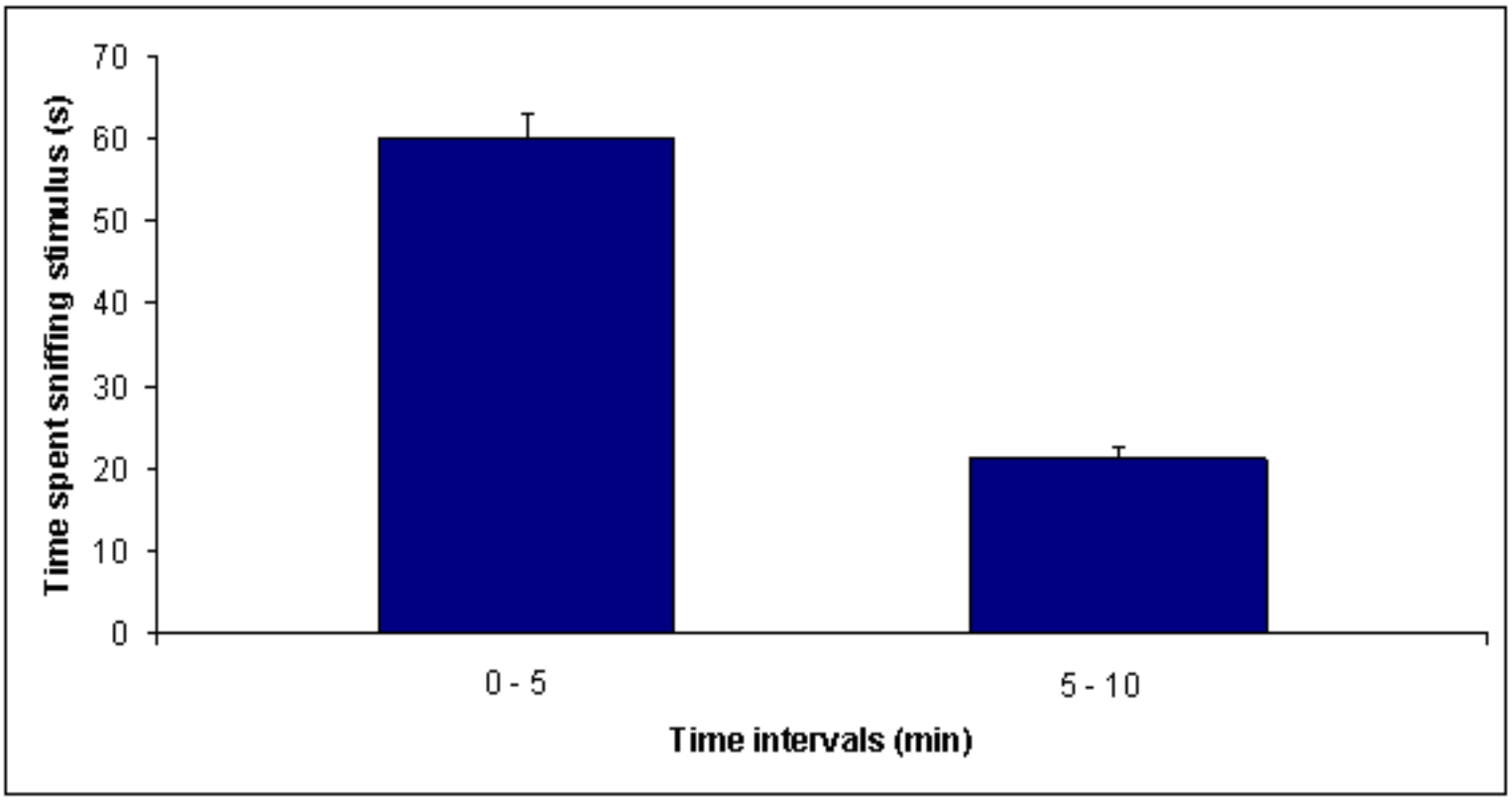

Figure 6. Habituation to stimuli during a total of 150 habituation-discrimination and habituation-generalization tests. Mean (+SE) time in seconds spent sniffing habituation odour during two five-minute segments.

A) Differences in odour and female perception across taxa

A summary of the results of the habituation-discrimination and habituation-generalisation experiments is given in Table 4. The *R. pumilio* populations (Jonkershoek

Table 4. Comparison of male odour as perceived by females of four *Rhabdomys* populations in habituation-discrimination and habituation-generalization experiments. Time given as mean (±SE) spent with each odour during the discrimination phase. Black dots = significant choices in discrimination tests (Wilcoxon tests).

