Reproductive isolation in the striped mouse
Rhabdomys: a case for reinforcement?

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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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Ahamed Mohaideen Riyas Ahamed
July 2006

TO

The thousands of people of my village

who lost their lives in the tsunami

Abstract

Reproductive isolation was investigated in two chromosomally distinct populations of *Rhabdomys* on the Gauteng highveld. The two populations, Midrand (2n = 48) and Irene (2n = 46), occur 15 km apart, with no known contact or hybrid zone between them. Behavioural experiments, comprising male-female dyadic encounters and female preference tests for same-and different-population male odour, were used to test for premating barriers. Aggression levels were highest in different-population than same-population dyads, and females spent more time with odours of males from their own population than of those of the other population. Breeding and postnatal development studies were conducted to establish postmating barriers. Compared to different-population pairs, reproductive success was markedly reduced in different-population pairings, and the few hybrids that were produced did not breed. My studies indicate that behavioural isolation is well-developed between the Midrand and Irene striped mice, and suggest that the mate recognition system has diverged in allopatry, which would reduce gene flow between the two populations. Such divergence supports the findings of mtDNA studies by other workers who proposed that the two chromosomal forms used in my study represent two subspecies of *R. dilectus*. Previous studies showed that distant striped mice populations (>900km) displayed behavioural divergence and intermediately located populations (~80km) were behavioural compatible but had hybrid failure; the Irene population was used in both studies. In comparison, the behavioural incompatibility between the closely-located Midrand and Irene populations provides support for the reinforcement of previous postmating isolation seen in the intermediately

located populations, particularly since no contact or hybrid zone exists

between the two forms. However, I cannot rule out other explanations, such

as dissimilar ecological conditions, influencing interfertility.

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1 INTRODUCTION

1.1 Reproductive isolation and speciation

The definition of a species has been hotly debated for over a century (e.g. Darwin, 1859; Dobzhansky, 1940; Mayr, 1963; Paterson 1978). Darwin (1859) and later Mayr (1963) proposed the Biological Species Concept (BSC) which maintained that species are units which are reproductively isolated from other species. The BSC maintains that members of a species do not interbreed with members of other closely-related species due to the different characteristics between them. This reproductive isolation between the populations is therefore a defining characteristic of the BSC (Albert and Shulter, 2004).

Proponents of the BSC maintain that there are several types of barriers or evolved characters which are likely to limit gene exchange within and between species, which can be grouped into premating and postmating barriers (Howard, 1993). Premating barriers may occur through ecological/habitat isolation, seasonal/temporal isolation, sexual isolation, ethological isolation, mechanical isolation and/or genetic isolation and result in no zygote formation (Mayr, 1963). Postzygotic barriers occur when the zygote is formed, but the offspring (hybrids) are not viable, are sterile or are of poor quality (Mayr, 1963).

While the BSC is widely accepted (Ridley, 2003), the concept has been criticised on several grounds. Paterson (1985) argued that the concept is mechanistic, is theoretical and not easily observed or supported by empirical evidence, whereas others, such as Bush (1982) and Mallet (1995), suggest

that reproductive isolation is a simplistic way to view species and speciation because it does not consider factors such as Mendelian, molecular and biochemical genetics. Mallet (1995) added that we should be looking at levels of gene flow and genetic structure to define species instead of isolation, and questioned the existence of reproductive isolating mechanisms.

As an alternative to the BSC, Paterson (1978, 1982, 1985) proposed the recognition concept (RC), which suggests that a group of organisms share a specific mate recognition system (SMRS). The SMRS is the result of co-evolved systems of signal transmission and perception (i.e. recognition and preference; Ryan and Rand, 1990; Butlin and Ritchie, 1994). These systems may work through one or more of several modalities (i.e. visual, tactile, auditory and/or olfactory cues; Butlin and Ritchie, 1994).

Inter-individual recognition, which consists of complex bicommunication systems, is important in mate choice (Alberts, 1992; Littlejohn, 1993). The signal response sequence which is important for initiating mating promotes mating between compatible races/forms (Butlin and Ritchie, 1994). Divergence in the SMRS leads to assortative mating only between mates sharing similar SMRS (Butlin, 1994), which ultimately leads to gene flow between similar populations and a reduction of gene flow and speciation between dissimilar populations (Hartfield and Schulter, 1996; Ritchie *et al.*, 1989).

Even though the BSC and SMRS concepts seem to contradict each other in the manner by which speciation occurs, both can be used to explain interfertility and gene flow between closely-related species and ultimately speciation. The BSC explains the concept of a species in terms of reproductive isolation, whereas SMRS explains it in terms of mate recognition, regardless of

whether breeding occurs. In this regard, the concepts differ mainly in how they view behaviour during courtship. In the BSC, ethological barriers function as premating isolating mechanisms that lead to a breakdown in courtship, thereby preventing mating (Heth and Nevo, 1981). In contrast, the recognition concept postulates that behavioural compatibility during courtship promotes interfertility between populations. The BSC is also concerned with postmating phenomena (e.g. hybrid failure) which are not considered in the RC, which instead maintains that species integrity is achieved at mating (Paterson, 1993; Heth and Nevo, 1981)

In this study, I have adopted an operational view of the behavioural phenomena associated with the BSC and RC, and view these as being opposite sides of the same coin (Pillay, 1993), since both maintain that behaviour promotes species cohesion as a consequence of or a contributor to gene flow between and within populations (Mayr, 1963; Littlejohn, 1993). The difference being in the one (RC) species are viewed as having similar mate recognition systems and in the other (BSC) species are behaviourally incompatible. In practice, both recognition and incompatibility may have biological significance in revealing patterns of interfertility among members of closely related species, since both processes require a initial cessation of gene flow. Therefore, reproductive isolation, as revealed by interfertility or the lack thereof, can be used as an indirect measure of gene flow and adaptations to local conditions, as suggested by Mallet (1995).

Several studies on small mammals have shown how discrimination between populations may be indicated by behavioural studies (e.g. Smith, 1965; Cox, 1989; Ganem and Searle, 1996; Smith *et al*., 1997). In South

Africa, Pillay *et al*. (1995a) showed that different vlei rat *Otomys irroratus* populations were characterised by increased aggression during courtship, which is predicted to occur when there is a breakdown in courtship behaviour (Bernard and Fitzsimmons, 1989). Such discrimination may lead to assortative mating between races or forms, which could restrict gene flow and ultimately lead to speciation.

1.1.1 Behaviour and speciation

The importance of behaviour in evolution and speciation is widely acknowledged (Dagley *et al*., 1994; Benedix and Howard, 1991; Sperling and Spence, 1991; Baker and Baker, 1990; Littlejohn and Watson, 1985; Mayr, 1963), but the way in which behaviour brings about speciation appears to be tied to the species concepts (BSC and RC) reviewed above. Some argue that the RC better explains the role of behaviour in speciation since it is easier to demonstrate that animals mate with individuals they recognise as mates, particularly since deviations in the co-evolved signal-perception system can be easily demonstrated (Paterson, 1980; Butlin and Ritchie, 1994).

The Biological Species Concept states that premating reproductive isolation can occur among individuals of different populations if there is breakdown in courtship behaviour (Barnard and Fitzsimmons, 1989; Boyd and Blaustein, 1985), leading to behavioural discrimination and assortative mating. Discrimination is shown in various ways, including, for example, higher aggression and lower amicability towards different population mates (Pillay *et al*., 1995a, b, c; Ganem *et al*., 1996; Pillay 2000a; Dempster, 1996).

Whether divergence occurs through recognition or discrimination is still contentious. However, both processes will ultimately lead to assortative mating (Ganem and Searle, 1996), and may explain the non-mixing of individuals (Ganem, 1998).

1.1.2 Chromosomal differences and interfertility

Rearrangement of chromosomes may contribute to speciation (White, 1978), and chromosomal mutations can be an efficient isolation mechanism, especially if chromosomal arrangements are complex (Britton-Davidian *et al*., 2000). Meester (1988) proposed a model of chromosomal speciation where chromosomal rearrangements result in postmating reproductive isolation, creating sibling species.

The role of chromosomal rearrangements in limiting gene flow between populations and promoting speciation is regularly debated (King, 1993). It is generally agreed that chromosomes are not directly linked to speciation (Meester, 1988). Nonetheless, chromosomal rearrangements are likely to inhibit interbreeding success mainly as a result of chromosomal heterozygosity leading to a reduction in hybrid fertility and even to sterility owing to mal-segregation and germ cell death during gametogenesis (Searle, 1993; Nevo, 1991; Meester, 1988; White, 1978). Several studies have shown that rodent populations that have undergone chromosomal divergence display severe interbreeding problems (Pillay *et al*., 1995c; Nevo, 1991). However, there are instances where chromosomal variation can occur without interbreeding problems, yet gene exchange between populations may be limited through other factors, such as behavioural breakdown (Fraguedakis-Tsolis *et al*., 1997;

Ganem and Searle, 1996; Pillay *et al*., 1995a, b; Corti and Thorpe, 1989; Capanna and Corti, 1982).

