Conservation Genetics of Australian Quolls

Maria J. Cardoso

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A dissertation presented to the University of New South Wales in fulfillment of requirement for the degree of Doctorate of Philosophy in Biological Sciences
Abstract 350 words maximum: (PLEASE TYPE)

As carnivorous marsupials, quolls are keystone species crucial to ecosystem health. Although opportunistic and adaptable, anthropogenic disturbance has led to severe population declines. In response, captive breeding and translocation programs were implemented in an attempt to mitigate extinction risks.

This thesis provides genetic (microsatellite) data to guide management of the four Australian quoll species. Parameters of interest to conservation were examined. Differences between translocated and endemic, island and mainland, and central and peripheral populations were also investigated. In addition, analysis of paternity was performed in a spotted-tailed quoll population.

Despite severe range contraction, western quolls (chuditch) have higher genetic variation than other Australian quoll species. Conversely, genetic variation is lowest in eastern quolls, but also in northern quolls restricted to islands. Evidence of founder effects was detected in translocated island, but not in translocated mainland populations, emphasizing that greater losses in genetic diversity occur on islands compared to mainland populations due to isolation from sources of gene flow.

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Although genetic differentiation is significant among populations within each quoll species, moderate levels of admixture were detected, suggesting that gene flow is playing a role in maintaining connectedness among populations. However, given that eastern quolls are less mobile than other Australian quoll species, they may be more susceptible to anthropogenic disturbance.

Finally, variation in spotted-tailed quoll population was detected in a spotted-tailed quoll population and appears to be a strategy that has evolved to maintain genetic variation and reduce inbreeding within populations.

These data provide invaluable information to guide and improve the conservation of the four Australian quoll species. Ongoing management of these threatened species, such as population monitoring, supplantations and translocations will help ensure that quolls remain as keystone species in the Australian environment.
“Another [quadruped] was called by the natives Je-Quoll: it is about the size and something like a polecat, of a light brown spotted with white on the back and white under the belly.”

- Journal entry by Joseph Banks on the Endeavour, 1770

“In those early days ... we domesticated quolls (“native cats”).”

- Reference in Nightwatchmen of Bush and Plain written by David Fleay in 1968.

To a world where all creatures may live without fear

May the QUOLL be with you

Image on front cover: Dasyurus maculatus (spotted-tailed quoll), reproduced from an illustration by Rosemary Woodford Ganf included in the book A Fragile Balance by Christopher Dickman © 2007.
Declaration of Originality

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at UNSW or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.

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Preface

This thesis consists of four stand-alone papers (chapter 2 to chapter 5). Two papers (chapter 2 and chapter 5) have been published in scientific journals. Chapter 2 was published in *Conservation Genetics* (Cardoso et al. 2009) as a first author paper and chapter 5 was published in the *Biological Journal of the Linnean Society* (Glen et al. 2009) as a second author paper. I contributed 50% to the analysis and writing of this paper (see letter following preface). The other two papers (chapter 3 and chapter 4) are being prepared for submission to journals of high standing.

The four Australian quoll species were examined separately in each of the chapters. The baseline genetic data on spotted-tailed quolls is not presented in a chapter format, but is included in Appendix 4. Raw data was compiled and is presented electronically, attached to this thesis. Due to the stand-alone nature of each chapter and the use of similar methods of genetic analyses for each species, some repetition does occur. In addition, to prevent unnecessary duplication, a single reference list is provided at the end of the thesis, formatted in the style of *Molecular Ecology*.

The work contained in this thesis was performed by myself, with guidance from my principal supervisors, Assoc. Prof. William B. Sherwin and Dr. Karen B. Firestone. Field work and sample collection were conducted by or in collaboration with State government industry partners and other independent researchers (see Raw Data 1, acknowledgements and Table 1-1). The contributions of co-authors to the conception, data analysis and writing of each chapter are detailed below:

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MJC - Maria J. Cardoso; WBS - William B. Sherwin; KBF - Karen B. Firestone; ASG - Alistair S. Glen; MDBE - Mark D. B. Eldridge; MO - Meri Oakwood
7th July 2010

To whom it may concern,

This letter is to certify that Maria Cardoso and Alistair Glen made an equal 50-50 contribution to the analysis and writing of the following manuscript:


The contributing authors can be contacted on the details below.

Yours sincerely

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Abstract

As carnivorous marsupials, quolls are keystone species crucial to ecosystem health. Although opportunistic and adaptable, anthropogenic disturbance has led to severe population declines. In response, captive breeding and translocation programs were implemented in an attempt to mitigate extinction risks.

This thesis provides genetic (microsatellite) data to guide management of the four Australian quoll species. Parameters of interest to conservation were examined. Differences between translocated and endemic, island and mainland, and central and peripheral populations were also investigated. In addition, analysis of paternity was performed in a spotted-tailed quoll population.

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Finally, multiple paternity was detected in a spotted-tailed quoll population and appears to be a strategy that has evolved to maintain genetic variation and reduce inbreeding within populations.

These data provide invaluable information to guide and improve the conservation of the four Australian quoll species. Ongoing management of these threatened species, such as population monitoring, supplementations and translocations, will help ensure that quolls remain as keystone species in the Australian environment.
Acknowledgements

It does not seem like that long ago that I was writing an honours thesis describing a novel herpesvirus discovered in Indian *Gyps* vultures. Six years later, vulture populations have not fully recovered, not from herpesvirus infections, but from lethal toxicity associated with environmental accumulation of a synthetic drug. Sadly, this is merely another shocking example of human-induced environmental change resulting in devastating impacts on ecosystem function. The dire predicament of so many of the world’s precious organisms is partly what inspired me to undertake a career in the biological sciences, which has over the years strengthened my passion for making a useful contribution to global biodiversity. So here I am now, writing a PhD dissertation on the conservation genetics of Australian quolls. The journey has been exciting, but not always smooth sailing! There were many challenges, but also numerous accomplishments and I am immensely grateful to all the wonderful people who supported and encouraged me along the way.

My list of acknowledgements seems virtually endless, so I sincerely apologise if anyone is overlooked. I must begin by crediting my former supervisor Dr. Karen Firestone for designing such an amazing project and providing me the opportunity to become involved. It is unfortunate however, that she could not see it to fruition. For that I am greatly indebted to Associate Professor Bill Sherwin, who kindly incorporated me onto his fast-growing list of students so that I could continue working on the project. Bill, I dearly appreciate your patience and ability to explain complex concepts of population genetics in a way that a molecular virologist, with little background in genetics, could understand. Your brilliant lateral thinking, quirky sense of humour and ability to put things into perspective helped me persevere through the tough times in order to complete this PhD. Thank you.

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Chapter 1

General Introduction

Many species are threatened with extinction worldwide and are likely to require human intervention to ensure their survival (Bonn and Gaston 2005; Allendorf and Luikart 2007). Mammals appear particularly prone to extinction (Cardillo et al. 2004) and Australian mammals in particular, with 22 species driven to extinction over the last 200 years: a further eight species are now restricted to islands or captive populations and many others have substantially reduced population sizes and distributions (Short and Smith 1994; Burbidge 1999; Fisher et al. 2003). The cause of species declines is often attributed to the growth of human populations and associated impacts such as the degradation and fragmentation of habitat, hunting, changed fire regimes, the introduction of exotic competitors, predators and pathogens, and climate change (Reed 2004; Pedersen et al. 2007; Sinervo et al. 2010). Extinction risk is thought to generally increase with body size, with many declining species being large mammalian carnivores (Cardillo et al. 2005). By contrast, terrestrial Australian species within the critical weight range (CWR, 35-5500g) have declined more severely since European settlement (Johnson and Isaac 2009).

Threatening processes and conservation requirements vary widely among species and must be managed accordingly if extinction risks are to be successfully mitigated. Wildlife managers aim to halt the loss of global biodiversity by linking patterns of threatened species distribution and abundance with the occurrence of threatening processes. In Australia, predation and competition by the introduced European red fox (*Vulpes vulpes*) and feral cat (*Felis catus*) has been implicated in many species declines (Dickman 1996). However, alongside external threats like habitat fragmentation and predation, intrinsic factors such as life-history traits may also determine extinction risk (Fisher et al. 2003). For instance, the risk of extinction for species at high trophic levels may be exacerbated by their need for large hunting areas that may be fragmented, but also a high abundance of prey species that may themselves be threatened. Therefore, those species that possess biological traits that allow ecological flexibility may be better adapted to deal with external threats (Cardillo et al. 2004; Cardillo et al. 2005).
1.1 Wildlife Management and Conservation

Population monitoring, captive breeding and translocations have become important conservation strategies used in the management of threatened species (Maudet et al. 2002). Monitoring wild populations is a necessary step to establish the conservation status of species thought to be at risk, because the disappearance of populations is a prelude to species extinction (Ceballos and Ehrlich 2002). Ongoing monitoring of wild, captive and translocated populations is important for tracking changes over time so that issues can be readily detected and management strategies implemented accordingly (Seddon et al. 2007). Historically, the translocation of species that have suffered severe range reductions has had limited success in establishing self-sustaining populations. Failure has often resulted from poor planning, inappropriate founder animals, low sample sizes, inadequate control of threatening processes, limited post-release monitoring and a general lack of resources (Wolf et al. 1998; Mock et al. 2004; Sigg et al. 2005; Seddon et al. 2007). In addition, success rates are thought to be low when captive-bred animals are used as the source for translocations, because inbreeding, loss of genetic diversity and genetic adaptations to captivity may lead to the loss of fitness and evolutionary potential of the translocated populations (Woodworth et al. 2002; Mathews et al. 2005). Translocation success is therefore dependent on factors such as habitat quality and size of the release site, the number and genetic composition of the animals released, and the management of threatening processes (Eldridge et al. 2004; Bouzat et al. 2009). There are of course exceptions where well planned management actions have led to successful translocations (Morris et al. 2003; Olsson 2007; Taylor and Jamieson 2008). Therefore, translocation programs may benefit threatened species if managed effectively to include monitoring of genetic variability, effective population size, population structure and dispersal. These parameters may elucidate the extent to which populations naturally support one another without management intervention, and in extreme cases, identify whether there may be a risk of genetic incompatibilities arising when animals are moved between locations (Storfer 1999; Bouzat et al. 2009).

1.2 Genetics in Conservation

Conservation programs should, as much as possible, draw on theory from disciplines such as ecology and genetics in order to be successful (Seddon et al. 2007). Although population persistence is often influenced by demographic and environmental
stochasticity, the evidence for genetic effects on fitness and persistence in wild populations is now irrefutable (Bouzat et al. 1998; Reed and Frankham 2003; Spielman et al. 2004). Despite such evidence, the role of genetics in conservation has been questioned (Caughley 1994) and genetic issues have often been neglected when planning management strategies (Maudet et al. 2002). Furthermore, the misconception regarding the role of genetics in conservation has led to a delay in the integration of genetic tools in wildlife management in Australia (Banks and Taylor 2004; Sarre and Georges 2009). However, advances in the rapidly expanding field of molecular genetics have allowed genetic data to be more readily applied to problems in wildlife management and conservation of Australian fauna (Sherwin et al. 1991; Taylor et al. 1994; Moritz et al. 1997; Firestone et al. 2000; Eldridge et al. 2004; Jones et al. 2004a; Cardoso et al. 2009).

Molecular data can be used to monitor parameters of interest to wildlife managers such as genetic variability, effective population size, and patterns of genetic structure and dispersal among populations. Microsatellites have become the marker of choice for many conservation studies, because of their high polymorphism, assumed neutrality and random distribution across genomes (Bruford and Wayne 1993). The use of field techniques such as mark-recapture and telemetry to gather population data can be limited by logistic and resource constraints (Banks et al. 2005; Beck et al. 2008). As a result, detailed demographic information on threatened species may be lacking or non-existent (Banks and Taylor 2004). The alternative is to use population genetics methods that are less reliant on intensive data collection to gather surrogate or complementary information to field data (Eldridge et al. 2004; Broquet et al. 2009). Importantly, baseline genetic data gathered through monitoring programs before and after population declines occur have the potential to shape and inform the conservation of threatened species by providing crucial information upon which management actions can be instigated (Banks and Taylor 2004; How et al. 2009). Population genetic data therefore takes some of the guesswork out of conservation planning, because it allows wildlife managers to be more confident in making informed decisions concerning the future of threatened species.
1.2.1 Genetic variation

Maintaining genetic variation is a common goal for the conservation of threatened species. Genetic variation is important for both the short- and long-term viability of populations, because it is the raw material for evolutionary change, allowing populations to evolve and adapt in response to changes in their environment (Frankel and Soulé 1981). Consequently, a lack of diversity is typically considered as evidence of a small or declining and potentially endangered population, with abundant species being, on average, more genetically diverse than scarce ones (Amos and Balmford 2001; Spielman et al. 2004). Management strategies such as translocations, which are often used to maximize species persistence, may sometimes lead to undesirable genetic consequences, such as bottlenecks, founder effects and the introduction of alleles maladapted to the local environment (Storfer 1999; Mock et al. 2004). As a result, translocated populations, particularly those founded by a small number of animals, may have lower genetic variation than their source populations. Therefore, the release of at least 30 founders from high-diversity rather than low-diversity source populations is recommended in order to maximise translocation success (Miller et al. 2009). The introduction of new genetic material into a population via the translocation of large diverse founder groups has been shown to boost genetic variation, fitness, persistence and potential for evolutionary adaptation to a changing environment (Westemeier et al. 1998; Bouzat 2001), thus reversing some of the effects of population declines. It has also been suggested that losses in genetic variation may be minimized when populations expand rapidly after translocation (Allendorf and Luikart 2007).

1.2.2 Island versus mainland populations

The high rate of recent global extinctions has resulted in the fragmentation and isolation of many mammal species (Short and Smith 1994; Cardillo et al. 2004). Those species that historically occurred on both the mainland and off-shore islands have persisted more frequently on islands due to their insulation from the anthropogenic disturbances that extirpated or reduced the size of the mainland populations (Channell and Lomolino 2000a). Islands are therefore seen as important refuges for the conservation of threatened species, providing opportunities for relatively inexpensive, although short-term management, compared to remnant mainland populations (Moro 2003; Eldridge et al. 2004; How et al. 2009). Consequently, translocations to islands have become an important management strategy to increase the survival of many
species (Frankham et al. 2002). However, the genetic effects of translocations can sometimes be exacerbated when animals are moved to islands (Eldridge et al. 1999), with the major concern being that island populations, through isolation, small population size and genetic drift, are more prone to extinction due to genetic erosion than their mainland counterparts (Frankham 1997; Burbidge and Manly 2002; Eldridge et al. 2004; Mills et al. 2004), which themselves are more likely to retain the evolutionary potential necessary for long-term persistence (Lacy 1997; Frankham et al. 2002). Furthermore, island populations can potentially become genetically valuable and warrant separate management due to endemism caused by divergence from their mainland relatives (Crandall et al. 2000; Wilson et al. 2009). However, the potential endemism of island populations, which is often associated with reduced genetic diversity, increased levels of inbreeding and the evolution of adaptations to island environments, may also limit their suitability as sources for translocations to mainland sites (Eldridge et al. 2004). Consequently, the translocation of Australian mammals from islands to the mainland have had limited success (Burbidge 1999).

1.2.3 Bottlenecks and founder effects

The detection of genetic bottlenecks is an important issue for conservation, because bottlenecked populations may be more vulnerable to extinction due to genetic drift, inbreeding and the loss of genetic variability and evolutionary potential (Frankham 1995c; Frankham et al. 1999). A bottleneck occurs when a population is reduced to a minimum size, either as the result of a population crash or through a founder event. The founder effect, as defined by Ernst Mayr (1963), is the effect of establishing a new population by a small number of individuals, carrying only a small fraction of the original population’s genetic variation. As a result, the new population may be distinctively different from the source population from which it was derived, both genetically where variation may be lower and phenotypically where particular traits may prevail over others. Founder effects are common in island populations, but may also occur in mainland populations due to isolation, range expansions and translocations. Environmental changes such habitat fragmentation, human persecution or over-exploitation, disease, wildfires and other catastrophes may all cause population bottlenecks (Frankham et al. 1999).
Bottlenecked populations may either become extinct or remain at a small size for some time before increasing in size once again, either gradually or rapidly, albeit often containing a reduced level of genetic variation (Lambert et al. 2005). Furthermore, the variation resulting from population bottlenecks can lead to an increase in deleterious alleles rather than variation that allows adaptive evolutionary change (Frankham et al. 1999). Population genetics theory predicts that heterozygosity will be reduced by $1/(2Ne)$ during a single generation bottleneck, where $Ne$ is the effective size or the effective number of adult breeders in the bottlenecked population (James 1971). However, the allelic diversity is reduced faster than the heterozygosity so that the observed heterozygosity is larger than the heterozygosity expected from the observed allele number at mutation-drift equilibrium (Luikart and Cornuet 1998). Genetic bottlenecks may sometimes be cryptic and difficult to detect. Therefore, statistical programs such as BOTTLENECK were developed to use heterozygosity excess to detect recent reductions in effective population size from allele frequency data (Piry et al. 1999). Having access to such molecular tools allows wildlife managers to detect population bottlenecks when managing populations. For example, knowledge about bottlenecks may be used when founding captive, reintroduced or translocated populations, or to identify populations with reduced ability for evolutionary potential, such as is often the case with island populations (Frankham et al. 1999).

### 1.2.4 Effective population size

Estimates of effective population size ($Ne$) or the number of effective breeding adults in a generation (Wright 1931) are important to conservation management, with the average ratio between effective and census population size ($Ne/N$) estimated to be approximately 0.1 for wildlife species (Frankham 1995b). Such estimates may be used when selecting founding individuals for translocations, assessing affects associated with bottlenecks and founder effects, but also for monitoring and early detection of changes in population size before declines are detectable by other means. The rate of loss of genetic variation via genetic drift is greater when the effective population size is small (Frankham 1995b). Consequently, as population size decreases, a number of genetic and environmental factors are likely to drive individual populations to extinction (Frankham et al. 2002). Franklin (1980) suggested that in order for a species or population to be viable in the long-term, it must have an effective population size of
at least 500. This however is a simple assumption that does not apply to all organisms, because precise Ne estimation is complex and largely dependent on the biology of the species (Nunney and Campbell 1993). Estimation of Ne is particularly difficult in populations or species with overlapping generations and short life spans, but also when there are temporal fluctuations in population size and confounding effects linked to immigration and population subdivision (Nunney 1993; Nunney and Elam 1994; Waples 2002). Consequently, many methods of estimating Ne rely on assumptions that are unrealistic in natural populations, such as drift-mutation equilibrium and constant population size, but are still valuable for monitoring the genetic structure of populations (Waples 2005, 2007).

The effective size of a population can be estimated in several ways using molecular genotype data. Temporal methods are widely used, but have the disadvantage of requiring two or more temporally-spaced sampling efforts (Wang and Whitlock 2003; Tallmon et al. 2004; Waples 2006), which is not often possible for many threatened species. For this reason, methods of estimating Ne based on single sampling efforts have attracted increasing interest in conservation genetics (Beebee 2009). However, because these methods have only been partially tested by simulations and empirical data, there are concerns regarding their precision (England et al. 2006). A recent study by Beebee (2009), which used empirical data to compare the precision of four different single-sample Ne estimators found that the Bayesian (Tallmon et al. 2008) and sibship (Wang 2009) based methods performed better than methods based on heterozygote excess (Luikart and Cornuet 1999; Peel et al. 2004) and linkage disequilibrium (Waples and Do 2008) when tested on empirical data and compared to census size estimates.

1.2.5 Dispersal and genetic structure

Successful implementation of conservation actions must be guided by knowledge regarding the genetic composition and structure of threatened populations. The rate of dispersal and gene flow among populations determines their potential for genetic differentiation (Wright 1951) and plays a critical role in the genetic structure, dynamics and local adaptation of populations. Dispersal is related to an organism’s mobility, but also the distance between subpopulations, the probability of survival during dispersal and the suitability of the surrounding landscape that is available for colonization (Vandermeer and Carvajal 2001). Genetic estimates of dispersal and gene flow are
therefore critical to species conservation, because they allow the identification of natural and/or anthropogenic discontinuities among populations. It is thought that species have a lower risk of extinction when maintained as a series of subpopulations linked by high levels of gene flow (Reed 2004), but the cohesion among populations may be disrupted if gene flow is reduced to fewer than one disperser per generation (Crow and Kimura 1970; Morjan and Rieseberg 2004; Bouchy et al. 2005).

Although very useful, dispersal is often one of the most difficult population parameters to measure (Nathan 2005), with both demographic- and genetic-based methods of estimating dispersal criticised for lack of accuracy (Vandewoestijne and Baguette 2004). Traditional direct (demographic) estimates of dispersal based on mark-recapture techniques can be biased by the size of the study area due to spatial and temporal restrictions (Franzen and Nilsson 2007), while indirect (genetic) estimates of dispersal are based on assumptions, such as mutation-drift equilibrium and constant population size, that may be unrealistic in natural populations (Whitlock and McCauley 1999) and do not differentiate between historical and contemporary patterns of dispersal (Bossart and Prowell 1998). However, different time frames can be selected for analysis based on which method is used to measure dispersal. For instance, widely used equilibrium-based methods such as Wright’s F-statistics (Wright 1951; Holsinger and Weir 2009) reflect historical dispersal processes, while individual-based assignment tests (Cornuet et al. 1999; Pritchard et al. 2000; Piry et al. 2004) estimate contemporary patterns of dispersal (Berry et al. 2004). A comparison of both approaches may be useful to conservation, because it may allow natural historical processes to be distinguished from changes in population structure triggered by more recent anthropogenic disturbance.

1.2.6 Defining Conservation Units

Restricted dispersal among populations is expected to give rise to local genetic structure, with relatedness between individuals declining with increasing geographical distance (Beck et al. 2008). Determining when groups of individuals are different enough to be considered separate populations is important to conservation management so that genetic incompatibilities can be avoided when animals are moved between locations (Waples and Gaggiotti 2006). As a result, many statistical methods have been developed to allow the identification of the number of populations and/or assign individuals to their population of origin (Paetkau et al. 1995; Rannala and Mountain
Identifying the boundaries of natural populations remains, however, a challenging and hotly debated topic that may have far-reaching management (and legal) implications, particularly when dealing with threatened species (Moritz 1994; Waples and Gaggiotti 2006). The challenge focuses on the protection of sufficient viable populations to enable a species to survive in the short- and long-term (Moritz 1999). Consequently, it is important to base management decisions concerning priorities for conservation on the frequency of dispersal observed between regions rather than on imaginary cartographical boundaries to which organisms do not generally adhere.

Theoretically, targets or units of conservation are commonly known as Evolutionarily Significant Units (ESUs) and Management Units (MUs). ESUs are defined as historically isolated and independently evolving sets of populations within a species that should be preserved as separate entities, because they are ‘reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci’ (Moritz 1994). MUs are the ecological components of ESUs represented by populations that are demographically independent and show divergence in allele frequencies at mtDNA and/or nuclear loci. MUs should be managed, but do not need to be preserved as separate entities in order to maintain the integrity of the larger ESUs (Moritz 1994, 1999; Hedrick et al. 2001b; Frankham et al. 2002).

1.2.7 Central versus peripheral populations

Knowledge about the genetic composition and structure of central and peripheral populations is important to conservation, because it helps to identify populations that should be prioritized for management. In addition, such knowledge may provide useful insights into processes associated with the contraction of species’ distributions. Studies of range contraction have found a tendency for species to persist in the periphery of their historical range, because these regions are often more isolated from the threatening processes that caused the species to contract in the first place (Channell and Lomolino 2000b). Furthermore, within a species range, the ‘central-peripheral’ hypothesis predicts that peripheral populations should exhibit lower genetic diversity and higher genetic differentiation than populations at the core of a species range (Eckert et al. 2008). The ‘central-peripheral’ hypothesis has a long history in the ecological literature, but its validity has recently been challenged (Sagarin et al. 2006).
The main argument is that most studies do not provide enough coverage across a species’ geographical range and may therefore not accurately interpret patterns of geographical variation in population genetic structure (Sagarin and Gaines 2002). These patterns may be influenced by both historical and contemporary changes to population size and gene flow, but also by spatial patterns in habitat quality (Vucetich and Waite 2003).

There is also considerable disagreement among biologists regarding priorities for the management of peripheral populations (Garner et al. 2004). Due to their isolation from central sources of dispersers, peripheral populations are thought to be more prone to temporal variability in abundance, bottlenecks and losses of genetic variation, and may therefore be of limited conservation value (Rowe and Beebee 2003; Eckert et al. 2008). If however, populations at the range periphery maintain substantial genetic variation, they may adaptively diverge from more central populations due to different selective pressures and reduced gene flow. Consequently, peripheral populations, although unstable compared to more diverse central populations, may be well adapted to local environmental conditions and should be regarded as evolutionarily significant (Vucetich and Waite 2003). Depending on their demographic and genetic properties, peripheral populations may provide a source of locally adapted significant variation upon which natural selection may act in the face of stochastic events that may drive less adaptable populations to extinction or trigger shifts in species’ geographical distributions (Garcia-Ramos and Kirkpatrick 1997; Eckert et al. 2008). Therefore, conservation strategies should aim to preserve those populations that possess the maximum genetic variation representative of the species, but less diverse peripheral populations may also be worth preserving.

1.4 Study species

The four species of Australian quoll (Dasyuridae: Marsupialia; Figures 1-1 to 1-4), previously described in detail by Firestone (1999), have experienced substantial declines in range and abundance since European settlement (Table 1-1; Figure 1-5). Coincidently, the three species of introduced eutherian carnivores, dogs (Canis lupus), foxes and cats, have expanded their range and now occupy most of Australia, including Tasmania (Dickman 1996). Furthermore, quolls remained the largest native marsupial carnivores on the Australian mainland following the extinction of the thylacin
Thylacinus cynocephalus) and the restriction of the Tasmanian devil (Sarcophilus harrisii) to Tasmania (Jones et al. 2003) ~2000 and ~600 years ago, respectively. Quoll species are endemic to different Australian regions ranging from the tropics in the north, deserts in the west and temperate and subalpine habitats in the south (Figure 1-5). They therefore occupy important ecological niches, but may also be regarded as keystone species whose presence represents ecosystem health (Morris et al. 2003; How et al. 2009).

Quolls are generalist predators and opportunistic scavengers whose diet may consist of insects, small to medium sized mammals, reptiles, amphibians and birds, but also carrion and some vegetable matter (Soderquist and Serena 1994; Jones and Barmuta 1998; Jones and Barmuta 2000). This opportunistic nature allows quolls to adapt to a wide range of habitats including rainforest, wet and dry sclerophyll forest, heathland and woodland (Jones et al. 2003; Glen and Dickman 2006). They are also known to forage near roads and human habitation, which can sometimes lead to mortality by motor vehicle impact or human persecution (Serena et al. 1991; Jones et al. 2003; Morris et al. 2003). Despite their adaptability, quolls have been affected by anthropogenic disturbance in various ways, ranging from habitat destruction to unregulated fire regimes and introduced species. As a result, three of the four Australian quoll species are now listed as threatened (Table 1-1).