Experiment subject	Habituation	Discrimination	Time (s)	Wilcoxon matched pairs
A) Differences in odour and female perception across taxa:				
a) *R. pumilio* (Jonkershoek)	*R. pumilio* (Jonkershoek)	*R. pumilio* (Goegap) *R. d. dilectus* (Irene)	73 (13.64) 112.80 (23.05)	T = 13, *P* = 0.139
b) *R. pumilio* (Goegap)	*R. pumilio* (Goegap)	*R. d. chakae* (Suikerbosrand) *R. pumilio* (Goegap)	96.52 (7.78) • 14.50 (3.04)	T = 1, *P* = 0.004
c) *R. pumilio* (Goegap)	*R. pumilio* (Goegap)	*R. d. dilectus* (Irene) *R. pumilio* (Goegap)	90.36 (4.86) • 20.24 (2.10)	T = 0, *P* = 0.002
d) *R. d. dilectus* (Irene)	*R. d. dilectus* (Irene)	*R. d. chakae* (Suikerbosrand) *R. d. dilectus* (Irene)	45.68 (5.42) • 16.40 (1.96)	T = 1, *P* = 0.004
e) *R. d. chakae* (Suikerbosrand)	*R. d. chakae* (Suikerbosrand)	*R. d. dilectus* (Irene) *R. d. chakae* (Suikerbosrand)	70.04 (3.41) • 28.51 (8.31)	T = 1, *P* = 0.004
f) *R. pumilio* (Goegap)	*R. d. chakae* (Suikerbosrand)	*R. d. dilectus* (Irene) *R. d. chakae* (Suikerbosrand)	24.69 (4.34) 29.70 (6.01)	T = 33, *P* = 0.625
g) *R. pumilio* (Goegap)	*R. pumilio* (Goegap)	*R. pumilio* (Jonkershoek) *R. d. dilectus* (Irene)	36.10 (7.91) 61.60 (6.83) •	T = 8, *P* = 0.047
h) *R. pumilio* (Jonkershoek)	*R. pumilio* (Goegap)	*R. pumilio* (Jonkershoek) *R. d. dilectus* (Irene)	31.40 (4.81) 77.30 (7.13) •	T = 0, *P* = 0.005
i) *R. d. dilectus* (Irene)	*R. d. dilectus* (Irene)	*R. pumilio* (Goegap) *R. d. dilectus* (Irene)	65.8 (10.21) • 21.5 (4.46)	T = -3.7, *P* = 0.005
j) *R. pumilio* (Jonkershoek)	*R. pumilio* (Jonkershoek)	*R. pumilio* (Jonkershoek) *R. d. dilectus* (Irene)	55.30 (11.01) 103.90 (7.48) •	T = 2, *P* = 0.009
k) *R. d. chakae* (Suikerbosrand)	*R. d. chakae* (Suikerbosrand)	*R. d. chakae* (Suikerbosrand) *R. pumilio* (Jonkershoek)	26.2 (6.47) 56 (8.48) •	T = 5, *P* = 0.022
l) *R. d. chakae* (Suikerbosrand)	*R. d. chakae* (Suikerbosrand)	*R. pumilio* (Goegap) *R. pumilio* (Jonkershoek)	52.4 (11.63) 63 (19.14)	T = 24, *P* = 0.721
m) *R. d. chakae* (Suikerbosrand)	*R. d. chakae* (Suikerbosrand)	*R. d. dilectus* (Irene) *R. pumilio* (Jonkershoek)	28.2 (5.02) 61.6 (11.06) •	T = 0, *P* = 0.005
n) *R. pumilio* (Jonkershoek)	*R. pumilio* (Jonkershoek)	*R. d. chakae* (Suikerbosrand) *R. pumilio* (Jonkershoek)	55.8 (9.25) • 21.2 (3.92)	T = 3.5, *P* = 0.007
o) *R. pumilio* (Jonkershoek)	*R. pumilio* (Jonkershoek)	*R. pumilio* (Goegap) *R. d. chakae* (Suikerbosrand)	18.9 (2.78) 66.8 (9.04) •	T = 5.2, *P* < 0.001
B) Similarities of odour and female perception across taxa:				
p) *R. pumilio* (Jonkershoek)	*R. pumilio* (Jonkershoek)	*R. d. chakae* (Suikerbosrand) *R. d. dilectus* (Irene)	50.4 (6.40) 55.8 (5.06)	T = -0.9, *P* < 0.001
q) *R. pumilio* (Goegap)	*R. d. dilectus* (Irene)	*R. pumilio* (Goegap) *R. d. chakae* (Suikerbosrand)	91.73 (13.39) • 13.50 (1.66)	T = 0, *P* = 0.002
r) *R. pumilio* (Goegap)	*R. d. chakae* (Suikerbosrand)	*R. pumilio* (Goegap) *R. d. dilectus* (Irene)	87.56 (10.38) • 14.94 (1.78)	T = 0, *P* = 0.002
s) *R. pumilio* (Goegap)	*R. pumilio* (Goegap)	*R. d. chakae* (Suikerbosrand) *R. d. dilectus* (Irene)	56.59 (3.74) 69.74 (8.46)	T = 33, *P* = 0.625
t) *R. d. chakae* (Suikerbosrand)	*R. pumilio* (Goegap)	*R. d. chakae* (Suikerbosrand) *R. d. dilectus* (Irene)	81.34 (6.99) 95.02 (11.13)	T = 19, *P* = 0.432
u) *R. d. dilectus* (Irene)	*R. d. dilectus* (Irene)	*R. pumilio* (Jonkershoek) *R. pumilio* (Goegap)	22.50 (4.86) 52.40 (11.63) •	T = 4, *P* = 0.017
v) *R. d. dilectus* (Irene)	*R. d. dilectus* (Irene)	*R. pumilio* (Jonkershoek) *R. d. dilectus* (Irene)	52.20 (11.44) 40.70 (10.03)	T = 19, *P* = 0.386
w) *R. d. dilectus* (Irene)	*R. pumilio* (Jonkershoek)	*R. d. dilectus* (Irene) *R. pumilio* (Goegap)	60 (10.0) • 23.5 (5.60)	T = 0, *P* = 0.005
C) Similarities in odour qualities and female perception within taxa:				
x) *R. pumilio* (Goegap)	*R. pumilio* (Goegap)	*R. pumilio* (Jonkershoek) *R. pumilio* (Goegap)	35.10 (4.74) 26.80 (4.73)	T = 9.50, *P* = 0.067
D) Differences in odour qualities and female perception within taxa:				
y) *R. pumilio* (Jonkershoek)	*R. pumilio* (Jonkershoek)	*R. pumilio* (Jonkershoek) *R. pumilio* (Goegap)	46.1 (6.66) 98.6 (6.89) •	T = 0, *P* = 0.005

and Goegap) could discriminate between odour stimuli of male *R. pumilio* and those of *R.d. dilectus* (Irene; lines c, h and j) and *R.d. chakae* (Suikerbosrand; line b). The Jonkershoek (*R. pumilio*) females could also discriminate their own population scent from that of Goegap (*R. pumilio*; line a). Goegap (*R. pumilio*) females could not distinguish between Irene (*R. d. dilectus*) and Suikerbosrand odours (*R. d. chakae*; line f). Irene (*R. d. dilectus*) females could discriminate between the odours of their own population and those of Suikerbosrand (*R. d. chakae*; line d) and Goegap (*R. pumilio*; line i). Suikerbosrand (*R. d. chakae*) females could discriminate between odours of their own population males and those of Irene (*R. d. dilectus*; line e) and Jonkershoek (*R. pumilio*; line k). Suikerbosrand (*R. d. chakae*) females could not discriminate between the two *R. pumilio* populations (line l). The Irene (*R. d. dilectus*) females, however, did not treat the two *R. pumilio* population odours as the same. They could discriminate between the Goegap odour and their own (line i) but not between the Jonkershoek odour and their own (line s). The two *R. pumilio* populations (Jonkershoek and Goegap) and the *R. d. chakae* population (Suikerbosrand) perceived odours of males of both subspecies, *R. d. chakae* and *R. d. dilectus* (Irene) as being equally different from the *R. pumilio* odours. The two subspecies, *R. d. chakae* (Suikerbosrand) and *R. d. dilectus* (Irene), could also differentiate between each other's odours (line d and e). Jonkershoek (*R. pumilio*) females could discriminate between the *R. pumilio* population odours and the Suikerbosrand (*R. d. chakae*) population odour (line n and o).