1.2 Reproductive isolation in rodents

Rodents make ideal models to test theories in gene flow and reproductive isolation, largely because of chromosomal and genetic polymorphisms in closely related species or populations. I have provided some of the most influential studies conducted on reproductive isolation in rodents in the succeeding paragraphs.

House mice have been a premier model for many studies on reproductive isolation. In one subspecies of house mice (*Mus musculus domesticus*) from the Orkney Archipelago, Scotland, discrimination between individuals of the same and different population occurred via behavioural displays, leading to assortative mating, even though the populations were closely-related morphologically and genetically (Ganem, 1998; Ganem and Searle, 1996). Other studies (e.g. Cox, 1984; Christophe and Baudoin, 1998; Britton-Davidian *et al*., 2000) showed that reproductive isolation occurs between two house mouse subspecies (*M. m. musculus and M. m. domesticus*), as a result behavioural (olfactory) divergence.

In contrast, Capanna *et al*. (1985), in an extensive study of chromosomally-different *M. m. domesticus* populations (2n=22, 24, 26) in Northern Italy, found that pairs from different populations experienced reduced interbreeding success. Such postmating isolation is most likely the result of Robertsonian mutations and is reinforced by ethological isolation and the absence of a hybrid zone, thus indicating premating isolation. These data are

substantiated by both electrophoretic and morphometric studies Capanna *et al*. (1985).

There are four chromosomal races (2n= 52, 54, 58, 60) of the mole rat (*Spalax ehrenbergi*), in Israel which are separated by narrow areas of hybridisation (Heth and Nevo, 1981; Nevo and Heth, 1976). Postzygotic isolation is incidental to evolutionary divergence in these chromosomal forms, because homospecific versus heterospecific mate selection, through a combination of aggression, olfaction and vocalisation, is likely to be the primary isolating mechanism in *S. ehrenbergi* (Heth and Nevo, 1981; Nevo and Heth, 1976).

Bowers *et al*. (1973) studied reproductive isolation in genetically and chromosomally different species of deer mice *Peromyscus maniculatus* and *P. melanantis*. Breeding studies revealed reduced reproductive success between species.

In chromosomally different but visually indistinguishable phyllotine rodent *Graomys griseoflavus* populations, Theiler and Blanco (1996a, b) found F2 hybids were sterile, resulting in postmating isolation. Moreover, premating isolation was indicated by olfactory discrimination with preferences for individuals from the same population and diploid number. Both premating and postmating isolation perhaps explains the lack of a hybrid zone between the forms.

In southern Africa, two rodent groups have been extensively-studied. Gerbils (*Tatera* spp.*, Gerbillurus* spp.) recognise conspecifics using species-specific patterns of behaviour (Dempster, 1996; Dempster *et al*., 1992, 1993).

In this group, premating isolation was accompanied by postmating isolation, as hybrids were inviable (i.e. suffered high mortality rates).

Populations of the vlei rat *Otomys irroratus* demonstrate remarkable karyotypic variability (Contrafatto *et al*., 1992), although there appears to be little or no interpopulation genetic or morphological variation (Taylor *et al*., 1992). Behavioural and breeding studies indicate pre- and postmating isolation (Pillay *et al*., 1995a, b, c). Chromosomally different populations occurring in close geographic proximity (<100km) showed reduced breeding success because of damaging fights and hybrid inviability (i.e. high pre-weaning mortality of offspring; Pillay *et al*., 1995b). Population-specific courtship behaviour, high aggression and olfactory preferences for same population mates were suggested to cause premating isolation (Pillay *et al*., 1995a, c).

1.3 Reinforcement

Reinforcement refers to a process where selection favours the evolution of premating isolation mechanisms that would reduce hybridisation (Dobzhansky, 1940; Howard, 1993; Butlin, 1995; Noor, 1999), and is sometimes viewed as a way of understanding the completion of the speciation (Servedio, 2004). Consider, for example, two divergent populations that are kept apart in allopatry by a geographical barrier. Both populations may have diverged genetically, due to a variety of reasons including differences in the local environment (Paterson, 1985; Verrel, 1988), random genetic drift (Rubinoff and Rubinoff, 1971), or as a result of the pleiotrophic effect of genes (Dobzhansky *et al*., 1968). If the populations make secondary contact later, the populations may be incompatible (Arnqvist *et al*., 2000; Servedio, 2004),

resulting in hybrid disadvantage (e.g. sterile or inviable hybrids). Selection may then operate against the energetic wastage of failed reproduction (i.e. hybrid breakdown), which would favour reinforcement (Dobzhansky, 1940) or reproductive character displacement (e.g. sexual signals and preferences; Butlin, 1987, 1989) of previously developed premating barriers (discussed later).

Because of the assumptions made about postmating isolation, reinforcement is supported in the BSC but not the RC. Moreover, reinforcement has been questioned (Paterson, 1978; Barton and Hewitt, 1981), mainly because of a lack of experimental support (Howard, 1993; Littlejohn, 1981; Phelan and Baker, 1987). Another important objection of reinforcement is that if two populations meet, and are of different sizes, those of the rarer population are more likely to hybridize more often than members of the other population, since members of rarer population will have more chance to mate with members of the larger population than members of their own population (Liou and Price, 1994). However, if the rarer population is significantly smaller or has a lower growth rate, and there is wasted reproductive effort due to the production of hybrid offspring, then the smaller population will become extinct before the formation of isolation barriers by reinforcement has occurred (Liou and Price, 1994).

Although it is unclear what role reinforcement plays in explaining contemporary biological diversity (Servedio, 2004), there have been several attempts to study reinforcement in several taxa. Following studies on acoustic insects (Walker, 1974) and Australian field crickets (Hill *et al*., 1972), many other studies were conducted from the 1980's (e.g. Littlejohn, 1981; Phelan

and Baker, 1987), including theoretical works (Sved, 1981; Spencer *et al.,* 1986; Liou and Price, 1994; Servedio and Kirkpatrick, 1997), taxonomic surveys (Coyne and Orr, 1989; Coyne and Orr, 1997; Howard, 1993), and numerous empirical studies. The latter ranged from flies to birds and provided evidence for hybrid failure and preference for the mating between same species (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). These empirical studies used various ways of assessing reinforcement including behavioural mate choice tests and breeding trials.

Much evidence for reinforcement has come from studies on the genus *Drosophila*. Coyne and Orr (1989) surveyed 119 incidences of hybridization between species of *Drosophila*, and later broadened this into 171 hybridizations (Coyne and Orr, 1997). They measured indices for premating and postzygotic isolation that range from 0 to 1 and indicate the degree of isolation for each type of mechanism; 0 being no reproductive isolation and 1 being complete reproductive isolation. According to their study, the rate of increase in reproductive isolation (both premating and postzygotic) correlates with genetic distances between species in allopatric populations. However, the evolution of premating isolation occurs at a faster rate than postzygotic isolation in sympatric populations, indicating that premating isolation may be reinforced by selection. Noor (1995) added to this dataset by showing that female *D. pseudoobscura* evolved increased sexual isolation from their sibling species *D. persimilis* by selection against detrimental hybridization.

There are some other notable studies. Gerhardt (1994) studied the call structure of chromosomally-different species of grey treefrogs *Hyla versicolor*

and *H. chrysoscelis* in two allopatric areas and a sympatric area, and found that female preferences for conspecific stimulus increased from allopatric to sympatry. In choice tests, male Amazon mollies *Poecilia latipinna* and *P. mexicana* apparently evolved preferences for females of same species (Ryan *et al*., 1996). European flycatchers *Ficedula hypoleuca* and *F. albicollis* displayed reproductive character displacement, in which females from both sympatric populations preferred same-species males (Saetre *et al*., 1997).

More recently, Servedio and Noor (2003) expanded the definition by suggesting that reinforcement represents an increase in premating isolation between hybridizing populations in response to any type of selection against interspecific matings, regardless of whether or not hybrids themselves are unfit, and includes situations where for example females interbreeding with males from other populations have lower fertility or higher mortality.