All the Australian quoll species are solitary, cryptic and predominantly nocturnal, which makes them difficult to detect in the wild. Furthermore, they are known to undergo seasonal fluctuations in population size due to life history strategies, which may complicate attempts to accurately monitor the size of populations. For instance, generations overlap, with juveniles becoming reproductively mature in their first year and being known to successfully breed in their final year (Kortner et al. 2004). In addition, the life span of each species is relatively short, ranging between one year (northern quolls) and four years (other quolls) in the wild. Furthermore, semelparity or post-breeding male die-off has been reported in northern quolls (Oakwood 2000). Although quolls tend to be successful seasonal breeders with five to seven young produced each winter, juvenile survival may be low following dispersal, primarily due to predation, but also because of failure to locate vacant territories suitable for colonization (Soderquist and Serena 2000). With the exception of eastern quolls, which
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may occur at high densities, disperse small distances (1km) and occupy non-territorial small home ranges with extensive overlap among both sexes (Godsell 1983), the other three species occur at low densities, disperse long distances (particularly males in the breeding season) and are territorial, with large intra-sexually exclusive female home ranges and male ranges that overlap with females and other males (Serena and Soderquist 1989; Oakwood 2002; Belcher and Darrant 2004; Kortner et al. 2004; Claridge et al. 2005).

Theoretically, connectivity among most quoll populations should be high given their dispersal ability, but because dispersal success is largely dependent on the availability of large areas of suitable habitat, gene flow and population structure may be disrupted by threatening processes such as habitat fragmentation, human-induced mortality and the presence of introduced predators and competitors. Therefore, genetic estimates of dispersal and gene flow may be useful to quoll conservation, because they may identify natural and/or anthropogenic discontinuities among populations that may be difficult to detect by other means. Separate State government agencies are currently responsible for the conservation of the four species of Australian quoll (Table 1-1). It is their intention to use population genetic data to monitor parameters of interest to conservation, such as genetic variability, effective population size and the extent of gene flow among populations in an attempt to reduce the extinction risk of each species (Dunlop and Morris 2008; Rankmore et al. 2008; Long and Nelson 2010b).

1.4.1 The northern quoll Dasyurus hallucatus Gould 1842

The northern quoll is the smallest of the Australian quoll species (Figure 1-1; Table 1-1). It formerly occurred across most of northern Australia, including some offshore islands (Oakwood 1997; Woinarski et al. 1999b; How et al. 2009). Over the last few decades the northern quoll has declined across much of its range (Figure 1-5A), probably through a combination of factors, such as fire and habitat degradation (Braithwaite and Griffiths 1994; TSSC 2005). Current threatening processes include changed fire regimes, predation by feral carnivores (Oakwood 2000; Woinarski et al. 2004a) and more recently, poisoning through ingestion of toxins when attempting to predate on the exotic cane toad Chaunus [Bufo] marinus (Burnett 1997; Oakwood 2004), a species that is now spreading rapidly across the northern quoll’s range. The northern quoll is ‘Endangered’ under the Australian Environment Protection and
Biodiversity Conservation Act (EPBC) and ‘Critically Endangered’ under Northern Territory legislation (Woinarski et al. 2007).

![Northern quoll (Dasyurus hallucatus)](image)

**Figure 1-1** Northern quoll (*Dasyurus hallucatus*). Photo courtesy of Meri Oakwood and Peter Foster.

In 2003, the Department of Natural Resources, Environment and the Arts (NRETA) of the Northern Territory captured 64 northern quolls from wild Northern Territory mainland populations and translocated them to two offshore islands to establish insurance populations and reduce the species’ risk of extinction (Rankmore et al. 2008). Samples for genetic analysis were taken from the original translocation founders and through monitoring programs of the islands from 2004-2006. Samples were also collected from two endemic islands and three mainland populations. The resulting molecular data were analysed to answer the following questions:

1) What is the genetic composition of the translocated island populations and how has it changed over time?
2) Were the translocations successful in establishing genetically viable populations? Are there any detectable genetic effects associated with the translocations?
3) How does the genetic composition of the translocated island populations compare to that of the endemic island and mainland populations? Is there any evidence of genetic erosion?

4) Will the translocated island populations be suitable for use in the future supplementation of threatened and locally extinct mainland populations?

1.4.2 The western quoll *Dasyurus geoffroii* Gould 1841

The western quoll, better known as the chuditch, is the largest native mammalian predator in Western Australia (Figure 1-2; Table 1-1). At the time of European settlement, chuditch were common and occupied nearly 70% of the Australian continent, occurring in every state and the Northern Territory on the Australian mainland. However, severe declines in the mid to late 1800s saw their distribution contract to approximately 5% of their former range (Figure 1-5C), with an estimated 6000 individuals remaining in the wild by the late 1980s (Serena et al. 1991). Major threats to chuditch populations include land clearing and habitat fragmentation, predation and competition from introduced predators and human-induced mortality such as poisoning, trapping, illegal shooting and road accidents. The species is currently listed as ‘Vulnerable’ under the *EPBC Act 1999* (Morris et al. 2003; Dunlop and Morris 2008).

A Recovery Plan that included control of introduced predators, captive breeding and translocations was prepared by the Department of Environment and Conservation of Western Australia (DEC, previously known as CALM) to mitigate threats and improve the conservation status of chuditch in Western Australia (Serena et al. 1991; Orell and Morris 1994; Dunlop and Morris 2008). Samples for genetic analysis were taken from three translocated and five remnant populations to answer the following questions:

1) What is the genetic composition of the translocated populations and how does it compare to that of the remnant populations? Is there any evidence of genetic erosion?

2) Were the translocations successful in establishing genetically viable populations? Are there any detectable genetic effects associated with the translocations?
3) What are the patterns of gene flow and genetic structure among the sampled populations? Are there any significant genetic discontinuities?

Figure 1-2 Male chuditch (*Dasyurus geoffroii*). Photo courtesy of Al Glen.

1.4.3 The eastern quoll *Dasyurus viverrinus* (Shaw 1800)

The eastern quoll is the third largest extant native carnivorous marsupial in Tasmania (Jones and Barmuta 2000; Figure 1-3; Table 1-1). It was once widely distributed throughout southeastern Australia from New South Wales to South Australia (Godsell 1982), but like the Tasmanian devil, it is now restricted to the island of Tasmania (Figure 1-5D). The combined pressures of a disease epidemic at the turn of the 20th century (Burbidge and McKenzie 1989), competition and predation from introduced species, and human encroachment and persecution led to a dramatic range decline and eventual extinction on the Australian mainland in the 1960s (NPWS 1999; Jones et al. 2003). The species is currently listed as ‘Near Threatened’ (IUCN 2008), because it is still common and widespread in many habitat types in Tasmania (Rounsevell et al. 1991). Eastern quolls may however become threatened by the introduced European red fox, which has recently become established in Tasmania (Berry et al. 2007; DPIW 2010). Coincidentally, some eastern quoll populations may already be at risk, with recent evidence suggesting that populations in the species’ core distribution (Tasmanian Midlands) may be in decline (RFA 1996).
Despite these threats, a Management Plan has not been prepared for the species (NPWS 1999). However, targeted and opportunistic monitoring of eastern quoll populations has been carried out by independent researchers and the Department of Primary Industries, Parks, Water and Environment (DPIPWE) of Tasmania. Samples for genetic analysis were collected from ten populations across the current eastern quoll distribution in order to acquire baseline data and answer the following questions:

1) What is the genetic composition of the sampled populations? Is there any evidence of genetic erosion associated with the reported population declines?

2) Given that sampling covered a large proportion of eastern quoll distribution within Tasmania, does the pattern of genetic variation among populations agree with the ‘central-peripheral’ hypothesis?

3) What are the patterns of gene flow and genetic structure among the sampled populations? Are there any significant genetic discontinuities?

4) Which populations may be suitable for establishing insurance populations on islands and the Australian mainland if widespread declines occur in the near future?

Figure 1-3 Male black and fawn eastern quolls (*Dasyurus viverrinus*) captured in the Huon Valley, Tasmania. Photos courtesy of Andrew Peters.
1.4.4 The spotted-tailed quoll *Dasyurus maculatus* (Kerr 1792)

The spotted-tailed quoll is the largest of the Australian quoll species and the largest carnivorous marsupial remaining on the Australian mainland (Figure 1-4; Table 1-1). In contrast to eastern quolls, spotted-tailed quolls were uncommon across their former range which included Tasmania, South Australia, Victoria, New South Wales and Queensland (Mansergh 1984). The species was in fact reported as one of the first to become locally extinct (or very rare) following colonization by Europeans (Lunney and Leary 1988). Spotted-tailed quolls are now presumed to be extinct in South Australia and their distribution is fragmented throughout much of their present range (Figure 1-5B), with small isolated populations likely to face local extinction (Backhouse 2003). Suggested threatening processes affecting spotted-tailed quolls include habitat destruction for agriculture, forestry and urban development, competition with introduced predators and human-induced mortality such as hunting, poisoning campaigns and motor vehicle collisions (Jones et al. 2003).

Two subspecies of spotted-tailed quoll are currently recognized. However, subdivisions based on morphology (Burnett 2001) and genetic data (Firestone et al. 1999) do not agree. *D. m. gracilis* is the smallest of the subspecies and is confined to the Wet Tropics bioregion of northeastern Queensland (Burnett and Marsh 2004). *D. m. maculatus* is distributed from southern Queensland to Tasmania. Based on genetic data, the subspecies division should be between the Tasmanian and Australian mainland forms and not between the north Queensland and the southern forms. Nationally, *D. m. gracilis* and *D. m. maculatus* (Australian mainland) are listed as ‘Endangered’, while *D. m. maculatus* from Tasmania is listed as ‘Vulnerable’ under the *EPBC Act 1999*.

In New South Wales, spotted-tailed quoll records are widely distributed within large areas of contiguous forest from the Queensland border in the north, to Kosciuszko National Park in the south (Long and Nelson 2010a). Despite the range decline of spotted-tailed quolls, some regions of northern NSW and East Gippsland in Victoria, where sufficient habitat remains and foxes are in low numbers or absent, are still strongholds for the species (Long and Nelson 2010a). However, the ongoing survival of spotted-tailed quolls relies on the conservation of extensive areas containing adequate resources, because the species exists at low densities and occupies large home ranges.
A national Recovery Plan for the spotted-tailed quoll is being developed by the Australian Federal government in partnership with State conservation agencies in Queensland, New South Wales, Victoria and Tasmania. The document outlines that given the threatened status of the spotted-tailed quoll throughout its range, all the currently known populations are considered to be important and should be prioritized for conservation (Long and Nelson 2010a, 2010b). As a result, targeted and opportunistic monitoring of spotted-tailed quoll populations has been carried out by conservation agencies and independent researchers (Table 1-1). Samples for genetic analysis were taken from 18 populations throughout spotted-tailed quoll range in Southern Queensland and New South Wales in order to acquire baseline data and answer the following questions:

1) What is the genetic composition of the sampled populations? Is there any evidence of genetic erosion?
2) What are the patterns of gene flow and genetic structure among the sampled populations? Are there any significant genetic discontinuities?
3) Is there evidence of multiple paternity in spotted-tailed quolls?

Figure 1-4  Spotted-tailed quoll (*Dasyurus maculatus*) captured at Butterleaf National Park, northern NSW. Photo courtesy of Meri Oakwood and Peter Foster.
1.5 Study aims

Genetic studies of Australian quoll species are relatively scarce due to difficulties in detecting individuals in the wild, but also due to the limited resources available for the conservation of these species. The microsatellite markers used in this study were isolated and characterized by Firestone (1999) and Spencer et al. (2007). Subsequent studies found these markers to be useful in defining differences among quoll populations (Firestone et al. 1999; Firestone et al. 2000; Belcher 2006) and in the non-invasive identification of individuals within a spotted-tailed quoll population (Ruibal et al. 2010). In addition, northern quoll populations on offshore islands were found to have less genetic variation than their mainland counterparts (How et al. 2009).

This thesis aims to expand current knowledge of the conservation genetics of the four Australian quoll species. The broad aims were to apply techniques commonly used in population genetics to determine the genetic composition and structure of populations of northern quolls (chapter 2; Cardoso et al. 2009), western quolls (chapter 3), eastern quolls (chapter 4) and spotted-tailed quolls (Appendix 4). In addition, the last research chapter (chapter 5; Glen et al. 2009) used paternity analysis to investigate aspects of breeding success and relatedness in a population of spotted-tailed quolls.

Genetic variability is expected to be highest in western quolls in accordance with previous results (Firestone et al. 2000) and lowest in island populations of some species, such as northern (How et al. 2009) and eastern quolls. Genetic population structure is expected to be more significant among eastern quolls, because this species is less mobile and occurs at higher densities than the other three species (Table 1-1). This study also provided an opportunity to assess and compare the genetic effects associated with the management practice of translocating animals to islands (northern quolls; chapter 2) and the mainland (western quolls; chapter 3). These results are expected to differ, because small island populations are thought to be more susceptible to the effects of isolation and genetic drift than mainland populations, which may be sustained by gene flow from neighbouring populations.
Table 2-1 Comparison of biological, demographic and conservation information for the four species of Australian quoll, northern quoll (*Dasyurus hallucatus*), western quoll (*Dasyurus geoffroii*), eastern quoll (*Dasyurus viverrinus*) and spotted-tailed quoll (*Dasyurus maculatus*).

<table>
<thead>
<tr>
<th>Species</th>
<th>northern quoll, <em>D. hallucatus</em></th>
<th>western quoll, <em>D. geoffroii</em></th>
<th>eastern quoll, <em>D. viverrinus</em></th>
<th>spotted-tailed quoll, <em>D. maculatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body size</td>
<td>300-1200g</td>
<td>900-1300g</td>
<td>700-1500g</td>
<td>1.8-3.5kg</td>
</tr>
<tr>
<td>Habitat</td>
<td>Rocky escarpment, open forest and woodland of lowland savanna</td>
<td>Predominantly in jarrah (<em>E. marginata</em>) and marri (<em>E. calaphylla</em>) forest and woodland</td>
<td>Variety of habitats including dry sclerophyll forest, scrub, heathland, grassland and cultivated land</td>
<td>Variety of habitats including dry sclerophyll forest, rainforest, scrub, woodland, grassland and cultivated land</td>
</tr>
<tr>
<td>Home range</td>
<td>3.8ha, ( \delta &gt; 100 )ha in the breeding season</td>
<td>300-800ha. Males have larger ranges than females</td>
<td>40ha. Extensive overlap among both sexes</td>
<td>300-1000ha. Males have larger ranges than females</td>
</tr>
<tr>
<td>Former distribution</td>
<td>Once common across most of northern Australia, including some offshore islands</td>
<td>Once common in every state and the Northern Territory on the Australian mainland</td>
<td>Formerly abundant in Tasmania and mainland Australia in NSW, VIC and SA</td>
<td>Once common throughout southeastern Australia from QLD to SA and Tasmania</td>
</tr>
<tr>
<td>Current distribution</td>
<td>Northern Australia from southeastern QLD to southwest Kimberley, with distinct population in the Pilbara (50% of former range)</td>
<td>South-western Western Australia (5% of former range)</td>
<td>Widely distributed and abundant throughout Tasmania, including Bruny Island (20% of former range)</td>
<td>Fragmented populations throughout southeastern Australia from QLD to VIC and Tasmania (50% of former range)</td>
</tr>
<tr>
<td>Current threats</td>
<td>Frequent fire regimes, predation by introduced carnivores, poisoning by the cane toad <em>Bufo marinus</em></td>
<td>Land clearing and habitat destruction, predation and competition by introduced predators, human persecution</td>
<td>Human persecution and road mortality, competition and predation by introduced predators</td>
<td>Habitat destruction, mortality linked to conflict with humans, competition and predation by introduced predators</td>
</tr>
<tr>
<td>Management actions</td>
<td>Captive breeding and translocations to offshore islands; Department of Natural Resources, Environment, the Arts and Sport of the Northern Territory (NRETAS)</td>
<td>Captive breeding, translocations and monitoring; Department of Environment and Conservation of Western Australia (DEC)</td>
<td>Opportunistic and targeted monitoring; Department of Primary Industries, Parks, Water and Environment of Tasmania (DPIPWE)</td>
<td>Population monitoring; DECC NSW, NPWS, DSE VIC, DPIPWE TAS, QPWS, Environment ACT</td>
</tr>
</tbody>
</table>
Figure 1-5 Historical (light shade) and present (dark shade) geographical ranges of Australian quoll species. A. northern quoll (*Dasyurus hallucatus*); B. spotted-tailed quoll (*Dasyurus maculatus*); C. western quoll (*Dasyurus geoffroii*); D. eastern quoll (*Dasyurus viverrinus*).
Chapter 2

Effects of founder events on the genetic variation of translocated island populations – Implications for conservation management of the northern quoll.

Abstract
Translocation is a strategy commonly used to maximize the persistence of threatened species, but it may sometimes lead to undesirable genetic consequences. The northern quoll (*Dasyurus hallucatus*) is a carnivorous marsupial that is critically endangered in Australia’s Northern Territory due to rapid population declines in areas recently colonized by the exotic cane toad *Chaunus [Bufo] marinus*. In 2003, 64 quolls were translocated to two offshore islands to establish insurance populations and reduce the species’ risk of extinction. In this study, we assessed genetic diversity at five microsatellite loci in the translocated populations, two endemic islands and three mainland populations. In the short-term (three generations), the translocated populations showed a slight but non-significant reduction in genetic diversity ($A = 4.1–4.2; He = 0.56–0.59$) compared to the mainland source populations ($A = 5.0–8.4; He = 0.56–0.71$). In comparison, high genetic erosion was observed in the endemic island populations ($A = 1.5–2.9; He = 0.11–0.34$). Genetic bottlenecks were detected on both endemic islands and in one mainland population, indicating recent reductions in population size. Our results are consistent with previous studies describing greater losses of genetic diversity on islands compared to mainland populations. Divergence from ancestral allele frequencies in the translocated populations also suggests effects due to founder events. This study, although short-term, highlights the importance of continued monitoring for detecting changes in genetic diversity over time and makes a significant contribution to our understanding of the effects of founder events on island populations.
Chapter 2 Genetic monitoring of northern quoll populations

2.1 Introduction

The translocation of species that have suffered severe range reductions has often had limited success in establishing self-sustaining populations (Wolf et al. 1998; Mock et al. 2004; Sigg et al. 2005). There are of course exceptions where well planned management actions have led to successful translocations (Morris et al. 2003; Olsson 2007; Taylor and Jamieson 2008). Translocation success is dependent on factors such as habitat quality and size of the release site, the number of founders and the management of threatening processes (Wolf et al. 1998). Despite genetic issues not always being the ultimate cause of translocation failure, they have often been neglected when assessing translocation success. Thus, the number, origin and genetic diversity of populations should always be considered in the planning of translocation programs (Maudet et al. 2002; Jamieson et al. 2006).

Baseline genetic data is important for tracking changes in populations over time so that problems can be readily detected and management strategies adapted accordingly. Although not always implemented, genetic monitoring should complement demographic studies of translocated populations in order to assess their long-term chances of survival. Translocations may lead to reductions in genetic diversity through founder effects and genetic bottlenecks, which are of concern to the continued persistence of endangered species (Hedrick et al. 2001a). Genetic variation is important, because it is considered to be the raw material for evolutionary change (Frankel and Soulé 1981), allowing populations to evolve and adapt in response to changes in their environment. As population size decreases, a number of genetic and environmental factors are likely to drive individual populations to extinction (Frankham et al. 2002). The genetic effects of translocations can sometimes be exacerbated when animals are taken to islands (Eldridge et al. 1999), with the major concern being that island populations, through isolation and genetic drift, are more prone to extinction due to genetic erosion than their mainland counterparts (Frankham 1997; Eldridge et al. 2004; Mills et al. 2004).

Anthropogenic disturbance has threatened the survival of many species worldwide (Reed et al. 2002; Cardillo et al. 2004; Bonn and Gaston 2005). Australian mammals have fared particularly poorly, with 22 species driven to extinction over the last 200 years, a further eight species are restricted to islands or captive populations, and many others are now
substantially reduced in population size and distribution (Short and Smith 1994; Burbidge 1999; Fisher et al. 2003). The northern quoll (*Dasyurus hallucatus*) is a carnivorous marsupial that formerly occurred across most of northern Australia, including some offshore islands (Woinarski et al. 1999b). Over the last few decades *D. hallucatus* has declined across much of its range (Braithwaite and Griffiths 1994), probably through a combination of factors, such as fire and habitat degradation. Current threatening processes include changed fire regimes, predation by feral carnivores (Oakwood 2000; Woinarski et al. 2004a) and more recently, poisoning through ingestion of toxins when attempting to predate on the exotic cane toad *Chaunus [Bufo] marinus* (Burnett 1997; Oakwood 2004), a species that is now spreading rapidly across the northern quoll’s range.

Aspects of the northern quoll’s biology and demography, such as short life spans (one to two years for females and one year for males), male semelparity (post-breeding male die-off), large home ranges and low density distributions can often result in seasonal fluctuations in population size, which may render the species particularly susceptible to stochastic events (Oakwood 2002). Recent local extinctions of northern quoll populations, even in areas within large conservation reserves such as Kakadu National Park (Oakwood 2004), clearly demonstrate that natural refuges for northern quolls are now severely under threat. These rapid population declines highlight the urgency to successfully implement effective management strategies for the conservation of the species (Hill and Ward 2008). The northern quoll is ‘endangered’ under the Australian *Environment Protection and Biodiversity Conservation Act* and ‘critically endangered’ under Northern Territory legislation (Woinarski et al. 2007). In 2003, to increase the probability of the species’ survival, northern quolls captured from wild Northern Territory mainland populations were translocated to Astell Island (1264ha) and Pobassoo Island (390ha) (Rankmore et al. 2008). Extensive surveys did not detect northern quolls on these islands prior to the translocations. Quolls are thought to have become locally extinct on these islands sometime after rapid sea level rises isolated them from the mainland approximately 8-12,000 years ago (Woinarski et al. 1999b).

As part of the translocation program, six microsatellite markers previously developed in quoll species (Firestone 1999) were used to measure the genetic composition of the
founder populations and to monitor changes in genetic variation in the translocated populations over time. Levels of genetic variation in the translocated populations were compared to those found on two endemic islands and three mainland populations (Figure 2-1). For each population we calculated estimates of genetic diversity, effective population size and observed reductions in allelic diversity compared to heterozygosity, indicative of recent genetic bottlenecks. To identify the current genetic structure among northern quoll populations in the Northern Territory, we assessed patterns of genetic differentiation between populations using AMOVA, pairwise $F_{st}$ and a Bayesian clustering method. Temporal genetic monitoring of the translocated populations was useful in identifying genetic effects as a result of the translocations. Appropriate genetic management of these populations will maximise their suitability for use in future supplementations of those mainland populations that are currently threatened with extinction. Conservation measures appropriate for maintaining a high level of genetic variation in the translocated and endemic island populations are discussed.

### 2.2 Materials and Methods

#### 2.2.1 Source of genetic samples

The samples used in this study are listed in Table 2-1 and geographic locations shown in Figure 2-1. For Astell Island (ATL; 1264ha), 45 founders (11 males, 34 females) were sourced from five locations within a 150km radius of Darwin and the East Alligator region of Kakadu National Park, approximately 500km north east of Darwin. For Pobassoo Island (PBO; 390ha), 19 founders (8 males, 11 females) were sourced from four locations within a 50km radius of Darwin. Details of island selection criteria, selection of founders, trapping and handling techniques are described elsewhere (Rankmore et al. 2008).
Prior to release, animals were fitted with passive integrated transponders (PIT) to facilitate tracking and recording of capture-recapture history once on the islands and ear tissue biopsies were taken for genetic analysis (stored in 70% ethanol). Ear biopsies were also taken from new individuals caught during subsequent monitoring surveys of the islands in 2005 and 2006 and from naturally occurring northern quoll populations on Groote Eylandt (GTE; 228,522ha) and Marchinbar Island (MBA; 20,860ha). These islands are thought to have become isolated from the mainland approximately 8000 years ago (Woinarski et al.)
Three Northern Territory mainland populations, which were the source of the animals translocated to the islands, were also sampled. These included the Darwin region (DWN) and two sites from Kakadu National Park: East Alligator (KNPER) and Kapalga (KNPKA) (Table 2-1, Figure 2-1). The Darwin sample was composed of animals captured from locations within a 50km radius of Darwin. The KNPER site comprises high quality quoll habitat including rugged sandstone and open woodlands and is known to have sustained an abundant quoll population prior to the recent declines (Oakwood 2004). The samples taken from this site were from quolls found either to be killed on roads or poisoned by cane toads. The KNPKA site is approximately 80km south west of East Alligator on the western edge of the park. The habitat in this area is predominantly tropical savanna comprised of open forest/woodland with occasional low rocky slopes. This region is particularly prone to fire (Woinarski et al. 2004b) with quoll numbers reported to have declined over the period 1986-1993 (Braithwaite and Griffiths 1994). This low density quoll population was sampled during an ecological survey conducted from 1992-1995.

2.2.2 DNA extraction and microsatellite genotyping

A ‘salting out’ method (Sunnucks and Hales 1996) was used to extract genomic DNA from all the samples, which were screened for the six microsatellite loci described by Firestone (1999). These markers were the only ones available at the time of analysis. Five additional markers were subsequently published (Spencer et al. 2007), but were not included in this study due to time constraints. The KNPKA samples (Table 2-1) were previously screened using radioactively-labeled markers (Firestone et al. 2000), but were re-analyzed along with other samples in the present study in order to make direct comparisons more accurate. Polymerase chain reactions (PCR), using fluorescently-labeled forward primers (Applied Biosystems), were carried out separately for each locus due to the different annealing temperatures and concentrations of MgCl₂ required for each primer set (1.3-VIC: 50°C, 2.5mM; 3.1.2-VIC/3.3.1-NED: 60°C, 2.0mM; 3.3.2-PET: 50°C, 2.0mM; 4.4.10-NED: 55°C, 2.5mM; 4.4.2-FAM: 55°C, 2.0mM). Reactions were made up in a total volume of 10μl containing 1x PCR buffer, 2.0-2.5mM of MgCl₂ (as above), 0.2mM of each dNTP (Amresco), 0.5μM of each forward and reverse primer, 1U of AmpliTaq Gold® (Applied Biosystems) and 25-50ng of template DNA. Amplifications were carried out in a Dyad™
thermal cycler (MJ Research) with an initial denaturation step of 95°C for 5 min followed by 39 cycles of 95°C for 30s, 50-60°C (as above) for 30s, 72°C for 30s and a final extension of 72°C for 30 min. The resulting PCR products were pooled based on size differences and alleles were separated electrophoretically on an Applied Biosystems 3730 automated sequencer. Allelic sizes were scored against the size standard GS500 LIZ (Applied Biosystems) and analyzed using the GeneMapper® v.3.7 software (Applied Biosystems).

Table 2-1 Source of northern quoll genetic samples included in this study. Reference numbers correspond to the geographic locations shown in Figure 2-1.

