Conservation genetics of the endangered southern brown bandicoot (*Isoodon obesulus*) in South Australia

You Li

Submitted for the degree of Doctor of Philosophy

School of Earth and Environmental Sciences

The University of Adelaide

August 2013

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Abstract

Habitat loss and fragmentation are major threats to the world's biodiversity. Throughout Australia, land has been extensively cleared and modified through agriculture, forestry and urbanisation. In South Australia, less than 20% of native forests and woodlands remain and many of these have been severely fragmented into smaller and isolated patches. Species inhabiting fragmented habitats can suffer from decreased population size, reduced or inhibited dispersal and a series of genetic risks, including inbreeding, reduced genetic diversity, increased genetic differentiation among populations and potentially increased extinction risk. The southern brown bandicoot (*Isoodon obesulus*), the focus of the current thesis, is a rabbit-sized ground-dwelling marsupial, which has declined in number dramatically over the last 220 years. The subspecies *I. o. obesulus* is listed as nationally endangered under the Australian Environment Protection and Biodiversity Conservation Act 1999. Habitat loss and fragmentation has become one of the main processes threatening the survival of *I. obesulus*, leading to a contracted distribution and local population extinctions throughout Australia. In this thesis, a combination of microsatellite, nuclear and mitochondrial markers have been applied to investigate several questions relating to population genetic structure, gene flow, dispersal and genetic distinctiveness of populations of *I. o. obesulus* in southern Australia. The results obtained in this thesis have increased our knowledge of the genetic connectivity of *I. o. obesulus* populations in fragmented landscapes and provided valuable baseline genetic information for the conservation management of the species. This thesis was structured into four distinct data chapters as explained below.

Chapter Two comprises a published primer note, in which nine polymorphic microsatellite markers were developed using a next generation sequencing approach. The markers were genotyped in 59 individuals from two distinct locations (the Mount Burr Range and the Mount Lofty Ranges) in South Australia. These markers, in addition to six microsatellite markers from a previous study, were used for the following thesis chapters and provide a valuable resource for future molecular ecological studies of *I. obesulus*.

In Chapter Three, I investigate population structure and gene flow/dispersal of *I. o. obesulus* within a fragmented forest system in south-east South Australia – the Mount Burr Range. In this fragmented habitat, native forest patches are surrounded by matrices of either *Pinus radiata* plantations or cleared agricultural land. A total of 147 samples from 14 native forest patches were genotyped at 15 microsatellite loci. The results showed significant population genetic structuring at a fine spatial scale, with strong genetic differentiation among patches. Gene flow and dispersal was limited and generally only among neighbouring patches. The findings contribute valuable information on the positioning of habitat corridors in this area, and enable the effectiveness of these corridors to be assessed in the future.

In the fourth chapter I utilise 14 microsatellite markers to genotype 284 individuals from 15 sites in a heavily modified peri-urban landscape in South Australia – the Mount Lofty Ranges. The results showed significant genetic differentiation among sites. Sites in the central Mount Lofty Ranges were also more genetically differentiated than sites distributed over a similar spatial scale in the Mount Burr Range, with evidence for a dispersal threshold of 1km (the Mount Burr populations had a ~2.5 km dispersal threshold), and with two sites appearing to be genetically isolated. These analyses suggested that gene flow/dispersal was limited to a higher degree in the Mount Lofty Ranges compared to Mount Burr, possibly due to the heavily modified landscape in the former area (e.g. a mixture of matrix of urban constructs and agricultural land).

The final data chapter (Chapter Five) investigates the phylogeography and population structure of the *I. o. obesulus* populations in South Australia and south-western Victoria using a combination of 14 microsatellite markers, two mitochondrial sequence markers (control region and *ND2*) and three nuclear sequence markers (*BRCA1*, *RAG1*, and *vWF*). This chapter aimed to identify any potential evolutionarily significant units (ESUs) in the study region. All markers supported two distinct genetic lineages of *I. o. obesulus* in South Australia and south-western Victoria. The first lineage consisted of individuals from the Mount Lofty Ranges and Kangaroo Island. Samples from the lower south-east of South Australia and south-western Victoria (the Grampians and Lower Glenelg) represented the second lineage. These two lineages should be considered as separate evolutionarily significant units and managed separately for conservation purposes. An expanded phylogenetic analysis was conducted using additional samples of *I. obesulus* from other regions in Australia and samples of *I. auratus* (the golden bandicoot, distributed in the Northern Territory and Western Australia). The results raise the issue of the taxonomic status of the two lineages and also suggest that current subspecies and species classification within *I. obesulus*/*I. auratus* may not adequately reflect the existing major genetic lineages.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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You Li

Acknowledgements

First of all, I would like to thank my supervisors Dr Melanie Lancaster, Professor Steve Cooper and Professor Sue Carthew for taking me in as their student and providing me with such an interesting project on the amazing bandicoot. Working with them and their students is a chance of a lifetime for me to get to experience the world of the incredible marsupials of Australia! I am deeply grateful to my supervisors for their patience, encouragement, enthusiasm, motivation, professional instructions and immense expert knowledge to walk me through the four-year's PhD. Massive thanks to them for quick and inspiring feedbacks on my thesis writing during the last couple of months before my candidature ends. Without their support and guidance, the completion of my PhD would not have been possible.

I would like to mention my colleagues. Thanks to Jasmin, with whom I experienced the first field trip of trapping bandicoots. She taught me all the skills I needed for trapping and handling and gave me an unforgettable memory. Thanks to Amanda who volunteered on my field trips whenever I needed her and provided excellent help in the field. Thanks to Nik for his patience and assistance with data processing. I also want to thank Bec and Casey for their amazing baking that always brought so much joy to the office. Their friendships have been invaluable and helped me through all the difficult times of my life in Australia.

I would like to thank all my volunteers who came to help me in the field. Thanks to Peter Hatcliffe, Clive Carlyle and Catherine Carlyle, Janet Tan, Samuel Clarke, Kyle Holland, Jess Rowe, Jamie Kohler, Paula Bertsch, Nathan Stavridis, Amy White, Andrew Wiewel, Inta Chambers, Anthony Hay, Toni Bellingham, Orazio Cultreri, and Ben Stevenson and Michelle Le Duff from the Department of Environment, Water and Natural Resources (DEWNR) for their excellent field assistance. I also thank Troy Horn, Bryan Haywood and Robert Mengler from ForestrySA for their advice and assistance in the field. Thanks to Troy, who helped me get familiar with the bandicoot habitat in the south-east and rescued me once when the car got bogged in the mud and thanks to Bryan who provided his assistance whenever I needed. Thanks to Luke Price from DEWNR for sharing cage traps and bandicoot samples and his kind assistance with creating maps.

I would also like to thank Kathy Saint for laboratory assistance and Alison Fitch and Mike Gardner for their assistance with 454 sequencing data analysis.

A very special thanks to Sandy McConachy, who has been putting so much effort into developing collaborations between various universities in China and the University of Adelaide. Without her help and encouragement, my adventure in the fantastic Australia would not have been possible. I also want to thank Professor Richard Russell for taking care of me and other Chinese Scholarship holders during our life in Australia. I'm looking forward to seeing them in China in the future.

My special thanks is to my boyfriend Bo Li and my family. Thanks to Bo for accompanying and supporting me to get through all the difficulties and enduring the bad temper of me during the hard writing period. Thanks to my Mum, Dad and brother for caring and having faith in me.

Lastly, I would like to thank the China Scholarship Council for providing a living allowance. I also want to thank all my funding bodies: the University of Adelaide, the Australian Research Council Linkage grant (LP0668987), Native Vegetation Council, Wildlife Conservation Fund, DEWNR, the Department of Environment and Primary Industries (Victoria) and The Roy and Marjory Edwards Scholarship provided by Nature Foundation SA.

Chapter One: Introduction

A photo showing the fragmented landscape through pine plantations in the south-east of South Australia. Photo by B. Haywood (ForestrySA)

Species decline and extinction in Australia

We are now facing a rapid loss of biological diversity, called the sixth extinction, a mass extinction that "compares with that of the other five mass extinctions revealed in the geological record" (Frankham 2002). Of the numerous threats to biological diversity, human activities are responsible for the majority, including habitat loss and fragmentation, invasive alien species, over-utilisation, pollution and diseases, incidental mortality, and climate change (Baillie *et al.* 2004).

Australia is rich in biodiversity with one of the 25 world's biodiversity hotspots occurring within this country (Mittermeier *et al.* 1998). It has thus been recognised as one of the 17 megadiverse countries that harbour more than 70% of the Earth's species (Conservation International 2000). In addition, a large number of Australia's species is endemic, with more than 80% of terrestrial mammals, flowering plants, reptiles and frogs and ectomycorrhizal fungi being found only in Australia (Castellano & Bougher 1994; Chapman 2009; Lindenmayer 2007). This continent also has most (around 70%) of the world's marsupial species (Dickman & Ganf 2007). Unfortunately, Australia has experienced dramatic species decline and extinction since European settlement, with the number of extinct mammals in Australia surpassing that of any other continent in the world. Twenty-two mammal species have become extinct in Australia since 1600, which comprise one third of the world's mammal extinctions (McKenzie & Burbidge 2002). A further 24% of Australia's mammals, 13% of birds, 6% of reptiles, and 13% of frogs are listed as critically endangered, endangered or vulnerable under the Australian Environment Protection and Biodiversity Conservation Act (Steffen *et al.* 2009).

Habitat loss and fragmentation

Among all the human-induced drivers of biodiversity loss worldwide, habitat loss and fragmentation (the division of a large continuous habitat area into several smaller units with decreased total habitat area) are considered the major and most widespread (Baillie *et al.* 2004; World Conservation Monitoring Centre 1992).

Dispersal and habitat fragmentation

A major effect of habitat loss and fragmentation is its potential negative impacts on dispersal of species (Banks *et al.* 2005b; Cushman 2006; Frankham 2002; Stow *et al.* 2001). Dispersal is an important process for a species' ecology (e.g. species persistence, distribution, population and community structure and reproduction) and evolution (e.g. gene flow between populations, maintenance of genetic diversity and speciation). As Dieckmann (1999) stated, "it is difficult to imagine any ecological or evolutionary problem that would not be affected by dispersal".

Dispersal is associated closely with the persistence of populations in isolated or fragmented habitats, since the possibility of reduced dispersal can lead to a series of problems (Barnett *et al.* 2008; Castellon & Sieving 2006; Stouffer *et al.* 2006). First, lowered dispersal will usually have a negative impact on genetic diversity. Limited dispersal means that individuals in a fragment may not have the opportunity to exchange genetic information with those in other fragments, resulting in decreased gene flow, increased probability of fixation of alleles by genetic drift and thus a loss of genetic diversity (Frankham 2002). Genetic diversity is required for evolution. It is related to adaptation, speciation and an organism's ability to respond to threats such as disease, environmental change, predators and parasites. Genetic diversity can affect many ecological processes at the population, community and ecosystem level (i.e. productivity of populations, interspecific competition and community structure) (Hughes *et al.* 2008). In fragmented populations, enhancing the movement of individuals may help to maintain or increase genetic diversity within subpopulations and high gene flow can diminish the negative effects of habitat fragmentation (see below; Amos & Harwood 1998).

Second, isolated or fragmented populations may suffer from the effects of inbreeding. Inbreeding is the mating between individuals related by ancestry (Frankham 2002). It can affect mating success, viability, sterility and fecundity, and thus reduce individual and population reproductive fitness; termed inbreeding depression (Frankham 2002; Ryan *et al.* 2003; Taylor 2003). Additionally, inbreeding may in turn reduce the level of genetic diversity within populations (Amos & Harwood 1998; Kristensen & Sorensen 2005). One mechanism for avoiding the problems associated with inbreeding is natal dispersal; the dispersal of individuals away from the place they were born (Greenwood 1980; Howard 1960; Szulkin & Sheldon 2008; Wright 1943). Another related mechanism is sex-biased dispersal (Bull & Cooper 1999; Costello *et al.* 2008; Pusey & Wolf 1996; Szulkin *et al.* 2013). For example, it is common in birds and mammals for members of one sex (usually males) to disperse or disperse further than the other sex (Pusey & Wolf 1996). Inbreeding avoidance mechanisms via dispersal can separate close relatives and thus reduce the chance of mating between them. In species where dispersal is used to avoid inbreeding, habitat fragmentation and reduced dispersal are more likely to result in inbreeding problems than in species that use other mechanisms of inbreeding avoidance such as extra-pair/extra-group copulation, delayed maturation or suppressed sexuality, active choice of unrelated mates, and other mechanisms of kin recognition (Blouin & Blouin 1988; Bretman *et al.* 2004; Pusey & Wolf 1996).

Matrix in fragmented habitat

The term "matrix" has been widely used in conservation biology and landscape ecology with varied definitions and usages. Throughout this thesis, the term "matrix" is used for landscape areas that are assumed not to be suitable habitat for the species of interest (Prugh *et al.* 2008). This definition

recognises that the matrix can include various forms of landscapes (e.g. natural or disturbed) and also fits with the broader definition theorised by Lindenmayer & Franklin (2002).

The influence of different types of matrix in fragmented landscapes (e.g. how they shape landscape connectivity, affect the magnitude of edge effects, provide alternative or secondary habitats and regulate the use of corridors and stepping stones) has been investigated in numerous studies (see review by Prevedello & Vieira 2010). In fragmented landscapes, the extent of connectivity between populations largely depends on the ability of animals to move through the landscape and the influence of landscape features on that movement, which can be negative or positive (Antolin *et al.* 2006; Baguette & Van Dyck 2007; Frankham 2002; Lindenmayer *et al.* 1999). For example, barriers such as roads, mountains, rivers or other matrices with poor habitat can limit animal movement (e.g. Arens *et al.* 2007; Clark *et al.* 2010; Levy *et al.* 2010; Quemere *et al.* 2010). Alternatively habitat corridors of native vegetation can promote movement (e.g. Antolin *et al.* 2006; Wilmer *et al.* 2008). Moreover, the effects of the matrix on organisms' movements between habitat patches are usually species-specific (Lindenmayer & Fischer 2007; Prevedello & Vieira 2010). Even within a species, the extent of dispersal and genetic structure will likely vary depending on the type of matrix encountered, their demographic history and the geographical location within which the organisms are distributed (e.g. populations at lower latitude tend to have greater genetic divergence than that of populations at higher latitude within species, Martin & McKay 2004) (Jensen *et al.* 2013). Consequently, measurements of dispersal have sometimes been correlated with environmental characteristics in fragmented landscapes; hence, the field of landscape genetics has developed in recent years to provide valuable information for this purpose (Manel & Holderegger 2013; Manel *et al.* 2003).

Genetic management of threatened species

Three levels of biological diversity are normally considered for conservation purposes: gene, species and ecosystem diversity (McNeely *et al.* 1990). Genetics is a feature of all these levels. Genetic factors not only influence ecosystem survival and function (Crutsinger *et al.* 2006; Reusch *et al.* 2005) but can also lead to potentially increased extinction risk through reduced genetic diversity, increased genetic differentiation, inbreeding depression and loss of evolutionary potential (Frankham 2005). Genetic factors, therefore, are considered necessary and crucial to the conservation of biodiversity (Allendorf *et al.* 2013; Frankel & Soulé 1981; Frankham 2002). Major genetic approaches in biological conservation include resolving taxonomic uncertainties and conservation units; management of wild populations; captive management and reintroduction; management of invasive species; the application of molecular tools in forensics, and understanding species biology and integrating demographic and environmental information with genetics to

predict extinction risks (Frankham 2002, 2010a). Here I will review the first two aspects which relate to this thesis.

Resolving taxonomic uncertainties

Species are fundamental to any consideration of biology, and thus the description and delineation of species is the first step in conservation biology (Costello *et al.* 2013; Frankham 2002). Besides the traditionally used morphological characteristics to define species, the development of molecular tools has greatly facilitated the resolution of taxonomic uncertainties (e.g. Anderson & Thompson 2002; Astrin *et al.* 2012; Avise 2000; Lefébure *et al.* 2006; Liu *et al.* 2009; Pons *et al.* 2006). For example, DNA barcoding methods can now assign unknown individuals to existing species using one or a few reference genes and thus provide additional promising information for species identification (Abdo & Golding 2007; Hajibabaei *et al.* 2007; Hebert & Gregory 2005). The recently arisen discipline of conservation genomics also provides an opportunity to search for "speciation genes" which can be used to predict reproductive isolation (e.g. Coleman 2009).

Within species, populations that are significantly genetically divergent and have evolved independently also require separate management for conservation purposes. Delineating conservation units within species using genetic data is particularly valuable for the management of endangered species where there may be a requirement for translocations or captive breeding to maintain viable population sizes (Frankham 2010b; Moritz 2002). Inappropriate conservation decisions, such as mixing populations that are genetically and evolutionarily distinct may result in detrimental consequences (e.g. outbreeding depression) (Frankham 2002; Frankham *et al.* 2011; Moritz 1999). Furthermore, conservation management approaches to maximise genetic diversity within species will enhance their potential for adaptation and resilience in the face of changing environments or the presence of new pathogens (Frankham 2002).

The term "evolutionarily significant unit" (ESU) is one of the most frequently discussed conservation units. The concept of an ESU was first introduced by Ryder (1986) as an operational unit for conservation purposes to avoid the use of units such as "subspecies" that often did not reflect independently evolving populations. With the development of molecular tools, a criterion for defining ESUs as "reciprocally monophyletic sister groups at mitochondrial loci" (mtDNA) and also those that "show significant divergent allele frequencies at nuclear loci" (nDNA) was proposed by Moritz (1994) and this criterion has been widely used since. Despite the debate over criteria to define ESUs (e.g. Crandall *et al.* 2000), an ESU is generally recognised as a group of organisms with high genetic and ecological distinctiveness that warrants separate management for conservation (Allendorf *et al.* 2013; Crandall *et al.* 2000; Funk *et al.* 2012; Moritz 1994; Ryder 1986; Waples 1991). A second tier conservation unit known as a "management unit" (MU) was

also proposed by Moritz (1994) to be a population showing significant allele frequency variation in mitochondrial and nuclear genetic markers, largely indicative of populations with significantly reduced gene flow between them.

The management of wild populations

Effective conservation genetic management strategies require pre-existing biological and genetic knowledge of the target species. Among these, knowledge of functional connectivity ("the degree to which the landscape facilitates or impedes movement among resource patches", Taylor *et al.* 1993) is a key factor for conservation management of threatened species in fragmented landscapes. Such information can be used for future landscape planning and management, such as for the design of habitat corridors, modelling population persistence and predicting how organisms respond to landscape changes (Sunnucks & Taylor 2008). In addition, genetic information can be used to guide conservation decisions such as translocations and reintroduction (Weeks *et al.* 2011). However, conservation management plans that have included genetic data from fragmented populations are limited. High-resolution molecular markers, based on microsatellite or simple sequence repeat DNA, have been widely used to evaluate levels of gene flow among wild populations (e.g. Aars *et al.* 2006; Banks *et al.* 2005a; Banks *et al.* 2005b; Schregel *et al.* 2012; Stow *et al.* 2001). These low-cost polymorphic markers provide powerful approaches to assess gene flow and dispersal among populations due to the difficulties in measuring dispersal using direct tracking methods in the wild, especially for threatened species (Casado-Amezua *et al.* 2012; Haag *et al.* 2010; Lindsay *et al.* 2008; Ugelvig *et al.* 2012). Molecular techniques also allow non-invasive sampling approaches (e.g. from faecal and hair samples) to be employed to study populations of threatened species. In addition to analyses for population genetics, the technique is powerful enough to provide individual genetic fingerprints that enable mating systems and social organisation to be studied (e.g. Banks *et al.* 2005c; Blyton *et al.* 2012; Kronauer *et al.* 2011).

The Southern Brown Bandicoot

Biology and ecology

The southern brown bandicoot, *Isoodon obesulus,* is a medium-sized, solitary, ground dwelling marsupial. Its guard hairs are coarse and brownish-grey and its underfur is soft, with a yellowish grey to pale grey colour (Jones 1924). It has small, round ears and a short pointed tail. The forelimbs are shorter than the hind limbs, with strong, curved claws and the hind feet show a fusion of the second and third toes. The average head and body length is 33 cm for males and 30 cm for females. The average body weight is 850 g for males and 700 g for females (Strahan 1983). *I. obesulus* is an omnivore, eating a variety of food. Subterranean invertebrates comprise its main diet, but it is also known to feed on fungi, plants and small vertebrates such as skinks and frogs

(Claridge 1988; Heinsohn 1966). Although juveniles are known to disperse away from their birthplace (Heinsohn 1966; Stoddart & Braithwaite 1979), it is not known how far the juveniles disperse. Home range studies of the species give varying estimates ranging from 0.5 to 9.0 ha, depending on gender and habitat structure (Broughton & Dickman 1991; Copley *et al.* 1990; Heinsohn 1966; Lobert 1990; McKenzie 1967; Moloney 1982; Paull 1993; Wilson 2004).

A variety of habitats are used by *I. obesulus* throughout its range. These include shrublands, swamp habitat, open forest, heathlands, sedgelands, grasslands, dry sclerophyll forest with heathy understorey and woodlands (Haby *et al.* 2013; Paull 1993; Rees 1997; Zenger *et al.* 2005). Although the vegetation types used by *I. obesulus* seem to vary in different regions, this species appears to generally prefer sandy textured soil, scrubby vegetation and mid-dense to dense ground cover (Paull 1995, 2003; Strahan 1983). The sandy soil preferred by *I. obesulus* is thought to provide better conditions for invertebrates than compact soil and also saves energy required for digging (Paull 2003). Dense ground cover can provide shelter for nests and protection from predators (including introduced predators such as the red fox and feral cat and natural predators such as quolls, snakes and a variety of raptors) (Paull 2003). Some plant species are particularly important for sheltering, such as *Xanthorrhoea australis* (Paull 1993, 2003). Besides these preferences, fire is another factor that is important for this species, with new regeneration of burnt habitats enhancing the abundance of both food (insects) and ground cover vegetation (Braithwaite & Gullan 1978; Stoddart & Braithwaite 1979; Strahan 1983).

Typical bandicoot habitat (Mount Burr Range in South Australia). Photo by Y. Li.

Distribution and conservation status

Populations of *I. obesulus* are found in South Australia, southern Victoria, Tasmania, south-western Western Australia, south-eastern New South Wales, and the Cape York in north Queensland. *I. obesulus* is the only member of the family Peramelidae surviving in South Australia, following the extinction of seven other species of this family.

Based on geographical distribution and morphological variation, five subspecies of *I. obesulus* have been identified: *I. o. obesulus* (the subject of this thesis), (south-east mainland Australia – the coastal fringe of New South Wales, southern South Australia, and southern Victoria), *I. o. nauticus* (Nuyts Archipelago, South Australia), *I. o. peninsulae* (north Queensland), *I. o. fusciventer* (south-western Western Australia), and *I. o. affinis* (Tasmania) (Figure 1-1, Paull *et al.* 2013). Studies based on modern molecular markers suggest that there is little genetic differentiation between *I. obesulus* and *I. auratus* (the golden bandicoot, distributed in the Northern Territory and Western Australia) and that they should be treated as a single species (Pope *et al.* 2001; Zenger *et al.* 2005). On the contrary, Westerman *et al.* (2012) confirmed the genetic distinctiveness of *I. auratus* from *I. obesulus* and *I. macrourus* (the closely related northern brown bandicoot) using phylogenetic and dating analyses of combined mitochondrial and nuclear sequences. More work needs to be done to further investigate the systematic relationships between *I. obesulus* and *I. auratus*.

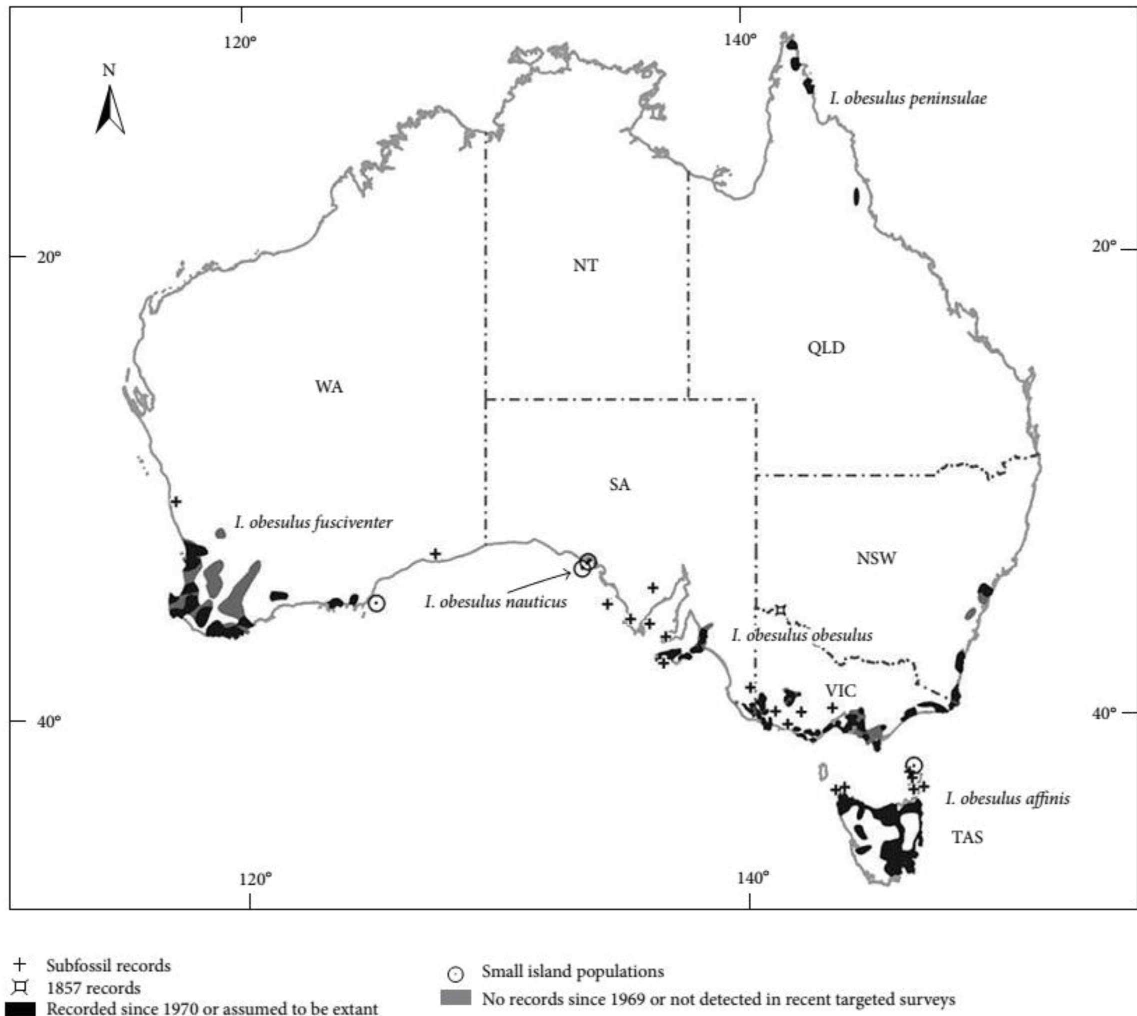

Figure 1-1 Distribution of *I. obesulus*. The map was adopted from Paull *et al.* (2013).