1.3.1 Studying reinforcement

Testing for reinforcement has posed a challenge for many workers, mainly because of the assumption that very strong selection is required to compensate for the negative effects of recombination and gene flow (Paterson, 1978; Spencer *et al*., 1986). Nonetheless, Howard (1993) proposed that there are three main prerequisites for reinforcement in nature: evidence of natural hybridisation, selection against hybridisation, and reproductive character displacement between populations that make contact compared to the populations in allopatry (Howard, 1993). Reproductive character displacement is defined as a pattern of greater divergence of an isolating trait (e.g. olfactory signature) in areas of sympatry between taxa than in areas of allopatry.

In reviewing 48 studies focusing on the presence or absence of reproductive character displacements, Howard (1993) found that in a variety of different orders and phyla, the cost of production of hybrids ranges from moderate (viable hybrid offspring of reduced fertility) to extremely high (no viable offspring). Of the 48 cases, 33 explained reproductive character displacement and of these, six studies provided evidence of reinforcement by demonstrating reproductive character displacement, intraspecific mating, and selection against hybridization, providing powerful support for reinforcement.

Smadja (2003) maintained that reinforcement can occur without reproductive character displacement. Indeed, several computer-based modelling studies maintain that for reinforcement to occur, there needs to be sufficiently lower hybrid fitness and sufficient divergence in the recognition system between the populations before secondary contact (Sved 1981; Spencer *et al.* 1986; Liou and Price 1994; Servedio and Kirkpatrick 1997). Furthermore, many population characters increase the occurrence of reinforcement, such as high carrying capacity, homospecific female choices, higher potential growth rates of populations, and high heritability of the SMRS (Liou and Price, 1994).

According to Noor (1999) and Coyne and Orr (2004), testing reinforcement needs many alternative hypotheses, which are themselves not easy to measure. Exciting progress has been achieved with the use of genetics in reinforcement in recent years (Servedio and Noor, 2003). These developments have also been enhanced by a new understanding of how chromosome rearrangements may allow incompatibilities to be maintained

despite hybridization in sympatry (Rieseberg, 2001; Navarro and Barton, 2003; Brown *et al.*, 2004).

1.4 The study animal *Rhabdomys*

The African striped mouse *Rhabdomys pumilio* (Sparrman, 1784) is a murid rodent that has a wide distribution south of the Sahara (Skinner and Smithers, 1990). Its economic importance (it can become an agricultural pest) and overall abundance has meant that more research has been done on it than most other African rodents (De Graaff, 1981). *Rhabdomys pumilio* inhabits many habitat types, although they prefer grasslands (De Graaff, 1981). It is an opportunistic omnivore with a varied diet (De Graaff, 1981). Adult males and females weigh 43.1g and 41.4 g respectively (De Graaff, 1981).

Striped mice breed from September to April (the wet summer months) on the highveld, followed by four months of anoestrus (Brooks, 1974). Following an average gestation period of approximately 25 days (Brooks, 1974; Pillay, 1999), 2-12 pups/litter (Skinner and Smithers, 1990; Kingdon, 1974; Brooks, 1974) are produced, with a mean of 5.9 on the highveld region of South Africa (Brooks, 1974). Weaning occurs at day 16 (Brooks, 1974; De Graaff, 1981). The age of sexual maturity varies across studies, with Pillay (1999) reporting maturity at 60 days and Kingdon (1974) reporting maturity at three months.

1.4.1 Taxonomy and interfertility

There are two karyotypic forms of *Rhabdomys pumilio* in South Africa (Mahida *et al.*, 1999). The 2N = 48 form is widespread whereas the 2N = 46

form, which is the result of a Robertsonian fusion, is restricted to the northern parts of the species distribution, particularly in the highveld region of the country (Ducroz *et al*., 1999; Rambau *et al*., 2003). Though karyotypic differences are found in this species, *Rhabdomys* was historically considered as a monospecific genus (Roberts, 1951; De Graaff, 1981). An allozyme electrophoresis study showed that gene flow is limited between widely spaced *R. pumilio* populations in South Africa (Mahida *et al*., 1999), and that the taxon conforms to an isolation by distance population genetic structure, which predicts that the genetic similarity between populations will decrease exponentially as the geographic distance between the populations increases (Wright, 1943).

The differences in chromosomal number have been useful to distinguish between races and have provided an opportunity to investigate interfertility between the races. Pillay (2000a) studied the relationship between chromosomal differences and interfertility in three widely spaced (>900 km) *Rhabdomys pumilio* populations, two with the 2n = 48 chromosomal form and one with the 2n = 46 form, and reported that females preferred males (or their olfactory signature) of their own population vs those of another population. A subsequent study by Pillay (2000b) demonstrated that behavioural incompatibility between mates severely reduced interpopulation breeding. The few hybrids that were produced were inviable and/or infertile. Taken together, Pillay's studies indicate that premating (behavioural) reproductive barriers are well-established, regardless of chromosomal difference (but see below).

Pillay's group then looked for areas where the two races were in close contact. In a subsequent MSc study of the chromosomal races on the highveld

of South Africa, Lancaster (2001) showed that for closely-occurring populations (~80km), neither sex showed a preference for same or different population mates. Interbreeding was successful, but there was F2 hybrid failure (i.e. they were sterile). Using the data from Pillay (2000a, b), Lancaster (2001) concluded that the results of these behavioural and breeding studies indicated the occurrence of geographical variation in mate recognition system and that the single Robersonian fusion that differentiates the two chromosomal populations could not cause postmating divergence between populations, suggesting that perhaps genomic variation must occur within the taxon. Importantly, behavioural incompatibility between distant populations but not between close populations concurred with the isolation by distance genetic model proposed by Mahida (*et al*., 1999)

In contrast to the earlier pronouncements on *Rhabdomys pumilio* being a monotypic genus, a recent phylogeographical study, using complete sequences of the mtDNA cytochrome b gene and cytogenetic approaches, revealed two major lineages within *Rhabdomys* (mean sequence divergence = 12%), strongly suggesting the existence of two species: *Rhabdomys pumilio* (clade 2) which has a westerly (i.e. xeric) distribution in South Africa and is characterised by the ancestral karyotype of the genus (2n = 48); and *Rhabdomys dilectus* (clade 1) which occurs in mesic areas (Rambau *et al*., 2003). Within clade 1, diploid number dichotomy coupled with sequence divergence (mean = 6%) suggested the distinction of two subspecies: *R. d. chakae* (2n = 48) which occurs in the east of South Africa, and *R. d. dilectus* which is characterised by the derived karyotype (2n = 46), and is found in the northern extent of the *Rhabdomys* distribution range (Rambau *et al*., 2003).

It is believed that divergence and speciation in the genus is associated with extensive paleoclimatic oscillation and habitat fragmentation through its distribution range (Rambau *et al*., 2003). Current ecological differences between the two clades may also explain differences in social organization (Schradin & Pillay 2005). *R. pumilio* in the arid succulent karoo forms social groups, comprising multiple adults of both sexes that share a nest and the same territory. In contrast, *R. d. chakae* in the moist grasslands of South Africa is solitary, territorial, and association between the sexes may be restricted to mating (Schradin & Pillay 2005). The social organisation of *R. d. dilectus* seems to be similar to that of *R. d. chakae* (Brooks 1974).

Assuming that the species and subspecies proposed by Rambau *et al*. (2003) are valid, this means that Pillay (2000a, b) studied all three groups and that Lancaster (2001) studied interfertility between the *R. dilectus* subspecies. To avoid confusion in my study, I will refer to striped mice by the genus name (*Rhabdomys*) from this point onwards.

The two *R. dilectus* subspecies are parapatric in part of their range in northern South Africa. However, no contact zone or naturally-occurring hybrids have been located despite intensive field work by us. The closest distance between the two forms is 15 km on the highveld in Gauteng, South Africa. The primary aim of my study is to assess whether these two forms (i.e. chromosomal races) are reproductively isolated, and a secondary aim is to ascertain whether reinforcement occurs. To assess the existence of premating reproductive isolation, I investigated male-female social interactions in intrapopulation and interpopulation dyadic encounters and female choice of odours of males from the same population and different population. Breeding

studies of intrapopulation and interpopulation pairs were used to test for the occurrence of postmating (and postzygotic) reproductive isolation. To ascertain whether reinforcement occurs, I compared my findings with those for distant populations (Pillay, 2000a, b) and populations occurring closer together Lancaster (2001).

1.5 Hypotheses and predictions

Hypothesis 1. The populations will be behaviourally compatible because of insufficient divergence in allopatry (Lancaster, 2001). Prediction 1. Levels of socionegative (aggression) and sociopositive (amicable) behaviours will be similar in different-population and same-population male-female dyadic encounters.

Prediction 2. Females will visit the odours of homotype (same population) males to the same extent as that of heterotype (different population) males in olfactory choice tests.