<table>
<thead>
<tr>
<th>Location</th>
<th>Population type</th>
<th>Date</th>
<th>Source location</th>
<th>N</th>
<th>Ref.</th>
<th>Fig 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astell Island (ATL)</td>
<td>Translocation</td>
<td>Mar 2003</td>
<td></td>
<td>45</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Darwin River Dam</td>
<td>12</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>East Alligator - Kakadu</td>
<td>14</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hayes Creek</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Humpty Doo</td>
<td>10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Litchfield</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-translocation</td>
<td>Dec 2005</td>
<td></td>
<td>74</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-translocation</td>
<td>July 2006</td>
<td></td>
<td>77</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pobassoo Island (PBO)</td>
<td>Translocation</td>
<td>Feb 2003</td>
<td></td>
<td>19</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Berry Springs</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Humpty Doo</td>
<td>13</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lambells Lagoon</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Noonamah</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-translocation</td>
<td>Dec 2005</td>
<td></td>
<td>28</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-translocation</td>
<td>Aug 2006</td>
<td></td>
<td>34</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Groote Eylandt (GTE)</td>
<td>Endemic island</td>
<td>2002-2006</td>
<td></td>
<td>12</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Marchinbar Island (MBA)</td>
<td>Endemic island</td>
<td>2004/2006</td>
<td></td>
<td>27</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Darwin region (DWN)</td>
<td>Mainland</td>
<td>1999-2004</td>
<td></td>
<td>21</td>
<td>1, 4, 6, 7, 8</td>
<td></td>
</tr>
<tr>
<td>Kakadu National Park - East Alligator (KNPER)</td>
<td>Mainland</td>
<td>2003-2004</td>
<td></td>
<td>15</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

2.2.3 Genetic diversity and differentiation

The 2006 sample from the translocated populations was used when making comparisons among populations. This procedure was used to reflect the current genetic variation present in these populations. For the endemic island and mainland populations, samples from different years were pooled (Table 2-1) to increase sample sizes. Deviations from Hardy-Weinberg equilibrium ($HWE$) within populations and Linkage Disequilibrium ($LD$) between loci were assessed by exact tests (Guo and Thompson 1992) (1000 dememorizations, 1000 batches, 1000 iterations) using the program GENEPOP 3.4.
Results obtained using multiple tests were corrected using the sequential Bonferroni procedure at $\alpha = 0.05$ (Rice 1989). Genetic diversity variables such as allele frequencies per locus, observed ($H_o$) and expected ($H_e$) heterozygosity, total number of alleles ($A$) and the mean number of unique ($uA$) and rare alleles ($rA$; frequency < 0.05) were estimated using the program GenAlex 6 (Peakall and Smouse 2006). FSTAT 2.9.3.2 (Goudet 1995) was used to estimate allelic richness ($AR$), the average number of alleles per locus standardized for unequal sample size (Petit et al. 1998) and the inbreeding coefficient ($F_{is}$), which is used to test the significance of the differences between the observed and expected heterozygosity (Weir and Cockerham 1984; Hartl and Clark 1997). Differences in $H_e$, $AR$ and $rA$ between population pairs were assessed using a Wilcoxon rank sign test (Sokal and Rohlf 1995). The frequency of null alleles ($r$) present in the data was estimated using the method of Brookfield (1996) where $r = (H_e-H_o)/(1+H_e)$. The program BOTTLENECK ver.1.2.02 (Piry et al. 1999) was used to investigate whether any of the sampled populations carried the molecular signature of a recent genetic bottleneck. Data were examined using the Wilcoxon’s heterozygosity excess test (Piry et al. 1999) and the allele frequency distribution Mode Shift indicator (Luikart et al. 1998) with 10,000 iterations under the stepwise mutation (SMM) and two phase mutation (TPM) models. These models were chosen, because they are considered to be the most appropriate for microsatellite data (Cornuet and Luikart 1996; Luikart and Cornuet 1998; Piry et al. 1999).

Population genetic theory predicts that (in the absence of mutation and migration) small, isolated populations will lose variation over time through genetic drift as a result of their finite size. The rate of loss of variation will depend on the effective population size ($N_e$) and the number of generations the population is isolated (Frankham 1997; Eldridge et al. 1999). Temporal samples (2003 and 2006) from the translocated populations (Table 2-1) provided a good opportunity to assess the genetic effect of translocations by identifying changes in allele frequency distributions due to genetic drift, while eliminating the confounding impacts of migration and gene flow (Hinten et al. 2003). Estimates of $N_e$ were calculated using the Moment and Maximum Likelihood temporal methods (Wang 2001) implemented in the program MLNE 1.1 (Wang and Whitlock 2003). Generation time, which can be approximated as the average age of reproductively-active individuals in a population (Sherwin and Murray 1990), was taken to be one year given that both males
and females are only known to breed in their first year (Oakwood 2000). However, recent results from demographic data show that survival rates may be higher on the islands (Rankmore et al. 2008). When serial temporal samples are not available, a single sample can be used to estimate the effective number of parents that produced the progeny from which the sample was drawn (Waples 2005). The Linkage Disequilibrium method (Hill 1981; Bartley et al. 1992) implemented in the program NeEstimator 1.3 (Peel et al. 2004) was used to estimate $Ne$ for each of the populations based on a single sample.

In order to examine the influence of genetic drift and subsequent divergence between populations, genetic differentiation was assessed using three different analyses: Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992), pairwise $F_{ST}$ (Weir and Cockerham 1984) and a Bayesian clustering method implemented in the program STRUCTURE 2.2 (Pritchard et al. 2000; Falush et al. 2007). AMOVA and pairwise $F_{ST}$ calculations were carried out in GenAlex 6.0 (Peakall and Smouse 2006), with 999 permutations used to estimate significance probabilities. Patterns of population structure based on $F_{ST}$ values were visualized using Principal Coordinates Analysis (PCA) (Orloci 1978) using GenAlex 6.0. STRUCTURE was used to define the number of distinct population groups ($K$) inferred from the genotypic data, without a priori information about populations. STRUCTURE uses a model-based Markov chain Monte Carlo (MCMC) algorithm to cluster individuals into populations while minimizing Hardy-Weinberg and gametic phase disequilibrium among loci within groups (Manel et al. 2005). Five independent runs of models $K = 1$ to 8 were used to infer the number of genetic populations deduced by posterior probabilities $\text{LnP(D)}$. Analyses were run using a 50,000 burn-in period, 100,000 iterations and an admixture model assuming correlated allele frequencies. STRUCTURE also reports a ‘membership coefficient’ for each individual in each of the inferred populations based on the degree of ancestry ($q$) that could be attributed to a given subpopulation. The best fit model (highest $\Delta K$) was selected using the criterion defined by Evanno et al. (2005).
2.3 Results

2.3.1 Genetic diversity

A total of 51 alleles were detected across the seven populations at six loci. This number was lower than the 62 alleles previously amplified in northern quolls (Firestone et al. 2000). Two and three monomorphic loci were detected in the GTE and MBA populations, respectively. All the other populations were polymorphic at all six loci. Initial exact tests found the two translocated populations and the DWN sample to deviate significantly from $HWE$ at one locus (4.4.2). $F_{is}$ was also found to be significantly greater than expected at this locus across all populations, indicating a deficiency of heterozygotes. Significant heterozygote deficiencies can sometimes be due to the presence of null alleles (Kalinowski and Taper 2006). Analysis of family data (known mother-offspring genotypes) from the translocated populations confirmed the presence of a null allele at this locus (data not shown). Using the method of Brookfield (1996), the frequency of the null allele was estimated to be 0.1 across the seven populations. A null allele at this same locus was also detected in western quolls, *Dasyurus geoffroii* (Cardoso, unpublished). Consequently, locus 4.4.2 was removed from subsequent analyses to prevent biasing the results. Exact tests performed on the remaining five loci detected significant deviation from $HWE$ at one locus (3.3.2) for ATL and at two loci (3.1.2 and 3.3.2) for KNPER. Significant $LD$ was detected at one locus pair for ATL, two locus pairs for KNPKA and eight locus pairs for KNPER. None of the other populations deviated significantly from $HWE$ or $LD$. Significant values of $F_{is}$ were only detected for GTE (heterozygote deficiency) and KNPER (heterozygote excess), indicating significant deviation from $HWE$ for these two populations (Table 2-2).

Genetic diversity was highest in KNPKA (Table 2-2), which was consistent with results previously obtained for this population (Firestone et al. 2000). KNPKA also possessed the greatest proportion of $uA$ and $rA$. A large proportion of $uA$ and $rA$ was also detected in the translocated populations, with genetic diversity ($He$ and $AR$) found to be moderate, although lower than the source mainland populations. Changes in $He$ and $AR$ for each translocated population are shown in Figure 2-2A. Although values were observed to decrease over time, they were not significantly different ($p > 0.05$). Genetic diversity was
lowest on MBA and GTE. However, only MBA had significantly lower \((p < 0.05)\) values of \(He\), \(AR\) and \(rA\) compared to the other populations. The significant \(F_{is}\) observed for GTE (Table 2-2) is likely due to sampling bias, because most of the samples were collected along a relatively short transect and may include animals that were more related to each other than expected by chance (S. Ward, pers. comm.). Monomorphic loci in the GTE (one locus) and MBA (two loci) samples were due to the fixation of high frequency alleles. Two other loci (3.1.2 and 3.3.2) were found to be close to fixation in the MBA population. Significant \((p < 0.05)\) heterozygosity excess was detected for GTE, MBA and KNPER under TPM, indicating evidence of recent genetic bottlenecks in these populations. MBA also showed significant heterozygosity excess under SMM and further evidence of a population bottleneck with the Mode Shift indicator.

Table 2-2 Genetic diversity values estimated at five microsatellite markers in 11 sample populations of northern quolls. \(N\), sample size; \(A\), total number of alleles; \(uA\), unique alleles; \(rA\), rare alleles (frequency < 0.05); \(AR\), allelic richness standardized for sample size; Expected and observed heterozygosity (\(Ho\) and \(He\)); \(Fis\), inbreeding coefficient. Significant values \((p<0.05)\) are shown in bold. Average values reported ± 95% confidence intervals. * \(p<0.05\) (pairwise Wilcoxon rank test).

<table>
<thead>
<tr>
<th>Population</th>
<th>(N)</th>
<th>(A)</th>
<th>(uA)</th>
<th>(rA)</th>
<th>(AR)</th>
<th>(He)</th>
<th>(Ho)</th>
<th>(Fis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astell Island founders (ATL03)</td>
<td>45</td>
<td>40</td>
<td>0.02±0.20</td>
<td>0.40±0.37</td>
<td>5.09±2.29</td>
<td>0.61±0.204</td>
<td>0.587±0.217</td>
<td>0.053</td>
</tr>
<tr>
<td>Astell Island 2005 sample (ATL05)</td>
<td>74</td>
<td>42</td>
<td>0.02±0.20</td>
<td>0.40±0.24</td>
<td>4.42±1.99</td>
<td>0.59±0.241</td>
<td>0.569±0.250</td>
<td>0.067</td>
</tr>
<tr>
<td>Astell Island 2006 sample (ATL06)</td>
<td>77</td>
<td>35</td>
<td>0.02±0.24</td>
<td>4.19±1.84</td>
<td>0.59±0.188</td>
<td>0.56±0.169</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Pobassoo Island founders (PBO03)</td>
<td>19</td>
<td>12</td>
<td>0.12±0.24</td>
<td>4.69±2.09</td>
<td>0.57±0.172</td>
<td>0.56±0.165</td>
<td>0.098</td>
<td></td>
</tr>
<tr>
<td>Pobassoo Island 2005 sample (PBO05)</td>
<td>28</td>
<td>30</td>
<td>0.02±0.24</td>
<td>0.20±0.40</td>
<td>4.27±1.61</td>
<td>0.61±0.180</td>
<td>0.57±0.165</td>
<td>0.081</td>
</tr>
<tr>
<td>Pobassoo Island 2006 sample (PBO06)</td>
<td>34</td>
<td>24</td>
<td>0.08±0.24</td>
<td>4.09±1.51</td>
<td>0.58±0.152</td>
<td>0.59±0.151</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Darwin region (DWN)</td>
<td>21</td>
<td>30</td>
<td>0.17±0.40</td>
<td>5.02±2.49</td>
<td>0.58±0.220</td>
<td>0.56±0.175</td>
<td>0.062</td>
<td></td>
</tr>
<tr>
<td>Kakadu NP East Alligator (KNPER)</td>
<td>15</td>
<td>25</td>
<td>0.12±0.24</td>
<td>4.59±1.37</td>
<td>0.61±0.162</td>
<td>0.56±0.190</td>
<td>-0.125</td>
<td></td>
</tr>
<tr>
<td>Kakadu NP Kapalga (KNPKA)</td>
<td>26</td>
<td>42</td>
<td>0.10±0.45</td>
<td>0.42±0.37</td>
<td>6.02±2.48</td>
<td>0.64±0.175</td>
<td>0.51±0.072</td>
<td>0.063</td>
</tr>
<tr>
<td>Groote Eylandt (GTE)</td>
<td>12</td>
<td>15</td>
<td>0.06±0.01</td>
<td>2.93±1.19</td>
<td>0.43±0.242</td>
<td>0.34±0.188</td>
<td>0.248</td>
<td></td>
</tr>
<tr>
<td>Marchinbar Island (MBA)</td>
<td>27</td>
<td>8</td>
<td>0.02±0.20</td>
<td>1.50±0.41</td>
<td>0.13±0.169</td>
<td>0.11±0.126</td>
<td>0.173</td>
<td></td>
</tr>
</tbody>
</table>

The lowest values of \(Ne\) were estimated for KNPER, GTE and MBA (Table 2-3). The Moment and Maximum Likelihood methods of calculating \(Ne\) from temporal samples gave similar results for each translocated population, with estimates comparable to the results obtained with the single-sample method. \(Ne\) was found to decrease on both translocated populations between 2003 and 2006 (Figure 2-2B). Interestingly, the single sample estimates for the founders of the islands were greater than that of the mainland source
Chapter 2 Genetic monitoring of northern quoll populations

populations (Table 2-3), indicating differences due to substructure of the original founder populations.

2.3.2 Genetic differentiation among island and mainland populations

Based on the results from the AMOVA and $F_{st}$ analyses, a large proportion of the genetic differentiation detected was due to differences between MBA, GTE and the rest of the populations ($F_{st} = 0.190 - 0.666, p = 0.001$; Table 2-4), with the largest $F_{st}$ found between MBA and GTE. All the pairwise $F_{st}$ comparisons were significant ($p < 0.05$) after Bonferroni corrections, except between DWN, KNPKA and the founders of the two translocated populations (Table 2-4B). The pattern of genetic structure among populations based on $F_{st}$ was displayed using PCA, with 83.4% of the variation captured on two axes (Figure 2-3). This result indicated the presence of three main genetic clusters corresponding to MBA, GTE and the mainland plus translocated populations. Within the 2003 founders of ATL (Table 2-1), treated separately according to geographic location (data not shown), $F_{st}$ was significant between all samples ($F_{st} = 0.067 – 0.143, p = < 0.05$) except between Humpty Doo and Litchfield ($F_{st} = 0.042, p = 0.118$). There was no significant genetic differentiation between the samples used to found PBO ($F_{st} = 0.012 – 0.020, p > 0.05$).

The Bayesian model-based clustering analysis in STRUCTURE supported a model with two genetic subpopulations based on 186 individuals from six geographic locations (Table 2-5A). As the correlated allele frequencies model can sometimes achieve better inference when excluding a population that is quite divergent from the others (Pritchard et al. 2000), the model was re-run without individuals from MBA. In this case, the analysis supported a model with three genetic subpopulations, suggesting substructure between GTE and the mainland populations. Comparisons of the known geographic origin and the average degree of ancestry ($q$) from each genetic subpopulation cluster showed that, at $K = 2$, MBA individuals grouped together in one cluster with a high average membership value of 0.974 (Table 2-5B). At $K = 3$, the GTE individuals grouped together in one cluster with a high average membership value of 0.956. The other individuals mainly grouped into cluster 2 at $K = 2$, with some co-ancestry in cluster 1 for ATL, DWN and KNPER. At $K = 3$,
membership values of individuals from the other four populations were distributed among
the three clusters, representing varying levels of admixture.

![Graph A](image)

**Figure 2-2** Observed changes in genetic diversity in the translocated northern quoll island
populations between the founders (2003) and the most current sample (2006); **A.** Allelic richness
(*AR*) and expected heterozygosity (*He*); **B.** Effective population size (*Ne*).
Table 2-3 Effective population size ($N_e$) estimates for each northern quoll population sample; N, sample size; Moments (M) and Maximum likelihood (ML) estimates with temporal samples were calculated using MLNE 1.1 (Wang and Whitlock 2003); Linkage Disequilibrium (LD) estimates with single samples were calculated using NeEstimator 1.3 (Peel et al. 2004).

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>M Ne</th>
<th>ML Ne</th>
<th>LD Ne (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astell Island founders (ATL03)</td>
<td>45</td>
<td>-</td>
<td>-</td>
<td>155.5 (73.4, 6367.4)</td>
</tr>
<tr>
<td>Astell Island 2005 sample (ATL05)</td>
<td>74</td>
<td>-</td>
<td>-</td>
<td>60.2 (42.8, 92.0)</td>
</tr>
<tr>
<td>Astell Island 2006 sample (ATL06)</td>
<td>77</td>
<td>64.32</td>
<td>65.32</td>
<td>73.7 (50.5, 120.6)</td>
</tr>
<tr>
<td>Pobassoo Island founders (PBO03)</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>58.1 (22.1, ∞)</td>
</tr>
<tr>
<td>Pobassoo Island 2005 sample (PBO05)</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>12.9 (9.1, 19.2)</td>
</tr>
<tr>
<td>Pobassoo Island 2006 sample (PBO06)</td>
<td>34</td>
<td>21.05</td>
<td>22.55</td>
<td>29 (17.9, 56.9)</td>
</tr>
<tr>
<td>Darwin region (DWN)</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td>42.5 (20.9, 297.1)</td>
</tr>
<tr>
<td>Kakadu NP East Alligator (KNPER)</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>4.5 (3.5, 6.0)</td>
</tr>
<tr>
<td>Kakadu NP Kapalga (KNPKA)</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>36.5 (24.2, 66.2)</td>
</tr>
<tr>
<td>Groote Eylandt (GTE)</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>8.1 (4.1, 23.7)</td>
</tr>
<tr>
<td>Marchinbar Island (MBA)</td>
<td>27</td>
<td>-</td>
<td>-</td>
<td>9.3 (9.1, ∞)</td>
</tr>
</tbody>
</table>

N, sample size; $N_e$, effective population size; M $N_e$, Moments estimate of $N_e$; ML $N_e$, Maximum Likelihood estimate of $N_e$; LD $N_e$, $N_e$ estimated using Linkage Disequilibrium with 95% confidence intervals (CI).

Table 2-4 Genetic differentiation results calculated in GenAlex 6 (Peakall and Smouse 2006); A. Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992); WP, within population variance; AP, among population variance; *** $p < 0.001$; MBA, Marchinbar Island; GTE, Groote Eylandt; B. Pairwise $F_{st}$ estimates (Weir and Cockerham 1984) calculated using AMOVA; $F_{st}$ values are listed below the diagonal; probability values are listed above the diagonal; significant ($p < 0.05$) Bonferoni-corrected $F_{st}$ values are shown in bold.

A

<table>
<thead>
<tr>
<th>Analysis of Molecular Variance (AMOVA)</th>
<th>WP</th>
<th>AP</th>
<th>Mean Fst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seven populations included</td>
<td>80%</td>
<td>20%</td>
<td>0.197***</td>
</tr>
<tr>
<td>Six populations (MBA excluded)</td>
<td>90%</td>
<td>10%</td>
<td>0.195***</td>
</tr>
<tr>
<td>Five populations (MBA and GTE excluded)</td>
<td>93%</td>
<td>7%</td>
<td>0.065***</td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th></th>
<th>ATL03</th>
<th>ATL05</th>
<th>PBC03</th>
<th>PBC05</th>
<th>PBC06</th>
<th>DWN</th>
<th>KNPER</th>
<th>KNPKA</th>
<th>GTE</th>
<th>MBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astell Island founders (ATL03)</td>
<td>-</td>
<td>0.043</td>
<td>0.147</td>
<td>0.001</td>
<td>0.124</td>
<td>0.002</td>
<td>0.020</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Astell Island (ATL05)</td>
<td>0.008</td>
<td>-</td>
<td>0.027</td>
<td>0.001</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Pobassoo Island founders (PBC03)</td>
<td>0.007</td>
<td>0.017</td>
<td>-</td>
<td>0.004</td>
<td>0.430</td>
<td>0.001</td>
<td>0.048</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Pobassoo Island (PBC05)</td>
<td>0.070</td>
<td>0.069</td>
<td>0.029</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Darwin region (DWN)</td>
<td>0.007</td>
<td>0.032</td>
<td>0.000</td>
<td>0.059</td>
<td>-</td>
<td>0.001</td>
<td>0.183</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Kakadu NP East Alligator (KNPER)</td>
<td>0.041</td>
<td>0.088</td>
<td>0.055</td>
<td>0.113</td>
<td>0.054</td>
<td>-</td>
<td>0.007</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Kakadu NP Kapalga (KNPKA)</td>
<td>0.016</td>
<td>0.046</td>
<td>0.014</td>
<td>0.074</td>
<td>0.005</td>
<td>0.029</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Groote Eylandt (GTE)</td>
<td>0.219</td>
<td>0.252</td>
<td>0.224</td>
<td>0.244</td>
<td>0.190</td>
<td>0.195</td>
<td>0.191</td>
<td>-</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Marchinbar Island (MBA)</td>
<td>0.287</td>
<td>0.292</td>
<td>0.367</td>
<td>0.438</td>
<td>0.369</td>
<td>0.468</td>
<td>0.356</td>
<td>0.666</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2-3 Pairwise $F_{st}$ patterns visualized using Principal Coordinates Analysis (PCA) (Orloci 1978). The axes explain 83.33 % of the total variation. ATL, Astell Island; PBO, Pobassoo Island; DWN, Darwin region; KNPER, Kakadu National Park East Alligator; KNPKA, Kakadu National Park Kapalga; GTE, Groote Eylandt; MBA, Marchinbar Island.
Table 2-5 Population genetic Bayesian clustering results calculated with STRUCTURE 2.2 (Pritchard et al. 2000); A. Ln P(D) and ΔK of individuals from six and five geographic locations. Best fit models based on ΔK are highlighted; B. Average proportion of membership (q) for each subpopulation structure compared to the sampled populations at K = 2 and K = 3.

<table>
<thead>
<tr>
<th>K</th>
<th>Ln P(D)</th>
<th>Var Ln P(D)</th>
<th>ΔK</th>
<th>Ln P(D)</th>
<th>Var Ln P(D)</th>
<th>ΔK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-2452.8</td>
<td>20.9</td>
<td></td>
<td>-2149.7</td>
<td>19.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-2191.9</td>
<td>122.1</td>
<td>147.1</td>
<td>-2034.9</td>
<td>332.3</td>
<td>9.3</td>
</tr>
<tr>
<td>3</td>
<td>-2066.9</td>
<td>261.9</td>
<td>21.5</td>
<td>-1913.0</td>
<td>283.2</td>
<td>60.5</td>
</tr>
<tr>
<td>4</td>
<td>-1961.9</td>
<td>348.1</td>
<td>55.3</td>
<td>-1858.1</td>
<td>370.4</td>
<td>18.2</td>
</tr>
<tr>
<td>5</td>
<td>-1916.7</td>
<td>400.4</td>
<td>18.9</td>
<td>-1817.3</td>
<td>504.8</td>
<td>5.1</td>
</tr>
<tr>
<td>6</td>
<td>-1876.1</td>
<td>552.7</td>
<td>9.6</td>
<td>-1798.7</td>
<td>872.7</td>
<td>3.2</td>
</tr>
<tr>
<td>7</td>
<td>-1844.4</td>
<td>774.6</td>
<td>6.8</td>
<td>-1767.7</td>
<td>872.7</td>
<td>2.8</td>
</tr>
<tr>
<td>8</td>
<td>-1819.9</td>
<td>914.0</td>
<td>6.4</td>
<td>-1747.7</td>
<td>1167.6</td>
<td>3.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sampled population</th>
<th>Subpopulation K=2</th>
<th>Clusters</th>
<th>K=3</th>
<th>Clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astell Island (ATL)</td>
<td>0.177</td>
<td>0.322</td>
<td>0.117</td>
<td>0.561</td>
</tr>
<tr>
<td>Pobassoo Island (PBO)</td>
<td>0.055</td>
<td>0.715</td>
<td>0.127</td>
<td>0.158</td>
</tr>
<tr>
<td>Groote Eylandt (GTE)</td>
<td>0.027</td>
<td>0.022</td>
<td>0.956</td>
<td>0.022</td>
</tr>
<tr>
<td>Marchinbar Island (MBA)</td>
<td>0.974</td>
<td>0.026</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Darwin region (DWN)</td>
<td>0.140</td>
<td>0.264</td>
<td>0.339</td>
<td>0.397</td>
</tr>
<tr>
<td>Kakadu East Alligator (KNPER)</td>
<td>0.104</td>
<td>0.169</td>
<td>0.527</td>
<td>0.304</td>
</tr>
</tbody>
</table>

2.4 Discussion
Our results indicate that the current management practice of translocating northern quolls to establish insurance populations on islands has so far not led to severe genetic effects. Due to the short-term nature of the analysis (3 generations), continued monitoring will be necessary to track the long-term progress of the translocation program. Although not significant, levels of diversity in the translocated populations were observed to slowly decrease over time since the translocations. This effect was more pronounced on ATL than on PBO and is thought to be due to differences in the substructure of the original founder populations. The mixing of individuals from subdivided mainland populations used to found ATL was observed to increase the overall effective population size of the original founders. This is a desirable conservation outcome, because it may benefit the long-term persistence of this population. By comparison, northern quolls endemic to islands have suffered severe genetic erosion, a result that is consistent with other studies of island and
mainland fauna (Eldridge et al. 1999; 2004). The high genetic differentiation observed between the endemic islands and the other populations is likely due to the effects of long-term isolation and random genetic drift that are common in many island populations. The genetic diversity in the remnant mainland populations was found to be higher than in the translocated and endemic island populations, although a reduction in effective population size has led to a genetic bottleneck in one of the populations from Kakadu National Park. This result is consistent with recent extensive declines of northern quoll populations concurrent with the arrival of cane toads in this region.

2.4.1 Genetic effects of translocations

The results of this study confirm that populations may be vulnerable to genetic effects associated with translocations. These effects may in turn be amplified when animals are translocated to islands due to their continued isolation and associated adaptations to island ecosystems (Eldridge et al. 1999; 2004). This study also demonstrates the importance of collecting samples from the original founders of populations as baseline data for long-term genetic monitoring. Tracking changes in diversity since the foundation of island populations allows the genetic effects of translocations to be readily detected, while eliminating the potentially confounding influence of migration (Hinten et al. 2003). Thus, this type of data makes a significant contribution to our understanding of the effects of founder events on populations. Translocations may lead to reductions in genetic diversity due to founder events and bottlenecks (Hedrick et al. 2001a). Founder events may in turn result in new populations that are distinctly different from the parent population from which they derived (Mayr 1963). These effects are due to the random selection of founders that may only carry a small fraction of the original population's genetic variation. The levels of genetic differentiation observed between the translocated and mainland populations show that some genotypic differentiation from ancestral allele frequencies has occurred even within the short span of three generations (Table 2-5). The short generation time and lifespan of northern quolls, resulting in a high turnover of individuals within populations, may in turn accelerate genetic divergence from ancestral genotypes in the long-term.
Although recent genetic bottlenecks were not detected in the translocated populations, reductions in genetic variation were observed compared to the source populations, with values of genetic diversity found to slowly decrease over time since the translocations (Figure 2-2A). The significant linkage disequilibrium detected for ATL suggests that this population may not currently be at drift-mutation equilibrium (Nei and Le 1973). Significant linkage disequilibrium may be due to the recent admixture of populations with differing gametic frequencies (Ohta 1982; Mueller 2004). Recent admixture may lead to reductions in observed heterozygosity relative to that expected (Wahlund 1928), although in this case, $F_{is}$ was not found to be significant (Table 2-2). The significant genetic differentiation detected among founders of ATL, but not PBO (see results) supports admixture of the ATL population at the time of foundation. Non-random mating may have occurred on ATL due to genetic or behavioural incompatibilities between animals from different geographic regions (Sigg et al. 2005), resulting in loss of observed heterozygosity. The relatively stable levels of heterozygosity on PBO (Table 2-2, Figure 2-2A) may be due to the more homogenous genetic composition of the original founders (non-significant $F_{st}$ between founders). A lower number of founders may have also reduced selection pressures on this population by decreasing competition for resources at the time of foundation. Fortunately, recent mark-recapture data showed evidence of high survival and reproductive rates in both translocated populations (Rankmore et al. 2008), indicating that northern quolls are currently breeding successfully on these islands.