I. obesulus was very common before the settlement of Europeans, but the populations of this species have declined dramatically during the past 220 years. Its range is now contracted and patchily distributed. For example, two of three strongholds of *I. obesulus* in South Australia (the Mount Lofty Ranges and the south-east) have experienced extensive native vegetation clearance through pine plantation, agriculture or urban development, leading to highly fragmented bandicoot habitats in these regions. Of all the subspecies of *I. obesulus*, only *I. o. obesulus* has been listed as nationally endangered under the *Environment Protection and Biodiversity Conservation Act 1999*. In individual states, *I. o. obesulus* is listed as endangered in New South Wales, vulnerable in South Australia and regarded as “near threatened” in Victoria.

Among all the Australian mammal superfamilies, the bandicoot superfamily (Perameloidea) has the highest faunal attrition index (Fitzgibbon *et al.* 2011; McKenzie *et al.* 2007). As the only surviving species of this family in the state of South Australia, *I. obesulus* has gained much attention from

researchers and wildlife managers for its conservation challenges (Brown & Main 2010; Department of Sustainability, Environment, Water, Population and Communities 2013; Haby *et al.* 2013; Haby & Long 2005). However, little is known about the genetic information (i.e. population genetic structure and gene flow) of *I. o. obesulus* populations in South Australia. There is an urgent need for these issues to be assessed in order to design more appropriate management strategies for the conservation of this species.

Study aims

The overall aim of this thesis was to increase our knowledge of population structure and gene flow of *I. o. obesulus* populations in South Australia and use this information to assist with conservation management of the species. Specifically, this study aims to:

- Develop additional microsatellite markers as a complement to eight previously developed markers (Zenger & Johnston 2001) to facilitate comprehensive population genetic and future behavioural ecological studies of the species (Chapter Two).
- Assess the extent of gene flow of *I. o. obesulus* among 14 native forest patches in the Mount Burr region of south-east South Australia using 15 microsatellite markers (Chapter Three).
- Investigate the extent of gene flow of *I. o. obesulus* in the Mount Lofty Ranges of South Australia. The results are also used to compare with the results from Chapter Three to infer the impacts of different forms of matrix on dispersal and gene flow of *I. o. obesulus* (Chapter Four).
- Investigate the genetic distinctiveness of *I. o. obesulus* populations across its range in South Australia and south-western Victoria using a combination of microsatellite markers, mtDNA and nDNA sequencing (Chapter Five).

Thesis structure

The data chapters of this PhD thesis have been written in a publication format, with Chapter Two already published (Li *et al*. 2013). The contents of the chapters are as follows:

<u>Chapter Two – Characterization of nine microsatellite loci from the endangered southern brown bandicoot (*Isoodon obesulus*) using 454 pyrosequencing</u>

In this published manuscript, nine polymorphic microsatellite markers were developed for *I. obesulus* using a next generation sequencing approach. The nine markers were genotyped in 59 individuals from two distinct locations (the Mount Burr Range and the Mount Lofty Ranges) in

South Australia. These markers form part of the microsatellite panel used in subsequent chapters. The citation is:

Li Y, Lancaster ML, Cooper SJB, Packer JG, Carthew SM (2013) Characterization of nine microsatellite loci from the endangered southern brown bandicoot (*Isoodon obesulus*) using 454 pyrosequencing. *Conservation Genetics Resources* **5**, 105-107.

Chapter Three – Population structure and gene flow in the endangered southern brown bandicoot (*Isoodon obesulus obesulus*) across a fragmented landscape

This manuscript investigates genetic connectivity of *I. o. obesulus* from a fragmented forest system in south-east South Australia. A total of 147 samples from 14 native forest patches, each surrounded by a matrix of either *Pinus radiata* plantations and/or cleared agricultural land, were genotyped using 15 microsatellite loci. The findings contribute crucial information regarding the need for and position of habitat corridors in this area, and will enable the effectiveness of these corridors to be assessed in the future.

Chapter Four – Population genetic structure of the endangered southern brown bandicoot (*Isoodon obesulus obesulus*) in a heavily modified landscape

This manuscript investigates gene flow and dispersal in *I. o. obesulus* in a heavily modified peri-urban landscape in the Mount Lofty Ranges of South Australia. A total of 14 microsatellite markers were used to genotype 284 individuals which were sampled from 15 sites. The results were compared to those in Chapter Three in which gene flow was examined in populations of the same species in a different fragmented landscape where plantations (*Pinus radiata*) were the dominant matrix between native forest patches. The findings highlight the importance of studying genetic connectivity at an individual landscape level and how different conservation management plans may be required for threatened species inhabiting distinct landscapes.

Chapter Five – Delineation of conservation units in an endangered marsupial, the southern brown bandicoot (*Isoodon obesulus obesulus*) in South Australia/Western Victoria, Australia

This chapter reports a population genetic/ phylogeographic analysis of *I. o. obesulus* from South Australia and south-western Victoria, using a combination of 14 microsatellite markers, two mitochondrial markers and three nuclear markers, to identify conservation units (ESU and MU) of *I. o. obesulus* populations. To further investigate the taxonomic status of the identified conservation units and the systematic relationship between *I. obesulus* and *I. auratus*, an expanded maximum likelihood analysis using additional samples of *I. obesulus* and *I. auratus* was carried out to place the above analysis of *I. o. obesulus* in the broader context of the proposed distribution of these two species in Australia.

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Chapter Two: Characterization of nine microsatellite loci from the endangered southern brown bandicoot (*Isoodon obesulus*) using 454 pyrosequencing

You Li[1, 2], Melanie L. Lancaster[1, 2, 3], Steven J. B. Cooper[1, 2, 4], Jasmin G. Packer[1] and Susan M. Carthew[1, 5]

(1) School of Earth and Environmental Sciences, the University of Adelaide, Adelaide, SA, 5005, Australia

(2) Australian Centre for Evolutionary Biology and Biodiversity, the University of Adelaide, Adelaide, SA, 5005, Australia

(3) Healesville Sanctuary, Healesville, VIC, 3777, Australia

(4) Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA, 5000, Australia

(5) Research Institute for Environment and Livelihoods, Charles Darwin University, Darwin, NT, 0909, Australia

Photo by Y. Li

Statement of Authorship

Title of Paper	Characterization of nine microsatellite loci from the endangered southern brown bandicoot (*Isoodon obesulus*) using 454 pyrosequencing
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
Publication Details	**Li Y**, Lancaster ML, Cooper SJB, Packer JG, Carthew SM (2013) Characterization of nine microsatellite loci from the endangered southern brown bandicoot (*Isoodon obesulus*) using 454 pyrosequencing. *Conservation Genetics Resources* **5**, 105-107.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	You Li		
Contribution to the Paper	Collected samples, performed DNA extraction, PCR amplification and genetic analyses on all samples, interpreted data, wrote manuscript and acted as corresponding author.		
Signature		Date	20/02/2014

Name of Co-Author	Melanie L. Lancaster		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	28/08/2013

Name of Co-Author	Steven J. B. Cooper		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	23/8/2013

Name of Co-Author	Susan M. Carthew		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	23/8/13

Name of Co-Author	Jasmin G. Packer		
Contribution to the Paper	Provided samples and reviewed manuscript.		
Signature		Date	26/8/13

Abstract

Nine polymorphic microsatellite markers were developed for a nationally endangered marsupial, the southern brown bandicoot (*Isoodon obesulus*) using a next generation sequencing approach. The nine markers were genotyped in 59 individuals from two distinct locations (the Mount Burr Range and the Mount Lofty Ranges) in South Australia. All loci showed Hardy-Weinberg equilibrium with the exception of one locus in the Mount Lofty population, possibly because of null alleles. No evidence of linkage disequilibrium was detected. These markers will provide valuable resources for future projects on the conservation genetics of southern brown bandicoots in Australia.

Keywords: Isoodon obesulus, endangered marsupial, microsatellites, partial 454 shotgun pyrosequencing

The southern brown bandicoot (*Isoodon obesulus*) is a medium sized Australian marsupial, which has declined in number dramatically over the last 220 years, resulting in the subspecies *I. o. obesulus* being listed as endangered under the Australian Environment Protection and Biodiversity Conservation Act 1999. In South Australia, *I. obesulus* is the only surviving member of the family Peramelidae. The remaining populations of the southern brown bandicoot in south-eastern South Australia are confined to small, seemingly isolated native forest fragments due to extensive forest clearance and the subsequent planting of pine trees. Habitat fragmentation has become one of the main processes threatening the survival of the southern brown bandicoot in south-eastern South Australia (Claridge & Barry 2000; Department of Environment and Conservation 2006). However, little is known about the genetic effects of fragmentation on this species and the potential for connectivity/gene flow among forest fragments. Here we developed nine microsatellite loci as a complement to eight previously developed markers (Zenger & Johnston 2001) to facilitate comprehensive population genetic and future behavioural ecological studies of the species.

A total of 2 261 microsatellite markers were identified from 59 949 sequenced reads produced by partial 454 shotgun pyrosequencing on a Titanium GS-FLX platform (Australian Genome Research Facility, AGRF, Brisbane, Australia) using the approach outlined in Gardner *et al.* (2011). Of these, 46 markers were selected for PCR trials on a single individual. Genomic DNA was extracted from ear tissue using the Gentra Puregene extraction kit (Gentra Systems Inc.). PCR-amplifications were performed using a method known as ‘‘multiplex-ready technology’’ (MRT) in which locus-specific primers were designed with a generic tag at their 5’ ends (Hayden *et al.* 2008). PCR-amplifications were performed in a final volume of 12 µl, containing 10 ng of DNA, 75 nM each of fluorescently labelled forward (MRT-generic) primer (HEX) and unlabelled reverse primer, an appropriate concentration of locus-specific primers (10, 20, 40 or 60 nM), 0.15 U Immolase DNA polymerase

(Bioline, Luckenwalde, Germany) and 2.4 µl of 5 × ImmoBuffer (Bioline). PCR amplifications were performed in two phases using the following cycles after a 10 min initial denaturation at 95 °C: first phase consisted of 5 cycles of 60 s at 92 °C, 90 s at 50 °C, 60 s at 72 °C and 20 cycles of 30 s at 92 °C, 90 s at 63 °C, 60 s at 72 °C; second phase consisted of 40 cycles of 15 s at 92 °C, 30 s at 54 °C, 30 s at 72 °C, and a final extension for 30 min at 65 °C. PCR products were visualized on a 6 % polyacrylamide gel.

Of the 46 loci, 18 amplified unambiguous product and the 18 loci were then screened for polymorphism in four individuals. Either VIC, FAM, NED or PET-labelled MRT generic primer was added to each PCR to produce fluorescently labelled products. PCR products were genotyped on an ABI 3730 DNA Analyser and alleles were scored using GeneMapper 4.0 (Applied Biosystems). From 18 loci nine were polymorphic and reliably scored (Table 2-1).

The nine loci were screened for variation in a subset of 59 individuals in South Australia (16 from one subpopulation in the Mount Burr Range and 43 from a second subpopulation in the Mount Lofty Ranges). Arlequin 3.11 (Excoffier & Lischer 2010) was used to examine allelic diversity, observed and expected heterozygosity, deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium. MICRO-CHECKER v. 2.2.3 (Van Oosterhout *et al.* 2004) was used to estimate null allele frequencies. Sequential Bonferroni corrections (Rice 1989) were applied to adjust significance values for multiple comparisons.

All loci were polymorphic, with the exception that marker Ioo6 was monomorphic in the Mount Lofty population (Table 2-1). However, this locus was polymorphic in the Mount Burr population and revealed multiple alleles when we expanded the number of samples from the Mount Lofty Ranges (N = 320) in a subsequent analysis (size range = 210-240 bp). Across all nine loci, the number of alleles ranged from 4 to 7 per locus (mean of 5). Estimates of observed and expected heterozygosity ranged respectively, from 0.06-0.60 to 0.06-0.74 for the Mount Burr population, and 0.07-0.63 and 0.11-0.60 for the Mount Lofty population. No evidence of deviation from HWE was detected after sequential Bonferroni correction with the exception of marker Ioo7 in the Mount Lofty population. This deviation possibly resulted from the presence of null alleles (r = 0.172). No pairs of loci showed significant linkage disequilibrium. In addition, we did not detect any evidence of linkage disequilibrium between the nine loci and six (B3-2, B15-1, B20-5, B34-2, B35-3 and B38-1) of the eight loci reported in Zenger & Johnston (2001) (the other two loci failed to amplify reliably in either population).

Table 2-1 Characterisation of nine microsatellite markers for the southern brown bandicoot

Locus	Repeat motif	Size range	Primer sequences (5'-3')	Genbank Accession number	Pop	N	N_A	H_O/H_E	HWE
Ioo2	(GAT)16	260-280	F:TTGCTATCAAATAACTATCAGGGG	JX188445	Mount Burr	16	4	0.40/0.50	0.168
			R:ACTGTGTGACATGCTGAAATCC		Mount Lofty	43	2	0.41/0.48	0.691
Ioo3	(CATATA)16	160-300	F:CAGTTGCAAGTAAATTCATTCATGG	JX188446	Mount Burr	16	4	0.27/0.51	0.023
			R:ATACATTCACACAGCATCCAC		Mount Lofty	43	2	0.07/0.12	0.121
Ioo4	(GATA)17	220-280	F:GTTTTAGTCCATGGGGTCCTG	JX188447	Mount Burr	16	4	0.60/0.67	0.876
			R:AGCTGGTCTATATCAACTTTGAGG		Mount Lofty	43	3	0.63/0.60	0.911
Ioo5	(GTATAT)14(GTGTAT)4	130-250	F:TCCTTGACTTAGACAGTGTTTCTC	JX188448	Mount Burr	16	2	0.06/0.06	1.000
			R:TGGGCTAGGATGTTTAAGGG		Mount Lofty	43	2	0.15/0.14	1.000
Ioo6	(GAT)13GAG(GAT)7	210-240	F:AGAAAAGGATGGTTTGCGGG	JX188449	Mount Burr	16	2	0.06/0.06	1.000
			R:CTTTGCCCTGGGATTCACG		Mount Lofty	43	1	NA	NA
Ioo7	(AT)8GT(AT)9	170-200	F:TGTGCCTCCTCCTAAAGGC	JX188450	Mount Burr	16	2	0.25/0.31	0.434
			R:TGAGGAGACTGAGGTTCAAAG		Mount Lofty	43	3	0.29/0.57	0.000*
Ioo8	(GGAA)17	270-290	F:AGGAATTTCTCTTGATTCCACTTG	JX188451	Mount Burr	16	4	0.56/0.65	0.524
			R:AAGTAGTAAATTTTGGAGGCAGG		Mount Lofty	43	2	0.43/0.37	0.405
Ioo10	(GTATA)20	240-290	F:TGTGTGATGCTGCACAAGTC	JX188452	Mount Burr	16	4	0.60/0.74	0.482
			R:CTCAGCCTCAATCTCTAACTGC		Mount Lofty	43	2	0.40/0.34	0.528
Ioo16	(AAC)26	220-270	F:TGGCCAATGGGTGGATGTG	JX188453	Mount Burr	16	3	0.25/0.51	0.027
			R:ACTTCTACTGCTTTCTGTTCCG		Mount Lofty	43	2	0.23/0.20	1.000

N number of individuals screened, N_A number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, HWE Hardy-Weinberg equilibrium P values.

* Significant deviation from Hardy-Weinberg equilibrium after sequential Bonferroni correction.

The microsatellite markers developed here, in addition to the loci from Zenger & Johnston (2001), provide a valuable resource for future research of the conservation biology and behavioural ecology of southern brown bandicoots in Australia.

Acknowledgements

This project was funded by the Australian Research Council Linkage grant (LP0668987), Native Vegetation Council, Wildlife Conservation Fund, and Nature Foundation SA. We thank Department of Environment and Natural Resources and Forestry SA for field assistance. We also thank Kathy Saint for laboratory assistance and Alison Fitch and Mike Gardner for their assistance with 454 sequencing data analysis.

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Chapter Three: Population structure and gene flow in the endangered southern brown bandicoot (*Isoodon obesulus obesulus*) across a fragmented landscape

You Li[1, 2], Melanie L. Lancaster[1, 2, 3], Susan M. Carthew[1, 5] and Steven J. B. Cooper[1, 2, 4]

(1) School of Earth and Environmental Sciences, the University of Adelaide, Adelaide, SA, 5005, Australia

(2) Australian Centre for Evolutionary Biology and Biodiversity, the University of Adelaide, Adelaide, SA, 5005, Australia

(3) Healesville Sanctuary, Healesville, VIC, 3777, Australia

(4) Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA, 5000, Australia

(5) Research Institute for Environment and Livelihoods, Charles Darwin University, Darwin, NT, 0909, Australia

Photo: Native Wells Forest Reserve in the Mount Burr Range. Left of the track shows the edge of the patch, right of the track shows the edge of the pine plantation. Photo by Y. Li.

Statement of Authorship

Title of Paper	Population structure and gene flow in the endangered southern brown bandicoot (*Isoodon obesulus obesulus*) across a fragmented landscape
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Publication Style

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	You Li		
Contribution to the Paper	Collected samples, performed DNA extraction, PCR amplification and genetic analyses on all samples, interpreted data, wrote manuscript and acted as corresponding author.		
Signature		Date	20/02/2014

Name of Co-Author	Melanie L. Lancaster		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	28/08/2013

Name of Co-Author	Susan M. Carthew		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	23/8/13

Name of Co-Author	Steven J. B. Cooper		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	23/8/2013

Abstract

Habitat destruction is one of the leading threats to biodiversity, resulting in the contraction and fragmentation of species' distributions and enhancing their potential for extinction in small populations. For conservation management of threatened species in such landscapes it is important to assess how fragmentation impacts on genetic connectivity of populations. This is also dependent on the biology of individual species and the nature of the intervening matrix. In this study, we investigated genetic connectivity in an endangered marsupial, the southern brown bandicoot (*Isoodon obesulus obesulus*) from a fragmented forest system in south-east South Australia. We genotyped 15 microsatellite loci in 147 samples from 14 native forest patches, each surrounded by a matrix of either *Pinus radiata* plantation or cleared agricultural land. Our results showed significant population genetic structuring at a fine spatial scale in the 520 km^2 Mount Burr region, with strong genetic differentiation among patches. Gene flow and dispersal was limited and generally only occurred among neighbouring patches. Our findings contribute crucial information to the physical positioning of habitat corridors in this area and provide baseline data to enable the effectiveness of these corridors to be assessed in the future.

Keywords: population connectivity, dispersal, gene flow, habitat fragmentation, genetic management, southern brown bandicoot, Isoodon obesulus

Introduction

The ongoing loss of habitat and fragmentation has become a focus of conservation ecology because of its serious threat to biodiversity (Fahrig 2003; Fazey *et al.* 2005; Foley *et al.* 2005; Frankham 2002). Habitat fragmentation results in decreased population sizes, reduced or inhibited dispersal and a series of genetic consequences, including inbreeding, reduced genetic diversity, increased genetic differentiation among populations and potentially increased extinction risk (Frankham 2002). Thus, the re-establishment of gene flow between fragmented populations is seen as a crucial action in the conservation management of threatened species (Frankham 2002, 2010; Storfer 1999; Van Dyke 2008). For example, dispersal among fragmented habitats can be facilitated by creating or enhancing existing landscape corridors. Despite the debate about species' actual use of corridors (e.g. Carthew *et al.* 2009; Falcy & Estades 2007; Haddad 2008), their enhancement of dispersal has been confirmed in several studies (Beier & Noss 1998; Gilbert-Norton *et al.* 2010). Nonetheless, in real-world landscapes, the effectiveness and need for habitat corridors can be difficult to measure or assess due to a lack of data on population genetic structure and gene flow among habitat patches prior to the construction of corridors.

Rational conservation genetic management strategies require pre-existing biological and genetic knowledge of the target species. Among these, knowledge of genetic connectivity is a key factor for conservation management of threatened species in fragmented landscapes. Such information can be used for future landscape planning and management, including the design of habitat corridors, for modelling population persistence and for predicting how organisms respond to landscape changes (Sunnucks & Taylor 2008). However, conservation management plans that have included genetic data from fragmented populations are limited (Weeks *et al.* 2011). High-resolution molecular markers, based on microsatellite or simple sequence repeat DNA, have been widely used to evaluate levels of gene flow among wild populations (e.g. Aars *et al.* 2006; Banks *et al.* 2005a; Banks *et al.* 2005c; Schregel *et al.* 2012; Stow *et al.* 2001). These low-cost polymorphic markers provide powerful approaches to assess gene flow and dispersal among populations, especially for threatened species due to the difficulties in measuring dispersal using direct tracking methods in the wild (Casado-Amezua *et al.* 2012; Haag *et al.* 2010; Lindsay *et al.* 2008; Ugelvig *et al.* 2012).

In Australia, habitat destruction and fragmentation have resulted in the decline of many animal species, leading to concerns about how they might be best managed in the future (Department of the Environment and Heritage 2004; Lindenmayer 2009; Lindenmayer *et al.* 2008; McKenzie *et al.* 2007). In south-eastern South Australia, only 14% of native forests and woodlands remain and many of these have been severely fragmented into smaller, isolated patches of native forest (henceforth referred to as “patches”) surrounded by pine plantations or pastoral land. The size of these patches ranges from a few to over 1 000 hectares (ha), but most are less than 300 ha (Carthew 2004). We have studied this fragmented forest system with an overall aim to explore the impacts of fragmentation on population processes in a suite of native mammal species, including the yellow-footed antechinus (*Antechinus flavipes*; McLean 2009, in prep.), Gould's long-eared bat (*Nyctophilus gouldi*) and lesser long-eared bat (*Nyctophilus geoffroyi*) (Fuller 2012, in prep.), common ringtail possum (*Pseudocheirus peregrinus*; Lancaster *et al.* 2011) and sugar glider (*Petaurus breviceps*; Malekian 2007, in prep.). Results for *P. peregrinus* showed reduced ecological connectivity among native forest patches compared to continuous forest, despite their ability to use the pine matrix for foraging and nesting (Lancaster *et al.* 2011). In the current study, we add an additional taxon to this multi-species study of fragmentation; the southern brown bandicoot (*Isoodon obesulus*).

I. obesulus is a rabbit-sized ground-dwelling marsupial, which has declined in numbers dramatically over the last 220 years, leading to its contracted distribution and local population extinctions (Coates *et al.* 2008; Department of Environment and Conservation 2006; Paull 1993, 1995). The subspecies *I. o. obesulus,* which is the focus of this study, is listed as nationally endangered (the Australian Environment Protection and Biodiversity Conservation Act 1999). In South Australia, *I. obesulus* is the only surviving member of the family Peramelidae. Remaining

populations of the species in south-eastern South Australia are confined to small, seemingly isolated native forest fragments due to extensive forest clearance and the subsequent planting of pine trees or use of land for agriculture. Habitat fragmentation has become one of the main processes threatening the survival of *I. o. obesulus* in south-eastern South Australia (Claridge & Barry 2000; Department of Environment and Conservation 2006). However, little is known about the genetic effects of fragmentation on this species and the potential for connectivity/gene flow among forest fragments.

One of the current strongholds of *I. o. obesulus* in South Australia is the Green Triangle Forest region, a major softwood plantation region for Australia. This region contains about 50 individual patches ranging in size from 5 ha to 2 200 ha that are largely separated from each other by non-native plantation forest (*Pinus radiata*) or cleared agricultural land (see Figure 3-1). The area is managed by a state government based organisation (ForestrySA) for commercial plantations and for flora and fauna (e.g. southern brown bandicoot, sugar glider, and yellow-bellied glider) conservation. ForestrySA is implementing a biodiversity corridor program (the Lower South-East Biodiversity Corridors Project) to construct 21 biodiversity corridors in the region (Horn 2003). Twelve of the proposed corridors are located in the Mount Burr Range in the middle of the Green Triangle Forest. Most patches in the Mount Burr Range are known to contain *I. o. obesulus* from past survey work (ForestrySA 2010, 2011; Paull 2003; Department for Environment and Natural Resources 2010) and geographic isolation of the patches ranges from 0.5 km to 2.5 km. The Mount Burr Range therefore represents an ideal system to study gene flow/dispersal of *I. o. obesulus* in a fragmented landscape. Importantly, it will provide baseline data prior to the construction of habitat corridors, which can subsequently be used to assess the effectiveness of this management technique.

Our study specifically aimed to assess the extent of gene flow/dispersal among *I. o. obesulus* from 14 native forest patches in the Mount Burr region using 15 microsatellite markers. These analyses were used to test the hypothesis that native forest patches are genetically isolated for *I. o. obesulus* due to a lack of dispersal through the agricultural and pine matrices.

Methods

Study area and sample collection

Sampling was carried out in 14 native forest patches located in the Mount Burr Range (20 $\times$ 26 km scale) of south-east South Australia (37°30'S, 140°24'E to 37°46'S, 140°47'E, Figure 3-1; Table 3-1) in the Green Triangle Forest. Each patch was visited at least twice to obtain sufficient samples for genetic analyses. A total of 147 bandicoot tissue samples were collected during 2009-2011 (mean trap success was 1.92% over 5 627 trap nights).