B) Similarities of odour and female perception across taxa

Neither Jonkershoek (*R. pumilio*) nor Goegap (*R. pumilio*) females could distinguish between the two sub-species, Irene (*R. d. dilectus*) and Suikerbosrand (*R. d. chakae*; lines p, q, r and s). Suikerbosrand (*R. d. chakae*) females perceived Irene (*R. d. dilectus*) and Suikerbosrand (*R. d. chakae*) scents as equally different to Goegap (*R. pumilio*; line t). Irene (*R. d. dilectus*) females could distinguish between Jonkershoek (*R. pumilio*) and Goegap (*R. pumilio*) scents but not between its own population scent and Jonkershoek (lines u and v). Therefore, it seems that Irene (*R. d. dilectus*) females perceived the Jonkershoek (*R. pumilio*) odour as being similar to its own. However, Irene (*R. d. dilectus*) females still perceived the Jonkershoek (*R. pumilio*) and Goegap (*R.

pumilio) odours as being similar to each other when compared to its own population scent (line w). Overall, the odour stimuli of *R. d. dilectus* and *R. d. chakae* males were perceived as more similar to each other than to those of male *R. pumilio* (e.g. lines m, s, w and h).

C) Similarities in odour qualities and female perception within taxa

Goegap (*R. pumilio*) females could not distinguish between male odours of their own population and those from Jonkershoek (*R. pumilio*; line x).

D) Differences in odour qualities and female perception within taxa

Jonkershoek females could differentiate between the odour of males of their own population and of males of distant populations of the same taxon (Goegap, *R. pumilio*), whereas the Goegap females could not (line x and y).

3.3) Preference

As seen in Figure 7a, Jonkershoek females (*R. pumilio*) preferred the odour of their own population males to those of different population males (Jonkershoek-Goegap: t_9 = 2.38, P = 0.042; Jonkershoek-Irene: t_9 = 2.41, P = 0.040; Jonkershoek-Suikerbosrand: t_9 = 2.65, P = 0.027). When given the choice between the odour of the Goegap males, which are also *R. pumilio*, and the Suikerbosrand (*R. d. chakae*) and Irene males (*R. d. dilectus*), the Jonkershoek females preferred the Goegap scent (Goegap-Irene: t_9 = 2.39, P = 0.041; Goegap-Suikerbosrand: t_9 = 4.78 2.41, P < 0.001). When only given a choice between the Irene and Suikerbosrand male scents, the Jonkershoek females showed no preference for either (Irene-Suikerbosrand: t_9 = 0.55, P = 0.593).

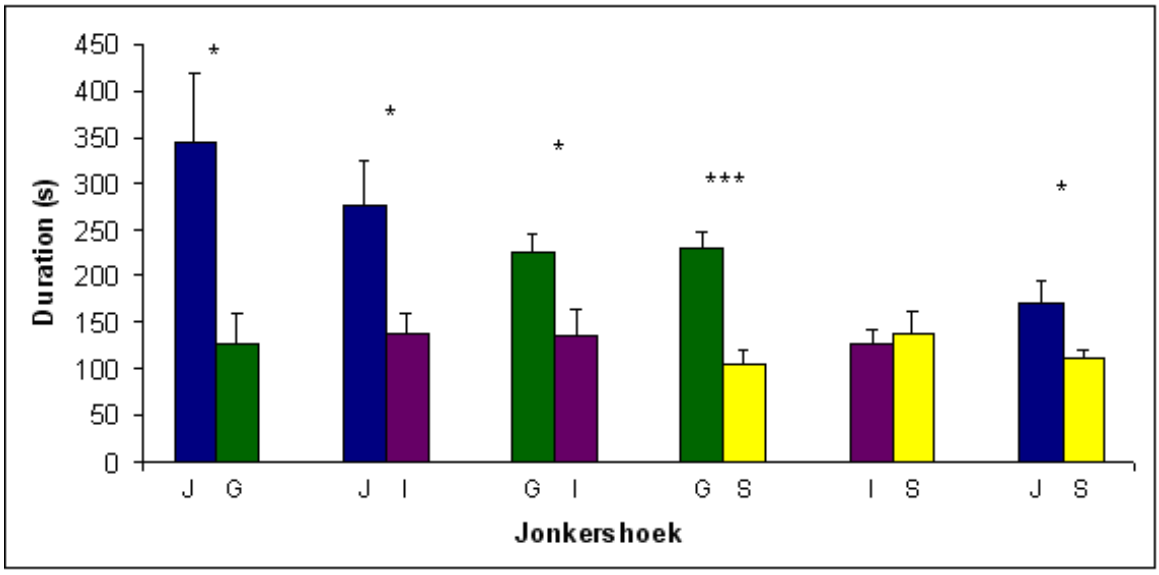

Figure 7a. Mean (+ SE) duration of visits made by Jonkershoek females to odour sources of males from four *Rhabdomys* populations in two-way choice tests. J = Jonkershoek, G = Goegap, I = Irene and S = Suikerbosrand. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

When given a choice between the scent of their own population males and those of the *R. dilectus* (Suikerbosrand and Irene) males (Figure 7b), the Goegap (*R. pumilio*) females followed the same trend as the Jonkershoek (*R. pumilio*) females and showed a preference for their own population males (Goegap-Irene: $t_9 = 7.66$, $P < 0.001$; Goegap-Suikerbosrand: $t_9 = 6.38$, $P < 0.001$). However, unlike the Jonkershoek females, Goegap females had no preference when given the choice between their own population males and the Jonkershoek males ($t_9 = 0.09$, $P = 0.927$). The Goegap females also chose *R. pumilio* (Jonkershoek) males over the *R. dilectus* males (Jonkershoek-Irene: $t_9 = 2.27$, $P = 0.049$; Jonkershoek-Suikerbosrand: $t_9 = 2.48$, $P = 0.035$). When given a choice only between the Irene (*R. d. dilectus*) and Suikerbosrand (*R. d. chakae*) males, the Goegap females showed no preference for either (Irene-Suikerbosrand: $t_9 = 0.89$, $P = 0.396$), the same result as the Jonkershoek females.

