Gene-flow in the rock hyrax (*Procavia capensis*) at different spatial scales

by
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Dissertation presented for the degree of Master of Science in the Faculty of Science at Stellenbosch University

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December 2013

Declaration

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Abstract

Limited dispersal, territoriality and the occupation of patchy habitats; characters that typify most African rock-dwelling (saxicolous or rupicolous) species, often result in structured genetic patterns with little or no gene-flow among populations (e.g., velvet worms, net-winged midges, elephant shrews, red rock rabbits and a variety of lizards and agamas). In an attempt to deepen our understanding of taxa that inhabit these "terrestrial islands" the distribution of genetic variation was studied at several spatial scales in the rock hyrax, *Procavia capensis*. This species has a polygynous social system that is unusual among taxa with similar ecological requirements, and a morphology that would intuitively be associated with poor dispersal capabilities (short limbs and a squat, heavy body). Possibly as a consequence of these considerations, few studies have attempted to determine the distance of migration by rock hyraxes and the influence that their social system and the surrounding landscape has on dispersal success. This investigation therefore tests hypotheses of how the ecology, distribution, social structure and the connectivity of the surrounding landscape have contributed to shaping the structure of rock hyrax genetic variation across the Namaqualand and western Fynbos regions. To do so, mitochondrial and microsatellite markers were used to document gene-flow at a fine spatial scale (an isolated population comprising 5 koppies), an intermediate spatial scale (across known geographic barriers to saxicolous taxa - the Cape Flats and Knersvlakte), and a regional spatial scale (across the Namaqualand/western Fynbos regions of South Africa - regions exhibiting contrasting landscape connectivity). In addition the genetic diversity, spatial clustering, sex-biased dispersal and relatedness (fine-scale) of colonies is described and the major genetic breaks detected in the investigation dated using a relaxed molecular clock approach. Finally, these results were compared to other studies that identified the Cape Flats and Knersvlakte as phylogeographic disruptors.

The genetic patterns at a fine spatial scale were complex: Gene-flow was restricted by the social structure of the rock hyrax rather than geographic distance, dispersal was female-biased and there was significant genetic structure. Genetic structure was also evident at the intermediate and regional spatial scales. In the Hottentots Holland Mountains and Cape Peninsula gene-flow was restricted (in both data sets) in comparison to localities that traversed the Cape Flats. In contrast, gene-flow across the Knersvlakte was restricted in the mitochondrial DNA data set but not so with microsatellites. A similar pattern was observed at a regional scale pointing to male-biased dispersal within this species - a result of its

polygynous social structure. In addition to sex-biased dispersal, landscape connectivity also influenced gene-flow on a regional spatial scale as the Namaqualand region, which has greater intermediate suitable habitat compared to the western Fynbos region, displayed significantly higher levels of gene-flow between sampling localities. Consequently, colonies in Namaqualand were genetically more diverse compared to those of the western Fynbos region. Two major matrilineal clades were evident on both side of the Knersvlakte - one to the north of this biogeographic break (Namaqualand), and the other to the south (western Fynbos). This was not, however, evident from the microsatellite data (reflecting the influence of male dispersal) where seven nuclear clusters were found. In keeping with other studies on saxicolous vertebrate taxa straddling the same region, this area of low connectivity has acted (and probably still does) as a barrier to gene-flow. Importantly, unlike in many other (admittedly invertebrate) species, no evidence of a genetic break was detected among hyrax populations across the Cape Flats. Colonies across the Hottentots Holland Mountains and Cape Peninsula regions may have been subject to founder-events and breeding isolation.

This investigation demonstrated the importance of using a well-structured sampling regime that included both mitochondrial and nuclear markers and it underscores the need to apply appropriate statistical programmes for inferring genetic patterns. It shows that landscape genetics may be useful in a conservation context and should be taken into account when planning conservation initiatives that include the implementation of corridors. In brief, the information contained in this study advances our knowledge of the dispersal capability and genetic diversity of contemporary rock hyrax populations.

Opsomming

‘n Beperkte spreidingsvermoë, territorialiteit en die bewoning van yl-verspreide habitat is kenmerkend van die meeste klip-bewonende spesies in Afrika en dit veroorsaak gereeld gestruktureerde genetiese patrone met min of geen genevloei tussen populasies (bv., die velvetwurms, net-vlerk muggies, klipklaasneuse, klipkonyne en ‘n verskeidenheid akkedisse en koggelmanders). In ‘n poging om kennis oor taksa wat hierdie “terrestriële eilande” bewoon te verdiep, het ons die die verspreiding van genetiese variasie bestudeer oor verskeie ruimtelike skale in die klipdassie, *Procavia capensis*. Hierdie spesie het ‘n veelwywige sosiale sisteem, wat vreemd is onder taksa met soortgelyke ekologiese vereistes, en ‘n morfologie wat intuïtief verbind kan word met swak spreidingsvermoëns (kort bene en ‘n kort, dik liggaam). As ‘n moontlike resultaat van hierdie oorwegings het min studies tot dusver daarop gefokus om die migrasie-afstand van klipdassies en die invloede van hulle sosiale sisteem en die omliggende landskap op spreidings-sukses te bepaal. Hierdie studie toets daarom hipoteses oor hoe die ekologie, verspreiding, sosiale struktuur en die konnektiwiteit (verbindheid) van die omliggende landskap bydra om die struktuur van genetiese variasie in klipdassies oor die Namakwaland en westelike Fynbos streke te beïnvloed. Derhalwe is mitochondriale en mikrosatelliet merkers gebruik om genevloei te bepaal op ‘n fyn ruimtelike skaal (‘n geïsoleerde populasie bestaande uit 5 koppies), ‘n gemiddelde ruimtelike skaal (oor bekende geografiese grense vir klipbewonende taksa - die Kaapse Vlakte en die Knersvlakte), en op ‘n streeks (groot) ruimtelike skaal (oor die Namakwaland/westelike Fynbos streke van Suid-Afrika - streke met kontrasterende konnektiwiteit van die landskap). Bykomend is die genetiese diversiteit, ruimtelike groepering, seksuele eensydigheid in spreiding en genetiese verwantskappe (fyn skaal) van kolonies beskryf en die hoof genetiese skeiding gedateer deur gebruik te maak van ‘n ontspanne molekulêre klok. Laastens het is die resultate van hierdie studie vergelyk met dié van ander studies wat die Kaapse Vlakte en Knersvlakte as filogeografiese skeidings gevind het.

Die genetiese patrone op ‘n fyn ruimtelike skaal was kompleks: Genevloei is beperk deur die sosiale struktuur van die klipdassie eerder as geografiese afstand, migrasie was wyfie-spesifiek en daar was beduidende genetiese struktuur tussen kolonies. Genevloei was beperk in die Hottentots Holland berge en die Kaapse Skiereiland (in beide datastelle) in vergelyking met lokaliteite oor die Kaapse Vlakte. In kontras was genevloei oor die Knersvlakte beperk in

die mitochondriale DNA, maar nie in die mikrosatelliete nie. ‘n Soortgelyke patroon is waargeneem op ‘n streeks skaal wat dui op mannetjie-spesifieke spreiding in hiérdie spesie - ‘n resultaat van die veelwywige sosiale struktuur. Bykomend, saam met geslag-spesifieke spreiding, het landskaps konnektiwiteit ook genevloei beïnvloed op ‘n streeks skaal omdat die Namakwaland streek, wat meer tussenleggende geskikte habitat bevat in vergelyking met die westelike Fynbos streek, beduidende hoër vlakke van genevloei tussen lokaliteite getoon het. Gevolglik was kolonies in Namakwaland geneties meer divers in vergelyking met dié van die westelike Fynbos streek. Twee hoof moederlike genetiese groepe is waargeneem op elke kant van die Knersvlakte - een aan die noorde van hierdie biogeografiese skeiding (Namakwaland) en een in die suide (westelike Fynbos). Dieselfde patroon was egter nie waarneembaar in die mikrosatelliet data nie (wat die invloed van mannetjie-spesifieke spreiding toon) waar sewe nukluêre groepe gevind is. In ag genome ander studies op klipbewonende gewerwelde taksa oor dieselfde verspreiding, het hierdie area van lae konnektiwiteit histories (en heelmoontlik ook huidiglik) as ‘n grens vir genevloei gedien. Belangrik, anders as in ander (hoewel ongewerwelde) spesies, kon ons geen bewyse verskaf van ‘n genetiese skeiding tussen klipdassie populasies oor die Kaapse Vlakte nie. Kolonies in die Hottentots Holland berge en Kaapse Skiereiland is dus onderhewig aan moontlike vestigings-effekte en telings-isolasie.

Hiérdie studie demonstreer die belang van die gebruik van ‘n goed-gestruktureerde monsternemingskema, die insluiting van beide mitochondriale en nukluêre merkers en dit beklemtoon ook die noodsaaklikheid van die gebruik van toepaslike statistiese programme vir gevolgtrekkings oor genetiese patrone. Dit toon ook dat landskapsgenetika nuttig mag wees in ‘n bewaringskonteks en in ag geneem moet word in die beplanning van bewarings inisiatiewe wat die implementering van korridors insluit. Kortliks, die informasie in hierdie studie bevorder ons kennis oor die spreidingsvermoë en genetiese diversiteit van kontemporêre klipdassie populasies.

Dedication

"Nothing in biology makes sense,
except in the light of evolution" –
Theodosius Dobzhansky

Dedicated to Sophia Louisa Visser (1914 – 1997) and Jacob Genis Visser (1946 – 2008).

Acknowledgements

My sincere gratitude to my supervisors, Professors Bettine van Vuuren and Terry Robinson for their ideas, encouragement and unfailing support. Their advice and enthusiasm are greatly appreciated.

I am indebted to the following people who assisted in various ways, especially the landowners and conservation agencies who allowed me access to their properties. Without their generosity this study would not have been possible: Alpie van den Heever (Springbok), Hannes and Brenda du Toit (Garies), Pikkie and Lida Rossouw (Brand-se-Baai), Gert van der Westhuizen (Nuwerus), Henry (Snr), Debbie and Henry (Jnr) Turner (Kliprand), Hugo van Niekerk (Nieuwoudt-ville), Wimpie and Susan Basson (Klawer), Kosie Greeff (Doringrivier), Sakkie and Helena Rossouw (Donkiesbaai), Theunis Smit (Elandsbaai), Klaas van Zyl (Vredenburg), André Visser (Ceres), Willie Mostert (Paardeberg), Mike Williams and his team (Table Mountain Arial Cableway), Justin Buchman (SANParks, Boulders Penguin Colony), Douglas Harding (SANParks, Boulders Penguin Colony), Cuan McGeorge (Bettiesbay Penguin Colony), Jannie du Plessis (SANParks, Table Mountain National Park) and Pia Galardi (SANParks).

All specimen collections were done so under permits from CapeNature (AAA-004-00714-0035) and SANParks (Issued 18 April 2011). Ethical clearance for the project was provided by the Stellenbosch University Ethics Committee (Ethics clearance 11NP_JAN01).

Special thanks to those who provided field assistance: Daniël Viljoen, André Visser, Robert Stolk, Schoeman Laubscher, Danny Potgieter, Cuan McGeorge, Josia Basson and Douglas Harding. I would like to express special gratitude to Sakkie and Helena Rossouw, Willie Mostert, Mike Williams and the staff at the Table Mountain Cableway who went out of their way to extend a helping hand in times when everything did not proceed smoothly.
Financial support was provided by the National Research Foundation (NRF Scarce Skills Scholarship) and Stellenbosch University (Post-graduate Merit Bursary).

Finally, I would like to express my sincerest gratitude and appreciation to my parents and friends for their love, encouragement and understanding throughout the course of this project.

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CHAPTER 1

Background to the study and study animal

1.1. Introduction

Biodiversity science has as one of its aims the explanation of spatial and temporal patterns of biotic diversity across different spatial scales. Of particular importance is the extent of gene-flow between isolates since this can directly affect species' integrity by increasing or decreasing population distinctiveness (Whitlock and McCauley, 1999). This has become critical in the face of rapid environmental change and biodiversity loss where the identification of populations, or groups with independent evolutionary histories, provide conservation agencies with valuable information for establishing suitable management strategies that may reduce the chances of local depletion (Moritz *et al*. 2000).

The life-history of a species contributes to the distribution of genetic variation across the landscape (Smit *et al*. 2011). Life-history attributes such as limited dispersal capability, territoriality and the occupation of patchy habitats characterize African rock-dwelling (saxicolous or rupicolous) mammals (see examples in Skinner and Chimimba, 2005). A structured genetic pattern with absence of (or limited) gene-flow has therefore been found in various southern African mountain-dwelling or rocky outcrop-specialist species (Prinsloo and Robinson, 1992; Prinsloo, 1993; Branch *et al*. 1995; Matthee and Robinson, 1996; Daniels *et al*. 2001; Lamb and Bauer, 2000; Wishart and Hughes, 2001, 2003; Matthee and Flemming, 2002; Gouws *et al*. 2004; Smit *et al*. 2007; Swart *et al*. 2009; Daniels *et al*. 2010; Gouws *et al*. 2010; Portik *et al*. 2011; Smit *et al*. 2011; McDonald and Daniels, 2012).

In this study the aim is to extend and build on existing knowledge of taxa that inhabit these ecologically important regions (Mares, 1997) within South Africa. Using both mitochondrial and nuclear markers, the distribution of genetic variation was determined at several spatial scales in the rock hyrax, *Procavia capensis*. These saxicolous, medium-sized afrotheres have a polygynous social system (which is not common among rock-dwelling mammals; Skinner and Chimimba, 2005) and their morphology should intuitively result in poor dispersal. In spite of this, few studies have attempted to determine the distance of migration by rock hyraxes (but see Hoeck, 1982, 1989; Gerlach and Hoeck, 2001) and how this is influenced by

their social system and the surrounding landscape (e.g., barriers to gene-flow which will invariably affect genetic structure). Hypotheses were therefore tested as to how the ecology, distribution, social structure and the connectivity of the surrounding landscape have shaped rock hyrax genetic variation across the Namaqualand and western Fynbos regions. Populations were selected (although not exclusively) in the Cape Peninsula/Hottentots Holland Mountains (across the Cape Flats), the Knersvlakte and across the Namaqualand/western Fynbos regions.

1.1.1. Species background

Fossil data from Egypt suggest that the Order Hyracoidea originated in Africa in the upper Eocene - lower Oligocene (Olds and Shoshani, 1982). The Hyracoidea are regarded as sharing a close phylogenetic affinity with Proboscidea (elephants) and Sirenia (dugongs and the manatee) to form the Paenangulata (Simpson, 1945; Novacek and Wyss, 1986; Shoshani, 1991; Kuntner *et al*. 2011; Meredith *et al*. 2011). There are three extant genera within Hyracoidea: the strictly saxicolous *Procavia*, the rock dwelling and partially arboreal *Heterohyrax*, and the strictly arboreal *Dendrohyrax* (Prinsloo, 1993; Skinner and Chimimba, 2005; Stuart and Stuart, 2007). The taxonomy of *Procavia* is somewhat uncertain with some authors regarding it as monotypic (Ellerman and Morrison-Scott, 1951; Olds and Shoshani, 1982; Meester *et al*. 1986; Skinner and Chimimba, 2005), while others recognize four or five species distributed across its African and middle eastern distribution (Allen, 1939; Hahn, 1934; Bothma, 1971; Roche, 1972; Corbet, 1978). Although the subdivisions within *Procavia* have more recently been regarded as reflecting sub-specific differences (Honacki *et al.* 1982; Meester *et al*. 1986), mitochondrial data suggests the presence of two distinct species within South Africa (Prinsloo and Robinson, 1992; Prinsloo, 1993) which correspond, respectively, to the south-western (Karoo) and north-eastern (Soutpansberg and Magaliesberg) regions of the country.

The rock hyrax is gregarious with a colony usually comprising a dominant territorial male accompanied by a harem of females (Sale, 1965; Hoeck, 1975; Olds and Shoshani, 1982; Fourie, 1983; Rubsamen *et al*. 1982; Prinsloo, 1993; Gerlach and Hoeck, 2001; Skinner and Chimimba, 2005; Druce *et al*. 2006; Aroch *et al*. 2007). Peripheral males may also be resident in the vicinity of a colony (Fourie, 1983; Gerlach and Hoeck, 2001; Skinner and Chimimba, 2005). A hierarchy exists in the rock hyrax social system where aggressive

behaviour is exhibited between the territorial male and peripheral males, although no similar social rankings exist between females (Fourie, 1983). All males disperse as sub-adults before or during the breeding season, or as adults during breeding dispersal whereas the females are largely phylopatric (Hoeck, 1982; Fourie, 1983; Aroch *et al*. 2007) although they may voluntarily disperse at a later stage (Fourie, 1983; Skinner and Chimimba, 2005). Dispersal should therefore be male-biased, although Gerlach and Hoeck (2001) found no sex-biased in the dispersal of *P. johnstoni*. During times of high population numbers, or food shortages, rock hyrax may disperse to other suitable rocky areas, sometimes over considerable distances (~ 20 kilometres; Skinner and Chimimba, 2005). At this time they have been recorded as using shelters such as holes in walls, culverts under roads, and even aardvark burrows (Olds and Shoshani, 1982; Rubsamen *et al*. 1982). Dispersal during times of over-population or unfavourable conditions may, therefore, contribute to higher than anticipated connectivity among geographically distant colonies (Kolbe, 1967; Prinsloo, 1993). The occupancy of suitable habitat by hyrax is a dynamic process and depends on several abiotic (rainfall and suitable crevices) and biotic (competition, predation and pathogens) factors (Sale, 1965; Hoeck, 1975; Olds and Shoshani, 1982; Hoeck, 1989).

Hyrax population sizes are known to fluctuate. These fluctuations occur in the face of predation, droughts, and infectious disease (Wagner and Bokkenheuser, 1961; Fairall *et al*. 1986; Fairall and Hanekom, 1987; Kotler *et al*. 1999; Cavanagh *et al*. 2002; Skinner and Chimimba, 2005; Druce *et al*. 2006; Lutze-Wallace *et al*. 2006; Chiweshe, 2007; Parsons *et al*. 2008; Parsons, 2010) and can, selectively or in concert, have significant impact on genetic structure. Bottlenecks that result from these perturbations (and especially those that persist for several generations) can cause a loss of genetic diversity and may even homogenize genetic structure (Prinsloo and Robinson, 1992; Prinsloo, 1993; Gerlach and Hoeck, 2001).

Major population crashes have been reported in South Africa for the rock hyrax (Bloomer, 2009). The widely held but unsubstantiated view is that there is currently a general decline in the numbers of rock hyrax across most of the Western Cape including populations in the Table Mountain National Park, Piketberg Mountains and the South Coast mountains in the vicinity of Oudtshoorn (Guy Palmer, Western Cape Nature Conservation; Melodie McGeoch, SANParks); hyrax historically occurred in high numbers in these areas (Skead *et al*. 2011). Interestingly, a similar decline has not been observed in the Northern Cape where high densities prevail.

Rock hyrax may be seen as a keystone species (Chiweshe, 2007) as they are a source of food for many predators such as the Verreaux (black) eagle (the rock hyrax forms 98% of its diet), the martial eagle, crowned eagle, leopard, caracal, jackal, African wild and various snake species (Turner and Watson, 1965; Olds and Shoshani, 1982; Davies, 1989; Gargett, 1990; Davies, 1994; Barry and Barry, 1996; Klein and Cruze-Uribe, 1996; Davies, 1999; Kotler *et al*. 1999; Skinner and Chimimba, 2005; Druce *et al*. 2006; Chiweshe, 2007; Stuart and Stuart, 2007; Kruger, 2010).

1.1.2. Genetic and adaptive differences

Since rock hyrax are restricted to rocky areas it is expected that dispersal between isolated rocky outcrops may be problematic. Previous work by Prinsloo and Robinson (1992) using mitochondrial DNA restriction-fragment-length polymorphisms (RFLPs) revealed significant genetic structure in populations from the Soutpansberg and northern parts of South Africa as well as those in the Karoo, leading to suggestions of two separate routes of dispersal - one along the Great Escarpment (their south-western clade) and the other along the Soutpansberg-Magaliesberg axis (their north-eastern clade). In addition, the gain or loss of specific restriction sites was unique to each studied population. However, whether distinct genetic groups exist in the Namaqualand (Northern Cape) and western Fynbos (Western Cape) regions is unknown. The Prinsloo and Robinson (1992) study included single specimens from the Western Cape (De Hoop Nature Reserve) and Northern Cape regions (Springbok) respectively, precluding a robust assessment of these geographic areas.

In addition to the available spatial genetic information on hyrax referred to above, an unpublished study by Palmer (Cape Nature; G. Palmer, personal communication) documented large differences in the maxillary tooth length and structure among certain populations in the Western Cape raising the prospect of subdivision in these areas. However, whether these differences have an underlying genetic basis is unknown since dental differences might also be correlated to food choice (Yom-Tov, 1993). Interestingly, Gerlach and Hoeck (2001) reported that the extralimital *P. johnstoni* similarly displays metapopulation dynamics in the Serengeti National Park with extinctions and subsequent recolonization among populations (or koppies - their “kopjes”). In this study overall genetic diversity was low, inbreeding was high and dispersal was not gender-biased.

1.1.3. Interpretive tools

Historical geographic processes acting on populations produce a distinct pattern in the distribution of alleles among populations (Irwin, 2002) provided sufficient time has elapsed (i.e., generation turn-over) for these differences to manifest and be detected with the markers employed. In the present study both mitochondrial and microsatellite markers are used to characterize connectivity (i.e., gene-flow) among contiguous (connected by intermediate suitable habitat) hyrax colonies, as well as between those from distinctly different geographic localities across the Namaqualand/western Fynbos regions.

Mitochondrial DNA has traditionally been used as marker of choice in phylogeographic studies as it is non-recombinant and inherited uniparentally (Avise, 1994, 1998; Irwin, 2002). The assignment of individuals to particular clades is relatively straight-forward and the partitioning of genetic variation, if present, is easily detected. Phylogeographic structure, resulting from barriers to gene-flow or isolation-by-distance, may be detected as genealogical gaps (Irwin, 2002). Mitochondrial DNA is therefore useful for disentangling genealogical relationships. It has many drawbacks, however, foremost its unimodal pattern of inheritance. Consequently the inclusion of nuclear markers is of critical importance (Zhang and Hewitt, 2003). Microsatellites (short tandem repeats scattered throughout the nuclear genome; Ingram, 2005) are invaluable in detecting genetic partitions within and among populations and are thus fundamental to elucidating gene-flow, population subdivision, migration patterns and genetic distance (Burland *et al*. 2001; Ingram, 2005). Social structure and mating patterns may also be inferred from microsatellite data and microsatellites are therefore frequently used in landscape genetics (Storfer *et al*. 2010). The inclusion of both microsatellite and mitochondrial markers allow a holistic assessment of population structure and landscape evolution.

Markers with different modes of inheritance (uniparental vs. biparental) are frequently employed to compare dispersal capabilities between the sexes in vertebrate organisms (as reviewed by Prugnolle and De Meeus, 2002). The basis of this approach is that for uniparentally inherited markers the one sex does not contribute to the genetic make-up of the offspring, while both sexes contribute in the case of biparentally inherited markers. Should sex-biased dispersal occur, a difference in the genetic structure of the two types of markers

will be evident (Goudet *et al*. 2002). Consequently, most molecular studies have relied on mitochondrial DNA data in conjunction with nuclear markers (such as microsatellites, allozymes or nuclear RFLPs) to investigate sex-biased dispersal (Quinn and White, 1987; Avise *et al*. 1992; Bowen *et al*. 1992; Melnick and Hoelzer, 1992; FitzSimmons *et al*. 1997; Wilmer *et al*. 1999; Nyakaana and Arctander, 1999; Escorza-Treviño and Dizon, 2000; Gibbs *et al*. 2000; Castella *et al*. 2001; Helbig *et al*. 2001; Doums *et al*. 2002; Kerth *et al*. 2002; Cegelski *et al*. 2003; Van Hooft *et al*. 2003; Zenger *et al*. 2003; Ujvari *et al*. 2008; Caparroz *et al*. 2009).

1.1.4. Sex-biased dispersal

Sex-biased dispersal (where individuals from one sex exhibit site phylopatry i.e., stay in or return to their natal site to breed or show reduced dispersal relative to the other sex which is more prone to disperse) is a frequent phenomenon in social vertebrate taxa (Prugnolle and De Meeus, 2002). The pattern resulting from sex-biased dispersal depends to a large extent on the breeding system of the species concerned (Greenwood, 1980; Pusey, 1987; Handley and Perrin, 2007); for example, male-biased dispersal is usually characteristic of polygynous species, while female-biased dispersal is predominantly found in monogamous taxa (Prugnolle and De Meeus, 2002). Most mammals exhibit male-biased dispersal (i.e., polygynous breeding systems; Greenwood, 1980; Dobson, 1982; Handley and Perrin, 2007), whereas dispersal in birds is female-biased (i.e., monogamous breeding systems; Greenwood, 1980).