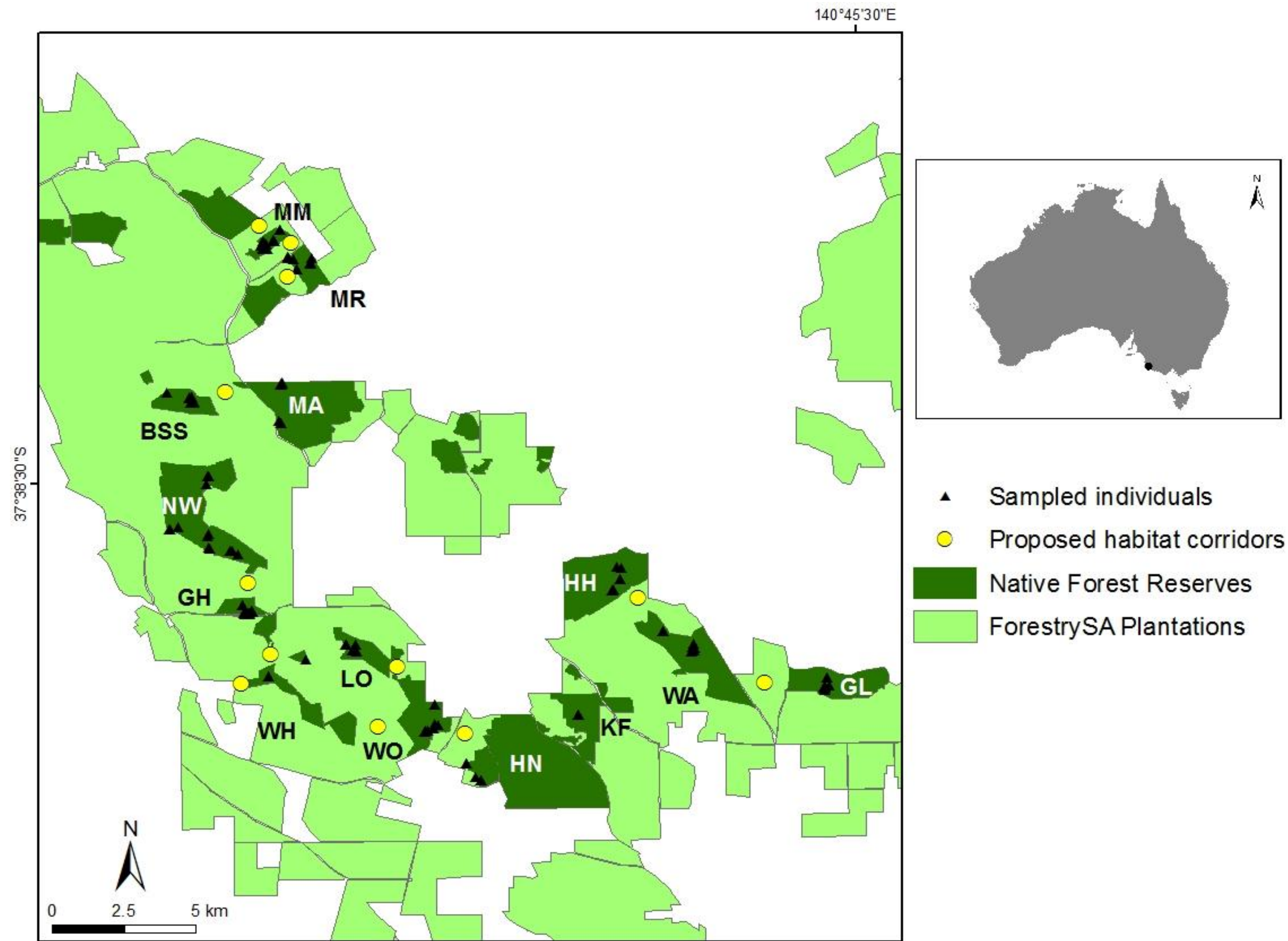

Figure 3-1 Detailed map of the Mount Burr Range in the Green Triangle Forest in the south-east of South Australia, Australia. Native forest patches are represented in dark green, pine plantations are represented in light green, and agriculture lands are represented in white. Names of the 14 sampled patches were marked in or next to the corresponding dark green areas (see Table 3-1 for full names). Sampled bandicoot individuals were marked with dark triangles. Twelve habitat corridors were proposed in this area to connect proximate patches (see text for details) and they were marked with yellow circles in the map.

Table 3-1 Sampling information and genetic diversity parameters for 14 patches

Patch name	Patch abbreviation	Approximate isolation time	Patch size (ha)	Distance to nearest patch* (km)	*N*	H_O	H_E	F_{IS}	*A*	*AR*	*IR*
Mount McIntyre	MM	mid 1930's	63.1	0.53	16	0.402	0.566	**0.298**	3.80	2.210	0.398
Mc Rosties	MR	mid 1930's	111.2	0.53	11	0.464	0.548	0.160	3.07	2.140	0.296
The Marshes	MA	mid 1930's	596.5	1.77	7	0.384	0.488	0.225	2.73	2.025	0.375
Burr Slopes South	BSS	1969-1972	135.5	1.77	15	0.338	0.425	**0.210**	3.00	1.878	0.402
Native Wells	NW	1969-1973	619.5	1.28	15	0.367	0.289	**0.159**	3.73	2.122	0.221
Glencoe Hill	GH	1972-1973	66.6	1.28	15	0.427	0.471	0.159	3.13	2.019	0.322
Long	LO	1963	147.1	0.13	8	0.534	0.566	0.054	3.13	2.212	0.166
Windy Hill	WH	1965-1969	139.6	1.32	3	0.267	0.433	0.461	1.93	1.840	0.588
Woolwash	WO	late 1940's	263.6	0.13	7	0.399	0.539	**0.331**	2.87	2.145	0.368
Honan	HN	late 1940's	1026.8	0.06	8	0.360	0.448	0.209	2.47	1.935	0.393
Kangaroo Flat	KF	early 1930's	302.8	0.06	6	0.577	0.564	-0.028	3.07	2.263	0.029
Hacket Hill	HH	1962	493.1	1.53	9	0.445	0.480	0.076	3.07	2.034	0.201
Wandilo	WA	1962	425.2	1.53	16	0.343	0.466	**0.271**	3.47	1.993	0.401
Grundy's Lane	GL	late 1940's - 1956	287.7	1.79	11	0.279	0.446	**0.390**	2.73	1.895	0.481

Sample size (*N*), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), allelic diversity (*A*), allelic richness (*AR*), and internal relatedness (*IR*).

Significant F_{IS} values were denoted in bold. $P < 0.05$.

* The "distance to nearest patch" was measured in ArcGIS 10 as straight-line distance (edge to edge).

Traps were set as grids that consisted of 3-5 parallel transects with five traps per transect. Traps were spaced 20 m apart along each transect and transects were 40 m apart. Trap grids were situated at different locations within each patch to ensure spatial coverage. Trap locations within patches were based on knowledge of *I. o. obesulus* presence obtained from a previous study (Paull 2003), a recent digging survey (Paull and colleagues, Department of Environment and Natural Resources, ForestrySA, 2007, unpublished data) and our own field observations of fresh bandicoot diggings (conical-shaped holes dug by bandicoots when foraging; Paull 2003) and vegetation characteristics, as the species is known to be associated with the grass tree *Xanthorrhoea australis* (Paull 2003). Cage traps (55 × 25 × 25 cm treadle) were covered with plastic and hessian bags to protect animals from rain and sun, bedded with small pieces of hessian to keep animals warm, and baited with a mixture of peanut butter and oats. Traps were opened before sunset every day and checked at first light the next morning. Captured bandicoots were processed on site. After measuring body weight and checking reproductive condition, a small notch of skin was removed from the ear for individual identity, with the tissue stored in a 50: 50 solution containing ethanol and saline for genetic analyses.

DNA extraction and genotyping

DNA was extracted using the Gentra Puregene extraction kit and methods specified by the manufacturer (Gentra Systems Inc.). Samples were genotyped at 15 microsatellite loci that had been developed for *I. obesulus* [six (B3-2, B15-1, B20-5, B34-2, B35-3 and B38-1) by Zenger & Johnston (2001), and nine by Li *et al.* (2013)] following PCR protocols in Li *et al.* (2013). Approximately 10% of all 147 samples collected were genotyped repeatedly at all 15 loci to check error rates. We expressed error rates as the number of errors per allele, which was calculated as the number of incorrect alleles divided by the total number of genotyped alleles (Hoffman & Amos 2005). Repeat genotyping error rate was very low, with an average of 0.0009 across all loci. Amplified products were run on an ABI 3730 DNA Analyser and alleles were scored using GeneMapper 4.0 (Applied Biosystems).

Population genetic analyses

Deviations from Hardy-Weinberg equilibrium (HWE) across loci and patches and assessment of linkage disequilibrium among loci were analysed using Genepop 4.1.0 (Raymond & Rousset 1995). Sequential Bonferroni corrections (Rice 1989) were applied to adjust significance values for multiple comparisons. Genetic diversity was estimated from the number of alleles per locus (A), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}) and allelic richness (AR) corrected for sample size. Calculation of F_{IS} and AR were performed in FSTAT 2.9.3.2 (Goudet 2001) and the remaining calculations were performed in Arlequin 3.11 (Excoffier

& Lischer 2010). Individual internal relatedness (*IR*) was also calculated as an estimate of parental relatedness (Amos *et al.* 2001) in an R extension package, Rhh (Alho *et al.* 2010).

The degree of genetic differentiation amongst bandicoots across different patches was measured first with pairwise F_{ST} in Arlequin 3.11(Excoffier & Lischer 2010) with 10 000 permutations. We also calculated the estimator of actual differentiation D_{EST} (Jost 2008) using an R package – DEMEtics (Gerlach *et al.* 2010), as F_{ST} may underestimate differentiation when using highly polymorphic markers such as microsatellites (Jost 2008).

Population genetic structure was further assessed using Bayesian clustering analyses in three different ways. First, we used STRUCTURE 2.3.3 (Pritchard *et al.* 2000) to detect the number of genetic clusters (*K*) in our data set. We ran the analysis with a burn-in of 100 000 and 100 000 MCMC steps after the burn-in, using an admixture model and correlated allele frequencies without providing any prior information on geographic location of individuals. The value of *K* was set from 1 to 14 due to the possibility of each patch representing a distinct genetic cluster, and ten replicates of each *K* were run. The most likely *K* was determined using STRUCTURE HARVESTER 0.6.92 (Earl & vonHoldt 2012) based on the method described in Evanno *et al*. (2005). We used CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) to average the membership probabilities for the ten runs of the most likely *K* and used DISTRUCT 1.1 (Rosenberg 2004) for visualising the final results.

Analyses were also implemented in two other programs, TESS 2.3.1 (Chen *et al.* 2007; Francois *et al.* 2006) and BAPS 5.2 (Corander *et al.* 2008). Both use a spatial Bayesian clustering and may perform better than STRUCTURE when overall F_{ST} is small, which we found to be the case for some pairs of patches (Table 3-2, Chen *et al.* 2007; Latch *et al.* 2006). Parameter settings for TESS used an admixture model with 10 000 burn-in and 50 000 sweeps. The BAPS analysis was run at the level of individuals with a spatial model. The value of *K* ranged from 2 to 14 with ten replicates of each *K* for both programs. The optimal *K* for TESS was chosen as the one with the stabilized value of the Deviance Information Criterion (DIC).

Spatial scale of genetic differentiation

Correlation between genetic distance and logarithm of geographical distance was investigated using Mantel tests (Isolde, Genepop 4.1.0, Raymond & Rousset 1995) with 10 000 permutations. Tests were done for the whole study area at both individual and patch levels [genetic distance: $\hat{a}$ statistic (Rousset 2000) for individual level, and F_{ST} / (1 - F_{ST}) and D_{EST} / (1 - D_{EST}) for patch level]. To assess possible influences of pine plantations on genetic structure, a partial Mantel test was also run to estimate the partial correlation of genetic distance and intervening forest type (0 = native forest, 1 = pine) for all individuals (see Figure 3-1 for locations of the sampled individuals) when

controlling for geographical distance. The partial Mantel test was performed in IBDWS v. 3.23 (Jensen *et al.* 2005) with 1 000 randomizations.

To further study the spatial scale of genetic variation, spatial autocorrelation analyses were performed in GenAlEx 6.41 (Peakall & Smouse 2006) using 0.5 km and 1.0 km distance class sizes. The analysis was also done for females (n = 58) and males (n = 69) separately to assess the possibility of sex-biased dispersal (20 samples with unknown gender information were excluded). Statistical testing of the analyses was based on 95% confidence intervals defined by 1 000 random permutations. Under limited gene flow, populations should show positive spatial genetic structure at short distances if sampling has covered the spatial scale of genetic structure (Smouse & Peakall 1999) and the correlograms should flatten out at the scale where gene flow is not connecting subpopulations (Aars *et al.* 2006; Gauffre *et al.* 2008).

Migrants and individuals with mixed ancestry were detected in both STRUCTURE 2.3.3 (Pritchard *et al.* 2000) and GENECLASS 2.0 (Piry *et al.* 2004). In STRUCTURE, the analysis was performed using sampling locations (i.e. patches) as prior population information with 100 000 burn-in and 100 000 MCMC steps after the burn-in. In GENECLASS, the test of first-generation migrants was performed using a Bayesian approach (Rannala & Mountain 1997) and the Monte Carlo re-sampling method of Paetkau *et al*. (2004) with 10 000 simulated individuals and an alpha of 0.05. For the likelihood computation, we used the likelihood ratio L_home / L_max which is the ratio of the likelihood of a given individual within the population where it was sampled to the highest likelihood value among all available populations (Paetkau *et al.* 2004).

Results

Hardy-Weinberg equilibrium, linkage disequilibrium and genetic diversity

Departure from HWE proportions was detected in 9 of 210 locus/patches tests after sequential Bonferroni correction. However, none of the 15 loci showed significant HWE deviations in more than two patches, indicating that there were unlikely to be problems with null alleles in the data set. Significant linkage disequilibrium between loci was found in 7 of 1470 pairwise comparisons (15 loci at each of the 14 patches) after correction for multiple comparisons, and none of the pairwise comparisons were significant for two patches at the same time. These 15 loci were therefore regarded as independently segregating loci.

The number of alleles varied from 2 (B20-5) to 10 (Ioo18) with a mean number of 5.93. Mean observed (H_O) and expected (H_E) heterozygosity across the 14 patches ranged from 0.267 to 0.577 and from 0.289 to 0.566 respectively (Table 3-1). Inbreeding coefficient (F_{IS}) ranged from -0.028 in KF to 0.461 in WH (Table 3-1). Allelic diversity (the average number of alleles per locus, A)

ranged from 1.93 to 3.80, with a mean of 3.01 (Table 3-1). Allelic richness (*AR*) ranged from 1.840 to 2.263, and internal relatedness (*IR*) ranged from 0.029 to 0.588 (Table 3-1). Statistical tests showed that there was no significant variation in H_O, H_E or *AR* among the 14 patches (ANOVA, F [13, 196] = 1.547, 0.678, and 0.854, $P = 0.104$, 0.783, and 0.603, respectively). Values of internal relatedness (*IR*) across patches were similar, with the exception of patch KF, where it was significantly lower ($IR = 0.029$) than that in patches BSS, GL, MM, WA or WH (*IR* 0.398-0.588; Tukey HSD: $P = 0.044$, 0.008, 0.044, 0.040 and 0.034, respectively; Table 3-1).

Genetic differentiation and population structure

Pairwise F_{ST} values among patches ranged from 0.021 (KF-HH, $P = 0.218$) to 0.361 (MR-GH, $P < 0.001$) and most comparisons were highly significant ($P < 0.001$; Table 3-2). Pairwise D_{EST} values ranged from 0.021 (KF-NW, $P = 0.170$) to 0.393 (MR-HN, $P < 0.001$), with generally higher values than F_{ST}, as expected (Table 3-2).

Bayesian clustering analysis using STRUCTURE found three distinct clusters ($K = 3$, Figure 3-2a) using the ΔK criterion (Evanno *et al.* 2005). Eighty-two percent of the individuals were assigned with a probability > 80% to one of the three clusters, with the patches MA, NW, WH, WO and KF showing marked admixture (patch's *q* in STRUCTURE: MA-0.38, NW-0.53, WH-0.59, WO-0.45, KF-0.52). TESS also identified three clusters. Population assignments resulting from these two analyses were very similar, with the exception that patches MA, WO, LO were assigned to cluster 2 in TESS, whereas they were assigned to cluster 3 in STRUCTURE (Figure 3-2a, b). The number of clusters inferred by BAPS was four (posterior probability was 0.9986). However, the fourth cluster in BAPS contained only seven individuals and it was not specific to any patch; this cluster was therefore disregarded. The result of population assignment in BAPS was generally concordant with that found by TESS, with only one patch HN being assigned to a different cluster in BAPS. Despite the finding that several patches were assigned differently in STRUCTURE/TESS/BAPS, the three clusters generally corresponded to groups of geographically proximate sampling localities (Figure 3-2). The two northern patches (MM and MR), which are the most geographically isolated ones, formed a discrete population (cluster 1) that is clearly differentiated from the remaining patches. Similarly, the eastern-most patches (HH, WA and GL) grouped into cluster 3 and are also genetically distinct. Cluster 2 consisted of patches from the middle area of the study, and these showed more admixture than the northern and eastern regions.

Table 3-2 Pairwise F_{ST} values (below diagonal) and pairwise D_{EST} values (above diagonal) for the 14 bandicoot patches (following Sequential Bonferroni correction)

	MM	MR	MA	BSS	NW	GH	LO	WH	WO	HN	KF	HH	WA	GL
MM	-	0.106**	0.287**	0.379**	0.298**	0.375**	0.235**	0.368	0.307**	0.342**	0.243**	0.351**	0.291**	0.360**
MR	0.068	-	0.319**	0.375**	0.307**	0.380**	0.251**	0.392**	0.374**	0.393**	0.235**	0.277**	0.292**	0.344**
MA	0.241***	0.243***	-	0.243**	0.158**	0.265**	0.206**	0.252	0.146**	0.229**	0.121*	0.151**	0.220**	0.158**
BSS	0.326***	0.328***	0.220***	-	0.092**	0.080**	0.144**	0.305**	0.186**	0.172**	0.072*	0.169**	0.179**	0.194**
NW	0.252***	0.235***	0.101	0.08	-	0.101**	0.088*	0.198**	0.161**	0.130**	0.021	0.146**	0.104**	0.169**
GH	0.351***	0.361***	0.226***	0.098***	0.101***	-	0.152**	0.335**	0.201**	0.238**	0.121**	0.173**	0.208**	0.274**
LO	0.172***	0.175***	0.145***	0.158***	0.058	0.185***	-	0.149	0.061	0.103**	0.044	0.116**	0.068*	0.131**
WH	0.277	0.286	0.285	0.344	0.166***	0.324	0.149	-	0.201**	0.213**	0.149	0.207**	0.185	0.195**
WO	0.222***	0.268	0.105	0.179***	0.125***	0.188***	0.051	0.216	-	0.141**	0.098	0.137**	0.162**	0.148**
HN	0.282***	0.291***	0.193***	0.190***	0.107	0.252***	0.083	0.307	0.144***	-	0.055	0.206**	0.132**	0.089**
KF	0.197***	0.172***	0.072	0.079	0.025	0.147***	0.023	0.172	0.076	0.044	-	0.032	0.047	0.080*
HH	0.264***	0.213***	0.093	0.148***	0.091	0.149***	0.08	0.208	0.103	0.153***	0.021	-	0.064	0.166**
WA	0.244***	0.240***	0.157***	0.170***	0.086***	0.190***	0.064	0.226	0.132***	0.113***	0.042	0.054	-	0.160**
GL	0.258***	0.247***	0.129***	0.210***	0.126***	0.275***	0.102	0.245	0.132	0.094	0.057	0.126	0.135***	-

Significant values at the 0.05, 0.01 and 0.001 levels are denoted by *, ** and *** respectively.

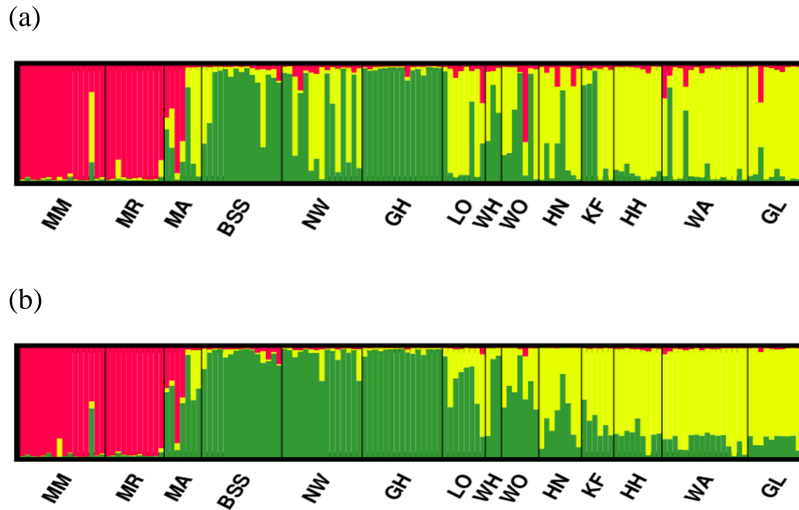

Figure 3-2 Genetic structuring in 14 bandicoot patches: (a) proportional membership (*q*) of each bandicoot individual to a genetic cluster identified by STRUCTURE; (b) proportional membership (*q*) of each bandicoot individual to a genetic cluster identified by TESS. Each vertical bar represents a bandicoot, and the length of each bar represents the probability of membership in each cluster (cluster 1 in red, cluster 2 in green, and cluster 3 in yellow).

Spatial scale of genetic differentiation

Isolation by distance (IBD) analyses showed significant and positive correlations between genetic distance ($\hat{a}$ statistic for individual level and Fst / (1 - Fst) for patch level) and geographical distance in the whole study area (individual level: r = 0.07, P = 0.028; patch level: r = 0.12, P = 0.011). The result for IBD was also significant when using D_{EST} as the measure of genetic distance (r = 0.19, P = 0.004). A partial Mantel test showed a significant correlation of genetic distance and intervening forest type (r = 0.16, P < 0.001), indicating there were generally higher genetic distances among individuals separated by plantation than those separated by native forest.

Spatial genetic autocorrelation analysis further illustrated the scale of genetic variation between individuals. An autocorrelogram with a distance class size of 0.5 km showed significantly positive genetic correlation values (r) for individuals up to 2.5 km, and significantly negative values only for a few classes above 5.5 km (Figure 3-3a). Genetic similarities between bandicoots generally decreased up to a distance of 2.5 km and then stabilised at a value near zero. The results of analyses using a larger distance class of 1.0 km showed a very similar pattern of correlations to that for the 0.5 km distance class (data not shown). The correlograms for both females and males showed similar patterns to that for the whole data set (Figure 3-3b and c).

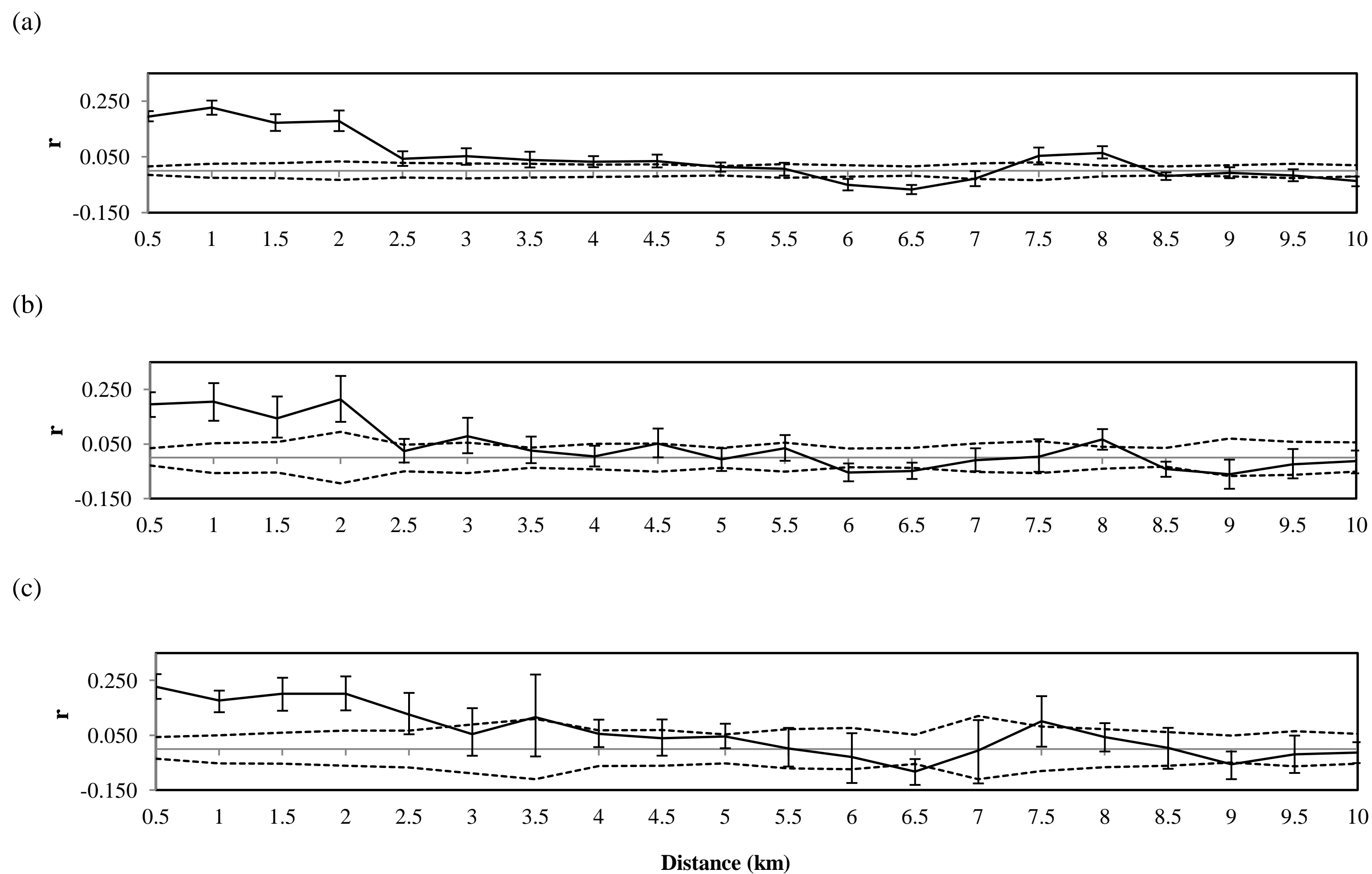

Figure 3-3 Correlograms showing genetic correlation (r) as a function of distance (0.5 km distance classes). The 95% confidence intervals (dashed lines) were determined by 1 000 permutations. Error bars of each estimate of r bound the 95% confidence intervals were determined by 1 000 bootstraps. (a) Whole data set; (b) Males only and (c) Females only.

Identification of migrants

A total of 24 individuals were identified as potential migrants (nine by STRUCTURE and 23 by GENECLASS, Table 3-3). Eight individuals were identified as migrants by both methods and were thus classified as such (Table 3-3).Of the remaining 16 individuals, 11 had a probability of >80% of belonging to their sampled patches in STRUCTURE and were thus classified as residents (Table 3-3). The remaining five individuals had *q*-values between 0.2-0.8 and were thus classified as individuals with mixed ancestry (Table 3-3) (Bergl & Vigilant 2007; Lecis *et al.* 2006; Reddy *et al.* 2012; Vaha & Primmer 2006). Six of the eight migrant events purportedly occurred between adjacent patches (samples 96, 117, 144, 307, 46, and 126) and two occurred as long distance dispersals [sample 61 (GL-KF, 5.5 km apart), and sample 141 (MM-GL, >20 km apart)].