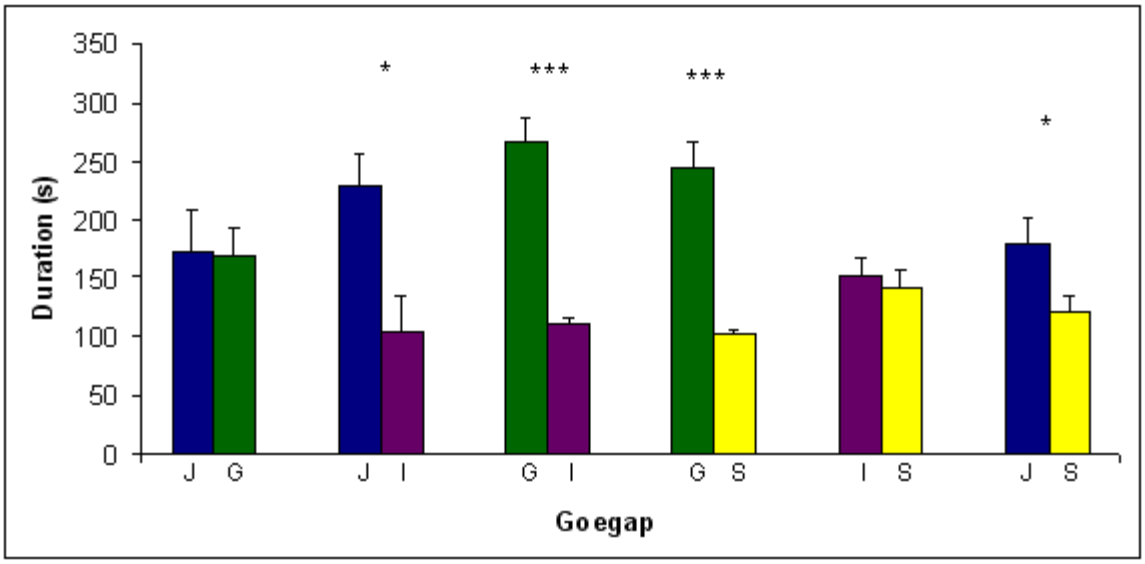

Figure 7b. Mean (+ SE) duration of visits of Goegap females to odour sources of males from four *Rhabdomys* populations in two-way choice tests. J = Jonkershoek, G = Goegap, I = Irene and S = Suikerbosrand. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

The Irene (*R. d. dilectus*) females preferred the scent of their own population males (Figure 7c) to the other population males (Irene-Jonkershoek: $t_9 = 2.34$, $P = 0.044$; Irene-Goegap: $t_9 = 3.71$, $P = 0.004$; Irene-Suikerbosrand: $t_9 = 5.53$, $P < 0.001$). Irene females showed no preference when given a choice between the Jonkershoek and Goegap (both *R. pumilio*) males ($t_9 = 0.48$, $P = 0.642$). When given the choice between the scent of the other *R. dilectus* population (Suikerbosrand) and the two *R. pumilio* populations (Jonkershoek and Goegap), the Irene females showed no preference (Jonkershoek-Suikerbosrand: $t_9 = 0.62$, $P = 0.548$; Goegap-Suikerbosrand: $t_9 = 0.20$, $P = 0.844$). This result is contrary to the Jonkershoek and Goegap females, which both belong to the *R. pumilio* taxon and chose *R. pumilio* males to the *R. dilectus* males.

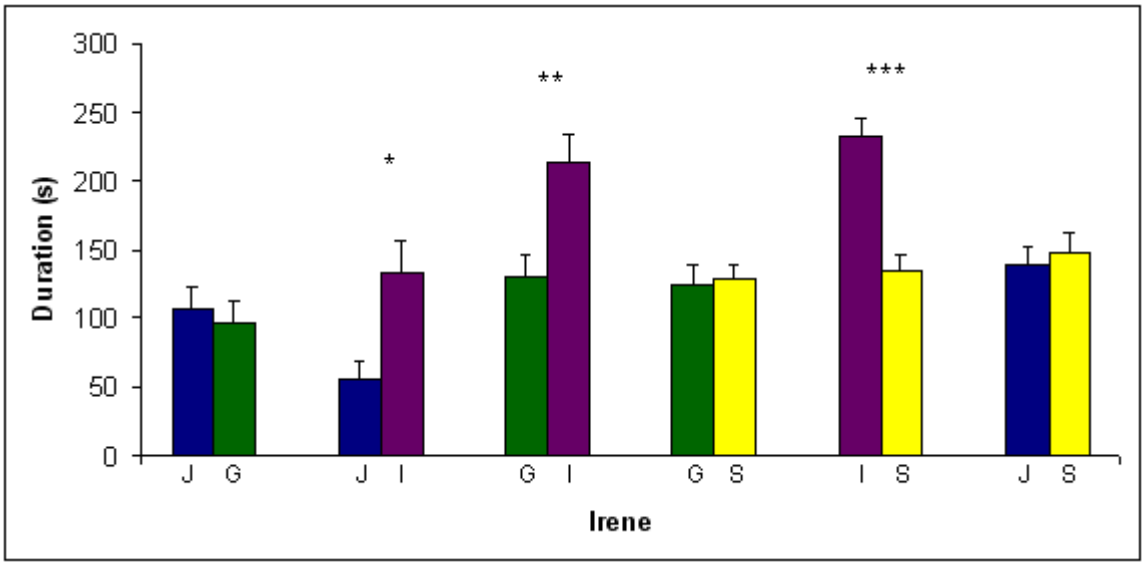

Figure 7c. Mean (+ SE) duration of visits of Irene females to odour sources of males from four *Rhabdomys* populations in two-way choice tests. J = Jonkershoek, G = Goegap, I = Irene and S = Suikerbosrand. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Following the same trend as all the other populations, Suikerbosrand (*R. d. chakae*) females showed a preference for their own population males (Figure 7d) to the other population males (Suikerbosrand-Jonkershoek: $t_9 = 4.18$, $P = 0.002$; Suikerbosrand-Goegap: $t_9 = 4.05$, $P = 0.003$; Suikerbosrand-Irene: $t_9 = 11.16$, $P < 0.001$). Unlike the other *R. dilectus* population (Irene), Suikerbosrand females chose its fellow *R. dilectus* population males to the two *R. pumilio* (Jonkershoek and Goegap) males (Irene-Jonkershoek: $t_9 = 5.11$, $P < 0.001$; Irene-Goegap: $t_9 = 4.68$, $P = 0.001$). As with the Goegap (*R. pumilio*) and Irene females, Suikerbosrand females showed no preference when given the choice between the Goegap males and the Jonkershoek (*R. pumilio*) males ($t_9 = 0.42$, $P = 0.682$).