2.4.2 Genetic bottlenecks and effective population sizes

The number of founders required to capture allelic diversity depends on the number and frequencies of alleles, so a large number of founders are needed if rare alleles are to be sampled (Frankham et al. 2002). The number of northern quolls translocated to ATL and PBO were sufficient to capture a large proportion of rare alleles (Table 2-2), particularly for ATL. The lower number of rare alleles detected on PBO may be of concern, because there is a greater chance that these alleles will be lost due to random genetic drift, increasing the risk of fixation of high frequency alleles and the ultimate loss of genetic diversity in the long-term. The loss of rare alleles due to genetic drift is therefore a better indicator than the expected heterozygosity for detecting recent reductions in effective
population size (Spencer et al. 2000), because the loss of alleles reflects an immediate consequence underlined by genetic bottlenecks (Nei et al. 1975).

Due to their isolation, the maintenance of genetic variation on islands is dependent on genetic drift, mutation and small and fluctuating population sizes (Frankham 1997). High genetic erosion was observed in the endemic northern quoll island populations (Table 2-2) and the heterozygosity excess test detected recent genetic bottlenecks on both islands. Similar results have been reported in other island species (Frankham 1997; Eldridge et al. 1999; Hinten et al. 2003; Eldridge et al. 2004; MacAvoy et al. 2007). The lower expected heterozygosity retained on MBA compared to GTE is concordant with the stronger genetic signal of a recent genetic bottleneck. Genetic erosion was also more significant on MBA than on GTE, which is most likely due to its smaller size (20,860ha versus 228,522ha) and hence smaller population size. The Bayesian clustering and $F_{st}$ results were consistent in identifying the two endemic island populations as genetically different (Table 2-4, Figure 2-3). This result was expected, because the islands have been isolated from the mainland since the last glacial maximum approximately 8,000 years ago (Woinarski 1999b). Shifts in allele frequency distributions were more pronounced for MBA than for GTE, which is likely a reflection of island size and long-term persistence at a low population size. The low effective population size detected on both islands (Table 2-3) may make them more susceptible to genetic effects, such as the fixation of alleles, non-random mating and extinction due to stochastic events (Frankham 1997).

Despite some loss in genetic variation in the translocated populations, the effective population sizes ($N_e$) were relatively high and comparable to the source populations (Table 2-3). Comparable estimates of $N_e$ for each translocated population obtained using both the single-sample and temporal methods gave us some confidence about the accuracy of these estimates. However, given the low number of loci and the large confidence limits of some of the $N_e$ estimates (Table 2-3), these results should be interpreted with some caution. The serial collection of samples from these populations, including original founders, gave us the opportunity to track changes in $N_e$ over time. Although $N_e$ decreased in both populations two years post-translocation, values have since begun to increase and appear to be converging over time (Figure 2-2B). The larger $N_e$ of the ATL founder population
compared to the source mainland populations was an interesting result. This perhaps suggests that the pooling of founders from geographically distinct populations may be beneficial to the persistence of translocated populations by increasing $N_e$. It is important to note here that this may only be true for species such as the northern quoll for which populations are not highly differentiated, thus avoiding effects associated with outbreeding depression.

2.4.3 Mainland populations

The mainland northern quoll populations possess higher levels of genetic diversity than the island populations (Table 2-2). This observation is consistent with previous findings in other species (Eldridge et al. 2004; Mills et al. 2004; Boessenkool et al. 2007), emphasizing that mainland populations are likely to retain more of the evolutionary potential necessary for long-term persistence (Frankham 1997; Lacy 1997; Frankham et al. 2002). The low genetic differentiation observed between the DWN and Kakadu National Park populations (Table 2-4B, Figure 2-3) suggests a continuous distribution of genotypes throughout these regions. This population may in future become more fragmented due to localized extinctions and the low probability of successful recolonisation of previously occupied habitats in the presence of existing threatening processes. Recent local extinctions of northern quolls due to the presence of cane toads in areas of Kakadu National Park (Oakwood 2004) clearly demonstrate that populations that were once natural refuges for northern quolls are now severely threatened with extinction. The KNPKA population has been declining severely since the early 1990s (Braithwaite and Griffiths 1994), with low population densities observed at the time of sampling in 1995 (Oakwood 2000). A relatively high $N_e$ estimate (Table 2-3) and failure to detect a genetic bottleneck suggests that this was still a healthy population at the time of sampling. Unfortunately, this population is now presumed to be locally extinct, with declines exacerbated since the arrival of cane toads in the area in 2001. The KNPER population has been declining rapidly since late 2003 due to the arrival of cane toads (Oakwood 2004). The heterozygosity excess test detected a recent genetic bottleneck in this population and $N_e$ for the sample taken in late 2003-04 was estimated to be a low 4.5 (Table 2-3), suggesting a severe decline in population size. Thus, the high genetic diversity in northern quoll
populations that was once found in Kakadu National Park is rapidly diminishing and will continue to do so unless the original threats responsible for the population declines are mitigated or eliminated.

2.4.4 Implications for conservation management

Wildlife managers need to be aware that the translocation of threatened species to islands for safe keeping is only beneficial in the short-term and that continued management efforts are required to maximize the persistence of marooned populations. Although the translocated northern quoll populations currently possess a moderate level of genetic diversity representative of the source mainland populations, without subsequent genetic management, they may succumb to the effects of genetic drift and isolation associated with small population size (Frankham 1998). The small size of the islands are suboptimal relative to the home ranges known for the species within their mainland habitat (35ha for females and over 100ha for males) (Oakwood 2002). The ecological carrying capacity of these islands may rapidly be reached due to the high reproductive capacity of the species. Most of the females sampled during post-translocation surveys were found to be carrying an average of six to eight young (Rankmore et al. 2008), which highlights the potential for high population growth on these islands. In the long-term, these islands may not be able to support a sustainable population of northern quolls, which may lead to severe population crashes and the exacerbation of genetic erosion due to population bottlenecks. Given that a low number of loci were used in this study, it is recommended that additional long-term genetic monitoring include a greater number of genetic markers such as additional microsatellite loci and an examination of mitochondrial DNA. Further analyses would help to better define long-term changes in the translocated, endemic and mainland populations.

Based on the results of this study, a number of management strategies are recommended:

- Continue to monitor changes in the genetic variation of the translocated populations over time, particularly the loss of alleles and effective population size (Table 2-2, Figure 2-2).
• If available, supplement the translocated populations with unrelated individuals from appropriate mainland or captive populations (Table 2-4B, Table 2-5, Figure 2-3).

• To prevent genetic divergence between the islands, rotate animals between the two translocated populations, particularly when there are more males in the population (just before the annual breeding season) or with 7-8 month old females that have not yet established territories (Oakwood 2000).

• Maximise the genetic diversity in future captive populations by selecting unrelated individuals from appropriate mainland and island populations (Table 2-4B, Table 2-5, Figure 2-3).

• Continue genetic monitoring of GTE and MBA in order to detect signs of further genetic erosion and supplement with unrelated animals from appropriate mainland, captive and island populations if available (Table 2-4B, Table 2-5, Figure 2-3).

These actions will attempt to maximize and maintain high genetic variation in the island populations, prevent further genotypic divergence from ancestral genotypes and increase the chance that these populations will remain more suitable for use in future supplementations of those mainland populations that are currently threatened with extinction.
Chapter 3

Genetic monitoring of translocated and remnant chuditch (Dasyurus geoffroii) populations from southwest Western Australia.

Abstract

Translocations are becoming an increasingly popular management tool for the conservation of threatened species. Our results supported the observation that translocations implemented as part of the Recovery Plan for the threatened chuditch (Dasyurus geoffroii) were successful in supplementing wild populations in areas of their former range in Western Australia. This success is thought to be due to the combination of an effective captive breeding program and coordinated fox control at translocation sites. In this study, levels of genetic variation were high in the translocated and remnant chuditch populations, and although some genetic bottlenecks were detected, effective population sizes were considered adequate for the species. Significant genetic differentiation was detected among most sample locations, but gene flow was moderate, with populations largely isolated by distance. Interestingly and in agreement with demographic data, genetic admixture was detected among some translocated and remnant populations. The combined evidence from ecological and genetic data aided our understanding of the population structure of this threatened species. While chuditch populations are currently doing well in southwest Western Australia, dispersal into areas of their former distribution will only be possible if conservation strategies such as introduced predator control and habitat conservation continue to be implemented.

3.1 Introduction

Translocations are becoming an increasingly popular management tool for the conservation of threatened species (Moritz 1999; Maudet et al. 2002). Translocation success is dependent on factors such as habitat quality and size of the release site, the number and genetic composition of the animals released, and the management of threatening processes (Eldridge et al. 2004; Bouzat et al. 2009). Success rates are thought to be low when captive-bred animals are used as the source for translocations.
(Mathews et al. 2005), because inbreeding, loss of genetic diversity and genetic adaptations to captivity may lead to the loss of fitness and evolutionary potential of the translocated populations (Woodworth et al. 2002). However, if managed effectively translocations are likely to benefit threatened species (Bouzat et al. 2009). The introduction of new genetic material into a population via translocation has been shown to boost genetic variation, fitness, persistence and potential for evolutionary adaptation to a changing environment (Westemeier et al. 1998; Bouzat 2001), thus reversing some of the effects of population declines.

Conservation translocations almost inevitably deal with populations that have experienced “bottlenecks” or severe reductions in size. Such populations may be more vulnerable to extinction due to genetic drift, inbreeding and the loss of genetic variability and evolutionary potential (Frankham 1995c). The rate of loss of genetic diversity via genetic drift is greater when the effective population size ($N_e$) is small (Frankham 1995b). Estimates of $N_e$ are important when monitoring translocations, because they may allow early detection of changes in population size before the decline is detectable by other means. Unfortunately, accurately estimating the effective size of natural populations is difficult (Kalinowski and Waples 2002; Luikart et al. 2010) and many of the assumptions that apply to methods of estimating $N_e$ are unrealistic in natural populations (Fraser et al. 2007). When temporal samples are not available for estimating $N_e$ due to poor sampling effort and/or lack of resources, a single sample can be used (Waples 2005). Although one-sample $N_e$ estimators have often been biased and imprecise, recent improvements have made these estimators more useful. However, it remains important to compare different methods, while being cautious to treat results as approximations (Waples 2006).

Knowing the genetic composition and population structure of a threatened species is beneficial to translocation management for several reasons, including understanding the extent to which populations naturally support one another, and in extreme cases, because there may be a risk of genetic incompatibilities arising when animals are moved between locations (Storfer 1999). It is widely accepted that many species occur as metapopulations in fragmented landscapes where discrete local populations are connected by dispersal (Hanski and Gaggiotti 2004). The rate of dispersal among populations determines their potential for genetic differentiation (Wright 1951) and the
likelihood that they will demographically and genetically support one another without management intervention. Dispersal is related to the organism’s mobility, but also the distance between subpopulations, the probability of survival during dispersal and the suitability of the surrounding landscape that is available for colonization (Vandermeer and Carvajal 2001). Consequently, many wide-ranging and mobile species are known to exhibit low levels of genetic structure and little or no signal of isolation by distance (Leblois et al. 2000; Kyle and Strobeck 2001; Jones et al. 2004a; Broquet et al. 2006). Although several genetic criteria may be used to determine when groups of individuals are isolated enough to be managed separately (Waples and Gaggiotti 2006), prioritizing populations for conservation remains challenging (Moritz 1999). As with \( N_e \) estimation, it is also useful to compare different methods when analyzing patterns of genetic population structure, particularly when making decisions regarding the conservation of threatened species.

In Australia, many mammal species have been driven to extinction over the last 200 years or are now substantially reduced in population size and distribution (Short and Smith 1994). The cause of species declines is often attributed to the growth of human populations and associated impacts such as the degradation and fragmentation of habitat, hunting, changed fire regimes and the introduction of exotic competitors, predators and pathogens (Reed 2004; Pedersen et al. 2007). Western Australia has experienced one of the greatest levels of regional mammal extinctions anywhere in Australia (Burbidge and McKenzie 1989). Losses were greatest in the wheatbelt, pastoral and desert areas, while mesic regions containing a higher abundance of remnant vegetation were less affected (Woinarski and Braithwaite 1990). The western quoll (\( Dasyurus geoffroii \), Gould 1841), better known as the chuditch, is the largest native mammalian predator in Western Australia. Similar to other large native marsupial predators (Jones and Barmuta 2000; Oakwood 2000; Kortner et al. 2004), chuditch are solitary, terrestrial and exist at low densities. Average home ranges are large (females, 300ha; males, 800ha) and chuditch are known to travel long distances in search of mates and resources (Serena and Soderquist 1989; Soderquist and Serena 2000). As a top predator, chuditch are an indicator species whose presence reflects ecosystem health (Morris et al. 2003). Their opportunistic and generalist diet makes them well adapted to a variety of different habitats (Soderquist and Serena 1994). At the time of European settlement, chuditch were common and occupied nearly 70% of
the Australian continent, occurring in every state and the Northern Territory on the Australian mainland. However, severe declines in the mid to late 1800s saw their distribution contract to approximately 5% of their former range (Figure 3-1a) with an estimated 6000 individuals remaining in the wild by the late 1980s (Serena et al. 1991). The species is currently listed as ‘Vulnerable’ under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 (Morris et al. 2003; Dunlop and Morris 2008).

Declining species often contract to portions of their range most isolated from disturbances thought to have led to declines (Channell and Lomolino 2000b). Similarly, chuditch disappeared from most of their former range, but were able to persist in the jarrah (*Eucalyptus marginata*) and marri (*Corymbia calophylla*) forests and woodlands of southwest Western Australia (Figure 3-1a). The jarrah forests are part of an important Australian bioregion, because they continue to support many species that have disappeared from elsewhere in their range (Wardell-Johnson and Horwitz 1996), resulting in a high abundance of prey species that are able to sustain healthy chuditch populations. These forests have been subjected to a variety of timber harvesting and burning regimes, but these practices do not appear to be detrimental to chuditch and may even be beneficial in areas where introduced predator control is also implemented (Morris et al. 2003). However, because chuditch have a short average life span of three years in the wild and are patchily distributed at low densities within their present range, populations may be vulnerable to local extinction in the face of stochastic events (Serena et al. 1991). Major threats to chuditch populations include land clearing and habitat fragmentation, predation and competition from the introduced European red fox (*Vulpes vulpes*) and feral cat (*Felis catus*), and human-induced mortality such as poisoning, trapping, illegal shooting and road accidents. A Recovery Plan for the chuditch was prepared to mitigate some of these threats and improve the conservation status of the species (Orell and Morris 1994; Dunlop and Morris 2008). Actions outlined in the Recovery Plan included the implementation of fox control over large areas of jarrah forest, as well as captive breeding and translocations of chuditch to areas of their former range. An increase in chuditch distribution and abundance was subsequently recorded within their present range in southwest Western Australia, but was not sufficient to improve the official conservation status of the species (Morris et al. 2003; Dunlop and Morris 2008).
In this study six microsatellite markers were used to determine the genetic composition and population structure of translocated and remnant chuditch populations across their present range in southwest Western Australia. We measured and compared the genetic variation among translocated and remnant populations in order to ascertain translocation success and look for evidence of genetic bottlenecks and genetic subdivisions and/or admixture among populations. Combined with ecological data, genetic information will be essential to our understanding of the population structure of the threatened chuditch and will allow well informed decisions to be made regarding the future conservation management of the species in Western Australia.

**Figure 3-1** Chuditch distribution maps. a. Chuditch distribution on the Australian mainland; State and Territory borders are marked; former distribution shaded light grey (dates shown are dates of last record); present distribution shaded dark grey; insert shows known chuditch distribution records from 1975-1990. Chuditch records are shown within (shaded stripes) and outside (numbers) the jarrah forest. Figure adapted from Serena et al. (1991) and Morris et al. (2003). b. Geographic location of chuditch populations included in this study. Source location for the translocations is circled; arrows indicate translocation sites.
3.2 Materials and Methods

3.2.1 Captive breeding and translocations

The geographic locations of all the populations included in this study are shown in Figure 3-1b. The chuditch captive breeding program was initiated in 1986 using nine wild-caught individuals sourced from jarrah forest in southwest Western Australia. In order to maintain genetic variation, the captive colony was regularly supplemented with wild-caught animals sourced from Batalling State Forest (BTG), a jarrah forest site. The captive colony provided animals to six translocation sites between 1987 and 2001 (Appendix 2). The chuditch Recovery Plan required two separate breeding colonies to be established: one consisting of animals from the jarrah forest and the other of animals from a semi-arid region. However, because a suitable semi-arid population source was not identified, all the translocations consisted of animals from jarrah forest (Morris et al. 2003). Three translocations appear to have been successful based on capture rates and survival data (Dunlop and Morris 2008) (Table 3-1): Julimar Conservation Park (JLM; 24,000ha), Lake Magenta Nature Reserve (MGA; 108,000ha) and Kalbarri National Park (KBI; 186,000ha).

The JLM site consists of jarrah/marri/wandoo forest/woodland and occurs at the northern edge of the jarrah’s distribution. The MGA site lies within the semi-arid wheatbelt of Western Australia and consists of salmon gum (*Eucalyptus salmonphloia*) woodlands and mallee shrublands. The KBI site consists of heath and Banksia shrublands (Orell and Morris 1994), with primary chuditch habitat identified in rocky sandstone gorge terrain. Although chuditch were known to have occurred in JLM, MGA and KBI before population declines took place, surveys undertaken prior to the translocations failed to detect chuditch at these sites. Interestingly, unmarked individuals were captured at MGA and JLM during post-translocation surveys, suggesting that some resident animals may have been present prior to the translocations (Morris et al. 2003). Other chuditch populations monitored by the Department of Environment and Conservation of Western Australia (Table 3-1) included Bindoon (BDN), Dwellingup State Forest (DGP), Kingston State Forest (KGN), Perup Nature Reserve (PRP) and Ravensthorpe (RVP). The methods used during monitoring surveys are described elsewhere (Morris et al. 2003).
3.2.2 DNA samples and microsatellite genotyping

Ear tissue biopsies collected during monitoring surveys (Table 3-1) were stored in DMSO solution (20% DMSO, 0.25M EDTA, saturated NaCl) or 70% ethanol. A ‘salting out’ method (Sunnucks and Hales 1996) was used to extract genomic DNA from the 280 samples, which were subsequently screened at six microsatellite loci (1.3, 3.1.2, 3.3.1, 3.3.2, 4.4.10, 4.4.2) (Firestone 1999) as described by Glen et al. (2009). The chuditch Recovery Plan required DNA fingerprinting of all captive breeding stock. As a result, hair samples were taken, but were deemed unsuitable for DNA analysis due to inappropriate storage conditions. Consequently, the only suitable samples available for genetic analysis of the captive colony were taken from 26 individuals between 1992 and 1994 (BTG). This sample is therefore a small subset of the animals used as a source for the translocations. Samples from the original founders of the translocations were also not available for genetic analysis, reducing the ability to accurately track long-term changes in genetic diversity since the foundation of these populations.

Table 3-1 Details of captive, translocated and remnant chuditch populations from southwest Western Australia; N, sample size; M, male; F, female.

<table>
<thead>
<tr>
<th>Location</th>
<th>Population</th>
<th>Lat/Long</th>
<th>Sample Dates</th>
<th>N</th>
<th>M/F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batalling State Forest *</td>
<td>Remnant</td>
<td>33º 21' S, 116º 34' E</td>
<td>1984-2000</td>
<td>54</td>
<td>202/198</td>
</tr>
<tr>
<td>Captive population (BTG) **</td>
<td>Captive</td>
<td></td>
<td>1986-2001</td>
<td>490</td>
<td>7/6</td>
</tr>
<tr>
<td>Bindoon (BDN)</td>
<td></td>
<td>31º 17' S, 116º 17' E</td>
<td></td>
<td>2014</td>
<td>13</td>
</tr>
<tr>
<td>Jan - Jun 2005</td>
<td>18/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake Magenta Conservation Reserve (MGA)</td>
<td>Translocation 1996-1998</td>
<td>33º 30' S, 119º 2' E</td>
<td></td>
<td>2017</td>
<td>8/1</td>
</tr>
<tr>
<td>Jan - Jul 2007</td>
<td>40/41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dwellingup State Forest (DGP)</td>
<td>Remnant</td>
<td>32º 50' S, 116º 19' E</td>
<td>Mar 2004</td>
<td>6</td>
<td>5/1</td>
</tr>
<tr>
<td>Jun - Jul 2007</td>
<td>34/27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ravensthorpe (RVP)</td>
<td>Remnant</td>
<td>33º 27' S, 114º 3' E</td>
<td>Mar 2007</td>
<td>7</td>
<td>0/7</td>
</tr>
<tr>
<td>Kingston State Forest (KGN)</td>
<td>Remnant</td>
<td>34º 5' S, 116º 20' E</td>
<td>Apr - May 1998</td>
<td>31</td>
<td>25/6</td>
</tr>
<tr>
<td>Perup - Upper Warren (PRP)</td>
<td>Remnant</td>
<td>34º 5' S, 116º 33' E</td>
<td>Oct - Dec 2006</td>
<td>33</td>
<td>21/12</td>
</tr>
</tbody>
</table>

* source location for captive population
** source population for reintroductions (total of 400 chuditch bred 1986-2001)
**bold**: translocated populations and samples included in these analyses
italic: samples from the founders of the translocated populations which were not suitable for genetic analysis
3.2.3 Analysis of genetic variation

Deviations from Hardy-Weinberg equilibrium (HWE) within populations and Linkage Disequilibrium (LD) between loci were assessed by exact tests (Guo and Thompson 1992) (1000 dememorizations, 1000 batches, 1000 iterations) using the program GENEPOP 3.4. (Raymond and Rousset 1995). Results obtained using multiple tests were corrected using the sequential Bonferroni procedure at $\alpha = 0.05$ (Rice 1989). The frequency of null alleles ($r$) present in the data was estimated using the method of Brookfield (1996) where $r = (H_e - H_o)/(1 + H_e)$. Allele frequencies per locus, total number of alleles ($A$), mean number of unique ($uA$) and rare alleles ($rA$; frequency < 0.05), and observed ($H_o$) and expected ($H_e$) heterozygosity were estimated using the program GenAlex 6 (Peakall and Smouse 2006). FSTAT 2.9.3.2 (Goudet 1995) was used to estimate allelic richness ($AR$), the average number of alleles per locus standardized for unequal sample size (Petit et al. 1998) and the inbreeding coefficient ($F_{is}$), which is used to test the significance of the differences between the observed and expected heterozygosity at 95% confidence intervals (Weir and Cockerham 1984). Differences in $H_e$, $AR$ and $rA$ between population pairs were assessed using a Wilcoxon rank sign test (Sokal and Rohlf 1995).

3.2.4 Genetic bottlenecks and effective population size

The program BOTTLENECK ver.1.2.02 (Piry et al. 1999) was used to investigate whether any of the sampled populations carried the molecular signature of a recent genetic bottleneck. Data were examined using the Wilcoxon’s heterozygosity excess test and the allele frequency distribution Mode Shift indicator under 10,000 iterations (Luikart et al. 1998). The Two Phase Mutation Model (TPM) with 95% Stepwise Mutations (SMM or strict single step mutations) and 5% multistep mutations were used following recommendations by Piry et al. (1999). TPM was chosen, because it is considered to be most appropriate for microsatellite data (Luikart and Cornuet 1998). Given that the number of chuditch caught during different sampling efforts was low (<20 individuals) or the time between sampling was short (<2 generations or 4-5 years), the samples from each population were pooled (Table 3-1) and estimates of effective population size ($N_e$) were obtained using one-sample methods, not temporal methods. Three different methods were compared, two of which were based on Linkage Disequilibrium (Hill 1981). The first, implemented in the program NeEstimator 1.3 (Peel et al. 2004), is thought to lead to severe downward bias due to sampling artefacts
such as small sample sizes and mixing of cohorts (England et al. 2006). The second, implemented in the program LDNE (Waples and Do 2008), incorporates the bias-correction algorithm described in Waples (2006). The third method, implemented in the program ONeSAMP (Tallmon et al. 2008), uses approximate Bayesian computation to estimate $Ne$ from a sample of microsatellite genotypes.

3.2.5 Population structure and gene flow

Genetic population structure was analyzed using six different methods: Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992), pairwise $F_{ST}$ (Weir and Cockerham 1984), Principal Coordinates Analysis (PCA) (Orloci 1978), Mantel tests (Mantel 1967) and two Bayesian assignment methods, one implemented in the program STRUCTURE 2.2 (Pritchard et al. 2000) and the other in the program GENECLASS2 (Piry et al. 2004). AMOVA, pairwise $F_{ST}$, PCA and Mantel tests were carried out in GenAlex 6.0 (Peakall and Smouse 2006), with 9999 permutations used to estimate significance probabilities. Patterns of population structure based on pairwise $F_{ST}$ estimates were visually represented using PCA. Mantel tests were performed following the methods of Smouse et al. (1986) to test for a statistical relationship between pairwise population genetic and geographic distance matrices. STRUCTURE 2.2 was used to define the number of distinct population groups ($K$) inferred from the genotypic data, without a priori information about populations (Manel et al. 2005). The admixture and correlated allele frequencies model was run five times at $K = 1$ to 12 with a 50 000 burn-in period and 100 000 iterations. The best fit model (highest $K$ or $\Delta K$) was then selected using the criterion defined by Evanno et al. (2005). STRUCTURE 2.2 was also used to detect dispersers among populations based on the degree of ancestry ($q$) that could be attributed to an individual in a given subpopulation. GENECLASS2 was used to assign individuals to the population in which their genotype is most likely to occur using Bayesian-based computation (Rannala and Mountain 1997) and the probability algorithm described in Paetkau et al. (2004), based on 10000 simulations and an assignment threshold of 0.01.

STRUCTURE 2.2 and GENECLASS2 extract information about recent dispersal from transient disequilibrium observed at individual multilocus genotypes of dispersers or individuals recently descended from dispersers, but unlike STRUCTURE 2.2, GENECLASS2 does not assume that all source populations have been sampled (Piry et
al. 2004). The effective number of dispersers or gene flow between population pairs was also estimated using Wright’s (1951) island model of population structure ($F_{ST} = 1/(4Nm + 1)$; $Nm$=effective number of individuals exchanged per generation) and the private allele method of Slatkin (1985). Wright’s island model has been widely criticized for its underlying assumptions including constant and infinite population size, symmetrical dispersal and drift-mutation equilibrium (Whitlock and McCauley 1999). These assumptions are often violated in natural populations, but Wright’s island model is nonetheless useful in providing a benchmark for comparison to other methods (Fraser et al. 2007). The private allele method estimates gene flow from the spatial distribution of rare alleles (Slatkin 1985). Slatkin’s method can also be used to find isolated populations by omitting one sample at a time and looking for differences in the estimates. Assumptions of the private alleles approach include discrete, non-overlapping generations and drift-mutation equilibrium.