Table 3-3 Results of migrants identified by STRUCTURE and GENECLASS analyses

Sample ID	Sampled patch	Sex	STRUCTURE probability to sampled patch	GENECLASS *P* value	likely origin patch in GENECLASS	Final migrant/admixture/resident classification	distance between origin patch and sampled patch* (km)
96	BSS	M	0.321	0.001	NW	MG	1.8
101	BSS	M	0.942	0.020	KF	RD	-
108	BSS	F	0.285	0.061	NW	AD	-
142	BSS	M	0.971	0.046	KF	RD	-
11	GH	F	0.851	0.006	MA	RD	-
42	GH	F	0.994	0.030	NW	RD	-
61	GL	M	0.217	<0.001	KF	MG	5.5
117	HH	F	0.025	0.003	KF	MG	1.6
144	HH	F	0.007	0.003	WA	MG	1.5
24	KF	Unknown	0.934	0.029	BSS	RD	-
73	LO	F	0.616	0.010	NW	AD	-
102	MA	M	0.92	0.018	HN	RD	-
137	MM	M	0.73	0.016	MR	AD	-
141	MM	M	0.007	0.002	GL	MG	>20
307	MM	F	0.105	0.005	MR	MG	0.5
12	MR	F	0.889	<0.001	MM	RD	-
103	MR	F	0.986	0.026	MM	RD	-
46	NW	F	0.438	0.001	BSS	MG	1.8
52	NW	M	0.935	0.035	WA	RD	-
53	NW	M	0.929	0.040	GH	RD	-
306	NW	M	0.784	0.004	LO	AD	-
69	WA	M	0.648	0.019	GL	AD	-
126	WH	F	0.179	<0.001	LO	MG	2
7	WO	M	0.869	0.012	WH	RD	-

MG, migrant; AD, admixture individual; RD, resident.

* The “distance between origin patch and sampled patch” was measured in ArcGIS 10 as straight-line distance (edge to edge).

Discussion

In the current study, we used 15 microsatellite markers to investigate the population structure and level of gene flow/dispersal of the endangered *I. o. obesulus* in the Mount Burr Range. We found significant genetic structure across the study region, with geographically proximate patches often being genetically more similar. Our results also revealed generally low levels of bandicoot dispersal and a tendency for dispersal usually between neighbouring patches only. While these analyses are not consistent with the hypothesis that native forest patches in this region are completely genetically isolated, they reveal that gene flow is limited to the extent that significant population genetic structure is evident at a fine spatial scale.

Our results incorporating both non-spatial (STRUCTURE) and spatial (TESS and BAPS) analyses revealed that the 14 patches formed three distinct genetic clusters or populations that generally corresponded to groups of proximate sampling localities. The results of genetic differentiation analyses also indicated that patches that were located closer together were less differentiated; suggesting gene flow was generally limited to proximate patches. However, sample numbers were small in some of these patches [e.g. WH (3) and KF (6)], so the lack of differentiation involving some of these sites could be because of a lack of power in the analyses.

In general concordance with the population structure analyses, there was only a small number (~8 to 13) of migrants identified, which further showed that effective dispersal of bandicoots was reduced and limited mostly to neighbouring patches (the two long-distance dispersals detected may have occurred via intermediate patches in a stepping-stone manner). In the spatial autocorrelation analyses, we did not detect any signature of sex-biased dispersal. The correlograms for the whole data set, and males and females individually all flattened out after 2.5 km, indicating that dispersal generally may not connect bandicoot populations from patches over distances longer than 2.5 km. This suggests that 2.5 km may be a threshold distance for dispersal of bandicoots in the Mount Burr Range, in agreement with findings from STRUCTURE/GENECLASS analyses where there was a tendency for migration between neighbouring patches. Short dispersal distances and a tendency to disperse between proximate patches was also observed for the common ringtail possum in the same region (Lancaster *et al.* 2011), and previously for *I. obesulus*'s close relative northern brown bandicoot (*I. macrourus*) in urban habitat fragments in Brisbane (Fitzgibbon *et al.* 2011), as well as several other mammals in fragmented landscapes (e.g. Bergl & Vigilant 2007; Peacock & Smith 1997; Taylor *et al.* 2007).

One possible explanation for the strong genetic differentiation of patches MM and MR in the northern part of the Mount Burr Range from other patches in the middle/south might be that they have been isolated for a longer time period. These two patches have been isolated from patches in

the middle region of the Mount Burr Range (henceforth referred to as “middle patches”) for almost 77 years, earlier than the time that most of the other patches were isolated from each other (see Table 3-1, Haywood, ForestrySA, 2012, data from historic aerial imagery). However, patch KF was isolated from eastern patches even earlier (over 80 years), but does not show significant genetic differentiation from its neighbouring patches (Table 3-2). It is notable that patch KF has a small sample size, which could weaken the power of our analyses to detect genetic differentiation. Nonetheless, the more closely located patches MM and MR (0.53 km) were genetically similar despite being separated from each other for over 70 years. In addition, these two patches did not show reduced genetic diversity compared with the other patches, suggesting gene flow was not limited between these two patches. These findings suggest that the extent of isolation of the patches (i.e. the distance between proximate patches), rather than the terms of historical isolation, may be more important for explaining the observed genetic structure. Indeed, patches MM and MR, located in the north of the Mount Burr Range, are geographically separated from the middle patches by a large distance of plantation (MM-BSS: ~ 6km) and agricultural land (MR-MA: ~ 4km), whereas the remaining patches are isolated from each other by relatively shorter distances (0.06-1.79km, about 1.1km on average) (Figure 3-1).

Detailed knowledge of dispersal capabilities of *I. obesulus* is currently limited. Although they are known to exhibit a pattern of juvenile dispersal, with newly independent bandicoots rapidly moving away from their birthplace (Heinsohn 1966; Stoddart & Braithwaite 1979), it is still unknown how far the juveniles disperse. Home range studies of the species have produced varying estimates of area, ranging from 0.5 to 9.0 ha, depending on gender and habitat structure (Broughton & Dickman 1991; Copley *et al.* 1990; Heinsohn 1966; Lobert 1990; McKenzie 1967; Moloney 1982; Paull 1993; Wilson 2004). *I. obesulus* is capable of moving hundreds of metres per day within their home range (Heinsohn 1966) and individuals have been shown to move short distances in cleared areas using vegetation along roadsides or watercourses as corridors (Paull 1993). In a study of the effect of prescribed burning on *I. o. obesulus* in South Australia, Long (2009) recorded post-fire movements of a male bandicoot of ~300 m out of its home range and 440 m back into its home range. A reintroduction study of the related but much smaller western barred bandicoot (*Perameles bougainville*, the smallest of the family Peramelidae, mean body weight 219 g) reported movements of up to 730 m in females and 4 km in males within a 12 km^2 conservation zone in Western Australia (Richards & Short 2003). Bowman *et al.* (2002) found that the vagility of mammals is better predicted from home range size than body size. Using Bowman’s (2002) method, O’Malley (2011) calculated the median dispersal distance (0.5-2.1 km) and the maximum dispersal distance (2.83-12 km) for *I. o. obesulus* based on its home range varying from 0.5 to 9.0 ha. Since most home range studies estimated a range from 0.5 to 5 ha, O’Malley (2011) concluded that *I. o. obesulus* has a median dispersal distance of 0.49 to 1.57 km, and a maximum dispersal distance of

2.83 km as an appropriate estimate of long distance dispersal. Our study concurs well with this, with our findings of detected migrants and admixture suggesting that bandicoot movement between proximate patches of up to a couple of kilometres apart is possible. It is notable that the presence of bandicoots in one of our studied patches (BSS), which was believed to be locally extinct for bandicoots according to a previous survey (ForestrySA, unpublished data, 2008), also confirms the bandicoots' capabilities of re-colonising patches separated by distances of 2 km.

Without a thorough comparison of population connectivity between continuous forest and fragmented habitat, it is difficult to verify that the current genetic structure we have observed in *I. o. obesulus* is the result of fragmentation versus an alternative hypothesis that *I. o. obesulus* normally shows isolation by distance over small spatial scales, sufficient to generate significant genetic differentiation across the landscape. Based on our findings and O'Malley's (2011) estimates of bandicoot dispersal distance, we would assume that *I. obesulus* would be mobile with the capability of moving several kilometres in natural continuous habitat (most likely achieved by dispersing juveniles). If this was the case then over the 20 $\times$ 26 km scale of the Mount Burr Range there would most likely be a single panmictic population. Hence the genetic structuring we have observed is likely to have resulted from fragmentation of the landscape. Indeed, fragmentation caused reduced population connectivity between isolated patches compared to a continuous native forest in the same region in southeast South Australia and western Victoria (37°30'S, 140°25'E to 38°00'S, 141°00'E) for an arboreal marsupial, the common ringtail possum (*P. peregrinus*) (Lancaster *et al.* 2011). Another landscape that includes a large component of pine plantations is the Tumut region in New South Wales, Australia. The impact of fragmentation that resulted from the development of pines on population connectivity in that region has been studied in a number of species [agile antechinus (*Antechinus agilis*), bush rat (*Rattus fuscipes*), greater glider (*Petauroides volans*) and two log-dwelling beetles(*Adelium calosomoides* and *Apasis puncticeps*)]. These studies all showed restricted gene flow and dispersal due to the pine matrix, despite the finding that individual species respond to fragmentation differently (Banks *et al.* 2005a; Banks *et al.* 2005b; Peakall *et al.* 2006; Schmuki *et al.* 2006; Taylor *et al.* 2007).

Implications for conservation management

In south-eastern South Australia, a primary conservation management action currently being implemented is the construction of biodiversity corridors (the Lower South-East Biodiversity Corridors Project) (Horn 2003). Twelve corridors were proposed in the Mount Burr Range to connect proximate patches, with none of them connecting northern-middle patches or middle-eastern patches (see Figure 3-1for locations of the twelve proposed corridors). In addition, two existing road reserves of native vegetation currently link MA with one northern patch (Overland Track Native Forest Reserve, not marked on map) (Horn 2003). Based on the current genetic

structure of the bandicoots in this area, we recommend adding more corridors to connect middle and eastern patches (e.g. between patch KF and HH). Our genetic analyses, conducted prior to the construction of these corridors, provide a baseline for assessing whether the corridors have successfully promoted gene flow among the patches (it is expected that most corridors will be completed by 2015 / 2016).

Acknowledgements

We would like to thank Bo Li, Amanda McLean, Jasmin Packer, Peter Hatcliffe, Clive Carlyle and Catherine Carlyle, Janet Tan, Samuel Clarke, Kyle Holland, Jess Rowe, Jamie Kohler, Paula Bertsch, Nathan Stavridis, Amy White, Andrew Wiewel, Inta Chambers, Anthony Hay, Toni Bellingham, Orazio Cultreri, and Ben Stevenson and Michelle Le Duff from the Department of Environment, Water and Natural Resources (DEWNR) for their excellent field assistance and thank Nicholas Fuller for his assistance with data processing. We also thank Troy Horn, Bryan Haywood and Robert Mengler from ForestrySA for their advice and assistance. This project was funded by the Australian Research Council Linkage grant (LP0668987), Native Vegetation Council, Wildlife Conservation Fund, and The Roy and Marjory Edwards Scholarship provided by Nature Foundation SA.

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Chapter Four: Population genetic structure of the endangered southern brown bandicoot (*Isoodon obesulus obesulus*) in a heavily modified landscape

You Li[1, 2], Steven J. B. Cooper[1, 2, 4], Melanie L. Lancaster[1, 2, 3], Jasmin G. Packer[1] and Susan M. Carthew[1, 5]

(1) School of Earth and Environmental Sciences, the University of Adelaide, Adelaide, SA, 5005, Australia

(2) Australian Centre for Evolutionary Biology and Biodiversity, the University of Adelaide, Adelaide, SA, 5005, Australia

(3) Healesville Sanctuary, Healesville, VIC, 3777, Australia

(4) Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA, 5000, Australia

(5) Research Institute for Environment and Livelihoods, Charles Darwin University, Darwin, NT, 0909, Australia

A photo showing fragmented native vegetation in the central Mount Lofty Ranges. Photo by Y. Li.

Statement of Authorship

Title of Paper	Population genetic structure of the endangered southern brown bandicoot (*Isoodon obesulus obesulus*) in a heavily modified landscape
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Publication Style

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	You Li		
Contribution to the Paper	Performed DNA extraction, PCR amplification and genetic analyses on all samples, interpreted data, wrote manuscript and acted as corresponding author.		
Signature		Date	20/02/2014

Name of Co-Author	Steven J. B. Cooper		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	23/8/2013

Name of Co-Author	Melanie L. Lancaster		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	28/08/2013

Name of Co-Author	Susan M. Carthew		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	23/8/13

Name of Co-Author	Jasmin G. Packer		
Contribution to the Paper	Provided samples and reviewed manuscript.		
Signature		Date	26/8/13

Abstract

Genetic connectivity is a key factor for maintaining the persistence of populations in fragmented landscapes. In highly modified landscapes such us urban areas, organisms' dispersal among fragmented habitat patches can be reduced due to the surrounding matrix, leading to subsequent decreased gene flow and increased potential extinction risk. However, few studies have compared within species how dispersal/gene flow varies between regions and among different forms of matrix that might be encountered. In the current study, we investigated gene flow and dispersal in an endangered marsupial, the southern brown bandicoot (*Isoodon obesulus obesulus*) in a heavily modified peri-urban landscape in South Australia, Australia. We used 14 microsatellite markers to genotype 284 individuals which were sampled from 15 sites. Analyses revealed significant genetic structure, with evidence that two individual sites were genetically isolated from other sites. Our analyses also indicated that dispersal was mostly limited to neighbouring sites, with a distance of 1 km being a likely threshold distance to bandicoots' dispersal. Comparisons of these results with analyses of a different population of the same species revealed that gene flow/dispersal was more limited in this peri-urban landscape than in a pine plantation landscape approximately 300 km to the south-east. These findings increase our understanding of how the nature of fragmentation can lead to profound differences in levels of genetic connectivity among populations of the same species.

Keywords: dispersal, gene flow, habitat fragmentation, southern brown bandicoot, Isoodon obesulus

Introduction

Habitat loss and fragmentation are the leading threats to biological diversity worldwide (Lindenmayer & Fischer 2006; Myers *et al.* 2000), and the rapid spread of urbanisation is a major driver of landscape degradation and fragmentation. In urban landscapes, once-continuous habitat is largely being replaced with fragmented remnants surrounded by a heterogeneous matrix of variable human constructs including buildings, roads, parks, gardens and even agricultural land in some peri- or semi-urban areas.

The importance of different types of matrices in fragmented landscapes has been recognised and their influences on biodiversity (e.g. isolation effects, being alternative or secondary habitats and regulating corridors and stepping stones) have been investigated in numerous studies (see review by Prevedello & Vieira 2010). In highly modified landscapes with complex matrices, movements of organisms and subsequent gene flow between habitat patches can be limited by landscape features such as roads, rivers or other matrices with unsuitable habitat (e.g. Clark *et al.* 2010; Levy

et al. 2010; Quemere *et al.* 2010). Reduced gene flow can lead to a range of consequences, including increased levels of inbreeding, loss of genetic diversity through genetic drift and potentially an increased extinction risk for remnant populations (Frankham 2002; Johansson *et al.* 2007). Thus, the degree to which organisms are able to move across a heterogeneous matrix is crucial for the persistence of populations in fragmented landscapes (Cushman 2006; Fahrig 2003; Lindenmayer *et al.* 2008; Lindenmayer & Fischer 2007).

In practice, the effects of matrix on organisms' movement between habitat patches are species-specific (Lindenmayer & Fischer 2007; Prevedello & Vieira 2010), and a species' dispersal capacity and genetic structure can vary depending on the type of matrix they encounter, their demographic history and the geographical location within which the organisms are distributed (e.g. within species, populations at lower latitudes tend to have greater genetic divergence than that of populations at higher latitudes within species; Martin & McKay 2004) (Jensen *et al.* 2013). Although a large body of work is now available on gene flow/dispersal capability between natural and anthropogenically modified landscapes (e.g. Banks *et al.* 2005; Dixo *et al.* 2009; Lancaster *et al.* 2011; Levy *et al.* 2010; Moore *et al.* 2011; Taylor *et al.* 2007), few studies have investigated how different forms of fragmentation with distinct matrices influence patterns of population connectivity within the same species (but see Arens *et al.* 2007; Berry *et al.* 2005). Such studies are useful because they improve our understanding of dispersal dynamics and help with decision-making for management (i.e. identifying priority areas for habitat restoration) in the case of species requiring conservation management.

Southern brown bandicoot (*Isoodon obesulus*) populations in South Australia represent an ideal system to explore this issue. *I. obesulus* is a rabbit-sized ground-dwelling marsupial, which has dramatically declined in number over the last 220 years, with studies providing evidence for a contracted distribution and local population extinctions (Coates *et al.* 2008; Department of Environment and Conservation 2006; Paull 1993, 1995). The subspecies *I. o. obesulus,* which is the focus of this study, is listed as Nationally Endangered (Australian Environment Protection and Biodiversity Conservation Act 1999). In South Australia, *I. obesulus* is the only surviving member of the eight Australian species of the family Peramelidae.

Three current strongholds of *I. o. obesulus* persist in South Australia (SA) – the Mount Lofty Ranges, the south-east region and Kangaroo Island. Current records for south-east SA indicate that *I. o. obesulus* is only located in the Green Triangle Forest region managed by a state government-based organisation (ForestrySA). This region is also one of Australia's major softwood plantation regions. The Green Triangle Forest region consists of numerous individual patches of native forest reserves embedded in matrices comprised of pine (*Pinus radiata*) plantations or agricultural land. Genetic connectivity of *I. o. obesulus* was previously investigated in this landscape (the Mount

Burr Range) with results showing significant population genetic structuring and restricted gene flow and dispersal to neighbouring patches, with 2.5km being identified as a likely dispersal threshold (Li *et al.*, in prep., see Chapter Three).

The Mount Lofty Ranges are another stronghold of *I. o. obesulus*. With its biodiversity richness, it was identified as one of the 15 Australian biodiversity hotspots by the Commonwealth Government in 2003. However, the region has experienced extensive native vegetation clearance and only 13% of the original vegetation remains (Department for Environment and Heritage 2009). The Mount Lofty Ranges region is highly fragmented with few relatively intact areas and variable amounts of degraded native vegetation embedded in a heterogeneous matrix of urban and agricultural land uses.

This study aimed to investigate population structure and the extent of gene flow of *I. o. obesulus* in the central Mount Lofty Ranges. Results obtained here were then compared to the study of gene flow in *I. o. obesulus* in the Mount Burr Range (Li, 2013, in prep., Chapter Three) to examine connectivity of bandicoot populations in different matrix systems. We propose a hypothesis that the region will be genetically structured due to reduced dispersal and gene flow through the matrix.

Methods

Study area

We surveyed 15 sites within the distribution of *I. o. obesulus* in the central Mount Lofty Ranges for sampling (Figure 4-1 and Table 4-1). At each site, traps were set as two parallel transects with 10 cage traps per transect. Cage traps (55 $\times$25 $\times$25 cm treadle) were covered with plastic and hessian bags to protect animals from rain and sun, bedded with small pieces of hessian to keep animals warm, and baited with a mixture of peanut butter and oats. Traps were set and checked in the morning and late afternoon during summer, autumn and spring and in the morning during winter. Captured bandicoots were processed on site. After measuring body weight and checking reproductive condition, a small notch of skin was removed from the ear for individual identity and stored in a 50: 50 solution containing ethanol and saline for genetic analysis. A total of 284 bandicoot tissue samples were collected during 11 field trips between 2008 and 2011.

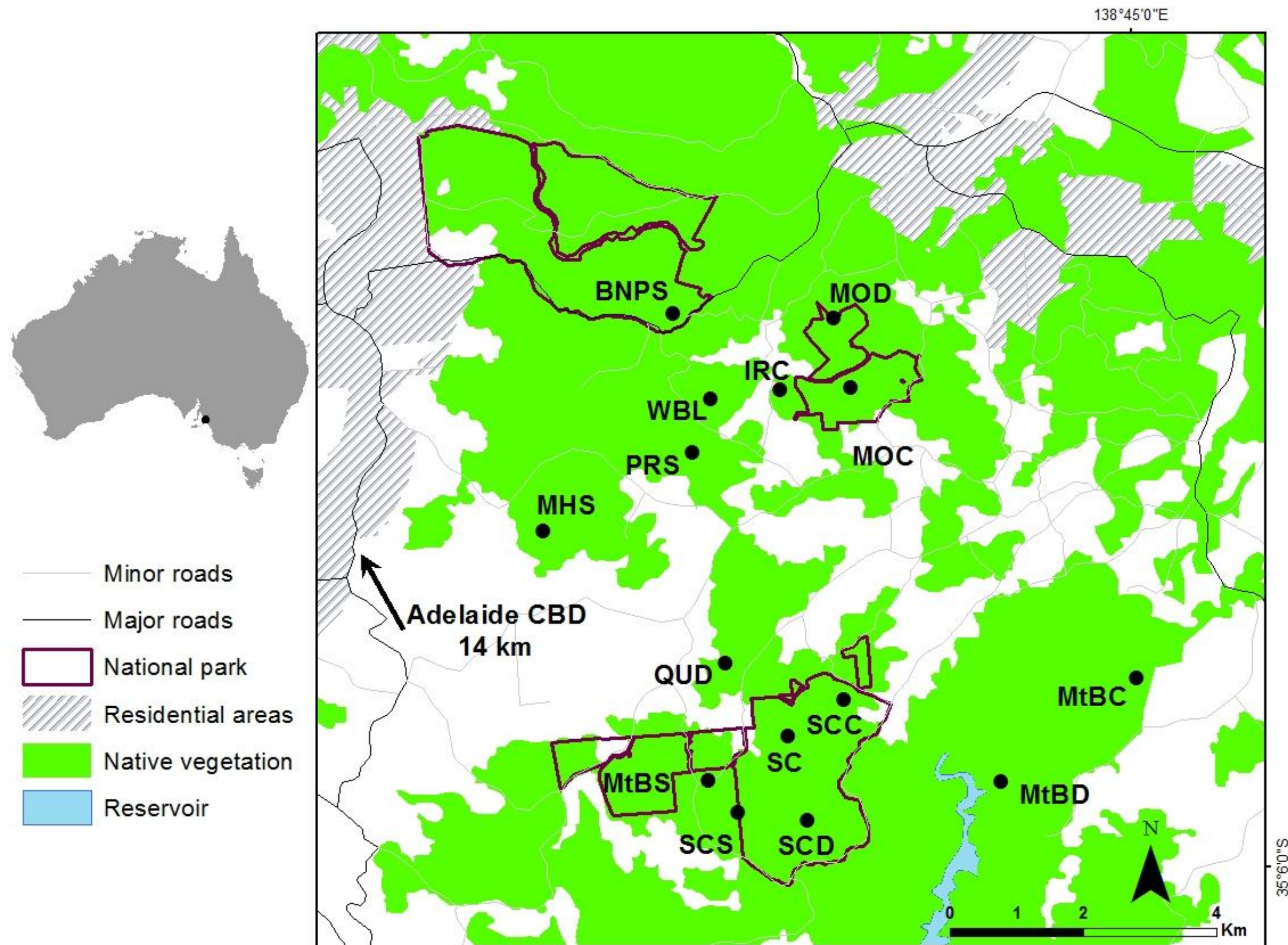

Figure 4-1 Detailed map of the study sites in the central Mount Lofty Ranges of South Australia, Australia. Outlined areas in the map denote three national parks in our study area (Belair National Park, Mark Oliphant Conservation Park and Scott Creek Conservation Park). White areas denote agricultural land. Sampled sites were marked with dark dots and labelled with letters (see Table 4-1 for full names).

Table 4-1 Sampling information and genetic diversity parameters for 15 sites

Site name	Site abbreviation	Distance to nearest sampled site* (km)	N	H_O	H_E	F_{IS}	A	AR	IR
Belair National Park	BNPS	1.39	43	0.371	0.393	0.057	2.29	1.610	0.485
Ackland Hill Rd Coromandel (Mud Hut)	MHS	2.53	41	0.497	0.502	0.010	3.64	1.961	0.206
Pole Rd	PRS	0.92	17	0.407	0.495	0.183	3.36	2.030	0.310
Wirra Birra low	WBL	0.92	12	0.529	0.546	0.032	2.93	2.054	0.173
Ironbank Rd	IRC	1.07	15	0.464	0.550	0.163	3.50	2.017	0.314
Mark Oliphant Conservation Park Site 1	MOD	1.08	17	0.532	0.540	0.016	2.93	1.950	0.179
Mark Oliphant Conservation Park Site 2	MOC	1.07	22	0.475	0.550	0.141	3.14	2.041	0.238
Dorset Vale Road	QUD	1.45	26	0.463	0.567	**0.186**	4.14	2.208	0.241
Mount Bold Reserve Site 1	MtBS	0.63	19	0.422	0.477	0.118	3.57	1.932	0.333
Scott Creek Conservation Park Site 1	SCS	0.63	11	0.607	0.581	-0.047	2.93	2.191	0.042
Scott Creek Conservation Park Site 2	SC	0.97	15	0.412	0.576	**0.293**	3.93	2.257	0.337
Scott Creek Conservation Park Site 3	SCC	0.97	8	0.658	0.530	**-0.264**	2.86	2.007	-0.040
Scott Creek Conservation Park Site 4	SCD	1.05	8	0.532	0.578	0.086	3.07	2.206	0.183
Mount Bold Reserve Site 2	MtBC	2.59	12	0.446	0.520	0.147	3.29	2.084	0.263
Mount Bold Reserve Site 3	MtBD	2.59	18	0.530	0.567	0.070	3.36	2.108	0.194

Sample size (N), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), allelic diversity (A), allelic richness (AR), and internal relatedness (IR).

Significant F_{IS} values were denoted in bold. $P < 0.05$.

* The "distance to nearest sampled site" was measured in ArcGIS 10 as straight-line distance.

DNA extraction and genotyping

DNA was isolated using the Gentra Puregene extraction kit, following the manufacturer's instructions (Gentra Systems Inc.). All individuals were genotyped at 14 microsatellite loci which had been developed for *I. obesulus*: five (B3-2, B15-1, B20-5, B34-2, and B38-1) developed by Zenger & Johnston (2001), and nine developed by Li *et al.* (2013). PCR amplifications followed the protocols in Li *et al.* (2013). Approximately 10% of all samples (n = 284) were genotyped twice at all 14 loci to check error rates. We expressed error rates as the number of errors per allele, which was calculated as the number of incorrect alleles divided by the total number of genotyped alleles (Hoffman & Amos 2005). Repeat genotyping error rate was very low, with an average of 0.0009 across all loci. Amplified products were run on an ABI 3730 DNA Analyser and alleles were scored using GeneMapper 4.0 (Applied Biosystems).

