Abstract

Using traditional ecological approaches to investigate species biology has often proven difficult, labour intensive and time consuming. Species/population boundaries and patterns of dispersal are often impossible to measure, particularly in shy and cryptic species, whilst inferences on reproductive success and mate choice, as well as relatedness are notoriously difficult and often prone to error. Molecular methods are being increasingly used in ecological research, including macropod research. However, only a limited number of species have been investigated. The investigation of macropod ecology at the molecular level is capable of overcoming some of the restrictions associated with more traditional techniques. Similarly the use of genetic techniques allows new methods of sample collection, including remote and non invasive sampling. The application of molecular genetic data, particularly when used in conjunction with information obtained via direct methods produces a powerful tool capable of elucidating several facets of species biology and ecology. Furthermore, its application to parentage and relatedness, population structure and dispersal patterns, as well as population size is providing new insights into macropod biology and ecology.

Introduction

Population or species boundaries, sociality, population size, dispersal patterns and reproduction are all fundamental aspects of macropod research. Traditionally these have been investigated via morphometric, captive and field based studies or less sensitive genetic methods. Research at the molecular level offers new insights into the population biology and ecology of macropods, whilst overcoming some of the limitations associated with more traditional techniques. Yet, relatively little genetic information has so far been incorporated into macropod research or management.

Direct observation and traditional ecological techniques are often limited in time and space and as a result may overlook important facets of population structure. These studies only provide a contemporary 'snapshot' (Moritz and Lavery, 1996), incapable of examining historic events and only rarely encompassing stochastic events, both of which may have long lasting effects on the structure of the species. Furthermore, these studies are typically confined to a small part of the distribution or community and conclusions based on a single site may not be directly transferable to other areas of the species distribution, particularly if the distribution encompasses environmental variation. In these instances molecular methodologies may prove beneficial as they are capable of investigating across multiple spatial scales, from individual to distributional variation, as well as temporal scales both historic and contemporary. Moreover, traditional ecological approaches are often limited in the number and type (sex or age class) of individuals investigated due to practical considerations associated with the capture of animals for the fitting of transmitters or tags. However, molecular approaches have expanded the methods available for the collection of data, many of which no longer require the capture of animals (Piggott and Taylor, 2003a; Piggott and Taylor, 2003b; Taberlet *et al.*, 1999).

Genetic studies provide a complimentary investigatory technique, capable of reducing some of the limitations associated with more traditional methodologies. Applications include estimates of population size and genetic diversity, the delineation of population and species boundaries and the examination of parentage, mating systems and group formation. Furthermore, the results have practical

applications in the identification of appropriate units and scales for management and monitoring, as well as the resolution of taxonomic uncertainties. Relatively few investigations of macropods have incorporated genetic analyses compared with the ecological foundation on which current knowledge is based. Herein the genetic techniques currently available and their recent application to macropods are reviewed.

Molecular Genetic Techniques

Molecular genetic techniques assess variation present in the DNA sequences within the genomes of individual organisms. Variants of heritable traits (i.e. alleles), which are assessed via specific genetic markers, exist across species, populations and within individuals. The fate of these variants across time and space is influenced by several factors including reproductive success, migration, population size and natural selection, as well as historical and stochastic events. Hence DNA sequences provide a traceable history of an individual, population or species, which molecular methodologies aid in unravelling.

Three levels of molecular change, genotypic, genic and genealogical assess this genetic variation at different evolutionary and geographic levels and scales (Sunnucks, 2000). The most sensitive of these levels are the genotypic arrays, a composite of genotypes from multiple loci in an individual, such as multiple microsatellite loci. This level investigates fine scale structure and individual relationships, such as parentage, movements or the relatedness of interacting individuals. The next level, genic data makes use of haplotypic and/or allelic frequencies from multiple loci (mtDNA or nuclear) assessing the variation on a broader geographic scale, that of populations. Finally, gene genealogies are comprised of sequence data, either mtDNA or nuclear. The variation at this level allows historic and longer term processes such as systematics, phylogeography and evolution to be investigated. The comparison of how this genetic variation changes across both time and space and correlates with features in the landscape, environmental variables including topography, rainfall and climate as well as dispersal abilities and behaviour creates a powerful tool.

Genetics in Macropods

The macropods encompass a variety of species ranging from small hare wallabies (1kg) to the large kangaroos (reaching 90kg) and exhibit an array of dispersal capabilities and mating systems (Strahan, 1995). Furthermore these species inhabit a variety of different ecological niches and habitats. However, compared with many other mammalian groups and despite being one of the largest groups of marsupials (~62 species) relatively few genetic studies exist.

The preponderance of genetic studies in macropods has been conducted on the rock-wallabies. In these species genetic analyses have integrated the study of mating systems (Hazlitt *et al.*, 2006; Spencer *et al.*, 1998), population structure (Eldridge, 1997; Piggott *et al.*, 2006b; Pope *et al.*, 1996), species boundaries (Eldridge and Close, 1992; Eldridge *et al.*, 2001b), sociality and relatedness (Hazlitt *et al.*, 2004), hybridisation (Bee and Close, 1993; Briscoe *et al.*, 1982; Eldridge and Close, 1992), dispersal and the source of migrant individuals (Eldridge *et al.*, 2001a), the levels of genetic diversity in both island (Eldridge *et al.*, 1999; Eldridge *et al.*, 2004a) and remnant mainland (Eldridge *et al.*, 2004b; Spencer *et al.*, 1997) populations, as well as estimates of census size via remote sampling (Piggott *et al.*, 2006a). The results of these studies have dramatically increased the understanding of several rock-wallaby species and provided information for management regimes, allowing the identification of several new species (Eldridge and Close, 1992; Eldridge *et al.*, 2001b).

The studies in rock-wallabies as well as in other small macropods (e.g. the rufous bettong, Pope *et al.*, 2005; and potoroos, Johnston *et al.*, 1984) have proven crucial to our understanding of species biology as they are typically cryptic and difficult to observe. However, while potentially equally informative, molecular studies of the larger macropods, such as the kangaroos where a large ecological knowledge base exists, remain limited. For instance only in the eastern grey kangaroo, *Macropus giganteus* (Zenger *et al.*, 2003a) and the red kangaroo, *Macropus rufus* (Clegg *et al.*, 1998) have studies been conducted to examine phylogeography and population structure. Many aspects of sociality, hybridisation and mating systems have not yet been investigated via molecular techniques and remain uncertain.

The range of species present within the macropod group exhibits variation for ecological and behavioural traits, such as population structure and mating systems. However, the limited number of species examined thus far fails to adequately sample this variation and many characteristics remain unexplored. Therefore, the macropod group presents an opportunity to increase our understanding of the evolution of various traits in mammals.

Genetic tools facilitating macropod research

Over the past few decades there have been several technological advances which have permitted the increased use of molecular methodologies. The advent of the polymerase chain reaction (PCR) in particular has been crucial to the increased application of genetic techniques as it allows the amplification of specific regions of DNA in usable concentrations. This has resulted in the development of suits of PCR-based genetic markers for both hypervariable regions, such as microsatellites, capable of individual identification (Goldstein *et al.*, 1999; Jarne and Lagoda, 1996), and evolutionary conserved regions enabling examination of longer term processes, including evolutionary relationships (Fumagalli *et al.*, 1999; Janke *et al.*, 1994; Simon *et al.*, 1994). The use of rapid and cost effective screens, including single strand conformational polymorphism (SSCP, Sunnucks *et al.*, 2000) and examination of single nucleotide polymorphisms (SNP, Morin *et al.*, 2004) has greatly improved the convenience with which genetic techniques could assay large sample sizes. Similarly the relative ease with which routine DNA sequencing can now be performed has further enhanced the application of molecular techniques (Sunnucks, 2000).

Sample Collection

In addition to samples taken during capture a vast array of techniques for the collection of genetic data exists. The advent of PCR significantly expanded the methods available for the collection of genetic data, as usable concentrations of DNA may now be extracted from a variety of sources including hair and even potentially degraded samples. The commercial harvest of large macropods such as

kangaroos may be used to facilitate broad distributional sampling strategies. Similarly tissue obtained from carcasses (e.g. road kills) or museum specimens greatly expands the potential sample sizes and sampling localities. Furthermore, in these large species specific individuals may be targeted without capture via the use of biopsy darts.

However, it is the application of non-invasive or remote sampling techniques, such as hair and scat collection which provide the greatest benefits (Goossens *et al.*, 1998; Kohn *et al.*, 1999; Sloane *et al.*, 2000), particularly among the many shy and cryptic macropod species (Piggott *et al.*, 2006a; Piggott and Taylor, 2003a; Piggott and Taylor, 2003b). Naturally with degraded samples some difficulties may be encountered, such as genotyping errors resulting from allelic drop out, the failure of PCR to amplify all alleles (Taberlet *et al.*, 1999). However, this may be overcome by multiple PCR amplifications (a minimum of 3), while methodologies employed in storing and preserving collected samples also increases the amount of DNA recovered, greatly improving the usability of degraded samples by reducing potential errors (Piggott and Taylor, 2003a)

Molecular Markers

An array of molecular markers suitable for assessing genetic variation at various levels and scales is available. The choice of genetic marker is dictated by the questions being addressed, sensitivity required and the geographic or evolutionary scale/level under investigation. Markers with differing mutation rates examine diversity on different time scales and hence may result in potentially contradictory results. For example in *M. rufus*, high long-term gene flow across the range is evident at slow mutating markers (e.g. mtDNA), while structuring is evident at markers with high mutation rates, resulting from transient population sub-divisions occurring at a local level.

Among the most commonly used markers are the highly variable microsatellite markers as they are capable of individual identification in addition to population level studies. Successful cross-species amplification in macropods means that most studies could successfully make use of the suite of already existing markers,

particularly since markers for a variety of species are already available (Zenger *et al.*, 2003b). While microsatellite loci are used in the examination of contemporary aspects, mtDNA sequences, with a slower mutation rate are suitable for examining historic relationships. Sequence analysis allows the reconstruction of phylogenetic relationships as well as detecting the occurrence of expansion and vicariance events within species. Not only is sequence data capable of elucidating the relationships between taxonomic units within species but also the evolutionary relationships between species.

In many macropods, as it is in many mammals, dispersal is male biased (Johnson, 1989). Thus, the sexes differentially influence genetic structure and markers suitable to investigate these differences also exist. In addition to the bi-parentally inherited makers (e.g. microsatellites) sex-specific markers are also accessible. Maternally inherited, mtDNA allows female dispersal to be inferred and maternal lineages to be traced. Conversely the paternally inherited, Y-linked markers provide information on male specific traits such as dispersal and paternity (Petit *et al.*, 2002). Recently Y-linked macropod microsatellite markers were isolated in *M. eugenii* and like nuclear microsatellites, some cross species amplifications are successful (Macdonald *et al.*, 2006)

Statistical Analysis

An increasingly sophisticated array of statistical methods, theoretical models and computer software capable of analysing genetic data exists. The assessment of genetic variation is often a complicated process, some methods are akin to ecological statistics, analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) for example but numerous additional methods are available for different aspects. Many of the methods of analysis have been reviewed in detail elsewhere (see Luikart and England, 1999; Luikart *et al.*, 1998; Manel *et al.*, 2005; Manel *et al.*, 2003; Prugnolle and de Meeus, 2002) and only a brief overview of some of the techniques commonly employed is provided here.