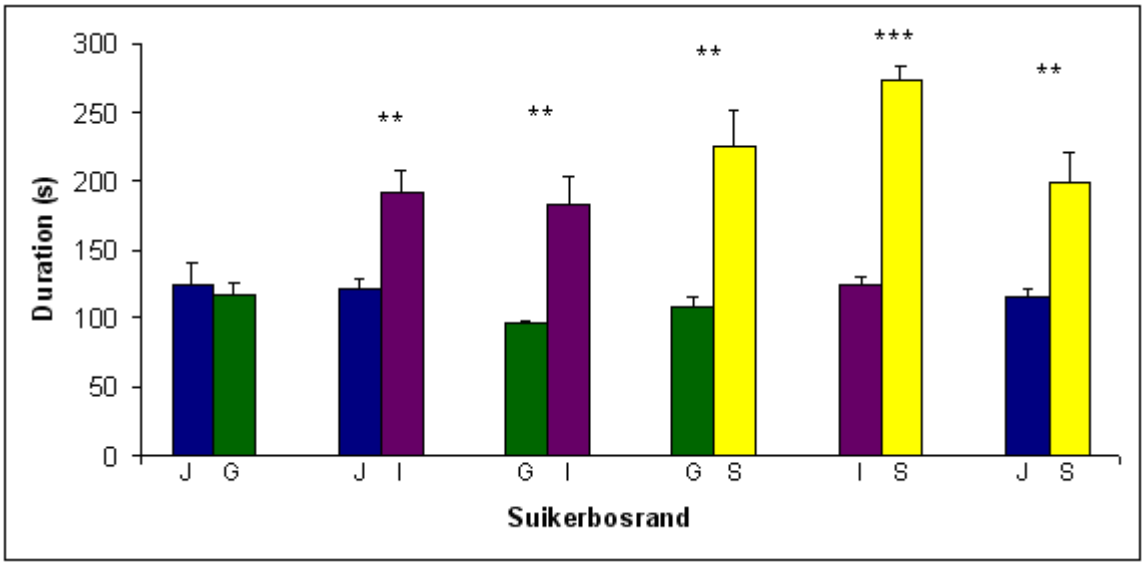

Figure 7d. Mean (+ SE) duration of visits of Suikerbosrand females to odour sources of males from four *Rhabdomys* populations in two-way choice tests. J = Jonkershoek, G = Goegap, I = Irene and S = Suikerbosrand. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Chapter 4: Discussion

4.1) Breeding studies

I expected that pairings that involved individuals of the same species would have higher reproductive success than those that involved individuals from different species or different sub-species. In my study, the Jonkershoek and Goegap populations belonged to the same species (*Rhabdomys pumilio*). I also predicted that as the Jonkershoek and Goegap populations are situated greater than 900 km apart, geographic distance could limit gene flow, so that the two populations would be reproductively isolated. The first prediction was shown to be correct as almost none of the different species or sub-species pairings produced viable hybrid offspring while the Jonkershoek-Goegap pairings (67% success) had slightly lower reproductive success than the same population pairings (Jonkershoek-76%, Goegap-93%, Irene-87%, Suikerbosrand-92%). My study does not support the geographic distance prediction, and my data suggest that interfertility is not influenced by geographic distance between the Jonkershoek and Goegap populations, as pre-mating (behavioural compatibility) and post-mating (viable hybrids) reproductive isolation was not apparent. These results also indicate divergence of mate recognition and no interfertility between the Jonkershoek, Irene and Suikerbosrand populations, between the Goegap, Irene and Suikerbosrand populations, and between the Irene and Suikerbosrand populations.

While breeding studies are most useful for revealing post-mating isolation, mainly as a result of hybrid sterility or infertility, they may also give an indication of pre-mating isolation through behavioural incompatibilities, such as through damaging fights (see Pillay *et al*. 1995c). In my study, aggression does not seem to be the main pre-mating barrier for populations of different species (Goegap or Jonkershoek with Suikerbosrand or Irene) or sub-species (Irene with Suikerbosrand). Only the Jonkershoek (*R. pumilio*) – Irene (*R. d. dilectus*) pairings showed a high level of fighting. Interestingly, this outcome was uni-directional, since fighting was observed in 50% of Jonkershoek female x Irene male pairs, but no incidences of fighting were recorded in Jonkershoek males x Irene female pairs. Similarly, allopatric populations of the vlei rat *Otomys irroratus* display significantly higher levels of aggression in different-population dyadic encounters (Pillay *et al*., 1995c).

While the Jonkershoek and Goegap populations interbred, litter sizes were significantly lower than that of intrapopulation pairs. Such a reduction in litter size has been found previously in a study on reproductive isolation in three distant *Rhabdomys* populations (Alice- *R. d. chakae*, Goegap- *R. pumilio*, Irene- *R. dilectus*) (Pillay, 2000b). However, unlike the Pillay (2000b) study where the hybrids also suffered retarded pre-weaning growth and low reproductive success, the hybrids in my study had similar pre-weaning growth to the offspring intrapopulation pairings. This could be attributed to the fact that any hybrids produced between the three populations in Pillay's (2000b) study would have been between different species or sub-species whereas in my study they were produced between two populations of the same species.

4.2) Mate recognition

I predicted that females from Jonkershoek and Goegap would perceive signals of males of each other's population as being more similar to signals of Irene and Suikerbosrand males. I also predicted that both Jonkershoek and Goegap females would perceive the olfactory cues of males from each other's population as being different from males of their own population due to divergence in these geographically distant populations. The first prediction was shown to be correct for the Goegap population but not for the Jonkershoek population, since Jonkershoek (*R. pumilio*) females perceived Goegap (*R. pumilio*) and Irene (*R. d. dilectus*) male scent as similar to their own male scent.

The second prediction was true for the Jonkershoek population but not the Goegap population. Although Jonkershoek females, that were habituated to their own males scent and then had a choice between Irene and Goegap males scent, seemed to view the two populations scent as similar to their own, they could still tell the difference when the choice was between their own males scent and either of the other two population scents. In contrast, Goegap (*R. pumilio*) females perceived Jonkershoek (*R. pumilio*) males as being more similar to their own population males than Irene (*R. d. dilectus*) males. Despite the fact that they are geographically separated, the Goegap females did not discriminate between their own male scent and that of Jonkershoek males. Although it is not known which of the Jonkershoek or the Goegap populations is the ancestral population, Kaneshiro (1976) proposed that asymmetric reproductive isolation may be due to discrimination by

ancestral females for the behaviour of ancestral and derived males, whereas derived females may not show a preference between the males. This would suggest that the Jonkershoek population is the ancestral one as their females discriminated between their own males and Goegap males, whereas the Goegap females did not discriminate. It is also possible that as a result of living in different habitats the females of the two populations have diverged in odour perception. Female mating preferences for a given male trait (such as odour) are influenced by adaptations and constraints not necessarily connected to female responses to that particular trait, such as selection for finding prey and avoiding predators (Ryan, 1998). It is also possible that the difference seen here can be attributed to genetic drift, whereby small founder populations create new and unusual gene combinations which are then sorted by selection (Templeton, 1980). This is unlikely, however, as even weak selection for a particular phenotype would greatly restrict the possibility for drift away from that phenotype (Lande, 1980). There has been very little empirical evidence supporting genetic drift as an important role in the evolution of phenotypic traits (Coyne *et al*., 1997).