Microsatellite analyses

Before pooling genotyping data across years, the variation in allele frequencies of the same site (e.g. site BNPS) across four years (2008-2011) was examined. No significant variation in allele frequencies (observed heterozygosity, H_O) was found for site BNPS (ANOVA, $F [3, 52] = 0.253$, $P = 0.859$). Other sites that had multiple year sampling were also checked for the variation of allele frequencies between years, and no significant variation was found (data not shown). We were therefore confident to pool data across years and conducted further analyses.

Conformation with Hardy-Weinberg equilibrium (HWE) across loci and sites was assessed in Genepop 4.1.0 (Raymond & Rousset 1995) and a test for linkage disequilibrium among loci was conducted in Arlequin 3.11 (Excoffier & Lischer 2010). MICRO-CHECKER v. 2.2.3 (Van Oosterhout *et al.* 2004) was used to estimate null allele frequencies. Sequential Bonferroni corrections (Rice 1989) were applied to adjust significance values for multiple comparisons.

Genetic diversity

Number of alleles per locus (A), observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated in Arlequin 3.11 (Excoffier & Lischer 2010), and inbreeding coefficient (F_{IS}) and allelic richness (AR, corrected for sample size) were estimated in FSTAT 2.9.3.2 (Goudet 2001). An estimate of parental relatedness was calculated using internal relatedness (IR) (Amos *et al.* 2001) in an R extension package, Rhh (Alho *et al.* 2010).

Genetic differentiation and population structure

To assess the degree of genetic differentiation of bandicoots across sites, we measured pairwise F_{ST} in Arlequin 3.11 (Excoffier & Lischer 2010) and also calculated pairwise D_{EST} as a measurement of

actual differentiation in the package DEMEtics (Gerlach *et al.* 2010) implemented in R, with 1 000 bootstrap iterations to determine statistical significance. The estimate of D_{EST} takes account of the effective number of alleles and may perform better than F_{ST} in the case of highly polymorphic markers such as microsatellites (Meirmans & Hedrick 2011).

We implemented Bayesian clustering analysis in STRUCTURE 2.3.3 (Pritchard *et al.* 2000) and TESS 2.3.1 (Chen *et al.* 2007; Francois *et al.* 2006) to characterize population genetic structure. STRUCTURE uses non-spatial Bayesian algorithm, while TESS incorporates spatial information into the analysis and thus increases the power of modelling genetic structure (Corander *et al.* 2008). The analysis in STRUCTURE used an admixture model with correlated allele frequencies, with a burn-in of 100 000 and 100 000 MCMC steps after the burn-in. The value of K was set from 1 to 15 with ten replicates of each K. STRUCTURE HARVESTER 0.6.92 (Earl & vonHoldt 2012) was used to determine the most likely K, using the *ad-hoc* ΔK method described in Evanno *et al.* (2005). For TESS, we ran the analysis under an admixture model using K ranging from 2 to 15 (10 replicates per K), with 10 000 burn-in and 50 000 sweeps. The value of the interaction parameter ψ (the strength of the spatial autocorrelation) was set to the default value, 0.6. The optimal K for TESS was chosen as the one with the stabilized value of the Deviance Information Criterion (DIC). For both analyses, CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) was used to average the membership probabilities for the ten runs of the most likely K and DISTRUCT 1.1 (Rosenberg 2004) was used to display the averaged results.

Spatial scale of genetic differentiation

To investigate the effect of isolation by distance (IBD), we ran Mantel tests (at both individual and site level) between linearised genetic distance [F_{ST} / (1-F_{ST}) and D_{EST} / (1- D_{EST})] and the logarithm of geographical distance using the subprogram Isolde of Genepop 4.1.0 (Raymond & Rousset 1995) with 10 000 permutations.

Spatial autocorrelation analyses were also performed in GenAlEx 6.41 (Peakall & Smouse 2006) to further study the spatial scale of genetic variation. We used 0.5 km as the distance class size and ran the analysis for males (n = 149) and females (n = 114) separately to check for signs of sex-biased dispersal (21 samples with unknown gender information were excluded). Statistical testing of the analysis was based on the 95% confidence interval defined by 1 000 random permutations. Under limited gene flow, populations should show positive spatial genetic structure at short distances if sampling has covered the spatial scale of genetic structure (Smouse & Peakall 1999) and the correlograms should flatten out at the scale where gene flow is not connecting subpopulations (Aars *et al.* 2006; Gauffre *et al.* 2008).

Identification of migrants

We used GENECLASS 2.0 (Piry *et al.* 2004) to identify first-generation migrants, using a Bayesian approach (Rannala & Mountain 1997) and the Monte Carlo re-sampling method of Paetkau *et al.* (2004) with 10 000 simulated individuals and an alpha of 0.05. In GENECLASS, we used the likelihood ratio L_home (the likelihood of a given individual being from the population where it was sampled) because it is more appropriate than other estimations if not all source populations were sampled (Paetkau *et al.* 2004).

Results

Genetic variability

Significant linkage disequilibrium was detected in 36 of the 1365 (2.6%) pairwise locus combinations and none of these were consistent across sites. Close physical linkage between any of the 14 loci was therefore considered unlikely. Twenty-five of the 210 locus $\times$ sites tests departed significantly from HWE after sequential Bonferroni correction, involving six loci (Ioo7, Ioo5, Ioo4, Ioo3, Ioo2, B15-1, B20-5 and B3-2). Three loci (Ioo7, Ioo3 and B3-2) deviated from HWE at more than two sites. Micro-checker detected that these three loci might contain null alleles. However, the presence of null alleles at these loci was not consistent across sites. In addition, deviations from HWE can result from inbreeding or the Wahlund effect (the reduction of heterozygosity due to population subdivision). To be cautious, we ran all the analyses (F_{ST}, clustering analysis, Mantel test, spatial autocorrelation) without the three loci and the results showed a similar pattern to that found for the full 14 locus data set. For this reason, we did not apply a correction for null alleles and retained the three loci for the analyses presented here.

Number of alleles ranged from 3 (Ioo6, Ioo16, and B38-1) to 11 (Ioo5) with an average number of 6.2. At each site, mean observed heterozygosity across loci ranged from 0.371 (site BNPS) to 0.658 (site SCC) and expected heterozygosity ranged from 0.393 (BNPS) to 0.581 (SCS) (see Table 4-1 for site codes and heterozygosity values). F_{IS} values ranged from -0.264 in SCC to 0.293 in SC (Table 4-1). Allelic diversity (the average number of alleles per locus, *A*) was lowest in BNPS (2.29) and highest in QUD (4.14), with a mean number of 3.26 (Table 4-1). Allelic richness ranged from 1.610 (BNPS) to 2.257 (SC) (Table 4-1). Statistical tests showed no evidence of variation in H_O, H_E and *AR* among all sites (ANOVA, F [14, 195] = 1.339, 1.542, and 1.277, P = 0.188, 0.099 and 0.225 respectively). Internal relatedness (*IR*) was highest in BNPS (0.485) and lowest in SCC (-0.040) (Table 4-1). Post hoc tests showed that bandicoots in BNPS had significantly higher values of *IR* when compared to the bandicoots from nine other sites (Tukey HSD, *P* value: BNPS-MHS <0.001, BNPS-MOC 0.001, BNPS-MOD <0.001, BNPS-MtBD <0.001, BNPS-QUD 0.001, BNPS-SCC <0.001, BNPS-SCD 0.023, BNPS-SCS <0.001, BNPS-WBL 0.001). *IR* in SCC was

significantly lower than five other sites (Tukey HSD, *P* value: SCC-BNPS <0.001, SCC-IRC 0.016, SCC-PRS 0.014, SCC-MtBS 0.004, SCC-SC 0.006).

Table 4-2 Pairwise F_{ST} values (below diagonal) and pairwise D_{EST} values (above diagonal) for the 15 bandicoot sites (following Sequential Bonferroni correction)

	BNPS	MHS	PRS	WBL	IRC	MOD	MOC	QUD	MtBS	SCS	SC	SCC	SCD	MtBC	MtBD
BNPS	-	0.481***	0.327***	0.187***	0.439***	0.372***	0.355***	0.415***	0.445***	0.314***	0.367***	0.368***	0.406***	0.384***	0.373***
MHS	0.412***	-	0.195***	0.353***	0.237***	0.211***	0.149***	0.216***	0.349***	0.333***	0.322***	0.300***	0.207***	0.253***	0.374***
PRS	0.365***	0.096***	-	0.172***	0.194***	0.212***	0.130***	0.335***	0.411***	0.333***	0.301***	0.367^{NA}	0.337***	0.135***	0.352***
WBL	0.257***	0.211***	0.121***	-	0.164***	0.213***	0.201***	0.319***	0.343***	0.255***	0.229***	0.331***	0.361***	0.255***	0.351***
IRC	0.420***	0.178***	0.111***	0.091***	-	0.168***	0.190***	0.251***	0.325***	0.447***	0.306***	0.312***	0.342^{NA}	0.257***	0.428***
MOD	0.329***	0.179***	0.129***	0.089***	0.139***	-	0.084***	0.253***	0.327***	0.297***	0.353***	0.313^{NA}	0.291^{NA}	0.251***	0.269***
MOC	0.344***	0.117***	0.069***	0.116***	0.155***	0.065***	-	0.313***	0.364***	0.277***	0.334***	0.328^{NA}	0.301^{NA}	0.213***	0.300***
QUD	0.361***	0.159***	0.193***	0.181***	0.182***	0.153***	0.210***	-	0.121***	0.245***	0.174***	0.149***	0.094***	0.244***	0.233***
MtBS	0.418***	0.234***	0.263***	0.201***	0.230***	0.194***	0.254***	0.065***	-	0.272***	0.128***	0.122***	0.184***	0.290***	0.289***
SCS	0.367***	0.224***	0.210***	0.189***	0.277***	0.151***	0.170***	0.145***	0.214***	-	0.180***	0.220***	0.208***	0.216***	0.196***
SC	0.371***	0.171***	0.194***	0.148***	0.190***	0.180***	0.202***	0.055***	0.054***	0.115***	-	0.126***	0.159***	0.223***	0.300***
SCC	0.339***	0.205***	0.224***	0.168***	0.237***	0.189***	0.195***	0.093***	0.097***	0.127***	0.041	-	0.061^{NA}	0.289***	0.216***
SCD	0.358***	0.141***	0.188***	0.157***	0.197***	0.122***	0.155***	0.032	0.122***	0.116***	0.055	0.051	-	0.293***	0.189^{NA}
MtBC	0.415***	0.142***	0.062*	0.200***	0.183***	0.159***	0.126***	0.153***	0.215***	0.157***	0.142***	0.175***	0.176***	-	0.275***
MtBD	0.309***	0.190***	0.179***	0.195***	0.258***	0.115***	0.136***	0.146***	0.218***	0.085***	0.180***	0.144***	0.104***	0.155***	-

*** 0.01 significance level

*0.05 significance level

NA = not available

Genetic population structure and gene flow

Pairwise F_{ST} values were significant for 101 of the 105 comparisons (Table 4-2). The highest pairwise F_{ST} was between BNPS and IRC ($F_{ST} = 0.420$, $P < 0.001$), and the lowest value was between QUD and SCD ($F_{ST} = 0.032$, $P = 0.050$). Pairwise D_{EST} values were generally higher than F_{ST}, with the highest value between BNPS and MHS ($D_{EST} = 0.481$, $P < 0.001$) and the lowest between SCC and SCD ($D_{EST} = 0.061$, no available P value). Mantel tests showed a significant association between genetic distance and geographical distance [measured as logarithm of (1 + geographical distance)] at both individual and site level (individual level: $\hat{a}$ statistic, $P < 0.001$; site level: F_{ST}, $P < 0.001$, D_{EST}, $P < 0.001$).

Using the program STRUCTURE, three clusters ($K = 3$) were identified based on the ΔK criteria (Evanno *et al.* 2005). Samples from BNPS grouped in cluster 1(hereafter 'BNPS cluster'), MHS, PRS, WBL, IRC, MOC, MOD and MtBC in cluster 2, and QUD, MtBS, SCS, SC, SCC, SCD, and MtBD in cluster 3 (hereafter 'southern cluster') (Figure 4-2a). Seventy-five percent of the individuals were assigned with a probability > 80% to one of the three clusters.

In the Bayesian clustering analysis computed by the program TESS, five clusters ($K = 5$) were identified, with the BNPS cluster and the southern cluster being identical to that found using STRUCTURE (Figure 4-2b). In TESS, samples from MHS were distinct and grouped in a separate cluster (hereafter 'MHS cluster') (Figure 4-2b). The fourth cluster contained samples from PRS, WBL, IRC, MOC, MOD and MtBC (hereafter 'northern cluster'). The fifth cluster detected in TESS contained only three individuals and it was not specific to any site. This cluster was therefore disregarded. Because MHS was clustered with six other sites in STRUCTURE but not in TESS, a second STRUCTURE run was then conducted using the data set containing sites MHS, PRS, WBL, IRC, MOC, MOD and MtBC. The analysis provided high support for $K = 2$, where MHS was distinct from other sites, as indicated in TESS (Figure 4-2c). We thus referred to $K = 4$ as the most likely number of genetic clusters in our dataset: the BNPS cluster, the MHS cluster, the northern cluster (PRS, WBL, IRC, MOC, MOD and MtBC) and the southern cluster (QUD, MtBS, SCS, SC, SCC, SCD, and MtBD).

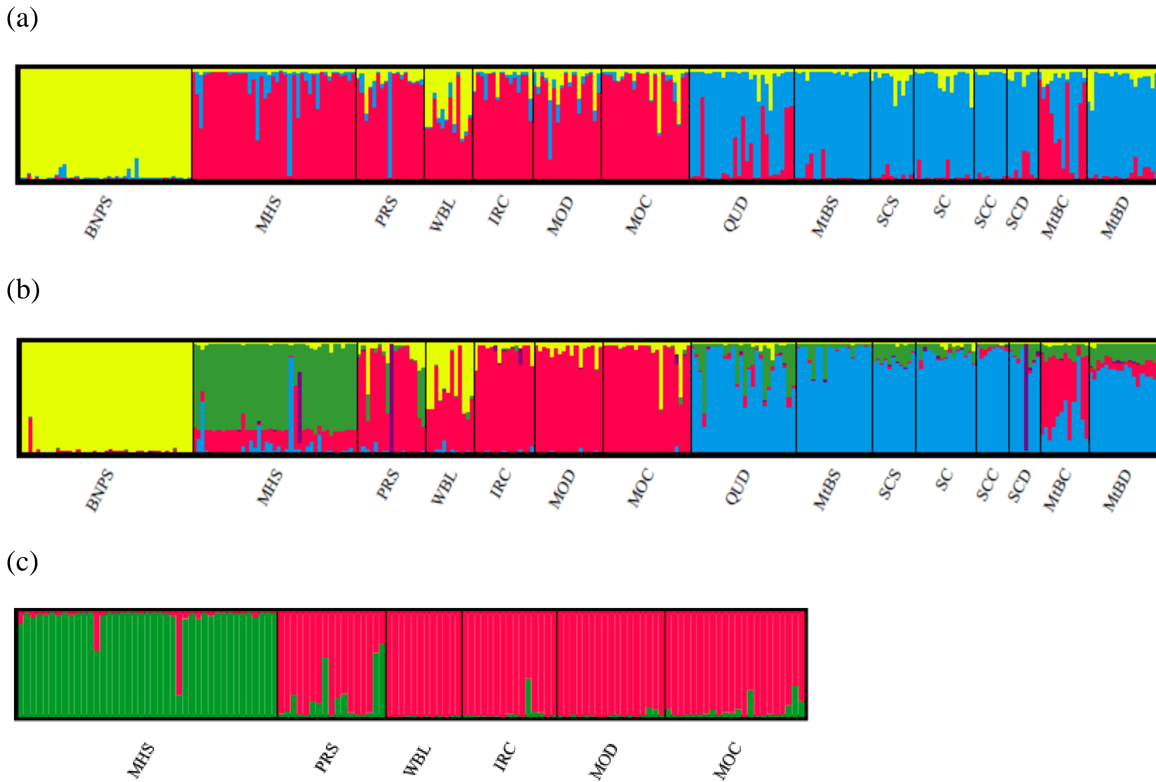

Figure 4-2 Genetic structure of 15 bandicoot sites. Proportional membership (*q*) of each bandicoot individual to a genetic cluster for the whole data set: (a) identified by STRUCTURE, (b) identified by TESS. Each vertical bar represents a bandicoot, and the length of each bar represents the probability of membership in each cluster (cluster 1 in yellow, cluster 2 in green, cluster 3 in red and cluster 4 in blue); (c) proportional membership (*q*) of each bandicoot individual to a genetic cluster identified by the second STRUCTURE run, using the data set containing sites MHS, PRS, WBL, IRC, MOC, MOD and MtBC.

For the whole data set (males and females together), spatial autocorrelation analysis revealed a significant and positive correlation for individuals up to 1 km at 0.5 km distance classes and the genetic similarities (r) then stabilized at a value around zero (Figure 4-3a). When analysed separately, males and females showed a similar relationship to each other and to the whole dataset (Figure 4-3b, c).

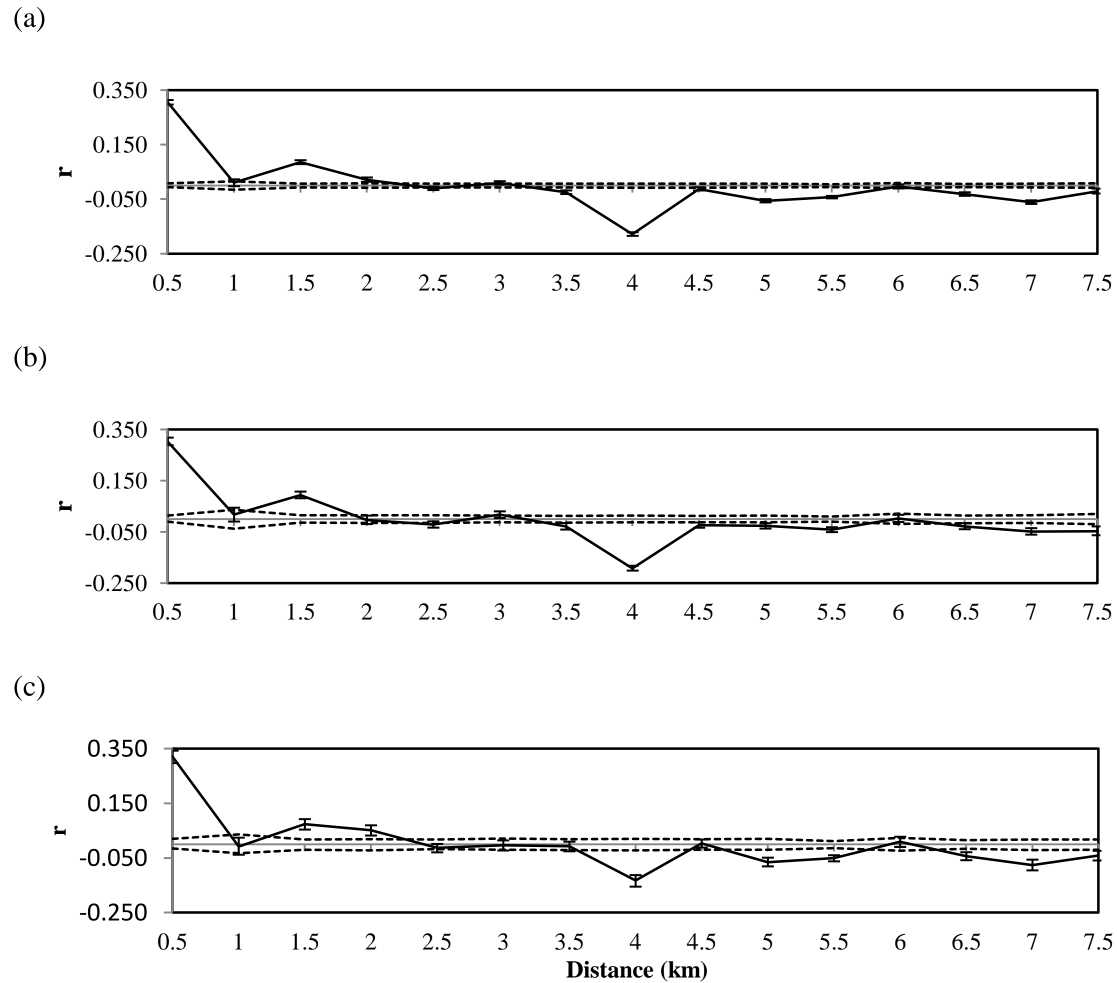

Figure 4-3 Correlograms showing genetic correlation (r) as a function of distance (0.5 km distance classes). The 95% confidence intervals (dashed lines) were determined by 1 000 permutations. Error bars of each estimate of r bound the 95% confidence intervals were determined by 1 000 bootstraps. (a) Whole data set; (b) Males only (n = 149) and (c) Females only (n =114).

Assignment tests computed by GENECLASS identified 13 first-generation migrants (5.4% of all individuals; Table 4-3). Of the 13 detected migrants, 11 occurred as short distance migrations (1-2 km; samples 595, 504, 833, 624, 742, 601, 675, 645, 648, 661, and 748), and two were examples of longer distance dispersal (~6 km, samples 635 and 794).

Table 4-3 Results of first generation migrants identified by GENECLASS

Sample ID	Sex	Sampled site	Origin site identified by Geneclass	Distance between origin site and sampled site (km)	GENECLASS *P* value
595	M	WBL	MOC	2.1	0.0102
504	F	IRC	PRS	1.6	0.0185
833	F	MOD	MOC	1.1	0.0137
624	M	MOC	PRS	1.1	0.0082
742	M	MOC	WBL	2.1	0.015
601	M	QUD	SCD	2.7	0.0149
675	F	QUD	SC	1.4	0.0026
645	F	MtBS	SC	1.4	0.0204
648	M	MtBS	SC	1.4	0.03
661	M	MtBS	SC	1.4	< 0.001
748	F	SCC	SC	1	0.0056
635	M	MtBC	QUD	6.2	0.0225
794	F	MtBC	MtBS	6.6	< 0.001

Discussion

In the current study, we used 14 microsatellite markers to investigate the population structure and level of gene flow and dispersal of the endangered *I. o. obesulus* in the central Mount Lofty Ranges. We found significant genetic structure in a relatively small geographic region that is highly modified and fragmented. The results revealed generally low levels of bandicoot dispersal and a tendency for dispersal usually between neighbouring sites. These analyses are consistent with the hypothesis we proposed that gene flow is severely limited to the extent that significant population genetic structure is evident at a fine spatial scale. In addition, the results we obtained here were compared to the genetic patterns (gene flow, population genetic structure and dispersal threshold) of the same species in another fragmented forest system (the Mount Burr Range in the south-east of South Australia).

Bayesian clustering analysis revealed that the 15 sites formed four distinct genetic clusters or populations, with two individual sites (BNPS and MHS) being genetically isolated from other sites. The results of genetic differentiation analyses also suggested gene flow was limited across the landscape. In agreement with this, no bandicoots were observed moving among sites that were grouped into different genetic clusters. The 13 genetically detected first generation migrants were restricted to moving between neighbouring sites of the same population cluster (the two long-

distance dispersals detected may have occurred via intermediate sites in a stepping-stone manner). Our spatial autocorrelation analyses suggest a 1-km threshold distance to dispersal of the bandicoot in the central Mount Lofty Ranges. Evidence for short distance dispersal and a tendency of dispersing only between proximate sites was also observed for the Mount Burr (south-east South Australia) population of *I. o. obesulus* in a fragmented forest system (2.5 km threshold distance, Li *et al.*, in prep, see Chapter Three).

Scott Creek Conservation Park (712 ha, sites SCC, SC and SCD are located within this park) is one of the three national parks in our study area, but much larger than Mark Oliphant (189 ha, containing sites MOC and MOD). It is one of the few relatively intact native vegetation areas in the Mount Lofty Ranges, with more homogenous and denser vegetation sites compared to the native vegetation surrounding the park. Compared to other studied sites, the three sites within Scott Creek Conservation Park showed non-significant pairwise F_{ST}, lower individual relatedness and evidence for more migration events (five bandicoots) out of the park. Large patches are considered important and critical in fragmented landscapes because they can reduce extinction proneness of populations of individual species, and increase species richness, vegetation diversity and immigration rates (Lindenmayer *et al.* 2008; Simberloff 1988). Our results suggest that Scott Creek Conservation Park may be a source population for dispersal and it should therefore be considered as a high priority for conservation.

By comparing our results to the previous study of population genetic structure and gene flow of *I. o. obesulus* from Mount Burr in the south-east of South Australia (Li *et al.*, in prep., see Chapter Three), we found that the populations in the central Mount Lofty Ranges were genetically structured over a much smaller spatial scale (~ 80 km^2) than the south-east populations (~520 km^2). Given the results from Mount Burr, where dispersal was detected between sites up to 2.5 km apart, we predicted that the scale of genetic differentiation at Mount Lofty Ranges would be similar or even lower than that of Mount Burr. Yet, we observed that sites appear to be genetically isolated at an even higher level than the Mount Burr population at a similar spatial scale, and found a shorter dispersal threshold (1 km). This suggests that gene flow/dispersal was limited to a higher degree in the current study compared to the Mount Burr study. Indeed, the landscape of the Mount Lofty Ranges has been heavily modified, with a matrix mixture of urban constructs and agricultural land and heterogeneous native vegetation with various level of degradation. The habitat within native forest fragments at Mount Burr is generally less disturbed and relatively more homogenous, with *Pinus radiata* plantations being the dominant matrix. Moreover, according to our findings, it is likely that the bandicoots may better utilise the pine forest to move among forest fragments. First, pine plantations can be used as habitat by a range of invertebrate taxa since they provide shelter and moist microhabitats due to plantation practices such as windrowing, mound ploughing, pruning and thinning (Bonham *et al.* 2002; Lindenmayer & Hobbs 2004). In particular, beetle species, a

major dietary food for *I. obesulus* (Jones 1924; Opie 1980; Quin 1988), have been found in pine plantations at greater levels of taxon diversity than in the native eucalypt forests (Neumann 1979). Hence, bandicoots are likely to enter pine plantations from adjacent patches for foraging. Second, accumulated fallen debris such as tree stumps and bark may provide better shelter for *I. obesulus* when dispersing in pine plantations, compared to open agricultural land. Moreover, the central Mount Lofty Ranges have greater traffic volumes than that in the Mount Burr Range (assessed based on Annual Average Daily Traffic estimates, produced by Road Asset Management Section, Government of South Australia), which can cause more traffic mortalities of *I. obesulus*. Deceased bandicoots along roads in the Mount Lofty Ranges have often been observed by local residents and staff of Department of Environment, Water, and Natural Resources (DEWNR). Another possible factor that could affect the dispersal of bandicoots in the two landscapes is predation by introduced animals (red fox and feral cat). However, to date it is not known whether the central Mount Lofty Ranges have more predators than the Mount Burr Range, though cats, in particular, are likely to be more prevalent given the higher number of dwellings and human population density in the Mount Lofty Ranges compared to the Mount Burr Range. Nonetheless, the more genetically structured population of *I. obesulus* in the Mount Lofty Ranges may be the result of a lower permeability of the matrix in this landscape. Similar findings that a matrix with poorer permeability leads to a lower level of gene flow among populations of the same species have been reported for other species, e.g. the Moor frog (*Rana arvalis*, Arens *et al.* 2007), the grand skink (*Oligosoma grande*, Berry *et al.* 2005) and a wing-dimorphic bush cricket (*Metrioptera bicolour*, Heidinger *et al.* 2013). More sophisticated analyses on the same dataset (i.e. using landscape genetic approaches) are needed to further investigate how different matrices affect genetic connectivity of *I. o. obesulus* populations.