Hypothesis 2. Based on the findings by Lancaster (2001), I expect that the populations will not be interfertile. Specifically, I predict that same- and different-population breeding pairs will have high reproductive success, but F2 hybrids are expected to be infertile or inviable (i.e. have poor growth and reduced survival).

Hypothesis 3. The hybrid disadvantage reported by Lancaster (2001) suggests that reinforcement could occur in populations occurring closer together. If so, in

contradiction to hypothesis 1, I expect that the populations will display premating isolation, mainly as a result of behavioural incompatibility.

1.5.1 A theoretical model to test Hypothesis 3

Although theoretical models have provided support for the occurrence of reinforcement at various spatial scales, such as on islands (Servidio and Kirkpatrick, 1997), in sympatry (Kawecki, 1997) or in secondary hybrid zones (Liou and Price, 1994), reinforcement in nature has been mostly observed in sympatry (Smadja, 2003). The two *Rhabdomys* populations used in my study do not make contact, despite extensive searches for a contact zone. Nonetheless, the two forms could have made contact in the past, since the habitat between the two forms was more or less homogeneous grasslands historically, and in their recent evolutionary history, there were no geographical barriers between the two populations (Pillay pers com). Currently, anthropogenic activity may contribute to a lack of contact. If my assumptions about previous contact are correct, I propose that if I compare my results with those of Pillay (2000a, b) and Lancaster (2001), both of which included one population also represented in my study, premating reproductive isolation will follow a U-shaped function, being most developed in the distant populations because of geographic distance (Pillay, 2000a, b) and in my study because of reinforcement; behavioural compatibility has been established in intermediate populations by Lancaster (2001).

2 MATERIALS AND METHODS

2.1 General

Animals used in the study were live-trapped at Midrand (2N = 48) (26°05' S,
28°10' E) and Irene (2N = 46) (25°09' S 25°20' E), Gauteng Province; the
distance between the populations is approximately 15km. The vegetation type
is the same in both populations (i.e. grassland; Cowling *et al*., 1997).
Rhabdomys was trapped in late 2004, using PVC live traps (25 x 8 x 8 cm),
baited with a mixture of raisins, oats, salt and cooking oil. Traps were covered
with grass to buffer extreme temperature variations, and checked every
morning. Forty adult (20 males and 20 females) *Rhabdomys* from each locality
were brought back to the Milner Park Animal Unit, University of the
Witwatersrand and housed by population in separate rooms before
experiments. This was to ensure that animals had not been exposed to other
population members before experiments, which occurred following an
acclimatisation period of one month.

Animals were housed under partially controlled environmental
conditions (light regime of 14L:10D, lights on at 05h00, 23-26°C; 30-50%
relative humidity). Epol® mouse cubes were provided *ad libitum*, and the diet
was supplemented with commercial parrot seeds twice a week. Water was
available at all times.

2.2 Chromosomal studies

Yeast preparation. After the behavioural and breeding studies, a
random selection of 10 males and 10 females from each population were
euthanased for karyotypic studies. The technique used followed the yeast

stimulation of bone marrow mitosis method (Lee and Elder, 1980). A yeast suspension (2-3 g fresh yeast and 5-6 g dextrose mixed in 25 ml warm tap water) was incubated at 40° C for 20 – 40 min until active (indicated by vigorous foaming). The animals were injected subcutaneously with this mixture in the dorsal region (0.5 ml yeast suspension per 25 g body weight). This was repeated after 24 hours.

Cell harvest. Colcemid, a mitotic arresting agent, was injected (0.1 ml 0.002% 10 g body weight) intraperitoneally 24 hours after the last yeast injection. Animals were euthanased by CO_2 inhalation 20 – 40 min later, and the femur removed and cleaned. The bone marrow was removed with a syringe using a tissue culture medium (Dulbbeco's Modified Eagles Medium) and centrifuged for 10 min at 1000 rpm. The supernatant was removed and 10 ml of 0.075M KCl (which had been incubated at 37° C) added. After a 10 min incubation period, the mixture was centrifuged for 5 – 10 min at 1000 rpm. The supernatant was removed, and the cells incubated and centrifuged in 0.075M KCl as described above another 2 times. Each time, the cells were re-suspended by vigorously agitating the pellet. After the final centrifugation, the supernatant was removed and 6–8 ml Carnoy's fixative (3 methanol: 1 glacial acetic acid) was added. The cell suspension was placed in a fridge overnight. The cells were suspended and centrifuged for 10 min at 1000 rpm. The supernatant was discarded, the cells re-suspended and 6 – 8 ml Carnoy's fixative added. This was repeated twice after which the cells were re-suspended in 1 – 2 ml of fixative.

Slide preparation. Three to 4 drops of the final suspension were dropped from a height of about 60 cm onto dry slides. The slides were left to

dry and then stained with Giemsa. Poly-lycine L was used in the fixation of the chromosome onto the slide. The slides were then viewed under a light microscope and the number of chromosomes in each of 10 spreads per individual were counted and averaged to determine its diploid number.

2.3 Behavioural studies

Behavioural experiments were conducted between 08h00 and 11h00, which is the period of maximum diurnal activity in *Rhabdomys* (Pillay, 1999). Behaviours were video recorded using a Sony Handycam camera recorder and a Sharp video recorder. About 2h prior to experiments, vaginal smears were taken to determine the reproductive state of females. For this, vaginal swabs were taken using cotton wool covered tooth picks and transferred onto a microscope slide, which were stained with both haemotoxylin and eosin. Based on cell types present in the smears, only receptive (oestrus) females were used.

2.3.1 Dyadic encounters

The interaction of 20 same- and 20 different-population male-female pairs were studied in neutral-arena dyadic encounters. Different population pairings were bi-directional (i.e. Midrand female x Irene male and Irene female x Midrand male). Each animal was used twice, once in same and once in different population dyads. There was a 1-2 week break between the first and second use of a particular individual in dyads.

Tests were done in glass aquaria (46 x 36 x 39 cm) which were covered with white paper on the outside of three of sides (to reduce the amount of

external light during video-taping). The floors of aquaria were covered with 2 cm wood shavings. Dyad partners were marked using non-toxic paint to facilitate individual identification. Mice in each dyad were separated initially with an opaque barrier which was lifted after 5 min (after Lancaster, 2001). At the end of each test, the glass aquaria were cleaned with water and alcohol to eliminate the odours of the previous occupants. Animals were video-recorded for 15 min. The behaviour of each dyad member was analysed using continuous sampling (Martin and Bateson, 1993).

The duration of the following four behaviours (behavioural components given in brackets; after Lancaster, 2001) were scored: socionegative (avoiding, chasing, defending, fighting, lunging, submissive, threatening, wrestling); sociopositive (allogrooming, huddling, naso-anal sniffing and naso-nasal sniffing); non-contact (avoidance, exploring, inactive); and sexual (attempt mounting, mounting, presenting). Changes in behavioural components were scored if the behaviour performed exceeded 2 seconds (Dempster, 1996). A multivariate analysis of variance (MANOVA) was used to test for statistical differences for all behaviours between populations and different dyad types. The sexes were subjected to separate analyses. Tukey HSD post-hoc tests were used to identify specific trends. The α was set at 0.05, and all tests were two-tailed.

2.3.2 Olfactory choice tests

A two-way olfactory choice test was used to assess the preferences of females for the odour stimuli of males from the same and different population. We tested female subjects since mate choice was more easily detected in

female *Rhabdomys* (Bennett & Pillay 2001). The stimuli used were one week old soiled bedding, containing faeces, urine and other body secretions. Olfactory instead of whole animal cues were used since Pillay (2000a) showed that females respond in the same way to males and their odour. Females were exposed to odour of unfamiliar males only.

Soiled bedding was collected from several males in each population and pooled to produce a population odour stimulus. This was done to decrease the bias such as familiarity, dominance and other social factors, which are known to influence preference (Pillay *et al*., submitted). The bedding was frozen at -15° C, at which temperature odour is retained (Christophe and Baudoin, 1998). Bedding was defrosted 15 min before tests and placed in a choice apparatus built from transparent plexiglas material, comprising a start box (length = 36 cm, width = 20 cm, height = 16 cm) connected via a Y maze (Ø 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).

At the beginning of each test, a female was placed in the start box and 30g of soiled bedding was placed in each choice chamber. After familiarisation for a few min, the test animal was allowed to enter the Y maze, and video recording was started once the female entered the maze. Recordings were made for 18 min (Pillay *et al*., submitted). The time a female spent in each arm and corresponding choice chamber as well as the time spent in contact with stimuli (i.e. sniffing, licking stimuli) was recorded. I controlled for laterality by alternating the position of the two stimuli (left and right) between tests.

Paired t-tests were used to test the hypothesis that females prefer the odour of the same population than the other population males. The α was set at 0.05, and all tests were two-tailed.