Within populations, genotypes from multiple loci allow the levels of genetic variation to be examined and the extent to which alleles are shared between

individuals. From this information the levels of relatedness between associated individuals (R, Queller and Goodnight, 1989) and inbreeding (Frankham, 1995) may be calculated. The effective population size, N_e as well as the occurrence of recent and historic bottlenecks may be assessed using this information (Cornuet and Luikart, 1996). Furthermore since offspring inherit an allele from each parent at nuclear loci (excluding Y-linked) parentage may also be inferred (Queller and Goodnight, 1989).

Spatial structuring and examination of dispersal and gene flow facilitate our understanding of how geographical and environmental features influence genetic variation at both the population and individual levels. Techniques range from measures of differentiation such as Wright's F_{ST}, which reflect the extent of gene flow between sampled localities, to assignment methods, which indicate the probability the individuals originated in the sampled population (Manel *et al.*, 2003). Bayesian clustering methods allow the identification of genetic units within species, whether the distribution is continuous or disjunct (Pritchard *et al.*, 2000). Correlation with geographic distance and genetic differentiation may be obtained via linear regression, Mantel's test or on a finer scale, spatial autocorrelation (Bertorelle and Barbujani, 1995; Peakall and Smouse, 2006). However, the interaction between dispersing individuals and the environment may in some instance be far more complex, correlating with other environmental variables. Under these circumstances rather than testing for direct geographic distance between populations, partial Mantel's tests allow other variables to be examined. For example, population structure may be determined by individuals dispersing along mountain ranges, rather than descending to lower altitudes and crossing between ranges.

The examination of sequence data (typically mtDNA) which examines historical relationships requires a different set of statistical tools. These relationships may be examined via the construction of evolutionary trees (e.g. PHYLIP, Felsenstien, 1989; MEGA, Kumar *et al.*, 2004) or, additionally within species via haplotype networks (e.g. TCS, Clement *et al.*, 2000). Historical events, such as vicariance events are also often apparent within these trees. However, other methods of analysis, such as mismatch analysis can elucidate historic expansion events and

demography (Excoffier, 2004; Schneider *et al.*, 2000). Finally nested clade analyses (NCA) are available for determining the geographical relationship between sampled localities (e.g. GEODIS, Posada *et al.*, 2000; Templeton, 2004) in addition to the previous mentioned correlation methods.

Application of genetic information to macropods

The combination of genetic data with information on behaviour, environmental and climatic variables allows the influence of these variables on the population to be inferred. Although only relatively few macropods have been examined via molecular techniques, the studies which have been conducted encompass a variety of facets including population size, parentage and mating systems and population structure as well as speciation and hybridisation.

Population Size and Genetic Diversity

Genetic diversity and population size are important facets of conservation biology, as they have been shown to influence fitness and the risk of extinction (Frankham, 1995). The effective population size, N_e is typically less than the census size as not all individual reproduce, contributing to the diversity in successive generations. Populations may suffer reductions in population size and genetic diversity for many reasons, including the impact of humans. Hunting and habitat modification by humans may dramatically reduce population sizes, resulting in a reduction in genetic diversity (Eldridge *et al.*, 2004b). Similarly, island populations are predicted to suffer the effects of inbreeding and small population size as they are either initially colonised by small groups or are incapable of sustaining large long term effective population sizes (Frankham, 1998). This erosion of genetic diversity is evident in many island macropods (Eldridge *et al.*, 2004a), for instance the black-footed rock-wallaby (*Petrogale lateralis*) which displays the lowest levels of genetic diversity reported in a wild marsupial population (Eldridge *et al.*, 1999).

The heritable traits which genetic markers represent may be used as a tagging method, akin to physical tags and markings used to traditionally census populations. This technique is particularly useful in species, such as the shy and cryptic

macropods that are difficult to observe or tag (Kohn *et al.*, 1999). The use of molecular tags are similar to ecological mark-recapture techniques which take a sample from the population and then use the number of times these marked individuals occur in subsequent samples relative to unmarked animals. This system has proven particularly useful in cryptic rock-wallaby populations when combined with non-invasive sampling techniques, such as faecal DNA as a tool for monitoring population sizes (Piggott *et al.*, 2006a).

Parentage and Relatedness

Previously mate choice and parentage were based on the observation of mating behaviours and copulation or associated with territory and social bonds. Mating systems within the macropods appear to range from promiscuous to monogamous. However, only three molecular studies of macropod species exist. Allied rock wallabies, *Petrogale assimilis* for instance appear socially monogamous, forming strong, long term bonds. However, some females appear to solicit extra-pair copulations, with genetic data revealing the social partner was not always the father of her offspring (Spencer *et al.*, 1998). Hence females appeared to maximise overall lifetime reproductive fitness with young more likely to be fathered by another male if the previous young, fathered by her social partner failed to survive to maturity. Conversely in the rufous bettong, *Aepyprymnus rufescens* there were indications the system was not purely promiscuous, with low variation between males' success and roughly half of females mating with the same male as in the previous breeding season (Pope *et al.*, 2005). These results have implication on how we view not only these species but the broader evolution of mating systems. Furthermore, with accurate assessment of parentage, new lines of investigation into the influence of genetic factors such as the major histo-compatibility complex (MHC), olfactory cues as well as physiological or social rank may have on mate choice are opened.

The forming of social groups in many macropods is often presumed to reflect relatedness, particularly among females (Jarman and Bayne, 1997; Johnson, 1986; Laws and Goldizen, 2003). The sharing of home ranges between mother and offspring, particularly female offspring has been documented in several macropod

species (Johnson, 1989), although in larger macropods associations may be temporary (Arnold *et al.*, 1990). In rock-wallabies, for instance females exhibit philopatry and there is distinct spatial clustering among closely related females (Hazlitt *et al.*, 2004). The ability to elucidate the degree of relatedness between individuals coupled with information on behaviour may allow further investigation of the complex sociality observed in captive macropods (Blumstein *et al.*, 2002).

Contemporary Population Structure

Given the range of species within macropods, from small 1kg hare-wallabies to large 60+ kg kangaroos, it is not surprising that a similarly degree of variation in genetic structuring is also apparent. In the large, highly mobile macropods only weak genetic structuring is evident (Clegg *et al.*, 1998; Zenger *et al.*, 2003a). However, even within these highly mobile species, distinct genetic units may be detected (Zenger *et al.*, 2003a). In species with specific habitat requirement, discontinuities in the environment may impede the free movement of dispersing individuals, resulting in high levels of structuring within a species. For example, rock-wallaby species, which are largely restricted to rocky outcrops, have proven to be among the most highly structured mammalian taxa, with colonies often representing distinct units for management (Browning *et al.*, 2001; Eldridge *et al.*, 2001a; Hazlitt *et al.*, 2004; Piggott *et al.*, 2006b; Pope *et al.*, 1996).

Not all species exhibit distinct units and dispersal in many species correlates with geographic distance, as dispersal abilities are typically less than the distributional range (Pope *et al.*, 2005). Furthermore, these patterns may be exhibited within colonies or populations (Hazlitt *et al.*, 2004). In addition to geographical distance other environmental variants have been shown to influence the scale of structuring across the distribution. In *M. rufus* for instance the geographical scale of genetic structuring is associated with variation in rainfall and topographic complexity (Clegg *et al.*, 1998). Furthermore, although not thoroughly examined, this provides some support to the hypothesis that river systems provide a conduit for migration in species which inhabit drought prone areas (Norbury *et al.*, 1994).

Most macropod species are hypothesised to exhibit male biased dispersal (Johnson, 1989) and this is evident in many of the species so far examined, including *A. rufescens* (Pope *et al.*, 2005), *Petrogale penicillata* (Hazlitt *et al.*, 2004; Piggott *et al.*, 2006b) and *M. giganteus* (Zenger *et al.*, 2003a). The inability to distinguish sex biased dispersal using the relatedness within populations in at least one instance (*M. giganteus*) may reflect differences in the reproductive success of dispersing sexes and hence requires further investigation.

Historical Population Structure

Historical or rare stochastic events may have long lasting effects on the population structure of a species, which may be elucidated via genetic analyses. The effects of rainforest contractions in northern Queensland, due to the climatic instability of the Pleistocene are evident in the northern bettong (*Bettongia tropica*) for instance, with a northern and southern lineage present (Pope *et al.*, 2000). These lineages are separated geographically by a mountainous region known as the Black Mountain barrier across which rainforest was absent until approximately 8,000 years ago (Kershaw *et al.*, 2003). This same division is also apparent in several other species including invertebrates (Hugall *et al.*, 2002), birds (Joseph and Moritz, 1994; Joseph *et al.*, 1995; Schneider *et al.*, 1998) and lizards (Cunningham and Moritz, 1998; Stuart-Fox *et al.*, 2001). Conversely, species whose current ecology indicates they are less specialised, do not display the same impacts on phylogenetic structure (Joseph and Moritz, 1994).

Speciation and Hybridisation

Speciation and hybridisation are often difficult to identify without the aid of molecular techniques. Morphological variation, for instance, has in several cases proven unreliable in the delineation of taxonomic boundaries, failing to reflect genetic partitioning (e.g. Juste *et al.*, 2003; Moro *et al.*, 1998; Orr, 2001). In taxa which appear morphological similar or are shy and difficult to study using ecological techniques, such as rock-wallabies (Eldridge and Close, 1992; Eldridge *et al.*, 2001b) and potoroos (Seebeck and Johnston, 1980) cryptic species may go

unnoticed. Conversely, other factors, such as environmental variation may influence morphology without significant partitioning of genetic diversity, as is often observed on island populations, *M. giganteus tasmaniensis* for example (Zenger *et al.*, 2003a).

The occurrence of hybridisation and its role in the evolution of vertebrates has only recently become apparent due to increased sensitivity in the techniques for detecting introgressive hybridisation (Seehausen, 2004). Although the inability to hybridise forms the basis of the biological species concept (Dobzanhsky, 1937; Mayr, 1940), it does not necessarily reflect the extent of genetic differences, with species capable of hybridising, displaying similar levels of differentiation to those which are reproductively isolated. Several macropod species have been shown to readily hybridise in captivity, although many display reduced fertility (Close and Lowry, 1990). While relatively few species have been investigated in the wild, several parapatric rock-wallaby species have been shown to hybridise in discreet contact zones (Bee and Close, 1993; Briscoe *et al.*, 1982; Eldridge and Close, 1992) although wild hybrids have also been reported in other groups (Coulson and Coulson, 2001).

Practical Implications: Management and Conservation

As a group the macropods present a dichotomy of management options. The vast majority of species have been disadvantaged by European colonisation, with population declines resulting in 37 species threatened or near threatened, more than half the number of currently recognised macropod species (IUCN, 2006). Human modification of the environment has also resulted in the substantial increases in the population size of many large macropods such as the kangaroos, which may become locally over abundant and problematic (Coulson, 1998; Coulson, 2001; Viggers and Hearn, 2005). Finally many of these kangaroo species are also harvested throughout most of their range as part of a commercial industry for meat and skin. The information produced by genetic analyses, has implications for the management and conservation of macropod species, particularly in addressing the scale at which management and monitoring should occur (Moritz, 1999).