The Goegap females could discriminate between their own population males and the two *R. dilectus* (Irene and Suikerbosrand) males but could not discriminate between the two sub-species males when compared with each other. Irene (*R. d. dilectus*) females could discriminate between their own males and Suikerbosrand (*R. d. chakae*) males as well as between their own and Goegap (*R. pumilio*) males. Surprisingly, not only could the Irene (*R. d. dilectus*) females perceive the Jonkershoek (*R. pumilio*) males as more similar to their own males than the Goegap (*R. pumilio*) males, they also did not discriminate between their own males from those of Jonkershoek. Suikerbosrand (*R. d. chakae*) females could discriminate between their own males and those of Irene (*R. d. dilectus*). The Suikerbosrand females, however, perceived the Irene males as being more similar to their own males than the two *R. pumilio* population (Jonkershoek and Goegap) males. In a previous study on *Rhabdomys*, Pillay *et al*. (2006) found no within-taxon variation in odour perception. In my study, there was variation in the perception of the Irene odour by the Jonkershoek and Goegap populations, both of which are in the *R. pumilio* taxon. The Jonkershoek and Goegap populations also showed variation in how they perceived each other's odour. While both studies considered the Goegap population, the major difference between my study and the Pillay *et al*. (2006) study is that I have looked at the Jonkershoek population while the

latter investigated a *R. pumilio* population from Gariep Dam. As has been stated above, it is possible that the Jonkershoek is the ancestral population while the Gariep Dam and Goegap populations could both be derived. Thus, it is not surprising that the Gariep Dam and Alice populations could not discriminate between each other's scent. In addition, the populations are distributed over great geographic distances which may account for some of the differences between my study and that of Pillay *et al*. (2006). Despite some confusion in the perception of each other's odours by the Jonkershoek and Irene populations, the *R. dilectus* subspecies were perceived as more similar to each other than to that of *R. pumilio*, and were equally different from that of *R. pumilio*. A similar result was obtained by Pillay *et al*. (2006), who suggested that the two sub-species share a common odour characteristic, distinct from that of *R. pumilio*. My study supports this suggestion. The confusion that arose between Jonkershoek and Irene could be attributed to the possibility that isolation between *Rhabdomys* populations is relatively recent, as suggested by Pillay *et al*. (2006) and therefore the Jonkershoek and Irene males could still have a similar smell.

4.3) Mate choice

A directional preference in a two-way choice could be a positive response to the specific signals carried by the preferred mate, or either a negative or a non response to those carried by the non-preferred individuals (Smadja *et al*., 2004). There were definite trends in preference showed by the female *Rhabdomys* based on population and species. Patterns of preference were consistent within a taxon but differed between the three taxa. I predicted that females would prefer males of their own population to that of a different population, and with one exception, the results obtained show that females of all populations preferred the odour of homotype (same population) to heterotype (different population) males. Similar findings have been reported in species/populations of several rodent genera, such as *Gerbillurus* (Dempster & Perrin, 1990), *Graomys* (Theiler & Blanco, 1996), *Otomys* (Pillay *et al.*, 1995a, b, c), *Peromyscus* (Moore, 1965; Smith, 1965), *Spalax* (Nevo *et al.*, 1976) and *Rhabdomys* (Pillay, 2000b). Non rodent examples which demonstrate preference for same population/species over a different population/species, include red-legged *Alectoris rufa* and rock partridges *A. graeca* (Ceugniet & Aubin, 2001), fly catchers *Ficedula* spp. (Sætre *et al*., 1997), *Drosophila* spp. (Noor, 1995), Amazon

mollies *Poecilia* spp. (Ryan *et al*., 1996), heliconius butterflies *Heliconius* spp. (Jiggins *et al*., 2001), rough periwinkle *Littorina saxatilis* (Rolan-Alvarez *et al*., 1999), and the meadow grasshopper *Chorthippus parallelus* (Ritchie *et al*., 1989).

The one exception was the Goegap (*R. pumilio*) females, which showed no preference for their own population scent over that of Jonkershoek (the other *R. pumilio* population). In contrast, the Jonkershoek females did show a preference for their own males over that of the Goegap males. This result is not surprising as it has already been shown in the habituation-discrimination tests (above) that Goegap females could not detect a difference in odour between the two *R. pumilio* populations while the Jonkershoek females could. Therefore, Goegap females did not show a preference for their own scent as they could not distinguish it from the Jonkershoek scent.

I also predicted that females would prefer phylogenetically more similar males than more distant males. Both *R. pumilio* populations, Jonkershoek and Goegap females, chose each other's males odour over those of the *R. dilectus* (Irene and Suikerbosrand) males odour. While the Suikerbosrand population females preferred their fellow *R. dilectus* (Irene) population males odour to the two *R. pumilio* population males odour, the Irene females did not, showing no preference between Suikerbosrand population males odour and the two *R. pumilio* population males odour. Similarly, house mice *Mus mus musculus* females spent more time sniffing homosubspecific odour than they did a heterosubspecific stimulus (*M. m. domesticus*), but female *M. m. domesticus* did not show a preference between the two subspecific signals (Ganem *et al*., 2005). In my study, both *Rhabdomys* subspecies preferred homosubspecific odours. The Irene females also showed no preference between the Suikerbosrand and Jonkershoek or Goegap males odours. The *R. pumilio* females showed no preference between the *R. dilectus* populations and the *R. dilectus* females showed no preference between the *R. pumilio* populations. Thus, Jonkershoek and Goegap (*R. pumilio*) females displayed no preference between Irene and Suikerbosrand (both *R. dilectus*) males. Equally, Irene and Suikerbosrand females showed no preference between male scents from Jonkershoek and Goegap. Subspecies of the house mouse *M. musculus* have been found to have different strengths in patterns of mate preference, which indicated an asymmetrical pattern of divergence (Smadja & Ganem, 2005). The sub-species of *R. dilectus* in this study showed a similar pattern of mate preference and this suggests a

more symmetrical pattern of divergence. It was also shown in the house mouse that preference was significantly assortative in populations from a contact zone, as opposed to nondirectional in allopatry (Smadja & Ganem, 2005). In my study, all four of the populations are allopatric, but in contradiction to the house mouse, these *Rhabdomys* populations all displayed assortative preference.