2.4 Breeding studies

2.4.1. Breeding

Thirty within-population (15 per population) and 22 between-population breeding pairs were established. Between-population pairs were bi-directional (i.e. 12 Midrand female x Irene male and 10 Irene female x Midrand male). For each between-population pairing, the offspring produced were backcrossed with individuals of the parent stock (after Pillay *et al*., 1995a; Pillay 2000a; Lancaster, 2001). Backcross pairings were used to assess hybrid fertility.

Breeding took place in 40 x 25 x 12 cm Lab-o-tec cages. Each pair was allowed to produce at least three litters or separated after 60 days, whichever occurred first. Pairs which did not reproduce were separated after 50 days (Lancaster, 2001) as were any which engaged in damaging fights (after Pillay *et al*., 1995a). The interval between pairing and date of birth of the first litter, the interlitter interval, number of litters born per pair, and litter size were noted for successful breeding pairs. Litters and the mother were weighed on the day of birth (day 0) and again on day 20, and the preweaning growth rates of litters were calculated using the formula [ln(mass time 1)–ln(mass time 2)]/(time 2–time 1); the growth rate of litters rather than individuals was considered since offspring in a related group are not statistically independent of each other (Boonstra & Hochachka, 1997). The sex ratio of litters was ascertained at birth, and the proportion of offspring in a litter that survived to weaning was recorded.

χ^2 analyses were used to compare the numbers of pairs reproducing and the number of pairs separated because of damaging fights. Due to the lack of data in the different-population pairs (see Results), further statistical analyses were restricted to same-population Midrand and Irene pairs. Percentage offspring surviving to weaning and growth rate were compared using the Mann Whitney U test. T-tests were used to compare the interval between pairing and production of the first litter, inter-litter interval and total number of young per pair. χ^2 contingency table was used to compare sex ratio. An analysis of covariance (ANCOVA) was used to compare litter size (maternal mass as a covariate). In addition to growth rate (paired t test), I also compared changes in litter mass from day 0 to day 20 using a repeated measures design. Tukey HSD post-hoc tests were used to identify specific trends. The α was set at 0.05, and all test were two-tailed.

3 RESULTS

3.1 Chromosomal studies

All individuals from the Irene population that were karyotyped had a diploid number of 46, whereas all those from Midrand populations had a 2n = 48 chromosomal number.

3.2 Behavioural studies

3.2.1 Dyadic encounters

Female behaviour

There were significant differences in the behavioural components among the females across the dyad types ($F_{12,87}$ = 8.35, p<0.001; Figure 3.1a). Socionegative behaviours were significantly greater during different-population than same-population dyads (Tukey post hoc tests). Sociopositive behaviours were observed only in same-population dyads, with Midrand pairs showing significantly higher levels of these behaviours than Irene dyads. Sexual behaviours were low or absent across all dyad types, but tended to be more common in same-population dyads. Non-contact behaviours varied significantly across dyad types, being lowest in Midrand same-populations dyad.

Male behaviour

Like females, males also showed significant differences in behaviours across dyad types ($F_{12,87}$ = 4.98, p<0.001; Figure 3.1b). Patterns of social behaviour in the males were similar to those observed in females. Different-
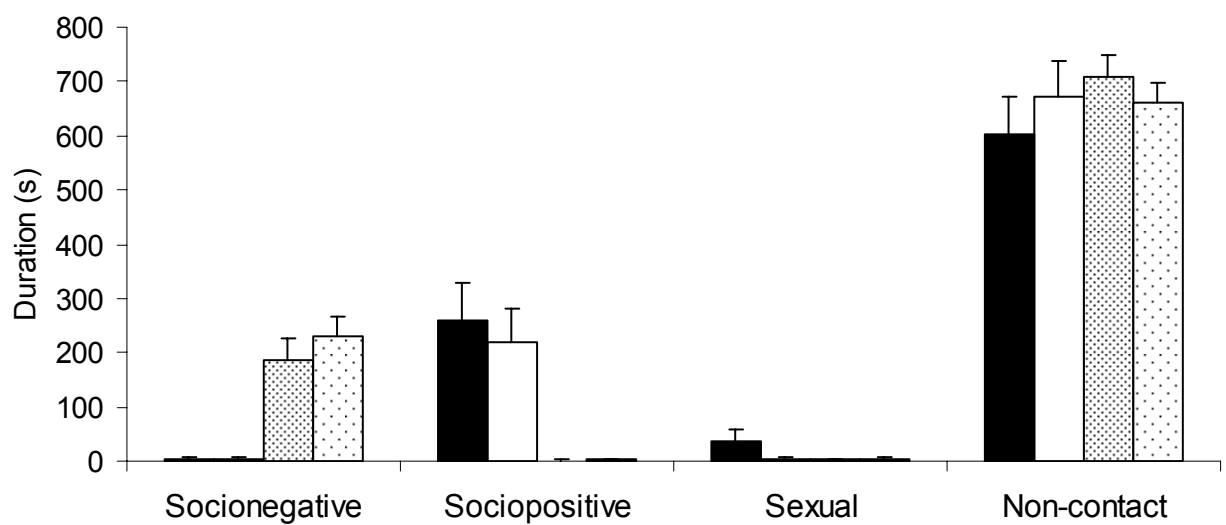
a) Females
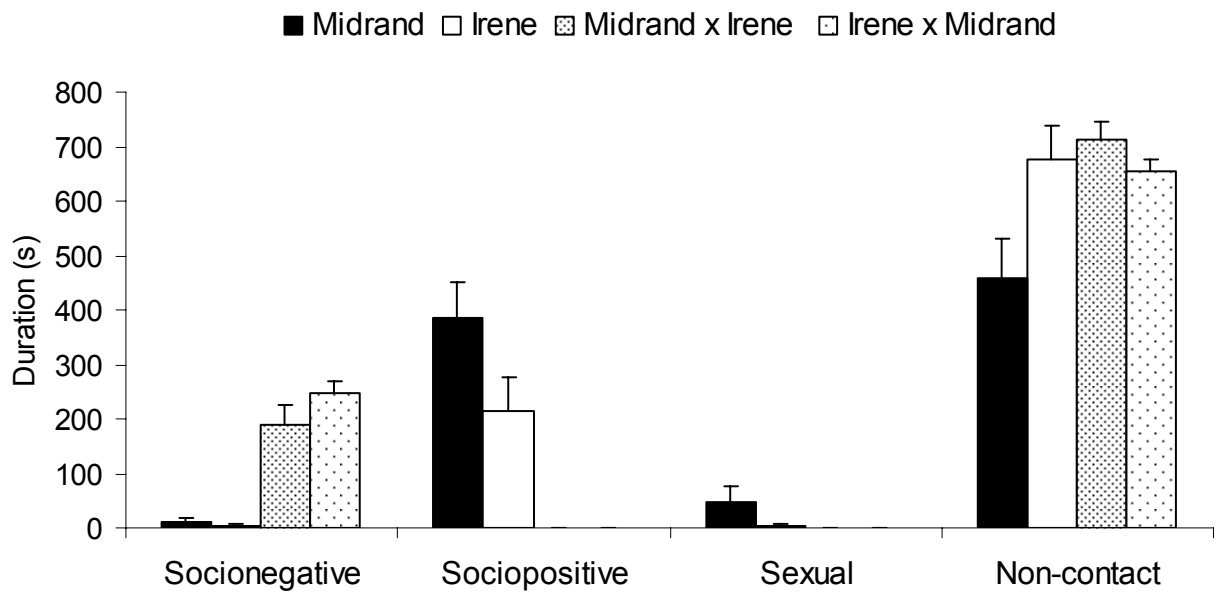
b) Males

Figure 3.1. Mean (±SE) duration of behavioural patterns of *Rhabdomys* a) females and b) males in dyadic encounters with opposite sex partners of the same and different populations. Between-population dyad types given as female x male.

population dyads displayed significantly higher socionegative and significantly lower sociopositive behaviours than same-population dyads (Tukey post hoc tests). Sexual behaviour was slightly more common in males than in females but were not significantly different across dyad types. Similar levels of non-contact behaviour occurred across dyad types.

3.2.2 Olfactory choice tests

Females from Midrand and Irene significantly preferred odour stimuli of males from their own population (Figure 3.2), both in terms of the time spent sniffing the odours (Midrand: t_9 = 4.52, p= 0.001, Irene: t_9 = 4.68, p= 0.001) and the time spent in the side of the Y maze with the odour source (Midrand: t_9 = 3.73, p= 0.005, Irene: t_9 = 3.08, p= 0.013).