Genetic information has become increasingly important in the resolution of taxonomic uncertainties and setting of conservation priorities. For instance, among the many shy and cryptic species of macropod, genetic analyses revealed the presence of a previously unrecognised species of potoroo (Seebeck and Johnston, 1980) and several rock-wallaby species (Eldridge and Close, 1992; Eldridge *et al.*, 2001b) as well as evidence of evolutionary significant units (ESU) within *P. penicillata* (Browning *et al.*, 2001). The ESU and management unit (MU) were designed to encapsulate the partitioning of genetic diversity and allow conservation to preserve the greatest degree of diversity below the species level. The designation of an ESU has important ramifications for the conservation priorities in many species, as it is recognised in the Australian Endangered Species Protection Act (Moritz, 1994), with similar acts worldwide (Waples, 1991). However, there is still debate in terms of how these units should be defined (for reviews see Crandall *et al.*, 2000; Fraser and Bernatchez, 2001; Goldstein *et al.*, 2000; Moritz, 1994; Moritz, 1999; Paetkau, 1999; Ryder, 1996; Waples, 1991). It is evident, however that both genetic and ecological examinations are important in the management and conservation of species (Crandall *et al.*, 2000).

The determination of genetic structuring also aids in the development of management strategies, identifying how genetic diversity is partitioned across the distribution of a species. This information allows the origin and dispersal route of individuals between sites to be inferred (Eldridge *et al.*, 2001a), an important facet in maintaining gene flow between fragmented populations. Identifying the source of individuals, even of introduced pest populations may also prove important. For example, an introduced population of tammar wallaby, *M. eugenii* in New Zealand proved particularly significant , as it originated from a now extinct Australian population and as such represent a considerable resource to conservation (Taylor and Cooper, 1999).

Six species of macropod have become extinct since European colonisation, due to habitat fragmentation, predation and competition, while at least 10 other species have experienced widespread population declines and extinctions (IUCN, 2006). As a result reintroduction schemes have become an important facet of conservation biology. Ensuring reintroduced populations possess, and are capable of retaining

sufficient genetic diversity for long-term survival is difficult, particularly whist attempting to maintain the natural population structure of the species but crucial to the successful establishment of new populations (Sigg, 2006). Some species of macropods which have become extinct on the mainland, have escaped threatening processes on islands. However, as previously discussed these island populations typically suffer from the erosion of genetic diversity, increasing the risk of extinction (Frankham, 1998; Frankham, 2003). Genetic analyses reveal these 'pristine' populations are not a conservation stronghold for these species and many often smaller mainland populations possess a greater extent of the genetic diversity remaining within the species (Eldridge *et al.*, 2004a). Therefore, while the conservation of the small remaining mainland populations may be difficult the relatively high levels of genetic diversity mean these populations have the greatest chance of survival.

Prospects

The macropods are a highly diverse iconic group, yet relatively few species have been the subject for genetic studies. Therefore there are still many unanswered questions in the majority of species, which may be addressed using current molecular techniques. The population structure, mating system and group formation in kangaroo species for instance remain uncertain as is the occurrence of hybridisation.

Advances continue to alter and expand the potential applications of available genetic techniques. Genomics is becomingly increasingly employed as the number of species with sequenced genomes and 'genome enabled' species, increase (Kohn *et al.*, 2006; Luikart *et al.*, 2003). In addition to expanding the number of neutral markers, typically examined by current techniques, genomics has several advantages. The examination of the DNA sequence data of functional genes increases our ability to investigate aspects of adaptation and evolution. Eventually the genomes of hundreds of species will be sequenced. As of 2006, the examination of the genomes of 75 vertebrate species, which may result in close to 1000 IUCN red listed mammalian species becoming 'genome-enabled' is in progress (Kohn *et al.*, 2006). However, the majority of these species are eutherian mammals and only

two marsupial genomes, the tammar wallaby *M. eugenii* and the American opossum *Monodelphis domestica* are currently under examination. Although *M. eugenii* is an Australian model marsupial species, used widely in a variety of research disciplines the coverage of the genome is comparatively low (Wakefield and Graves, 2005).

Although examination of the macropod genome is limited, the assessment of functional genes, notably at MHC loci (Browning *et al.*, 2004; Siddle *et al.*, 2006) is already in progress. The examination of functional markers allows the extent of variation at loci where selection may be expected to maintain diversity, to be quantified as well as the investigation of adaptive and detrimental genetic variation. Several macropod species on islands have been shown to display significant reductions in diversity at neutral markers, yet little is known if this is reflective of functional regions of the genome. Furthermore, the investigation of certain loci such as the MHC, opens up new lines of investigation at the population level (Seddon and Baverstock, 1999), as this region of the genome has been implicated in disease resistance and mate choice in eutherian mammalian species (Bernatchez and Landry, 2003; Hedrick, 2004; Penn and Potts, 1998; Potts *et al.*, 1994; Tregenza and Wedell, 2000).

Conclusions

The use of molecular techniques to examine and infer various aspects of the biology, ecology and history of macropod species will continue to change and expand. Genetic techniques are capable of providing new insights whilst overcoming some of the difficulties and restrictions associated with more traditional techniques. However, relatively few macropod species have currently been examined and many questions still require investigation. Regardless of whether the macropod species is an obvious feature in the landscape, such as the red kangaroo, or far more cryptic and difficult to study using traditional techniques, genetic analyses are capable of providing new insights. Thus, genetic information is a valuable resource for macropod research, increasing our understanding of macropod ecology, biology and evolution whilst having practical implications for the continued management and conservation of the diverse range of macropod species.

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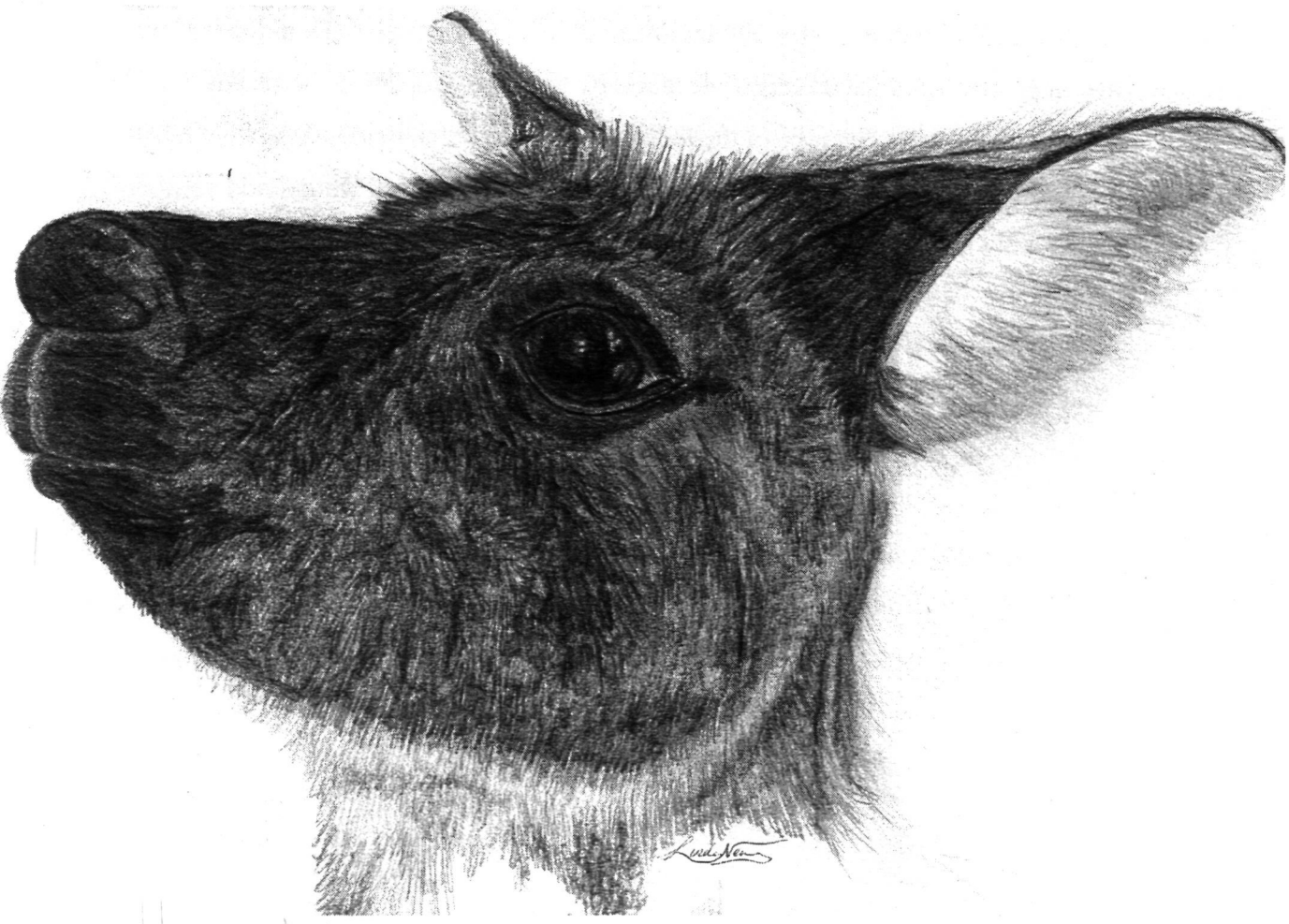
CHAPTER II:

Landscape Discontinuities Influence Gene flow and Genetic Structure in the Western Grey Kangaroo, *Macropus fuliginosus*.



Abstract

Examination at the molecular level has proven useful in inferring the population structure of species, particularly where distributions are large and encompass environmental or morphological variation. The western grey kangaroo, *Macropus fuliginosus* is distributed throughout most of southern Australia and displays substantial morphological variation. To examine genetic structuring, at various spatial scales, and dispersal patterns, a total of 478 individuals from 20 sampling sites throughout the trans-continental distribution were genotyped at 9 highly polymorphic microsatellite loci. Overall genetic diversity is among the highest observed in marsupials. However, the Kangaroo Island population, isolated for 9,500 years, possessed significantly reduced diversity. Five genetically and geographically distinct units, some associated with morphological variants were identified across the range of *M. fuliginosus*. The mountain range and salt lakes of the Lake Torrens/Flinders Range area and the treeless expanse of the Nullarbor Plain appear to influence genetic structuring in *M. fuliginosus* by significantly reducing dispersal and gene flow. However, some admixture is present at the geographical boundaries between mainland units. Only populations in the west of the range displayed the effects of isolation by distance, indicating the recent invasion of the eastern range or the influence of environmental/landscape variables on mobility and dispersal. Only minor differences in dispersal were noted between the sexes, with males responsible for the largest movements.

Introduction

In the past, inferences of population structure in widespread, highly mobile species with continuous distributions have been difficult. This is further confounded when their distribution encompasses a range of environmental conditions or habitats, potentially resulting in local adaptations or morphological variants. Analysis at the molecular level has helped to overcome these difficulties by enabling the delineation of species/population boundaries and providing a means for quantifying the degree of differentiation between units (Manel *et al.*, 2003; Sunnucks, 2000).