Several hypotheses articulate the evolutionary role of sex-biased dispersal in social systems (Handley and Perrin, 2007). These include (i) kin selection with resource competition (Clarke, 1978; Greenwood, 1980), (ii) local mate competition (Hamilton, 1967; Dobson, 1982; Perrin and Mazalov, 2000) and (iii) inbreeding avoidance (Bengtsson, 1978; Packer, 1979; Dobson, 1982; Waser *et al*. 1986; Pusey, 1987; Clutton-Brock, 1989; Wolff, 1993; Perrin and Mazalov, 2000). Male-biased dispersal is predicted when local mate competition exceeds local resource competition, as is the case in polygynous/promiscuous systems (e.g., mammals; Perrin and Mazalov, 2000) - a system characterizing the rock hyrax.

In polygynous/promiscuous systems males and females compete for different resources. Males have higher reproductive output compared to females (copulation is a short process

and can happen with multiple partners), and male fitness is therefore limited by female availability (Perrin and Mazalov, 2000; Lehmann and Perrin, 2003). Inbreeding is not deleterious to males as they do not forfeit other breeding opportunities. In contrast, females have a large parental investment in reproductive output (pregnancy, lactation and rearing) and female fitness is limited by the processing of resources. Sex-biased dispersal may therefore introduce sexual asymmetries to patterns of local competition in hyrax as males engage in local mate (male-male) competition (Clutton-Brock, 1989; Fourie, 1983 for rock hyrax), while females show local resource competition.

As a consequence, inbreeding incurs costs due to the reproductive investment of female hyrax (Lehmann and Perrin, 2003). Inbreeding can result in a loss of fitness through inbreeding depression. Intrasexual competition between related individuals for access to limited resources (to maximize reproductive success) directly influences kin selection and inbreeding avoidance - the main factors resulting in sex-biased dispersal between populations (Bengtsson, 1978; Packer, 1979; Dobson, 1982; Pusey, 1987; Clutton-Brock, 1989; Pusey and Wolf, 1996). Therefore, if members of one sex disperse (be it natal or breeding dispersal), there is less risk of inbreeding to the other (phylopatric) sex and reduced competition among kin, thus increasing inclusive fitness (Clarke, 1978; Perrin and Mazalov, 2000). In polygynous systems, females choose immigrants above a certain inbreeding threshold (depending on relatedness) that in turn boosts male dispersal. Theory predicts that females should therefore prefer immigrants over residents at high inbreeding loads, however at lower levels of inbreeding, more related males will be favoured (Lehmann and Perrin, 2003).

Like other polygynous mammals (as reviewed by Storz, 1999), the rock hyrax mating system should therefore partition populations into breeding groups that are maintained both by the phylopatry of females and the aggressive exclusion of immigrant males. While both sexes may have the potential to disperse (Handley and Perrin, 2007), long-distance gene-flow may be facilitated by males.

Male-biased dispersal has been demonstrated through the use of genetic markers in several mammalian taxa (in both uniparental and biparental situations; Melnick and Hoelzer, 1992; Nyakaana and Arctander, 1999; Wilmer *et al*. 1999; Escorza-Treviño and Dizon, 2000; Castella *et al*. 2001; Kerth *et al*. 2002; Cegelski *et al*. 2003; Van Hooft *et al*. 2003; Zenger *et*

al. 2003). Indeed, similar patterns of male dispersal were demonstrated in two species with identical social systems to the rock hyrax. The closest relatives of conies, the African elephant (*L. africana*; Nyakaana and Arctander, 1999), as well as the macaque monkey (*Macaca mulatta*; Melnick and Hoelzer, 1992) have systems where males leave their natal group before sexual maturity, whereas females remain for life. Consequently contrasting patterns were evident from the analysis of mitochondrial DNA versus microsatellites in these species.

Sex-biased dispersal due to a polygynous mating system influences the evolutionary potential of a species as it results in differing genetic patterns across the landscape, be it with uniparentally (mitochondrial DNA) or biparentally (microsatellites) inherited markers. In addition, the spatial scale of investigation also impacts on the genetic patterns observed. The following section will show how landscape structure may influence genetic substructuring between populations in conjunction with sex-biased dispersal.

1.1.5. Landscape Genetics

Landscape genetics is an emerging field which considers the actual movement of organisms (with respect to habitat connectivity) from that organism's perspective (Holderegger and Wagner, 2006). This is an important concept since an animal's perception of the landscape differs from the simplistic simulations incorporated in isolation-by-distance (IBD) analyses (Coulon *et al*. 2004). According to Storfer *et al*. (2007), landscape genetics may be defined as "*research that explicitly quantifies the effects of landscape composition, configuration and matrix quality on gene-flow and spatial genetic variation*". This field integrates ecology, spatial statistics and population genetics (Holderegger and Wagner, 2006; Storfer *et al*. 2007; Holderegger and Wagner, 2008; Storfer *et al*. 2010), and examines these in the context of evolutionary processes within species (Holderegger and Wagner, 2006). It aims to quantify the relationship between ecological variables, genetic variation and the actual spatial partitioning thereof (Storfer *et al*. 2007). The focus is on the degree to which the landscape structure facilitates the movement and subsequent gene-flow of certain organisms, i.e., landscape connectivity (Taylor *et al*. 1993).

Landscape connectivity refers to the connectivity of the surrounding matrix - defined by Holderegger and Wagner (2008) as "*the often hostile space that separates the patches of a*

species' habitat in a given landscape". Landscape connectivity is consequently a result of an animal's dispersal behaviour combined with the grain (penetrability/connectivity) of that particular landscape (Brooks, 2003; Baguette and Van Dyck, 2007). The matrix is a major factor determining biological and ecological processes as the quality and quantity of areas that separate suitable habitat affects the distribution of genetic variation, be it adaptive or non-adaptive (Holderegger and Wagner, 2008). Two types of landscape connectivity are evident. Gene-flow is an example of functional connectivity, whereas structural connectivity relates to how suitable habitat patches are distributed across the landscape (Brooks, 2003; Baguette and Van Dyck, 2007; Holderegger and Wagner, 2008).

In addition to the structural features (connectivity and habitat quality), gene-flow is also affected by ecological factors. These include habitat porosity, habitat persistence and population persistence (Peterson and Denno, 1998; Pérez-Espona *et al*. 2008). As the landscape imposes selective pressures on dispersal behaviour, populations of the same species inhabiting differing environmental regimes may evolve different behaviours (aggregated *versus* fragmented; as reviewed by Baguette and Van Dyck, 2007). Variation in the dispersal behaviours that characterize populations depend on how residents perceive the surrounding landscape. As each organism has a perceptual range (the range at which it is able to detect suitable habitat patches), dispersal will depend on suitable habitat patches that overlap within this range. If the distribution of suitable habitat patches is larger than the perceptual range (visual acuity) of an animal, a dispersal event will incur costs (through increased search time and possible predation). Reluctance to cross the boundary of suitable habitat patches has been reported for various taxa, especially when the habitat is fragmented. For example, Baguette and Van Dyck (2007) demonstrated that butterflies from fragmented systems were less inclined to cross habitat boundaries than those from aggregated landscapes.

The only spatial aspect included in traditional population genetic studies is isolation-by-distance (IBD) which is limited with regard to spatial inference (Storfer *et al*. 2007; Holderegger and Wagner, 2008). Since Manel *et al*. (2003) coined the term "*landscape genetics*", however, a substantial number of studies have incorporated geographic variables (such as coordinates and landscape features, Storfer *et al*. 2010) when evaluating the spatial distribution of genetic variation. Processes such as gene-flow are affected by the quality of a landscape and not just purely spatial distance (Holderegger and Wagner, 2006; McRae and Beier, 2007). Multivariate models that take landscape variables into account perform

significantly better at explaining genetic differences among populations than do simple isolation-by-distance tests (Michels *et al*. 2001; Coulon *et al*. 2004; Spear *et al*. 2005; Broquet *et al*. 2006; Pérez-Espona *et al*. 2008; Zalewski *et al*. 2009; Storfer *et al*. 2010). As a consequence, analyses of genetic structure are becoming increasingly spatial in nature. Landscape genetics is therefore much closer to the real-world situation as the actual dispersal of organisms may deviate from an abstract gene-flow index for populations or from an ecological connectivity index (Holderegger and Wagner, 2006).

Various questions have already been addressed through landscape genetic studies including the identification of barriers to gene-flow, quantifying diversity, the inference of landscape change, identification of migrants in relation to landscape condition, estimation of source-sink dynamics, invasive species and the spread of disease (as reviewed by Storfer *et al*. 2010). The field provides valuable information for disciplines such as evolutionary biology, ecology and conservation biology (Holderegger and Wagner, 2006).

Understanding the effects of landscape connectivity on the genetic structure of organisms provides insight into spatial genetic patterns such as barriers to gene-flow, metapopulations, isolation-by-distance and clines (Coulon *et al*. 2004), and can aid in identifying possible dispersal corridors (Coulon *et al*. 2004; Storfer *et al*. 2007). The field further sheds light on biological processes such as speciation and species' distributions (Storfer *et al*. 2007). Genetic variation may vary over different spatial scales as particular landscape variables may differentially affect gene-flow on differing scales - a process linked to a species' biology (Storfer *et al*. 2007). Landscape genetic approaches using spatial information may also permit the identification of barriers which are undetectable by conventional population genetic methods (Coulon *et al*. 2004) and where various structures across the landscape may cause abrupt breaks (Dupanloup *et al*. 2002), or more gradual transitions (Geffen *et al*. 2004) in gene-flow patterns. In addition, anthropogenic landscape changes may have a profound impact on the connectivity between suitable habitat patches through fragmentation and thus reduce gene-flow and genetic diversity (Coulon *et al*. 2004). Unsuitable habitat does not provide cover against predators and is often too extensive for a species to cross in one step; thus fragmented landscapes affect the dispersal capability of species. Direct observational data on the movement of species is difficult or not practical for many taxa. Landscape genetics thus offers a framework to examine variables that facilitate or impede dispersal and gene-flow. In a conservation context, the field may help elucidate dispersal routes between

populations in fragmented habitats which will allow the construction of corridors to facilitate gene-flow between fragmented areas (either reserves or anthropogenically fragmented areas).

1.1.6. Regional barriers to gene-flow

Genetic subdivision and phylogenetic gaps have been demonstrated in a myriad of mammalian taxa (see examples in Avise, 1994). These genetic gaps arise due to mutations becoming independently fixed in a species genome (through drift and/or selection) following its divergence and subsequent evolutionary trajectory from common ancestry. Concordant patterns should emerge in the genealogies of different (mitochondrial and nuclear) genetic markers when a barrier to gene-flow separates populations for protracted periods (Avise, 1994).

Comparative phylogeography aims to compare the contemporary spatial patterns of genetic variation in multiple co-distributed, unrelated taxa and to assesses the degree to which these taxa responded to historical biological, climatic and geographic (orogenic factors such as uplift) events (Bermingham and Martin, 1998; Schneider *et al*. 1998; Brunsfeld *et al*. 2000; Ditchfield, 2000; Arbogast and Kenagy, 2001; Hewitt, 2001; Zink, 2002; Dawson, 2005; Lapointe and Rissler, 2005; Cotterill, 2006; Feldman and Spicer, 2006; Soltis *et al*. 2006; Castoe *et al*. 2009; Wallis and Trewick, 2009; Yang *et al*. 2009; Rodríguez-Sánchez *et al*. 2010; Cotterill and De Wit, 2011; Goodier *et al*. 2011). If similar historical forces (geological or environmental) affected population processes in multiple taxa across the same geographic region, similar phylogeographic patterns are to be anticipated (see for example Sullivan *et al*. 2000; Zink, 2002; Lapointe and Rissler, 2005; Joseph and Omland, 2009).

Genetic data on a diverse array of taxa have facilitated comparative phylogeographic approaches in the northern hemisphere (Australia, Europe, China, eastern European Alps, the Caribbean, Canary islands, Hawaii, Pacific Northwest and North America, Central America, south-eastern United States, the California Floristic Region and South America; Avise, 1998; Bermingham and Martin, 1998; Avise, 2000; Brunsfeld *et al*. 2000; Ditchfield, 2000; Hewitt, 2001; Soltis *et al*. 2006; Joseph and Omland, 2009; Wallis and Trewick, 2009; Yang *et al*. 2009). Comparatively few studies have, however, been conducted in the southern hemisphere (Wallis and Trewick, 2009) and specifically in South Africa (but see Tolley *et al*. 2009).

For species confined to rocky habitats two notable landscape features significantly structure the spatial distribution of genetic variation. First, the Knersvlakte was documented as a geographic barrier in various taxa from different taxonomic groups and with different life-histories (Branch *et al*. 1995; Matthee and Robinson, 1996; Lamb and Bauer, 2000; Matthee and Flemming, 2002; Smit *et al*. 2007; Daniels *et al*. 2010; Portik *et al*. 2011). The Knersvlakte is a large, arid plain in the Namaqualand area situated between the Bokkeveld- and Kamiesberg Mountains (Kounov *et al*. 2008). The formation of this area has been attributed to uplift during the Miocene approximately 18 Mya (Moon and Dardis, 1988), however, more recent studies suggest that it has in fact been forming since ~ 90 Mya (Kounov *et al*. 2008). The Knersvlakte was once the outlet of the paleo-Karoo River drainage system during the Cretaceous (Kounov *et al*. 2008). Continued erosion following the end of the Cretaceous shaped the present-day topography of the area. This large, sparsely vegetated, flat area with an elevation of 109 - 153 metres above sea-level (Kounov *et al*. 2008), is approximately 40 - 100 km in width and offers little refuge for dispersing rock-dwelling taxa.

A second potential geographic barrier to gene-flow is situated further to the south and comprises a ~ 60 kilometres wide stretch of sand between the Cape Peninsula and the Hottentots Holland Mountains (Schalke, 1973; Adelana *et al*. 2010) that offers little suitable habitat and refuge to dispersing species with saxicolous requirements. This area is currently referred to as the Cape Flats. Although small disjunct outcrops occur on the Cape Flats (Schalke, 1973), these are quite rare (Adelana *et al*. 2010) and not deemed adequate in aiding dispersing animals. The area mainly consists of aeolian sands of marine origin (Walker, 1952; Siesser and Dingle, 1981; Adelana *et al*. 2010) and includes a large part of the existing Cape Town metropolitan area. This barrier has also repeatedly been enforced since the low-lying area is frequently inundated during marine transgressions.

1.1.7. The study

Five major research questions can be identified within a landscape genetics context: (1) quantifying influence of landscape variables and configuration on genetic variation; (2) identifying barriers to gene flow; (3) identifying source-sink dynamics and movement corridors; (4) understanding the spatial and temporal scale of an ecological process; and (5) testing species-specific ecological hypotheses (Storfer *et al*. 2007). This project addresses all

five of these questions using both mitochondrial and nuclear (microsatellite) markers in *P. capensis*.

Sampling was designed to examine questions of connectivity at different spatial scales. In addition to a stratified sampling regime across the South African Namaqualand/western Fynbos regions, sampling efforts were also focussed around presumed barriers to movement in rock hyrax. This is important as different landscape features may differentially affect genetic variation at various spatial scales and few landscape studies have been conducted using this approach. At a fine scale the spatial genetic structure, gene-flow and sex-biased migration of hyrax between five different koppies in an isolated population were investigated. To address intermediate spatial scales, genetic structure and gene-flow across and around known barriers such as the Cape Flats and Knersvlakte were targeted. At a regional spatial scale we investigated how landscape connectivity affects gene-flow and the distribution of genetic variation across the landscape in two regions with differing connectivities (Namaqualand and the western Fynbos region). Finally, these results were compared to other investigations that identified the Cape Flats and Knersvlakte as phylogeographic breaks along the South African west coast.

CHAPTER 2

Phylogeography of *Procavia capensis* across the Namaqualand and western Fynbos regions of South Africa – a mitochondrial and microsatellite perspective

2.1. Introduction

Habitat preference can impact on the distribution of genetic variation across landscapes (Smit *et al*. 2011). Saxicolous species are by definition habitat bound (Chapter 1) and one may anticipate that tracts of open terrain represent significant barriers to a species' successful dispersal. Consequently, it is no surprise that two such areas, the Knersvlakte (Branch *et al*. 1995; Matthee and Robinson, 1996; Lamb and Bauer, 2000; Matthee and Flemming, 2002; Smit *et al*. 2007; Daniels *et al*. 2010; Portik *et al*. 2011) and Cape Flats (Daniels *et al*. 2001; Wishart and Hughes, 2001, 2003; Gouws *et al*. 2004; Swart *et al*. 2009; Gouws *et al*. 2010; McDonald and Daniels, 2012), both depauperate in this type of habitat, have been found to structure the spatial distribution of genetic variation in rock-dwelling taxa.

Given its habitat preferences, similar patterns of genetic structure may consequently be anticipated in the rock hyrax (*P. capensis*). Prinsloo and Robinson (1992) identified significant genetic structure across the species' South African distribution. Based on mitochondrial DNA restriction-fragment-length polymorphisms (RFLPs) two major clades (a south-western and north-eastern clade) were detected, evidently tracking different dispersal routes. However, their study included only two collection sites in the current area of interest, one in the Western Cape (De Hoop Nature Reserve - Fynbos region) and one in the Northern Cape (Springbok - Namaqualand region). Consequently whether the Knersvlakte and the Cape Flats have impacted on the phylogeography of *P. capensis* in these regions is moot. Nonetheless, it is noteworthy that hyrax from the De Hoop Nature Reserve were genetically the most divergent of those sampled nationally by Prinsloo and Robinson (1992) possibly reflecting long periods of very limited gene-flow into this region from surrounding areas, coupled to the gradual accumulation of new mutations within the population.

This study extends what is known of the genetics and population structure of South African saxicolous species through the analysis of *P. capensis* collected at numerous sites across the Namaqualand and western Fynbos regions of South Africa (Figure 2.1). Conclusions were based on structure using both mitochondrial and nuclear markers and these data examined in the context of the species' ecology, social structure and life history parameters. In so doing, the sampling deficiencies inherent in the Prinsloo and Robinson (1992) and Prinsloo (1993) studies were addressed through the inclusion of larger sample sizes and the selection of multiple sampling localities across the Namaqualand and western Fynbos regions. Lastly, a search for correspondence with published phylogeographic patterns from other rock-dwelling specialist taxa across the same broad geographic area was undertaken. It was anticipated that this would give greater insight to factors influencing the distribution of genetic variation in these and other saxicolous species thereby informing conservation strategies for these ecologically important areas.

The inclusion of both microsatellite and mitochondrial markers allows for a holistic assessment of population structure, population dynamics and sex-biased dispersal. The approach also permits an assessment of the landscape on genetic patterns and processes. The mitochondrial cytochrome b gene is widely used in species identification (Parson *et al*. 2000; Bradley and Baker, 2001) due to its ease of amplification and unambiguous genetic assignment benefits (Parson *et al*. 2000). Consequently, a large database of cytochrome b sequences from multiple taxa is available on public databases such as GenBank (Parson *et al*. 2000) and representative sequences from taxa of interest may be obtained for genealogical and phylogeographic analyses. The cytochrome b segment has wide application in phylogeography (Avise, 1994, 1998; Irwin, 2002) as the assignment of individuals to particular clades is relatively straight-forward and therefore geographic patterning resulting from long-term barriers to gene-flow may be detected (Irwin, 2002). Its maternal pattern of inheritance also makes the investigation of female gene-flow possible. On the other hand microsatellites (variable tandem repeats scattered throughout the genome) are frequently used in landscape genetics (Storfer *et al.* 2010) as they are invaluable in detecting gene-flow, genetic partitions within and among populations, social structure and mating patterns (Burland *et al*. 2001; Ingram, 2005). The microsatellite markers used in this study were developed for *Procavia* (although they also show variability in *Heterohyrax*) to investigate population dynamics and genetic structure of hyrax populations in the Serengeti National Park (Gerlach *et al*. 2000; Gerlach and Hoeck, 2001). The selection of markers for use in the

present investigation was based on the Gerlach and Hoeck (2001) study with the aim of providing maximum resolution with respect to gene-flow, population substructuring, population dynamics and mating patterns of *P. capensis* at different several spatial scales.

2.2. Materials and Methods

2.2.1. Sample collection

The analysis of phylogeographic structure was based on a minimum of 20 animals per sampling site (Figure 2.1.) - the only exceptions were Klawer (n = 15), Loeriesfontein (n = 11) and Boulders (n = 12). Ear-clippings were taken and stored at room temperature in a saturated salt solution supplemented with 20% dimethyl sulfoxide (DMSO).

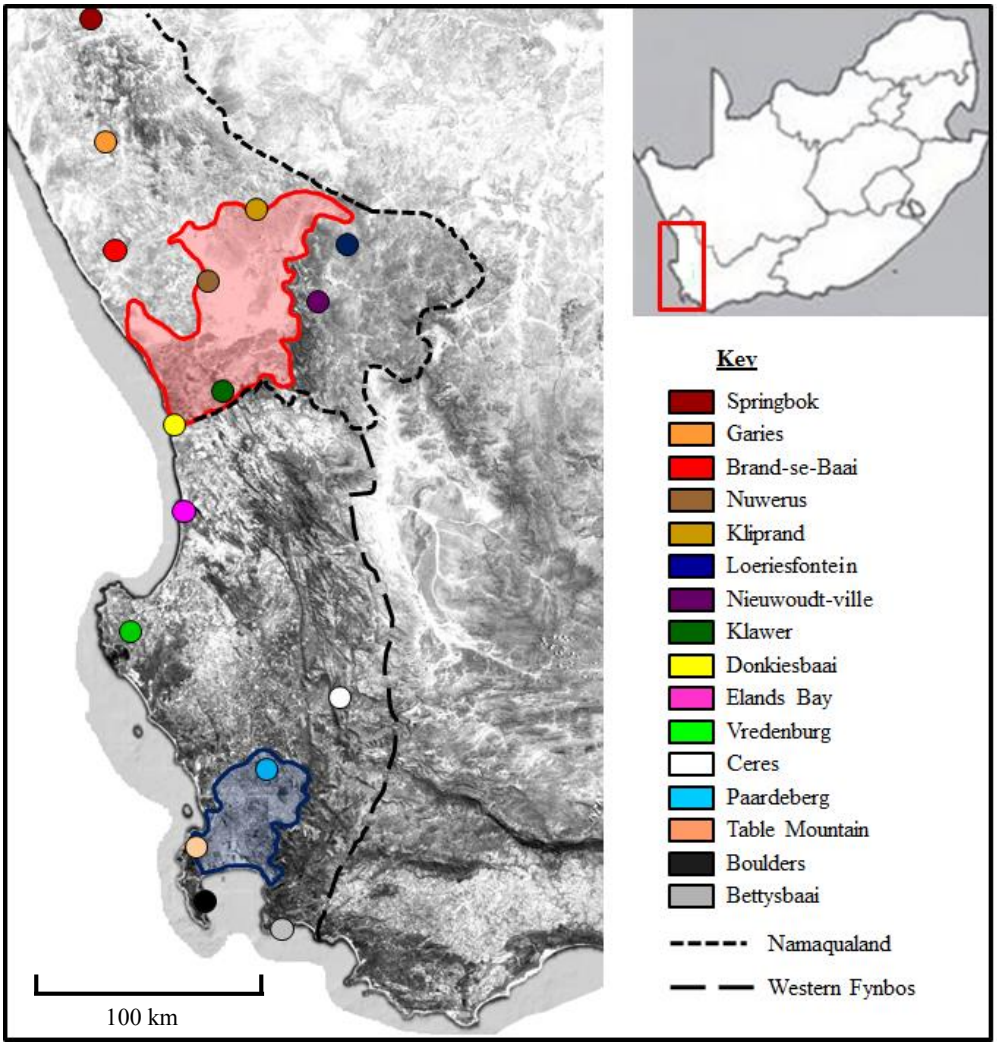

Figure 2.1. Map showing the 16 *P. capensis* collection sites sampled across the Namaqualand and western Fynbos regions of South Africa. The positions of the two known biogeographic breaks, the Knersvlakte (delineated in red) and Cape Flats (delineated in blue), are also indicated.