The poor permeability of the matrix in the Mount Lofty Ranges may also explain why BNPS and MHS are the most genetically isolated sites. Site BNPS also had the highest pairwise F_{ST} and D_{EST} and the highest internal relatedness which suggests that inbreeding may be occurring. MHS is separated from other bandicoot locations by large areas of cleared (agriculture) land (Figure 4-1), and dispersal of bandicoots from this site could be greatly restricted. For site BNPS, although it is seemingly embedded in native vegetation, the native vegetation beyond to the south of the sampling location appears to be too sparse to be suitable bandicoot habitat (aerial image, DEWNR, http://www.naturemaps.sa.gov.au). In addition, a road with high traffic volume adjacent to BNPS could also be a major barrier to dispersal of *I. o. obesulus*. Our results indicate that these sites are more vulnerable to local extinction and therefore should be managed with a specific strategy that enhances genetic variation within them (see below).

In the Mount Burr study, the inbreeding coefficient (F_{IS}) of the 14 patches ranged from -0.028 to 0.461 with about half of the values being significant (see Chapter Three). In the Mount Lofty

Ranges, F_{IS} values of the 15 sites ranged from -0.264 to 0.293 with generally lower values than the previous study and with only two values being significantly positive. This observation of heterozygosity deficiencies in the Mount Burr study might indicate the presence of non-random mating within the patches. Under the assumption that bandicoots in the Mount Lofty Ranges show limited dispersal to a higher degree than at Mount Burr, it is likely that partial inbreeding may have occurred, possibly due to low population density at the Mount Burr Range. Currently, there are no data on bandicoot population density and population abundance in the two study regions, though the much smaller sample size in the Mount Burr study, despite considerable sampling effort, lends support to this idea of a lower population density at Mount Burr compared to the Mount Lofty Ranges.

Implications for conservation

The construction of habitat corridors is one widely used approach to promote population connectivity in fragmented landscapes, which is also the primary conservation management action plan for the Mount Burr population in the south-east of South Australia. However, this approach may be impractical in some situations, such as in the Mount Lofty Ranges, due to the embedded human constructs and agricultural land in the landscape. Retention and management of native vegetation to maintain and improve suitable bandicoot habitat is therefore recommended as an alternative way to deal with habitat fragmentation in this area. In addition, in order to reduce the effects of inbreeding and increase long-term persistence of the numerous genetically distinct populations, the management of these populations would benefit from augmentation of gene flow between populations. Such genetic rescue and/or genetic restoration could be accomplished by moving individuals between populations (e.g. moving individuals into BNPS and MHS from their adjacent sites). If translocations were considered, we suggest managers should first evaluate the risks associated with translocations and consider potential mitigation strategies, as recommended by Weeks *et al*. (2011).

Acknowledgements

We would like to thank Luke Price from DEWNR for sharing bandicoot samples and his assistance with creating maps. We also thank Nicholas Fuller for his assistance with data processing. This project was funded by the Australian Research Council Linkage grant (LP0668987), Native Vegetation Council, Wildlife Conservation Fund, DEWNR and The Roy and Marjory Edwards Scholarship provided by Nature Foundation SA.

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Chapter Five: Delineation of conservation units in an endangered marsupial, the southern brown bandicoot (*Isoodon obesulus obesulus*) in South Australia/Western Victoria, Australia

You Li[1, 2], Melanie L. Lancaster[1, 2, 3], Susan M. Carthew[1, 5], Jasmin G. Packer[1], Mark Adams[4] and Steven J. B. Cooper[1, 2, 4]

(1) School of Earth and Environmental Sciences, the University of Adelaide, Adelaide, SA, 5005, Australia

(2) Australian Centre for Evolutionary Biology and Biodiversity, the University of Adelaide, Adelaide, SA, 5005, Australia

(3) Healesville Sanctuary, Healesville, VIC, 3777, Australia

(4) Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA, 5000, Australia

(5) Research Institute for Environment and Livelihoods, Charles Darwin University, Darwin, NT, 0909, Australia

Photo by Y. Li

Statement of Authorship

Title of Paper	Delineation of conservation units in an endangered marsupial, the southern brown bandicoot (*Isoodon obesulus obesulus*) in South Australia/Western Victoria, Australia
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Publication Style

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	You Li		
Contribution to the Paper	Collected samples, performed DNA extraction, PCR amplification and genetic analyses on all samples, interpreted data, wrote manuscript and acted as corresponding author.		
Signature		Date	20/02/2014

Name of Co-Author	Steven J. B. Cooper		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	23/8/2013

Name of Co-Author	Melanie L. Lancaster		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	28/08/2013

Name of Co-Author	Susan M. Carthew		
Contribution to the Paper	Supervised development of work, helped in data interpretation and critically reviewed manuscript.		
Signature		Date	23/8/13

Name of Co-Author	Mark Adams		
Contribution to the Paper	Provided samples, provided *CR* sequences and reviewed manuscript.		
Signature		Date	23/8/13

Name of Co-Author	Jasmin G. Packer		
Contribution to the Paper	Provided samples and reviewed manuscript.		
Signature		Date	26/8/13

Abstract

Conservation programs for threatened species are greatly benefiting from genetic data for its power in providing knowledge of levels of genetic diversity and population distinctiveness. Delineation of conservation units using genetic data in particular, is an important first step in devising management plans for threatened species. The endangered southern brown bandicoot (*Isoodon obesulus obesulus*) has a disjunct distribution range in South Australia, raising the possibility that populations of the subspecies may represent distinct conservation units. In the current study, we used a combination of 14 microsatellite markers and sequence data from two mitochondrial and three nuclear sequence markers to investigate the phylogeography and population structure of *I. o. obesulus* in South Australia and south-western Victoria, with the aim to identify any potential evolutionarily significant units (ESUs) relevant to conservation management. Our phylogenetic analyses of mtDNA and nuclear DNA sequences and population genetic analyses of microsatellite markers all supported the presence of two distinct evolutionary lineages of *I. o. obesulus* in South Australia and south-western Victoria. The first lineage consisted of individuals from the Mount Lofty Ranges and Kangaroo Island. Samples from the lower south-east of South Australia and south-western Victoria (the Grampians and Lower Glenelg) represented the second lineage. These two lineages should be considered as separate ESUs and therefore should be managed separately for conservation purposes. An expanded phylogenetic analysis was also conducted using additional samples of *I. obesulus* from other regions in Australia and samples of *I. auratus* (the golden bandicoot, distributed in the Northern Territory and Western Australia). The results raise the issue of the taxonomic status of the two lineages and also suggest that the current subspecies and species classification within *I. obesulus*/*I. auratus* may not adequately reflect the existing major genetic lineages.

Keywords: Isoodon obesulus, evolutionarily significant unit, management unit

Introduction

Defining units of management for conservation of species is an important step in conservation biology, particularly in the management of endangered species (Frankham 2010; Moritz 2002). Inappropriate conservation decisions, such as mixing populations that are genetically and evolutionarily distinct may have detrimental consequences (e.g. outbreeding depression, loss of local adaptation) (Frankham 2002; Moritz 1999). However, conservation management approaches to maximise genetic diversity within species will enhance their potential for adaptation and resilience in the face of changing environments or the presence of new pathogens.

The “evolutionarily significant unit” (ESU) is one of the most frequently discussed conservation units. The concept of an ESU was first introduced by Ryder (1986) as an operational unit for

conservation purposes to avoid the use of units such as "subspecies" that often did not reflect independently evolving populations. With the development of molecular tools, criteria for defining ESUs as "reciprocally monophyletic sister groups at mitochondrial loci" (mtDNA) and that they "show significant divergent allele frequencies at nuclear loci" (nDNA) were proposed by Moritz (1994) and have been widely used since. Despite the debate over criteria of defining ESU (e.g. Crandall *et al.* 2000), an ESU is generally recognised as a group of organisms with high genetic and ecological distinctiveness which warrants separate management for conservation (Allendorf *et al.* 2013; Crandall *et al.* 2000; Funk *et al.* 2012; Moritz 1994; Ryder 1986; Waples 1991). A second tier conservation unit known as a "management unit" (MU) was also proposed by Moritz (1994), relating to populations showing significant allele frequency variation in mitochondrial and nuclear genetic markers, largely indicative of significantly reduced gene flow between them.

Australia has experienced a dramatic mammal decline and extinctions since European settlement. The extinct mammals in Australia contribute to almost 50% of the world's vanished mammal species (Short & Smith 1994). The southern brown bandicoot (*Isoodon obesulus*), which was very common before the settlement of Europeans, has declined in number dramatically over the last 220 years, leading to a much contracted distribution and local population extinctions (Coates *et al.* 2008; Department of Environment and Conservation 2006; Paull 1993, 1995). In the state of South Australia, *I. obesulus* is the only surviving member of the family Peramelidae. The south-east mainland population (*I. o. obesulus*), which is the focus of this study, is listed as nationally endangered (the Australian Environment Protection and Biodiversity Conservation Act 1999). *I. o. obesulus* occurs in separate regions of southern South Australia, southern Victoria (primarily in coastal regions), and coastal fringes of New South Wales.

Three current strongholds of *I. o. obesulus* persist in South Australia: the Mount Lofty Ranges (including the Fleurieu Peninsula area), the lower south-east forest and Kangaroo Island. Indeed, these regions have been documented as the main area of distribution for *I. o. obesulus* since European settlement (one other distribution area was Eyre Peninsula, which has no modern records of *I. o. obesulus*) (Kemper 1990; Paull 1995; Paull *et al.* 2013). Populations of *I. o. obesulus* in these regions have become isolated and disjunct from each other (Figure 4-1). Significant genetic divergence may have resulted from such long-term isolation, leading to concerns about whether these populations are genetically distinct at some level and whether they represent separate conservation units. However, there is very limited knowledge regarding how populations within *I. o. obesulus* are genetically structured. Some translocations of individuals from South Australian to Victorian wildlife parks have occurred in the past (Zoos South Australia, unpublished data), raising the question of whether escaped animals may potentially impact local populations in Victoria, and whether this practice overall was sensible given potential genetic divergence between populations in Victoria and South Australia. There is an urgent need to identify conservation units (e.g. ESUs)

within *I. o. obesulus*, which will guide the development of appropriate management plans and priorities to conserve these populations.

In the current study, we used a combination of microsatellite markers and mtDNA and nDNA sequencing to investigate the genetic distinctiveness of *I. o. obesulus* populations across its range in South Australia and south-western Victoria. Specifically, we aimed to (1) identify evolutionarily significant and management units under the criteria proposed by Moritz (1994); and (2) use these results to address conservation issues for *I. o. obesulus.* Following the finding of two divergent genetic lineages within *I. o. obesulus*, we broadened the phylogenetic study to include representative samples of both *I. obesulus* and *I. auratus* (the golden bandicoot, distributed in the Northern Territory and Western Australia) from sites across Australia, to further investigate their systematic affinities.

Methods

Sampling and DNA extraction

A total of 522 individuals were collected from 40 locations in the state of South Australia, covering the three main regions of the distribution of *I. o. obesulus* in the state (the Mount Lofty Ranges, Kangaroo Island and the Mount Burr Range in the south east; Table 5-1, Figure 5-1). Another 39 individuals from six additional locations in two regions in Victoria (the Grampians and the Lower Glenelg National Park; Table 5-1, Figure 5-1) were also sampled. DNA was extracted using the Gentra Puregene extraction kit and methods specified by the manufacturer (Gentra Systems Inc.).

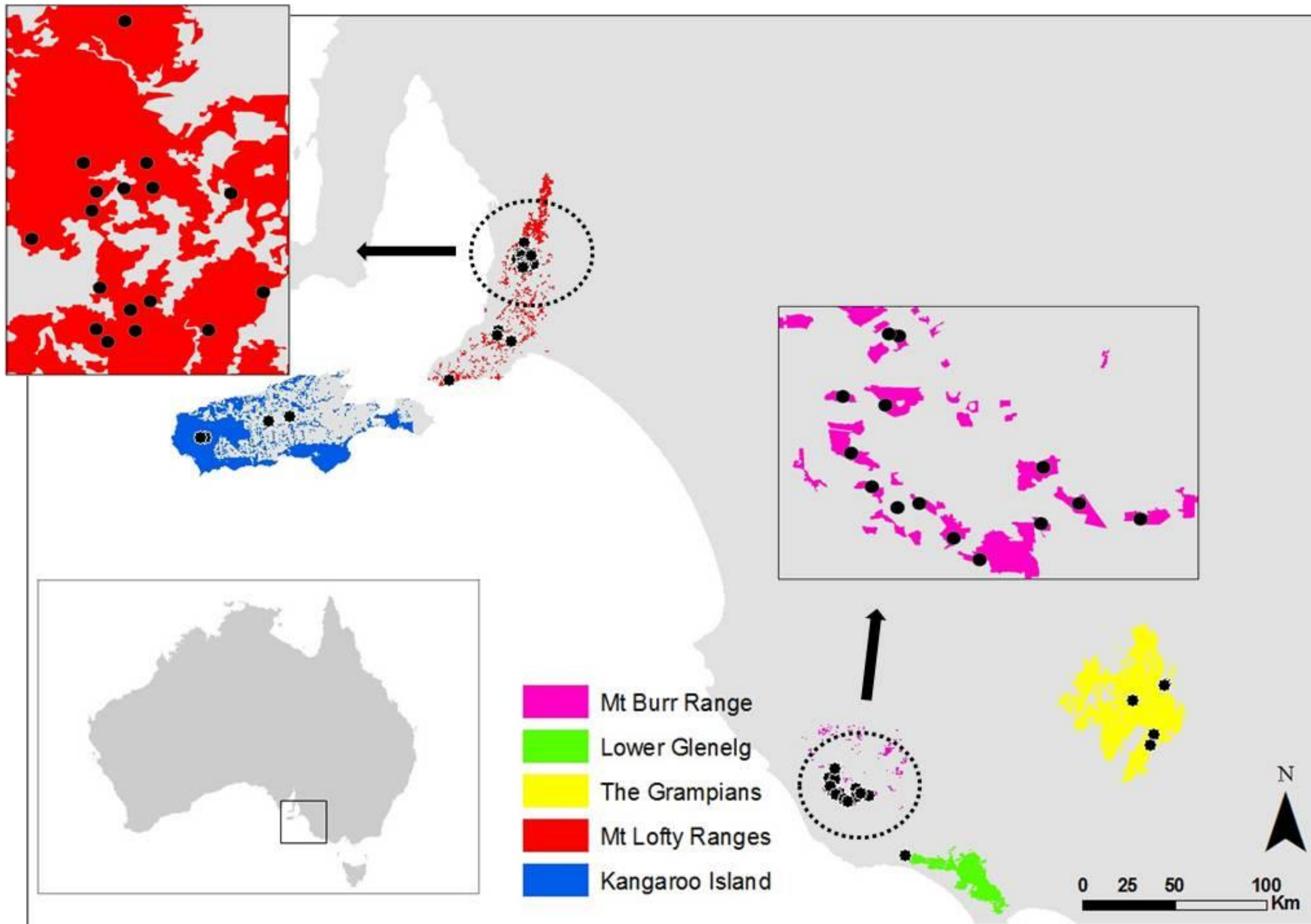


Figure 5-1 Distribution of the five sampled regions of *I. o. obesulus* in southern Australia. The coloured areas depict native vegetation. Sampled locations in each region are labelled with black dots. The Mount Burr Range and the central Mount Lofty Ranges are enlarged for better visualisation.

Table 5-1 Sampling information and genetic diversity parameters based on microsatellite data set

Region	Sampling Locality		N	H_O	H_E	AR
	Code	Name				
Mount Lofty Ranges (including Fleurieu Peninsula) (N = 358)	CCP	Cleland Conservation Park	9	0.275	0.488	1.418
	BNPS	Belair National Park	43	0.371	0.393	1.309
	MOC	Mark Oliphant Conservation Park Site 1	22	0.475	0.550	1.511
	MOD	Mark Oliphant Conservation Park Site 2	17	0.532	0.540	1.463
	MHS	Ackland Hill Rd Coromandel (Mud Hut)	41	0.497	0.502	1.466
	IRC	Ironbank Rd	15	0.464	0.550	1.472
	PRS	Pole Rd	17	0.407	0.495	1.495
	WBL	Wirra Birra low	12	0.529	0.546	1.507
	QUD	Dorset Vale Road	26	0.463	0.567	1.567
	MtBD	Mount Bold Reserve Site 1	18	0.530	0.567	1.527
	MtBC	Mount Bold Reserve Site 2	12	0.446	0.520	1.520
	MtBS	Mount Bold Reserve Site 3	19	0.422	0.477	1.443
	SC	Scott Creek Conservation Park Site 1	15	0.412	0.576	1.576
	SCC	Scott Creek Conservation Park Site 2	8	0.658	0.530	1.492
	SCD	Scott Creek Conservation Park Site 3	8	0.532	0.578	1.578
	SCS	Scott Creek Conservation Park Site 4	11	0.607	0.581	1.581
	WW	Warrawong	20	0.427	0.443	1.443
	MBCP	Mount Billy Conservation Park	12	0.427	0.494	1.459
	DCCP	Deep Creek Conservation Park	18	0.218	0.384	1.357
	ABGC	Adelaide Blue Gum property	9	0.491	0.510	1.366
	WADC	Wadnama property	6	0.472	0.528	1.226
Kangaroo Island (N = 17)	KI1	Kangaroo Island Site 1	4	0.438	0.543	1.310
	KI2	Kangaroo Island Site 2	1	1.000	1.000	1.357
	KI3	Kangaroo Island Site 3	3	0.396	0.537	1.307
	KI4	Kangaroo Island Site 4	5	0.408	0.506	1.362
	KI5	Kangaroo Island Site 5	4	0.375	0.498	1.356
Mount Burr Range (N = 147)	BSS	Burr Slopes South NFR	15	0.379	0.482	1.413
	GH	Glencoe Hill NFR	15	0.507	0.584	1.459
	GL	Grundy's Lane NFR	11	0.254	0.436	1.436
	HH	Hacket Hill NFR	9	0.471	0.498	1.463
	KF	Kangaroo Flat NFR	6	0.601	0.587	1.546
	LO	The Long NFR	8	0.552	0.595	1.553
	MA	The Marshes NFR	7	0.456	0.550	1.472
	MM	Mount McIntyre NFR	16	0.382	0.557	1.557
	MR	Mc Rosties NFR	11	0.508	0.583	1.542
	NW	Native Wells NFR	15	0.443	0.541	1.502
	WA	Wandilo NFR	16	0.325	0.443	1.443
	WH	Windy Hill NFR	3	0.367	0.617	1.440
	WO	Woolwash NFR	7	0.386	0.570	1.529
The Grampians (N = 27)	HN	Honan NFR	8	0.446	0.549	1.431
	JR	Read/Spinks Property	3	0.483	0.483	1.345
	YOGA	Joyce Weight's Property	7	0.250	0.508	1.327

	VR	Victoria Range	4	0.505	0.530	1.378
	JCK	Jimmy's CK	6	0.667	0.678	1.291
	WAN	Wannon	7	0.369	0.407	1.291
Lower Glenelg (N = 12)	LG	Lower Glenelg	12	0.449	0.605	1.317

N – Sample size, H_O – observed heterozygosity, H_E – expected heterozygosity, AR – allelic richness, NFR = native forest reserve

Microsatellite genotyping and analyses

All 561 samples were genotyped at 14 microsatellite loci that had been developed for *I. obesulus* [six (B3-2, B15-1, B20-5, B34-2 and B38-1) by Zenger & Johnston (2001), and nine by Li *et al.* (2013)] following PCR protocols in Li *et al.* (2013). Amplified products were run on an ABI 3730 DNA Analyser and alleles were scored using GeneMapper 4.0 (Applied Biosystems).

Genetic diversity was estimated by the number of alleles per locus (A), observed heterozygosity (H_O), expected heterozygosity (H_E) and allelic richness (AR) corrected for sample size. Calculation of AR was performed in FSTAT 2.9.3.2 (Goudet 2001) and the remaining calculations were performed in Arlequin 3.11 (Excoffier & Lischer 2010).

We implemented Bayesian clustering analysis in STRUCTURE 2.3.3 (Pritchard *et al.* 2000) to characterize population genetic structure across the five sampled sites. We used an admixture model with a burn-in of 100 000 and 100 000 MCMC steps after the burn-in. We also used correlated allele frequencies based on the assumption that these populations would have experienced some gene flow in the past. The value of K was set from 1 to 46 with ten replicates of each K. STRUCTURE HARVESTER 0.6.92 (Earl & vonHoldt 2012) was used to determine the most likely K, using the *ad-hoc* ΔK described in Evanno *et al.* (2005). CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) was used to average the membership probabilities for the ten runs of the most likely K and DISTRUCT 1.1 (Rosenberg 2004) was used to display the averaged results.

We also applied a spatial principal component analysis (sPCA) to investigate spatial patterns of genetic variation in the R package Adegenet version 1.3-7 (Jombart 2008). This multivariate method uses an index of spatial autocorrelation (Moran's I) to summarize the variance of allele frequencies between studied entities (Jombart *et al.* 2008). Unlike STRUCTURE, sPCA does not rely on a particular genetic model and thus does not require Hardy-Weinberg equilibrium or linkage equilibrium. We used the Delaunay triangulation method to construct the connection network between bandicoots. An sPCA analysis produces two types of structure: global structure (positive spatial autocorrelation) and local structure (negative spatial autocorrelation) (Jombart *et al.* 2008). An abrupt drop of the eigenvalues was determined as the boundary between strong and weak structures according to Jombart (2008). We also performed a Monte-Carlo test with 10 000 permutations to examine the significance of the observed structure.

Sequencing of mitochondrial and nuclear markers and data analyses

Two mitochondrial gene segments and three nuclear fragments were amplified (Table 5-2): the noncoding control region (*CR*, also called the "D-loop region" in vertebrates), the NADH dehydrogenase subunit 2 gene (*ND2*); protein coding portions of the breast and ovarian cancer susceptibility gene (*BRCA1*, exon 11), recombination activating gene-1 (*RAG1*, intronless) and vonWillebrand factor gene (*vWF*, exon 28). We chose a geographically representative subset of 51 individuals for mtDNA sequencing. For nDNA, a subset of 27 individual representatives from the five main sampling regions was used for sequencing. We also sequenced three individuals of the closely related northern brown bandicoot (*I. macrourus*) (from the states of New South Wales, Queensland, and Western Australia, respectively) at all these genes to include them as an outgroup for further analyses. The primers we used to amplify these genes and their annealing temperatures are listed in Table 5-2. PCR amplifications were carried out in 25μl volumes containing 0.1U AmpliTaq Gold® polymerase (Applied Biosystems), 10 × Gold Buffer (Applied Biosystems), 0.20 mM dNTPs, 2.5 mM MgCl2, 0.5 μM of each primers and approximately 100 ng genomic DNA. Thermocycling conditions were: initial activation at 94 °C for 3 minutes; 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 48-55 °C for 45 seconds, and extension at 72 °C for 60 seconds; and a final extension at 72 °C for 3 minutes. PCR products were purified using Millipore MultiScreen PCR_{384}Filter Plates (Millipore) and were sent to the Australian Genome Research Facility (AGRF) for sequencing.

Table 5-2 Primers used to amplify segments of *CR*, *ND2*, *BRCA1*, *RAG1*, and *vWF* within *I. o. obesulus*

Gene	Primer name	Source	Sequence (5' - 3')	Tm*
CR	m989 (L15999M)	Fumagalli *et al.* 1997	ACCATCAACACCCAAAGCTGA	55 °C
	m990 (H16498M)	Fumagalli *et al.* 1997	CCTGAAGTAGCAACCAGTAG	55 °C
ND2	m635 (mmND2.1)	Bulazel *et al.* 2007	AGGGTGTTATACCTTCATTTTTGG	48 °C
	m636 (mrND2c)	Osborne & Christidis 2001	GCACCATTCCACTTYTGAGT	48 °C
BRCA1	G1800 (F9)	Meredith *et al.* 2008	AGTTCTGAAAGTGGATTCTTT	50 °C
	G1801 (R-1MAC9-20)	Meredith *et al.* 2008	CTGACCTRCAGCCTGAGGATTTCAT	50 °C
RAG1	G2311 (F2204)	Amrine-Madsen *et al.* 2003	GCTTCTGGCTCWGTCTACATYTGTAC	50 °C
	G2312 (R2794)	Amrine-Madsen *et al.* 2003	AAACGCTGTGARTTGAAACT	50 °C
vWF	G2313 (MF119)	Amrine-Madsen *et al.* 2003	GACTTGGCYTTYCTSYTGGATGG	55 °C
	G2314 (MR1140)	Amrine-Madsen *et al.* 2003	TTGATCTCATCSGTRGCRGGATTGC	55 °C

* annealing temperature for PCR amplifications

DNA sequences were edited and aligned in Geneious 6.1.4 (www.geneious.com). Sequences were submitted to GenBank (xxxx-xxxx; see Appendix). Before concatenating the two mitochondrial genes (*CR* + *ND2*), we constructed separate phylogenetic trees to check their concordance. We used Arlequin 3.11 (Excoffier & Lischer 2010) to calculate the number of haplotypes (*Nh*), haplotype diversity (*h*) and nucleotide diversity (π) on the combined mitochondrial dataset and individual nuclear genes. Pairwise distances among haplotypes for concatenated mtDNA and individual nDNA was calculated using the *p*-distance method with 1 000 bootstrap replicates in Mega 5.10 (Tamura *et al.* 2011).