Traditionally, insights into dispersal and population structure were acquired via field based ecological techniques. While these techniques provide valuable insights into current demography, dispersal ability and behaviour, limitations restrict the precision with which inferences of population structure can be made, particularly in species with broad distributions (Moritz and Lavery, 1996). The information obtained on dispersal ability, for instance has often been confounded by the effects of environmental variables on mobility (Clegg *et al.*, 1998). Thus, unless multiple studies occur throughout the range, information obtained from one site may not be directly transferable to other regions, particularly when distributions encompass changing landforms, climate and habitat. Technical or practical requirements also usually limit examination to relatively mature individuals, in which natal dispersal may have already occurred. Furthermore, inferences of natal dispersal are difficult with animals often travelling outside the study area and rarely is information on the subsequent reproductive success of dispersing individuals acquired (Bensch *et al.*, 1998; Johnson and Gaines, 1990; Perrin and Mazalov, 1999; Wauters *et al.*, 1994), further confusing inferences of dispersal. Finally, ecological studies examine contemporary features and rarely encompass the rare stochastic events which may have long lasting impacts on population structure. The advent and application of high resolution molecular markers, which allow for individual identification have proven extremely effective and are capable of overcoming some of the limitations associated with past ecological techniques (Moritz and Lavery, 1996; Sunnucks, 2000).

Population genetic structure is largely dictated by the differentiating factors of genetic drift and selection and the counteracting influence of gene flow between populations (Slatkin, 1987). To a large extent genetic structure is dependent on the dispersal abilities of a species and hence distance is naturally expected to influence structure. However, discontinuities in the landscape and geographical barriers are equally likely to have an impact. The use of molecular markers provides an opportunity to quantify the hierarchy of genetic diversity, from localised populations to distributional variation, thus providing insights into the effect of geographic barriers (Carmichael *et al.*, 2001; Kyle and Strobeck, 2002; Paetkau *et al.*, 1999), the rate of dispersal (Waser and Strobeck, 1998) and even on the colonisation history of the species (Perez *et al.*, 2002). This information also has practical implications for the management of species in terms of conservation, effecting harvesting regimes and determining the location of illegally sourced material (Moritz, 1999; Primmer *et al.*, 2000).

A diverse array of patterns and scales of population structure exists across taxa, as each species is differently affected by geographical barriers and distance. The dispersal range of many species is less than the distributional range, often resulting in isolation by distance (IBD) in which population similarities decrease over geographic distance, while geographical barriers impede dispersal creating distinct genetic units (Slatkin, 1987; 1993). However, this may not apply to those species which are highly mobile and capable of dispersing across large distances and substantial geographic barriers. Furthermore, combinations of long and short distance movements by different individuals, often related to sex, may complicate the process of differentiation which typically occurs in less mobile species (Cooper *et al.*, 1995; Lansman *et al.*, 1981; Patton *et al.*, 1994). The large carnivores, such as wolves and bears typically possess a high dispersal potential and hence little structure is generally observed among populations (Forbes and Boyd, 1997; Forbes and Hogg, 1999; Lehman and Wayne, 1991; Roy *et al.*, 1994). Conversely species with more stringent habitat requirements display highly structured and well differentiated populations as observed in rock-wallaby species (Eldridge, 1997; Eldridge *et al.*, 2001; Piggott *et al.*, 2006; Pope *et al.*, 1996), and thin horned sheep (Worely *et al.*, 2004). Many large herbivores typically inhabit large ranges of relatively continuous habitat and display intermediate levels of structuring across

these ranges. The plains ungulates, for instance display moderate to low structure as they are often capable of utilising or migrating through these large tracts of continuous habitat (Broders *et al.*, 1999; Polziehn *et al.*, 2000; Wilson and Strobeck, 1999). Similarly several of the large macropods are characterised by high genetic diversity with relatively little obvious structure even on a continental scale (Clegg *et al.*, 1998; Zenger *et al.*, 2003).

Despite its initial discovery in 1802 (Flinders, 1814), the western grey kangaroo, *Macropus fuliginosus* was only recognised as a distinct species relatively recently by Kirsch and Poole (1967; 1972). Its range stretches continuously across much of southern Australia, from the west coast to almost the Great Dividing Range in the east, reaching as far north as Shark Bay in the west and into south-western Queensland in the east (see Figure II-1). The distributional limits are dictated by the winter dominated annual rainfall, greater than 200mm (Cairns *et al.*, 1991; Caughley *et al.*, 1987). *M. fuliginosus* typically inhabits heath vegetation moving onto open grasslands to feed. Not surprisingly, this transcontinental 3500km wide range encompasses large changes in landforms, climate and habitat, all of which may have a filtering effect on gene flow.

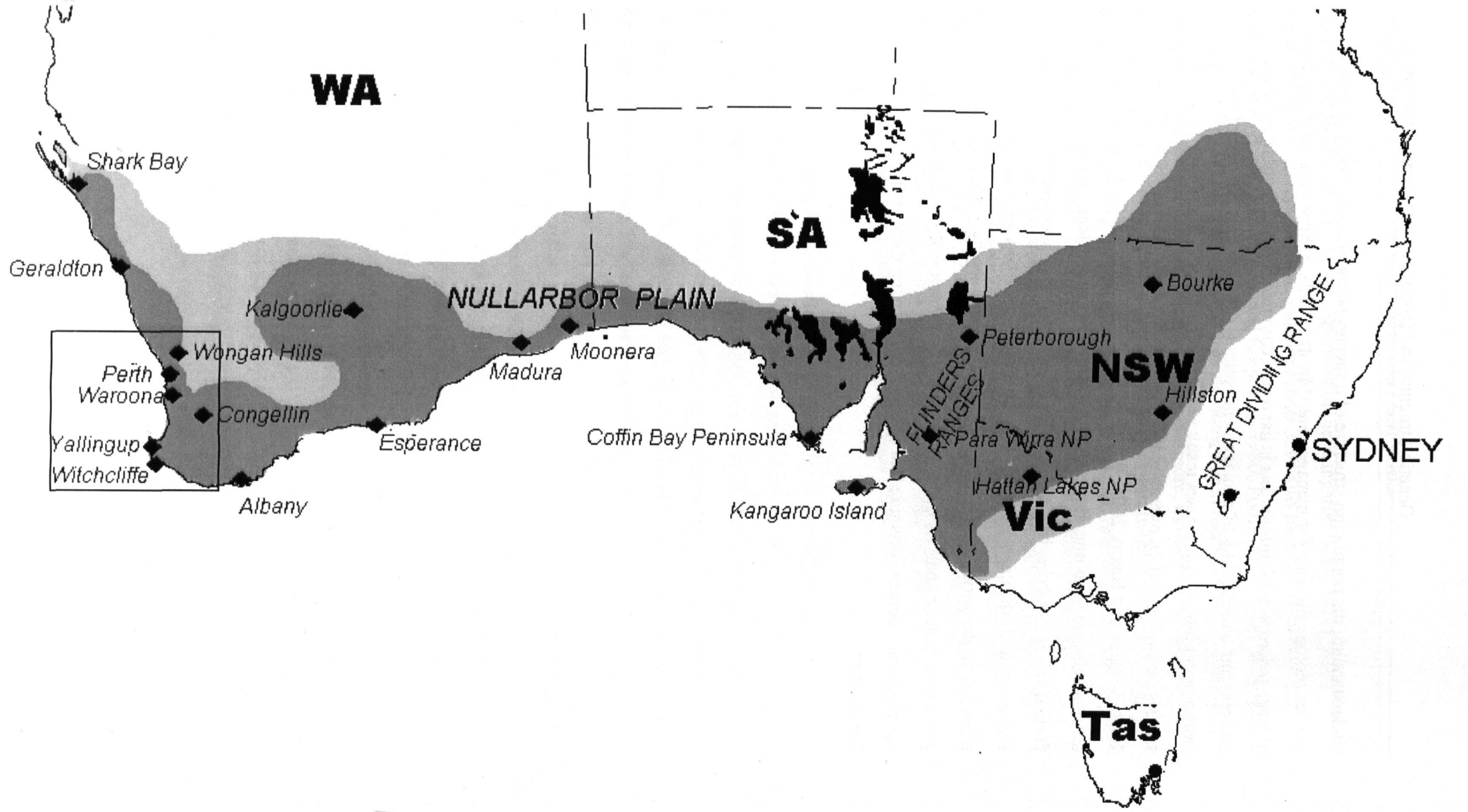
Currently two subspecies are recognised, an insular subspecies, *M. fuliginosus fuliginosus* on Kangaroo Island, South Australia, and the mainland subspecies, *M. fuliginosus melanops* (Kirsch and Poole, 1972). The two subspecies are distinguished morphologically with differences in colouration as well as in cranial and body measurements (Poole *et al.*, 1990). Significant differences are also present among reproductive traits including the timing and length of the oestrus cycle (Poole and Catling, 1974). In addition, differences in the length of pouch life (Poole, 1976) and the period from parturition to next lactation (Poole, 1975) are also apparent. None of these differences, however, present a barrier to reproduction (Poole, 1976).

While less spectacular than the differences between the Kangaroo Island and mainland populations, *M. f. melanops* from the western areas of the range differ morphologically to those from the east. These differences include a tendency to a slighter build in the forequarters and a longer, squarer muzzle in Western Australia

(Kirsch and Poole, 1972). As was observed in the Kangaroo Island populations, some differences in reproductive and other traits have also been noted, though to a lesser degree (Poole, 1975; Poole and Carpenter, 1980; Poole *et al.*, 1990; Poole and Catling, 1974). Whilst these variants had previously been recognised as subspecies, many of the differences are not significant. Furthermore, morphometric analyses failed to identify the presence of sharp discontinuities within the continuous range of this species, indicating the presence of a cline from one form to another (Poole, 1976; Poole *et al.*, 1990). However, while this may be indicative of a single panmictic population, the presence of admixture at the boundaries of distinct units would blur morphological differences resulting in a similar pattern. Despite considerable variation in *M. fuliginosus* for several traits and the availability of genetic markers suitable for assessing population boundaries and levels of admixture, little genetic information on *M. fuliginosus* exists.

Information on dispersal and hence potential gene flow in *M. fuliginosus* is limited, predominately coming from a handful of ecological studies conducted in the eastern parts of the range. Long-term home ranges vary from 221 to 449 hectares (Coulson, 1993), with females and small males occupying smaller ranges than large males (Priddel *et al.*, 1988a). However, the only study conducted in the western region of the range reported vastly smaller home ranges of only 39 to 70 ha (Arnold *et al.*, 1992). These differences may relate to specific attributes of the respective sites. However, they may also reflect differing dispersal tendencies occurring in different parts of the range. As has been found in other field based studies of large macropods (Croft, 1991; Jaremovic and Croft, 1991; Jarman and Taylor, 1983; Priddel *et al.*, 1988a), *M. fuliginosus* appears largely sedentary, displaying high site fidelity (Priddel *et al.*, 1988b). There are, however, reports of the presence of a mobile portion of the population ranging from 2 to 7% comprising of both males and females which display both short (18km) and long (85km) range movements, a pattern similar to that observed in red kangaroos (*Macropus rufus*, Arnold *et al.*, 1992). Distinguishing which movements, if any, represent dispersal is difficult, with some (particularly the shorter) appearing to be associated with travelling between seasonal home ranges (Arnold *et al.*, 1992).

Understanding the population structure of a species is important not only in terms of species biology but also for management (Moritz, 1999). Like several other species of large macropod, *M. fuliginosus* is harvested commercially throughout its range and presents a dichotomy of management options. Human modification of the environment has, in many cases resulted in increases in population sizes, with the species becoming locally overabundant and problematic (Coulson, 1998; Coulson, 2001; Viggers and Hearn, 2005; Wilson, 1991). Determining an appropriate scale for management is an important component of any management strategy, particularly in a species in which such a dichotomy of management options exists. Currently all of the knowledge, on which management plans are based, is largely restricted to the varied results of field based ecological studies. This project provides a genetic analysis of *M. fuliginosus* using microsatellite loci, to examine contemporary genetic structure and dispersal, addressing these gaps in current knowledge.