2.2.2. Experimental procedures

2.2.2.1. Mitochondrial DNA

Total genomic DNA was extracted from ear clippings using a commercial DNA extraction kit (DNeasy Blood & Tissue kit; Qiagen) following the manufacturer's protocols. The entire mitochondrial cytochrome b gene including forward and rear flanking regions was amplified and sequenced. Universal primers (*L14724* and *H15915*) were used for amplification and sequencing (Kocher *et al*. 1989; Irwin *et al*. 1991). The studies by Prinsloo and Robinson (1992) and Prinsloo (1993) were based largely on mitochondrial RFLP data (although cytochrome b sequence data was also included in the Prinsloo 1993 study). To facilitate comparison with the Prinsloo (1993) investigation (and because RFLP investigations have been superseded by sequencing approaches), mitochondrial cytochrome b sequence data was used in this study. In an attempt to investigate male-biased dispersal in the species, an attempt was made to amplify the Y specific locus, SRY (Menotti-Raymond *et al*. 2003), from all sampled males.

Amplification of the cytochrome b region was carried out for 10 adult animals (five males and five females) per sampling locality. PCR amplifications followed standard protocols. In short, amplifications were carried out in a GeneAmp PCR 2700 system (Applied Biosystems) with a thermal profile involving an initial denaturation step of 4 min at 96°C followed by 35 cycles of 96°C for 30 s, a region-specific annealing temperature of 50°C for cytochrome b for 30 s and 72°C for 1 min. A final extension step at 72°C for 5 min completed all reactions. Successful amplifications were visualized on a 1% agarose gel. Sequencing reactions were performed using BigDye chemistry (Applied Biosystems). Sequencing products were cleaned to remove unincorporated dye label using sephadex columns. Samples were analysed on an ABI 3170 (Applied Biosystems) automated sequencer at the Central Analytical Facility (Stellenbosch University). Electropherograms of the raw data were checked manually (Geneious Pro™ 5.0 software, Biomatters Ltd, New Zealand) and aligned in MacClade version 4.06 for OS X (Maddison and Maddison, 2003).

2.2.2.2. Microsatellites

Four microsatellite loci *Hy-D49, Hy-T12, Hy-T17* (Gerlach *et al*. 2000) and *HCA18* (P. Bloomer, personal communication) were selected (Appendix A). These loci were chosen for ease of amplification and levels of polymorphism detected in various populations. The forward primer of each primer pair was 5'-labelled with one of four fluorophores (6-FAM, HEX, VIC or NED). Genotyping was performed on all available specimens from each sampling locality. Following primer optimization, all loci were amplified at 48ºC; subsequent amplifications were performed in a multiplex at this annealing temperature. A Multiplex PCR Kit (Qiagen) was used for the amplification in a final reaction volume of 10 µl consisting of 5 µl Qiagen Multiplex Master Mix, 2 µl of primer mix (2 mM), 1 µl water and 2 µl of template DNA (~ 30 ng). PCR conditions were 15 min of initial denaturation at 95ºC, 35 cycles of 30 s of denaturation at 94ºC, 90 s of annealing at 48ºC, 90 s of extension at 72ºC and 10 min of final elongation at 72ºC. For genotyping, 1 µl of diluted (1/80) PCR product was combined with 15 µl of deionized formamide and 0.2 µl of the GS500LIZ size standard (Applied Biosystems). Samples were genotyped on an ABI3170 Prism (Applied Biosystems) and scored using ABI Prism Genemapper software 3.7 (Applied Biosystems).

2.2.3. Data analyses

2.2.3.1. Mitochondrial DNA

2.2.3.1.1. Genealogical and molecular dating analyses

Genealogical analyses were conducted to search for phylogeograhic patterns and to date divergence events between clades. An additional eight *P. capensis* sequences were available on GenBank; these were downloaded and aligned to the data generated in the present study. The outgroups for the genealogical analyses were members of the Afrotheria and include *Heterohyrax brucei*, *Dendrohyrax dorsalis*, *Loxodonta africana*, *Dugong dugong*, *Orycteropus afer*, *Elephantulus edwardii*, *Macroscelides proboscideus* and *Echinops telfairi*.

Phylogenetic trees were constructed using parsimony and Bayesian Inference approaches. For this, specimens sharing a specific sequence-based haplotype (genetically unique sequence) in sampling localities were collapsed into a single representative haplotype. Parsimony analyses

were executed in PAUP* version 4.0 (Swofford, 2003). Trees were generated with heuristic searches and TBR branch swapping using 100 random taxon additions. Statistical confidence in nodes was determined through 1 000 bootstrap replicates (Felsenstein, 1985). Bayesian Inference trees were constructed in MrBayes 3.2 (Ronquist *et al*. 2011). The model of evolution that best fitted the data ($GTR+I_{(0.485)}+G_{(0.232)}$) was determined by jModelTest version 2.0.2 (Posada, 2008) using the Akaike Information Criterion (AIC) (Akaike, 1973). The programme was run for 5 x 10^6 generations with sampling every 100 generations. After discarding the first 25% of the trees as burnin, a majority rule consensus tree with posterior probabilities was constructed. Posterior probabilities of >0.90, and bootstrap values >70% were considered statistically acceptable.

To obtain estimates of times of divergence for various clades, a relaxed molecular clock approach was adopted in BEAST version 1.7 (Drummond *et al*. 2007). Five calibration points were specified which included the root of the Afrotheria (80.9 ± 11.1 million years ago), the ages of the Paenangulata (64.3 ± 7.3 million years), the Macroscelidea (49.1 ± 9.8 million years), the Hyracoidea (6.1 ± 2.2 million years), and the Proboscidea (5.3 ± 3.1 million years) (Meredith *et al*. 2011). Runs were continued for 20 x 10^6 generations sampling every 1 000 generations (burnin = 2 000). Results were visualized in Tracer version 1.5 (Rambaut and Drummond, 2003).

Phylogenetic trees are not always sensitive enough to detect variation and relationships below the species level (Posada and Crandall, 2001). In addition, several assumptions underpinning phylogenetic tree construction (such as evolution is strictly bifurcating) are violated (Posada and Crandall, 2001). As an alternative to the tree approach, a haplotype network was constructed using TCS 1.21 (Clement *et al*. 2000).

To determine the amount of genetic divergence among groups identified in the phylogenetic analyses, as well as hyrax populations sampled in geographically distant localities, sequence divergences (uncorrected p-values) were calculated in DnaSP version 5.10.01 (Librado and Rozas, 2009).

2.2.3.1.2. Population analyses

To determine whether genetic variation was significantly structured, ϕ_{ST} was calculated with all sampling localities included. Additionally, pair-wise ϕ_{ST} values were calculated between localities. Significance was determined through 9 999 permutations of the data (Arlequin version 3.5; Excoffier and Lischer, 2010).

Distinct groups identified using phylogenetic approaches are often caused by barriers to migration and reflected by limited gene-flow across the landscape. To further investigate this, the programme Alleles In Space (AIS) version 1.0 (Miller, 2005) was used. AIS uses Monmonier's algorithm to search for barriers by searching for the greatest genetic distance between any two locations in a triangle. An interpolation-based graphic approach was used to detect genetic structure over the landscape. The default settings of "midpoint derived from Delaunay triangulation" and "residual genetic distances" were used; the "distance weight value" was set to 1.5 when the visual spatial approach was adopted.

2.2.3.2. Microsatellites

2.2.3.2.1. Summary statistics and inbreeding

The presence of null alleles introduces a potential bias to analyses. We used Microchecker version 2.2.3 (Van Oosterhout *et al*. 2004) to assess whether null alleles were present in the data and followed Okello *et al*. (2005) in viewing a value >0.2 as indicative of their presence. Linkage disequilibrium was investigated using Genepop version 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008) by running Markov chains for 10 000 iterations. We also assessed whether colonies conformed to Hardy-Weinberg equilibrium (HWE) (Genalex version 6.4; Peakall and Smouse, 2006).

2.2.3.2.2. Population and clustering analyses

Population differentiation was estimated through pairwise F_{ST} values (Arlequin version 3.5; Excoffier and Lischer, 2010). The spatial location of genetic clusters within the studied areas was determined using Bayesian assignment approaches implemented in Geneland version 2.0.10 (Guillot *et al*. 2005). This programme determines the spatial location of populations

(without prior input) from multi-locus genotypes through the simultaneous analysis of both genetic and geographical data (Guillot *et al*. 2005). A Reversible Jump (RJ) Markov Chain Monte Carlo (MCMC) algorithm was applied to estimate the number and location of genetic clusters (K) across the landscape (Guillot *et al*. 2005). Geneland also outperforms other spatial genetic clustering programmes when F_{ST} values are $\geq$0.04 (i.e., when the number of migrants between populations is low) and is efficient at detecting potential contact zones between populations (Chen *et al*. 2007). As allele frequencies were uncorrelated between sampling localities (calculated in Genalex; Peakall and Smouse, 2006) and gene-flow was low (Chapter 3), the "no admixture" model with "independent/uncorrelated allele frequencies" was selected in subsequent analyses. We ran 100 000 permutations with a thinning of every 100 trees to search the optimal spatial distribution of markers. Ten chains were run, and the one with the highest likelihood retained.

2.2.4. Phylogeography of regional saxicolous fauna: a comparative perspective

Comparative data were sourced from published genetic studies representative of the same broad geographic region that, importantly, included the Knersvlakte (Branch *et al*. 1995; Matthee and Robinson, 1996; Lamb and Bauer, 2000; Matthee and Flemming, 2002; Smit *et al*. 2007; Daniels *et al*. 2010; Portik *et al*. 2011) and the Cape Flats (Daniels *et al*. 2001; Wishart and Hughes, 2001, 2003; Gouws *et al*. 2004; Swart *et al*. 2009; Gouws *et al*. 2010; McDonald and Daniels, 2012). These data were compared to the phylogenetic analysis of hyrax drawn from the 16 sampling localities of the present study.

2.3. Results

2.3.1. Mitochondrial DNA

2.3.1.1 Population and clustering analyses

The trees generated by MP, BEAST and the Bayesian methods were largely congruent (Figure 2.2.) with two major well-supported *P. capensis* clades evident across the Namaqualand and western Fynbos regions (uncorrected sequence divergence separating these clades = 1.916%; Jukes-Cantor corrected = 1.941%). These can be assigned to two

vegetation types: a Namaqualand and a western Fynbos biome (Mucina and Rutherford, 2006). These two clades reflect the disruption caused by the Knersvlakte and this divergence is dated at ~ 8.9 Mya (Figure 2.2.). The same clades were evident in the haplotype network and could not be connected at the 95% confidence level (Figure 2.3.). Overall, shallow genetic structure was evident in the haplotype network with few mutational changes/missing haplotypes within clades (Figure 2.3.). The AIS analysis confirmed the Knersvlakte to be a major barrier to gene-flow for rock hyrax populations across the Namaqualand/western Fynbos regions (Figure 2.4.). In addition, significant genetic discontinuity was detected among populations across the western Fynbos region (Figure 2.4.). Significant pairwise differentiation was evident between all localities across the sampled distribution area (Table 2.1.). Amplification of the male-specific marker (SRY) proved unsuccessful, even with modifications to the protocol. Male-biased dispersal could thus not be directly estimated. The SRY marker used in this study was developed for the domestic cat (*Felis catus*; Menotti-Raymond *et al*. 2003) and it is probable that mutations in the primer binding site of *P. capensis* precluded the annealing of the primers.

Table 2.1. Pairwise ϕ_{ST}/F_{ST} values between the 16 *P. capensis* populations sampled across the Namaqualand and western Fynbos regions. Values above the diagonal are based on the microsatellite (F_{ST}) data and those below the diagonal represent the mitochondrial sequence data (ϕ_{ST}) (cytochrome b). Differentiation across the Knersvlakte (red block) and the Cape Flats (the blue block) is highlighted. All values were significant at $p < 0.05$ except those indicated in red.

Locality	Springbok	Garies	Brand-se-Baai	Nuwerus	Kliprand	Loeries-fontein	Nieuwoudt-ville	Klawer	Donkies-baai	Elands Bay	Vredenburg	Ceres	Paardeberg	Table Mountain	Boulders	Bettysbaai
Springbok	-	0.025	0.039	0.024	0.024	0.077	0.033	0.139	0.064	0.132	0.200	0.076	0.215	0.151	0.347	0.222
Garies	0.230	-	0.055	0.050	0.024	0.085	0.027	0.140	0.066	0.123	0.160	0.081	0.191	0.141	0.336	0.214
Brand-se-Baai	0.305	0.448	-	0.044	0.026	0.066	0.020	0.123	0.054	0.108	0.196	0.081	0.207	0.179	0.368	0.242
Nuwerus	0.108	0.280	0.377	-	0.036	0.083	0.038	0.145	0.065	0.120	0.182	0.062	0.203	0.102	0.33	0.224
Kliprand	0.113	0.030	0.218	0.129	-	0.069	0.013	0.151	0.054	0.121	0.201	0.059	0.208	0.141	0.375	0.218
Loeriesfontein	0.912	0.918	0.978	0.906	0.795	-	0.072	0.109	0.067	0.195	0.179	0.091	0.206	0.199	0.411	0.184
Nieuwoudt-ville	0.571	0.594	0.621	0.566	0.436	0.328	-	0.135	0.058	0.102	0.192	0.088	0.208	0.165	0.347	0.227
Klawer	0.619	0.635	0.658	0.615	0.501	0.411	0.125	-	0.050	0.220	0.186	0.092	0.115	0.198	0.379	0.118
Donkiesbaai	0.707	0.720	0.752	0.701	0.591	0.492	0.209	0.019	-	0.100	0.158	0.036	0.098	0.122	0.339	0.129
Elands Bay	0.799	0.810	0.857	0.793	0.686	0.782	0.457	0.110	0.107	-	0.241	0.122	0.209	0.194	0.434	0.286
Vredenburg	0.918	0.926	0.989	0.911	0.798	0.821	0.232	0.350	0.436	0.777	-	0.144	0.232	0.200	0.386	0.208
Ceres	0.860	0.869	0.926	0.854	0.748	0.894	0.566	0.214	0.307	0.079	0.903	-	0.151	0.087	0.354	0.139
Paardeberg	0.913	0.921	0.979	0.907	0.799	0.827	0.321	0.405	0.487	0.781	0.833	0.895	-	0.149	0.252	0.112
Table Mountain	0.918	0.926	0.989	0.911	0.808	0.979	0.624	0.471	0.535	0.701	1.000	0.855	0.982	-	0.258	0.189
Boulders	0.908	0.916	0.974	0.902	0.802	0.956	0.629	0.492	0.552	0.704	0.972	0.839	0.958	0.727	-	0.283
Bettysbaai	0.808	0.815	0.856	0.804	0.705	0.741	0.451	0.385	0.428	0.578	0.732	0.681	0.740	0.737	0.735	-/

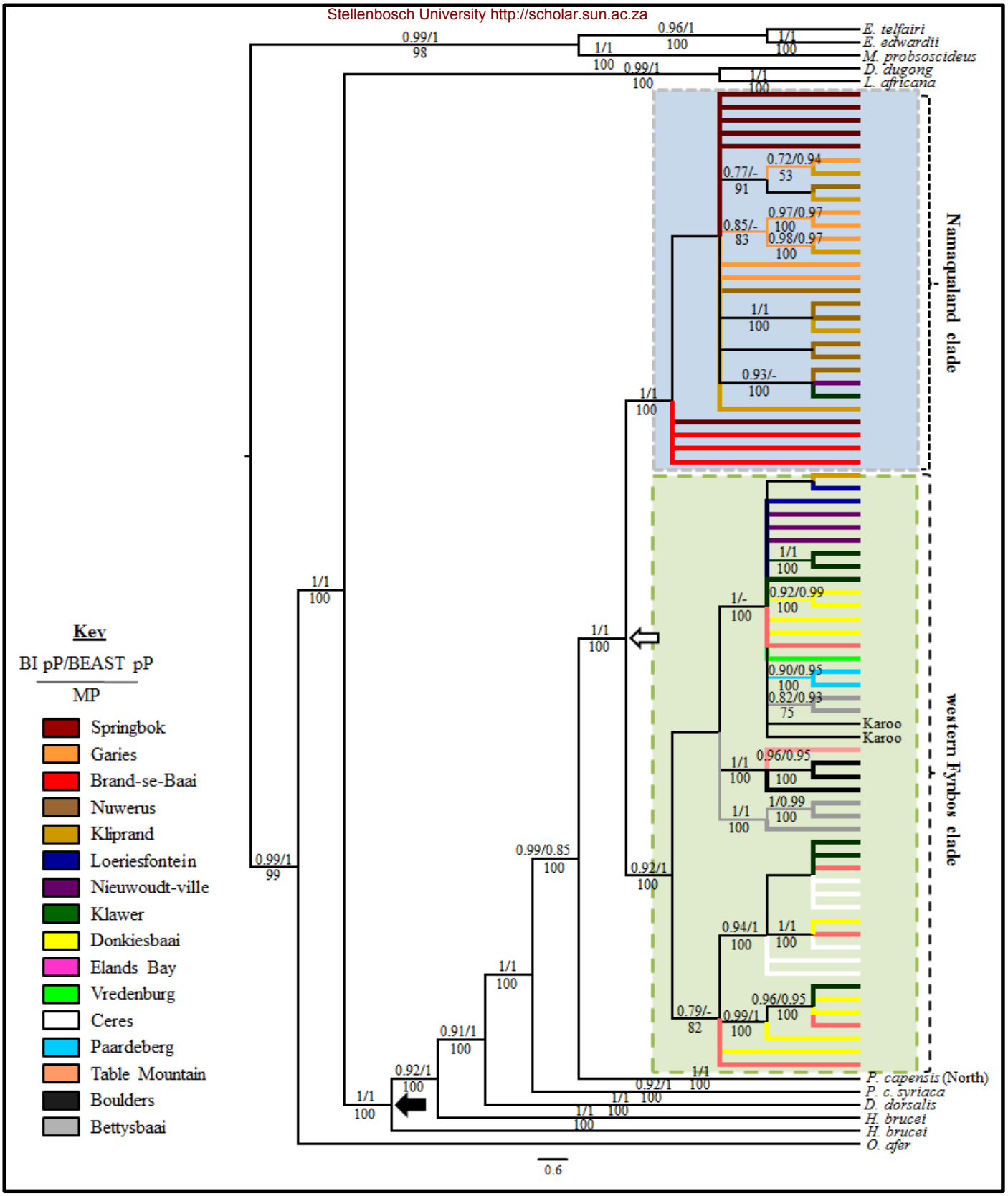

Figure 2.2. Bayesian phylogram obtained from the analyses of the cytochrome b haplotypes among the 16 *P. capensis* sample sites across the Namaqualand and western Fynbos regions of South Africa. The values above each node represent the posterior probability (pP) values derived from the Bayesian inference (MrBayes and BEAST) analyses and those below nodes are the Maximum Parsimony values ("-" indicate that the grouping was not found by the particular analysis). The populations comprising the Namaqualand and western Fynbos clades are shown. The divergence dates for two nodes, the Hyracoidea (14.4 ± 3.0 Mya; black arrow) and Knersvlakte-split (8.9 Mya; white arrow) are indicated.

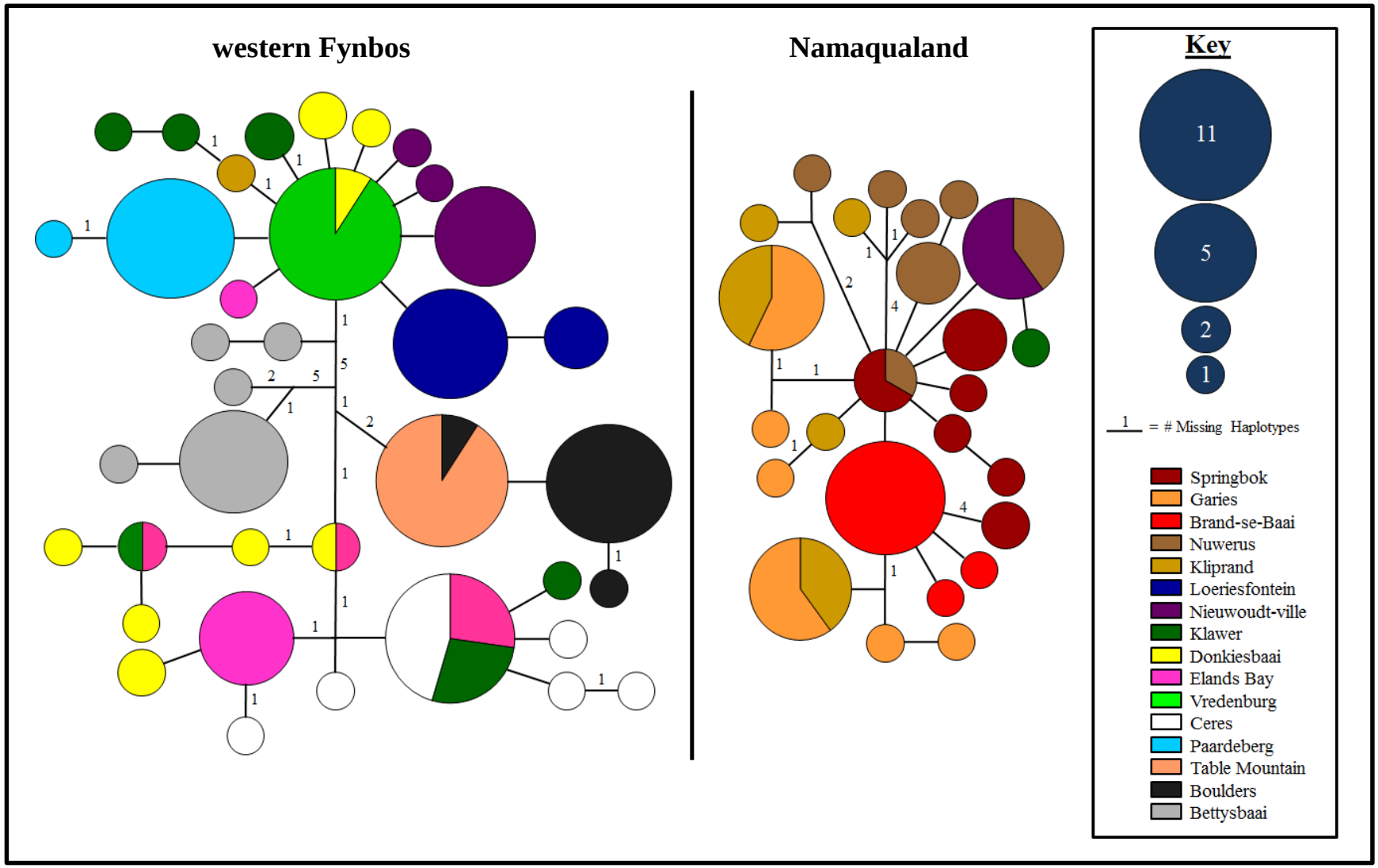

Figure 2.3. Haplotype networks based on cytochrome b gene sequences demonstrating the two mitochondrial DNA clades (western Fynbos and Namaqualand) detected in *P. capensis* from localities across the Namaqualand/western Fynbos regions South Africa. The size of each circle reflects the number of specimens with a particular haplotype. Numbers on branches represent the mutational steps separating haplotypes.