We used Mega 5.10 (Tamura *et al.* 2011) to construct the neighbour-joining (NJ) trees for the concatenated mtDNA and individual nDNA data, using the proportion (*p*) of nucleotide site changes as a distance estimate. The stability of the nodes in the trees was assessed by 1 000 bootstrap replicates. A pairwise distance matrix was also generated in Mega 5.10 (Tamura *et al.* 2011) using the *p*-distance method. Relationships among haplotypes for combined mtDNA and individual nDNA markers were represented as a median-joining network obtained by NETWORK version 4.6 (www.fluxus-engineering.com). We were not able to obtain a network for *RAG1* because this gene fragment only showed two haplotypes in our dataset. We thus used TCS 1.21 (Clement *et al.* 2000) to visualize relationships between haplotypes for *RAG1*.

We carried out an additional phylogenetic analysis including 11 samples of *I. obesulus* from WA, NSW, and TAS, and six samples of *I. auratus* to further investigate the systematic relationship between *I. obesulus* and *I. auratus*. Only a small number of these samples amplified successfully for the nDNA markers and thus only the mtDNA dataset was presented here. We performed a maximum likelihood (ML) analysis with RaxmlGUI 1.3 (Silvestro & Michalak 2012) which is a user friendly front-end for RAxML (Stamatakis 2006). ML analysis was performed using the mtDNA dataset which was partitioned by gene (*CR* and *ND2*), and employed a General Time Reversible (GTR) model (Tavare 1986) with gamma (Γ) rate heterogeneity (Yang 1994) for unequal rate variation at sites for each gene partition. The analysis was carried out with 1 000 bootstrap replicates with 100 searches per replicate.

Results

Microsatellite analyses

The number of alleles across microsatellites varied from 6 (Ioo2) to 17 (Ioo5) with a mean number of 11.64. Mean observed (H_O) and expected (H_E) heterozygosity across the 46 locations ranged from 0.218 to 1.000 and from 0.384 to 1.000 respectively (Table 5-1). Allelic richness (*AR*) ranged from 1.226 to 1.581 (Table 5-1).

Bayesian clustering analysis using STRUCTURE found two distinct clusters using the Δ*K* criterion (Evanno *et al.* 2005) ($K = 2$, Figure 5-2a, c). Ninety-nine percent of the individuals were assigned with a probability > 90% to one of the two clusters. The two clusters clearly corresponded to geographical locations: individuals from south-eastern South Australia and western Victoria (Mount Burr Range, Grampians and Lower Glenelg) formed one distinct cluster (hereafter the "east group") and individuals from the Mount Lofty Ranges and Kangaroo Island in South Australia were grouped together (hereafter the "west group") (Figure 5-2a). To further investigate the sub-structure within each group, we ran another two analyses using the same STRUCTURE settings. Four sub-clusters were identified within the west group and three were identified within the east group (Figure 5-2d, e). Within the east group, one cluster consisted of bandicoots from the Grampians and Lower Glenelg, and bandicoots from the Mount Burr Range structured into two clusters (Figure 5-2d). For the analysis of the west group, three sub-clusters were found within the central Mount Lofty Ranges and the fourth cluster consisted of bandicoots from the Fleurieu Peninsula and Kangaroo Island (Figure 5-2e).

When using sPCA, the analysis found a significant global structure ($P < 0.01$), but not local structure ($P = 1$) based on the whole dataset. The positive eigenvalues suddenly decreased after the first two values (data not shown) and thus these two principal axes were retained to account for the observed spatial genetic structure. We retrieved two distinct groups by combining the first two scores of global structure. These two groups matched the clusters we observed in STRUCTURE, in which the samples were separated into west group and east group, with Grampians population being genetically differentiated from other populations within the east group (Figure 5-2b).

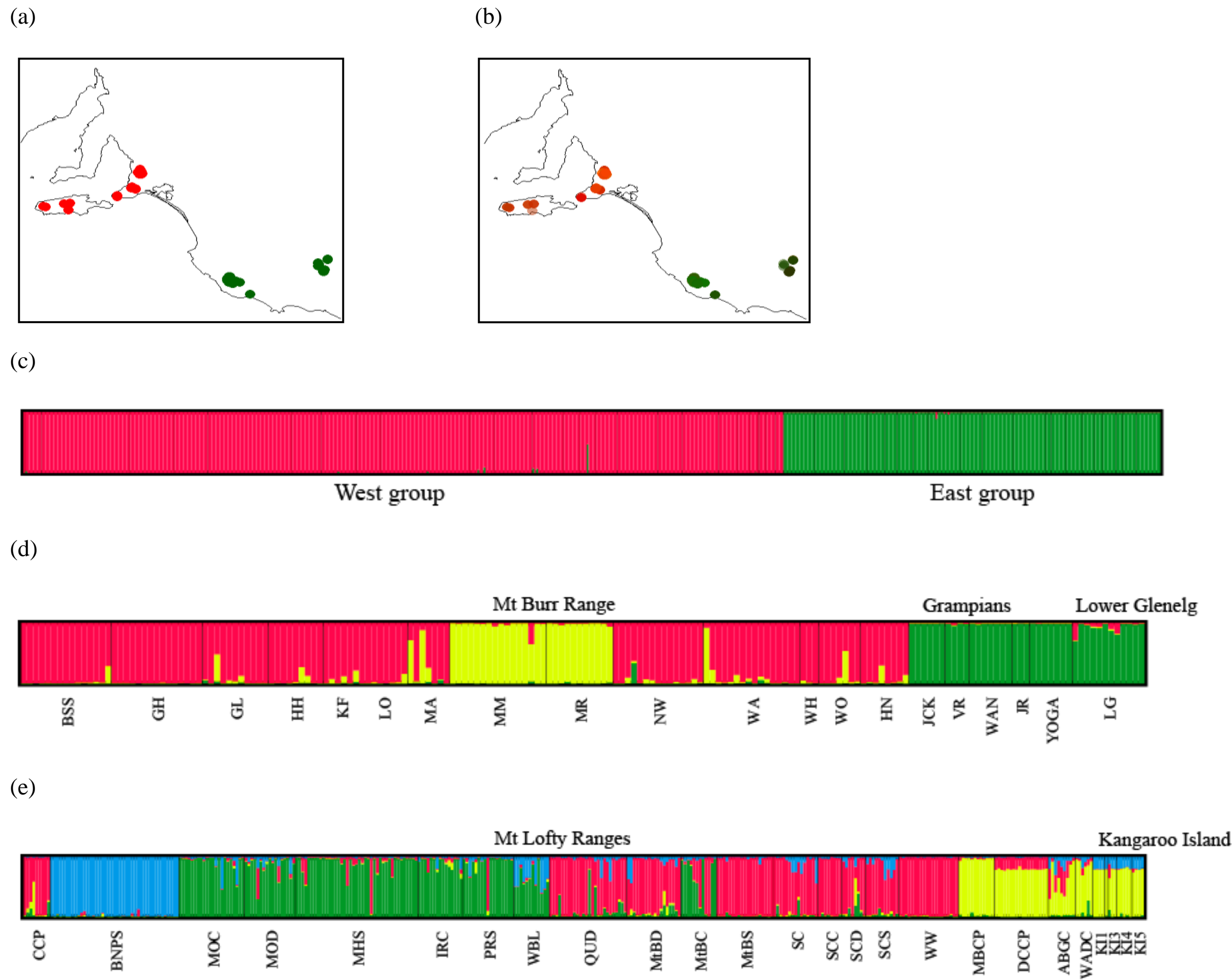


Figure 5-2 Bayesian clustering results obtained in STRUCTURE and results from sPCA analyses. (a) STRUCTURE clustering results for the whole data set: individuals were mapped based on coordinates and marked with circles in different colours representing clusters assigned by STRUCTURE (cluster1 – orange, cluster 2 - green); (b) colorplot of sPCA results: summarised are the first two scores of global structure and each score was transferred into a channel of colour. Circles represented individuals and were mapped based on coordinates; (c) proportional membership (q) of each bandicoot individual to a genetic cluster identified by STRUCTURE for the whole data set; (d) proportional membership (q) of each bandicoot individual to a genetic cluster identified by STRUCTURE for the east group; (e) proportional membership (q) of each bandicoot individual to a genetic cluster identified by STRUCTURE for the west group.

Sequencing analyses

A total of 1 287 bp mitochondrial DNA sequence was obtained (*CR* – 591 bp, *ND2* – 696 bp; GenBank accession nos. xxxxx-xxxxx; see Appendix) in 54 individuals (51 individuals of *I. o. obesulus* and three individuals of *I. macrourus*). Four indels were identified within the *CR* fragment in *I. o. obesulus*. No indels were found in the protein-coding gene *ND2*. In addition, phylogenetic trees for individual mtDNA genes generated concordant results (data not shown), suggesting the likely absence of nuclear pseudogenes in the dataset. We were thus confident to concatenate the sequences of the two genes. Within the 1 287-bp mtDNA fragment in *I. o. obesulus*, 79 polymorphic sites and 14 haplotypes were found (Table 5-3). Overall haplotype and nucleotide diversity was 0.844 and 0.021, respectively, with the highest haplotype and nucleotide diversity in the Mount Lofty Ranges ($h = 0.872$, $\pi = 0.009$; Table 5-3).

The nDNA alignment generated 581 and 517 bp for *BRCA1* and *RAG1*, respectively, in 27 individuals of *I. obesulus*. We obtained an 831-bp fragment of *vWF* from 21 individuals of *I. o. obesulus* as a result of difficulties in amplifying the other six individuals. In addition, two of the three samples of *I. macrourus* amplified successfully for *BRCA1* and only one of them worked for *RAG1* and *vWF*. Accession numbers in GenBank of these sequences are xxxx-xxxx (see Appendix). The nDNA was much less variable than the mtDNA. There were six polymorphic sites detected for *BRCA1*, only one for *RAG1* and five for *vWF*, with four, two and three haplotypes identified for *BRCA1*, *RAG1* and *vWF*, respectively (Table 5-3). Overall haplotype diversity of individual genes ranged from 0.556 in *BRCA1* to 0.462 in *RAG1*, and nucleotide diversity ranged from 0.003 in *BRCA1* to 0.001 in *RAG1* (Table 5-3). No indels were observed in any of the nDNA genes. The average distance among haplotypes was 0.023 and pairwise distances ranged from 0.000 (H1-H14, H4-H11, and H12-H13) to 0.040 (H6-H12 and H6-H13) for the mtDNA dataset (Supporting Information, Table S5-1a). For the individual nDNA dataset, the average distance among haplotypes was 0.005, 0.002, and 0.004, with pairwise distances ranging from 0.003-0.007, 0.002-0.002, and 0.002-0.006 for *BRCA1*, *RAG1*, and *vWF*, respectively (Supporting Information, Table S5-1b, c, and d).

Table 5-3 Diversity estimates for combined mitochondrial (*CR* and *ND2*) and nuclear markers (*BRCA1*, *RAG-1*, and *vWF*)

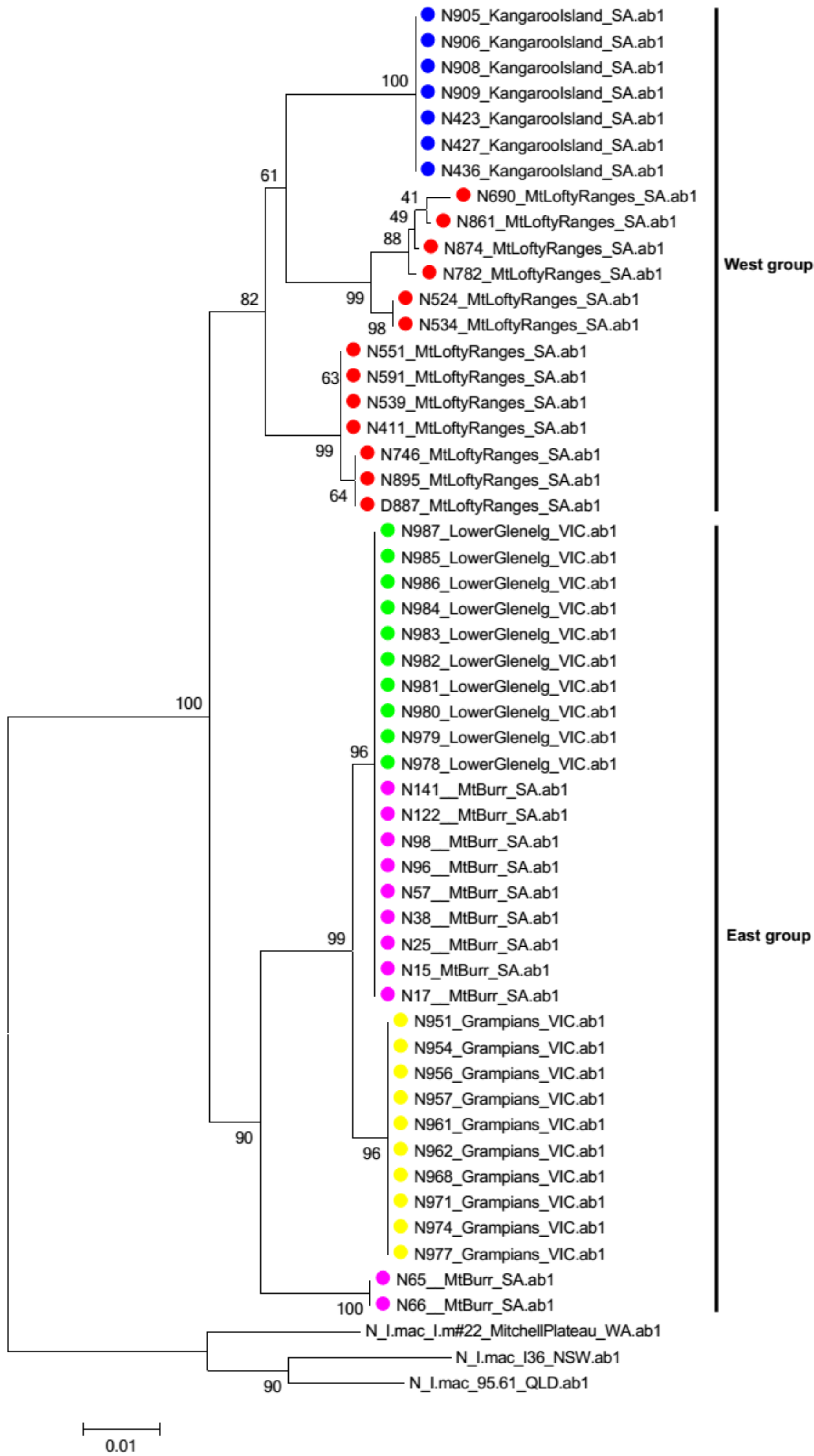
Region	Combined Mitochondrial dataset			*BRCA1*			*RAG-1*			*vWF*		
	N	*h* ±SD (*Nh*)	*π* ±SD	*N*	*h* ±SD (*Nh*)	*π* ±SD	*N*	*h* ±SD (*Nh*)	*π* ±SD	*N*	*h* ±SD (*Nh*)	*π* ±SD
Mount Lofty Ranges	13	0.872 ±0.067 (7)	0.009 ± 0.005	13	0.295 ±0.156 (3)	0.001 ± 0.001	13	0.000 ±0.000 (1)	0.000 ±0.000	9	0.000 ±0.000 (1)	0.000 ± 0.000
Kangaroo Island	7	0.286 ±0.196 (2)	0.000 ± 0.000	5	0.000 ±0.000 (1)	0.000 ± 0.000	5	0.000 ±0.000 (1)	0.000 ±0.000	4	0.000 ±0.000 (1)	0.000 ± 0.000
Mount Burr Range	11	0.327 ±0.153 (2)	0.006 ± 0.004	5	0.000 ±0.000 (1)	0.000 ± 0.000	5	0.000 ±0.000 (1)	0.000 ±0.000	5	0.000 ±0.000 (1)	0.000 ± 0.000
The Grampians	10	0.200 ±0.154 (2)	0.000 ± 0.000	2	0.000 ±0.000 (1)	0.000 ± 0.000	2	0.000 ±0.000 (1)	0.000 ±0.000	2	1.000 ±0.500 (2)	0.002 ± 0.003
Lower Glenelg	10	0.356 ±0.159 (2)	0.000 ± 0.000	2	0.000 ±0.000 (1)	0.000 ± 0.000	2	0.000 ±0.000 (1)	0.000 ±0.000	1	1.000 ±0.000 (1)	0.000 ± 0.000
Total	**51**	**0.844 ±0.036 (14)**	**0.021 ± 0.010**	**27**	**0.556 ±0.070 (4)**	**0.003 ± 0.002**	**27**	**0.462 ±0.065 (2)**	**0.001 ±0.001**	**21**	**0.529 ±0.079 (3)**	**0.002 ± 0.001**

N – sample size, *h* – haplotype diversity, *Nh* – number of haplotypes, *π* – nucleotide diversity, SD – standard deviation

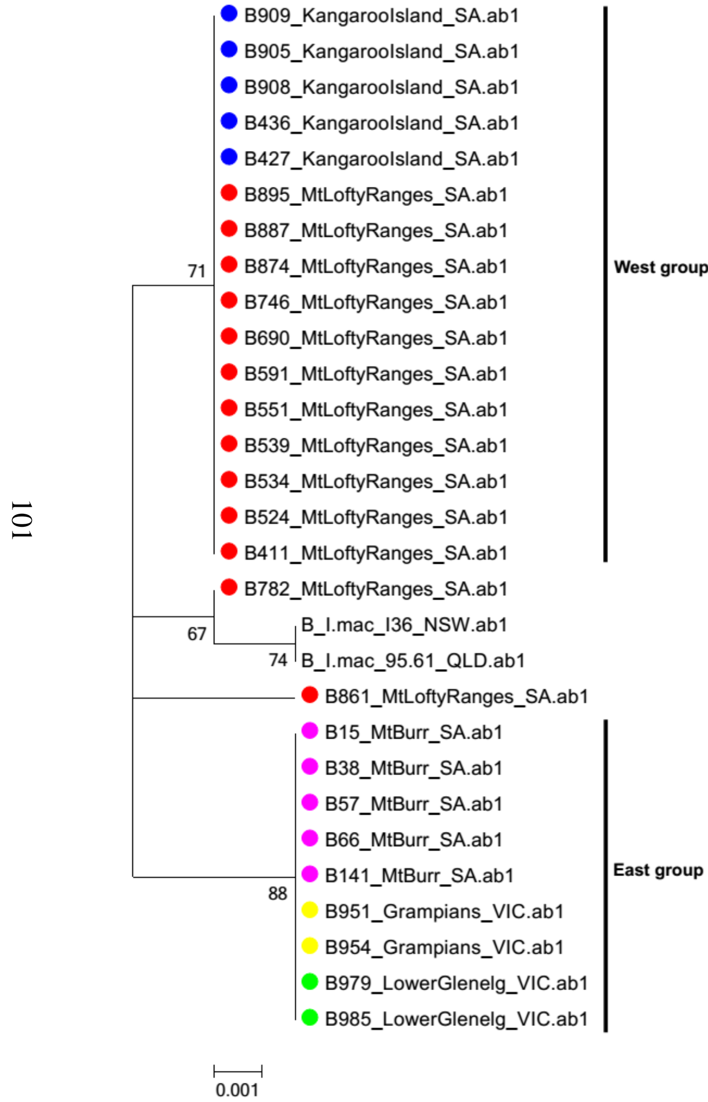
A neighbour-joining (NJ) tree based on the concatenated mtDNA dataset revealed two well-supported (100% bootstrap support) reciprocally monophyletic groups of haplotypes (Figure 5-3a). The same pattern, with regard to the presence of two main lineages, concordant with those found for the mtDNA tree, was also found in NJ trees constructed using nDNA data (Figure 5-3b, c and d). The first clade comprised populations from the west group (the Mount Lofty Ranges and Kangaroo Island) and the second group comprised the east group (the Mount Burr Range, Grampians and Lower Glenelg). For the mtDNA NJ tree, two samples from the Mount Burr Range were grouped together and were sister to all other samples within the east group. The rest of the samples in the Mount Burr Range were grouped with samples from Lower Glenelg, and together were sister to a lineage comprising haplotypes from the Grampians samples. Within the west group, samples from Kangaroo Island formed a reciprocally monophyletic clade with partial samples from the Mount Lofty Ranges (most of which were from the Fleurieu Peninsula). This clade was sister to the remaining samples from the Mount Lofty Ranges.

The result showing two distinct clades in NJ trees was further supported in our network analyses (using NETWORK and TCS). For the mtDNA haplotype network, none of the 14 haplotypes was shared between the west and east group (Figure 5-4a). The most common haplotype within the east group (H1) was found in 17 individuals (33.3% of 51 sequenced samples). H1 was the dominant haplotype representing samples from the Mount Burr Range and Lower Glenelg. The haplotypes representing Grampians samples were H12 and H13, and they were not found in any other samples from other locations. Within the west group, no haplotypes were shared between the two regions (Mount Lofty Ranges and Kangaroo Island). The pattern of haplotype structure between the west and east group was also evident for the three nDNA networks, in which the west and east group were each represented by a different dominant haplotype (Figure 5-4b, c, and d).

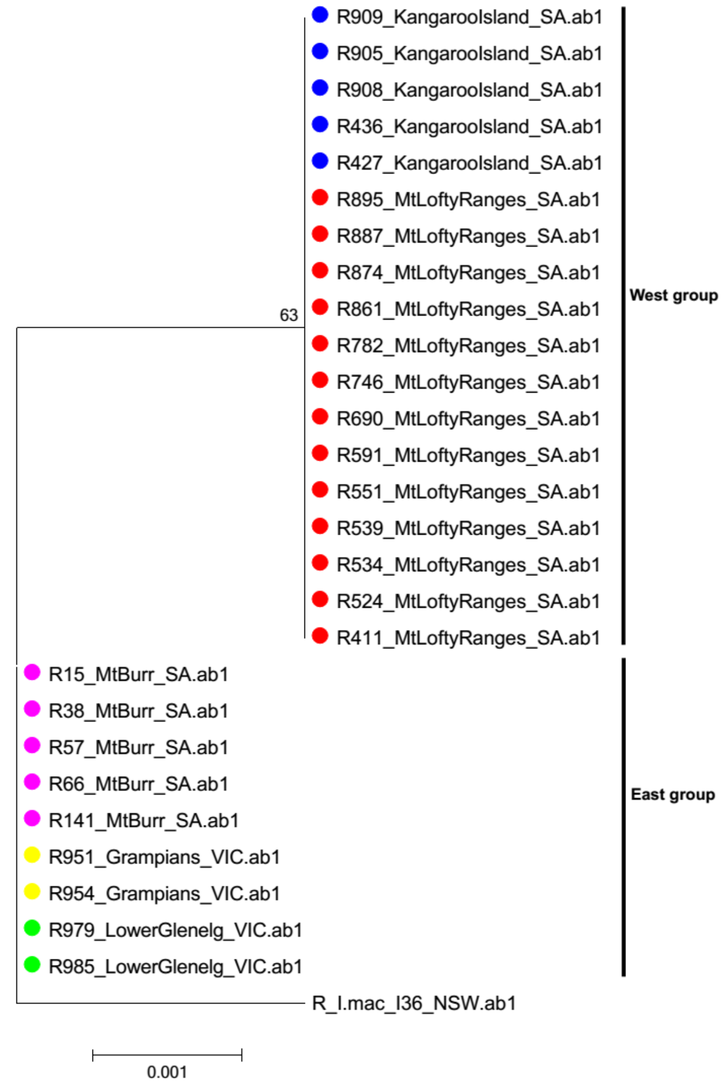
(a)



(b)



(c)



(d)

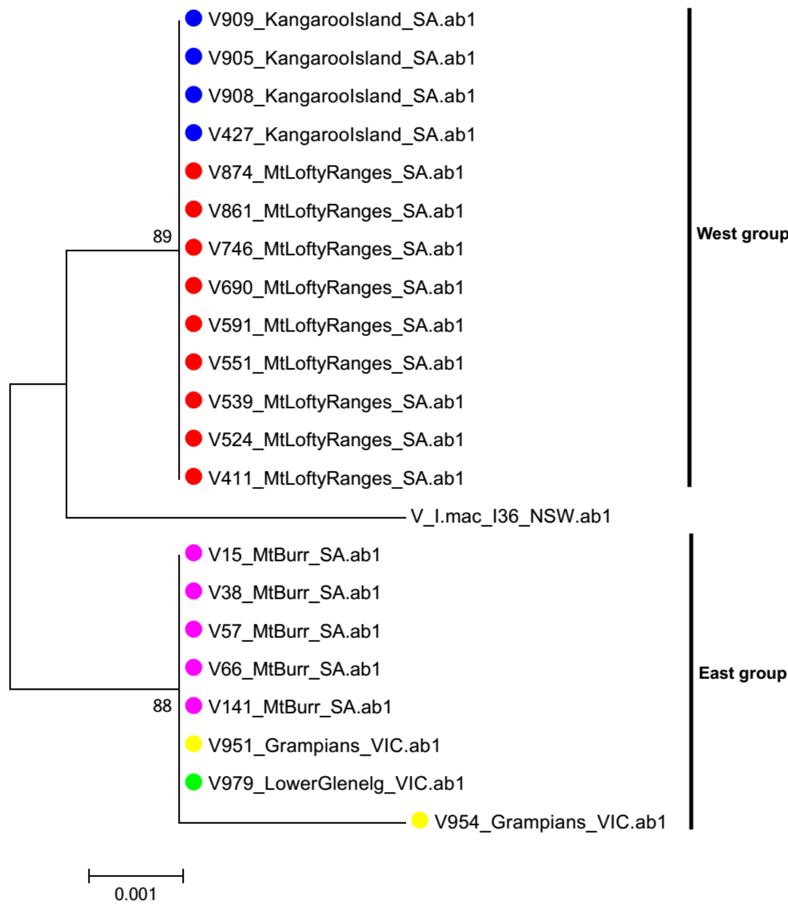


Figure 5-3 Neighbour-joining trees based on (a) concatenated mtDNA data, (b) *BRCA1*, (c) *RAG1*, and (d) *vWF* data. Bootstrap values are indicated on branches. Samples from the five main regions are labelled with coloured circles (Kangaroo Island – blue, the Mount Lofty Ranges – red, the Mount Burr Range – purple, Grampians – yellow, and Lower Glenelg – green). I. mac = *I. macrourus*

(a)

(b)

(c)

(d)

Figure 5-4 Haplotype network for combined mitochondrial markers (a) and nuclear markers [*BRCA1* (b), *RAG1* (c) and *vWF* (d)]. The sizes of the circles are proportional to the frequency of the haplotype. Pie slices represent the portion of samples to each sampling location (Kangaroo Island – blue, the Mount Lofty Ranges – red, the Mount Burr Range – purple, Grampians – yellow, and Lower Glenelg – green). Black squares represent median vectors and numbers on branches represent the number of mutation steps. The number of individuals for each haplotype is denoted in circles and the haplotypes with only one individual are left unnumbered.