Preferences were strong in both populations, with 9 out of 10 females (1 tie) Midrand females and all 10 Irene choosing homotype males. The level of interest of females was calculated by dividing the amount of time that females spent with both test samples by the total time of experiments (i.e. 18 min). Modderfotein females spent 22.45% (SE= 4.97) and Irene females spent 27.31% (SE=4.31) of time with test samples.

a) Odour source

b) Side of apparatus

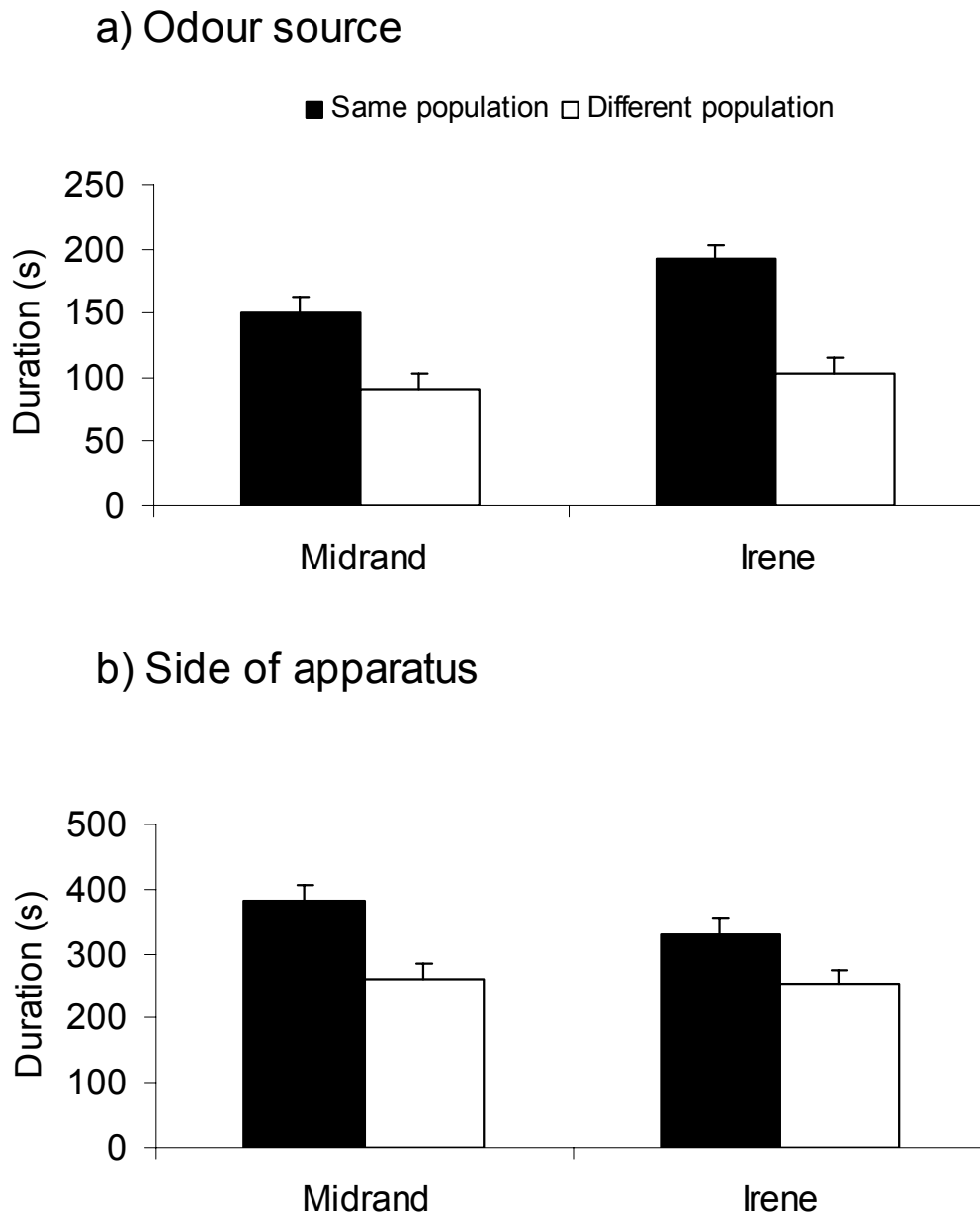Figure 3.2. Mean (+SE) duration of time that *Rhabdomys* females spent with a) the odour source and b) the side of the apparatus containing the odour source.

3.3 Breeding studies

A summary of the results of the breeding trials of same- and different-population pairs is given in Table 3.1. Reproductive success (numbers of pairs reproducing) decreased significantly across breeding pairs. Almost all (93%) of

same populations pairs produced offspring, whereas different population breeding success was significantly reduced. Only one Midrand x Irene pair (8%) and one Irene x Midrand pair (10%) produced offspring. No same-population pairs engaged in damaging fights, but several different-populations pairs actively engaged in damaging fights, and were separated.

Due to the lack of data in the different-population pairs, further statistical analyses were restricted to same-population pairs. Both the interval between pairing and production of the first litter and the interlitter interval were similar between the populations (Table 3.1).

Table 3.2 provides details of the litter characteristics of successful pairs. None of the parameters measured differed between same-population pairings, including the number of litters produced per pair, litter size, total number of young, sex ratio, offspring survival to weaning, and pre-weaning growth rate. In addition, there was no difference in litter mass on day 0 (Midrand = 24.99 ± 0.99 g; Irene = 22.65 ± 0.95 g) and day 20 (Midrand = 163.92 ± 7.76 g; Irene = 143.04 ± 6.20 g) between populations ($F_{1,67}$ = 2.96, p=0.09). As expected, in both Midrand and Irene population, litter mass on day 0 and day 20 increased due to growth ($F_{1,67}$ =786.87, p<0.001). There was no difference in maternal mass at weaning between the Midrand (n = 37, 71.50 ± 16.71 g) and Irene (n = 32, 69.03 ± 16.47 g; t_{67} = 0.62, p=0.539) populations, and maternal mass was not a good predictor of litter size ($F_{1,66}$ = 0.03, p=0.868).

Table 3.1. Mating success, pairs engaged in damaging fights, interval between pairing and production of first litter, and interlitter interval for the pairings indicated. Dashes indicate no data available. Different-population dyads are given as female x male; n = sample size.

Pairings	Proportion of successful pairs	Pairs fighting (%)	Mean ($\pm$SE) interval between pairing and first litter	Mean ($\pm$SE) interlitter interval
Same-population				
Midrand	14/15	0	27.21 (4.04); n = 14	27.35 (4.68); n = 26
Irene	14/15	0	29.79 (9.51); n = 14	25.14 (3.26); n = 22
Different-population				
Midrand x Irene	1/12	41.66	25 (0); n = 1	-
Irene x Midrand	1/10	40	33 (0); n = 1	-
Statistics	$\chi^2_3 = 16.39$, p<0.001	$\chi^2_3 = 14.85$, p=0.002	$t_{26} = 0.93$, p=0.361[*]	$t_{46} = 1.60$, p=0.118[*]

[*] – Only same-population pairings were compared because of no/few data for different-population pairings.

Table 3.2. Litter characteristics of successful pairings and mother's mass at weaning. Different-population dyads given as female x male; n = sample size.

Pairings	Mean (±SE) litters/pair	Mean (±SE) litter size	Mean (±SE) number of young/litter	Sex ratio F: M	Mean (±SE) Offspring surviving to weaning (%)	Mean (±SE) Growth rate
Same-population						
Midrand	2.60 (0.91); n = 14	19.53 (8.36); n = 39	19.53 (8.36); n = 14	149:151	98.66 (5.22); n = 39	0.10 (0.13); n = 37
Irene	2.47 (0.99); n = 14	16.00 (8.18); n = 35	16.00 (8.18); n = 14	120:117	98.68 (4.81); n = 35	0.09 (0.02); n = 32
Different- population						
Midrand x Irene	1 (0.29); n = 1	4.0 (0.00); n = 1	4.0 (0); n = 1	2:2	100 (0); n = 1	-
Irene x Midrand	1 (0.32); n = 1	6.0 (0.00); n = 1	6.0 (0); n = 1	4:2	83.33 (0); n = 1	
Statistics *	t_{28} = 0.70, p=0.384	$F_{1,66}$ = 2.70, p=0.105	t_{27} = 1.76, p=0.09	χ^2_1 = 0.04, p=0.824	U = 677, N = 39,35, p=0.955	U = 473, N = 37,32, p=0.154

* – Statistical comparisons were made for the same-population pairings only; different-populations were not included because of small sample sizes.