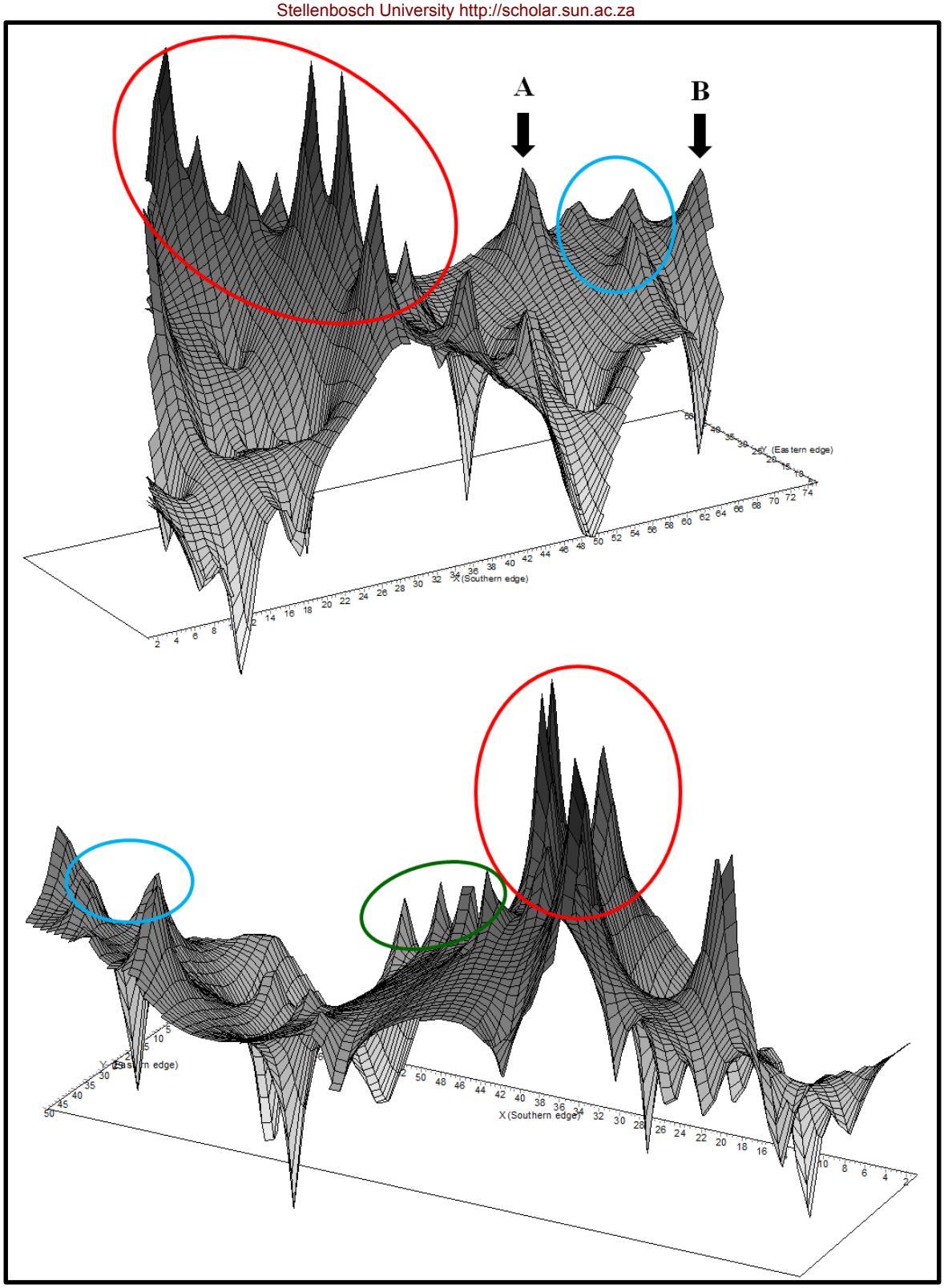

Figure 2.4. A graphical interpolation-based representation of the genetic structure in the cytochrome b sequence data over the Namaqualand and western Fynbos distribution of *P. capensis*. Peaks represent the genetic differentiation between sampled populations. The genetic breaks across the Knersvlakte (circled in red) and between the populations of the south-western Cape (circled in blue) are shown. Structure was also evident along the southern rim of the Knersvlakte (circled in green) and in the south-western Cape region between Paardeberg and Ceres (A), and Table Mountain and Boulders (B).

2.3.1.2. Phylogeography of regional rupicolous fauna: a comparative perspective

The comparison of the key findings of published phylogeographic studies that included the Knersvlakte and Cape Flats in their coverage may be found in Appendix C. These include the Smith's red rock rabbit (*Pronolagus rupestris*; Matthee and Robinson, 1996), southern rock agama (*Agama atra*; Matthee and Flemming, 2002; Swart *et al*. 2009), Cape rock elephant-shrew (*Elephantulus edwardii*; Smit *et al*. 2007), speckled padloper tortoise (*Homopus signatus*; Daniels *et al*. 2010), freshwater river crab (*Potamonautes brincki*; Daniels *et al*. 2001), net-winged midge (*Elporia barnardi*; Wishart and Hughes, 2001, 2003), freshwater phreatoicidean isopod (*Mesaphisopus capensis*; Gouws *et al*. 2004; Gouws *et al*. 2010) and Cape velvet worm (*Peripatopsis capensis*; McDonald and Daniels, 2012). Whereas studies that included the Knersvlakte surveyed mostly vertebrate taxa (Mammalia and Reptilia; Appendix C), those with coverage of the Cape Flats included mostly invertebrate taxa (Crustacea, Insecta and Euonycophera; Appendix C). The exception was the investigation by Swart *et al*. (2009) who studied *A. atra*. In the summary (Appendix C), particular attention was paid to the genetic markers used, the precise locality of phylogeographic breaks detected (if present), the amount of sequence divergence between clades, divergence time estimates between clades, type of phylogeographic pattern found and, finally, the factors that may have influenced the observed phylogeographic profiles (i.e., the phylogeographic interpretation).

2.3.4. Microsatellites

Four microsatellite marekers were amplified in this study; all four showed relatively high levels of polymorphism (i.e., comparable to those reported in the Gerlach and Hoeck, 2001 study; see Appendix B). Three additional markers were tested but these failed to amplify, even with modifications to the protocol.

2.3.4.1. Population and clustering analyses

Significant pairwise genetic differentiation was detected between all localities over the sampling range (Table 2.1.). The Geneland analysis retrieved seven genetic clusters over the landscape (Figure 2.5.). These clusters correspond to the following areas: 1.) Springbok and Garies, 2.) the northern rim of the Knerslakte also including Loeriesfontein and Nieuwoudt-

ville on the southern rim, 3.) Klawer, 4.) Donkiesbaai, 5.) Elands Bay, Ceres and Vredenburg, 6.) Table Mountain, Boulders and Paardeberg, 7.) Bettysbaai.

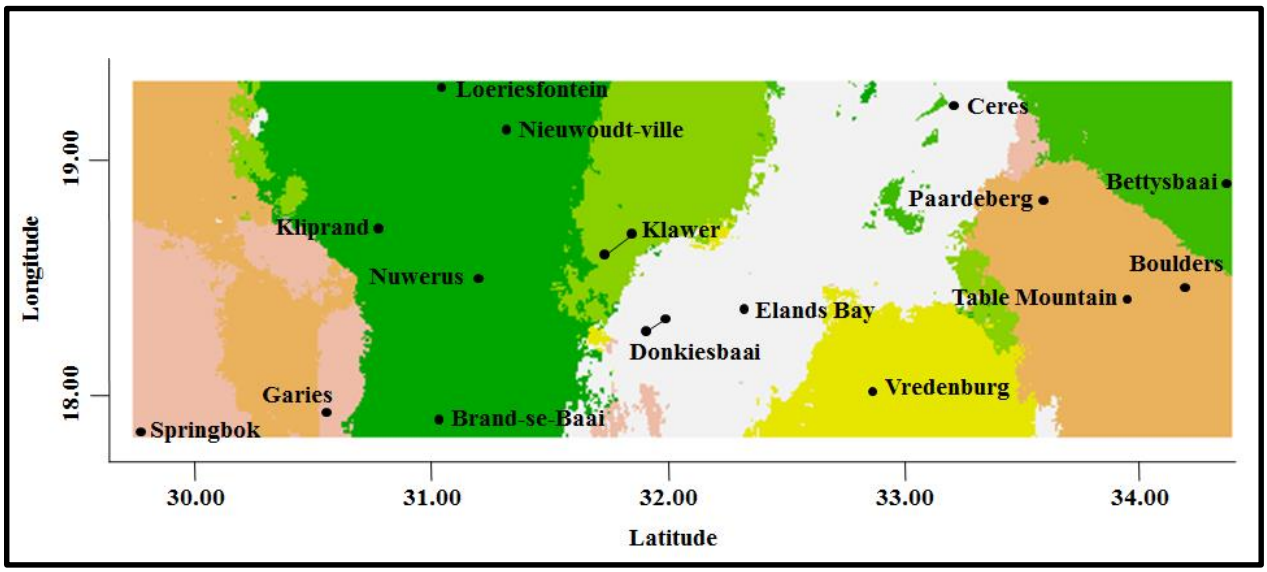

Figure 2.5. Genetic groupings revealed by the Geneland analysis of the microsatellite data. Dots reflect the location of each population and the colours correspond to each seperate genetic grouping.

2.4. Discussion

2.4.1. Population differentiation

Significant genetic differentiation was detected among rock hyrax colonies across the Namaqualand and western Fynbos regions in both the mitochondrial DNA and microsatellites (Table 2.1.). The pairwise F_{ST} values based on the microsatellite data were generally lower than the Φ_{ST} values obtained from the mitochondrial DNA. This is readily explained by the way in which the values were calculated. Population structure is commonly analysed through the use of Wright's F_{ST} with values close to 0 indicating little differentiation (Meirmans, 2006; Jost, 2008). F_{ST} values depend on the amount of within-population variation relative to the amount of total between-population variation (Jost, 2008), thus higher levels of genetic variation (higher polymorphism) usually lead to more within-population variation and consequently lower F_{ST}-values (Hedrick, 1999; Meirmans, 2006; Jost, 2008). It should be noted that the higher within-population variation in microsatellite markers compared with

lower within-population variation in the mitochondrial markers have somewhat biased (lowered) the F_{ST}-values compared with the Φ_{ST} values. Non-significance in some of the pairwise Φ_{ST} values (Table 2.1.) may be attributed to gene-flow into and from these localities subsequently decreasing the differentiation.

2.4.2. Genetic structure across the Cape Flats

Given reports of the Cape Flats acting as a biogeographic barrier to invertebrate dispersal (Daniels *et al*. 2001; Wishart and Hughes, 2001, 2003; Gouws *et al*. 2004; Gouws *et al*. 2010; McDonald and Daniels, 2012) an attempt was made to determine whether a similar pattern would possibly hold for *P. capensis* (although considered unlikely given its greater vagility). To address this, sampling localities were selected in the Hottentots Holland Mountains and Cape Peninsula (Figure 2.1.). No evidence of a geographic break was observed in either the genealogical (Figures 2.2. and 2.3.) or clustering analyses (Figure 2.5.).

Several factors other than vagility may have acted to cause this, one being that the separation of the Hottentots Holland and Cape Peninsula populations is too recent to result in reciprocal monophyly in the hyracoid mitochondrial lineages. The invertebrate taxa all have faster mutation rates due to their smaller body size, lower longevity and less effective DNA copying and repair mechanisms (Janecka *et al*. 2012; Leffler *et al*. 2012). It is to be expected, therefore that invertebrates will become reciprocally monophyletic in a shorter time-span across the same geographic barrier (in this case the Cape Flats).

Interestingly, in spite of lack of evidence of a geographic break, significant (and high) levels of genetic subdivision characterise the hyrax sampling localities in the Hottentots Holland Mountains and Cape Peninsula (Table 2.1.) - observations that are consistent with the AIS analysis which identified barriers to gene-flow between localities in each of these regions (Figure 2.4.). It is hypothesised that this reflects low landscape connectivity since little suitable habitat (crevices) is present in the sandstone formations of these areas (Mucina and Rutherford, 2006; Chapter 3). This makes movement between colonies problematic, resulting in elevated levels of divergence between populations over time.

2.4.3. Genetic structure across the Knersvlakte

Two major, well-supported matrilineal clades were evident that essentially comprise populations to the north (Namaqualand clade) and south (western Fynbos clade) of the Knersvlakte (see TCS, MP, Bayesian and BEAST analyses; Figures 2.2. and 2.3.). This finding supports the hypothesis that the Knersvlakte is the major biogeographic barrier (see AIS analysis; Figure 2.4.) to saxicolous species. It reflects the scarcity of suitable rock hyrax habitat that in turn has impacted on gene-flow across this region.

The lack of concordance with the microsatellite data (Figure 2.5.) can readily be explained when viewing these data in a geographic context. The cluster that includes the Springbok and Garies localities probably reflects the connected nature of these localities through the dispersal route provided by the Kamiesberg mountain range. The populations from the margins of the Knersvlakte region (Brand-se-Baai, Nuwerus, Kliprand, Loeriesfontein, Nieuwoudt-ville) similarly formed a single genetic cluster rather than reflecting the phylogeographic break evidenced by the mitochondrial DNA data. The Knersvlakte essentially consists of a large stretch of open habitat bracketed by a mountainous margin (escarpment) to the north (Kamiesberg Mountains) and south (Warm Bokkeveld Mountains) (Figure 2.1); the southern rim terminates at the Nieuwoudtville sampling site. These margins do not converge to the east; rather the escarpment (margins) is cleaved by a ~ 40 kilometre swaith of land that has little or no suitable habitat.

Although one may anticipate (based on the mitochondrial DNA data) that gene-flow measured by the microsatellites would similarly be impaired across the Knersvlakte, genetic exchange does exist between the northern and southern margins of this region. The most plausible explanation for these contrasting patterns (mitochondrial DNA versus microsatellites) is to invoke male-biased dispersal. The microsatellites thus show the movement of males across and around the Knersvlakte, whereas the genetic structure evident in the mitochondrial DNA is maintained by the lack of dispersal by the females (Figures 2.2. and 2.3.). Importantly, dispersing males carry the mitochondrial DNA of their mothers and strong geographic patterning of the mitochondrial DNA would therefore, in theory, point to the phylopatry of both males and females. Although strong geographic patterning is found across the Knersvlakte in the mitochondrial DNA, no similar patterns are evident along the margins of this region - factors pointing to the inability of females to disperse across this

barrier although not excluding the contribution of female (and male) movement along the margins. Although attempts to investigate population structure using a Y-specific locus proved unsuccessful (see Materials and Methods section), male-biased dispersal has been recorded in *P. capensis* using observational experiments (Fourie, 1983) and in *P. capensis*' close relative, *P. johstoni,* using mark-recapture experiments (Hoeck, 1982, 1989). Males of these species voluntarily disperse as subadults (12 – 30 months) (natal dispersal; Hoeck, 1982; Fourie, 1983) before and during the mating season, or as adults during the breeding season (breeding dispersal; Fourie, 1983) whereas females largely show site phylopatry (Hoeck, 1975, 1982; Fourie, 1983; Hoeck, 1989) as they are not under similar pressure (as males are) to leave the colony (Hoeck, 1982; Fourie, 1983; Hoeck, 1989; Gerlach and Hoeck, 2001). These are all important life history traits that impact on the genetic structure of *Procavia* populations.

Among the localities to the south of the Knersvlakte, the Klawer and Donkiesbaai/Elands Bay/Ceres areas (Figure 2.1.) clustered separately in the microsatellite survey (Figure 2.5.). The AIS analysis (using mitochondrial DNA sequences) also identified barriers between these areas (Figure 2.4.). The populations here are surrounded by the large expanses of unsuitable habitat that separate them from adjacent areas resulting in terrestrial islands. The Donkiesbaai/Elands Bay/Ceres genetic cluster is more complex but may also reflect landscape connectivity. For example, Donkiesbaai and Elands Bay are connected through the coastal belt that may serve as a dispersal route to hyrax in these regions, although this is speculative. These localities are, however, connected to Ceres by the Cederberg mountain range which contains ample suitable habitat. Nonetheless, explanations for the dispersal of animals (and hence gene-flow) from Elands Bay and Donkiesbaai to the Cederberg Mountains are not immediately obvious as both localities are surrounded by large tracts of unsuitable habitat to the south-east.

2.4.4. Divergence time between clades

The split between the Namaqualand and western Fynbos (mitochondrial) clades occurred ~ 8.9 Mya (Figure 2.2.). This coincides with a major marine transgression during the late Miocene that began in the middle Miocene and reached its greatest extent (~ 300 metres) in the late Miocene/early Pliocene (Siesser and Dingle, 1981). The upper reaches of the Knersvlakte today has an elevation of 109 - 153 metres above sea-level (Kounov *et al*. 2008),

and a marine transgression of this magnitude would have inundated this low-lying area thus effectively isolating the higher regions to the north and south. It is important to emphasize that the correspondence in divergence time and transgression may be purely coincidental. If the Miocene transgression was in large part the driver of the Namaqualand and western Fynbos divergence, multiple subsequent regression events (which have been recorded at Miocene/Pliocene boundary and in the late Pliocene; Siesser and Dingle, 1981) and the resultant pulses of low sea-levels, such as currently experienced, would have facilitated gene-flow across the Knersvlakte thus reversing differentiation. Such a pattern is, however, not found. A more plausible explanation probably entails gender-biased dispersal coupled to low connectivity across this region as the main factors influencing the integrity of the two mitochondrial DNA clades. Females appear to exhibit "site phylopatry" (unlike males; Figure 2.5.), which maintains the integrity of the two mitochondrial lineages.

Different routes of dispersal may also be a causal factor in the divergence between the Namaqualand and western Fynbos clades. Sequences from the Karoo specimens that were downloaded from Genbank were found to cluster within the western Fynbos clade in both the Bayesian and MP analyses (they form a sister assemblage to the Namaqualand/western Fynbos *P. capensis* in the BEAST analysis). This suggests the hyrax comprising the Namaqualand clade (specifically females) followed a different colonization route, whereafter they were separated from those of the western Fynbos clade by the poor connectivity of the Knersvlakte. Divergence due to different dispersal routes have been previously shown for *P. capensis* (see Prinsloo and Robinson, 1992; Prinsloo 1993).

2.4.5. Intraclade paraphyly

Protracted divergence times with low levels of gene-flow are considered to have been necessary to produce the reciprocal monophyly observed between the Namaqualand and western Fynbos clades. However, weak geographic patterning and paraphyly was observed within each phylogroup with few mutational steps characterizing the haplotypes (Figure 2.3.). This is surprising given the divergence of the Namaqualand and western Fynbos clades at ~ 8.9 Mya. This may reflect (i) a low rate of mutation in cytochrome b gene within this particular genus or (ii) the relatively short times of geographic isolation between sampling localities within each clade. In other words, connectivity between populations has decreased in relatively recent times. A conserved rate of mutation is, however, a more plausible

explanation. If it is assumed the dating estimates of the present study are accurate for the origin of the Hyracoidea (the divergence date in the present study is corroborated by recent molecular dating studies and fossil evidence; see Chapter 4), this gives a mutation rate of 0.003 (between *Procavia* and *Heterohyrax*) to 0.004 (between *Procavia* and *Dendrohyrax*) substitutions per site per million years at the third codon position. This mutation rate is indeed lower than reported for other mammals (0.007 - 0.008 substitutions per site per million years reported for e.g., Cetaceans; Nabholz *et al*. 2008; Leffler *et al*. 2012), thus confirming the slow rate of change in the *Procavia* mitochondrial genome.

2.4.6. Phylogeography of regional rupicolous fauna: a comparative perspective

As with the findings of this study, most other phylogeographic investigations covering the region of interest found significant differentiation ($F_{ST} > 0.5$) (Matthee and Flemming, 2002; Smit *et al*. 2007; Daniels *et al*. 2010) and relatively low sequence divergence estimates (below 2%) for vertebrate taxa whose distributions traversed the Knersvlakte (Smit *et al*. 2007; Daniels *et al*. 2010; Appendix C). Various factors have been proposed to explain genetic discontinuities across this barrier. These include glacial cycles during the Pleistocene/Pliocene, marine transgressions causing vicariant events, climate change resulting in refugia, isolation due to limited dispersal, habitat heterogeneity with intervening unsuitable habitat between suitable patches, geographic barriers and difference in elevation (Matthee and Robinson, 1996; Matthee and Flemming, 2002; Smit *et al*. 2007; Swart *et al*. 2009; Daniels *et al*. 2010). The factors influencing the genetic structure of various rock-dwelling taxa across the Namaqualand and western Fynbos regions are thus varied and not mutually exclusive. Of these the present study shows that habitat heterogeneity (unsuitable habitat between suitable habitat patches) and geographic barriers are the major factors influencing genetic substructuring between *P. capensis* populations across the sampled distribution. Importantly, however, none of the above studies has empirically tested the effects of landscape connectivity as a long-standing phylogeographic barrier to gene-flow across a landscape - a notion which is not frequently raised when comparing multiple taxa. The development of the Knersvlakte is dated at ~ 18 Mya (Moon and Dardis, 1988) although recent studies suggest a much more ancient date (~ 90 Mya; Kounov *et al*. 2008). Irrespective of this wide variance, the region has been an enduring and persistent geographic barrier that predates the distribution of all the species for which phylogeographic data are available (*P. capensis*, *P. rupestris*, *A. atra*, *E. edwardii*, *H. signatus*) and it is reasonable to expect that the

low connectivity of this area continues to influence the dispersal of saxicolous taxa through recent times. As is evident from the data presented thus far, *P. capensis* is a good model species to examine finer-scale genetic structure linked to the formation of the surrounding landscape (referred to as landscape genetics, Storfer *et al*. 2007) and this will form the substance of Chapter 3.

Phylogeographic congruence in co-distributed taxa is commonplace (Bermingham and Avise, 1986; Avise, 1992; Scribner and Avise, 1993; Avise, 2000; Arbogast and Kenagy, 2001; Lapointe and Rissler, 2005; Feldman and Spicer, 2006; Castoe *et al*. 2009; Tolley *et al*. 2009) suggesting that vicariance and dispersal occurred *in concert* due to common historical events. While the studies of Avise (1992; 2000), Lapointe and Rissler, (2005) and Tolley *et al*. (2009) among others included taxa with different life-histories and ecological requirements, similar patterns have been detected between co-distributed but ecologically similar taxa (Arbogast and Kenagy, 2001; Feldman and Spicer, 2006; Castoe *et al*. 2009). The present investigation was similarly based on taxonomically diverse yet ecologically similar (saxicolous) taxa that coincide in terms of distribution (Chapter 2) and not surprisingly, congruent phylogeographic patterns were also found, although the timing of the divergences was different.

A review of the literature revealed large differences in divergence time estimates across the Knersvlakte (Appendix A). Similarly, major differences were noted in the timing of genetic splits between co-distributed taxa worldwide (Zink, 1996; Brunsfeld *et al*. 2000; Zink, 2002). Timing of divergences may be tiered since expansions are not abrupt but periodic (Zink, 1996; Zink, 2002; Soltis *et al*. 2006; Yang *et al*. 2009). Another factor influencing the accuracy of molecular dating is that lineage-specific mutation rates are not homogenous (Bermingham and Moritz, 1998), something that was assumed in most of the studies included in this review. This has largely been solved through the use of new molecular dating techniques (Bayesian estimates) and multiple unlinked genetic markers (Bermingham and Moritz, 1998).

Zink (1996) proposed that the only prerequisite when comparing phylogeographic patterns is that species should be co-distributed. This somewhat narrow view ignores the possibility that differences in genetic patterns may also be subject to other factors. These include expansions from refugia that vary in space, time and extent due to different life-histories, the effects of

several isolating and dispersal events, recent population expansions, population histories, ancestral populations sizes, and dispersal capabilities (dispersal rates) of animals, as well as the effects of different barriers to gene-flow and mitochondrial DNA rate heterogeneity between taxa (Zink, 1996; Bernatchez and Wilson, 1998; Schneider *et al*. 1998; Brunsfeld *et al*. 2000; Zink, 2002; Arbogast and Kenagy, 2001; Dawson, 2005; Soltis *et al*. 2006; Wallis and Trewick, 2009; Yang *et al*. 2009). It is clearly evident that including taxa with a diverse array of ecological requirements may blur the effects of vicariance simply because different species respond differently to certain environmental barriers. Consequently to search for a common genetic pattern among a large diversity of taxa may prove problematic and a more focused approach that includes only taxa with similar ecological requirements may be more informative for comparative phylogeographic inference. Such a study would give a clearer and more biologically appropriate pattern since similar landscape features could, potentially, act as barriers to gene-flow. While South Africa has a complex climatic and orogenic history, this investigation shows that the comparison of ecologically similar species may, in fact, result in an improved understanding of the effects of landscape structure as a barrier to gene-flow.

CHAPTER 3

Connectivity of *Procavia capensis* colonies at various spatial scales with a specific focus on barriers to gene-flow

3.1. Introduction

Landscape connectivity refers to the connectivity of the surrounding matrix, defined by Holderegger and Wagner (2008) as "*the often hostile space that separates the patches of a species' habitat in a given landscape*". The matrix is a major factor determining the movement of animals with specific habitat requirements. Therefore the quality and quantity of areas that separate suitable habitat affect the distribution of both adaptive and non-adaptive genetic variation (Coulon *et al*. 2004; Storfer *et al*. 2007; Holderegger and Wagner, 2008). Two types of landscape connectivity are identified to assess the influence of the landscape on the movement of species. Gene-flow is an example of functional connectivity, whereas structural connectivity relates to how suitable habitat patches are distributed across the landscape (Holderegger and Wagner, 2008). These approaches are invaluable (Castella *et al*. 2001; Scribner *et al*. 2001; Hammond *et al*. 2006; Keogh *et al*. 2007; Ujvari *et al*. 2008) since assessing the movement of animals through direct observational methods, such as mark-recapture experiments or radio-telemetry, are often laborious, difficult or not feasible (Goudet *et al*. 2002; Broquet *et al*. 2006). Moreover these do not, for instance, show long-distance gene-flow events (Dallimer *et al*. 2002; Hammond *et al*. 2006). Landscape genetics thus offers a framework by which one can isolate the influence of landscape variables and their impact on genetic variation as well as the identification of barriers to gene flow, source-sink dynamics and movement corridors between populations. This informs our understanding of the spatial and temporal scales of an ecological process (Storfer *et al*. 2007).