The ML tree based on our expanded dataset showed the presence of two divergent lineages: one comprising the west group of samples, *I. obesulus* samples from WA, and samples of *I. auratus*, with polyphyly of haplotypes from each of the two species (i.e. one of the Mount Lofty Ranges groups is more closely related to a haplotype from *I. auratus* than it is to other haplotypes from *I. obesulus*); the other comprising the east group and *I. obesulus* from NSW and TAS (Figure 5-5).

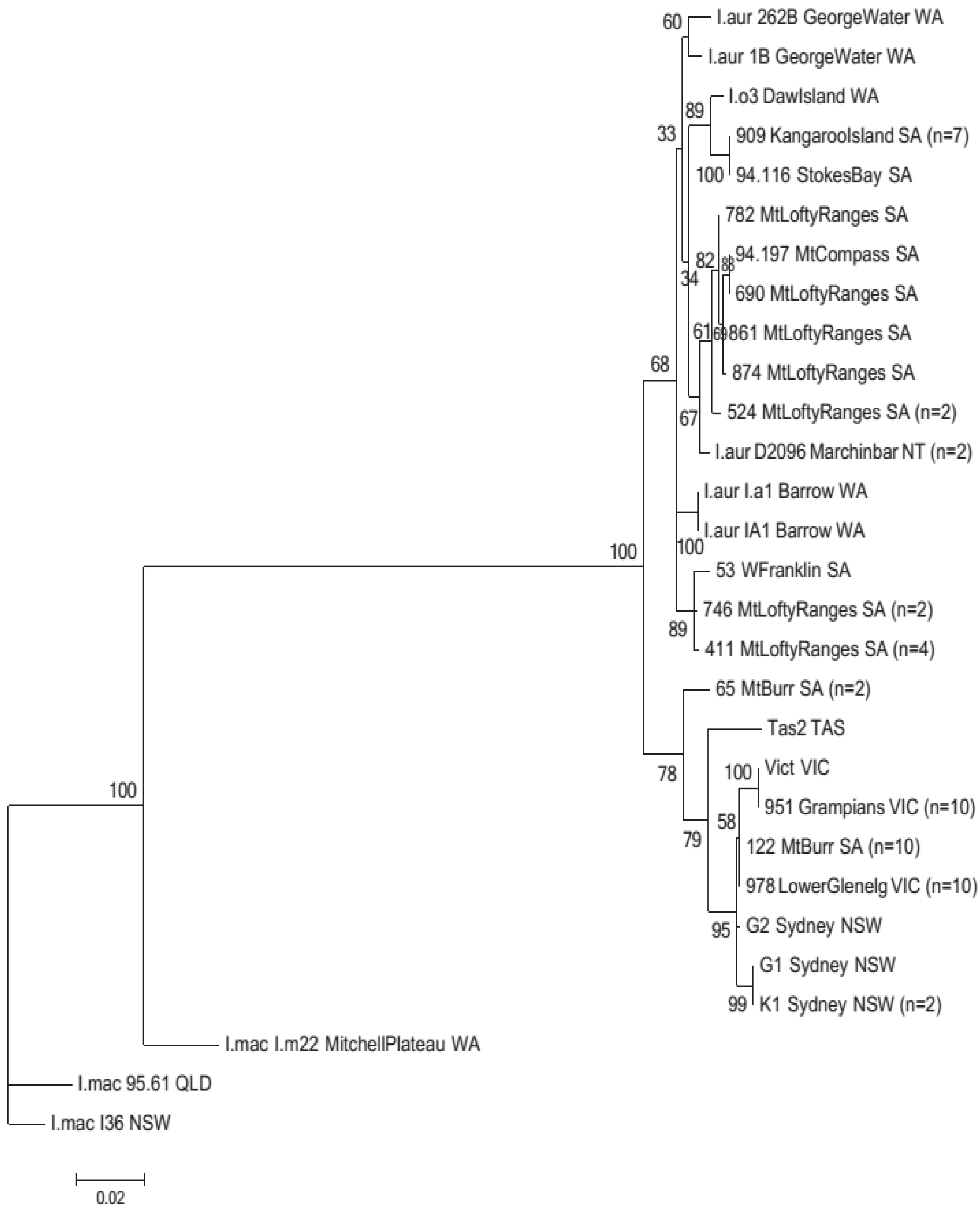


Figure 5-5 ML tree obtained from the partitioned mtDNA dataset. Bootstrap values are indicated on branches. I. mac = *I. macrourus*. I. aur = *I. auratus*. The remaining samples are *I. obesulus*.

Discussion

There was strong evidence for the existence within *I. o. obesulus* of two reciprocally monophyletic groups of mtDNA and nDNA haplotypes, one distributed in the Mount Lofty Ranges and Kangaroo Island and a second distributed in the south-east of SA and western Victoria. Under the criteria proposed by Moritz (1994) for defining ESUs (see introduction), our genetic analyses support the existence of at least two distinct ESUs within *I. o. obesulus*. Within each ESU, there are distinct populations showing significantly reduced gene flow to other nearby populations, which should be considered as different Management Units (MUs). The unique mtDNA haplotypes found within Grampians (H12 and H13) and Kangaroo Island (H4 and H11) populations were distinct from haplotypes from other populations. There is also evidence for a distinct haplotype within the Grampians population for the nuclear marker *vWF* and further evidence from sPCA analyse for genetic distinctiveness of the Grampians population. In addition, population genetic structure was also found within both the Mount Lofty Ranges and the Mount Burr Range using microsatellite markers. These results suggest that each ESU was comprised of numerous MUs (west ESU: Kangaroo Island population and four distinct populations in the Mount Lofty Ranges; east ESU: Grampians population, Lower Glenelg population and two distinct populations in the Mount Burr Range).

We found significant levels of divergence for mtDNA haplotypes between the west and east groups (mean 3.5% for concatenated mtDNA, mean 4.7% for control region, and mean 2.4% for *ND2*). The 4.7% control region divergence is similar to a previously published level of control region divergence between the subspecies of *I. obesulus* (mean 4.46%, Zenger *et al.* 2005) and the mean 4.8% divergence between *I. obesulus* and *I. auratus* (Zenger *et al.* 2005). It is also comparable to the level of control region divergence between populations of other marsupial species (e.g. 5.7% in the yellow-footed rock wallaby, *Petrogale xanthopus*, Pope *et al.* 1996; 4.0% in tiger quolls, *Dasyurus maculatus*, Firestone *et al.* 1999; 3.75% in red kangaroos, *Macropus rufus*, Clegg *et al.* 1998; and 5.0% in the black-footed rock-wallaby, *P. lateralis*, Eldridge *et al.* 2001). Overall, the level of divergence between the two ESUs reported here is at the high end of those reported for other intra-specific studies of marsupials and between a related species of bandicoot. This raises the question of the taxonomic status of the two ESUs of *I. o. obesulus* we report here, and their systematic relationship with *I. auratus.* Previous genetic studies of the taxonomic status of *I. obesulus* and *I. auratus* have led to conflicting conclusions. Westerman *et al.* (2012) confirmed the genetic distinctiveness of *I. auratus* from *I. obesulus* and *I. macrourus* using phylogenetic and dating analyses of combined mitochondrial and nuclear sequences based on six samples (two for each species). In contrast, Pope *et al.* (2001) and Zenger *et al.* (2005) suggested that *I. auratus* should be considered as a subspecies of *I. obesulus* instead of a separate species, based on the

apparent paraphyly of *I. auratus* with subspecies of *I. obesulus*. Our expanded ML analysis of the concatenated mitochondrial data showed two divergent lineages, with our west group of samples, *I. obesulus* samples from WA and samples of *I. auratus* comprising one lineage, and our east group and *I. obesulus* from NSW and TAS comprising the other lineage. These results suggest that current subspecies and species classification within *I. obesulus/I. auratus* may not adequately reflect the existing major genetic lineages. Furthermore, the polyphyly of our west group with *I. auratus* and the east group with *I. obesulus*, suggest the possibility that each group actually represents a distinct species of bandicoot. We are now carrying out a broader and more detailed phylogenetic/phylogeographic study of *I. obesulus* and *I. auratus* to further resolve their taxonomic status.

Implications for conservation

Our findings provide strong evidence that there are at least two ESUs existing within *I. o. obesulus*, and we thus recommend that these two ESUs should be managed separately. Unless either of the ESUs suffers a significant decline in genetic diversity that may warrant genetic rescue, any future translocation plans between them should not be considered, since such actions may lead to outbreeding depression and reduced reproductive fitness according to criteria given by Frankham *et al.* (2011).

Within each ESU, in order to minimise the potential for inbreeding and increase the long-term persistence of the numerous genetically distinct populations (MUs), the management of these populations may benefit from augmentation of gene flow among populations. Such genetic rescue and/or genetic restoration could be accomplished by constructing habitat corridors and/or moving individuals between populations within the same ESU (see Chapter Three and Four for detailed population genetic structure analyses within each ESU). Potential risks need to be evaluated when translocations are considered within a certain ESU (e.g. pathogen transmission, outbreeding depression, loss of local adaptation and replacement of recipient genetic background; Weeks *et al.* 2011). For example, although the population of Kangaroo Island did not show any evidence of reduced genetic diversity (microsatellite markers) compared to the mainland population, a result often found in island populations due to inbreeding and genetic drift (Eldridge *et al.* 1999; Frankham 1997; Jensen *et al.* 2013), the long period of isolation from the mainland, and the distinct mtDNA haplotypes in this population suggest that translocation between Kangaroo Island and the mainland population (the Mount Lofty Ranges) may potentially lead to outbreeding depression (Frankham *et al.* 2011). Therefore, strategies that can mitigate the risks associated with translocation should be considered. A risk-assessment framework has been proposed by Weeks *et al.* (2011) to guide conservation managers to make decisions about translocations, including mitigation strategies (for example, using translocated individuals from nearby sites of the recipient

population; evaluating preliminary crosses in captivity; using healthy individuals for translocation; and using a mix of source populations).

Acknowledgements

We would like to thank Luke Price and Mark Bachmann from DEWNR and ForestrySA for donating bandicoot samples. This project was funded by the Australian Research Council Linkage grant (LP0668987), Native Vegetation Council, Wildlife Conservation Fund, DEWNR, the Department of Environment and Primary Industries (Victoria) and The Roy and Marjory Edwards Scholarship provided by Nature Foundation.

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Supporting Information

Table S5-1 Pairwise distance among haplotypes for (a) concatenated mtDNA, (b) *BRCA1*, (c) *RAG1* and (d) *vWF*. The distance values were showed in lower left and the standard errors were showed in upper right.

(a)

mtDNA	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14
H1		0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.002	0.002	0.000
H2	0.019		0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.004	0.004	0.004
H3	0.032	0.028		0.004	0.003	0.004	0.001	0.003	0.003	0.003	0.004	0.005	0.005	0.005
H4	0.035	0.033	0.015		0.003	0.004	0.003	0.003	0.003	0.003	0.000	0.005	0.005	0.005
H5	0.033	0.030	0.014	0.015		0.002	0.003	0.002	0.002	0.002	0.003	0.005	0.005	0.005
H6	0.037	0.035	0.017	0.019	0.006		0.003	0.001	0.001	0.001	0.004	0.006	0.006	0.005
H7	0.031	0.027	0.002	0.015	0.013	0.016		0.003	0.003	0.003	0.003	0.005	0.005	0.005
H8	0.036	0.032	0.015	0.017	0.004	0.002	0.014		0.001	0.001	0.003	0.005	0.005	0.005
H9	0.036	0.033	0.015	0.018	0.005	0.002	0.015	0.001		0.001	0.003	0.005	0.005	0.005
H10	0.036	0.032	0.015	0.017	0.006	0.002	0.014	0.002	0.001		0.003	0.005	0.005	0.005
H11	0.035	0.033	0.015	0.000	0.015	0.019	0.015	0.017	0.018	0.017		0.005	0.005	0.005
H12	0.005	0.023	0.032	0.036	0.036	0.040	0.032	0.038	0.039	0.038	0.036		0.000	0.002
H13	0.005	0.023	0.032	0.036	0.036	0.040	0.032	0.038	0.039	0.038	0.036	0.000		0.002
H14	0.000	0.019	0.032	0.035	0.033	0.037	0.031	0.036	0.036	0.036	0.035	0.005	0.005	

(b)

BRCA1	H1	H2	H3	H4
H1		0.003	0.002	0.003
H2	0.005		0.003	0.003
H3	0.003	0.005		0.003
H4	0.005	0.007	0.005	

(c)

RAG1	H1	H2
H1		0.002
H2	0.002	

(d)

vWF	H1	H2	H3
H1		0.002	0.002
H2	0.004		0.003
H3	0.002	0.006	

Chapter Six: General Discussion

Photo by B. Li

This thesis contains four data chapters in the form of a published primer note, and manuscripts of three papers in the process of submission. Detailed discussions of presented studies are provided for each chapter. The closing chapter summaries the major results of the preceding chapters to emphasise the significance and implications of the findings for conservation management, and also considers the limitations of this study and points out future directions of the research.

Synthesis

Current management actions and recovery plans of the endangered *I. o. obesulus* focus on the following objectives: (1) retain and manage the existing bandicoot populations and their habitat; (2) identify the processes that are threats to bandicoot populations (i.e. habitat loss and fragmentation, fire regimes, and predatory/pest animal species) and implement threat abatement strategies; (3) increase the knowledge of the distribution, abundance, ecology and population structure of the bandicoot; (4) monitor and evaluate responses of bandicoot populations to recovery actions; (5) build a network of individuals, government and non-government organisations to facilitate the recovery actions; (6) enhance the public awareness of the bandicoot and encourage community participation in the recovery program; and (7) assess the possibility and requirement for captive populations (Brown & Main 2010; Haby & Long 2005). Results from this thesis contribute to many of the above objectives and can be used as baseline information or a guide when designing conservation plans (see below). In addition, this study involved close collaboration between several government and non-government conservation organisations, and thus will serve as a linkage network for the management and recovery of *I. o. obesulus*. For the conservation of bandicoot populations, several management actions could be carried out: the construction of habitat corridors, the management of native vegetation to increase bandicoot habitat, and the movement of individuals from one area to another (translocation).

Translocation

Translocation is considered a primary tool for conservation for the purpose of maintaining or increasing biodiversity and maximizing persistence and resilience for both common and threatened species. In the light of genetic implications, the purpose of translocations involves enhancing or maintaining genetic diversity, alleviating detrimental effects of inbreeding depression, and reducing genetic load (the reduction of mean population fitness) in threatened species (Hedrick & Fredrickson 2010; Pickup & Young 2007; Weeks *et al.* 2011). Three different types of translocation are recognised by the International Union for Conservation of Nature (IUCN): re-stocking (or augmentation, movement of individuals of a species into its original habitat), re-introduction (movement of an organism into a part of its native range from which it has disappeared), and introduction (movement of an organism outside its historically known range)

(IUCN 1987). If translocation was considered a priority for current management of *I. o. obesulus* in South Australia it would mainly involve augmentation and re-introduction.

The risks associated with translocation include outbreeding depression, loss of local adaptation, replacement of the recipient genetic background, and disease transmission (Weeks *et al.* 2011). Among these, outbreeding depression is often considered to be the issue of most concern. Outbreeding depression is defined as the "reduction in any pre- or post-mating aspect of reproductive fitness because of attempted crossing of distinct lines/populations, subspecies or even species" (Weeks *et al.* 2011). A framework has been developed by Frankham *et al.* (2011) to help predict the probability of outbreeding depression when translocation is proposed between two populations. The framework included five questions to help make the decision of translocation: (1) is the taxonomy resolved?; (2) are there fixed chromosomal differences among populations?; (3) has there been gene flow between populations within the last 500 years?; (4) are there substantial environmental differences?; and (5) are populations separated for more than 20 generations (Frankham *et al.* 2011).

This study is the first to provide strong genetic evidence for the existence of two distinct ESUs of *I. o. obesulus* in South Australia and south-western Victoria (populations in the Mount Lofty Ranges and Kangaroo Island versus populations in the south-east South Australia and south-western Victoria). According to Frankham's decision framework for translocation, the two ESUs should be managed separately based on their evident genetic differentiation resulting from long term isolation. Therefore, any future translocation plans between them should be avoided since such actions may lead to outbreeding depression. Within the Mount Lofty Ranges, two genetically isolated populations were identified (BNPS and MHS). Negligible dispersal into these patches suggests that both are potentially vulnerable to local extinction (particularly in the event of a fire). Based on the recovery action stating that all existing populations of *I. obesulus* should be retained (see above), translocation may be an appropriate strategy to restore the genetic diversity in these two sites. From Frankham and colleagues' decision framework, the probability of outbreeding depression is expected to be very low if translocation is sourced from sites within the Mount Lofty Ranges. Therefore, genetic rescue and/or restoration by moving individuals from nearby sites into BNPS and MHS could help to maintain these populations and increase their genetic diversity. However, other risks of translocation would need to be thoroughly assessed first, the most detrimental of which being the potential for pathogen transmission (Cunningham 1996; Meltzer 1993), since local adaptation may be less of an issue given the scale of proposed translocation. For bandicoot populations in other sites/patches, considerable population genetic structure is evident and the level of gene flow is insufficient to generate panmictic populations in both landscapes (the Mount Lofty Ranges and the Mount Burr Range). However, gene flow was found mostly between neighbouring sites/patches and genetic diversity appeared to be maintained at similar levels in both landscapes.

These results suggest that translocations among these sites/patches within each locality might not be necessary unless the number of populations is very low or re-introduction is needed in a site/patch with local extinction of bandicoots. Alternative approaches such as ecosystem management would be a better strategy for the conservation of these populations.

Ecosystem management

Single-species approaches to conservation research provide detailed knowledge of relationships between one species and its environment and useful information that can be applied directly for its conservation policy and management (Lindenmayer *et al.* 2007). However, management strategies derived from these studies may neglect their influence on other species and sometimes even have detrimental effects on the conservation of other species (Baker 2000; Pulliam 2000; Simberloff 1998). Ecosystem-based conservation research, on the contrary, identifies key management strategies that may benefit multiple species (Lindenmayer *et al.* 2007). Practically, however, it is impossible to study every single species due to limited funding and time. Therefore, a complementarity between single-species and ecosystem-based conservation research would be prudent to achieve successful conservation outcomes (Lindenmayer *et al.* 2007). Current suggestions for management of *I. o. obesulus*, by way of constructing habitat corridors and retention and maintaining native vegetation of natural habitat, is an ecosystem management approach. These management strategies will benefit the long-term sustainability of other species within the same fragmented landscape through restoring suitable habitat and increasing population connectivity.

At present, *I. o. obesulus* may be best managed using a combination of single-species management (i.e. translocation) and ecosystem management (e.g., habitat corridors, restoration of native vegetation) in South Australia. By comparing the effects of fragmentation on *I. o. obesulus* across two study systems (Chapter Three and Four), this thesis has highlighted that important population processes can be differently affected in the same species depending on the scale and type of fragmentation different populations are subjected to. In landscapes outside of South Australia with similar levels of fragmentation or similar matrices to that in the Mount Lofty Ranges or Mount Burr Range, one might predict that habitat fragmentation may impact bandicoot populations in a similar way to findings from this study. However, predictions about whether populations may be isolated or not need to be treated with caution, and preferably backed up by carrying out further empirical analyses. The markers developed in this study provide an excellent resource for such analyses. Moreover, it is difficult to predict the response of populations of *I. o. obesulus* in other fragmented landscapes with different or more complex matrices. Conservation management of these populations at the ecosystem level, therefore, may be a primary approach if a specific management strategy cannot be obtained due to a lack of ecological and genetic information.

Limitations of the study and future directions of the research

South Australia has experienced extensive native vegetation clearance for agriculture, forestry and urban land uses, leaving 14% of native forest or woodlands remaining in the south-east. In the Mount Burr Range, native vegetation has been severely fragmented into smaller, isolated patches surrounded by matrices of pine or open agricultural land, with no large tracts of natural continuous forest still persisting in this area. In the Mt Lofty Ranges, the native vegetation is much more heterogeneous than that in the Mt Burr Range. Suitable bandicoot habitats are patchily distributed in the Mt Lofty Ranges. There are also lots of open areas with very low cover of native vegetation. These open areas make it unlikely that any of our adjacent collection sites in the Mt Lofty Ranges are connected by continuous habitat. The evidence for limited gene flow also suggests that the habitat in the Mt Lofty Ranges is unlikely to be continuous. Therefore, information on the extent of gene flow and dispersal of the bandicoot in an undisturbed landscape was not possible to obtain in the current study. Without a thorough comparison of population connectivity between continuous forest and fragmented habitat, it is difficult to determine whether *I. o. obesulus* normally shows isolation by distance over small spatial scales or whether the observed genetic structure in *I. o. obesulus* is indeed the result of fragmentation. Comparison of *I. o. obesulus* between isolated patches and relatively larger continuous forest would be beneficial and could be carried out in other regions of Australia where continuous forest systems still occur (e.g Victoria, Rees & Paull 2000).

In addition, detailed knowledge of the dispersal capabilities of *I. obesulus* is limited. Although they are known to exhibit a pattern of juvenile dispersal, with newly independent bandicoots rapidly moving away from their birth place (Heinsohn 1966; Stoddart & Braithwaite 1979), it is still unknown how far the juveniles disperse and whether this pattern of juvenile dispersal is the case for all populations. According to O'Malley's (2011) vagility estimates of this species based on its home range studies, I would only assume that *I. obesulus* could be highly vagile with the capability of moving several kilometres in natural continuous habitat (most likely achieved by dispersing juveniles). In this case, the observed genetic structure is more likely the consequence of fragmentation of the landscape in the Mount Burr Range. Future research of dispersal patterns of *I. obesulus* (i.e. dispersal distance and their capability to move between different habitat types) using GPS or radio tracking technology is required. Such information will increase our knowledge of the behaviour of this species and help clarify the effects of fragmentation on population connectivity of bandicoot populations.

The construction of habitat corridors has become a widely used approach to promote population connectivity within fragmented landscapes. However, without the data on population genetic structure and gene flow among habitat patches prior to the construction of corridors, the effectiveness of habitat corridors can be difficult to measure. In the south-eastern South Australia, a

biodiversity corridor program has been proposed by a state government based organisation (ForestrySA), with twelve corridors to be implemented in the Mount Burr Range. My study of gene flow/dispersal of *I. o. obesulus* in this area provides a valuable opportunity for future research to assess the effectiveness of these habitat corridors.

A particular interest of this study was to investigate genetic connectivity of numerous *I. o. obesulus* populations within fragmented landscapes. The results of significant genetic structure and limited gene flow across two different landscapes raise the issue of functional connectivity ("the degree to which the landscape facilitates or impedes movement among resource patches", Taylor *et al.* 1993), which is the response of individual bandicoot populations to different landscape features (i.e. pines, pastoral lands, roads and urban constructs). Future research could correlate landscape characteristics with the estimates of gene flow using landscape genetics tools to further investigate how different matrices affect genetic connectivity of *I. o. obesulus* populations. The fragmented landscape in the south-east of South Australia has also been the focus of studies for other native mammal species (yellow-footed antechinus, Gould's long-eared bat, lesser long-eared bat, common ringtail possum and sugar glider), with an overall aim of exploring the impact of fragmentation on population processes. A multispecies landscape-genetic approach could be developed based on these studies to achieve more generalised conservation strategies for the management of biodiversity. The general pattern derived from this multispecies study may also apply to other ecosystems.

My study identified the presence of two distinct ESUs of *I. o. obesulus* in South Australia and raised the issue of whether the two ESUs represent different species. Furthermore, my study revealed a closer relationship between *I. auratus* and the Mount Lofty Ranges/Kangaroo Island ESU of *I. o. obesulus* than previously identified, suggesting species classification within *I. obesulus/I. auratus* requires clarification. Previous morphological work in *I. obesulus* suggested an association between geographical variation in body size and habitat structure (and also the amount of annual rainfall) (Cooper 1998, 1999). Morphological analyses of specimens from each ESU and the broader range of the species in Australia will thus complement genetic methods in resolving the taxonomic status of populations. In addition, a broader detailed phylogenetic study is also required to independently assess the systematics of the group. To cover the whole distribution range in Australia of this species, sampling sites should include Nuyts Archipelago of South Australia (*I. o. nauticus*), southern Victoria (*I. o. obesulus*), Tasmania (*I. o. affinis*), south-western Western Australia (*I. o. fusciventer*), south-eastern New South Wales (*I. o. obesulus*), and the Cape York of Queensland (*I. o. peninsulae*). Along with the three nuclear markers (*BRCA1*, *RAG1*, and *vWF*) used in this study, additional nuclear markers should be utilised in future phylogenetic studies of *I. obesulus* to conduct more thorough and more complicated analyses.

The Murray Basin is recognised as a significant biogeographic barrier in eastern Australia (Chapple *et al.* 2011a; Chapple *et al.* 2011b; Dickinson *et al.* 2002; Ford 1987a, b). Genetic breaks across the Murray Basin region have been observed in several species, including a dunnart (Cooper *et al.* 2000), two frogs (Schauble & Moritz 2001; Symula *et al.* 2008), two lizards (Chapple *et al.* 2005; Dubey & Shine 2010), a snake (Keogh *et al.* 2005), a skink (Chapple *et al.* 2011a) and grasshoppers (Kawakami *et al.* 2009). The Murray Basin may also have originally been responsible for the limited or lack of no gene flow in *I. obesulus*, resulting in the current genetic subdivision we observed. A recent study showed that climatic factors and the related natural vegetation patterns also play important roles in the distribution of *I. obesulus* (Paull *et al.* 2013). Climatic fluctuations during ice ages, therefore, may have had an influence on population structure by contracting the distribution range of the species. A comprehensive phylogeographic study with dating analyses would help to investigate these hypotheses further.

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Appendix

Microsatellite genotypes and sequence data were stored on CD

Chapter Three alignments: microsatellite genotypes for Chapter Three.

Chapter Four alignments: microsatellite genotypes for Chapter Four.

Chapter Five alignments: microsatellite genotypes and sequence data for Chapter Five.

NOTE:
The appendices are on a CD included with the print copy of the thesis held in the University of Adelaide Library.