WA
SA
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Tas
Shark Bay
Geraldton
Kalgoorlie
NULLARBOR PLAIN
Wongan Hills
Perth
Waroona
Congellin
Yallingup
Witchcliffe
Albany
Esperance
Madura
Moonera
Coffin Bay Peninsula
Kangaroo Island
Bourke
Peterborough
FLINDERS RANGES
Para Wirra NP
Hattah Lakes NP
Hillston
GREAT DIVIDING RANGE
SYDNEY

Figure II-1: Geographical distribution and sampling locations of *Macropus fuliginosus* in southern Australia. Light grey shading indicates the distributional limits, whilst the dark grey denotes regions where densities are greater than 0.1 kangaroos per square kilometre (after Caughley, 1987). The sampling localities are indicated by diamonds (refer to Table II-2 for sample sizes). Populations used to examine fine scale structure and dispersal patterns are shown boxed in Western Australia. The large salt lakes (and major tributaries) in South Australia are shown in black.

Materials and Methods

Sampling and DNA extraction

Tissue samples were solicited from commercial harvesters, government agencies involved in control measures and researchers. A total of 20 sites were sampled across the distribution of *M. fuliginosus*, including one population of the insular subspecies *M. f. fuliginosus* from Kangaroo Island, South Australia (Figure II-1). As much as possible sampling was confined to discreet areas (~10,000ha), although several social groups would have been incorporated in each sampling locality. The sampling design was hierarchical, allowing for examination of dispersal and population structure at three scales, local (30-150km), regional (150-1000km) and continental (1000-3200km). Typically, approximately 30 individuals, deemed sufficient to detect structuring (Cornuet *et al.*, 1999) in an equal sex ratio were sampled from each site (see Table II-2). Samples were usually skin tissue, although liver and kidney were used for some individuals. In the field samples were preserved in either 80% ethanol, a 20% DMSO $NaCl_2$ solution (Kilpatrick, 2002) or frozen at -20°C until DNA extraction took place. DNA extraction for all tissue types was carried out according to Sunnucks and Hale (1996) or Sigg *et al.* (2005).

Microsatellite Amplification and Screening

Nine macropod microsatellite loci were used to assess genetic diversity, 5 derived from the tammar wallaby (*Macropus eugenii*, T3-1T, T4-2, T15-1, T19-1, T31-1, T32-1 and T46-5, Zenger and Cooper, 2001a) and one from each of the eastern grey kangaroo (*Macropus giganteus*, G26-4, Zenger and Cooper, 2001b), allied rock-wallaby (*Petrogale assimilis*, Pa595, Spencer *et al.*, 1995) and bridled nailtail wallaby (*Onychogalea fraenata*, B90, Pope *et al.*, 2000). Amplification was via polymerase chain reaction (PCR) in 10 µl reaction volumes containing 100-200ng of genomic DNA, 2.5 mM $MgCl_2$, 16mM $(NH_4)_2SO_4$, 67mM Tris-HCl (pH 8.8) 0.1% Tween 20, 200µM of each dCTP, dGTP and dTTP, 20µl of dATP, 0.05µl of [^{33}P] dATP at 1000Ci/mol (Perkin Elmer), 1µM of each forward and reverse primer and 0.5U *Taq* polymerase (Bioline, Australia). PCR amplification was carried out in an MJ Research PTC100 thermocycler, with an initial 94°C denaturation for 3

minutes, followed by 'touchdown' cycles of 94°C denaturation for 30 seconds, annealing temperatures of 60°C, decreasing in 2°C increments each cycle to 50°C for 45 seconds (1°C increments were used from 60 to 55°C for both T15-1 and B90) and an extension step of 72°C for 1 minute. On completion of the last touchdown cycle another 30 cycles were performed with 50°C annealing temperature (55°C for T15-1 and B90), following which a final extension step of 72°C for 5 minutes occurred. Amplified PCR products were resolved on a 6% denaturing polyacrylamide gel (Sequa-gel 6, Geneworks). An M13 control standard size DNA reference markers was run adjacent to the samples to provide a reference size for microsatellite alleles (USB, T7 Sequenase kit; Amersham). Products were visualised by autoradiography according to Taylor *et al.* (1994).

Sexing PCR

The sex of sampled animals was either determined at the time of sample collection, or via PCR, where sex was determined by the simultaneous use of X (G6PD, Lobel *et al.*, 1995) and Y- linked (SRY, O'Neill *et al.*, 1998) primers. In order to determine if sexing had occurred correctly at the time of collection, the sex of a subset of the sample, 150 kangaroos, was determined both at the time of sampling and via PCR. Amplification occurred in 10 µl reaction volumes containing 100-200ng of genomic DNA, 2.5 mM $MgCl_2$, 16mM $(NH_4)_2SO_4$, 67mM Tris-HCl (pH 8.8) 0.1% Tween 20, 200µM of each dCTP, dGTP and dTTP, dATP, 0.16µM of each G6PD and 0.1µM of each SRY forward and reverse primers and 0.5U *Taq* polymerase (Bioline, Australia). PCR amplification was carried out in an MJ Research PTC100 thermocycler, with an initial 94°C denaturation for 3 minutes, followed by 35 cycles of 94°C denaturation for 30 seconds, annealing temperature of 60°C and an extension step of 72°C for 1 minute. On completion of the last cycle a final extension step of 72°C for 5 minutes took place. Male DNA produced two bands while female DNA only one.

Diversity Indices

The level of genetic polymorphism was measured as allelic diversity (*A*), observed heterozygosity (H_o) and the heterozygosity expected (H_e) from Hardy-Weinberg

equilibrium (HWE, Nei, 1978) using MICROSATELLITE TOOLKIT EXCEL ADD-IN (Park, 2001). Due to variation in some sample sizes allelic richness was also calculated using FSTAT version 2.9.3.2 (Goudet, 2000). Deviations from HWE were tested using 10 000 iterations of the Monte Carlo Marcov Chain (MCMC) simulation implemented by GENEPOP version 3.4 (Raymond and Rousset, 1995) on all loci, populations and globally. Linkage disequilibrium was also assessed using GENEPOP (10000 permutations). F_{IS}, a measure of inbreeding/presence of null alleles was calculated for all loci and populations, jack-knifing across populations or loci respectively via FSTAT.

An analysis of molecular variance (AMOVA) implemented by GENALEX 6 (1000 permutations, Peakall and Smouse, 2006) was used to investigate the significance of genetic partitioning between the two subspecies, *M. f. fuliginosus* and *M. f. melanops* and within *M. f. melanops* between the eastern and western regions of the range (see Table II-2). In addition, a hierarchical analysis of allelic richness between the same groupings was performed in FSTAT (1000 permutations). Sites in which less than 15 animals were sampled were excluded from this analysis

Gene Flow and Dispersal

Fine scale genetic structuring and dispersal was examined at both local and regional scales using two methods. Firstly all possible pairwise relatedness values were calculated according to Queller and Goodnight (1989) in IDENTIX version 1.0 (Belkhir *et al.*, 2002). These values were used to compare within, versus among group relatedness at two levels, male versus female and locally versus regionally. The significance of the comparisons was tested via randomisation, performed by RT version 2.1 (10,000 permutations, Manly, 1997). Secondly an assignment test was used to determine the likelihood of finding one of the observed genotypes in each of the populations. Assignment indices were calculated in GENECLASS version 2.0.g (Piry *et al.*, 2004), from which individuals were assigned to the population to which they had the highest probability of belonging. Values were determined by both direct and simulated analyses (10,000 simulations). To avoid likelihood values of zero, a frequency of 0.01 was assigned to all alleles absent in a population. As with the relatedness values comparisons between the relative assignments of males and

females were made. In addition, corrected assignment indices (AI_C) were calculated according to Fauvre *et al.*, (1997) in order to elucidate patterns of male and female dispersal. The AI_C mitigates population effects, potentially arising from differences in genetic diversity by subtracting population means following log transformations. Hence the AI_C values average zero in a population. Negative values characterise individuals with a lower probability of originating locally. The patterns of dispersal were produced for the species overall as well as within regional localities (Kangaroo Island, eastern and western regions).

Pairwise distance values were used to estimate the number of individuals that migrate between sampling locations per generation. The relationship $N_em = [(1/\theta_{st})-1]/4$ was used to estimate the net migration rate from the dataset (Wright, 1951).

Analyses of correlations between gene flow and geographic distance for all populations and separately for those in the eastern and western regions were examined. The significance of these relationships was calculated using Mantel's permutation test and with 10,000 permutations executed by IBD version 3.02 (Jensen *et al.*, 2005). The strength of this relationship was determined by regressing all pairwise genetic similarity values $F_{ST}(1- F_{ST})$ against the corresponding geographic distance. Mean relatedness (R) overall and separately for males and females were also compared to geographic distance between localities. Again comparisons between eastern and western regions were made.

Evidence of recent population bottlenecks were investigated using BOTTLENECK version 1.2.02 (Cornuet and Luikart, 1996) to test for an excess in heterozygosity using the Wilcoxon rank sign test. A mixed model of microsatellite mutation was assumed, with single-step mutations assumed to account for 90% of all mutation events and a variance among multiple steps of 12, as suggested by Piry *et al.* (1999) and Vernesi *et al.* (2003).

Population Structure

The various levels of population genetic structure were evaluated using three approaches, incorporating both genic and genotypic methods. Firstly, pairwise

values of θ_{ST} were calculated (1000 permutations) using FSTAT. When performing multiple simultaneous comparisons, the sequential Bonferroni procedure (Rice, 1989) with alpha set to 0.05, was used to adjust significance level. Secondly, neighbour-joining (NJ) topologies were constructed using Nei's (1978) unbiased distance and θ_{ST} (Weir and Cockerham, 1984) using the program MICROSAT version 1.5b (Minch *et al.*, 1995) and PHYLIP version 3.64 (Felsenstien, 1989). As a measure of support for the tree, 1000 bootstrapped distance matrices were generated using MICROSAT and a consensus tree of all the resulting trees was built using PHYLIP. Finally, multilocus genotypic data was used to infer population structure. The Bayesian clustering method implemented by STRUCTURE 2.1 (Pritchard *et al.*, 2000) was used to infer the population structure. The results generated were based on simulations from 1-20 inferred populations (k) using a burnin period of 50 000, found sufficient to minimise error (α), with 10^6 iterations. The inferred number of populations within the sample was deduced using both posterior probability (Pritchard *et al.*, 2000) and delta log likelihood (Evanno *et al.*, 2005). Each of the identified clusters was subsequently rerun to test for further stratification within the units. Another assignment method, GENECLASS was also employed to determine the percentage of individuals correctly assigned to each of the identified groups and confirmed structure. IDENTIX was also used to determine if the groups represented a panmictic population and hence lacked further structuring.

Results

Microsatellite Variability

A total of 478 *M. fuliginosus* from 20 localities spanning the species distribution were genotyped at 9 microsatellite loci. All loci were found to be highly polymorphic, exhibiting large numbers of alleles ranging from 13-57 (Table II-1). No significant deviations from HWE ($p>0.001$) were detected, nor was there evidence of linkage disequilibrium between loci.