4 DISCUSSION

My experiments indicate that behavioural divergence has occurred between the two *Rhabdomys* populations. Both Midrand and Irene females visited odour stimuli of males from their own population more often and for longer periods than odour of males from the other population. In addition, there were higher levels of socionegative (e.g. aggression) behaviours in different-population dyadic encounters than in same-population encounters, particularly by females. In contrast, higher levels of sociopositive (e.g. amicable) behaviours were observed in same-population encounters.

Breeding studies indicated that both populations had similar breeding success (93% in both Midrand and Irene) and reproductive performance. However, only two (9%) of different-population pairs produced offspring. None of the few hybrids produced bred. Interpopulation crosses were characterised by high levels of agonistic behaviours, leading to damaging fights. Taken together, the results of the behaviour and breeding studies indicate that premating reproductive isolation is well-developed between the Midrand and Irene populations. Postmating isolation was not detected using the protocol used.

4.1 Behavioural isolation

The results of my study resemble those of a study of the vlei rat *Otomys irroratus* (Pillay *et al*., 1995c). Allopatric populations of the vlei rat displayed significantly higher levels of aggression (socionegative) and significantly lower levels of amicability (sociopositive) in different-population dyadic encounters, and the frequency of sexual behaviours was markedly reduced in

interpopulation encounters (Pillay *et al.*, 1995c). Although sexual behaviours were low or absent across all dyad types in my study, these behaviours tended to be more common in females in same-population dyads. Pillay *et al.* (1995c) concluded that the populations in their study were behaviourally isolated, which appears to be the case in the Irene and Midrand striped mouse populations.

Various patterns of mate preference have been observed in different rodent taxa. Female house mice *Mus m. musculus* 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). Whole animal and olfactory choice experiments in *O. irroratus* (Pillay *et al.*, 1995b) and *Rhabdomys* populations (including the Irene population used in my study; Pillay 2000b) revealed that females significantly preferred same-population males. Olfactory preference for same population and karyotypically similar individuals was also reported in the phyllotine rodent *Graomys griseoflavus* (Theiler and Blanco, 1996a, b).

Other examples which demonstrate preference for same population/species over a different population/species, include red-legged *Alectoris rufa* and rock partridges *A. graeca* (Ceugniet and Aubin 2001), fly catchers *Ficedula* spp. (Saetre *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* (Ritchi *et al.*, 1989).

Mate recognition systems involve the co-evolution of two components (the emitter of the signal and the receptor), which is under strong stabilising selection. There are two theories about the routes to the co-evolution. First, the genetic coupling hypothesis assumes a common genetic basis for the two communication entities (Butlin, 1994). Second, an increasing number of studies also suggest that signal and receiver may co-evolve in an uncoupled fashion (Gerhardt, 2005; Schul and Bush 2002). This uncoupled (non-parallel) evolution may be the result of sensory drive (Endler, 1992) and/or sensory exploitation (Endler and Basolo, 1998; Ryan and Rand, 1990), both of which could explain the evolution of new signals. The generality of these mechanisms has been questioned, however (Schul and Bush, 2002). Understanding the evolution of mate recognition systems requires an assessment of both signal and perception characteristics. Visual and acoustic signals have received much attention (Borland, 1986; Butlin, 1994; Saetre *et al*., 1997; Rolan-Alvarez *et al*., 1999; Albert and Shulter, 2004; Gerhardt, 2005), while olfactory signals have received some attention (Singer, 1997; Kayali-Sayadi, 2003).

Pillay *et al*. (submitted) studied odour-based mate recognition in *R. pumilio* and both subspecies of *R. dilectus*, including the Irene population used here. By studying female responses (receptor) for male odour (signal) in two populations per taxon, Pillay *et al*. (submitted) showed that two subspecies of *R. dilectus* share a common odour signal, distinct from that of *R. pumilio*, and also that each subspecies carries different signals. Moreover, females of all three taxa displayed different perception characteristics (i.e. possessing different receptors). Interestingly, populations within each taxon seemed to have a similar signal-receptor system, despite occurring over large geographic

distances. Therefore, it seems plausible that the mate recognition systems of Irene and Midrand populations appear to have diverged, which may explain the homotypic choice and heightened aggression.

4.2 Breeding studies

The decreased reproductive success in interpopulation and backcross pairings observed in my study has been reported in other rodents. A study by Pillay *et al*. (1992) on three chromosomally distinct allopatric populations of *O. irroratus* revealed that all intrapopulation pairings reproduced, while reproductive success was reduced to 83% in interpopulation pairings. In another study on *O. irroratus*, Pillay *et al*. (1995a) showed that reproductive success was 100% in intrapopulation pairs and 48% in interpopulation. Pillay, (2000a) studied three allopatric populations of *Rhabdomys* (including the Irene population used here) and showed that reproductive success was high (74-87%) in intrapopulation pairings and extremely reduced (0-53%) in interpopulation pairings. A study on house mice reported reduced reproductive success between chromosomal races: 65% in intraracial and 50% in intertracial pairings (Chatti *et al*., 2005).

Reduced interspecific breeding success has also been reported in other taxa. Saetrae *et al*. (1997) reported that sympatric populations of European flycatchers of the genus *Ficedula* had a within-species reproductive success of 95% and a 26% between-species reproductive success. Noor (1995) reported qualitatively higher intraspecies reproductive success in *D. persimilis* and *D. pseudoobscura.*

My study suggests that the higher degree of damaging fights due to the pre-copulatory aggression may have delayed the production of the first and successive litters, as suggested by Pillay *et al*. (1995c) and Pillay (2000a). The underlying mechanisms promoting aggression is not known, but most likely involves olfactory cues (Dempster, 1996; Ganem *et al*., 1996; Pillay 2000a). There is some evidence that differences in morphology and behavioural displays between two subspecies of carabid beetles *Calleida viridipennis* leads to injuries during courtship (Sota and Kubota, 1998). Tynkkynen *et al*. (2004) showed that character displacement in wing spot size leads to the interspecific aggression between the banded demoiselle *Calopteryx splendens* and the beautiful demoiselle *C. virgo.* Interspecific aggression is an important evolutionary force (Pillay *et al*., 1995a, b, c; Dempster, 1996; Ganem *et al*., 1996; Pillay 2000a), often leading to assortative mating and premating isolation ultimately (Servedio and Noor, 2003).

4.2.1 Chromosomal variation and interfertility

The role of chromosomal differences in reproductive isolation and thus speciation is not clear. Karyotypic differences can cause interbreeding problems, and are thus important in keeping the populations isolated (Britton-Davidian *et al*., 2000). Alternatively, chromosomal differences may have arisen as a by-product of divergence in allopatry (Baker and Bickham, 1986). Several models have been proposed that relate chromosomal changes to reproductive isolation. Fischer *et al*. (2000) maintained that chromosomes might cause mis-segregation during meiosis resulting in reduced fertility or complete sterility of chromosomal hybrids. Baker and Bickham (1986) proposed a model whereby

chromosomal speciation occurs in small peripatric founder populations, and heterozygotes produced are selected against. The stasipatric model of speciation proposes that chromosomal speciation occurs within the species range (White, 1968); here, heterozyogtes have lower fitness and are out competed by homozygotes (parental type)

Britton-Davidian *et al*. (2000) maintained that if chromosomal mutations are complex enough, chromosomal evolution is an efficient isolation mechanism. Regardless of how chromosomal divergence occurs, theoretical models maintain that structural rearrangements are expected to result in problems with meiosis in the hybrids (Patton and Sherwood, 1983; Capanna *et al.*, 1985; Capanna and Redi, 1994), although there are exceptions (e.g. cotton rat *Sigmodon* fulviventer; Patton and Sherwood, 1983). In addition, Goulielmos and Zouros (1995) proposed that chromosomal mutations can disrupt gene interactions, resulting in reduced fertility and viability. Genetic imbalances themselves may promote physiological, behavioural and/or morphological (e.g. gamete) abnormalities may also cause hybrid failure (Mayr, 1963; Dobzhansky *et al*., 1968; Goulielmos and Zouros, 1995). Too few hybrids were produced in my study to evaluate the influence of chromosomal and/or genetical factors on hybrid fitness. Nonetheless, the single Robertsonian mutation in *Rhabdomys* was not expected to result in interbreeding problems (T. Robinson, pers. comm.; see also Ganem *et al*., 1996).

4.3 Assessing reinforcement

Reinforcement refers to a process where hybridisation between two sympatric taxa produces hybrids with reduced fitness, and subsequent

selection would operate against the energetically wasteful production of hybrids (Dobzhansky, 1940; Howard, 1993; Butlin, 1995; Noor, 1999). The Irene and Midrand populations occur in close geographic proximity but are not sympatric. For this reason, I proposed a U-shaped hypothesis of pre-mating isolation and geographic distance in *Rhabdomys* (see section1.5.1).