4.4) Pre- and post-zygotic reproductive isolation

One of the major factors that determine mate choice is the quality and compatibility of potential partners, and the signals that broadcast these two types of information often interact, and their relative importance may be context dependent (Roberts and Gosling, 2003). Within populations, pre-copulatory compatibility signals are believed to be rare or unreliable when compared to post-copulatory signals (Jennions, 2006) and in terms of quality, costly pre-copulatory signals are relatively common and seem to be honest and mostly reliable across environmental conditions (David *et al*., 2000). Therefore any populations in my study that are reproductively isolated could be so as a result of pre- or post-zygotic barriers, either is equally possible. In my study, pre-mating isolation was tested by assessing aggression during the breeding studies, differences in odour perception, and differences in odour preference. Postzygotic isolation was tested during the breeding studies by assessing the lack of viable hybrids. The traditional view of mating signal evolution suggests that certain features of the mate choice signals can be used for species recognition (Bimova *et al*., 2009) and in theory only individuals of the same species are able to possess the signal-response sequence necessary to achieve mating, and a breakdown in the signal-response sequence occurs in intraspecific pairs (Butlin & Richie, 1994). Thus, the species-specific mate recognition signals can serve as significant barriers between diverged genomes (Bimova *et al*., 2009). Odour signals can diverge and therefore act as pre-mating barriers between different populations. For this to have occurred in my study, two conditions must be achieved. Firstly, the *Rhabdomys* females must be able to perceive the scent of males of another population as different to their own and secondly they must have a preference for their own population scent over any others.

Jonkershoek (*R. pumilio*) females perceived differences between their own population males and those of the other three populations and always displayed a

preference for their own population males (Table 5). Jonkershoek females perceived the Suikerbosrand (*R. d. chakae*) population males as being the most different from its own

Table 5. Summary of the responses by female *Rhabdomys* of four populations in habituation-discrimination/-generalization and two-way choice tests. J = Jonkershoek, G = Goegap, I = Irene and S = Suikerbosrand.

Female subjects	*R. pumilio* (Jonkershoek) versus *R. pumilio* (Goegap)		*R. pumilio* (Jonkershoek and Goegap) versus *R. d. dilectus* (Irene)		*R. pumilio* (Jonkershoek and Goegap) versus *R. d. chakae* (Suikerbosrand)		*R. d. chakae* (Suikerbosrand) versus *R. d. dilectus* (Irene)	
	Perception	Prefers	Perception	Prefers	Perception	Prefers	Perception	Prefers
Jonkershoek (*R. pumilio*)	Different	J	Similar (Goegap more similar than Irene)	J G	Different	J G	Similar	Equal
Goegap (*R. pumilio*)	Similar	Equal	Different	J G	Different	J G	Similar	Equal
Irene (*R. d. dilectus*)	Different	Equal	Similar (Jonkershoek) Different (Goegap)	I	Different	Equal	Different	I
Suikerbosrand (*R. d. chakae*)	Similar	Equal	Different	I	Different	S	Different	S

males and the Goegap (*R. pumilio*) males as being the most similar. The Jonkershoek females did not discriminate between the Irene (*R. d. dilectus*) population and their own population, but they still showed a preference for their own population, indicating a mismatch between the results of the habituation-discrimination and choice tests. Thus, the Jonkershoek population may be reproductively isolated from the other three populations due to divergence in olfactory signals in the males and female perception of the male odours. The breeding tests revealed that there was no or limited interfertility between Jonkershoek (*R. pumilio*) and the Suikerbosrand (*R. d. chakae*) and the Irene (*R. d. dilectus*) populations but not the Goegap (*R. pumilio*) population. The odour tests indicated that the Jonkershoek striped mice were behaviourally compatible with the Irene and Goegap populations.

The fact that odour does not act as a reproductive barrier between the Goegap (*R. pumilio*) and Jonkershoek (*R. pumilio*) populations became even clearer when it was shown that unlike Jonkershoek females, Goegap females were not able to perceive a difference between the two populations and thus did not display a preference for their own population males to those of Jonkershoek (Table 5). Also unlike Jonkershoek females, Goegap females discriminated between their own population and both the Irene (*R. d. dilectus*) and Suikerbosrand (*R. d. chakae*) males. As the breeding studies show, there is reduced interfertility between the Goegap population and the Irene and Suikerbosrand populations, and it is entirely plausible that divergence in odour plays a large part in this isolation.

Irene females showed the same perception as the Jonkershoek females as they perceived the Jonkershoek males as similar to their own males and different from the Goegap males but preferred the Suikerbosrand males (which they perceived as different to their own males) to the Jonkershoek males (Table 5). As the Irene females perceived the Goegap males as different to their own males and they preferred their own males, it seems even more likely that divergence in odour plays a part in reproductively isolating these two populations (as the bidirectional pairings between these populations did not reproduce). Irene females also perceived a difference between their own males and Suikerbosrand males. They did not breed with any of the other populations. Therefore, it seems likely that divergence in odour plays a part in keeping these two populations reproductively isolated.

The Suikerbosrand females were the only females which perceived males of all of the other three populations as being different from their own males (Table 5). This suggests that the Suikerbosrand population has diverged the most from all the populations studied here. This is backed up by the breeding tests where no hybrids were produced between the Suikerbosrand population and any of the other three populations.

Odour is important in the house mouse where the main component of the signal–receptor system, which is assumed to be involved in assortative mate choice, includes chemical signals in the urine and faeces (Bimova *et al*., 2009). My results suggests the existence of population specific odour signals as three out of the four populations could distinguish their own population scent from the others and prefer their own population scent. In addition, even though Jonkershoek females could differentiate their males from the Goegap males, this did not inhibit their interfertility.