Allelic diversity ranged between 4.44 and 15.56 alleles per locus, averaging 9.68 while expected heterozygosity ranged from 0.60 to 0.90 (Table II-2), averaging 0.85

in the species overall, 0.86 on the mainland. The insular population, *M. f. fuliginosus* displayed the lowest levels in both allelic diversity, 4.44 and heterozygosity, 0.60. Several populations, Congelin, Coffin Bay Peninsula, Kalgoorlie, Madura, ParaWirra National Park and Kangaroo Island were found to deviate significantly from HWE assumptions ($p<0.05$) indicating a mild Walhund effect. At one of these sites, Madura this deviation was highly significant ($p<0.01$).

Allelic richness varied across mainland populations with the greatest variation occurring within the western populations, notably the central southern region of Western Australia (e.g. Congelin). Across the mainland populations the levels of allelic richness decreased significantly ($p<0.01$) from west to east, with the westerly precinct the most diverse at 12.6 alleles per 15 individuals, decreasing to 9.2 in the eastern parts of the range. The lowest levels of diversity occur on Kangaroo Island at 3.6 alleles per population (n=15) significantly less than that observed on the mainland ($p<0.05$).

Table II-1: Characteristics of 9 microsatellite loci calculated for all samples (n=478) across the range of *Macropus fuliginosus*.

Locus	Number of Alleles	Allele Size Range (bp)	F_{IS} (±SE)
T46-5	13	131-183	-0.022 (±0.02)
T3-1T	43	145-341	0.034 (±0.02)
T15-1	22	138-190	-0.006 (±0.03)
T31-1	18	108-200	-0.047 (±0.03)
B90	17	90-122	-0.004 (±0.03)
T19-1	20	123-187	0.004 (±0.02)
Pa595	33	100-268	0.024 (±0.02)
G26-4	57	207-463	0.016 (±0.02)
T32-1	16	138-170	-0.061 (±0.03)

Table II-2: Microsatellite diversity (9 loci) for sampled populations of *Macropus fuliginosus*. The average observed and expected heterozygosity (H_o and H_e), F_{IS}, average number of alleles and allelic richness are shown. * denotes significant deviations from HWE expectations ($p<0.05$)

	Location		Sample Sizes	Expected Heterozygosity (±SD)	Observed Heterozygosity (±SD)	Number of Alleles (±SD)	F_{IS}
Macropus fuliginosus fuliginosus	Insular	Kangaroo Island	25	0.60 (±0.06)	0.62 (±0.03)	4.44 (±2.24)	-0.043*
Macropus fuliginosus melanops	Eastern mainland	Bourke	14	0.83 (±0.03)	0.80 (±0.04)	8.22 (±2.77)	0.04
		Hillston	25	0.87 (±0.02)	0.87 (±0.02)	11.20 (±3.38)	-0.007
		Hattah Lakes	9	0.82 (±0.02)	0.74 (±0.05)	6.00 (±1.80)	0.097
		Peterborough	20	0.83 (±0.02)	0.84 (±0.03)	9.11 (±2.20)	-0.01
		ParaWirra NP	32	0.78 (±0.03)	0.79 (±0.02)	8.78 (±1.86)	-0.011
		Coffin Bay	36	0.85 (±0.03)	0.87 (±0.02)	12.89 (±6.88)	-0.03
	Western mainland	Madura	34	0.84 (±0.02)	0.90 (±0.02)	11.56 (±3.97)	-0.074*
		Moonera	21	0.87 (±0.02)	0.90 (±0.02)	11.22 (±4.44)	-0.038
		Kalgoorlie	30	0.86 (±0.02)	0.77 (±0.03)	11.11 (±3.98)	0.107
		Esperance	29	0.89 (±0.02)	0.92 (±0.02)	14.22 (±4.94)	-0.034
		Albany	30	0.89 (±0.01)	0.87 (±0.02)	13.78 (±4.29)	0.023
		Yallingup	8	0.88 (±0.02)	0.81 (±0.03)	8.44 (±1.81)	0.095

Witchcliffe	19	0.88 (±0.02)	0.85 (±0.05)	11.78 (±3.73)	0.035
Congelin	38	0.90 (±0.01)	0.91 (±0.02)	15.56 (±6.29)	-0.026*
Perth	27	0.87 (±0.02)	0.87 (±0.02)	13.67 (±6.04)	0.001
Waroona	7	0.90 (±0.03)	0.87 (±0.04)	8.78 (±2.28)	0.036
Geraldton	24	0.88 (±0.02)	0.84 (±0.02)	14.33 (±6.60)	0.048
Wongan Hills	30	0.88 (±0.02)	0.91 (±0.02)	13.89 (±5.56)	-0.026
Shark Bay	20	0.83 (±0.02)	0.78 (±0.03)	8.67 (±2.12)	0.072

A significant amount of genetic diversity ($p<0.05$) was partitioned between *M. f. fuliginosus* and *M. f. melanops*, with 17% of the variation explained and 75% occurring within populations. Similarly a significant amount of the genetic variation was partitioned between the three groups, Kangaroo Island, eastern and western mainland ($p<0.05$), with 6% of the variation occurring between the groups and 86% of the variation within populations.

Gene flow and Dispersal

The net number of migrants per generation across mainland populations ranged from 1.77 to 41.42 (Table II-3). The highest number of migrants was observed between populations separated by small distances, Madura and Moonera (50km) for example, whilst least migration was observed between distant populations (>2000km), such as Shark Bay and ParaWirra National Park. Reflecting these low migration rates, 66.7% of individuals (51.7% of individuals with simulation) could be correctly assigned to the population from which they were sampled. Comparisons of within versus among population relatedness displayed significant differences at both local ($p<0.01$) and regional scales ($p<0.01$).

A significant negative correlation was found between genetic distance F_{ST} ($1-F_{ST}$) and geographic distance across localities ($p<0.01$; $R^2=0.33$). However, following separation into eastern and western regions the relationship was evident only in the west ($p<0.01$; $R^2=0.39$; Figure II-2a) and not amongst eastern populations ($p=0.42$; Figure II-2b).

Table II-1: Genetic differentiation amongst *Macropus fuliginosus* populations. Pairwise F_{ST} values above diagonal and pairwise number of migrants per generation (N_em) below. * $p<0.05$ after Bonferroni Correction. ns- non significant

	Kangaroo Island	Bourke	Hillston	Coffin Bay	Peterborough	Para Wirra	Hattah Lakes	Albany	Congelin	Esperance	Kalgoorlie	Geraldton	Madura	Moonera	Perth	Shark Bay	Waroona	Witchcliffe	Wongan Hills	Yallingup
Kangaroo Island		0.2059*	0.1580*	0.1895*	0.2072*	0.1999*	0.2168*	0.1713*	0.1365*	0.1476*	0.1582*	0.1669*	0.1587*	0.1559*	0.1592*	0.2047*	0.1487*	0.1536*	0.1390*	0.1710*
Bourke	0.96		0.0216*	0.0814*	0.0599*	0.0728*	0.0717*	0.0646*	0.0462*	0.0556*	0.0597*	0.0621*	0.0755*	0.0538*	0.0652*	0.0736*	0.0527*	0.0784*	0.0576*	0.0538*
Hillston	1.33	11.32		0.0571*	0.0468*	0.0724*	0.0522*	0.0411*	0.0273*	0.0347*	0.0432*	0.0477*	0.0490*	0.0335*	0.0277*	0.0643*	0.0267*	0.0514*	0.0295*	0.0426*
Coffin Bay	1.07	2.82	4.13		0.0380*	0.1238*	0.0603*	0.0528*	0.0529*	0.0361*	0.0567*	0.0533*	0.0693*	0.0445*	0.0590*	0.0972*	0.0635*	0.0647*	0.0584*	0.0700*
Peterborough	0.96	3.92	5.09	6.33		0.1070*	0.0442*	0.0611*	0.0469*	0.0367*	0.0594*	0.0579*	0.0802*	0.0597*	0.0617*	0.1086*	0.0749*	0.0639*	0.0624*	0.0555*
Para Wirra	1.00	3.18	3.20	1.77	2.09		0.0773*	0.0886*	0.0740*	0.0729*	0.1049*	0.0736*	0.0975*	0.0929*	0.0707*	0.0952*	0.0898*	0.1079*	0.0830*	0.0847*
Hattah Lakes	0.90	3.24	4.54	3.90	5.41	2.98		0.0736*	0.0520*	0.0520*	0.0767*	0.0652*	0.0689*	0.0504*	0.0713*	0.1094*	0.0681	0.0709*	0.0632*	0.0824*
Albany	1.21	3.62	5.83	4.48	3.84	2.57	3.15		0.0223*	0.0172*	0.0421*	0.0349*	0.0481*	0.0338*	0.0233*	0.0662*	0.0254	0.0277*	0.0196*	0.0207
Congelin	1.58	5.16	8.91	4.48	5.08	3.13	4.56	10.96		0.0115	0.0401*	0.0272*	0.0375*	0.0254*	0.0253*	0.0659*	0.0166	0.0177*	0.0219*	0.0150
Esperance	1.44	4.25	6.95	6.68	6.56	3.18	4.56	14.28	21.49		0.0348*	0.0217*	0.0302*	0.0186*	0.0225*	0.0602*	0.0229	0.0296*	0.0269*	0.0278
Kalgoorlie	1.33	3.94	5.54	4.16	3.96	2.13	3.01	5.69	5.98	6.93		0.0616*	0.0336*	0.0134*	0.0453*	0.0852*	0.0385*	0.0386*	0.0425*	0.0298*
Geraldton	1.25	3.78	4.99	4.44	4.07	3.15	3.58	6.91	8.94	11.27	3.81		0.0531*	0.0401*	0.0274*	0.0590*	0.0221	0.0454*	0.0270*	0.0254
Madura	1.33	3.06	4.85	3.36	2.87	2.31	3.38	4.95	6.42	8.03	7.19	4.46		0.0060	0.0498*	0.0826*	0.0375*	0.0547*	0.0489*	0.0640*
Moonera	1.35	4.40	7.21	5.37	3.94	2.44	4.71	7.15	9.59	13.19	18.41	5.98	41.42		0.0404*	0.0728*	0.0206*	0.0390*	0.0325*	0.0438*
Perth	1.32	3.58	8.78	3.99	3.80	3.29	3.26	10.48	9.63	10.86	5.27	8.87	4.77	5.94		0.0577*	0.0295	0.0446*	0.0188*	0.0259
Shark Bay	0.97	3.15	3.64	2.32	2.05	2.38	2.04	3.53	3.54	3.90	2.68	3.99	2.78	3.18	4.08		0.0469*	0.0851*	0.0648*	0.0638*
Waroona	1.43	4.49	9.11	3.69	3.09	2.53	3.42	9.59	14.81	10.67	6.24	11.06	6.42	11.89	8.22	5.08		0.0191	0.0071*	0.0068^{ns}
Witchcliffe	1.38	2.94	4.61	3.61	3.66	2.07	3.28	8.78	13.87	8.20	6.23	5.26	4.32	6.16	5.36	2.69	12.84		0.0227	0.0100^{ns}
Wongan Hills	1.55	4.09	8.22	4.03	3.76	2.76	3.71	12.51	11.17	9.04	5.63	9.01	4.86	7.44	13.05	3.61	34.96	10.76		0.0195*
Yallingup	1.21	4.40	5.62	3.32	4.25	2.70	2.78	11.83	16.42	8.74	8.14	9.59	3.66	5.46	9.40	3.67	36.51	24.75	12.57	