3.3 Results

3.3.1 Genetic variation and effective population size

Initial exact tests found significant deviations from HWE at locus 1.3 for BTG, locus 4.4.10 for KGN and locus 4.4.2 for BDN, BTG, DGP, KGN and PRP. $F_{is}$ was also found to be significantly greater than expected for DGP, KGN, MGA and PRP, indicating a deficiency of heterozygotes in these populations. Significant heterozygote deficiencies can sometimes be due to the presence of null alleles (Kalinowski and Taper 2006). Null alleles with frequencies greater than 0.1 were detected at locus 4.4.2 in seven populations and at locus 4.4.10 in four populations. Null alleles were not detected in KBI and RVP. Interestingly, locus 4.4.2 was also found to possess null alleles in northern quolls (Cardoso et al. 2009). Locus 4.4.2 was therefore removed from subsequent analyses, because null alleles are known to cause bias to results (Chapuis and Estoup 2007). Significant $LD$ was detected at one locus pair (3.3.1/4.4.10) for JLM and two locus pairs (1.3/3.3.1 and 3.3.1/4.4.10) for PRP.

A total of 69 alleles were detected at five microsatellite loci across the nine populations. All the loci tested were polymorphic, with the number of alleles per locus ranging between five and fifteen. Genetic diversity was moderate (mean $AR = 5.87$, mean $He = 0.790$) across the nine populations (Table 3-2) and paired population tests
for AR, He and rA revealed no significant differences (p < 0.05). Rare alleles were detected in all the populations except KBI and RVP. Unique alleles were detected in DGP, RVP, KGN and PRP. The program BOTTLENECK detected a significant (p < 0.05) heterozygosity excess for BTG, MGA and DGP under TPM. Furthermore, a significant shift in allele frequency distributions was detected for RVP using the Mode Shift indicator. The highest Ne was estimated for DGP and PRP (mean = 100.1 and 72.4, respectively; Figure 3-2). The bottleneck results obtained in this study should be treated with caution due to limitations imposed on the program by low sample sizes and low number of loci (Piry et al. 1999). The Ne estimates obtained for BDN, JLM, MGA and KGN were similar (mean = 47.0), and BTG, KBI and RVP had the lowest Ne (mean = 11.3). Similarly, these Ne estimates should be treated as approximations due to possible bias caused by overlapping generations, admixture and immigration (Luikart et al. 2010).

Table 3-2 Genetic diversity estimated in nine sampled chuditch populations using five microsatellite loci. N, sample size; A, total number of alleles; AR, allelic richness; uA, unique (private) alleles; rA, rare alleles (frequency < 0.05); He, expected heterozygosity; Ho, observed heterozygosity; Fis, inbreeding coefficient; Average values reported ± confidence intervals; translocated populations are shown in bold; * signature of a genetic bottleneck detected.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>A</th>
<th>AR</th>
<th>uA</th>
<th>rA</th>
<th>He</th>
<th>Ho</th>
<th>Fis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTG *</td>
<td>26</td>
<td>34</td>
<td>5.31±0.68</td>
<td>0.000±0.000</td>
<td>0.101±0.011</td>
<td>0.774±0.043</td>
<td>0.817±0.074</td>
<td>-0.034±0.056</td>
</tr>
<tr>
<td>BDN</td>
<td>20</td>
<td>36</td>
<td>5.44±0.81</td>
<td>0.000±0.000</td>
<td>0.101±0.011</td>
<td>0.764±0.056</td>
<td>0.740±0.147</td>
<td>0.057±0.148</td>
</tr>
<tr>
<td>JLM</td>
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<td>43</td>
<td>5.70±1.19</td>
<td>0.000±0.000</td>
<td>0.246±0.019</td>
<td>0.792±0.055</td>
<td>0.824±0.147</td>
<td>-0.027±0.142</td>
</tr>
<tr>
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<td>44</td>
<td>6.20±1.27</td>
<td>0.000±0.000</td>
<td>0.188±0.023</td>
<td>0.817±0.056</td>
<td>0.765±0.098</td>
<td>0.082±0.123</td>
</tr>
<tr>
<td>DGP *</td>
<td>67</td>
<td>57</td>
<td>6.97±0.89</td>
<td>0.014±0.005</td>
<td>0.319±0.028</td>
<td>0.856±0.033</td>
<td>0.811±0.092</td>
<td>0.061±0.088</td>
</tr>
<tr>
<td>KBI</td>
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<td>30</td>
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<td>0.000±0.000</td>
<td>0.000±0.000</td>
<td>0.747±0.027</td>
<td>0.822±0.177</td>
<td>-0.042±0.232</td>
</tr>
<tr>
<td>RVP*</td>
<td>7</td>
<td>25</td>
<td>5.00±1.24</td>
<td>0.014±0.005</td>
<td>0.000±0.000</td>
<td>0.708±0.129</td>
<td>0.886±0.163</td>
<td>-0.177±0.079</td>
</tr>
<tr>
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<td>44</td>
<td>6.20±0.73</td>
<td>0.014±0.005</td>
<td>0.174±0.029</td>
<td>0.822±0.030</td>
<td>0.697±0.171</td>
<td>0.168±0.209</td>
</tr>
<tr>
<td>PRP</td>
<td>55</td>
<td>53</td>
<td>6.51±0.82</td>
<td>0.043±0.011</td>
<td>0.304±0.019</td>
<td>0.828±0.045</td>
<td>0.771±0.137</td>
<td>0.078±0.156</td>
</tr>
</tbody>
</table>

3.3.2 Genetic population structure and gene flow

Pairwise $F_{ST}$ estimates were low to moderate (0.001-0.165; Table 3-3a) and, except for the comparison between BDN and JLM (p=0.459) which are genetically identical, estimates were all statistically significant after Bonferoni corrections (p=0.001). The greatest genetic differentiation was detected between KBI and RVP ($F_{ST}$ =0.165). Mean $F_{ST}$ was 0.059 between all the sampled populations and 0.061 between the remnant
populations alone. Mean $F_{ST}$ values were lower in chuditch than in other quoll species (0.131-0.170; Appendix 1).

**Figure 3-2** Effective population size ($Ne$) estimates for nine populations of chuditch obtained using three single sample methods; 95% confidence intervals are shown; LD, linkage disequilibrium method of Hill (1981) implemented in the program NeEstimator 1.3 (Peel et al. 2004); LDNe, linkage disequilibrium method of Hill (1981) implemented in the program LDNE (Waples and Do 2008); 1samp, approximate Bayesian computation method implemented in the program ONeSAMP (Tallmon et al. 2008). Arrows mark populations in which the signature of a recent genetic bottleneck was detected using BOTTLENECK ver.1.2.02 (Piry et al. 1999).

Mantel tests found a positive significant relationship between genetic and geographic distance ($R_{xy} = 0.612$, $p = 0.05$). KBI, JLM and MGA were omitted from these analyses to eliminate bias due to shared reintroduction histories. The overall pattern of genetic structure among populations was visualized using PCA with 80.46% of the variation captured on two axes (Figure 3-3a). Three main genetic clusters were revealed, corresponding to northern (BDN, JLM), central (DGP, MGA) and southern (KGN, PRP) populations. BTG clustered closer to BDN and JLM, while KBI and RVP were genetically distinct to all the other populations. Molecular variance was 6% among the nine populations (mean $F_{ST} = 0.059$, $p = 0.001$; Table 3.3b) and was not significantly different when remnant and translocated populations were treated separately. JLM and BDN were found to be genetically identical, while MGA and DGP clustered together and were more similar to the northern than the southern populations. In general, the
Bayesian clustering analysis performed in STRUCTURE 2.2 agreed with the pattern of
genetic structure described above. The smallest values of $K$ and $\Delta K$ that captured the
major structure in the data were chosen as $K = 3$, corresponding to three main genetic
clusters (Figure 3-4). Interestingly, KBI and RVP clustered with DGP and MGA, and
MGA showed genetic admixture between northern and central populations.

Table 3-3 a. Genetic differentiation among chuditch populations presented as pairwise $F_{ST}$
(Weir and Cockerham 1984); significant ($p=0.001$) values after Bonferroni correction are shown
in bold; b. Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992); WP, within
population variance; AP, among population variance; *** $p < 0.001$.

<table>
<thead>
<tr>
<th></th>
<th>BTG</th>
<th>BDN</th>
<th>JLM</th>
<th>MGA</th>
<th>DGP</th>
<th>KBI</th>
<th>RVP</th>
<th>KGN</th>
<th>PRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTG</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDN</td>
<td><strong>0.058</strong></td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JLM</td>
<td>0.050</td>
<td>0.001</td>
<td>0.000</td>
<td></td>
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<tr>
<td>MGA</td>
<td>0.046</td>
<td>0.034</td>
<td><strong>0.019</strong></td>
<td>0.000</td>
<td></td>
<td></td>
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<tr>
<td>DGP</td>
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<td>0.035</td>
<td>0.024</td>
<td>0.000</td>
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<td>0.063</td>
<td>0.000</td>
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<tr>
<td>RVP</td>
<td><strong>0.128</strong></td>
<td>0.133</td>
<td>0.135</td>
<td>0.095</td>
<td><strong>0.088</strong></td>
<td><strong>0.165</strong></td>
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<td>0.084</td>
<td>0.063</td>
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<td><strong>0.089</strong></td>
<td><strong>0.128</strong></td>
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<tr>
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<td>0.092</td>
<td>0.090</td>
<td>0.079</td>
<td>0.058</td>
<td>0.052</td>
<td><strong>0.083</strong></td>
<td><strong>0.125</strong></td>
<td><strong>0.043</strong></td>
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<table>
<thead>
<tr>
<th>Analysis of Molecular Variance (AMOVA)</th>
<th>WP</th>
<th>AP</th>
<th>Mean $F_{ST}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All populations</td>
<td>94%</td>
<td>6%</td>
<td>0.059***</td>
</tr>
<tr>
<td>Remnant populations</td>
<td>94%</td>
<td>6%</td>
<td>0.061***</td>
</tr>
<tr>
<td>Translocated populations</td>
<td>95%</td>
<td>5%</td>
<td>0.047***</td>
</tr>
</tbody>
</table>

The analyses of dispersal performed in GENECLASS2 and STRUCTURE 2.2 detected
a total of 36 dispersers among the nine populations. A large number of dispersers were
detected between PRP and KGN, while DGP shared individuals with most of the
northern and southern populations. Interestingly, a disperser from MGA was identified
in RVP. The gene flow ($Nm$) estimates obtained using Wright’s Island model and
Slatkin’s private alleles method are shown in Table 3-4.
Figure 3-3 Pattern of population differentiation among chuditch visualized using Principal Coordinates Analysis (PCA) (Orloci 1978). The axes explain 80.46 % of the total genetic variation; The main genetic clusters are circled.

Figure 3-4 Proportion of membership of individual chuditch in each sample to each subpopulation cluster estimated using the Bayesian clustering analysis implemented in STRUCTURE 2.2 (Pritchard et al. 2000).

Mantel tests revealed a negative significant relationship ($p=0.001$) between gene flow and geographic distance using both methods. $Nm$ estimates obtained using Wright’s island model were all greater than one. Gene flow was particularly high ($Nm=1014.2$) between BDN and JLM. Slatkin’s private allele method also estimated high levels of gene flow between BDN and JLM and values of $Nm>1$ were detected between DGP and most other populations, except KBI, RVP and KGN. The average number of
dispersers obtained using Slatkin’s exclusion analysis was 0.585, but this value increased to 1.040 and 1.330 when the KBI and RVP samples was excluded, respectively.

Table 3-4 Estimates of gene flow among chuditch populations. Wright's island model estimates below diagonal; Slatkin's private allele estimates above diagonal; estimates > 1 dispersers per generation are shown in bold

<table>
<thead>
<tr>
<th></th>
<th>BTG</th>
<th>BDN</th>
<th>JLM</th>
<th>MGA</th>
<th>DGP</th>
<th>KBI</th>
<th>RVP</th>
<th>KGN</th>
<th>PRP</th>
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<tr>
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<td>0.718</td>
<td>0.545</td>
<td>0.876</td>
<td><strong>1.637</strong></td>
<td>0.468</td>
<td>0.272</td>
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<td>0.691</td>
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<tr>
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<td><strong>4.1</strong></td>
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<td>0.787</td>
<td><strong>1.215</strong></td>
<td>0.467</td>
<td>0.426</td>
<td>0.641</td>
<td>0.581</td>
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<tr>
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<td><strong>1014.2</strong></td>
<td>0.000</td>
<td><strong>1.053</strong></td>
<td><strong>1.185</strong></td>
<td>0.579</td>
<td>0.492</td>
<td>0.504</td>
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<td><strong>1.159</strong></td>
<td>0.559</td>
<td>0.761</td>
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<tr>
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<td>6.8</td>
<td>7.7</td>
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<td>0.824</td>
<td>0.650</td>
<td>0.901</td>
<td><strong>1.457</strong></td>
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<tr>
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<td>1.7</td>
<td>2.1</td>
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<td>0.000</td>
<td>0.681</td>
<td>0.412</td>
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<tr>
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<td>1.7</td>
<td>2.5</td>
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<td><strong>1.4</strong></td>
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<td>4.1</td>
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<td>2.7</td>
<td>1.9</td>
<td><strong>6.6</strong></td>
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</tbody>
</table>

3.4 Discussion
The genetic data presented in this study confirm that conservation strategies implemented as part of the chuditch Recovery Plan were successful in maintaining high genetic variation in wild and translocated chuditch populations throughout their present range in Western Australia. In addition, the levels of gene flow that were detected reflected the translocation program. Therefore, combined with existing ecological data (Morris et al. 2003; Dunlop and Morris 2008), the genetic data generated have greatly aided our understanding of the population structure of this threatened species.

3.4.1 Genetic variation, bottlenecks and effective population size
Genetic bottlenecks were detected in four populations, but genetic variation was high, and effective population sizes appear to currently be adequate for the persistence of the species, which is known to occur at low densities (Morris et al. 2003). Chuditch populations exhibited high genetic variability at microsatellite loci (Table 3-2) despite the species having experienced a severe historical range contraction (Figure 3-1a). In addition, the levels of genetic diversity in chuditch were high compared to all other Australian quoll species (Appendix 1), an observation also made by Firestone et al. (2000). The reasons behind the differences in diversity among species are unclear, but
may be due to the high adaptability of chuditch, which formerly occupied a wide range of habitats in all states and the Northern Territory of the Australian mainland. Unfortunately chuditch population declines were so severe that a captive breeding and translocation program were necessary to boost wild populations and reintroduce chuditch to areas of their former range in Western Australia. Three of the six attempted translocations were successful based on capture rates and survival data (Dunlop and Morris 2008). The reasons for some translocation failure are unknown, but limited fox control may have been a contributing factor (Morris et al. 2003). By contrast, coordinated fox control was implemented at the successful translocation sites. Approximately ten years post-translocation, the translocated populations have maintained levels of genetic variation similar to that of endemic remnant populations, which supports the success of the translocation program. Translocation programs often have low success rates, particularly when captive-bred animals are used (Mathews et al. 2005). In this case, regular supplementation of the breeding colony with wild chuditch (Appendix 2) allowed a high level of genetic diversity to be maintained within the captive population in preparation for the translocations. This, combined with the release of a relatively large number of founders (Table 3-1) would have allowed the founding populations to better adapt to their environment, increasing population persistence in the long-term.

Except for DGP, data on genetic bottlenecks and effective population size were in agreement, with bottlenecked populations having lower $Ne$ than non-bottlenecked populations (Figure 3-2). Encouragingly, similar estimates of $Ne$ were obtained using the different one-sample methods tested in this study. Genetic signatures of recent population bottlenecks were detected for BTG, MGA, DGP and RVP. The genetic bottleneck and low $Ne$ detected for BTG is likely to be the signature of past founder effects associated with captive breeding. For MGA, low numbers of chuditch were captured during post-translocation monitoring surveys (Table 3-1), suggesting that this population may have either bottlenecked due to maladaptation of the original founders to the semi-arid environment they were translocated into or succumbed to predation pressure due to ineffective fox control (K. Morris, pers. comm.). Fortunately, the MGA population now appears to be recovering (K. Morris, pers. comm.), with the current $Ne$ thought to be moderate given the demographics of this population. DGP was the only bottlenecked population for which high $Ne$ was estimated (Figure 3-2). Ecological data
shows this population to be spatially patchy but currently abundant (Glen 2008), a pattern that may be explained by the recent history of the region. Dwellingup was subjected to more intense logging and burning regimes than other parts of the jarrah forest and chuditch numbers were very low before fox baiting was initiated in the early 1990s. Persistence at low numbers may have constituted a genetic bottleneck in DGP, but efficient fox control allowed the effective population size to recover to high levels. The results showing a genetic bottleneck for RVP and low $N_e$ for RVP and KBI (Figure 3-2) must be interpreted with caution, because of low sample size. Although rare alleles were not detected in either population, levels of allelic richness and heterozygosity were not significantly different to the other populations (Table 3-2). Therefore, a larger sample from RVP and KBI should be tested before any definite conclusions can be drawn.

3.4.2 Population structure and gene flow

In contrast to other known wide-ranging and mobile species (Leblois et al. 2000; Kyle and Strobeck 2001; Jones et al. 2004a; Broquet et al. 2006), significant genetic structure and isolation by distance were detected among most chuditch populations (Table 3-3a). Interestingly, levels of differentiation are lower in chuditch than in other Australian quoll species (Appendix 1) and in contrast to northern quoll translocations (Cardoso et al. 2009; Cardoso unpublished), chuditch translocations did not have a significant effect on $F_{ST}$ (Table 3.3b), suggesting admixture between translocated and remnant individuals. The results obtained using pairwise $F_{ST}$ and assignment tests supported the presence of three main genetic clusters, with moderate gene flow connecting neighbouring populations (Figure 3-3 and Figure 3-4; Table 3-4). The central populations (DGP, MGA), although more genetically similar to northern populations (JLM, BDN), were also genetically admixed with the southern populations (KGN, PRP), indicating that except for geographic distance, there are currently no major barriers to gene flow among the chuditch populations sampled in this study. The high genetic differentiation detected between KBI, RVP and the other populations is more than likely an effect due to small sample size. Genetic dissimilarities were also observed between the translocated populations and BTG (Table 3-3a; Figure 3-3). A feasible explanation is that because BTG was only a subsample of the captive population, it was not a good representation of the animals released at the translocation sites. Pedigree data may have been useful in providing further insights, while samples
from the original founders of the translocations would have allowed more direct temporal comparisons to be made.

The results of this study supported ecological evidence of co-habitation between translocated and resident chuditch in some areas. Interestingly, MGA was found to be genetically admixed with DGP and the northern populations (Figure 3-4). Originally sourced from the same stock as JLM, individuals from MGA have partly diverged from their original founders via genetic drift, local adaptation to a semi-arid environment and/or gene flow with resident populations. The latter is most likely, because unmarked animals presumed to be local residents were found near the MGA site during post-translocation surveys (Morris et al. 2003). There is also evidence from demographic data that chuditch from RVP and surrounding regions may have mixed with animals from MGA (B. Johnson, pers. comm.). This observation was supported by the GENECLASS2 analysis, which detected a disperser from MGA in the RVP population.

Unmarked chuditch were also detected near the JLM site during post-translocation surveys, and consistent with previously acquired movement data (K. Morris, pers. comm.), genetic analyses found JLM and BDN to be genetically identical (Table 3-3a, Figure 3-3). Bindoon is a fenced army training reserve contiguous with the JLM site. The fencing would however not have prevented the dispersal/exchange of juveniles between these sites. The Bindoon population may therefore have been established by dispersal from the JLM translocation site (B. Johnson, pers. comm.) Genetic admixture was also detected between DGP, KBI and RVP (Figure 3-4). However, successful dispersal would be unlikely given the large geographic distances between these locations (Figure 3-1b). This apparent admixture may be an artefact of the differences in sample size; the probability of finding alleles common to all three populations is high due to the large sample size of DGP and the small size of the KBI and RVP samples. Therefore, as a precaution, analyses should be repeated with larger sample sizes for KBI and RVP before definite conclusions can be drawn.

3.4.3 Management of chuditch populations
The success of chuditch management appears due to the combination of an effective captive breeding program and coordinated introduced predator control at translocation sites. These conservation strategies have allowed chuditch numbers to increase from the 6000 individuals estimated to remain in the wild during the late 1980s (Serena et al.
1991; Morris et al. 2003). Populations will need to be maintained at sizes greater than 2000 individuals if long-term persistence of this iconic species is to be achieved (Reed et al. 2003). Even though chuditch populations are currently genetically diverse, they are often patchy and occur at very low densities, which makes them vulnerable to stochastic events (Morris et al. 2003). The persistence of chuditch populations and dispersal into areas of their former range are largely dependent on the ongoing implementation of successful conservation strategies such as introduced predator control and habitat conservation to maintain connectivity between populations. The potential downside of exchange between populations is disruption of local adaptation through outbreeding depression. However, this is thought to be less of a concern than inbreeding depression, particularly when populations have not been isolated for a long time on the evolutionary scale (Storfer 1999; Bouzat et al. 2009). In the case of Western Australian chuditch populations, the results of the translocations suggest that evolutionary separation has not been sufficient to prevent successful interbreeding between translocated individuals and local remnant populations.

Management recommendations resulting from this study are as follows:

1) Continue to regularly monitor the ecology, size and genetics of remnant and translocated populations so that unforeseen problems such as declining numbers and loss of genetic diversity can be readily detected;

2) Preserve connectivity between populations through habitat restoration and introduced predator control, while keeping in mind the possibility of disease transmission between locations that were previously separated. This will allow gene flow to maintain high levels of genetic variation and appropriate levels of genetic subdivision, particularly between the more isolated southern and semi-arid populations;

3) Ensure the persistence of significant populations, such as DGP and MGA, which possess a large amount of the total genetic variation found in chuditch. Animals sourced from these populations may therefore be used to supplement other populations, should it become necessary.
Chapter 4

Genetic variation and population structure in the carnivorous marsupial *Dasyurus viverrinus*, an Australian species at risk of extinction?

Abstract
Eastern quolls (*Dasyurus viverrinus*) are still abundant in many parts of Tasmania, but may become threatened by the introduced European red fox (*Vulpes vulpes*). Due to a lack of detailed demographic information on eastern quolls, molecular data becomes a crucial surrogate to inform the ongoing management of the species. The aim of this study was therefore to acquire baseline genetic data for use in current and future conservation strategies. Genetic variation was lower in Tasmanian eastern quolls than in quoll species from the Australian mainland. Within Tasmania, genetic variation was greater in central than in peripheral populations, with the lowest levels detected on Bruny Island. Significant genetic population structure, consistent with regional differentiation, was largely explained by geographic distance among populations. Levels of gene flow were moderate, with genetic admixture greatest among central populations. Eastern quolls from genetically diverse central Tasmanian populations will be an important source for translocations to islands and the Australian mainland if widespread declines begin to occur. Ongoing monitoring of population size and genetic variation of these populations will allow conservation strategies to be adapted accordingly. In order for translocations to be successful, managers must consider the genetic composition of founders, but also habitat-specific adaptations, disease implications and threatening processes at translocation sites.

4.1 Introduction
Anthropogenic disturbance threatens the survival of many species worldwide (Cardillo et al. 2004), with Australian mammals having fared particularly poorly since European settlement (Short and Smith 1994). The disappearance of populations is a prelude to
species extinction (Ceballos and Ehrlich 2002). Therefore, monitoring wild populations is a crucial step to establishing the conservation status of species thought to be at risk (Maudet et al. 2002). Although population persistence may be influenced by demographic and environmental stochastic factors, the evidence for genetic effects on fitness and persistence in wild populations now seems irrefutable (Bouzat et al. 1998; Reed and Frankham 2003). Baseline genetic data gathered before population declines occur have the potential to shape and inform the management of threatened species. The rapidly expanding field of molecular genetics has allowed genetic data to be more readily available for the conservation management of Australian fauna (Sherwin et al. 1991; Taylor et al. 1994; Moritz et al. 1997; Firestone et al. 2000; Eldridge et al. 2004; Jones et al. 2004a).

Island populations are often at greater risk of extinction than their mainland counterparts as a consequence of isolation and small population size (Frankham 1998; Eldridge et al. 2004). Populations that have experienced a bottleneck or severe reduction in size often display a reduction in both allele numbers ($N_A$) and gene diversity (heterozygosity, $H_e$), but allelic loss will occur more rapidly than loss of heterozygosity (Nei et al. 1975; Cornuet and Luikart 1996). The rate of loss of genetic variation via genetic drift is greater when the effective population size ($N_e$) is small (Frankham 1995b). Unfortunately, accurately estimating the effective size of natural populations is difficult (Kalinowski and Waples 2002). When temporal samples are not available for estimating $N_e$, a single sample can be used (Waples 2005) and although one-sample $N_e$ estimators have often been biased and imprecise, recent improvements have made these estimators more useful. However, it remains important to compare different methods, while being cautious to treat results as approximations (Waples 2006).

The ‘central-peripheral’ hypothesis predicts that populations at the core of a species range should exhibit greater genetic diversity and abundance than populations at the range periphery. Consequently, due to their isolation from central sources of dispersers, peripheral populations are thought to be more prone to temporal variability in abundance, bottlenecks and losses of genetic variation (Eckert et al. 2008). Many species occur as metapopulations in fragmented landscapes where discrete local populations are connected by dispersal (Hanski and Gaggiotti 2004). Genetic
discontinuities within and between populations in correlation to natural and/or anthropogenic environmental features become important when planning management strategies such as the translocation and supplementation of threatened populations (Manel et al. 2003). Supplementation may have a negative impact on population persistence by introducing diseases or parasites to previously uninfected populations (Reed 2004) and introducing alleles maladapted to the local environment. However, disruption of local adaptation through outbreeding depression is less of a concern than inbreeding depression, particularly for populations that have had high dispersal or have not been isolated for a long time on the evolutionary scale (Storfer 1999).

The rate of dispersal among populations determines their potential for genetic differentiation (Wright 1951) and is related to the organism’s mobility, but also the distance between subpopulations, the probability of survival during dispersal and the suitability of the surrounding landscape that is available for colonization (Vandermeer and Carvajal 2001). Acquiring demographic data on population size, dispersal and population structure using mark recapture methods can often be expensive and time consuming. Similar information can be obtained much more readily from population genetic data, which can be used to guide management strategies (Eldridge et al. 2004). However, as with $Ne$ estimation, it is also useful to compare different methods when analyzing patterns of genetic population structure.

The eastern quoll (*Dasyurus viverrinus* Dasyuridae: Marsupialia) is the third largest (0.7-1.5kg) extant native carnivorous marsupial in Tasmania (Jones and Barmuta 2000). It was once widely distributed throughout southeastern Australia (Godsell 1982), but is now restricted to Tasmania (165 000 km$^2$) and Bruny Island (362km$^2$), an island off the southeast coast of Tasmania. The combined pressures of a disease epidemic at the turn of the 20$^{th}$ century (Burbidge and McKenzie 1989), competition and predation from introduced species, human encroachment and persecution led to a dramatic range decline and eventual extinction on the Australian mainland in the 1960s (NPWS 1999; Jones et al. 2003). The species is currently listed as ‘Near Threatened’ (IUCN 2008), because it is still common and widespread in many habitat types in Tasmania (Rounsevell et al. 1991).
Chapter 4 Genetic monitoring of eastern quoll populations

Eastern quolls are a highly adaptable species which occupy an important ecological niche as predators and opportunistic scavengers, particularly now that a related species, the Tasmanian devil (*Sarcophilus harrisii*) has declined dramatically due to disease (Hawkins et al. 2006). Tasmanian devils may have helped to suppress the establishment of viable populations of the European red fox (*Vulpes vulpes*), which was recently introduced to Tasmania (DPIW 2008) and has the potential to threaten eastern quolls and other medium-sized species to extinction via predation and resource competition (Jones et al. 2004b; McKnight 2008). Furthermore, the short life-span (3-4 years) and seasonal population fluctuations of eastern quolls (Godsell 1983) may exacerbate their vulnerability to decline in the face of stochastic events (Oakwood 2002). Some eastern quoll populations may already be at risk, with recent evidence suggesting that populations in the species’ core distribution (Tasmanian Midlands) may be in decline (RFA 1996). Despite these threats, a Management Plan has not been prepared for the species (NPWS 1999).