For species restricted to rocky outcrops, the connectivity of the surrounding landscape should have a notable influence on the distribution of genetic diversity across the landscape (see Chapter 1 for examples). Put differently, this type of habitat is important in determining the connectivity between habitat patches since intervening unsuitable habitat may form a significant barrier to dispersal. The rock hyrax, *Procavia capensis*, is a case in point. Although *P. capensis* is relatively mobile (Chapter 2), there is a cost to dispersal as the

dispersal distance is largely constrained by predator density and availability of suitable refuge sites (Turner and Watson, 1965; Fairall *et al*. 1986; Fairall and Hanekom, 1987; Kotler *et al*. 1999; Druce *et al*. 2006). This makes *P. capensis* a suitable model species to investigate the effect of landscape connectivity on the distribution of genetic variation in a rock-dwelling vertebrate.

In this chapter both mitochondrial- and nuclear DNA (microsatellite) markers were used in a landscape genetics approach to show how habitat connectivity affects the genetic distinctiveness of rock hyrax populations at various spatial scales. At a fine scale, the spatial genetic structure, gene-flow and sex-bias in migration between five different koppies comprising an isolated population were ivestigated (see Figure 3.1. below). To address intermediate spatial scales, genetic structure and gene-flow were investigated focussing sampling across and around known barriers to other saxicolous species, the Cape Flats and Knersvlakte. Although these areas are known geographic barriers to gene-flow, their effects on connectivity between populations have never been explicitly investigated. At the largest spatial scale it was demonstrated that functional connectivity influences the distribution of genetic variation in two regions with contrasting connectivity; Namaqualand and the western Fynbos regions. This is also important as convention suggests that there is currently a general decline in the rock hyrax numbers at most monitored sites in the Western Cape (western Fynbos region) although no similar declines are evident in the north-lying Namaqualand region (Chapter 1). Finally, the data was examined for signatures typical of populations decline by comparison to hyrax from the Namaqualand region where no similar declines have been reported.

3.2. Materials and Methods

3.2.1. Sample collection

Sampling of *P. capensis* was conducted in a hierarchical fashion across the Namaqualand and western Fynbos regions focussing on known geographic barriers (Knersvlakte and Cape Flats) (Figure 2.1). Sampling procedures were similar to those outlined in Chapter 2. The following sampling schemes were adopted:

3.2.1.1. Fine scale

At a fine spatial scale, connectivity among koppies was assessed in the Vredenburg area. The Vredenburg study site is a single, isolated population comprising five koppies (Figure 3.1.). As relatedness and sex-bias in dispersal was investigated at this locality, the sex and a rough estimate of age (adult > 1 year, juvenile < 1 year; following Fourie, 1983) was recorded for each of the specimens.

Figure 3.1. Arial view of the Vredenburg population showing the five different koppies where animals were sampled.

3.2.1.2. Intermediate scale

Connectivity at intermediate spatial scales was assessed across and around two geographic barriers, the Cape Flats and Knersvlakte. Specimens were collected from four localities across the Cape Flats (colonies on the Cape Peninsula and those in the Hottentots Holland Mountains). Although five colonies occur along both the Atlantic and False Bay sides of the Cape Peninsula only two (Table Mountain on the Atlantic side and Boulders on the False Bay side) could be accessed and sampled in this study (Figure 2.1.). In addition, connectivity of populations (both around and across the Knersvlakte) was assessed by including eight

sampling localities from the mountainous margins (rims) of this geographic region (Figure 3.1.).

3.2.1.3. Regional scale

To address the regional spatial scale component, specimens were collected from sixteen localities across the Namaqualand (Springbok, Garies, Brand-se-Baai, Nuwerus, Kliprand, Loeriesfontein, Nieuwoudt-ville, Klawer and Donkiesbaai) and western Fynbos (Elands Bay, Vredenburg, Ceres, Paardeberg, Table Mountain, Boulders and Bettysbaai) regions (Figure 2.1.). This permitted the analysis of large-scale connectivity patterns between these regions comprising matrices of contrasting connectivity (Figure 3.2.).

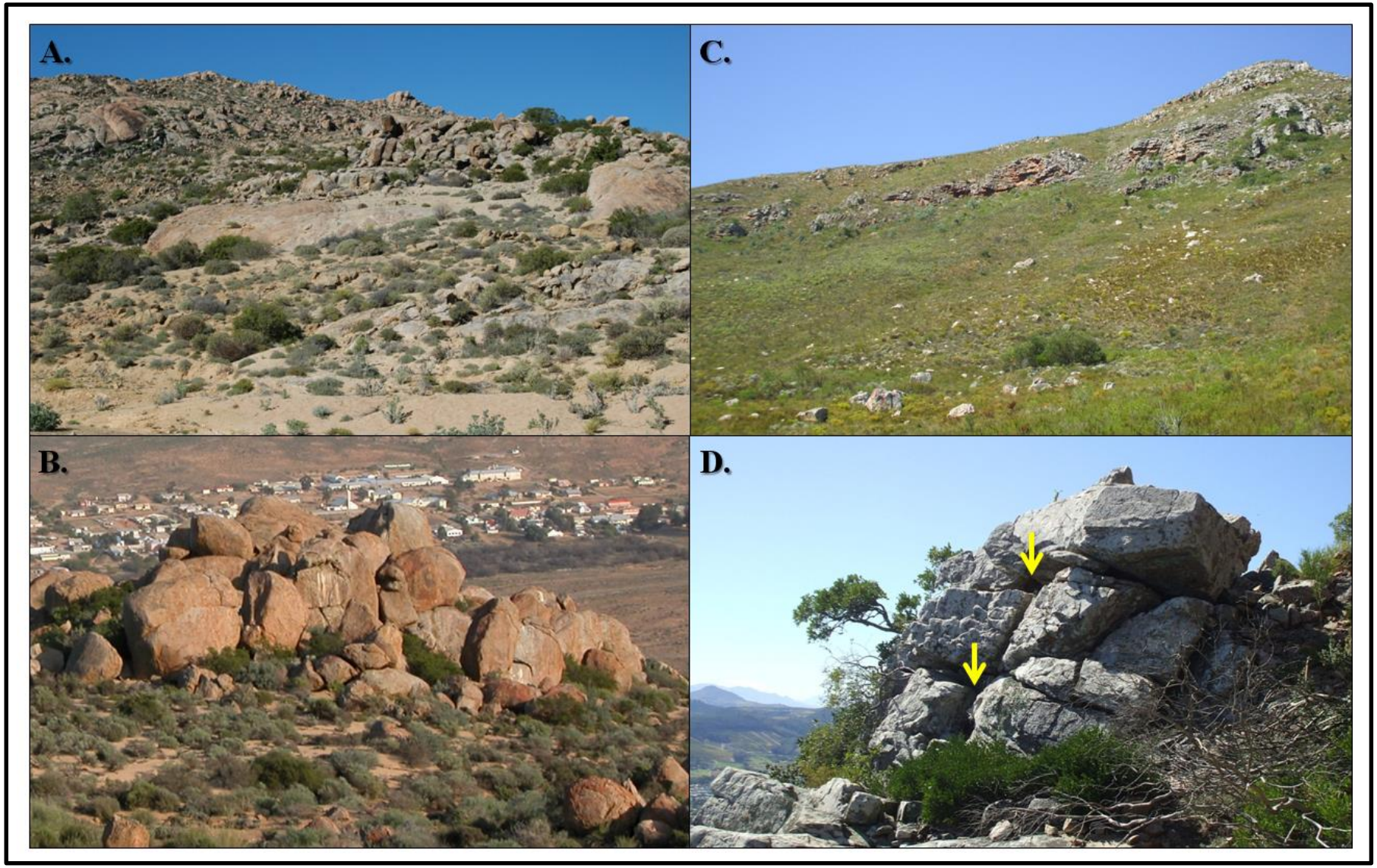

Figure 3.2. The Namaqualand landscape consists of multiple close-lying suitable habitat patches (A) with abundant suitable crevices (B). In contrast, the western Fynbos landscape contains quantitatively fewer and more dislocated habitat patches (C) with a limited number of suitable crevices (arrowed) (D).

3.2.2. Experimental procedures

Detailed protocols for both mitochondrial and microsatellites are presented in Chapter 2.

3.2.3. Data analyses

3.2.3.1. Summary statistics and inbreeding

Genetic diversity detected within each sampling locality and summary statistics for the mitochondrial DNA analyses (including number of haplotypes and nucleotide (π) diversity) were calculated in Arlequin version 3.5 (Excoffier and Lischer, 2010). Similar measures of genetic diversity resulting from the microsatellite analysis were also calculated; these included allelic diversity indices (total number of alleles and mean number of alleles per locus; FSTAT version 2.9.3.2; Goudet, 2001), observed as well as expected heterozygosities (Genalex version 6.4; Peakall and Smouse, 2006). These values were compared between the Namaqualand and western Fynbos regions using Statistica version 10 (Statsoft Inc. 2011). Inbreeding in each colony was assessed by Wright's F_{IS} (Genalex version 6.4; Peakall and Smouse, 2006).

3.2.3.2. Population analyses

Fluctuations in population size (based on the mitochondrial DNA data) in sampling localities in each of the two major clades (Namaqualand and western Fynbos; Chapter 2) were investigated. This was done using Fu's Fs (a statistic that evaluates population equilibrium; Fu, 1997) and mismatch distributions (plotting the various haplotypes against their respective frequencies) in DnaSP 5.10.01 (Librado and Rozas, 2009). To visualize this variability through time, Bayesian skyline plots were constructed in BEAST 1.4 (Drummond *et al*. 2007). The programme simulated 10 x 10^6 generations sampling every 1 000 generations (burnin = 1 000). Bottleneck version 1.2.02 (Cornuet and Luikart, 1996) was used to investigate whether demographic changes were evident in the history of each sampling locality based on the microsatellite data. This programme measures recent effective population size changes. A two-phased model of mutation was employed (recommended by Luikart *et al*. 1998 for microsatellite data) and the Wilcoxon sign-rank test value was applied

to assess the probability that an excess of heterozygosity existed at a significant number of loci in a colony.

The hierarchical spread of variation was assessed using an analysis of molecular variation (AMOVA implemented in Arlequin version 3.5; Excoffier and Lischer, 2010) between the Hottentots Holland Mountains and Cape Peninsula (Cape Flats), the northern (Brand-se-baai, Nuwerus, Kliprand) and southern rim (Loeriesfontein, Nieuwoudt-ville, Klawer, Donkiesbaai, Elands Bay) localities, and between the Namaqualand and western Fynbos clades (Chapter 2). Isolation-by-distance was evaluated using a Mantel test as employed in Arlequin version 3.5 (Excoffier and Lischer, 2010) for the mitochondrial DNA dataset. Geographical distances were determined "as the crow flies"; i.e., the shortest and most direct route between localities rather than along mountain ranges. A Mantel test (implemented in Genalex version 6.4; Peakall and Smouse, 2006) was also applied to estimate isolation-by-distance in the microsatellite data; geographical distances were calculated by the programme based on the coordinates of each sampling point.

The spatial locations of genetic clusters (based on microsatellite data) across the landscape were determined by Bayesian assignment implemented in Geneland version 2.0.10 (Guillot *et al*. 2005) using the same settings as those in Chapter 2. Gene-flow among sampling localities was estimated in Lamarc version 2.1.6 (Kuhner, 2006). Lamarc involves a Markov chain Monte Carlo coalescent genealogy sampling approach to calculate parameters such as effective population size, growth rate and immigration rate. The programme was run using the Bayesian search strategy under the GTR model for the mitochondrial DNA; 10 initial chains were run for 10 000 generations (burnin = 1 000) and 3 final chains of 5 x 10^6 generations (burnin = 10 000) completed the analysis. In the case of the microsatellite data, the "Brownian" model was selected and 10 initial chains were run for 10 000 generations (burnin = 1 000); two final chains of 1 x 10^6 generations (burnin = 10 000) completed the analysis. Statistically significant differences in gene-flow around the Knersvlakte versus across this region, as well as between the Namaqualand region versus the western Fynbos region, were determined (Statistica version 10, Statsoft Inc. 2011). Gene-flow values based on the mitochondrial DNA data were compared to values from the microsatellite data (Statistica version 10, Statsoft Inc. 2011) to test for a sex-bias in dispersal. This was done for the Namaqualand and western Fynbos regions respectively.

Additional analyses of the fine spatial scale microsatellite data from the Vredenburg sampling locality were performed. This was done to investigate relatedness and the population assignment of specimens within and between colonies. Relatedness was calculated in Coancestry version 1.0 (Wang, 2010). This permitted estimates of average relatedness of specimens within colonies, between colonies, and between genetic groups, and is thus also a measure of inbreeding. Differences between intracolony, intercolony and intergroup (Geneland cluster) relatedness were determined in Statistica version 10 (Statsoft Inc. 2011). An assignment test was performed to investigate sex-bias in dispersal and whether each animal originated from koppies other than the ones at which they were sampled (Genalex version 6.4; Peakall and Smouse, 2006). For this test, animals were placed into four groups: adult males, adult females, juvenile males and juvenile females. Assignment of specimens was done considering each colony as a separate population, and each "Geneland genetic cluster" as a separate population.

3.3. Results

3.3.1. Fine scale

The 10 specimens from the Vredenburg locality all had the same mitochondrial DNA haplotype (Table 3.2.). No further tests of gene-flow or genetic structure were performed.

Microsatellite data from 77 specimens was derived from five colonies in the Vredenburg area. Pairwise F_{ST} values between colonies ranged between 0 and 0.117 (Table 3.3.). Genetic diversity was significantly partitioned (F_{ST} = 0.568; $p < 0.001$) among colonies in the Vredenburg sampling locality. Colonies A and B were not significantly different; not surprising given the geographic proximity of the rocky outcrops to each other. All other colonies were significantly distinct with the exception of colony E. Although this may indicate the regular exchange of individuals, it may also simply reflect the much smaller sample size of this colony, and hence weaker statistical resolution.

Isolation-by-distances ($r = 0.165$; $p < 0.01$) was evident over the landscape in the Vredenburg sampling locality. Geneland retrieved three genetic clusters (Figure 3.3.). Colonies that grouped together were geographically adjacent. The highest levels of gene-flow (>10

individuals per generation) were observed between Colonies A and B and Colonies D and E respectively (Table 3.4.). Gene-flow between different koppies was comparatively low ranging from <1 individual per generation, to two individuals per generation. The gene-flow (Table 3.4.) results confirm that each genetic cluster (Figure 3.3.) may be regarded as a breeding colony. In the assignment test of population membership, dispersal among these three clusters (breeding colonies) was female-biased (40% of adult females assigned to different koppies from where they were sampled compared to 15% in males) in the case of adult animals; no sex-bias was evident for dispersing juveniles. In addition, intracolony and intragroup relatedness was significantly higher than intercolony relatedness when juveniles were included (Table 3.1; a.), however when only adult animals were tested, no similar patterns were evident for male, female or all adult animals overall (Table 3.1; b.). By including only juvenile animals, relatedness was again shown to be higher within colonies (Table 3.1; c.).

Table 3.1. Statistical tests of the hypotheses formulated to examine relatedness in the Vredenburg rock hyrax population, genetic diversity (including nucleotide diversity, haplotype diversity, microsatellite genetic diversity and expected heterozygosity) and gene-flow (around and across the Knersvlakte, within the Namaqualand and western fynbos region and between the mitochondrial DNA and microstallite datasets within each of these regions). The labels (a - l) refer to the specific hypothesis investigated (see text). For each variable the mean, the null hypothesis investigated, the test value, degrees of freedom and the p-value of the appropriate statistical test are presented. Values in red indicate non-significance.

Label	Variable	Mean ± S.D	Null hypothesis	Test value	df	p-value
		Relatedness (juveniles included)				
a.)	Intracolony relatedness	0.372 ± 0.026	Intracolony and intragroup relatedness do not differ significantly from inter-colony relatedness	3.957 (T-test)	13	< 0.01
	Intercolony relatedness	0.297 ± 0.050				
	Intragroup relatedness	0.398 ± 0.035				
		Relatedness (juveniles excluded)				
b.)	Male relatedness	0.083 ± 0.058	Intracolony and intragroup relatedness do not differ significantly from inter-colony relatedness	-0.15	8	> 0.05
		0.147 ± 0.066				
		0.153 ± 0.090				
	Female relatedness	0.155 ± 0.052	Intracolony and intragroup relatedness do not differ significantly from inter-colony relatedness	0.373 (T-test)	13	> 0.05
		0.152 ± 0.065				
		0.158 ± 0.043				
	All adults relatedness	0.164 ± 0.040	Intracolony and intragroup relatedness do not differ significantly from inter-colony relatedness	0.932 (T-test)	13	> 0.05
		0.156 ± 0.056				
		0.171 ± 0.039				
		Relatedness (juveniles only)				
c.)	Intracolony relatedness	0.194 ± 0.045	Intracolony does not differ significantly from inter-colony relatedness	10.000 (Mann-Whitney U)	13	< 0.05
	Intercolony relatedness	0.148 ± 0.051				
		Nucleotide diversity (mitochondrial DNA)				
d.)	Namaqualand	0.005 ± 0.004	Nucleotide diversity does not significantly differ between the Namaqualand and western Fynbos regions	2.093 (T-test)	14	> 0.05
	western Fynbos	0.002 ± 0.002				

		Number of haplotypes (mitochondrial DNA)				
e.)	Namaqualand western Fynbos	5.444 ± 2.007 3.286 ± 2.059	The number of haplotypes does not significantly differ between the Namaqualand and western Fynbos regions	2.358 (T-test)	14	< 0.05
		Genetic diversity (microsatellites)				
f.)	Namaqualand western Fynbos	27.222 ± 3.073 16.714 ± 5.345	Genetic diversity does not significantly differ between the Namaqualand and western Fynbos regions	4.199 (T-test)	14	> 0.001
		Expected heterozygosity (microsatellites)				
g.)	Namaqualand western Fynbos	0.798 ± 0.020 0.643 ± 0.130	Expected heterozygosity does not significantly differ between the Namaqualand and western Fynbos regions	2.816 (T-test)	14	< 0.05
		Gene-flow (Knersvlakte)				
h.)	Around Across	1.196 ± 0.876 0.430 ± 0.212	Gene-flow around the Knersvlakte does not differ significantly from gene-flow across this region	2.247 (T-test)	12	< 0.05
		Gene-flow (regional scale)				
	Mitochondrial DNA					
i.)	Namaqualand western Fynbos	0.290 ± 0.231 0.117 ± 0.069	Gene-flow levels do not significantly differ between the Namaqualand and western Fynbos regions	2.918 (T-test)	20	< 0.01
	Microsatellites					
j.)	Namaqualand western Fynbos	0.873 ± 0.432 0.378 ± 0.137	Gene-flow levels do not significantly differ between the Namaqualand and western Fynbos regions	3.084 (T-test)	20	< 0.01
		Gene-flow (regional scale)				
	Namaqualand					
k.)	Mitochondrial DNA Microsatellites	0.290 ± 0.231 0.873 ± 0.432	Gene-flow levels do not significantly differ between the mitochondrial DNA and microsatellite datasets	-5.379	36	< 0.001
	western Fynbos					
l.)	Mitochondrial DNA Microsatellites	0.117 ± 0.069 0.378 ± 0.137	Gene-flow levels do not significantly differ between the mitochondrial DNA and microsatellite datasets	-5.442	18	< 0.001

Table 3.2. Genetic diversity values for the mitochondrial DNA and microsatellites of the *P. capensis* populations sampled across the South African west coast region. In the case of mitochondrial DNA, the number of specimens (n), nucleotide diversity (π) and number of haplotypes in each population is presented, whereas the number of specimens (n), total number of alleles, expected heterozygosity within each population and the inbreeding coefficient (F_{IS}) of each population is shown for the microsatellite data. Localities in the vicinity of the Knersvlakte (red block) and Cape Flats (blue block) are indicated.

Locality	Mitochondrial DNA			Microsatellites			
	n	Nucleotide diversity (π)	No. Haplotypes	n	Total No. Alleles	Expected Heterozygosity	F_{IS}
Springbok	10	0.003 ± 0.002	6	21	28	0.703 ± 0.126	0.144
Garies	10	0.003 ± 0.002	6	30	31	0.679 ± 0.129	0.138
Brand-se-Baai	10	0.000 ± 0.000	3	29	26	0.669 ± 0.117	0.036
Nuwerus	10	0.004 ± 0.002	7	26	31	0.663 ± 0.140	0.157
Kliprand	10	0.008 ± 0.004	6	21	27	0.684 ± 0.104	0.054
Loeriesfontein	10	0.000 ± 0.000	2	11	21	0.740 ± 0.041	0.125
Nieuwoudt-ville	10	0.010 ± 0.006	4	21	26	0.661 ± 0.140	0.123
Klawer	10	0.011 ± 0.006	7	15	26	0.676 ± 0.089	0.186
Donkiesbaai	10	0.008 ± 0.005	8	21	29	0.743 ± 0.075	0.121
Elands Bay	10	0.004 ± 0.003	5	25	19	0.545 ± 0.096	0.098
Vredenburg	10	0.000 ± 0.000	1	77	16	0.617 ± 0.057	0.250
Ceres	10	0.002 ± 0.001	6	22	25	0.715 ± 0.079	0.258
Paardeberg	10	0.000 ± 0.000	2	18	16	0.574 ± 0.043	0.110
Table Mountain	10	0.000 ± 0.000	1	22	16	0.591 ± 0.108	0.100
Boulders	10	0.001 ± 0.001	3	12	7	0.233 ± 0.145	-0.122
Bettysbaai	10	0.006 ± 0.003	5	25	18	0.657 ± 0.044	0.202
Total	**160**	**0.004 ± 0.002**	**72**	**396**	**60**	**0.634 ± 0.027**	**0.098**

Table 3.3. Pairwise F_{ST} values between the five colonies in the Vredenburg *P. capensis* population based on the microsatellites data. Values in black were significant at $p < 0.05$; those in red were non-significant.

Colony	A	B	C	D	E
A	/				
B	0.029	/			
C	0.079	0.071	/		
D	0.086	0.117	0.038	/	
E	0.034	0.044	-0.034	-0.022	/

Table 3.4. Gene-flow between the five colonies in the Vredenburg *P. capensis* population based on the microsatellites data. Values in red are gene-flow levels >1 individual per generation (standard error included).

Colony	**Microsatellites** ----->	**Microsatellites** <-----
A-B	12.384 + 0.569 - 5.283	10.096 + 3.171 - 0.693
A-C	0.756 + 0.329 - 0.525	0.278 + 0.267 - 0.228
B-C	1.180 + 1.169 - 0.368	2.028 + 0.606 - 0.394
C-D	1.019 + 0.209 - 0.483	0.393 + 0.897 - 0.056
C-E	1.122 + 0.864 - 0.795	0.890 + 0.755 - 0.469
D-E	2.097 + 1.624 - 1.063	19.126 + 0.684 - 19.051
A-D	0.018 + 0.003 - 0.007	1.191 + 0.213 - 0.105
B-D	1.521 + 0.561 - 0.219	0.706 + 0.304 - 0.119
A-E	2.324 + 0.401 - 0.641	0.955 + 0.067 - 0.169
B-E	0.745 + 0.244 - 0.523	0.176 + 0.091 - 0.115

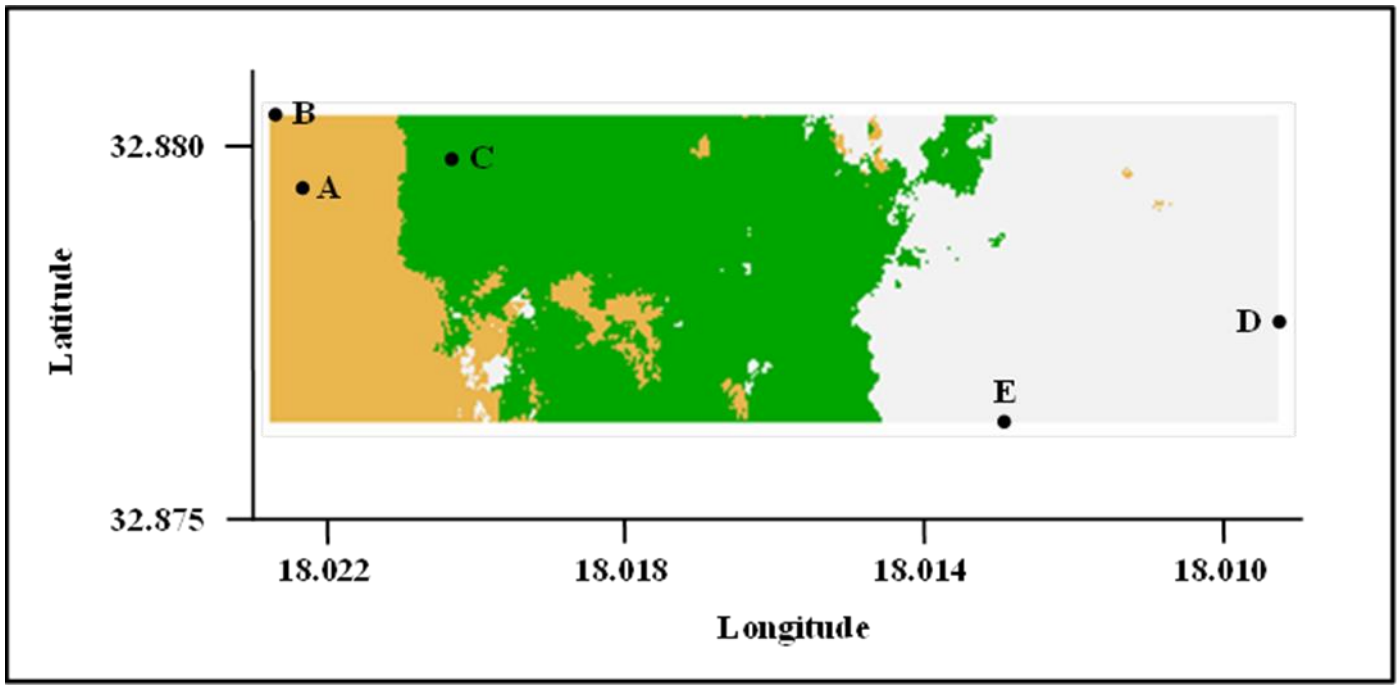

Figure 3.3. The three genetic groupings detected over the landscape in the Vredenburg population using microsatellites analysed by Geneland. Dots represent the location of each colony.