A comparison between my study and those of Pillay (2000a, b) and Lancaster (2001) indicate that patterns of behavioural compatibility are not consistent with geographic distance (Table 4.1). Aggression levels in dyads and breeding pairs were highest in distant populations (Pillay 2000b) and in my study. In contrast, Lancaster (2001) reported behavioural compatibility in populations occurring 80km apart. Olfactory choice tests revealed that females preferred odour stimuli of males from same populations, which concurred with findings of Pillay (2000a) in distant populations (Table 4.1). Again, the study by Lancaster (2001) contradicts the other two studies, since females did not show any preferences in populations located 80km apart. Lancaster (2001) found evidence of hybrid inviability in breeding studies, but in my study and that by Pillay (2000a), too few hybrids were produced to assess their fitness.

Taken together, these studies confirm my prediction of a U-shaped model of pre-mating isolation: well-developed in distant and close populations but does not appear to exist in intermediate populations. It is tempting to speculate that the enhanced premating isolation may function to reduce the production of inviable hybrids that were found by Lancaster (2001), which in turn would explain the lack of a hybrid zone. Similarly, behavioural incompatibility explains a lack of hybrid zones in populations of house mice (Capanna *et al.*, 1985; Cox, 1984).

Table 4.1. Comparison of the behavioural and breeding characteristics in three studies using *Rhabdomys* populations occurring at different geographic distances apart. Geographic location of populations is provided in Figure 4.1.

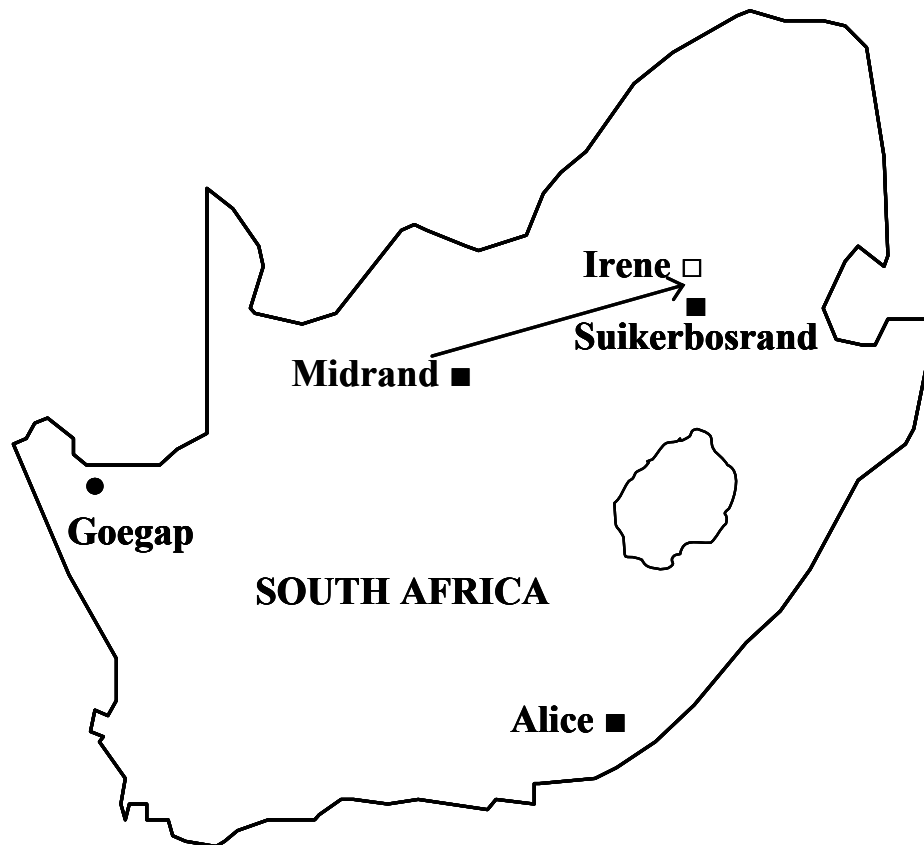| Parameters | Geographic distance | | |
	>900 km	80 km	15 km
Populations	Geogap, Alice and Irene	Irene, Suikerbosrand	Irene and Midrand
Male-female behaviour	Aggression higher in different-population than same-population pairs.	The behaviour of between-population dyads indistinguishable from same population dyads.	Different-population dyads displayed higher levels of aggression and lower levels of amicability than same-population dyads.
Olfactory choice tests	Females preferred homotype rather heterotype males.	Both males and females did not show a preference between same- and different-population mates	Test females preferred odour stimuli of males from same than the other population.
Breeding studies	• Different-population pairs had reduced breeding success and higher levels of damaging fights. • Hybrid survival compromised • Hybrids had slower growth rate.	• Damaging fights only in backcross pairings. • Mortality rates highest in the offspring of backcrosses. • Similar growth rate of offspring in different pairings.	• Markedly reduced breeding success and damaging fights in different-population pairs. • Very few hybrids produced and none reproduced.
Source	Pillay 2000a, b	Lancaster 2001	Present study

Figure 4.1. The geographic location of *Rhabdomys* populations used in behavioural and breeding studies. Pillay (2000a, b) studied Goegap, Irene and Alice, Lancaster (2001) studied Irene and Suikerbosrand, and I studied Irene and Midrand (indicated with an arrow). Symbols next to the location names show taxon and clade affinity (after Rambau *et al.*, 2003): circle = *R. pumilio*, clade 2, 2n = 48; closed square = *R. d. chakae*, clade 1. 2n = 48; and open square = *R. d. dilectus*, clade 1, 2n = 46.

Because the Irene and Midrand populations are not sympatric, there are likely to be alternative explanations for the behavioural incompatibility between the populations. It is possible that the populations are subjected to different ecological pressures, such as competition for signal space, predation, and habitat structure; mate recognition systems are sensitive to ecological variation in several taxa (Ryan, 1990; Butlin and Ritchie, 1994;

Nelson, 1989; Seddon, 2005). Other factors known to influence mate preference include sexual selection, genetic drift and genetic divergence due to founder events (Noor, 1999; Tregenza *et al.*, 2000)

4.4 Conclusions and future studies

The two populations used in the present study represented two chromosomal races, and possibly even two subspecies: *R. dilectus dilectus* (Irene) and *R. d. chakae* (Midrand; Rambau *et al.*, 2003). Sub-speciation within *R. dilectus* is suspected to have occurred some 600 000 years ago (Rambau *et al.*, 2003). Hence, one would hypothesise that isolating mechanisms would have evolved in allopatry. However, Lancaster (2001) showed that the subspecies are behaviourally compatible, indicating no divergence in allopatry. Nonetheless, behavioural compatibility does not imply that mate recognition signals are similar in the Irene and Suikerbosrand populations studied by Lancaster, since there is some evidence that the mate recognition system of the two *Rhabdomys* subspecies has diverged (Pillay *et al.*, submitted). Instead, it is possible that the individuals from the Irene and Suikerbosrand populations do not discriminate between each other. One reason could be a strong outbreeding tendency in *Rhabdomys* (Pillay, 1999).

Taking the above arguments to their logical conclusion, I assume that the Irene and Midrand populations have different mate recognition systems, based on subspecies differentiation reported by Pillay *et al.* (submitted). These differences could explain the mate preference findings and even the high levels of aggression in dyads and during breeding. It is still unclear why the differences in mate signals resulted in strong premating isolation in my

study but not in that of Lancaster (2001). I suggest that one explanation could be reinforcement of postmating isolation, although other explanations may apply. Ultimately, premating behavioural barriers would reduce gene flow between the populations.

Future studies could follow many directions. One of the most important would be to put more effort to find a contact zone between the two populations. In addition, I studied only two populations in close proximity, and it would be important to study more populations to assess if the patterns observed here are widely applicable.

Reasons for a lack of a contact zone and even the behavioural divergence could be because of geographical, climatic or ecological barriers. In recent evolutionary history, there were no geographical barriers between the two populations. The climatic conditions of the two regions are very similar with minimal differences in the area separating Irene and the Midrand. Additionally, *Rhabdomys* is a generalist which is tolerant of a wide variety of climatic conditions. Thus ecological correlates should be studied in more detail. A seemingly minor difference, such as the quantity or quality of food may have a major influence in keeping the populations apart (Noor, 1999).

The hypothesis that premating isolation may be due to reinforcement must be fully explored in future. In particular, the genetics of close populations need to be compared with populations occurring further apart to investigate contact during the historical past (Servedio and Noor, 2003). Reinforcement also occasionally leaves a signature referred to as reproductive character displacement' (Servedio, 2004), which should be investigated in close and distant striped mice populations.

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