In this study seven microsatellite markers were used to gather baseline genetic information on eastern quolls throughout their range in Tasmania. Specific aims were: 1) To estimate the genetic diversity of Tasmanian eastern quolls; 2) To look for evidence of genetic bottlenecks and estimate current effective population sizes; 3) To determine whether the pattern of genetic variation among eastern quoll populations agrees with the ‘central-peripheral’ hypothesis and 4) To look for significant population structure and estimate levels of gene flow among eastern quolls. These data will be essential to our understanding of the genetic composition and population structure of eastern quolls and will allow informed decisions to be made regarding the conservation of the species, particularly if as predicted, widespread declines occur in the near future.

4.2 Materials and Methods

4.2.1 Sample collection and microsatellite analysis

Eastern quolls (n=20-64) were sampled from 10 localities throughout Tasmania (Figure 4-1). Quolls from Cradle Mountain (CDL), Bronte Park (BTP) and Mount Field (MFD) were caught opportunistically during Tasmanian devil monitoring programs conducted by the Department of Primary Industries and Water (DPIW). Samples from Freycinet Peninsula (FCN) and Vale of Belvoir (VBV) were provided by other researchers (see
Chapter 4 Genetic monitoring of eastern quoll populations

Acknowledgements. Samples from Gladstone (GLD) were dried museum skins acquired from the Queen Victoria Museum in Launceston, which together with the VBV samples, were previously analysed (Firestone et al. 2000). The remaining four sites (Bruny Island, BNY; Huon Valley, HVL; Mathina, MTA and Mount William, MWM) were selected for sampling based on historical eastern quoll records (Rounsevell et al. 1991). Because MWM is located ~ 4km from GLD (D. Underhill, pers. comm.) the two locations were considered to represent the same population, sampled 45 years apart. Habitat types at sampling sites ranged from alpine meadow (CDL, VBV, MTA) to schlerophyll forest (BTP, MFD, HVL), coastal heath (MWM, FCN), pine plantation (MFD) and open pasture interspersed by remnant native vegetation (MWM, MTA, HVL, BNY). Eastern quolls were captured in cage (Mascot) and PVC traps (Mooney unpublished). Ear tissue biopsies were stored in 70% ethanol. A ‘salting out’ method (Sunnucks and Hales 1996) was used to extract genomic DNA from 380 samples. DNA from the 45 museum specimens had been previously extracted (Firestone et al. 2000). The 425 samples were screened at eleven microsatellite loci (1.3, 3.1.2, 3.3.1, 3.3.2, 4.4.10, 4.4.2, 1A1, 1H3, 6D5, 7F3 and 5G4) as described in Glen et al. (2009).

4.2.2 Genetic variation

Deviations from Hardy-Weinberg equilibrium (HWE) within populations and Linkage Disequilibrium (LD) between loci were assessed by exact tests (Guo and Thompson 1992) using the program GENEPOP 3.4 (Raymond and Rousset 1995) with 1000 dememorizations, batches and iterations. Results obtained using multiple tests were corrected using the sequential Bonferoni procedure at $\alpha = 0.05$ (Rice 1989). Allele frequencies per locus, total number of alleles ($A$), mean number of unique ($uA$) and rare alleles ($rA$; frequency < 0.05), and observed ($Ho$) and expected ($He$) heterozygosity were estimated using the program GenAlex 6 (Peakall and Smouse 2006). FSTAT 2.9.3.2 (Goudet 1995) was used to estimate allelic richness ($AR$), the average number of alleles per locus standardized for unequal sample size (Petit et al. 1998) and the inbreeding coefficient ($FIs$), which was used to test the significance of the differences between the observed and expected heterozygosity with 95% confidence (Weir and Cockerham 1984). Differences in $He$, $AR$ and $rA$ between population pairs were assessed using a Wilcoxon rank sign test (Sokal and Rohlf 1995).
Figure 4-1 Historical eastern quoll (*D. viverrinus*) distribution records (black circles). Figure adapted from Rounsevell (1991). Larger grey circles represent the sampled locations included in the present study: VBV, Vale of Belvoir (n=20); CDL, Cradle Mountain (n=29); BTP, Bronte Park (n=64); GLD, Gladstone (n=45); MWM, Mount William (n=45); MTA, Mathina (n=35); FCN, Freycinet peninsula (n=44); MFD, Mount Field (n=50); HVL, Huon Valley (n=30); BNY, Bruny Island (n=63).

4.2.3 Genetic bottlenecks and effective population size

The program BOTTLENECK ver.1.2.02 (Piry et al. 1999) was used to investigate whether any of the sampled populations carried the molecular signature of a recent genetic bottleneck. Data were examined using the Wilcoxon’s heterozygosity excess test and the allele frequency distribution Mode Shift indicator under 10,000 iterations (Luikart et al. 1998). The Two Phase Mutation Model (TPM) with 95% Stepwise
Chapter 4  Genetic monitoring of eastern quoll populations

4.2.4 Genetic variation in central and peripheral populations

Google Earth© 2008 was used to identify the geographic centre of the core eastern quoll distribution. Geographic distances were then measured between the core and each sampled location listed in Table 4-1. To reflect current eastern quoll distribution, only recent samples were included in this analysis; samples from GLD (1960s) and VBV (1995) were omitted, as were those from BNY due to the likely impact of long-term isolation on genetic diversity. Correlation and regression analyses were performed using ANOVA in SPSS© v.9.0. to examine relationships between genetic diversity and geographic distance from the core of eastern quoll distribution in Tasmania.

4.2.5 Population structure and gene flow

Genetic population structure was analyzed using six different methods: Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992), pairwise $F_{ST}$ (Weir and Cockerham 1984), Principal Coordinates Analysis (PCA) (Orloci 1978), Mantel tests (Mantel 1967) and two Bayesian assignment methods; one implemented in the program STRUCTURE 2.2 (Pritchard et al. 2000) and the other in the program GENECLASS2 (Piry et al. 2004). AMOVA, pairwise $F_{ST}$, PCA and Mantel tests were carried out in GenAlex 6.0 (Peakall and Smouse 2006), with 9999 permutations used to estimate significance probabilities. Patterns of population structure based on pairwise $F_{ST}$ estimates were visually represented using PCA. Mantel tests were performed following the methods of Smouse and co-workers (Smouse et al. 1986) to test for a statistical relationship between pairwise genetic and geographic distance matrices. STRUCTURE
2.2 was used to define the number of distinct population groups ($K$) inferred from the genotypic data, without a priori information about populations. (Manel et al. 2005). The admixture and correlated allele frequencies model was run five times at $K = 1$ to 12 with a 50 000 burn-in period and 100 000 iterations. The best fit model (highest $K$ or $\Delta K$) was then selected using the criterion defined by Evanno et al. (2005). STRUCTURE 2.2 was also used to detect dispersers among populations based on the degree of ancestry ($q$) that could be attributed to an individual in a given subpopulation. GENECLASS2 was used to assign individuals to the population in which their genotype is most likely to occur using Bayesian-based computation (Rannala and Mountain 1997) and the probability algorithm described in Paetkau et al. (2004), based on 10 000 simulations and an assignment threshold of 0.01. The effective number of dispersers or gene flow between population pairs ($N_m$) was also estimated using Wright’s (1951) island model of population structure ($F_{ST} = 1/(4N_m + 1); N_m=$gene flow) and the private allele method of Slatkin (1985). Wright’s island model has been widely criticized for its underlying assumptions including constant and infinite population size, symmetrical dispersal and drift-mutation equilibrium (Whitlock and McCauley 1999). These assumptions are often violated in natural populations, but Wright’s island model is nonetheless useful in providing a benchmark for comparison to other methods (Fraser et al. 2007). The private allele method estimates gene flow from the spatial distribution of rare alleles (Slatkin 1985). Slatkin’s method can also be used to find isolated populations by omitting one sample at a time and looking for differences in the estimates. Assumptions of the private alleles approach include discrete, non-overlapping generations and drift-mutation equilibrium.

### 4.3 Results

#### 4.3.1 Genetic variation

A subsample (n=50) of the 425 individuals sampled from 10 populations were initially genotyped at 11 microsatellite loci. As a result, four of these loci were excluded from further analyses, because three (1A1, 7F3 and 5G4) were monomorphic and one (3.3.2) was out of HWE for all the sampled locations. The remaining seven loci did not deviate significantly from HWE or LD after sequential Bonferroni correction. A total of 37 alleles were amplified in 425 samples and the number of alleles per locus ranged from 1-9 (mean=3.43) across the ten locations. Values of $AR$ and $He$ ranged from 1.86-3.97
(mean=3.18) and 0.299-0.556 (mean=0.467), respectively. Unique (private) alleles were detected in BTP, VBV, MWM and GLD. BNY was monomorphic at two loci (1.3 and 3.3.1) and genetic diversity ($AR$, $He$ and $rA$) was significantly lower ($p < 0.05$) for BNY than the other populations (Table 4-1).

4.3.2 Genetic bottlenecks and effective population size

The program BOTTLENECK ver.1.2.02 detected a significant ($p < 0.05$) heterozygosity excess for BNY, HVL, FCN and VBV under TPM, suggesting recent genetic bottlenecks at these locations. BNY also showed significant heterozygosity excess under SMM and further evidence of a population bottleneck with the Mode Shift indicator. There were some discrepancies between the $Ne$ estimates obtained using the three single-sample methods (Figure 4-2). For BNY, MFD and BTP, estimates were higher when the Bayesian method (ONEsAMP) was used, while for CDL and GLD, estimates were greater when the LDNe method was used. However, estimates were comparable among methods for HVL, FCN, VBV, MTA and MWM. The highest $Ne$ was estimated for BNY, followed by BTP and GLD. VBV and HVL had the lowest $Ne$. The Moment and Maximum Likelihood temporal method estimated $Ne$ to be 166.60 (89.56, 331.37) for the GLD/MWM population.

Table 4-1 Genetic diversity values estimated at seven microsatellite loci in 10 sampled populations of eastern quolls. $N$, sample size; $P$, number of polymorphic loci; $A$, total number of alleles; $AR$, allelic richness standardized for sample size; $rA$, rare alleles (frequency < 0.05); $uA$, unique (private) alleles; Expected and observed heterozygosity ($Ho$ and $He$); $Fis$, inbreeding coefficient; Average values reported ± 95% confidence intervals; Estimates found to be significantly different ($p<0.05$ after Bonferroni correction; pairwise Wilcoxon sign rank test) from other populations are shown in bold; populations with the signature of a genetic bottleneck are marked with an asterisk.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>P</th>
<th>A</th>
<th>AR</th>
<th>rA</th>
<th>uA</th>
<th>He</th>
<th>$He$</th>
<th>$Ho$</th>
<th>$Fis$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruny Island (BNY)</td>
<td>63</td>
<td>5</td>
<td>13</td>
<td>1.86±0.51</td>
<td>0.000±0.00</td>
<td>0.000±0.00</td>
<td>0.299±0.179</td>
<td>0.308±0.189</td>
<td>-0.024</td>
<td></td>
</tr>
<tr>
<td>Huon Valley (HVL)</td>
<td>30</td>
<td>7</td>
<td>23</td>
<td>3.18±0.89</td>
<td>0.081±0.01</td>
<td>0.000±0.00</td>
<td>0.474±0.128</td>
<td>0.494±0.170</td>
<td>-0.023</td>
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<tr>
<td>Mount Field (MFD)</td>
<td>50</td>
<td>7</td>
<td>31</td>
<td>3.71±1.10</td>
<td>0.243±0.03</td>
<td>0.000±0.00</td>
<td>0.540±0.098</td>
<td>0.517±0.093</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Freycinet (FCN)</td>
<td>44</td>
<td>7</td>
<td>26</td>
<td>3.43±1.12</td>
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<td>0.000±0.00</td>
<td>0.551±0.126</td>
<td>0.541±0.125</td>
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<td>Bronte Park (BTP)</td>
<td>64</td>
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<tr>
<td>Cradle Mountain (CDL)</td>
<td>29</td>
<td>7</td>
<td>21</td>
<td>2.85±0.76</td>
<td>0.108±0.02</td>
<td>0.000±0.00</td>
<td>0.443±0.092</td>
<td>0.429±0.118</td>
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<tr>
<td>Vale of Belvoir (VBV)</td>
<td>20</td>
<td>7</td>
<td>20</td>
<td>2.84±0.78</td>
<td>0.054±0.01</td>
<td>0.027±0.01</td>
<td>0.525±0.075</td>
<td>0.420±0.166</td>
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</tr>
<tr>
<td>Mathina (MTA)</td>
<td>35</td>
<td>7</td>
<td>24</td>
<td>3.38±1.31</td>
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<td>0.000±0.00</td>
<td>0.467±0.172</td>
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</tr>
<tr>
<td>Mount William (MWM)</td>
<td>45</td>
<td>7</td>
<td>24</td>
<td>3.16±1.02</td>
<td>0.081±0.02</td>
<td>0.027±0.01</td>
<td>0.386±0.152</td>
<td>0.402±0.162</td>
<td>-0.031</td>
<td></td>
</tr>
<tr>
<td>Gladstone (GLD)</td>
<td>45</td>
<td>7</td>
<td>27</td>
<td>3.44±1.32</td>
<td>0.243±0.02</td>
<td>0.027±0.01</td>
<td>0.427±0.163</td>
<td>0.419±0.160</td>
<td>0.031</td>
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</table>
Figure 4-2 Effective population size ($N_e$) estimates for eastern quoll populations obtained using three single sample methods; LD, linkage disequilibrium method of Hill (1981) implemented in the program NeEstimator 1.3 (Peel et al. 2004); LDNe, linkage disequilibrium method of Hill (1981) implemented in the program LDNE (Waples and Do 2008); ONeSAMP, approximate Bayesian computation method implemented in the program ONeSAMP (Tallmon et al. 2008); bottlenecked populations are marked with an arrow.

4.3.3 Genetic variation in central and peripheral populations

Geographic distances between the sampled locations ranged between 4 and 260km (Figure 4-1; data not shown). A core geographic position was arbitrarily assigned at coordinates 42°00′69″S, 147°17′10″E. For each population, values of $H_e$ and mean $N_e$ were then plotted against distance from the distribution core (Figure 4-3). Regression analyses revealed a significant linear relationship between $H_e$ and distance from the distribution core ($R^2 = 0.824$, $p = 0.004$) and a significant non-linear (polynomial) relationship between $N_e$ and distance from the distribution core ($R^2 = 0.754$, $p = 0.028$). The $N_e$ values obtained for CDL and MWM were the obvious outliers preventing this relationship from being linear. Thus, a linear relationship ($R^2 = 0.876$, $p = 0.002$) was obtained when CDL and MWM were removed from the analysis (data not shown). Furthermore, a significant correlation between $H_e$ and $N_e$ was not found ($R^2 = 0.0533$ $p = 0.920$) across all the sampled locations.
Figure 4-3 Populations in order of distance (km) from the arbitrarily assigned eastern quoll distribution core plotted against values of Heterozygosity ($H_e$) and mean effective population size ($N_e$); trendlines and relationship equations are shown.

### 4.3.4 Population structure and gene flow

AMOVA showed that 13% of the total genetic variation was found among the 10 eastern quoll populations ($F_{ST} = 0.131$, $p = 0.001$). Total genetic variation among populations decreased to 10% when BNY was excluded (mean $F_{ST} = 0.101$, $p = 0.001$). The pairwise $F_{ST}$ estimates supported significant ($p = 0.001$) population structure among eastern quolls (Table 4-2). The greatest genetic differentiation was found between BNY and populations in the northwest (CDL, VBV; $F_{ST}=0.359-0.426$) and the northeast (MWM, GLD, MTA; $F_{ST}=0.270-0.340$) of Tasmania. The BNY sample was most similar to HVL ($F_{ST}=0.074$). Differentiation between other population pairs was low to moderate, with $F_{ST}$ estimates ranging between 0.032 and 0.199. The overall pattern of genetic structure among populations was visualized using PCA, with 78.6% of the variation captured on two axes. Three main genetic clusters were revealed within the Tasmanian mainland, corresponding to the southern/central (BNY, HVL, MFD, BTP, FCN), northwestern (VBV, CDL) and northeastern populations (GLD, MWM, MTA) (Figure 4-4). This pattern did not change when BNY was excluded from the analysis (data not shown). Results obtained using Mantel tests showed a positive significant relationship between genetic and geographic distance ($R^{xy} = 0.608$, $p = 0.000$) for both males and females.
Table 4-2 Genetic differentiation among eastern quoll populations based on pairwise $F_{ST}$ (Weir and Cockerham 1984). Values above 0.2 (moderate to high differentiation) are shown in bold. All pairwise population comparisons were statistically significant after sequential Bonferroni correction ($p = 0.001$).

<table>
<thead>
<tr>
<th></th>
<th>BNY</th>
<th>HVL</th>
<th>MFD</th>
<th>FCN</th>
<th>BTP</th>
<th>CDL</th>
<th>VBV</th>
<th>MTA</th>
<th>MWM</th>
<th>GLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNY</td>
<td>0.000</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>HVL</td>
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<td>0.000</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>MFD</td>
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<td>0.000</td>
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<td>0.105</td>
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<td>0.359</td>
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<td>0.153</td>
<td>0.160</td>
<td>0.078</td>
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<td>MTA</td>
<td>0.339</td>
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<td>0.095</td>
<td>0.086</td>
<td>0.215</td>
<td>0.196</td>
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<td>MWM</td>
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<td>0.176</td>
<td>0.113</td>
<td>0.085</td>
<td>0.230</td>
<td>0.199</td>
<td>0.070</td>
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</tr>
<tr>
<td>GLD</td>
<td>0.340</td>
<td>0.224</td>
<td>0.217</td>
<td>0.153</td>
<td>0.132</td>
<td>0.221</td>
<td>0.180</td>
<td>0.060</td>
<td>0.033</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Figure 4-4 Patterns of population differentiation among eastern quolls visualized using Principal Coordinates Analysis (PCA) (Orloci 1978). The axes explain 78.6 % of the total genetic variation; the main genetic clusters are circled.

The pattern of genetic structure described above was generally supported by the Bayesian clustering analysis performed in STRUCTURE. The smallest values of $K$ that captured the major structure in the data was $K=4$ when BNY was included and $K=3$ when BNY was excluded (Figure 4-5A). The number of subpopulations inferred from $K$ therefore corresponded to genetic clusters including BNY, central, northeast and
northwest populations. Given that $K$ is sometimes known to overestimate the number of subpopulations within datasets (Evanno et al. 2005), $\Delta K$ was also estimated. In this case, the analysis supported a model with two subpopulations (Figure 4-5B). The genetic clusters inferred from $\Delta K$ corresponded to southern versus central and northern populations when BNY was included. However, excluding BNY from the analysis revealed a different subpopulation structure within the Tasmanian mainland, with the northeastern populations separating out from the other populations. The assignment results obtained using GENECLASS2 were consistent with the above. Cross-assignments with high confidence ($P > 0.90$), indicating gene flow, were observed between central populations, but also between more peripheral populations like MTA and MWM, and VBV and CDL (Appendix 3).

The dispersal analysis performed using Slatkin’s private allele method revealed a large estimate of gene flow ($Nm = 20.659$, Table 4-3) between BTP and MFD, suggesting that these locations are well connected by gene flow. Wright’s island model resulted in a greater number of estimates of $Nm > 1$ compared to Slatkin’s private allele method (30 and 9 population pairs, respectively; Table 4-3). All estimates of $Nm > 1$ calculated using Slatkin’s method were also $Nm > 1$ using Wright’s island model. Mantel tests revealed a negative, but non-significant relationship between gene flow and geographic distance on both accounts. The exclusion analysis performed using Slatkin’s private allele method estimated the average number of migrants exchanged between all the populations to be 4.405, but this value was reduced to 1.660 when the BTP sample was excluded. On the other hand, when MWM was excluded, the average number of migrants increased to 6.388, suggesting that this population may be isolated.
Figure 4-5 Proportion of membership of individuals in each sample for each subpopulation cluster estimated without *a priori* population information using the Bayesian clustering analysis implemented in STRUCTURE 2.2 (Pritchard et al. 2000). Analyses were performed with and without BNY. A. Membership to subpopulation clusters inferred using $K$; B. Membership to subpopulation clusters inferred using $\Delta K$.

Table 4-3 Estimates of gene flow among eastern quoll populations. Effective number of dispersers/generation ($Nm$) obtained using Wright’s (1951) island model are shown below the diagonal and $Nm$ estimated using Slatkin’s (1985) private allele method above the diagonal. Estimates of $Nm > 1$ disperser/generation are shown in bold.

<table>
<thead>
<tr>
<th></th>
<th>BNY</th>
<th>HVL</th>
<th>MFD</th>
<th>FCN</th>
<th>BTP</th>
<th>CDL</th>
<th>VBV</th>
<th>MTA</th>
<th>MWM</th>
<th>GLD</th>
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<tbody>
<tr>
<td>BNY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HVL</td>
<td>3.141</td>
<td>1.592</td>
<td>0.559</td>
<td>20.659</td>
<td>0.960</td>
<td>0.554</td>
<td>0.564</td>
<td>0.393</td>
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<td>2.026</td>
<td>0.740</td>
<td>20.659</td>
<td>0.968</td>
<td>0.840</td>
<td>0.521</td>
<td>0.782</td>
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<tr>
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<td>0.407</td>
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<td>0.537</td>
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<td>1.383</td>
<td>2.936</td>
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<td>0.477</td>
<td>0.624</td>
<td>0.635</td>
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<td>MTA</td>
<td>0.488</td>
<td>1.056</td>
<td>0.844</td>
<td>2.395</td>
<td>2.656</td>
<td>0.912</td>
<td>1.024</td>
<td>-</td>
<td>0.912</td>
<td>1.348</td>
</tr>
<tr>
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<td>1.237</td>
<td>1.172</td>
<td>1.953</td>
<td>2.683</td>
<td>0.838</td>
<td>1.007</td>
<td>3.297</td>
<td>-</td>
<td>1.376</td>
</tr>
<tr>
<td>GLD</td>
<td>0.486</td>
<td>0.864</td>
<td>0.904</td>
<td>1.382</td>
<td>1.648</td>
<td>0.882</td>
<td>1.142</td>
<td>3.913</td>
<td>7.363</td>
<td>-</td>
</tr>
</tbody>
</table>
4.4 Discussion

Although eastern quolls are currently still abundant in many parts of Tasmania, their survival is at risk from the recently introduced European red fox. Time and resource constraints have led to a lack of detailed demographic information on eastern quolls. Therefore, the baseline molecular data on genetic variability, population size and dispersal generated in this study become a crucial surrogate to inform the management of the species. Ongoing genetic monitoring will allow conservation strategies to be adapted accordingly, particularly if, as predicted, widespread declines occur in the future.

4.4.1 Genetic variation

Island populations often have lower levels of genetic variation than mainland populations of the same species due to founder events, island effects and genetic bottlenecks (Frankham 1997; Eldridge et al. 2004). Tasmania became isolated from the Australian continent by the post-glacial sea rise 12000-8000 years ago. The land-bridge between the Tasmanian mainland and Bruny Island is also thought to have disappeared at this time (Burbidge and Manly 2002). The levels of genetic variation detected in Tasmanian eastern quolls were low compared to those previously found in extant and extinct quoll species from the Australian mainland, but were similar to those found in the Tasmanian devil, a larger carnivorous marsupial now also restricted to Tasmania (Appendix 1) (Firestone et al. 2000; Jones et al. 2004a; Cardoso unpublished). Additionally, the BNY population possessed significantly lower genetic variation compared to populations from the Tasmanian mainland (Table 4-1). The low genetic diversity on BNY is likely due to genetic drift in a small isolated island population.

4.4.2 Population bottlenecks and effective population size

Population genetics theory predicts that genetic variability is lost at a rate dependent on the effective size of a population (Frankham 1995b). Encouragingly, similar estimates of Ne were obtained using the different one-sample methods tested in this study (Figure 4-2). The most significant discrepancy was the large Ne obtained for BNY, even though its genetic variability was lower than the other populations (Table 4-1). Ne estimates may sometimes be biased by a population’s life history and mating patterns (Frankham 1995a). Compared to mainland populations, BNY is isolated and the sex-ratio was strongly female-biased (pers. obs.). These factors may have biased the results and
affected the ability of the methods to accurately estimate $Ne$ for this population. The temporal $Ne$ estimate for the MWM/GLD sample was also more than two times greater than the one-sample method estimate obtained for the same populations. This overestimation may have been due to the method’s assumption that the population was isolated (Wang 2001). As expected, populations found to possess signatures of recent genetic bottlenecks (BNY, HVL, FCN, MWM and VBV) had lower allelic richness and proportion of rare alleles than the non-bottlenecked populations (Table 4-1). However, the proportion of rare alleles detected in MTA and MWM may be of concern, because there is a high probability that these alleles will be lost due to random genetic drift, leading to the ultimate loss of genetic diversity in the long-term. It is worth noting that even though a genetic bottleneck was not detected for MWM, a marked but not significant ($p > 0.05$) reduction in $He$ and $rA$ was observed between GLD and MWM (Table 4-1), indicating a decrease in the genetic variation of this population between the 1960s and 2005.

**4.4.3 Genetic variation in central and peripheral populations**

The significant correlation detected between heterozygosity and geographic distance from the core eastern quoll distribution (Figure 4-3) supports the theory that peripheral populations exhibit less genetic diversity than populations at the core (Eckert et al. 2008). Higher heterozygosity was detected in central (BTP, MFD, FCN) than in peripheral (CDL, MWM, HVL) eastern quoll populations (Table 4-1). Populations located at range margins are thought to be more isolated from the central source of migrants and are therefore more prone to genetic bottlenecks and consequent losses of genetic variation (Garner et al. 2004). In this study, the private allele method of Slatkin identified BTP, a central population, as the largest source of migrants to other populations. Conversely, MWM, a peripheral population, was found to be isolated from the other populations, making it more prone to genetic bottlenecks and losses of genetic diversity than more central populations.

The relationship between $Ne$ and geographic distance from the core of eastern quoll distribution was significant, but not linear, with peripheral populations like CDL and MWM exhibiting levels of $Ne$ greater than expected based on their distance from the distribution core (Figure 4-3). Peripheral populations, although thought to be more unstable and prone to temporal variability in abundance, are often well adapted to local
environmental conditions and are thus evolutionarily significant (Vucetich and Waite 2003). This may well be true for eastern quolls in the north of Tasmania which exhibit seasonal fluctuations in population size, but are also well adapted to habitat types quite different to those of more central populations.