3.3.2. Intermediate scale

3.3.2.1. Cape Flats

Cytochrome b sequence (n = 40 specimens) and microsatellite data (n = 77 specimens) were obtained from animals collected in the Cape Peninsula and Hottentots Holland Mountains (Paardeberg, Table Mountain, Boulders and Bettysbaai). Variation was significantly partitioned between the two groups (Hottentots Holland Mountains and Cape Peninsula) across the Cape Flats for both the mitochondrial DNA (F_{ST} = 0.434; $p < 0.001$) and microsatellites (F_{ST} = 0.148; $p < 0.001$). Gene-flow between all sampling localities was <1 individual per generation for both data sets (Table 3.5.). The Geneland analysis retrieved four genetic clusters over the Cape landscape corresponding to the sampling areas (Paardeberg, Bettysbaai, Table Mountain and Boulders; Figure 3.4.).

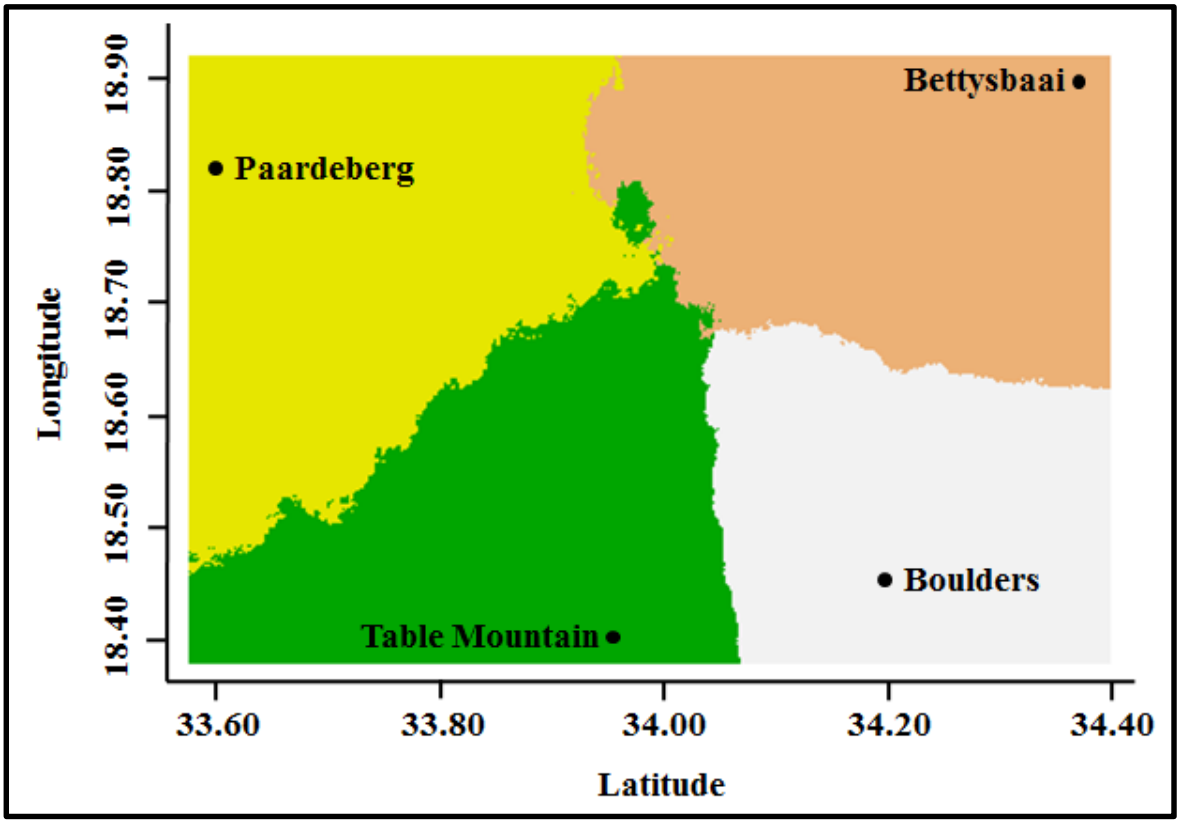

Figure 3.4. Genetic groups detected in the microsatellite data by Geneland analysis on an intermediate spatial scale across the Cape Flats. Dots represent the locations of each of the four sampling localities.

3.3.2.2. Knersvlakte

Cytochrome b (n = 80 specimens) and microsatellite data (n = 169 specimens) were drawn from localities across the Knersvlakte. Genetic diversity was significantly partitioned between *P. capensis* sampling localities on the northern and southern rims of the Knersvlakte in both the mitochondrial DNA (F_{ST} = 0.563; $p < 0.001$) and microsatellites (F_{ST} = 0.239; $p < 0.001$). Evidence of gene-flow (>1 individual per generation) was apparent around and across the Knersvlakte (Table 3.5.). Genetic exchange around the Knersvlakte was significantly higher (Table 3.1; h.) than across this region in the case of mitochondrial DNA. No similar significant pattern was evident from the microsatellites. The Geneland analysis retrieved three genetic clusters over the Knersvlakte landscape (Figure 3.5.). These pertain to the northern rim of the Knerslakte (but including two localities on the south rim - Loeriesfontein and Nieuwoudt-ville), the Klawer area, and the coastal localities of Donkiesbaai and Elands Bay respectively.

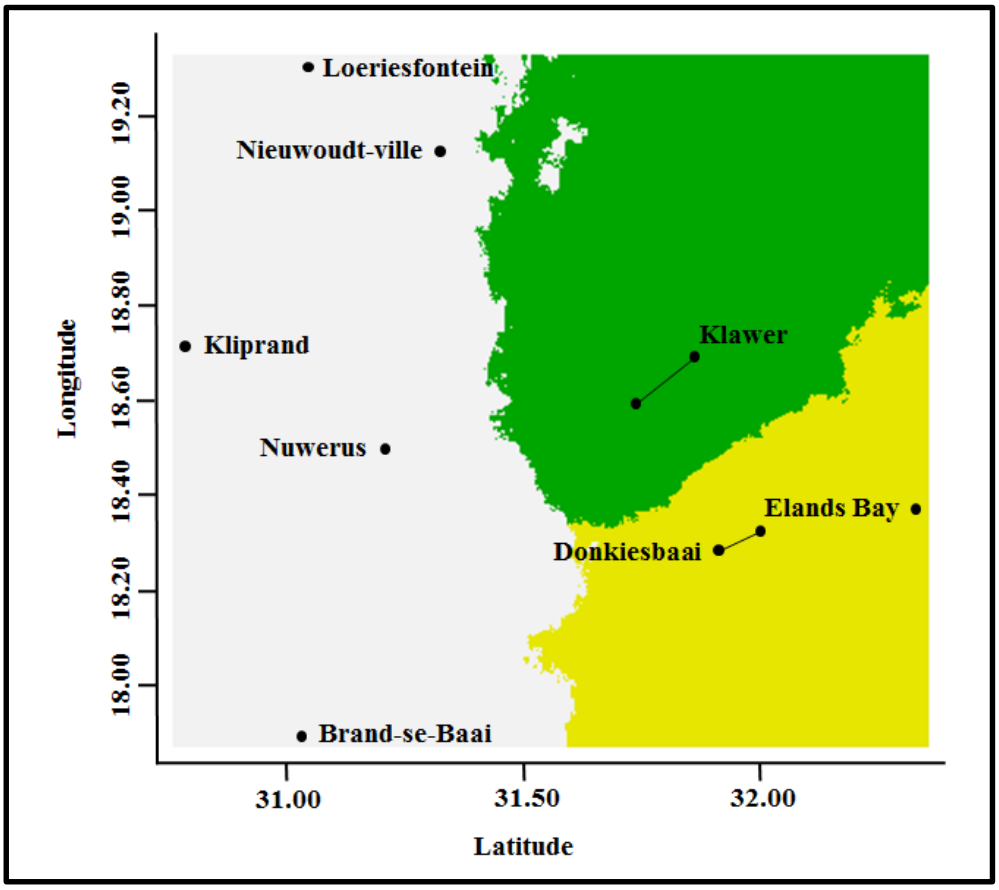

Figure 3.5. Genetic groups detected in the microsatellites at an intermediate spatial scale across the Knersvlakte landscape by Geneland analysis. Dots represent the locations of each of the sampling localities.

3.3.3. Regional scale

Overall 160 specimens were sequenced for the cytochrome b gene (characterized by 72 haplotypes i.e., genetically unique sequences) whereas microsatellite data were obtained for 396 *P. capensis* specimens sampled from populations across the Namaqualand and western Fynbos regions. Less than 4% of the microsatellite dataset comprised missing data. No linkage was detected between loci across the sampled distribution. Although null alleles were not detected, the *Hy-T12* locus had more than 50% of its alleles in the same size class. This precluded a binomial test for null alleles at this locus. All sampling localities, with exception of the Vredenburg sampling locality, fulfilled the requirements of Hardy Weinberg Equilibrium. In total 60 alleles were detected at the four loci (Table 3.2.). Allele diversity ranged between 1 (monomorphic) to 12 alleles.

Nucleotide diversity did not differ significantly (Table 3.1; d.) between the Namaqualand and western Fynbos regions; however, sampling localities in the western Fynbos region contained significantly fewer (Table 3.1; e.) haplotypes compared to those of Namaqualand (Table 3.2.). In the microsatellite data, significantly lower genetic diversity (Table 3.1; f.) and expected heterozygosity (Table 3.1; g.) was evident in the western Fynbos region compared to the Namaqualand region (Table 3.2.). Inbreeding was evident between colonies across the sampled distribution (average $F_{IS} = 0.098$; $P < 0.01$; Table 3.2.).

The partitioning of variance was maximized when the sampling localities were grouped into the two clades detected previously (i.e., Namaqualand and western Fynbos clades; Chapter 2). This held for both the mitochondrial DNA ($F_{ST} = 0.600$; $p < 0.001$) (21.31% of the variation among sampling localities and 18.71% within sampling localities) and microsatellites ($F_{ST} = 0.160$; $p < 0.001$) (5.68% of the variation among sampling localities and 94.32% of the variation within sampling localities). Isolation-by-distance was evident over the sampled distribution in both the mitochondrial DNA ($r = 0.434$; $p < 0.001$) and microsatellite data sets ($r = 0.208$; $p = 0.001$). Overall gene-flow levels between sampling localities were low (from <1 individual to 3 individuals per generation; Table 3.5.); these were, however, significantly higher between localities in the Namaqualand region relative to the western Fynbos region (Table 3.1; i.; j.). In addition, gene-flow estimates based on the microsatellites were significantly higher than for the mitochondrial DNA in both the Namaqualand (Table 3.1; k.) and western Fynbos (Table 3.1; l.) regions. The Geneland analysis retrieved seven genetic clusters over the landscape (see Chapter 2 discussion).

Table 3.5. Gene-flow between the 16 *P. capensis* populations sampled in the South African west coast region. Calculations are based on both mitochondrial DNA (cytochrome b) and microsatellite data. Gene-flow across three different areas, the Knersvlakte (red block) (around and across), the Cape Flats (blue block) and the Cape Peninsula (green block) are indicated. Values in red are gene-flow levels >1 individual per generation (standard error included).

Locality	Locality	mtDNA ----->	mtDNA <-----	Microsatellites ----->	Microsatellites <-----
Springbok	Garies	0.036 + 0.599 - 0.036	0.027 + 0.344 - 0.027	1.237 + 0.231 - 0.237	2.033 + 0.265 - 0.067
Garies	Brand-se-Baai	0.000 + 0.282 - 0.000	0.019 + 0.362 - 0.019	0.276 + 0.037 - 0.037	1.707 + 0.006 - 0.002
Nuwerus	Springbok	0.502 + 1.126 - 0.502	0.374 + 0.969 - 0.373	2.650 + 0.130 - 1.245	0.552 + 0.487 - 0.051
Garies	Nuwerus	0.000 + 0.683 - 0.000	0.124 + 1.935 - 0.124	0.695 + 0.032 - 0.062	1.379 + 0.003 - 0.403
Kliprand	Garies	0.276 + 0.656 - 0.267	1.046 + 1.688 - 1.007	0.424 + 0.188 - 0.028	0.766 + 0.003 - 0.001
Brand-se-Baai	Nuwerus	0.083 + 0.774 - 0.083	0.039 + 0.265 - 0.039	1.365 + 0.057 - 0.045	0.134 + 0.006 - 0.009
Nuwerus	Kliprand	0.840 + 0.699 - 0.822	0.731 + 1.183 - 0.557	0.664 + 0.002 - 0.001	1.042 + 0.003 - 0.136
Kliprand	Loeriesfontein	0.001 + 0.049 - 0.001	0.000 + 0.599 - 0.000	0.420 + 0.001 - 0.001	0.551 + 0.235 - 0.035
Loeriesfontein	Nieuwoudt-ville	0.116 + 0.683 - 0.116	0.016 + 0.187 - 0.016	1.899 + 0.066 - 0.836	1.551 + 0.107 - 0.738
Nieuwoudt-ville	Klawer	0.394 + 0.805 - 0.394	0.367 + 0.312 - 0.364	1.192 + 0.032 - 0.116	1.023 + 0.047 - 0.180
Klawer	Donkiesbaai	0.941 + 2.145 - 0.941	0.116 + 2.378 - 0.116	0.318 + 0.006 - 0.000	0.328 + 0.028 - 0.262
Donkiesbaai	Elands Bay	0.493 + 0.149 - 0.276	1.147 + 1.225 - 0.999	0.121 + 0.097 - 0.017	0.394 + 0.083 - 0.243
Loeriesfontein	Nuwerus	0.000 + 0.257 - 0.000	0.000 + 0.115 - 0.000	0.345 + 0.067 - 0.017	0.753 + 0.071 - 0.162
Nieuwoudt-ville	Nuwerus	0.131 + 1.146 - 0.131	0.113 + 0.150 - 0.113	0.631 + 0.076 - 0.037	1.052 + 0.005 - 0.717
Klawer	Nuwerus	0.001 + 0.237 - 0.001	0.026 + 0.591 - 0.026	0.872 + 0.068 - 0.017	0.975 + 0.115 - 0.166
Donkiesbaai	Nuwerus	0.002 + 0.290 - 0.002	0.002 + 0.328 - 0.002	0.475 + 0.230 - 0.001	0.456 + 0.001 - 0.001
Nieuwoudt-ville	Kliprand	0.021 + 0.485 - 0.021	0.006 + 0.068 - 0.006	0.965 + 0.193 - 0.270	0.889 + 0.036 - 0.566
Brand-se-Baai	Donkiesbaai	0.001 + 0.462 - 0.001	0.003 + 0.248 - 0.003	1.856 + 0.115 - 0.573	0.289 + 0.025 - 0.010
Klawer	Elands Bay	0.338 + 0.409 - 0.333	0.448 + 0.140 - 0.438	0.521 + 0.014 - 0.282	0.367 + 0.110 - 0.094
Vredenburg	Donkiesbaai	0.002 + 0.673 - 0.002	0.000 + 0.147 - 0.000	0.781 + 0.003 - 0.105	0.314 + 0.010 - 0.042
Elands Bay	Vredenburg	0.000 + 0.086 - 0.000	0.000 + 0.135 - 0.000	0.649 + 0.140 - 0.603	0.450 + 0.175 - 0.015
Ceres	Elands Bay	0.145 + 0.548 - 0.144	0.525 + 0.769 - 0.524	0.301 + 0.095 - 0.053	0.492 + 0.002 - 0.001
Vredenburg	Ceres	0.028 + 0.610 - 0.028	0.000 + 0.099 - 0.000	0.265 + 0.001 - 0.000	0.135 + 0.037 - 0.046
Ceres	Klawer	0.199 + 0.317 - 0.199	0.261 + 0.496 - 0.259	0.361 + 0.033 - 0.007	0.260 + 0.003 - 0.003

Table Mountain	Vredenburg	0.001 + 0.100 - 0.001	0.001 + 0.055 - 0.000	0.251 + 0.009 - 0.008	0.293 + 0.136 - 0.016
Ceres	Paardeberg	0.000 + 0.172 - 0.000	0.009 + 0.454 - 0.009	0.390 + 0.051 - 0.000	0.369 + 0.001 - 0.000
Paardeberg	Table Mountain	0.007 + 0.101 - 0.007	0.001 + 0.433 - 0.001	0.214 + 0.020 - 0.007	0.645 + 0.181 - 0.039
Boulders	Bettysbaai	0.065 + 0.688 - 0.065	0.012 + 0.475 - 0.012	0.473 + 0.042 - 0.217	0.563 + 0.007 - 0.005
Table Mountain	Boulders	0.106 + 0.031 - 0.106	0.014 + 0.606 - 0.014	0.202 + 0.018 - 0.007	0.156 + 0.062 - 0.061

3.3.3.1. Changes in population size

The Namaqualand clade showed a population expansion as evidenced by its highly negative and significant test value (Fu's F = -12.297, $p < 0.05$). No similar findings were evident in the western Fynbos clade. These results were supported by the mismatch distributions and Bayesian skyline plot analyses (Figure 3.6.). Additionally, no evidence of a population bottleneck was reflected in the history of any of the sampling localities.

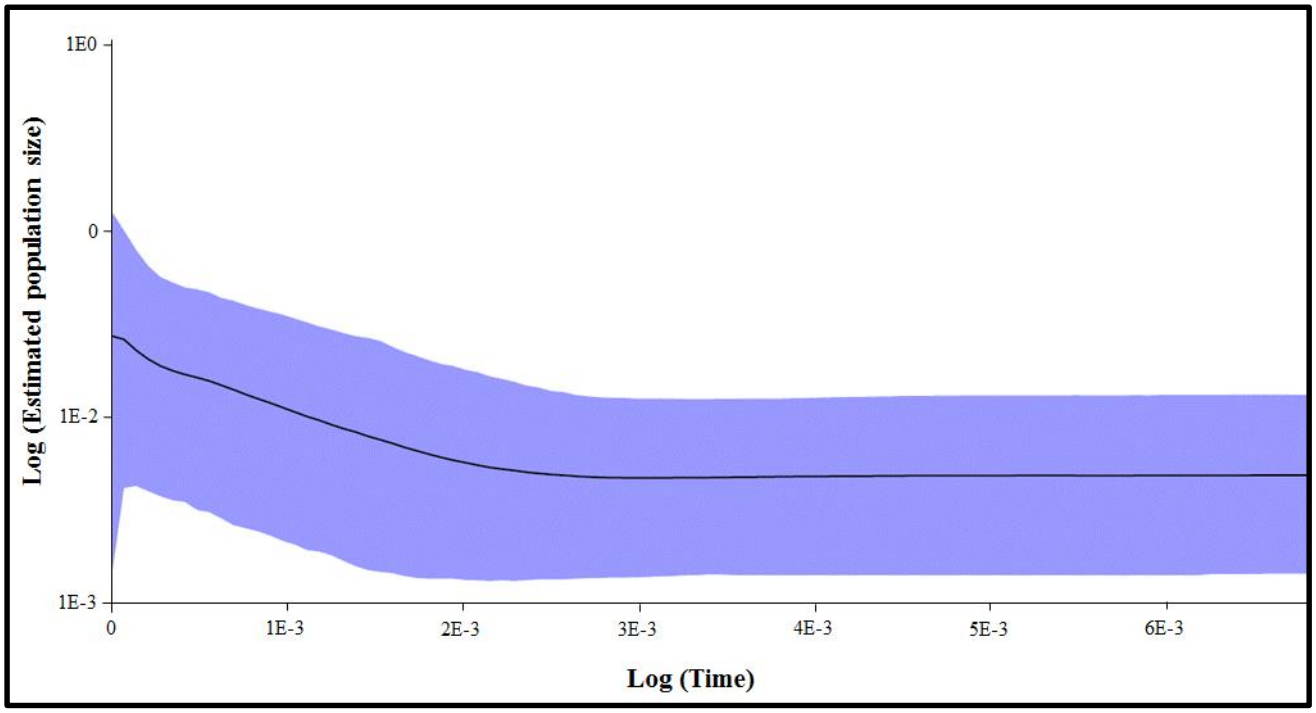

Figure 3.6. Bayesian skyline plot showing changes to population size over time in the Namaqualand clade.

3.4. Discussion

3.4.1. Genetic structure at a fine spatial scale

Significant genetic structure was evident between *P. capensis* colonies on a fine spatial scale (Table 3.3.). Data on fine-scale genetic structure within populations are comparatively scarce

(Aars and Ims, 1999; Vos *et al*. 2001; Arnaud, 2003; Holzhauer *et al*. 2006; Sander *et al*. 2006; Cabe *et al*. 2007; Pérez-Espona *et al*. 2008) and especially so for smaller mammals (but see Coltmann *et al*. 2003; Schweizer *et al*. 2007). The subjective nature of defining the spatial scale of an investigation as "fine" (these have varied from 50 metres to 14 kilometres - Coltmann *et al*. 2003; Brouat *et al*. 2003) has, to some extent, compromised direct comparisons with the results of the present study (spatial scale of 210m - 850m). Those with the closest spatial sampling regimes to that of the hyrax reported here are the Soay sheep (*Ovis aries*; > 50m; Coltmann *et al*. 2003), and the common vole (*Microtus arvalis*; 330m - 2560m; Schweizer *et al*. 2007) which yielded F_{ST} values of 0.003 - 0.010 in the case of the former and 0.013 - 0.054 for the latter. Consequently the finding in the present study of significant levels of differentiation (0.038 - 0.117; Table 3.3.) between colonies of rock hyrax is striking.

Three genetic groups (clusters) were evident over the landscape (Colonies A/B, colony C and colonies D/E; Figure 3.3.) that correspond to geographic positions of the different koppies. In line with this, gene-flow within the three Geneland groups (clusters) was higher than between them (see for instance genetic exchange between colonies A and B; Table 3.4.). Gene-flow between Colonies D and E (separated by 500 metres of open farmland) was also high, but biased. More animals are dispersing to Colony E from Colony D than *vice versa* (this was also confirmed by the assignment analysis where all of the animals from Colony E assigned to Colony D). Colony D is thus a source "population" and Colony E a sink as it consists of a large rock with only two crevices, thus representing marginal habitat. The large number of hyraxes in the area (up to 50 animals per colony were observed; personal observation) increases competition for food and shelter which results in assymetrical gene-flow from larger to smaller colonies (as reviewed by Palstra *et al*. 2007). Although significant isolation-by-distance was evident at a fine scale, the koppies are relatively near one another (5 koppies; 210m - 850m apart) and dispersal will therefore incur minimal predation costs (Turner and Watson, 1965; Fairall *et al*. 1986; Kotler *et al*. 1999; Druce *et al*. 2006).

Intragroup relatedness of the three Geneland groups was significantly higher than intergroup relatedness (Appendix F), a result biased by the inclusion of juveniles. Juvenile animals are probably mostly the offspring of a territorial male (peripheral males seldom mate; Fourie, 1983),

and sampling was done during the breeding season before natal dispersal - a factor which would have increased the intracolony relatedness. A parentage analysis would have confirmed whether juveniles are mostly fathered by a single male; however such analysis could not be performed as the relatively low genetic diversity in the microsatellites rendered the assignment of parents ambiguous. With juvenile animals excluded, no significant difference in the relatedness of adult male and female animals was observed between groups. Although we could provide no evidence of colony stability based on relatedness (kin selection), sex-biased dispersal was evident within the Vredenburg population. This would also structure the distribution of genetic variation since one sex remains largely philopatric (see e.g., Avise, 1994).