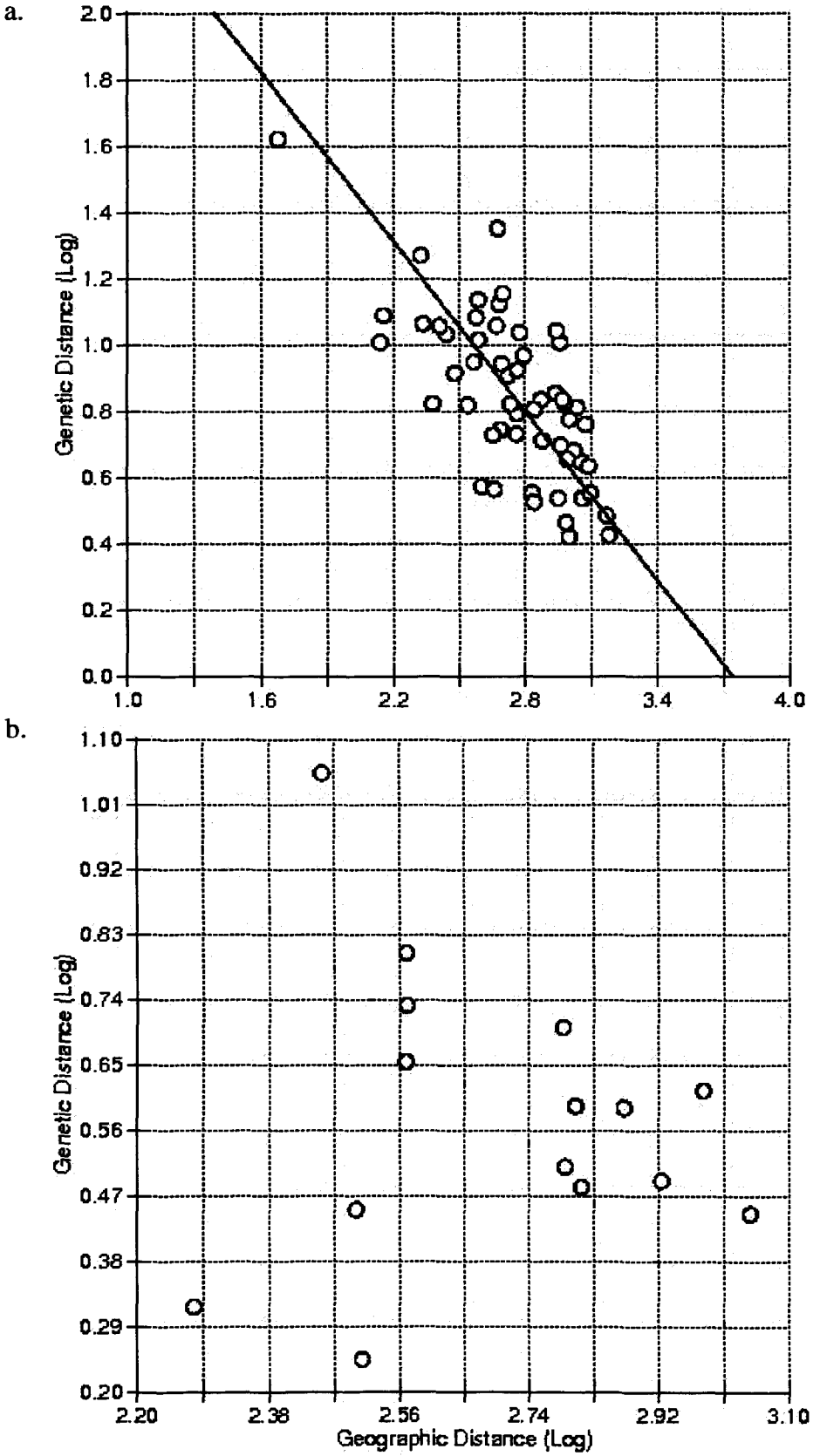

Figure II-2: Plots of genetic distance (log $F_{ST}(1- F_{ST})$) and geographic distance (log) between mainland *Macropus fuliginosus* populations for a) western and b) eastern region of the range. Only the western region displays a significant relationship ($p<0.01$; $R^2=0.388$). The line indicating the least squares regression estimate.

Comparisons of both male and female pairwise relatedness values revealed significant differences in within versus among population comparisons ($p<0.01$). Significant differences were not observed between male and female dispersal overall ($p=0.18$). Nor were differences evident between the sexes at local ($p=0.39$) or regional scales (east, $p= 0.1699$; west, $p=0.5387$). However, assignment did reveal a larger proportion of females (0.641 without simulation, 0.485 with simulation) being correctly assigned to the population from which they were sampled compared to males (0.561 without simulation, 0.434 with simulation). AI_C values showed a similar pattern of dispersal with no significant differences between the sexes. However, as Figure II-3 shows, the largest movements were attributable to males. Examination of relatedness, similarly failed to reveal any correlation with distance ($p>0.05$).

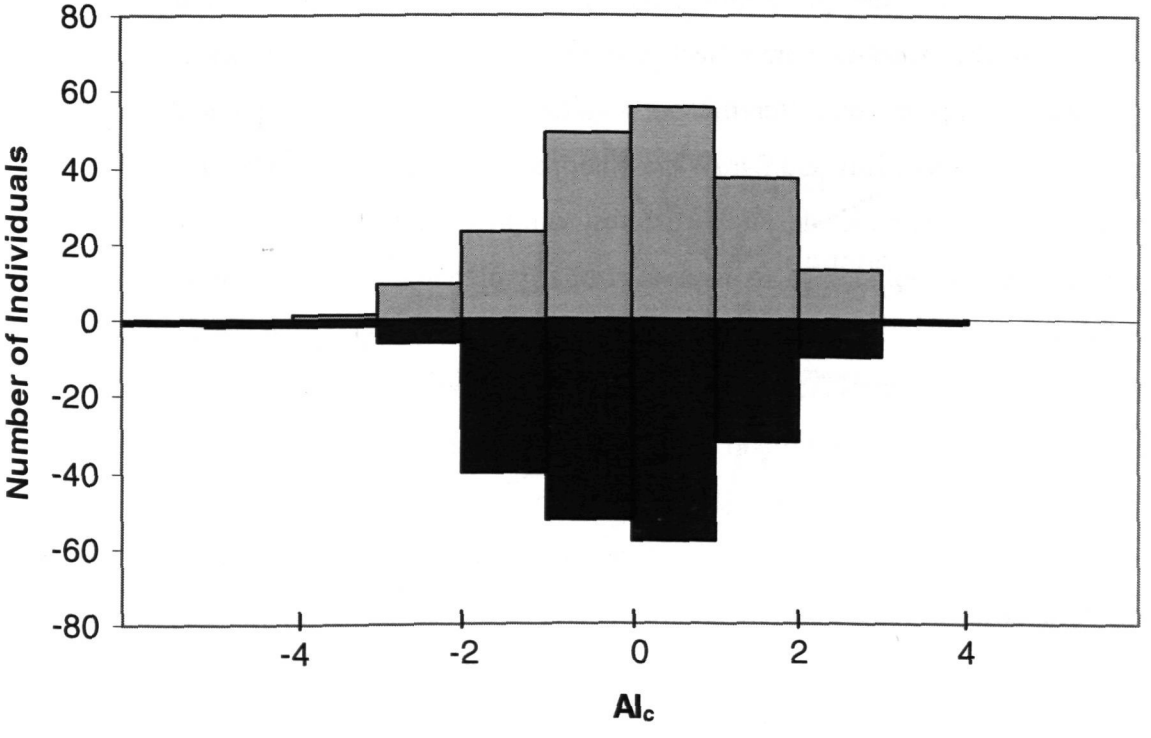

Figure II-3: Plot of the corrected assignment indices for all populations of *Macropus fuliginosus*. Females are shown above the line, while males are below. No significant differences between male and female dispersal were observed overall or in either the eastern or western regions of the continent, nor on Kangaroo Island ($p>0.05$).

None of the mainland populations revealed any evidence of recent bottlenecks. Kangaroo Island, however, displays a significant heterozygosity excess ($p<0.05$) and an associated mode shift indicating the population has suffered a recent bottleneck.

Population Structure

Several pairwise comparisons of F_{ST} revealed significant differences both before and after Bonferroni correction, with values varying between 0.006 and 0.217 (Table II-3). As expected the greatest differences occurred between Kangaroo Island and mainland populations. Within the mainland the largest differences existed between populations at the eastern and western extremities of the range, while the least difference was demonstrated by populations which were separated by small geographic distances (e.g. 50 km), such as Moonera and Madura at 0.006 (Table II-3). One population, Shark Bay displays relatively high levels of differentiation from all other populations, even those geographically close. These levels of genetic differentiation are reflected in the NJ tree (Figure II-4), in which most populations appear well differentiated, with the exception of sites separated by less than 100km. Shark Bay and ParaWirra National Park both appeared highly differentiated from other sites despite the nearest sampled site occurring less than 400km away. Kangaroo Island also displays similarly high levels of differentiation.

The Bayesian clustering of STRUCTURE indicates the presence of four distinct clusters within *M. fuliginosus* with all other values of K deemed highly improbable ($p=0.999$). Kangaroo Island appears distinct from the mainland, which is itself divided into 3 clusters. Cluster I consists of the eastern populations, Hillston, Bourke, ParaWirra National Park, Peterborough and Hattah Lakes National Park. The second cluster forms a western group comprising Congelin, Esperance, Shark Bay, Geraldton, Perth, Yallingup, Waroona, Wongan Hills and Witchcliffe. Finally connecting the two extremes of the range, is a central cluster containing Madura, Moonera, Kalgoorlie and Coffin Bay Peninsula. Where these clusters meet geographically, substantial admixture is evident (Figure II-5). Esperance for instance, displays a mixture of individuals with a high probability of belonging to western and central clusters as well as individuals displaying mixed ancestry (Figure

II-5). Similarly Peterborough and ParaWirra National Park, near the edges of the central and eastern clusters display evidence of admixture but to a lesser extent. When analysed separately, Kangaroo Island as well as the eastern and central clusters produced a pattern consistent with a single population. The western cluster, however, displayed evidence of further sub-structuring. Two geographic clusters were identified within the west, a northern grouping comprised of Perth, Wongan Hills, Waroona, Geraldton and Shark Bay, and a southern cluster incorporating Congelin, Esperance, Witchcliffe, Yallingup and Albany. No further stratification was evident within the north-western and south-western groups. This overall genetic structure was confirmed via assignment test, with 91.8% (78.1% with simulation) of individuals assigned to the same cluster as was identified in STRUCTURE. Similarly the relatedness between individuals within the five clusters was not significantly different from that expected in a panmictic population (p=0.14).