Post-zygotic reproductive isolation because of chromosomal variation has been shown in populations of several rodent species, such as the phyllotine rodent *Graomys griseoflavus* (Theiler & Blanco 1996), house mice *Mus musculus domesticus* (Capanna & Redi 1994), the vlei rat *Otomys irroratus* (Pillay *et al*. 1992, 1995c), and the mole rat *Spalax ehrenbergi* (Nevo, 1991). However, post-zygotic reproductive isolation can also occur without chromosomal variation as shown in two species of gerbils *Tatera afra* and *T. brantsii* which have identical karyotypes but breeding results in hybrid disadvantage (Dempster, 1996). *Rhabdomys* has two karyotypic forms, the more prevalent 2n = 48 form found in large parts of South Africa and the 2n = 46 form in the extreme northern parts, particularly in the highveld region of South Africa (Ducroz *et al*. 1999). Both forms were represented in this study (2n = 48: Goegap, Jonkershoek and Suikerbosrand; 2n = 46: Irene). Chromosome banding studies indicate that the difference between the chromosomal forms of *Rhabdomys* is the presence of a Robertsonian fusion in the 46 form (Taylor, 2000) and a single Robertsonian mutation is not expected to result in interbreeding problems (R.V. Rambau, pers. comm.). In my study, chromosomal variation does not appear to play a role in reproductive isolation. While there were no hybrid offspring produced between populations that had a different chromosomal form (Irene with any of the other three populations) there were also no viable offspring produced between some of the populations which had the same chromosomal form (Jonkershoek or Goegap with Suikerbosrand). The formation of hybrids in laboratory breeding experiments (Pillay, 2000a) and the low genotypic divergence (Mahida *et al*., 1999) are thought to indicate that the chromosomal change in *Rhabdomys* is fairly recent (Taylor, 2000) and thus would not be expected to play a significant role in interfertility.

Overall, my results indicate divergence in mate recognition and choice is an important pre-mating isolating mechanism and well developed between the two *Rhabdomys* species. Interfertility was found between the Jonkershoek and Goegap *R. pumilio*, and while the hybrids produced were viable and fertile, litter size was reduced in these interpopulation pairings, indicating post-zygotic failure. Therefore, my study also shows that the divergence in mate recognition signals between Jonkershoek and Goegap striped mice can be overcome in the confined space in captivity, resulting in interbreeding.

4.5) Geography and Phylogeny

Although it has been shown that the primary predictor of reproductive isolation in *Rhabdomys* is phylogeny, geographic distance is still expected to play a part as there should be no selection to maintain compatibility of mating behaviour between individuals of populations that are either geographically or ecologically separated (Charlesworth & Charlesworth, 2000). An allozyme electrophoretic study of *R. pumilio* by Mahida *et al*. (1999) showed that gene flow is restricted between widely spaced populations in southern Africa, and that the taxon shows a good correlation to the isolation by distance model, as proposed by Wright (1943). My study shows that phylogeny plays a greater role in predicting reproductive isolation between populations than geographic distance, which is contrary to the isolation by distance model. All of the interpopulation pairings that produced no viable hybrid offspring were between populations of different species or sub-species, and the only pairing that did have viable hybrid offspring were the two populations from the same species (Jonkershoek and Goegap, *R. pumilio*). Of course, all of the interpopulation pairings that failed reproductively were not only phylogenetically distant, but also separated by geographic distance (except the Irene and Suikerbosrand populations). Geographic influences on mate recognition would be expected to include genetic drift and sexual selection (West-Eberhard, 1983; Herring & Verrell, 1996). This does not seem to be the case in my study as the Jonkershoek-Goegap pairings produced viable hybrids despite being separated by > 900 km. The Jonkershoek and Goegap populations also occupy different habitats and ecological conditions, which can result in different foraging methods (Price, 2008) and interactions with predators (Endler & Basolo, 1998). However, this also does not seem to be enough to prevent breeding between the Jonkershoek and Goegap populations. Comparisons of between-taxa odour quality and perception showed there is a marked divergence between *R. dilectus* and *R. pumilio* and to a lesser extent between *R. d. dilectus* and *R.d. chakae* which corresponds with the proposal of *Rhabdomys* taxonomy based on an mtDNA phylogeny (Rambau *et al*., 2003). Therefore, the populations in this study exhibit a phylogenetic pattern of signal divergence and pattern rather than a geographic one.

4.6) Where does Jonkershoek fit in?

The Jonkershoek population differed from the Suikerbosrand and Irene populations in terms of geography, phylogeny and ecology and differed from the Goegap population in terms of geography and ecology.

Ecology can play an important role as mate attraction signals are subject to strong sexual selection which is imposed by environmental factors that determine which mode of communication is most effective, as well as affecting finer-scale characteristics of the behaviour and physiology of senders and receivers (e.g. Witte *et al*., 2005; Doucet *et al*., 2007). To maximize signal efficacy, both sender and receiver evolve traits that increase signal detectability (Milner *et al*., 2008). The signals therefore are a match to the environment in which they are produced, while receivers evolve sensory systems that compensate for environmentally induced signal degradation during transmission (Endler, 1992). An example of this is the Panamanian golden frog, *Atelopus zeteki*, which lives in an environment where noise from cascading water has led to the partial loss of the normal acoustic signals used to attract mates, and instead visual signals comprising limb movements are used as they are more effective in an acoustically noisy environment (Lindquist & Hetherington, 1996). While the environmental context of a signalling male is usually tested using visual or auditory signals it can be applied to any signal, including odour. Prezygotic isolation mechanisms relying on ecological differentiation has been shown before (Irises: Cruzan & Arnold, 1994; butterflies: Jiggins *et al*., 1996). Thus it is possible that the odour characteristics and perception of the Goegap and Jonkershoek females are different due to the fact that they live in different habitats and therefore face different ecological factors.

Choice tests to assess preference in laboratory conditions have been shown to be a good estimator of the tendency to mate in the house mouse (Laukaitis *et al*., 1997; Smadja & Ganem, 2002). However, in my study, Jonkershoek females discriminated between their own males and Goegap males, and preferred their own males but still successfully bred with Goegap males. It would appear that geographic distance and/or ecological differences may be limiting gene flow between these populations, since they have diverged in their odour perception and preference but there has just not been enough time for this divergence

to affect interfertility. It is possible that isolation between *Rhabdomys* populations is relatively recent, as suggested by Pillay *et al*. (2006).

4.7) Future studies

It has been shown that there can be a difference in the strength of mate choice between areas of contact between populations and areas where populations are in allopatry (Smadja & Ganem, 2005). Although no hybrid zones have yet been found for *Rhabdomys* in South Africa, studies should be done on populations close to the contact zones between the taxa. In addition, as ecology can influence mate recognition, either directly (e.g. predator-prey interactions) or indirectly (e.g. resource availability), studies should be done on individual *Rhabdomys* populations in an attempt to understand the types of ecological pressures they are under.

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