4.4.4 Population structure and gene flow

Significant population structure, largely explained by geographic distance, was detected among eastern quoll populations throughout their range on the Tasmanian mainland (Table 4-2, Figure 4-4). This finding was in contradiction to results obtained for a related species, the Tasmanian devil, which was shown to have very little genetic structure and no evidence of isolation by distance (Jones et al. 2004a). Many other wide-ranging mobile species exhibit low levels of genetic structure and little or no signal of isolation by distance (Leblois et al. 2000; Kyle and Strobeck 2001; Broquet et al. 2006). Conversely, less mobile species often exhibit more significant population structure (Pope et al. 2006). Due to their high-density, social structure, small body size and limited dispersal ability, geographic distance may be a barrier large enough to induce regional differentiation among eastern quolls. However, assignment tests detected high levels of genetic admixture among some of the more closely located central populations (Table 4-3), which may explain the differences observed between the partitioning based on $K$ and $\Delta K$ obtained using STRUCTURE 2.2 (Figure 4-5). In agreement with the ‘central-peripheral’ hypothesis (Eckert et al. 2008), differentiation was lower (Table 4-2) and gene flow higher (Table 4-3) among central populations than among central and peripheral populations. Mountain ranges in the north of Tasmania may limit dispersal towards more southern populations, while water is an obvious physical barrier restricting dispersal between Bruny Island and the Tasmanian mainland. Although BNY is genetically different to northern mainland populations, it maintains similarities to HVL, the most southern population sampled on the Tasmanian mainland (Figure 4-1). This pattern is likely due to the historical north-south expansion of eastern quolls along a continuous distribution before Bruny Island became isolated from the Tasmanian mainland.

4.4.5 Conservation management of eastern quolls

The relatively large sample sizes obtained in this study support the observation that eastern quolls are still abundant in parts of Tasmania. The species is currently thriving
on Bruny Island where the combination of suitable habitat and lack of predators and major competitors have allowed the opportunistic and highly adaptable eastern quoll to establish high densities. Some Bruny Island residents have suggested that eastern quolls are in plague proportions and that their numbers should be reduced. However, anthropogenic disturbance such as selective culling, may severely disrupt eastern quoll population dynamics, further reducing genetic variation and limiting the long term persistence of the BNY population. Culling is therefore not recommended for Bruny Island eastern quolls, which should instead be conserved as an insurance population, because they are protected from threats present on the Tasmanian mainland.

In general, eastern quoll populations in the northeast, northwest and southeast of Tasmania have lower genetic variation than more central populations (Table 4-1; Figure 4-3). Furthermore, a monitoring survey conducted in 2005 failed to capture eastern quolls in the VBV population (pers. obs). It is a concern that this population may already be locally extinct and highlights the need for ongoing monitoring of the nearby population in Cradle Mountain. In addition, results suggest that populations in the far north east may be isolated, with genetic variation having decreased since the 1960s. It is possible that isolation exacerbated by declines in the surrounding midlands populations may have disrupted gene flow and subsequently reduced the genetic diversity of the far northeastern populations. Populations at the periphery of species distributions may possess unique habitat adaptations worth protecting, because they may be beneficial to the species when dealing with environmental change. On the other hand, more genetically diverse and admixed central populations such as BTP and MFD are also worth protecting, because they appear to be the main source of migrants to other populations, making them suitable for future translocations if declines begin to occur.

Management recommendations such as population monitoring, habitat protection and feral predator control have been made for the conservation of eastern quolls, but these recommendations are yet to be implemented (NPWS 1999; TAS 2000). However, the threats currently facing eastern quolls strongly warrant the development of a management plan for the species and as highlighted by this study, genetic data provide invaluable information that should be incorporated into conservation strategies. Due to the inherent low genetic variability often found in island populations, the maintenance
of genetic diversity should be a high priority for wildlife managers. Recommendations resulting from this study include:

1) Ongoing population monitoring, including health assessment and genetic sampling of known eastern quoll populations, particularly those identified with low genetic diversity. Future information can be compared to the baseline genetic data obtained in this study;

2) Attempt supplementation from genetically diverse central populations if recolonization from surrounding populations does not naturally occur in the northeast and northwest of Tasmania;

3) Treat Bruny Island eastern quolls as an important insurance population. Attempt supplementation from suitable mainland populations to enhance genetic diversity;

4) Attempt translocations to other islands and mainland Australia to establish insurance populations if widespread declines are detected in mainland Tasmanian populations. In order to increase the success rate of translocations, the genetic composition of source individuals, habitat-specific adaptations, disease implications and threatening processes should always be considered.
Chapter 5

Who’s your daddy? Paternity testing reveals promiscuity and multiple paternity in the carnivorous marsupial *Dasyurus maculatus* (Marsupialia: Dasyuridae)

**Abstract**

Female promiscuity is common among mammals but its advantages, particularly for marsupials, remain unclear. Using microsatellite DNA from pouch young of known mothers, we identified the most likely fathers of 25 wild spotted-tailed quolls (*Dasyurus maculatus*) from six litters. We aimed to determine whether young within the same litter had different fathers, and whether breeding success of males was associated with large body mass (consistent with inter-male competition) or scrotal width (consistent with sperm competition). We also explored the possible influence of promiscuity on relatedness within litters. Finally, we used data on paternity and relatedness to make inferences regarding movement and dispersal.

Four litters were sired by more than one male, and three males sired offspring in more than one litter. Known fathers had higher body mass, but not scrotal width, than males of unknown paternity status, suggesting that males may compete for access to females. Sires were less related to dams than expected by chance, and litters with multiple paternity had lower relatedness than litters sired by a single male.

**5.1 Introduction**

Promiscuous mating systems are common in the marsupial family Dasyuridae, with multiple paternity and sperm competition known or suspected to occur in some species (Shimmin et al. 2000; Kraaijveld-Smit et al. 2002; Taggart et al. 2003; Holleley et al.)
Promiscuous mating by females, or polyandry, is also common among eutherian mammals, and litters of young are often fathered by more than one male. Confusion of paternity to prevent infanticide appears to be a common reason for the evolution of polyandry, but this explanation is unlikely to apply to marsupials, in which infanticide is uncommon (Wolff and Macdonald 2004). Other possible advantages include increased quality of paternal genes (Kirkpatrick 1996), avoidance of genetic incompatibilities between parental genotypes through sperm competition (Jennions and Petrie 2000), and genetic bet-hedging (Yasui 2001). Multiple paternity in wild populations of marsupials has been shown in the honey possum, *Tarsipes rostratus* Gervais & Verraux (Wooller et al. 2000), agile antechinus, *Antechinus agilis* Dickman *et al.* (Kraaijveld-Smit et al. 2002), feathertail glider, *Acrobates pygmaeus* Shaw (Parrott et al. 2005) and brown antechinus, *Antechinus stuartii* Macleay (Holleley et al. 2006).

The spotted-tailed quoll, *Dasyurus maculatus* Kerr, is a medium-sized carnivorous marsupial that bears a single litter of 5-6 young each year. Several aspects of its biology led us to suspect that polyandry and multiple paternity may occur in this species. First, a promiscuous mating system has been inferred from the species’ spatial organization. Females occupy relatively small, exclusive territories while males occupy much larger home ranges, overlapping with several other males and females (Glen and Dickman 2006). Second, polyandry also seemed likely based on physical characteristics. *D. maculatus* shows strong sexual dimorphism in body mass (Jones and Barmuta 2000; Taggart et al. 2003; Glen 2005), and an increase in scrotal widths during the annual mating season (Glen 2005). These traits are common among species with promiscuous mating (Taggart et al. 2003). Sexual dimorphism, with males being larger, is a likely indicator of inter-male competition for copulations (Taggart et al. 2003). Large testes relative to body mass may be an adaptation for sperm competition, as paternity success in such systems is related (among other factors) to numbers of sperm (Snook 2005).

The aims of our study were to 1) determine the paternity of pouch young of known mothers in a wild population of *D. maculatus*, 2) investigate whether young within the same litter had different fathers, 3) investigate whether breeding success of males was associated with large body mass (implying inter-male competition) or scrotal width (consistent with sperm competition), and 4) explore the possible influence of
promiscuous mating on levels of relatedness. We also use data on paternity and relatedness to make inferences about movement and dispersal in the study population.

5.2 Materials and Methods

5.2.1 Trapping and handling of quolls

Spotted-tailed quolls were trapped in adjacent areas of Marengo and Chaelundi State Forests in north-eastern New South Wales, Australia (30° 07’S, 152° 23’E). We trapped quolls over four nights each month from January 2003 to October 2004, except for February 2003. Up to 40 cage traps (30 x 30 x 60 cm, Mascot Wireworks, Sydney) were set at intervals of 500 m along a network of roads. Each trap was wrapped in plastic sheeting, insulated with coconut fibre, and baited with chicken wings. Traps were checked in the early hours after dawn each morning.

Captured quolls were weighed with a spring balance, and measurements taken of scrotal width where applicable. Tissue samples were collected by removing a small strip of skin from the edge of the ear with surgical scissors, having first swabbed the area with ethanol. Samples were stored in 70 % ethanol prior to genetic analysis. Tissue samples from juveniles in the mother’s pouch were taken from the tip of the tail. Each adult animal was implanted with a subcutaneous microchip (Destron Fearing Corp., Saint Paul) between the shoulder blades for individual identification. The study area and trapping methods are described in detail by Glen & Dickman (2006).

5.2.2 DNA extraction and microsatellite genotyping

The ‘salting out’ method of Sunnucks & Hales (1996) was used to extract genomic DNA from the tissue samples and genotypes were scored at 11 microsatellite loci. Polymerase chain reactions (PCR), using fluorescently labelled forward primers (Applied Biosystems), were carried out separately for the six loci described by Firestone (1999) due to the different annealing temperatures and concentrations of MgCl₂ required for each primer set (1.3-VIC: 50°C, 2.5mM; 3.1.2-VIC/3.3.1-NED: 60°C, 2.0mM; 3.3.2-PET: 50°C, 2.0mM; 4.4.10-NED: 55°C, 2.5mM; 4.4.2-FAM: 55°C, 2.0mM). Reactions were made up in a total volume of 10µl containing 1x PCR buffer, 2.0-2.5mM of MgCl₂ (as above), 0.2mM of each dNTP (Amresco), 0.5µM of each
forward and reverse primer, 1U of AmpliTaq Gold® (Applied Biosystems) and 25-50ng of template DNA. Amplifications were carried out in a Dyad™ thermal cycler (MJ Research) with an initial denaturation step of 95°C for 5 min followed by 39 cycles of 95°C for 30 sec, 50-60°C (as above) for 30 sec, 72°C for 30 sec and a final extension of 72°C for 30 min. The other five microsatellite loci (1A1, 1H3, 5G4, 6D5 and 7F3) were multiplexed and amplified following the conditions described in Spencer et al. (2007).

The resulting PCR products were diluted, pooled together and separated electrophoretically on an Applied Biosystems 3730 automated sequencer. Allelic sizes were scored against the size standard GS500 LIZ (Applied Biosystems) and analyzed using GeneMapper® v.3.7 (Applied Biosystems).

5.2.3 Relatedness and paternity analysis

Deviations from Hardy-Weinberg equilibrium (HWE) and Linkage Disequilibrium (LD) between loci were assessed by exact tests (1000 dememorizations, 1000 batches, 1000 iterations) using the program GENEPOP 3.4 (Raymond and Rousset 1995). Genetic relatedness (R) between all possible pairs of individuals was calculated using the program MER V3 (Wang 2002). Relatedness coefficients range between -1 and +1. A positive R-value between two individuals indicates that they are more related than expected by chance, and a negative R-value indicates that they are less related than expected by chance (Wang 2002). A Mantel test (Mantel 1967), incorporated in the program GenAlex 6 (Peakall and Smouse 2006) was used to test for a statistical relationship between genetic relatedness and geographic distance matrices of all individuals in the population. Separate tests were carried out for males and females using 1000 random permutations. GenAlex 6 was also used to calculate the expected heterozygosity (He) or genetic diversity (Hartl and Clark 1997) of individuals in the population at 11 microsatellite loci.

Paternity was assigned using the program CERVUS 3.0 (Kalinowski et al. 2007). The method derives likelihood of paternity from microsatellite data taking into account the number of candidate males, the proportion of males sampled and gaps and errors in the genetic data. A statistic Delta (Δ) is then used to resolve paternity with criteria that allow assignment of paternity to the most likely male with a known level of statistical confidence (Marshall et al. 1998). In order to assess the resolving power of the loci to assign parentage, simulations were performed using 10,000 iterations, 35 candidate
males, 0.85 as the proportion of fathers sampled, 0.98 as the proportion of loci successfully typed (observed value) and a standard mis-scoring error rate of 0.01 (Marshall et al. 1998; Holleley et al. 2006). Likelihood ratios in the form of LOD scores were calculated for each candidate parent, based on the genotypes of the candidate parents, offspring and known mothers. Negative, zero, or positive LOD scores respectively imply that the candidate parent is less likely, equally likely, or more likely to be the true parent than a randomly-chosen individual. The most likely candidate parent thus has the highest or most positive LOD score. The statistic $\Delta$ was used to calculate the confidence of paternity assignment at 80% and 95% levels. It is defined as the difference in LOD scores between the most likely and second most likely candidate parent. Only LOD scores greater than zero are considered when calculating $\Delta$ and if only one candidate parent has a LOD score exceeding zero, $\Delta$ is defined to be that LOD score (Marshall et al. 1998).

5.2.4 Morphometric comparisons

In order to determine whether paternity success was associated with body mass or scrotal width, we compared measurements taken from known fathers (identified by genetic analysis) to those from males of unknown paternity status. We used measurements of body mass and scrotal width taken from the fathers during or shortly before the mating season in which the offspring were sired. As scrotal width varies significantly with season (Glen 2005), we compared the scrotal widths of known fathers with those of males in the broader population using only measurements taken during the mating season. Our hypotheses were that 1) mean body mass, and 2) mean scrotal width, would be higher among known fathers than among males of unknown paternity status. We tested these hypotheses using 1-tailed $t$-tests. Data were first checked for normality using box plots, and for equal variance using $F$-tests.

Among known fathers, we also tested for correlations between body mass or scrotal width and number of offspring sired in each litter.

5.3 Results

We collected tissue samples from 52 mature quolls (35 males, 17 females), as well as 30 pouch young from six litters. None of the loci were found to deviate from Hardy-
Weinberg and Linkage Disequilibrium. Genetic diversity within the population was comparable to that of other populations in New South Wales (Cardoso unpublished data).

We were able to identify the fathers of 25 (83%) of the pouch young. Three samples from pouch young continuously failed to amplify, possibly due to the low quantity or quality of the DNA present. The other two samples could not accurately be assigned to a known father using CERVUS, indicating that the actual father(s) may not have been sampled. The cumulative number of males encountered during the trapping study reached a plateau between November 2003 and March 2004. Subsequently, a newly-independent juvenile male (body mass 900 g) was captured in March 2004. New males were encountered sporadically thereafter, and were assumed to be immigrant or transient individuals. We are therefore confident that the population of candidate fathers was effectively trapped. However, we failed to obtain full genotypes for six males because samples failed to amplify at some loci.

Following the methods of Holleley et al. (2006), we chose *a priori* to assign paternity at a confidence of 80%. However, all but one of the known fathers were assigned with 95% confidence. Two of the six litters were sired by a single male, while four were sired by more than one male. Three males sired offspring in more than one litter by separate females (Table 5-1).
Table 5-1 Most likely fathers of 25 newborn spotted-tailed quolls *Dasyurus maculatus* from 6 litters in Marengo and Chaelundi State Forests.

<table>
<thead>
<tr>
<th>Litter</th>
<th>Most likely father</th>
<th>Offspring ID</th>
<th>LOD</th>
<th>Δ</th>
<th>Confidence</th>
<th>Paternal body mass (g)</th>
<th>Paternal scrotal width (mm)</th>
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</thead>
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<td>1</td>
<td>M201 1</td>
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<td>0.458</td>
<td>+</td>
<td>80%</td>
<td>4200</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>M201 2</td>
<td>4.78</td>
<td>4.78</td>
<td>*</td>
<td>95%</td>
<td>4200</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>M198 3</td>
<td>11.6</td>
<td>11.6</td>
<td>*</td>
<td>95%</td>
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<td>34</td>
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<tr>
<td></td>
<td>M182 4</td>
<td>7.80</td>
<td>7.80</td>
<td>*</td>
<td>95%</td>
<td>3720</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>M184 1</td>
<td>7.31</td>
<td>6.84</td>
<td>*</td>
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<td>3600</td>
<td>28</td>
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<tr>
<td></td>
<td>M184 2</td>
<td>8.01</td>
<td>8.01</td>
<td>*</td>
<td>95%</td>
<td>3600</td>
<td>28</td>
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<td>8.87</td>
<td>*</td>
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<td>3.74</td>
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<td>32</td>
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<tr>
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<td>*</td>
<td>95%</td>
<td>3600</td>
<td>28</td>
</tr>
<tr>
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<td>1.03</td>
<td>1.03</td>
<td>*</td>
<td>95%</td>
<td>3600</td>
<td>28</td>
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<tr>
<td></td>
<td>M184 5</td>
<td>3.38</td>
<td>3.38</td>
<td>*</td>
<td>95%</td>
<td>3600</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
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<td>2.89</td>
<td>2.89</td>
<td>*</td>
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<td>3.38</td>
<td>3.38</td>
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<td>8.55</td>
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<td>95%</td>
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(+)= 80% confidence; (*) = 95% confidence

Thus, eight male quolls were identified as having fathered offspring. Comparison using a box plot (Figure 5-1a) shows that the mean body mass of known fathers was higher than that of males of unknown paternity status. The plot shows that the data conform with the assumption of normality, but that variances are unequal, as confirmed by an *F*-test (*F*_{26,7} = 3.26, *P* = 0.11). We therefore used a Welch 2-sample *t*-test, which allows for unequal variances. This showed that the mean body mass of known fathers exceeded that of other males (*t* = -3.8, df = 21.6, *P* = 0.0005). The mean scrotal width
of known fathers did not differ greatly from that of other males (Figure 5-1b), although variance was higher among the former ($F_{19,8} = 0.55, P = 0.27$). A Welch 2-sample $t$-test showed no difference ($t = -0.39, \text{df} = 12.1, P = 0.35$). Among known fathers, the number of offspring sired in each litter was not related to body mass ($r = 0.28, P = 0.4$) or to scrotal width ($r = -0.353, P = 0.3$).

**Figure 5-1** Mean body mass (a) and scrotal width (b) of spotted-tailed quolls known to have fathered offspring in comparison with males of unknown paternity status.

Except for one litter (litter 4), successful fathers were less related to the females than other males in the population, with most relatedness coefficients for sires and non-sires below the population mean of $R = -0.0265$ (Figure 5-2). Offspring in litters with multiple paternity, except for litter 4, had lower relatedness coefficients than offspring in litters sired by a single male (Figure 5-3). The high level of relatedness observed in litter 4 was due to one of the sires being more related to the female than expected by chance. This male was possibly the son of the female from a previous litter ($r = 0.47$). Mantel tests revealed a significant correlation between genetic relatedness and geographic distance for females ($r = -0.191, P = 0.050$), but not for males ($r = -0.096, P = 0.070$).
Figure 5-2 Relatedness (R) of the mother of each litter to sires and non-sires respectively. Dashed line indicates the mean level of relatedness (-0.0265) for the population.

Figure 5-3 Relatedness within litters with one (open bars) and two sires (solid bars).

5.4 Discussion

Our results demonstrate not only that multiple paternity occurs within litters of *D. maculatus*, but also that large males are much more likely to father offspring than smaller males. This is consistent with a system in which males compete aggressively for mating opportunities. As well as gaining more copulations, large males may also be more successful at guarding mates, thus avoiding sperm competition (Holleley et al.)
In a system where breeding success of males is strongly dependent on size, the body mass of males will be subject to strong selective pressure. This may explain why, according to Taggart et al. (2003), sexual size dimorphism in *D. maculatus* is among the most extreme within the Dasyuridae. As age is likely to be a strong correlate of body size, paternity may also be biased towards older males.

On the question of sperm competition, our results are inconclusive. The known fathers in our population did not have consistently larger scrotal widths than other males and larger scrotal width did not correlate with a larger number of offspring sired (although the number of litters sampled may have been too small to assess this rigorously). However, comparing scrotal widths is likely to give only a coarse indication of the likelihood of sperm competition. More reliable inferences could be based on such data as testis mass or number of sperm per ejaculate. The large increase in scrotal size during the annual mating season (Glen 2005) does support the possibility of sperm competition. Although the ability of female *D. maculatus* to store sperm is unknown, sperm storage has been recorded in the female reproductive tract of many dasyurids. For example, female *D. viverrinus* Shaw can store sperm for 14 days (Taggart et al. 2003). It seems probable that *D. maculatus* also has this ability. If large males are more successful at guarding mates, they might avoid sperm competition from smaller males simply by preventing further copulations. Conversely, if small males are incapable of guarding mates from larger rivals, they may face sperm competition. Thus, mate guarding could make sperm competition difficult to observe in the wild. Captive experiments may be informative, if females are mated with males of similar body size, so that the effect of testis size could be studied without the confounding effects of body size and mate guarding. Captive experiments might also investigate whether post-copulatory selection by females biases paternity towards males with lower relatedness, and whether there is a subsequent effect on the viability of offspring (Fisher et al. 2006).

Finally, our genetic results also revealed that breeding had taken place between two radio-collared quolls whose home ranges (as revealed by radio telemetry and trapping records) showed no overlap (A. S. Glen and C R Dickman 2006). This further supports the observation that male *D. maculatus* roam over increased distances during the mating season, presumably in search of females (A. S. Glen and C R Dickman 2006).
The greater mobility of males is also supported by our Mantel tests. No significant correlation was found between the relatedness of males and their geographic distance within the study area. By contrast, there was a significant negative correlation between female relatedness and geographic distance. This supports the theory of female natal philopatry described previously in this species (Firestone et al. 1999).
Chapter 6

General Discussion:

Summary of Results and Conservation Implications for Australian quolls

6.1 Introduction

Threatened species often require active *in situ* and/or *ex situ* management to enhance their survival. While anthropogenic disturbance may be the ultimate cause of species’ extinction, certain life-history traits can determine how well populations deal with threatening processes (Fisher et al. 2003). A thorough understanding of a species’ biology is therefore of benefit to management, because it provides insights into previously unrecognized species-specific survival strategies that determine their risk of extinction. In the case of large carnivorous marsupials such as quolls, adaptable and opportunistic behaviour, high reproductive output and short generation lengths can to some extent counteract the negative effects of human disturbance. However, disadvantageous life history traits such as low juvenile survival and short life spans, in combination with human-induced mortality, may exacerbate the extinction risk of susceptible populations (Jones et al. 2003).

Extensive knowledge of the occurrence, distribution and abundance of Australian quoll species is lacking due to difficulties with detection of individuals in the wild. Quolls are elusive and nocturnal, and like other large carnivores, are known to occupy large home ranges at low population densities. Quoll persistence and abundance is therefore dependent on the availability of resources able to sustain populations within large areas of suitable habitat. This becomes particularly important for the larger species like the western and spotted-tailed quolls (Dunlop and Morris 2008; Long and Nelson 2010a). Demographic data important for developing population viability analyses for quoll species, such as population size and dispersal, can be hard to obtain using ecological methods. Population genetic data used to complement or as a surrogate to ecological data, may therefore be beneficial in helping to fill in some crucial gaps in our current knowledge of quoll population dynamics.
The aim of this thesis was to provide sound genetic data and advice to assist and improve the ongoing management of Australian quoll species. The genetic monitoring of translocated western quoll populations (chapter 3) and the examination of parentage and relatedness in a spotted-tailed quoll population (chapter 5; Glen et al. 2009) were carried out following recommendations outlined by Firestone (1999). Additional genetic data obtained for northern quolls (chapter 2; Cardoso et al. 2009), eastern quolls (chapter 4) and spotted-tailed quolls (Appendix 4) were beneficial in further defining population structure within these species (Firestone et al. 1999; Firestone et al. 2000; Belcher 2006).

6.2 Summary of Results

Samples are often difficult to obtain in the wild due to the elusive nature and low population densities of most quoll species. Large sample sizes are important for increasing the statistical power of genetic analyses and instilling confidence in the accuracy of the data generated. This study benefited from relatively large sample sizes for most of the populations examined, which was primarily due to the coordinated effort of government agencies responsible for the conservation of quoll species in their respective States (Table 1-1). It is however important to note that only a small number of samples (less than 20 individuals) were available for some populations (Tables 2-1, 3-1 and 4-1), the results of which should be interpreted with caution. In addition, certain analyses, such as the estimation of effective population size and the detection of genetic bottlenecks may be biased when only a small number of genetic markers are included (Piry et al. 1999; Waples 2005). Unfortunately, additional markers were not available for use in this study, which is why in some cases, multiple tests and analysis methods were compared to ensure accuracy. Nonetheless, I am confident that the genetic data presented in this thesis are useful in providing invaluable information to help guide the conservation of the four Australian quoll species.

It is important for wildlife managers to monitor the success of implemented conservation strategies (Maudet et al. 2002). The results presented in chapter 2 (Cardoso et al. 2009) indicate that the current management practice of translocating northern quolls to establish insurance island populations has, in the short-term, not led to severe genetic effects. However, the results do confirm that populations are
vulnerable to genetic effects associated with translocations to islands. Thus, founder events resulted in the divergence from ancestral allele frequencies observed in the translocated island populations, but also in reductions in genetic diversity compared to mainland source populations. Interestingly, current estimates of effective population size were greater in the population sourced from geographically subdivided mainland populations than in the one sourced from closely located mainland populations. Therefore, for a species such as the northern quoll, whose populations are not significantly divergent on an evolutionarily scale, translocating a variety of different genotypes may reduce competition for resources at foundation. This may in turn be beneficial to population persistence by allowing rapid population expansion and long-term evolutionary potential. Compared to the translocated island populations, low effective population sizes and high genetic erosion were detected in the endemic island populations which have been isolated from mainland populations for approximately 8-12000 years (Woinarski et al. 1999b). This result was consistent with previous findings of other island species and emphasizes that greater losses in genetic diversity occur on islands compared to mainland populations due to isolation from sources of gene flow and associated adaptations to island ecosystems (Frankham 1997; Eldridge et al. 2004; How et al. 2009).

When known threatening processes are mitigated, translocations to mainland sites tend to have a greater rate of success compared to island translocations, because mainland populations are often sustained by dispersal and gene flow from surrounding populations (Frankham 1997). The results presented in chapter 3 support the observation that the captive breeding and translocation programs implemented as part of the chuditch Recovery Plan were successful in establishing three viable wild populations in areas of their former range in Western Australia (Dunlop and Morris 2008). Although some genetic bottlenecks were detected, effective population sizes were considered adequate for the persistence of the species, which is known to occur at low densities. Genetic variation was high and not significantly different between the translocated and the remnant populations, suggesting that gene flow is playing an important role in maintaining genetic diversity. Genetic admixture was observed among some translocated and neighbouring remnant populations, which is consistent with demographic data (Morris et al. 2003). However, significant regional differentiation and isolation by distance were detected among the populations examined.
Genetic monitoring is important in helping to define the extinction risk of species thought to be at risk (How et al. 2009). Although eastern quolls are currently still abundant in many parts of Tasmania, there are perceived risks to their future survival posed by the introduced European red fox (DPIW 2010). The results presented in chapter 4 revealed that genetic variation was lower in Tasmanian eastern quolls than in quoll species from the Australian mainland. Furthermore, within Tasmania, the lowest levels of genetic variation were detected on Bruny Island. These observations once again highlight that greater loss of genetic diversity occurs on islands compared to mainland populations (Eldridge et al. 2004; How et al. 2009). In agreement with the ‘central-peripheral’ hypothesis (Eckert et al. 2008), genetic variation was greater in central than in peripheral mainland populations. In addition, genetic admixture was greatest among the more closely located central populations, which were identified as a significant source of dispersers to neighbouring populations. Despite moderate levels of gene flow, regional genetic differentiation and isolation by distance were significant, with northern populations found to be isolated relative to the more central and southern populations.