Sex-bias is most likely a result of the social structure of the rock hyrax in addition to the high hyrax population density at this sampling locality, a situation that would deter the random immigration of animals (Barocas *et al*. 2011) and in this case, specifically males. Due to social hierarchy (Chapter 1) females receive less resistance when moving between colonies at a fine spatial scale (female dispersal occurs between neighbouring colonies at <500 metres; Fourie, 1983). At higher densities, however, males would confront resistance when dispersing due to aggressive exclusion by other males (and will thus remain in their natal group area; Hoeck, 1982; Fourie, 1983). Female biased dispersal is not frequently encountered although it has been detected in pikas (*Ochotona princeps*; Smith, 1974), African wild dogs (*Lycaon pictus*; Frame and Frame, 1976), chimpanzees (*Pan troglodytes*; Sugiyama, 1973), mountain gorillas (*Gorilla gorilla*; Harcourt *et al*. 1976), the hamadryas baboon (*Papio hamadrys*; Hammond *et al*. 2006) and in the white-lined bat (*Saccoteryx bilineata*; Bradbury and Vehrencamp, 1976). In these social systems males acquire and defend resources (as reviewed by Handley and Perrin, 2007). It is not clear how long a territorial male hyrax holds tenure in a colony, however, they are long-lived (Hoeck, 1989) and probably remain in a dominant position for several generations. As a consequence, daughters may disperse at sexual maturity to prevent mating (inbreeding) with their father (Handley and Perrin, 2007). As females are not constrained by hierarchy (Fourie, 1983), they are accepted into nearby colonies and this short-distance dispersal is sufficient to prevent inbreeding and kin competition. The social system and population structure of the rock hyrax thus seem to be the main factors influencing genetic differentiation between colonies at a fine spatial scale.

Clearly, it would appear that behavioural attributes rather than landscape features shape fine-scale genetic patterns in highly mobile species (Carlsson *et al*. 1999; Coltmann *et al*. 2003; Schweizer *et al*. 2007), in contrast to geographical (Carlsson *et al*. 1999; Brouat *et al*. 2003; Lampert *et al*. 2003; Pampoulie *et al*. 2004; Cabe *et al*. 2007) and anthropogenic features (Vos *et al*. 2001; Arnaud, 2003; Holzhauer *et al*. 2006) which act as major barriers to poorly dispersing taxa. Both the Soay sheep and common vole have polygynous/promiscuous social systems similar to the rock hyrax (Coltmann *et al*. 2003; Schweizer *et al*. 2007; Chapter 3). As behavioural attributes including dispersal are closely linked to the social system of a particular species (Schweizer *et al*. 2007), it is no surprise that the behaviour of the rock hyrax significantly structures the distribution of genetic variation at a fine spatial scale between colonies only a few hundred metres apart. Usually a social system such as this leads to sex-biased dispersal (Greenwood, 1980; Dobson, 1982; Handley and Perrin, 2007).

3.4.2. Genetic structure at intermediate and regional spatial scales

Given genetic structure at a scale of a few hundred metres (previous section), it is no surprise that a structured genetic pattern was evident between rock hyrax colonies at larger (intermediate and regional) spatial scales. At an intermediate spatial scale (within and between the Hottentots Holland Mountains and Cape Peninsula) each sampling locality formed a distinct genetic group in the clustering analyses (Figure 3.4.). Similarly, significant genetic differentiation was detected across the Knersvlakte in both the data sets, although it was more pronounced on the southern side of this region (Figure 3.5.). In summary, landscape connectivity appears to have a significant role in influencing genetic patterns and this forms the substance of the following section.

3.4.3. Landscape connectivity

Regionally, gene-flow in Namaqualand was significantly higher compared to the western Fynbos region in both the data sets reflecting differences in the connectivity of these areas. The western Fynbos region has quantitatively fewer suitable habitat patches (rocky outcrops) between

sampled areas compared to Namaqualand which comprises a matrix of abundant rocky outcrops connected to mountainous areas (Kamiesberg Mountains, Warm Bokkeveld Mountains and Cederberg Mountains) (Figure 3.2.). In addition, the granitic mountains and koppies of the Namaqualand region are characterized by suitable crevices, whereas the sandstone Cape Fold Mountains offers little in the way of similar suitable habitat (Figure 3.2.). As a result, movement of hyrax is facilitated in the aggregated Namaqualand landscape, a situation not found in the fragmented western Fynbos region. Consequently, rock hyrax occur in "terrestrial islands" that are more pronounced in the western Fynbos region. In this system, populations are relatively isolated from one another (impaired genetic exchange), hence within-population variability is diminished (due to inbreeding under breeding isolation) and between-population variability exaggerated. This has been demonstrated for various taxa (Selander, 1970; Avise *et al*. 1975; Ashley and Wills, 1987; Gerlach and Hoeck, 2001).

Given that habitat quantity (connectivity) and quality affect dispersal (Coulon *et al*. 2004; Neville *et al*. 2006; Pérez-Espona *et al*. 2008; Spear *et al*. 2005; Smit *et al*. 2010; Spear *et al*. 2010), open areas devoid of suitable habitat such as the Knersvlakte and Cape Flats are expected to impair gene-flow. Although the Cape Flats does not constitute a barrier to gene-flow in *P. capensis* (Chapter 2), the pronounced genetic differentiation across the surrounding Hottentots Holland Mountains and Cape Peninsula regions is puzzling. The low connectivity within and between (across the Cape Flats) the Hottentots Holland Mountains and Cape Peninsula must therefore impede gene-flow between hyrax colonies, resulting in breeding isolation.

Genetic exchange across and around the Knersvlakte was more complex. Some gene-flow (>1 individual per generation) was evident from the analysis of populations around and across this region, although genetic exchange around the Knersvlakte was significantly higher than across it (in the mitochondrial DNA but not the microsatellites). Dispersal around the Knersvlakte may occur around the margins of this region which are mountainous (Kamiesberg- and Warm Bokkeveld Mountains) and have abundant suitable habitat. Dispersal across the Knersvlakte is, however, less obvious since this large arid plain (40 - 100 kilometres in breadth) contains no suitable habitat. Although Hoeck (1989) postulated that hyrax dispersal to koppies 15 kilometres apart was unlikely, rock hyraxes have been observed to cross large distances. For example, in the

Steytlerville area a hyrax was found to occupy a springhare burrow >40 kilometres from the nearest rocky habitat suggesting that they are able to utilize a large variety of cover types (Kingdon, 1971; Olds and Shoshani, 1982; Rubsamen *et al*. 1982).

The presence of a genetic break at the Knersvlakte suggested by the mitochondrial DNA indicates that while it serves as a semipermeable barrier to gene-flow, the levels of gene-flow are insufficient to reverse the genetic differentiation in the mitochondrial DNA over time. Indeed, differentiation between subpopulations been reported even if the gene-flow is >1 individual per generation (Jost, 2008). Given that the microsatellites show evidence of gene-flow (reflecting both male and female dispersal) but mitochondrial DNA does not, this would suggest that females are less inclined to cross this barrier (Knersvlakte) compared to males. Female site phylopatry (although not complete site fidelity) also influences genetic structure in highly mobile species such as the green turtle (*Chelonia mydas*) in the Indian-Pacific and Atlantic-Mediterranean Oceans (Meylan *et al*. 1990; Bowen *et al*. 1992), Atlantic salmon (*Salmo salar*) from North America and Europe (Bermingham *et al*. 1991; Davidson *et al*. 1989), seaside sparrow (*Ammodramus maritimus*), and the Canada goose (*Branta Canadensis*; Avise and Nelson, 1989; Van Wagner and Baker, 1990; Zink, 1991).

Males thus mediate the spread of genetic diversity (nuclear DNA) at larger (intermediate and regional) spatial scales (also see Chapter 2). We thus propose the following scenario: Male rock hyraxes that disperse across relatively large distances probably establish as peripheral males near colonies where they constantly try to replace the territorial male (Fourie, 1983). After the territorial male dies or when its condition deteriorates to such an extent that it is unable to defend its territory, it is replaced by the peripheral male (also see Fourie, 1983; Hoeck, 1989). In addition, inbreeding is evident in rock hyrax populations, especially in isolated areas (also see Gerlach and Hoeck, 2001). Consequently dispersal will be a major driving force to prevent inbreeding depression. In terms of mate choice, the findings of the present study are in agreement with data on other species (Lehmann and Perrin, 2003) since the rock hyrax displays inbreeding above the threshold value and consequently the dispersal of males is favoured. Sexual selection is clearly an influencing factor in driving sex-biased dispersal in the rock hyrax.

3.4.4. Genetic diversity

The most genetically diverse hyrax colonies were found in the Namaqualand region - an observation underscored by both the data sets. Genetic diversity in the western Fynbos region was, by comparison, lower. Importantly, we could provide no statistical evidence of past bottlenecks at any of the sampling localities (and in this respect the results of the present study mirror those of Gerlach and Hoeck, 2001 for hyrax populations in the Serengeti); all colonies were at equilibrium (i.e., not expanding or contracting).

The amount of genetic diversity within populations (under a constant population size) thus appears to be linked to the connectivity of the surrounding matrix. Consequently, in fragmented areas (such as the western Fynbos region), few animals successfully migrate and this may result in limited genetic diversity being represented in the founding population. Moreover, little new genetic diversity is accumulated over time since gene-flow with surrounding areas is low; populations consequently persist under conditions of breeding isolation. In the Namaqualand area, on the other hand, the aggregated landscape facilitates greater dispersal success and hence higher levels of gene-flow that in turn results in higher genetic diversity.

These findings show a link between landscape connectivity and genetic diversity in the western Fynbos region where sampling included geographically isolated localities as well as localities connected to mountain ranges. Genetic patterns suggesting small founder populations (although we could provide no empirical evidence of founder effects) were detected in the poorly connected localities of Vredenburg, the Cape Peninsula and Hottentots Holland Mountains (Paardeberg). Even though animals were sampled from multiple colonies at each locality, low genetic diversity was evident in both the mitochondrial DNA (Vredenburg, Table Mountain and Paardeberg) and microsatellites (Boulders) indicating the possibility of small founding populations of females or males respectively. The deviation from Hardy-Weinberg in the Vredenburg population is most likely because of inbreeding (Table 3.2.). Although the overall sample size for this locality is large, the population itself is isolated given its location. Cluster analysis confirms this with the Vredenburg population grouping as a distinct cluster (Figure 2.5.) - a consequence of lack of gene flow into this site (Table 3.5.). Colonies in the above-mentioned

areas thus show breeding isolation. In contrast, localities connected to mountain ranges, for example Bettysbaai (connected to the Kogelberg and Rooi-Els) and Ceres (connected to the Witzenberg), displayed relatively higher genetic diversity resulting from exchange with colonies from surrounding areas.

Finally, unlike other regions, the hyrax populations from the Namaqualand region were found to be expanding (Figure 3.6.) reflecting the high connectivity and an abundance of intermediate "bridging" habitat in this region. These conditions and the high population densities have facilitated the spread of genetic diversity across the Namaqualand landscape.

CHAPTER 4

Conclusions and future prospects

Several factors influence genetic structure within species including mobility, behavioural attributes such as habitat choice, barriers to dispersal, gender-biased dispersal as well as historical demographic events that remove populations from equilibrium (Avise, 1994). This thesis employed a multi-disciplinary approach and showed that the genetic structure of rock hyrax populations across the Namaqualand/western Fynbos regions is influenced by the behaviour and social structure of the species, the connectivity of its habitat, its dispersal capability and the presence of long-term barriers to gene-flow. Here I discuss how this complex system influences genetic patterns across the landscape and relate the findings to patterns found in other taxa.

4.1. This study

4.1.1. Quantifying the influence of landscape variables and configuration on genetic variation

Several studies have compared genetic structure between aggregated and fragmented areas (Pither and Taylor, 1998; Keyghobadi *et al*. 2005; Broquet *et al*. 2006; Baguette and Van Dyck, 2007). These all demonstrate that the propensity for dispersal decreases with increasing fragmentation. A similar result was obtained in the rock hyrax, a species which largely depends on habitat connectivity for dispersal (Chapter 3). By comparing aggregated and fragmented landscapes it was shown that aggregated (high connectivity) areas facilitate gene-flow among populations, whereas fragmented (low connectivity) areas result in reproductive isolation of populations (Chapter 3; Gerlach and Hoeck, 2001).

In addition to habitat quantity, its quality also impacts on hyrax genetic structure. Rock hyrax utilize crevices within rocks (Sale, 1966; Hoeck, 1975; Olds and Shoshani, 1982; Hoeck, 1989). Sandstone (which is dominant in the western Fynbos region) contains few suitable crevices when compared to the granite extrusions that are common in the Namaqualand region (Chapter 3).

Habitat quality is thus an important contributing factor to the lower levels of gene-flow and to genetic diversity detected among hyrax populations in the western Fynbos region.

In summary therefore, the present study focuses on two areas frequently investigated in landscape genetics, (i) quantifying genetic diversity and (ii) identifying migrants in relation to landscape condition (as reviewed by Storfer *et al*. 2010). It is clear that higher habitat quality and quantity promotes population stability and results in higher genetic diversity and gene-flow in natural systems (Pérez-Espona *et al*. 2008; Keyghobadi *et al*. 2005). Surprisingly this is a relationship that remains poorly explored in the field of landscape genetics.

4.1.2. Testing species-specific ecological hypotheses

The landscape matrix in conjunction with an individual's acuity (an awareness of surrounding areas) may influence dispersal behaviour of species (Spear *et al*. 2005). For instance, fragmented landscapes may negatively affect dispersal as animals are unable to identify nearby habitat patches and are thus reluctant to disperse. Consequently to fully appreciate these factors' influence on gene-flow, and ultimately their role in evolution, a thorough understanding of the autecological characteristics of a species is necessary.

It is possible that the dispersal behaviour of rock hyrax is influenced by its perception of the surrounding landscape. The Namaqualand area (aggregated) comprises numerous close-lying granite koppies that fall well within the perceptual range of this small mammal. In addition, the ground-cover comprises "open" vegetation (Namaqualand Hardeveld; Mucina and Rutherford, 2006) which does not hinder perception of nearby habitat patches. In sharp contrast, the western Fynbos region has few suitable habitat patches and these are surrounded by thick stands of Fynbos (Sandstone Fynbos; Mucina and Rutherford, 2006). This would cause suitable habitat patches to fall outside the perceptual range of resident rock hyraxes and increase the reluctance of animals to disperse. The Knersvlakte Bioregion provides additional support for the perception-hypothesis in the rock hyrax. This sparsely vegetated, flat-plain is apparently crossed readily by males (Chapters 2 and 3), even though it comprises a relatively large stretch of unsuitable habitat (~ 40 kilometres). The adjacent mountainous margins are clearly visible across

the Knersvlakte (at least to the human eye) and it seems reasonable to speculate that male hyrax are aware of these areas when dispersing. In effect, the rock hyrax social system promotes the long distance dispersal of males, while perceptual range determines the distance of dispersal events.

4.1.3 Identifying barriers to gene flow

Although the Knersvlakte, a long-term barrier to gene flow, has caused historic isolation (due to low habitat connectivity) in *P. capensis*, this was only evident for the matrilineal genetic line (Chapter 2), a phenomenon attributable to sex-biased dispersal (Chapters 2 and 3). Regions of low connectivity act as barriers to dispersal of females, more so than they do in males (Chapter 3). Consequently, it may be anticipated that large regions of low connectivity act as significant barriers to gene-flow in female rock hyrax.

Given that suitable rocky habitat is often sparsely distributed across the landscape, it is no surprise that a structured genetic pattern between habitat patches has been demonstrated in rock hyrax (Chapter 2) and other saxicolous taxa (King, 1987; Wyatt *et al*. 1992; Lovich, 2001). Areas devoid of rocky habitat act to limit dispersal between colonies occupying these habitat islands (Kim *et al*. 1998; Gerlach and Hoeck, 2001; Lovich, 2001). Various factors have been proposed to explain the formation of phylogeographic breaks across certain geographic barriers (Chapter 2; Arnaud, 2003; Berthier *et al*. 2004; Pérez-Espona *et al*. 2008), although few studies have focussed on addressing the influence of the landscape matrix (connectivity) on spatial genetic variation. This is surprising given that the identification of barriers to gene-flow has been crucial to the development of landscape genetics (Keyghobadi *et al*. 2005; Vos *et al*. 2001). Landscape connectivity is inextricably tied to geographic barriers since reduced connectivity over protracted periods will impact on the differentiation of populations straddling poorly connected areas thus contributing to evolutionary processes (Lovich, 2001; Keyghobadi *et al*. 2005).

4.1.4. Understanding the spatial and temporal scale of an ecological process

In addition to comparing dispersal patterns between broad geographic regions, a stratified approach to determine connectivity at various spatial scales was also undertaken. Dispersal capability in conjunction with environmental patchiness (as perceived by the organism) is proposed to influence gene-flow and population genetic structure at differing spatial scales (Avise, 1994; Keyghobadi *et al*. 2005; Murphy *et al*. 2010; Storfer *et al*. 2010), however, few studies have applied this reasoning (see Keyghobadi *et al*. 2005 as an exception). Using this approach it was shown that landscape connectivity influenced the distribution of genetic variation in the rock hyrax at larger (intermediate and regional) spatial scales (Chapter 3), while at a fine spatial scale behavioural attributes come into play. In addition, a switch in sex-biased dispersal (from a fine to a regional scale) was detected - a pattern that would have been overlooked if only one spatial scale was studied.

4.1.5. Identifying source-sink dynamics and movement corridors

Source-sink dynamics and movement corridors determine the distribution of genetic variation in the rock hyrax (Chapter 3; Gerlach and Hoeck, 2001). Although the study did not provide empirical evidence of population bottlenecks, connectivity appears to be the major factor influencing the genetic diversity of contemporary hyrax populations (Chapter 3). Populations in the higher connectivity, Namaqualand landscape displayed increased levels of genetic diversity compared to their western Fynbos counterparts - a testament to the higher gene-flow in this region and the consequent buffering against loss of genetic diversity in these populations. In addition, mountain ranges act as movement corridors between rock hyrax populations (Chapter 2; Prinsloo and Robinson, 1992) thereby facilitating gene-flow to smaller populations neighbouring such areas (Chapter 3; Gerlach and Hoeck, 2001). It therefore seems likely that areas of higher connectivity and mountainous corridors influences the persistence of small satellite populations.

4.2. Conservation implications

Curbing genetic diversity loss is an important consideration in determining conservation strategies. Consequently studies that focus on habitat and population fragmentation are crucial to conservation and management decisions (Moritz *et al*. 2000; Storfer *et al*. 2010). The effect of habitat fragmentation, albeit natural, is important in shaping the distribution of genetic variation in the rock hyrax (Chapter 3). Populations in poorly connected areas show reduced genetic diversity and impaired genetic exchange. This poses a problem to current conservation initiatives as three of the sampling localities (Bettiesbay, Table Mountain National Park and Boulders) are in conservation areas. Fluctuations in the number of animals in isolated colonies will likely occur over time and cause a loss in the genetic diversity and homogenize genetic profiles across a landscape. In a conservation context this means that animal numbers should be augmented in at least one of the conservation areas (Boulders which has maximally 20 animals) through a breeding programme, or by translocation of genetically closely related animals (e.g., from the Cape Peninsula National Park or Table Mountain National Park).

Conservation planning should take cognizance of the lower landscape connectivity of certain areas under their management (Tankwa Karoo National Park, West Coast National Park, Table Mountain National Park, Silvermine National Park; Cape Peninsula National Park, Boulders and the Betties Bay Penguin colonies and the Kogelberg Nature Reserve) and consider strategies that would establish corridors (as with the Greater Cederberg Biodiversity Corridor; Low *et al*. 2004) to allow migration and gene-flow among them.

4.3. Limitations and future prospects

The present study used the latest genetic software to analyse datasets of an adequate size. Additionally, a well-structured, stratified sampling scheme was adopted. Some limitations, however, might have compromised the results, especially the number of genetic markers used and their variability. The conservative nature of the cytochrome b region (Chapters 2 and 3) impacted the detailed analysis of geographic patterning among populations. Although a more variable region such as the mitochondrial control region may have improved phylogeographic

signal, this may have compromised its iclusion with data from Prinsloo and Robinson (1992) and Prinsloo (1993) in an extensive taxonomic revision of *Procavia*. Additionally, the limited number of microsatellite markers available for procaviids precluded important information such as parentage; a greater number of variable markers would bolster estimations of gene-flow and genetic clustering.

From this study it is evident that the rock hyrax is an appropriate model to investigate the effects of landscape connectivity on a rock-dwelling vertebrate. Future studies should therefore aim to develop and employ a higher number of more variable markers such as microsatellites and SNPs. With the increasing availability, efficiency and increasingly lower costs of whole-genome sequencing and improved annotation technology and SNP recovery (G10KCOS, 2009; Ng and Kirkness, 2010), the volume of genetic information can be expected to increase exponentially in the following decade. The development of more powerful Bayesian coalescent-based programmes (Rosenberg and Nordberg, 2002; Beaumont and Rannala, 2004) coupled with new landscape genetic approaches that quantify landscape variables and/or rely on a GIS (Geographic Information Systems) basis will further this field and promote our understanding of species' distributions and the landscape's impact on genetic patterns. Furthermore, given the anticipated availability of increasingly large genetic data sets, the use of more holistic approaches that include factors such as the social system, mating patterns, biology, ecology and dispersal behaviour of taxa will be invaluable in unravelling intergeneric and intrageneric relationships and identifying regions of conservation importance.

In addition, these approaches will likely sharpen our understanding of what constitutes a "species". Currently, the various species concepts (as reviewed by De Queiroz, 2007) are are hotly debated the outcome of which will impact taxonomy and the way in which we classify animals. The Hyracoidea are unlikely to escape this since it is evident that a taxanomic revision of the group and especially Procavia and Heterohyrax is long overdue (Prinsloo 1993). New multi-faceted concepts of a "species" may be developed and fruitfully tested in *P. capensis*.

4.4. Conclusion

This study deals with the complex interplay of an organism's ecology, social system, behaviour, dispersal capability and the distribution and quality of the habitat it occupies. This interaction influences the distribution of genetic variation across the landscape. Behaviour data in conjunction with genetics is central to understanding how an animal's social system may influence dispersal patterns. This is especially so in social species where dispersal and gene-flow between the sexes may be reflected in differences between nuclear- and mitochondrial DNA patterns. Dispersal behaviour may, however, also depend on the perception by an animal of the surrounding landscape, and the distribution of suitable habitat within this matrix. Landscape connectivity may significantly impede or facilitate gene-flow at various spatial scales - a result exemplified by this study. Areas of low connectivity may act as barriers to gene-flow resulting in vicariance (especially pronounced in the Knersvlakte region in South Africa), although this has rarely been invoked to explain genetic patterns. In addition, this is one of few comparative phylogeographic studies that rely on taxa with similar ecological requirements. This allows a more accurate elucidation of historic events influencing a particular set of taxa. Finally, the investigation demonstrates the importance of using a well-structured sampling scheme, the inclusion of both mitochondrial and nuclear markers and the application of appropriate, powerful statistical programmes to infer genetic patterns. This shows that landscape genetics may be useful in a conservation context and should be taken into account when planning conservation initiatives and possible corridors.

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APPENDICES

Appendix A

Information on the microsatellite markers used in this study. The table includes the locus name, size of the marker, composition of the repeat making up the microsatellite and the reference from where the marker was sourced.

Locus	Size	Repeat	Reference
Hy-T12	147	$(TTA)_6$	Gerlach et al. 2000
Hy-T17	110	$(CAA)_5$	Gerlach et al. 2000
Hy-D49	242	$(AC)_{12}$	Gerlach et al. 2000
HCA18	140	$(ACT)_6$	P. Bloomer, personal communication

Appendix B

Allele frequencies per population for the four microsatellite loci used in this study.

Locus	Allele	Springbok	Garies	Brand-se-Baai	Nuwerus	Kliprand	Loeriesfontein	Nieuwoudt-ville	Klawer	Donkiesbaai	Elandsbaai	Vredenburg	Ceres	Paardeberg	Tafelberg	Boulders	Bettiesbaai
Hy-T12	137	0.214	0.074	0	0.058	0	0.273	0	0	0.071	0	0	0	0	0	0	0.280
	143	0	0.074	0.155	0.038	0.125	0.227	0.143	0.567	0.333	0.200	0.454	0.409	0.417	0.159	0	0.520
	147	0.786	0.833	0.810	0.865	0.775	0.5	0.857	0.433	0.595	0.800	0.546	0.591	0.583	0.841	1.000	0.200
	154	0	0.019	0.034	0.019	0.1	0	0	0	0	0	0	0	0	0	0	0
	164	0	0	0	0.019	0	0	0	0	0	0	0	0	0	0	0	0
Hy-T17	100	0.048	0	0	0	0.024	0	0	0	0	0	0	0	0	0	0	0
	102	0.333	0.233	0.345	0.231	0.333	0.136	0.286	0.100	0.150	0	0	0.091	0	0	0	0
	104	0	0	0.017	0.019	0.024	0	0	0	0	0	0	0	0	0.136	0.542	0.100
	105	0.310	0.350	0.103	0.365	0.238	0.182	0.262	0.167	0.125	0.240	0.396	0.386	0.167	0.477	0.250	0.260
	107	0	0.067	0.017	0.096	0.071	0.409	0.143	0	0.075	0	0	0.045	0.111	0	0	0.060
	108	0.190	0.200	0.207	0.192	0.190	0	0.071	0.067	0.250	0.640	0.013	0.227	0.278	0.136	0	0
	109	0	0	0	0	0	0	0	0	0	0	0.468	0.068	0	0.114	0	0.240
	110	0.119	0.133	0.224	0.096	0.119	0.182	0.143	0.633	0.375	0.120	0.123	0.182	0.444	0.136	0.208	0.340
	111	0	0.017	0.086	0	0	0.045	0.095	0	0.025	0	0	0	0	0	0	0
	112	0	0	0	0	0	0.045	0	0	0	0	0	0	0	0	0	0
	113	0	0	0	0	0	0	0	0.033	0	0	0	0	0	0	0	0
Hy-D49	227	0	0	0	0.023	0.025	0	0	0	0	0	0	0	0	0	0	0
	228	0.048	0.038	0	0.068	0	0	0.119	0.133	0	0.022	0	0.025	0	0	0	0
	229	0	0	0	0	0	0	0	0	0	0	0	0.025	0	0	0	0
	230	0	0	0.096	0	0	0.045	0.071	0	0.194	0.326	0.24	0.175	0.056	0	0	0
	231	0	0	0.058	0	0	0	0	0	0	0	0	0	0	0	0	0
	232	0	0	0	0	0	0	0	0	0	0	0	0	0	0.341	0	0
	233	0	0	0	0	0	0	0	0.033	0.028	0	0	0	0	0	0	0
	234	0.119	0	0.250	0.250	0.050	0.227	0.048	0.300	0.028	0.043	0	0.150	0	0	0	0.063
	235	0	0	0	0	0	0	0	0	0.028	0	0	0	0	0	0	0

	236	0.024	0.308	0.038	0.023	0.025	0	0	0	0.028	0	0.329	0	0	0	0	0
	237	0.095	0.135	0	0.045	0.200	0.182	0.024	0.067	0.083	0.043	0.164	0.050	0	0	0	0.021
	238	0.190	0.058	0.212	0.318	0.075	0.227	0.19	0.167	0.194	0.174	0.014	0.050	0.028	0	0	0
	239	0	0.019	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	240	0.262	0.192	0.250	0.159	0.275	0.091	0.262	0.100	0.167	0.152	0	0.300	0.028	0	0	0.313
	242	0	0.038	0.038	0	0.050	0	0	0.100	0.139	0.217	0	0.100	0.556	0.341	0.208	0.208
	243	0	0	0.019	0.023	0	0.182	0	0	0	0	0	0	0	0	0	0
	244	0.095	0.077	0.019	0.023	0.250	0.045	0.143	0.033	0.111	0.022	0	0	0.028	0.023	0	0
	245	0	0	0	0	0	0	0	0	0	0	0	0.050	0	0.045	0	0
	246	0	0.019	0	0	0	0	0.024	0.033	0	0	0.164	0	0	0	0	0.021
	247	0	0.019	0	0	0	0	0.048	0.033	0	0	0.089	0.050	0.306	0.250	0.792	0.375
	248	0	0	0	0.023	0	0	0	0	0	0	0	0	0	0	0	0
	249	0.048	0	0.019	0.045	0	0	0	0	0	0	0	0	0	0	0	0
	251	0	0.058	0	0	0	0	0	0	0	0	0	0.025	0	0	0	0
	253	0.024	0.038	0	0	0.025	0	0.071	0	0	0	0	0	0	0	0	0
	255	0	0	0	0	0.025	0	0	0	0	0	0	0	0	0	0	0
	257	0.095	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HCA18	131	0	0	0	0	0	0	0	0	0	0	0	0	0.194	0	0	0
	132	0	0	0	0.019	0	0	0	0	0	0	0	0	0	0	0	0
	133	0.024	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	136	0.119	0.241	0.036	0.058	0	0	0.143	0.214	0.029	0	0	0	0	0	0	0
	137	0	0	0	0	0	0	0	0	0.235	0	0	0	0	0	0	0
	138	0	0.034	0	0.077	0.029	0	0.048	0	0.029	0	0	0	0	0	0	0.260
	139	0.048	0.207	0.196	0	0.265	0.318	0.119	0.036	0.029	0	0	0.125	0	0	0	0.020
	140	0.024	0	0	0	0	0.091	0	0	0	0	0	0	0	0	0	0
	141	0	0	0	0.250	0.147	0	0	0	0.118	0.071	0.171	0.300	0	0.432	0	0.120
	142	0	0.103	0	0.135	0.029	0	0.048	0.286	0.235	0.143	0.143	0.100	0.639	0.295	1.000	0.600
	143	0.143	0.034	0.036	0.038	0.059	0	0	0.036	0.029	0.048	0	0.250	0	0.136	0	0
	145	0.048	0	0.214	0.154	0.147	0.182	0.048	0.036	0.118	0.048	0	0.175	0	0.136	0	0
	147	0.119	0.207	0.304	0.135	0.324	0.182	0.429	0.071	0.147	0.643	0.093	0.050	0.028	0	0	0
	149	0.119	0.103	0.214	0.135	0	0.227	0.119	0.143	0.029	0.048	0.593	0	0.139	0	0	0

151	0.143	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
152	0	0.017	0	0	0	0	0	0	0	0	0	0	0	0	0	0
153	0.119	0.052	0	0	0	0	0	0.036	0	0	0	0	0	0	0	0
155	0.095	0	0	0	0	0	0.048	0.143	0	0	0	0	0	0	0	0

Appendix C

Outcomes of the different genetic studies conducted on diverse organisms that show the Knersvlakte and Cape Flats shaped genetic profiles of extant taxa. The reference to each study, the species, taxonomic class, genetic markers used, the sequence divergence between clades, divergence time estimates between clades, phylogeographic patterns found, and the proposed factors thought to have shaped the phylogeographic structure are presented.

Reference	Species	Class	Markers	FST	Sequence Divergence (%)	Divergence Time (Mya)	Phylogeographic Pattern	Phylogeographic interpretation
Prinsloo and Robinson, 1992	*Procavia capensis*	Mammalia	10 RFLPs (mtDNA)	-	4.2 ± 0.9	2	Two major clades (species) were found across South Africa. Gain or loss of restriction sites was unique to each population and low levels of genetic divergence were evident. Same haplotypes were shared south and north of the Knersvlakte. De Hoop was the most genetically divergent population.	Clades were attributed to dispersal along two different routes. Low levels of divergence due to recent colonizations from the Karoo (due to migration out of this area following a population increase).
Knersvlakte								
Present study	*Procavia capensis*	Mammalia	Cytochrome b (mtDNA); 4 Microsatellites (nDNA)	0.72 (mtDNA); 0.16 (Microsatellites)	1.92	8.87	Significant structure was evident in both mtDNA and microsatellites over Knersvlakte (and whole sampling range) with two major matrilineal clades (Namaqualand and western Fynbos) evident on either side of this region. Same clades not evident in the microsatellite data (seven nuclear clusters).Significantly higher levels of gene-flow between sampling localities in Namaqualand compared to western Fynbos region. Consequently, colonies in Namaqualand were genetically more diverse compared to those of the western Fynbos region.	Low habitat connectivity coupled with male-biased dispersal and female site phylopatry (intermediate- and regional spatial scales) across the Knersvlakte caused vicariance event in matriline. Microsatellite groups were influenced by landscape connectivity. Higher gene-flow and genetic diversity in Namaqualand region may also be attributed to the connectivity of the landscape as the Namaqualand region has more suitable habitat patches between sampled populations, thus facilitating gene-flow and preventing bottlenecks.

Matthee and Robinson, 1996	*Pronolagus rupestris*	Mammalia	14 Allozymes (mtDNA)	0.246	7.94 ± 1.40	-	Two clades (south-eastern and north western) were evident across South Africa. South-eastern clade (relatively high sequence divergences and significant sub-structure.) is bound to the Great Escarpment region. North-western clade (contains two sub-clades) is not similarly constrained. North-western clade populations are not as distantly related, even though they are geographically far apart.	A difference in elevation created refugia during Pleistocene glacial cycles. Isolation of populations for long periods with limited gene-flow has caused high sequence divergences in south-eastern clade while subclades of north-western clade are due to vicariance. Populations that are not distantly related were influenced by recent dispersal.
Smit *et al*. 2007	*Elephantulus edwardii*	Mammalia	Cytochrome b (mtDNA); Control Region (mtDNA)	0.834	1.97	1.4 - 2.0	Three clades (Karoo, Namaqua, central Fynbos) were found over the distribution of which the central Fynbos clade could be subdivided into four sub-clades. Sympatric haplotypes were found and explained at the hand of ancestral polymorphism or secondary contact after differentiation and range expansion. There was an absence of gene-flow with no shared haplotypes between groups, however haplotypes were shared within groups.	Divergence of Namaqua and central Fynbos clades caused by Pliocene/Pleistocene glacial cycles that resulted in topographic and climatic changes which were repeatedly enforced through multiple events. A series of marine transgressions (2 Mya) inundated the western coastal plains in conjunction with changing flow patterns of rivers and harsh climate change on the western side of the continent. Additionally, the Namaqualand habitat is not homogenous as rocky outcrops are terrestrial islands. The central Fynbos clade lies in the safety of the CFM and the four subclades here resemble climatic differences and vegetation differences related to the position of mountains. This resulted in restricted gene-flow and IBD.
Matthee and Flemming, 2002	*Agama atra*	Reptilia	16S rRNA (mtDNA); Cytochrome b (mtDNA)	-	9.45	2.2 - 4.4	Three distinct clades (Southern Namibia, north-central S.A., south-eastern S.A.) were evident. On a fine scale, isolated habitat islands were genetically distinct from other populations in a clade.	Historic isolation (vicariance) between clades were caused by Pleiocene glacial cycles and subsequent climatic changes resulting in isolation in refugia. The distribution of mountains and koppies are also important as isolated populations were more genetic structured. Limited dispersal, long term isolation, and barriers to gene-flow (Knersvlakte, Orange River and Kalahari sand-flows) thus influenced genetic structure.

Daniels *et al*. 2010	*Homopus signatus*	Reptilia	Cytochrome b (mtDNA); ND4 (mtDNA); Prolactin (nDNA)	0.571	0.62 ± 0.13 (cyt b); 1.20 ± 0.32 (ND4)	1.0 - 2.6	Two clades (North-western and south-eastern) were evident which contain 3 distinct yet linked clades. Five groupings in haplotype network and phylogenetic tree (1.) North western Kamiesberg mountains and koppies including Loeriesfontein, 2.) koppies of the south western coast towards the Bokkeveld Mountains at Nieuwoudt-ville, 3.) south-east in the Cederberg, 4.) Hantam Mountains, 5.) Pofadder). High genetic divergence wasa evident between haplotypes of southern distribution. Geographic clustering largely characterized the haplotype network, although sympatric haplotypes were found.	North-western and south-eastern clades diverged across the Knersvlakte. The Cederberg was a historic refugium during Pliocene glacial cycles. High genetic divergence in south influenced by the heterogenous landscape where populations occur on mountains and are separated by valleys that restrict gene-flow.
Lamb and Bauer, 2000	*Pachydactylus rugosus*	Reptilia	16S rRNA (mtDNA)	-	22.95 ± 3.85	-	Two species, *P. barnardi* and *P. formosus* separated near Knersvlakte.	
Cape Flats								
Present study	*Procavia capensis*	Mammalia	Cytochrome b (mtDNA); Microsatellites (4)	0.79 (mtDNA); 0.15 (Microsatellites)	-	-	In the Hottentots Holland Mountains and Cape Peninsula, gene-flow was equally restricted (in both data sets) within these mountainous areas compared to localities that traversed the Cape Flats.	The fragmented landscape resulted in breeding isolation of populations.
Swart *et al*. 2009	*Agama atra*	Reptilia	Control Region (mtDNA); ND2 (mtDNA)	0.865	4.47 ± 0.94 (Control Region); 5.34 ± 0.88 (ND2)	0.64 - 2.36 (northern CFR - Limietberge); 0.64 - 1.67 (Cape Peninsula - Central CFR)	Four genetic provinces (Cape Peninsula, northern CFR, Limietberg, central CFR) were evident with no shared haplotypes in the Cape Flats; three of these provinces (northern CFR, Cape Peninsula and Limietberg) contact in the western CFR. Central CFR contained three subclades of which only one was well-supported. Shared haplotypes were found over long distances in two of the sub-clades.	There are no extant geographic barriers between the clades, but vicariance due to Pleistocene glacial cycles and resulting climate fluctuations is responsible for isolation; these resulted in fragmentation of mountain habitat. The CFR was fragmented into isolated dry areas (which *A. atra* prefer) during the Pleistocene. The Cape Flats also acted as a barrier as aridification reduced plant cover. This led to colonization of the Cape Peninsula whereafter Pleistocene marine interglacial transgressions inundated the Cape Flats and plant cover increased during mesic periods (thus isolating the Cape Peninsula).

Gouws *et al*. 2004	*Mesaphisopus capensis*	Insecta	12 Allozymes (nDNA); 12S rRNA (mtDNA)	0.673 (Allozymes)	17.67 ± 2.03 (12S rRNA)	14 (Allozymes); 6.8 - 8.0 (12S rRNA)	Two clades (Cape Peninsula and Hottentots Holland Mountains) were evident which could be sub-divided into five sub-clades (Table Mountain–Southern Peninsula, Silvermine, Franschhoek, Jonkershoek and Gordon's Bay). Populations were identical in Table Mountain but there was significant sequence divergence between Table Mountain and the Southern Peninsula.	Pleistocene glacial cycles caused climatic oscillations and sea-level fluctuations. Marine transgressions thus inundated the Cape Flats and gaps in the Cape Peninsula causing barriers to gene-flow. Climatic oscillations coupled with environmental changes (causing drainage evolution) may have caused divergence though extinctions and recolonizations. Forests also existed on the Cape Flats, but climate change caused it to become dry and the water unsuitable, thus restricting gene-flow.
Gouws *et al*. 2010	*Mesaphisopus capensis*	Insecta	12 Allozymes (nDNA); 12S rRNA (mtDNA); COI (mtDNA)	-	-	-	Four clades were evident of which two are found in the Cape (Cape Peninsula, Hottentots Holland Mountains). Four genetically divergent regions evident in the Cape (Bettysbaai, Hottentots Holland Mountains, Steenbras and Kogelberg).	The Cape Peninsula clade diverged from the Hottentots Holland Mountains clade over the Cape Flats. Pliocene/Pleistocene glacial cycles also caused allopatric divergence through refugia (e.g., Cape Fold Mountains outliers on the Agulhas Plain). Dispersal took place when the climate was favourable and divergence occurred when conditions deteriorated (intervening populations went extinct).
Wishart and Hughes, 2001	*Elporia barnardi*	Insecta	6 Allozymes (nDNA)	0.390	-	-	A large amount of differentiation was evident between streams in Table Mountain.	Limited dispersal and gene-flow coupled with high habitat fidelity caused differentiation.
Wishart and Hughes, 2003	*Elporia barnardi*	Insecta	COI (mtDNA)	0.791	~ 5	2.0 - 3.5	Two divergent clades (Table Mountain and Hottentots Holland Mountains) were evident and there was a large amount of differentiation (sub-structuring) between streams in Table Mountain on a small spatial scale.	Limited dispersal and gene-flow coupled with high habitat fidelity are biological attributes that shaped the genetic structure of this species. The period of isolation on Table Mountain was short, but animals are confined to catchments; however the Hottentots Holland Mountains and Table Mountain has had a long period of isolation as the connecting land-bridge between these areas has been eroding since the geologically stable Tertiary.
Daniels *et al*. 2001	*Potamonautes brincki*	Crustacea	13 Allozymes (nDNA)	0.655 (Cape Peninsula - southern Hottentots Holland); 0.825 (Cape Peninsula - northern Hottentots Holland	-	-	Four lineages were evident pertaining to the Cape Peninsula, Jonkershoek/Somerset West area, south-west coast area and Fernkloof respectively. Two of these clades (Cape Peninsula and Hottentots Holland Mountains) are situated in the Cape. Individuals from the Cape Peninsula were closely related to those of the Jonkershoek/Somerset West and individuals from the south-west coast grouped with those of Fernkloof.	Divergence caused by historical differences in rainfall and temperature brought on by the glacial climatic oscillations in the Miocene/Pliocene (divergence between the Cape Peninsula and south-west coast area due to regressions) and Pleiocene/Pleistocene (divergence between the south-west coast area and Hottentots Holland Mountains due to arid conditions).

McDonald and Daniels, 2012	*Peripatopsis capensis*	Euonychophora	COI (mtDNA); 18S rRNA (nDNA)	-	8.93	2.13 – 4.38	Three geographically discrete genetically distinct clades were evident (Cape Peninsula, Overberg, Theewaterskloof/Overstrand). Genetic differentiation was low among populations in Cape Peninsula (compared to other clades where there was considerable genetic structure). This conforms to an island-mainland system with low dispersal.	Cape Flats, Ruêns and Breede River valley basin act as barriers to gene-flow. Additionally, these animals are habitat (forest) specialists with a poor dispersal capability thus restricting dispersal. Forests were paleorefugia on Cape Peninsula in ravines and gorges, therefore Pliocene/Pleistocene glacial cycles caused climatic oscillations which affected afromontane forest contraction and expansion cycles. This led to allopatric diversification. Marine regressions also resulted in a decline in rainfall in mountains and decreased forest cover, thus causing a decline in the genetic diversity of the Cape Peninsula. The Cape Flats was a historic (today it is shrub-dominated) corridor (Podocarpus forest covered it in the Pleistocene) which allowed dispersal. The Theewaterskloof/Overstrand clade exhibits high genetic diversity due to a long evolutionary history in the heterogenous landscape of the Cape Fold Mountains.

Appendix D

Genetic diversity in the mitochondrial DNA and microsatellites of the 16 *P. capensis* populations sampled across the South African west coast region. The number of polymorphic sites in each population is shown for the mitochondrial DNA. In the case of the microsatellites, the number of alleles for each marker (*Hy-T12*, *Hy-T17*, *Hy-D49*, *HCA18*) and observed heterozygosity within each population are presented.

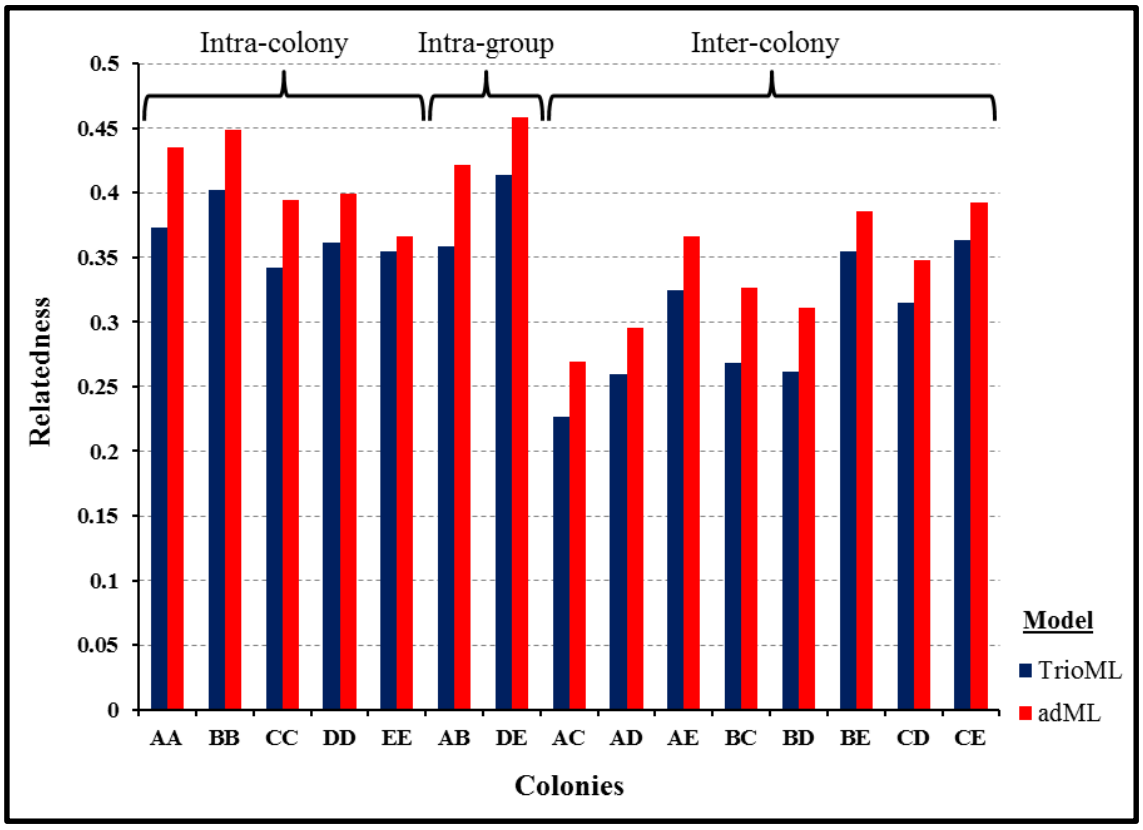
Locality	**Mitochondrial DNA** No. Polymorphic sites	**Microsatellites** *Hy-T12*	*Hy-T17*	*Hy-D49*	*HCA18*	Observed Heterozygosity
Springbok	10	2	5	10	11	0.619 ± 0.101 S.D.
Garies	9	4	6	12	9	0.597 ± 0.118 S.D.
Brand-se-Baai	2	3	7	10	6	0.657 ± 0.110 S.D.
Nuwerus	13	5	6	11	9	0.572 ± 0.137 S.D.
Kliprand	31	3	7	10	7	0.665 ± 0.083 S.D.
Loeriesfontein	1	3	6	7	5	0.682 ± 0.059 S.D.
Nieuwoudt-ville	23	2	6	10	8	0.595 ± 0.172 S.D.
Klawer	34	2	5	10	9	0.574 ± 0.075 S.D.
Donkiesbaai	20	3	6	10	10	0.674 ± 0.071 S.D.
Elandsbaai	17	2	3	8	6	0.504 ± 0.136 S.D.
Vredenburg	0	2	4	6	4	0.466 ± 0.084 S.D.
Ceres	8	2	6	11	6	0.547 ± 0.160 S.D.
Paardeberg	2	2	4	6	4	0.528 ± 0.066 S.D.
Table Mountain	0	2	5	5	4	0.545 ± 0.145 S.D.
Boulders	3	1	3	2	1	0.542 ± 0.164 S.D.
Bettiesbaai	18	3	5	6	4	0.537 ± 0.156 S.D.
Total	**191**	**5**	**11**	**26**	**18**	**0.565 ± 0.029 S.D.**

Appendix E

Microsatellite genetic diversity detected among the five colonies comprising the *P. capensis* Vredenburg population. For each colony the number of samples (n), number of alleles for each marker (*Hy-T12*, *Hy-T17*, *Hy-D49*, *HCA18*), total number of alleles, observed heterozygosity within the colony and the inbreeding coefficient (F_{IS}) is shown.

Colony	n	Hy-T12	Hy-T17	Hy-D49	HCA18	Total	Observed Heterozygosity	Expected Heterozygosity	F_{IS}
A	12	2	3	5	2	12	0.458 ± 0.070	0.528 ± 0.098	0.176
B	20	2	3	5	2	12	0.368 ± 0.137	0.523 ± 0.127	0.320
C	20	2	3	4	3	12	0.588 ± 0.103	0.593 ± 0.044	0.035
D	17	2	3	5	4	14	0.453 ± 0.037	0.638 ± 0.069	0.319
E	8	2	3	4	3	12	0.429 ± 0.160	0.556 ± 0.039	0.310
Total	**77**	**2**	**4**	**6**	**4**	**16**	**0.459 ± 0.047**	**0.568 ± 0.034**	**0.206**

Appendix F



Genetic relatedness based on microsatellite data between specimens collected from the five koppies comprising the Vredenburg population. The pairs of letters represent the colonies between which relatedness was tested. For instance, AA represents intracolony relatedness (relatedness within colony A) and AB intercolony relatedness (between colonies A and B). Intracolony, intragroup and intercolony relatedness is indicated.