These results emphasize that island populations are more prone to genetic erosion and founder effects than their mainland counterparts (Frankham 1997; Eldridge et al. 2004), because mainland populations are often sustained by gene flow from surrounding populations and are therefore more likely to retain the evolutionary potential necessary for long-term persistence (Frankham et al. 2002). Although gene flow was detected among eastern quoll populations, Tasmania is itself a large island where organisms may be more prone to reduced genetic diversity, increased levels of inbreeding and evolution of adaptations to island environments associated with long-term isolation and genetic drift (Frankham 1997). Consequently, eastern quolls possess lower genetic diversity relative to northern and western quolls. In addition, population structure was more significant in eastern quolls than in the other two species, which is possibly a result of their small size and reduced mobility (Table 1-1).

Baseline genetic data on spotted-tailed quolls from New South Wales were collected and analysed (see Appendix 4), but not written up as a chapter in this thesis. In chapter 5 (Glen et al. 2009), paternity analysis was performed on a population from central
New South Wales. Within this population, multiple paternity was identified in four out of the six litters sampled, with male reproductive success significantly correlated to body size. Furthermore, successful fathers were less related to the females than other males in the population and offspring in litters with multiple paternity were more genetically diverse and less related than offspring in litters sired by a single male. Multiple paternity in spotted-tailed quolls therefore appears to be a strategy that has evolved to maintain high genetic diversity and reduce inbreeding depression within populations. This study exemplifies the usefulness of genetic data in gaining insights into the social structure of a species that has proven difficult to study by other means.

6.3 Conservation Implications

Monitoring and maintaining high effective population sizes and genetic variability is of paramount importance to threatened species conservation, because these factors have the potential to enhance long-term evolutionary adaptation in response to environmental stochasticity (Frankham et al. 2002). It is important to note here that the estimates of effective population size obtained in this study must only be treated as approximations, because overlapping generations, short life-spans, temporal fluctuations in population size and recent genetic admixture (Fraser et al. 2007; Waples 2007) may bias Ne estimation in quoll species. For instance, the high Ne estimated for eastern quolls on Bruny Island may be an effect of temporal fluctuations in population size and skewed sex ratios, while recent genetic admixture associated with translocations may have biased Ne estimates in northern quolls translocated to islands.

Consistent with previous findings (Firestone et al. 2000) and despite a history of widespread population decline, chuditch have higher levels of genetic variability than other Australian quoll species (Appendix 1). Given that abundant species should be, on average, more polymorphic than scarce ones (Bazin et al. 2006), the high diversity in chuditch populations may be remnant of a once abundant species, before the onset of rapid range contraction. On this basis, eastern quolls, which were also very abundant in southeastern Australia, should have maintained high levels of genetic diversity comparable to chuditch, had they not become extinct on the mainland. The higher levels of diversity detected in extinct mainland eastern quoll populations (Firestone et al. 2000; Appendix 1) certainly reflect this assumption.
By contrast, genetic effects associated with island ecosystems have resulted in low diversity in eastern quoll populations restricted to Tasmania and Bruny Island, but also in northern quolls endemic to islands. Ongoing genetic monitoring of low diversity populations will be necessary to detect further losses of diversity so that supplementation may be considered before adverse genetic effects compromise population viability. In addition, due to the continued presence of threatening processes on the Australian and Tasmanian mainland, the long-term persistence of quoll populations can only be assured if introduced predator control and habitat conservation strategies continue to be implemented into the future (Jones et al. 2003). Similarly, the success of future translocations of northern and eastern quolls to supplement mainland populations and set up insurance populations, respectively, can only be maximized by mitigating those threats that caused populations to become threatened or extinct in the first place (Seddon et al. 2007).

The translocations carried out to aid the recovery of northern and western quolls have so far been successful in establishing viable populations. Large reductions in genetic variation in the translocated northern quoll island populations may have been minimised, because these populations expanded rapidly after translocation (Allendorf and Luikart 2007; Rankmore et al. 2008), while translocated western quoll populations may have been sustained by gene flow from surrounding remnant populations. Despite this success, ongoing monitoring to assess long-term population persistence is recommended, particularly on the islands isolated from mainland sources of gene flow (How et al. 2009). The success of these translocations does however highlight the benefits of implementing conservation strategies designed to control threatening processes, while also considering genetic implications (Sarre and Georges 2009). Management actions strategically aimed to maintain high genetic diversity, both in captivity by regular supplementation with wild animals (Appendix 2), but also at translocation sites through the release of a relatively large number of founders (Table 2-1; Table 3-1). In addition, the practice of mixing founding individuals from demographically isolated mainland populations of northern quolls was advantageous to population persistence by increasing the current effective population size. However, this observation could not have been made if founding individuals had not been sampled for genetic analyses, which emphasizes the importance of collecting such samples. These considerations may in the long run increase conservation success and
should be taken into account when planning future management programs for quoll species. For instance, given their small home ranges and ability to exist at high densities, at least 100 genetically diverse eastern quolls should be sourced from existing Tasmanian populations for use in translocations to islands or the Australian mainland if, as predicted, widespread population declines begin to occur.

Successful implementation of translocations and supplementations must be guided by knowledge regarding the genetic composition, but also the population structure of threatened species. For instance, understanding the extent to which populations naturally support one another both genetically and demographically will help to better target populations for conservation and to avoid risks associated with genetic incompatibilities that may arise when animals are moved between locations (Moritz 1999; Storfer 1999). Previous work suggested that populations of spotted-tailed, northern and eastern quolls were demographically isolated and should be treated as functionally separate units that should not be admixed unless population numbers drop to critical levels (Firestone 1999a). This thesis, which examined a larger number of populations within species, but also relatively larger sample sizes for most of the populations examined, did find some agreement to previous findings. For instance, endemic northern quoll island populations have significantly diverged from each other and should not be admixed. However, although populations of all four Australian quoll species are more genetically structured and isolated by distance than other known mobile species (Leblois et al. 2000; Kyle and Strobeck 2001; Jones et al. 2004a; Broquet et al. 2006), current levels of gene flow are likely to maintain connectedness and restrict significant divergence among populations. Therefore, the movement of individuals between current quoll populations is unlikely to cause adverse effects due to genetic incompatibilities, because differences among populations are relatively minor on an evolutionary scale.

Defining Management Units can be challenging and sometimes controversial (Moritz 1999; Waples and Gaggiotti 2006). Confidence in identifying separate Management Units may also be severely affected by how extensively the sampled populations are distributed across a species geographical range (Taylor and Dizon 1999). Given these constraints, it may be far more worthwhile to primarily consider the connectedness between localities, but also the availability of suitable habitat and aspects of local
adaptation when planning conservation programs for mobile and adaptable species such as quolls. Quolls are known to move large distances through existing remnant vegetation corridors on private and public land (K. Morris, G. Koertner, A. Glen, pers. comm.; pers. obs.). Therefore, protecting and enhancing existing corridors or creating new corridors between remnant habitat patches will benefit dispersing individuals and help prevent genetic divergence between populations by increasing gene flow. Genetic theory predicts that long-term species viability will be maintained if, in total, a system of connected subpopulations has a large enough effective size and each subpopulation receives at least one genetically effective disperser per generation (Slatkin 1987; Laikre et al. 2009). Therefore, it may be beneficial to protect those quoll populations that were identified as the main source of dispersers to neighbouring populations. The high levels of admixture detected among some quoll populations suggest that habitat fragmentation has so far not had a severe impact on quoll population structure. Eastern quolls may perhaps be the exception, because being less mobile than other quoll species, the dynamics and stability of populations may be more influenced not only by distance, but also by dispersal barriers such as fragmented habitat and the presence of introduced predators and competitors that may trigger shifts in abundance and distribution.

Therefore, management decisions regarding which populations should be prioritized for conservation should primarily be based on estimates of connectedness between regions rather than on imaginary cartographical boundaries to which organisms do not generally adhere. If however, there are requirements for conservation units to be defined within species, recommendations based on the levels of significant divergence at microsatellite loci detected in this study are as follow:

- The endemic northern quoll island populations (chapter 2; Cardoso et al. 2009) should each be considered as separate Management Units. These island populations may be valuable enough to warrant separate management, because they have become genetically unique due to their long-term isolation from the mainland. However, supplementation of the islands from mainland source populations may outweigh the cost of separate conservation, because ongoing genetic erosion and the threat of inbreeding may make these populations more prone to extinction in the long-term
• Northern and southern chuditch populations (chapter 3) should be considered as separate Management Units based on significant regional differentiation and isolation by distance. However, results from assignment tests show that these populations currently appear to be indirectly connected by gene flow through more centrally located populations.

• In Tasmania, northeastern, northwestern, southern and the Bruny Island eastern quoll populations (chapter 4) should be considered as separate Management Units based on the significant regional differentiation and isolation by distance detected by assignment and Mantel tests. However, supplementation of the Bruny Island population may outweigh the cost of separate conservation, because ongoing genetic erosion and the threat of inbreeding may make this population more prone to extinction in the long-term.

• Southern NSW spotted-tailed quoll populations should be treated as separate Management Units to northern populations.

It is also important to consider the contraction of species’ distributions when interpreting genetic structure, because populations may not only be influenced by spatial patterns in habitat quality, but also by historical and contemporary changes to population size and gene flow (Vucetich and Waite 2003). Therefore, observations pertaining to the ‘central-peripheral’ hypothesis, which predicts that peripheral populations should exhibit lower genetic diversity and higher genetic differentiation than populations at the core of a species range (Eckert et al. 2008), may provide useful insights into patterns of species’ range contraction and resulting population structure. The validity of the theory however, is constrained by how extensively the sampled populations are distributed across a species geographical range (Sagarin and Gaines 2002). One advantage of studying species restricted to islands is the opportunity to widely sample distributions. As a result, the ten eastern quoll populations examined in this study were sampled across most of the species current geographical range. As predicted by the ‘central-peripheral’ hypothesis, Tasmanian mainland populations (not Bruny Island) at the range periphery are less diverse and more genetically differentiated from central populations than populations at the core of the species’ range. Conversely, genetic diversity was not significantly different among sampled western quoll populations, be they central or peripheral. Due to severe population declines, the
western quoll is currently at the periphery of its much larger historical range (Figure 1-5C). Therefore, the ‘central-peripheral’ hypothesis can only be tested in western quolls if specimens from extinct central populations are also examined. Similarly, the geographical range of northern quolls has contracted significantly in recent years (Figure 1-5A). Given that only a small number of mainland northern quoll populations were sampled in this study, the ‘central-peripheral’ hypothesis cannot accurately be tested for this species. Sampling also failed to cover the entire mainland distribution of spotted-tailed quolls, but in agreement with the ‘central-peripheral’ hypothesis, genetic diversity is greater in northern than in more peripheral southern New South Wales populations.

6.4 Suggested Further Work

The present study represents a broad scale analysis of genetic variability and population structure within quoll species, based on microsatellite data. It would be useful to expand this work to include:

1) Ongoing genetic monitoring of translocated northern and western quoll populations to detect long-term effects associated with translocations. In addition, ongoing monitoring of translocated northern quoll island populations will help to determine their suitability for use in future supplementations of locally endangered and/or extinct mainland populations.

2) Fine-scale genetic analyses of populations for which extensive geographic data is available so that more detailed data on population structure and dispersal may be obtained. For instance, analysis of relatedness and dispersal within translocated northern quoll island populations and most of the sampled western and spotted-tailed quoll populations may provide valuable insights into population dynamics that may be difficult to obtain by other means. Unfortunately, extensive geographic data are not available for eastern quolls, because sampling was largely opportunistic for this species.

3) Additional sampling of mainland populations of all quoll species to achieve a more extensive coverage of current distributions. This will allow better estimates of connectedness to be obtained, but will also help to more accurately define Management
Units and identify differences between central and peripheral populations. Further samples from populations for which sample sizes were low in this study should also be obtained so that more accurate comparisons can confidently be made among populations.

4) Repeat the genetic analyses using additional microsatellite loci (Spencer et al. 2007) and markers such as mitochondrial DNA in order to increase data resolution and further define connectedness, genetic boundaries and conservation units within species. For instance, it may sometimes be difficult to correctly identify dispersers when genetic variation is high and not significantly different among populations, because dispersers are unlikely to be very different to resident animals. Identifying dispersers may be challenging, but such data are valuable to our understanding of population dynamics and demography. Therefore, a larger number of markers may provide the resolution needed to make more accurate distinctions and ensure that threatened populations are not more isolated than expected based on the results of this study. Mitochondrial DNA, being maternally inherited, may also be used to examine patterns of sex-biased dispersal that may be difficult to study by other means.

5) Compare current eastern quoll populations to extinct mainland specimens from museum collections in order to examine genetic subdivisions within this species using microsatellite and mitochondrial DNA markers. Such data will become important if translocations from Tasmania to mainland Australia are to be attempted, because genetic incompatibilities may occur if previously undetected genetically distinct eastern quoll populations are still in existence on the Australian mainland. However, outbreeding depression may be outweighed by the advantages of establishing sustainable populations in areas of the species’ former range.

In conclusion, the data presented in this thesis were useful in providing invaluable information to help guide the conservation of the four Australian quoll species. Ongoing monitoring and management of these threatened species will benefit their future survival and help ensure that they continue to function as keystone species crucial to ecosystem health.
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Appendix 1

Genetic diversity values for five large Australian carnivorous marsupials; *Dasyurus hallucatus*, northern quoll; *Dasyurus geoffroii*, western quoll (chuditch); *Dasyurus viverrinus*, eastern quoll; *Dasyurus maculatus*, spotted-tailed quoll; *Sarcophilus harrisii*, Tasmanian devil; *N*, sample size; *A*, total number of alleles; *NA*, mean number of alleles/locus; *He*, expected heterozygosity; *FST*, genetic differentiation amongst populations.

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<td><em>Dasyurus viverrinus</em></td>
<td>Firestone et al. 2000</td>
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<td>0.567</td>
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<tr>
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<td>Firestone et al. 2000</td>
<td>93</td>
<td>6</td>
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<td>3.97</td>
<td>0.567</td>
<td>3</td>
<td>0.136</td>
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<tr>
<td><em>Dasyurus viverrinus</em></td>
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<tr>
<td>- Aus mainland</td>
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</table>
Appendix 2

Supplementary data for chapter 3; A. Chuditch held in captivity between 1986 and 2001; Information taken from breeding records held at Perth Zoo (SPARKS; G. Gaikhorst, pers. comm.). Arrows indicate translocation events; B. Effective population size (Ne) estimates, along with lower (-CI) and upper (+CI) confidence limits obtained for chuditch populations using three single-sample methods; translocated populations shown in bold; populations with the signature of a genetic bottleneck are marked with an asterisk.

A

![Graph showing the number of animals caught and released between 1986 and 2001.]

B

<table>
<thead>
<tr>
<th>Population</th>
<th>NeEstimator</th>
<th>LDNe</th>
<th>OneSamp</th>
<th>Mean</th>
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<tbody>
<tr>
<td>Batalling State Forest (BTG) *</td>
<td></td>
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<tr>
<td>Bindoon (BDN)</td>
<td>20</td>
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<tr>
<td>Kalbarri National Park (KBI)</td>
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<td></td>
<td></td>
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</table>

Notes: NeEstimator, LDNe, OneSamp, Mean represent Ne estimates using different methods.
Appendix 3

Supplementary data for chapter 4;

A. Effective population size ($N_e$) estimates, along with lower (-CI) and upper (+CI) confidence limits obtained for eastern quoll populations using three single-sample methods; populations with the signature of a genetic bottleneck are marked with an asterisk;

B. Proportion of individuals assigned to eastern quoll populations estimated using GENECLASS2 (Piry et al. 2004). N, sample size; the last column shows the proportion of individuals from a given population that failed to be assigned; assignments to source populations are shaded. Significant cross-assignments to other populations with a proportion > 0.9 are shown in bold.

### A

<table>
<thead>
<tr>
<th>Population</th>
<th>NeEstimator</th>
<th>LDNe</th>
<th>OneSamp</th>
<th>Mean</th>
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</thead>
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<td>41.3 51.5</td>
<td>17.0 13.3</td>
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<tr>
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### B

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<th>MFD</th>
<th>FCN</th>
<th>BTP</th>
<th>CDL</th>
<th>VBV</th>
<th>MTA</th>
<th>MWM</th>
<th>GLD</th>
<th>unass.</th>
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<td>1.00</td>
<td>0.98</td>
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<td>1.00</td>
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<td>0.30</td>
<td>0.60</td>
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<td>0.86</td>
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<td>0.94</td>
<td>0.20</td>
<td>0.43</td>
<td>1.00</td>
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<td>0.64</td>
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<td>0.11</td>
<td>0.49</td>
<td>0.89</td>
<td>0.78</td>
<td>0.98</td>
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Appendix 4

Baseline genetic data for spotted-tailed quolls (*Dasyurus maculatus*) obtained from 18 locations in New South Wales.

These baseline genetic data are presented here to serve as a reference for future studies of spotted-tailed quoll populations. Within their distribution in New South Wales (A5-1), genetic variation was moderate, but significantly lower in the most southern population examined (A5-2). Isolation by distance was significant (A5-6), with genetic differentiation greatest between the southern populations and the other sampled locations (A5-4, A5-5 and A5-8). Estimates of effective population size were inconclusive, particularly for populations where sample sizes were low (A5-3). The high levels of genetic admixture detected amongst most populations (A5-7) may have had an effect on the estimation of effective population size.
A4-1. Spotted-tailed quoll (*Dasyurus maculatus*) populations analysed in this study; A. Sample names (abbreviations), *N*, sample size; and B. Geographic location of populations; sample distribution ranged from southern Queensland (Cherribah) to southern New South Wales (Byadbo).

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean Lat/Long</th>
<th>Sample Years</th>
<th>N</th>
</tr>
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<tbody>
<tr>
<td>Cherribah (CBA)</td>
<td>28° 26' 15&quot;/146° 6' 4&quot;</td>
<td>2004-2005</td>
<td>21</td>
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<tr>
<td>Boonoo Boonoo National Park (BOO)</td>
<td>28° 50' 38&quot;/146° 10' 10&quot;</td>
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<td>22</td>
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<td>Currawong/Torrington (CUR)</td>
<td>29° 11' 12&quot;/145° 54' 15&quot;</td>
<td>2006</td>
<td>13</td>
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<tr>
<td>Washpool National Park (WSP)</td>
<td>29° 26' 59&quot;/146° 11' 7&quot;</td>
<td>2002-2003, 2005</td>
<td>22</td>
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<tr>
<td>Capoompeta National Park (CAP)</td>
<td>29° 22' 27&quot;/146° 1' 14&quot;</td>
<td>2003, 2006</td>
<td>51</td>
</tr>
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<td>Butterleaf National Park (BTL)</td>
<td>29° 31' 4&quot;/146° 0' 45&quot;</td>
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<td>29° 17' 5&quot;/146° 8' 11&quot;</td>
<td>2003</td>
<td>6</td>
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<tr>
<td>Guy Fawkes River National Park (GFK)</td>
<td>29° 53' 59&quot;/146° 10' 27&quot;</td>
<td>2003</td>
<td>9</td>
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<tr>
<td>Marengo State Forest (MRG)</td>
<td>30° 6' 46&quot;/146° 23' 32&quot;</td>
<td>2003</td>
<td>53</td>
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<tr>
<td>Styx River State Forest (STR)</td>
<td>30° 33' 32&quot;/146° 14' 4&quot;</td>
<td>2005</td>
<td>22</td>
</tr>
<tr>
<td>Cunnawarra National Park (CUN)</td>
<td>30° 36' 8&quot;/146° 19' 1&quot;</td>
<td>2001</td>
<td>15</td>
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<tr>
<td>Werrikimbe National Park (WKB)</td>
<td>31° 14' 58&quot;/146° 9' 28&quot;</td>
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<tr>
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<td>31° 29' 26&quot;/145° 20' 27&quot;</td>
<td>2003</td>
<td>8</td>
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<tr>
<td>Tuggolo State Forest (TGL)</td>
<td>31° 32' 37&quot;/145° 31' 28&quot;</td>
<td>2003-2006</td>
<td>78</td>
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<td>Barrington region (BTN)</td>
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<tr>
<td>Gosford region (GFD)</td>
<td>33° 25' 34&quot;/145° 12' 13&quot;</td>
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</tr>
<tr>
<td>Byadbo Wilderness (BDB)</td>
<td>36° 42' 55&quot;/148° 26' 6&quot;</td>
<td>2002-2006</td>
<td>67</td>
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</tbody>
</table>

B

![Map showing the geographic location of populations](image)
A4-2. Genetic diversity values for each spotted-tailed quoll population were estimated at six microsatellite loci using GenAlex 6 and FSTAT 2.9.3.2 (Goudet 1995); \( N \), sample size; \( P \), number of polymorphic loci; \( A \), total number of alleles; \( AR \), allelic richness standardized for sample size; \( rA \), rare alleles (frequency < 0.05); \( uA \), unique (private) alleles; \( He \) and \( Ho \), expected and observed heterozygosity; \( Fis \), inbreeding coefficient; Average values reported ± 95% confidence intervals; Estimates found to be significantly different (\( p<0.05 \) after Bonferoni correction; pairwise Wilcoxon sign rank test) from other populations are shown in bold; populations with the signature of a genetic bottleneck are marked with an asterisk.

<table>
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<tr>
<th>Population</th>
<th>N</th>
<th>P</th>
<th>A</th>
<th>AR</th>
<th>uA</th>
<th>rA</th>
<th>He</th>
<th>Ho</th>
<th>Fis</th>
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<tr>
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<td>6</td>
<td>26</td>
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<td>0.182±0.022</td>
<td>0.594±0.077</td>
<td>0.656±0.097</td>
<td>-0.079±0.112</td>
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<td>BOO</td>
<td>22</td>
<td>6</td>
<td>28</td>
<td>3.39±1.01</td>
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<td>0.614±0.145</td>
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<td>0.637±0.166</td>
<td>0.023±0.186</td>
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<td>0.526±0.164</td>
<td>0.535±0.205</td>
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A4-3. Effective population size \((Ne)\) estimates for each spotted-tailed quoll population were obtained using three single sample methods; linkage disequilibrium methods of Hill (1981) implemented in the program LDNe (Waples and Do 2008) and NeEstimator 1.3 (Peel et al. 2004), and an approximate Bayesian computation method implemented in the program ONeSAMP (Tallmon et al. 2008); \(N\), sample size; populations showing the signature of a genetic bottleneck are marked with an asterisk; lower (-CI) and upper (+CI) confidence limits are shown.

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A4-4. Genetic differentiation among spotted-tailed quoll populations based on pairwise $F_{ST}$ (Weir and Cockerham 1984). Significant values ($p=0.001$) are shown in bold.

|       | CBA | BOO | CUR | WSP | CAP | BTL | SPB | GFK | MRG | STR | CUN | WKB | TMA | TGL | BTN | GFD | RTN |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| CBA   | 0.000 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| BOO   | 0.003  | 0.000 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| CUR   | 0.020  | 0.000  | 0.000 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| WSP   | 0.192  | 0.154  | 0.058  | 0.000 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| CAP   | 0.060  | 0.041  | 0.000  | 0.087  | 0.000 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| BTL   | 0.144  | 0.119  | 0.022  | 0.000  | 0.053  | 0.000 |     |     |     |     |     |     |     |     |     |     |     |     |
| SPB   | 0.089  | 0.065  | 0.006  | 0.060  | 0.050  | 0.013  | 0.000 |     |     |     |     |     |     |     |     |     |     |     |
| GFK   | 0.095  | 0.072  | 0.006  | 0.044  | 0.032  | 0.026  | 0.000  | 0.000 |     |     |     |     |     |     |     |     |     |     |
| MRG   | 0.087  | 0.080  | 0.035  | 0.083  | 0.065  | 0.066  | 0.025  | 0.011  | 0.000 |     |     |     |     |     |     |     |     |     |
| STR   | 0.166  | 0.136  | 0.056  | 0.067  | 0.077  | 0.050  | 0.065  | 0.030  | 0.037  | 0.000 |     |     |     |     |     |     |     |     |
| CUN   | 0.147  | 0.109  | 0.042  | 0.086  | 0.079  | 0.068  | 0.049  | 0.017  | 0.043  | 0.024  | 0.000 |     |     |     |     |     |     |     |
| WKB   | 0.164  | 0.131  | 0.061  | 0.062  | 0.090  | 0.051  | 0.049  | 0.017  | 0.038  | 0.014  | 0.017  | 0.000 |     |     |     |     |     |     |
| TMA   | 0.150  | 0.144  | 0.090  | 0.144  | 0.146  | 0.086  | 0.108  | 0.080  | 0.076  | 0.091  | 0.097  | 0.085  | 0.000 |     |     |     |     |     |
| TGL   | 0.116  | 0.104  | 0.042  | 0.069  | 0.078  | 0.037  | 0.040  | 0.008  | 0.033  | 0.040  | 0.045  | 0.021  | 0.046  | 0.000 |     |     |     |
| BTN   | 0.070  | 0.077  | 0.074  | 0.198  | 0.116  | 0.150  | 0.081  | 0.067  | 0.081  | 0.120  | 0.116  | 0.127  | 0.132  | 0.101  | 0.000 |     |     |
| GFD   | 0.116  | 0.118  | 0.084  | 0.235  | 0.138  | 0.180  | 0.152  | 0.098  | 0.104  | 0.178  | 0.123  | 0.155  | 0.152  | 0.126  | 0.092  | 0.000 |     |
| RTN   | 0.254  | 0.279  | 0.268  | 0.364  | 0.330  | 0.322  | 0.268  | 0.272  | 0.241  | 0.317  | 0.262  | 0.259  | 0.259  | 0.210  | 0.187  | 0.241  | 0.000 |
| BDB   | 0.249  | 0.272  | 0.265  | 0.364  | 0.304  | 0.335  | 0.277  | 0.276  | 0.290  | 0.335  | 0.274  | 0.302  | 0.321  | 0.256  | 0.199  | 0.285  | 0.177 |
A4-5. Pattern of population differentiation ($F_{ST}$) among spotted-tailed quoll populations visualized using Principal Coordinates Analysis (PCA) (Orloci 1978). The axes explain 65.5% of the total genetic variation.
A4-6. Relationship between genetic distance ($F_{ST}$) and geographic distance (km) among spotted-tailed quoll populations was calculated using Mantel tests (Mantel 1967) implemented in GenAlex 6 (Peakall and Smouse 2006).
A4-7. Estimates of gene flow among spotted-tailed quoll populations. Effective number of dispersers/generation \((N_m)\) obtained using Wright’s (1951) island model are shown below the diagonal and \(N_m\) estimated using Slatkin’s (1985) private alleles method above the diagonal. Estimates of \(N_m > 1\) disperser/generation are shown in bold.

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A4-8. Population Structure among spotted-tailed quolls. The proportion of membership of individuals in each sample for each subpopulation cluster was estimated using the Bayesian clustering analysis implemented in STRUCTURE 2.2 (Pritchard et al. 2000); A. Number of subpopulation clusters when $K=2$; B. Number of subpopulation clusters when $K=3$. 

![Diagram A](image1.png)

![Diagram B](image2.png)