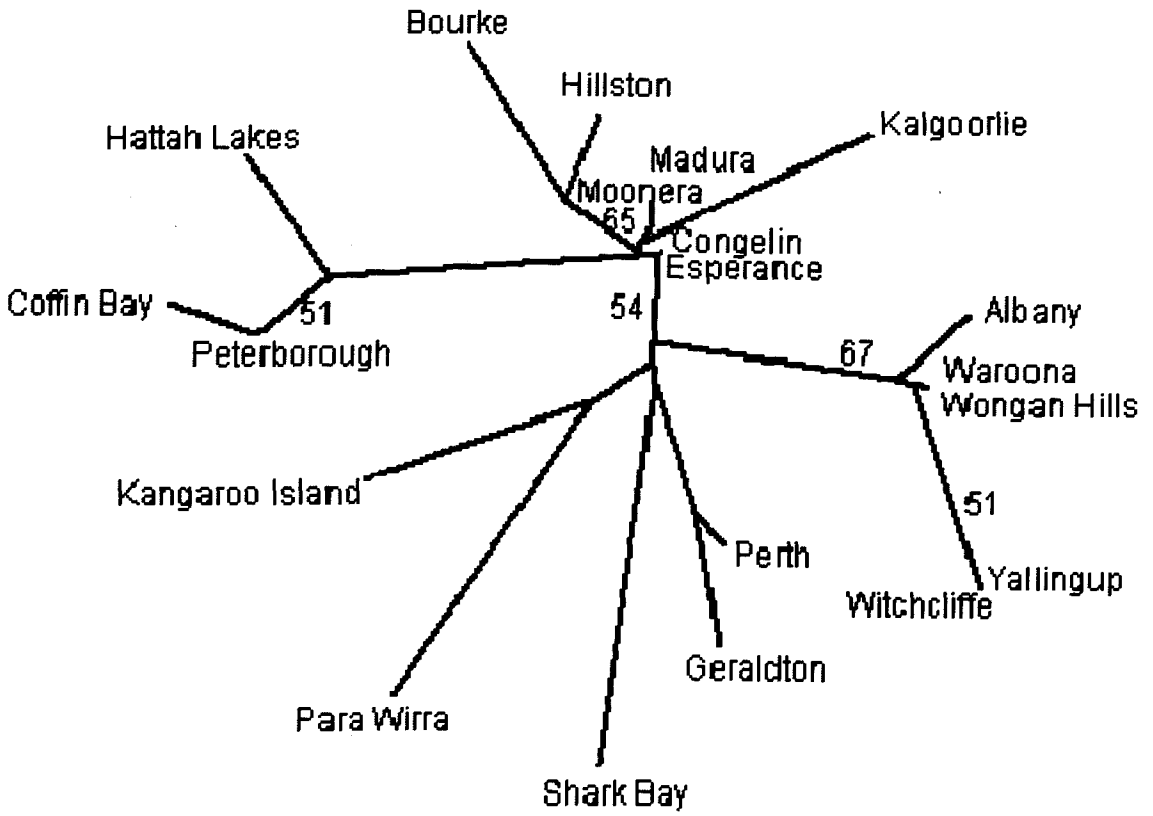

Figure II-4: Unrooted neighbour-joining tree based on Nei's (1978) unbiased genetic distance amongst populations for the microsatellite data. Bootstrap values <50% are not shown.

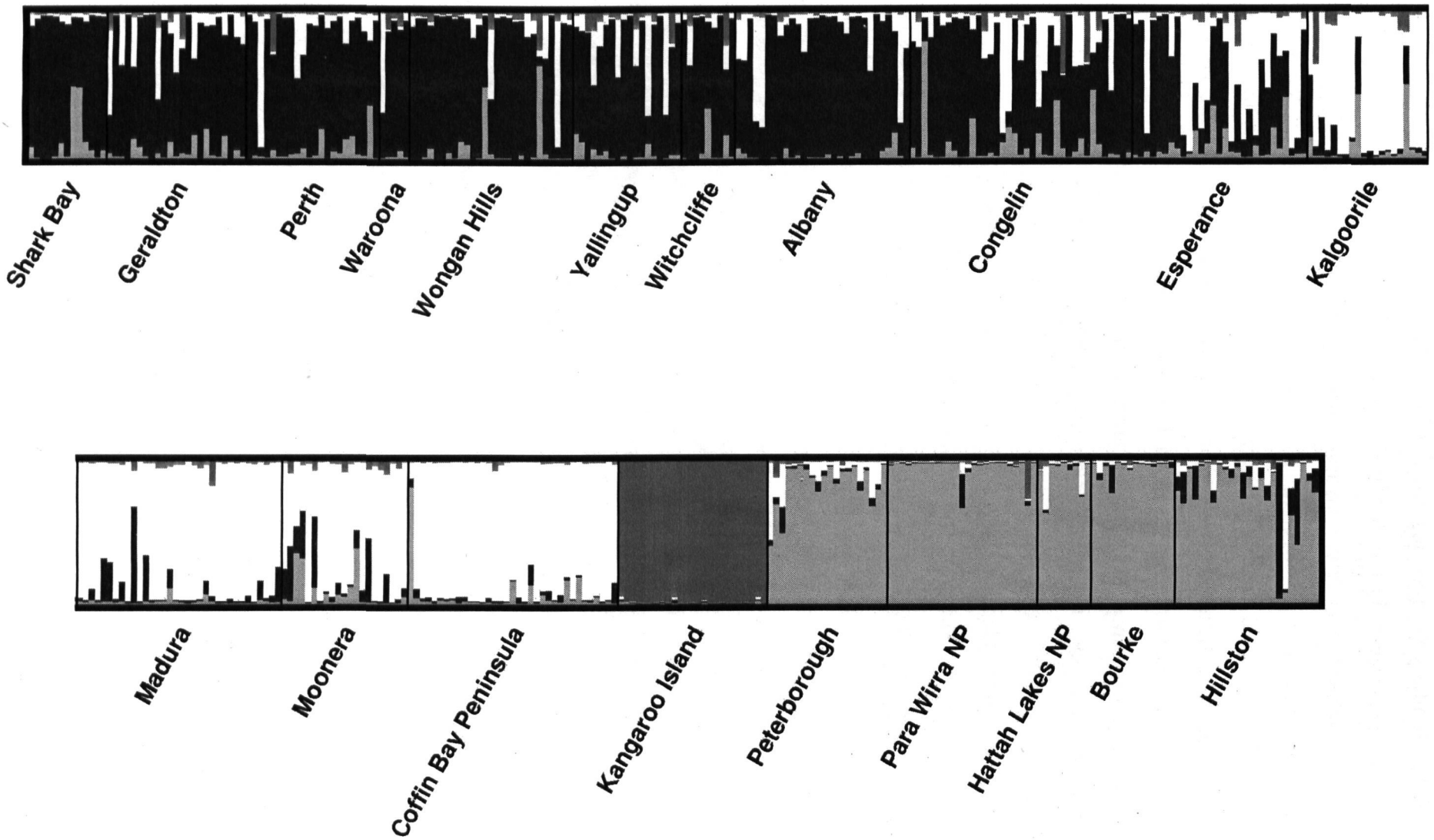
Shark Bay
Geraldton
Perth
Waroona
Wongan Hills
Yallingup
Witchcliffe
Albany
Congelin
Esperance
Kalgoorlie
Madura
Moonera
Coffin Bay Peninsula
Kangaroo Island
Peterborough
Para Wirra NP
Hattah Lakes NP
Bourke
Hillston

Figure II-5: Assignment probabilities calculated in STRUCTURE 2.1 for all *Macropus fuliginosus* individuals showing the four clusters, dark grey – western region (Perth, Shark Bay, Geraldton, Wongan Hills, Witchcliffe, Albany, Congelin, Yallingup, Esperance), white- central region (Kalgoorlie, Moonera, Madura, Coffin Bay Peninsula), hatched- Kangaroo Island, light grey- eastern region (ParaWirra National Park, Peterborough, Bourke, Hillston, Adelaide, Hattah Lakes National Park). Black lines separate populations. Admixture is evident in populations, particularly those occurring on the geographical edge of the groupings, e.g. Esperance and Peterborough (see Figure II-1)

Discussion

Overall genetic diversity in *M. fuliginosus* was high, except on Kangaroo Island where the erosion of genetic diversity, via drift and a recent genetic bottleneck is apparent. Four genetic units, which also form geographical groups, were identified across the distribution of *M. fuliginosus*, comprising Kangaroo Island, the eastern, Central/Nullarbor and western mainland regions. The latter was further divided into northern and south-western subregions. The effect of isolation by distance was evident in western but not eastern populations, suggesting that eastern populations are the result of a recent range expansion or influenced by environmental variables. Dispersal was not strongly sex biased, although males were apparently responsible for the largest movements.

Genetic Diversity

Macropus fuliginosus displays extensive genetic diversity across the nine examined microsatellite loci. Its average H_e of 0.85 is among the highest recorded for marsupials (0.05-0.86, n=24, Bowyer *et al.*, 2002), but is comparable to that observed in another large macropod *M. giganteus* (0.82±0.02, Zenger *et al.*, 2003). This indicates that overall *M. fuliginosus* has maintained long term, large effective population sizes. This is especially true of mainland populations. However Kangaroo Island is an exception which will be discussed shortly.

Levels of variation however are not uniform across the mainland and a significant reduction in allelic diversity is apparent from west to east. This is consistent with the hypothesis, *M. fuliginosus* originated in south-western Australia as indicated by its resistance to the toxin fluoroacetate (Mead, 1985; Oliver *et al.*, 1979). This resistance is the result of coevolution between the plant species which produce the toxin and herbivores which feed on it. Restricted to south-western Australia only 40 species are capable of producing fluoroacetate (Alplin, 1971; Twigg and King, 1991), allowing resistance to be used as a marker for tracing historic range changes (Oliver *et al.*, 1979). Regardless of where in the range an individual occurs all *M. fuliginosus* possess a resistance to fluoroacetate, while other species remain susceptible (Mead, 1985; Oliver *et al.*, 1979). Moreover, a progressive reduction in

allelic richness was evident when comparisons were extended to incorporate the eastern, western and central clusters identified by Bayesian analysis ($p<0.05$), indicating a stepping stone or infinite island model of expansion may have occurred. Expansion under this hypothesis would also result in the genetic units as groups become increasingly differentiated from the original (Austerlitz *et al.*, 1997). Further investigation at loci with slower mutation rates, such as mtDNA will be required to more accurately elucidate the historical movement patterns of *M. fuliginosus.*

Kangaroo Island

The Kangaroo Island population is atypical among *M. fuliginosus* in having significantly reduced genetic diversity (H_e reduced by 30%, allelic diversity by 54% on average compared with mainland populations). As a continental island (55 × 150 km), Kangaroo Island and its biota became separated from the mainland approximately 9,500 year ago, by rising sea levels (Thom and Chappel, 1975). The erosion of genetic diversity predicted for island populations due to their isolation and lack of gene flow (Frankham, 1997; 1998), is evident in many island macropod populations, even those with relatively large current census sizes (Eldridge *et al.*, 1999; Eldridge *et al.*, 2004). *M. f. fuliginosus* displays this reduction, possessing a high effective inbreeding co-efficient of 0.70 compared with other medium sized island mammals (Frankham, 1998). In contrast to *M. f. fuliginosus* and other island macropods, the Kangaroo Island population of the smaller tammar wallaby *M. eugenii*, the only other macropod inhabiting Kangaroo Island, does not display a reduction in genetic diversity (Taylor and Cooper, 1998) This may, in part be attributed to the extent of the available habitat maintaining a stable and large effective population size in this species (census size up to 10^6 individuals, Wright and Scott, 1999).

Currently Kangaroo Island supports an abundant population of *M. f. fuliginosus* (I. Beveridge personal communication). There is, however, evidence of a recent genetic bottleneck indicating the population has previously undergone a substantial reduction in population size. Prior to its isolation, Kangaroo Island formed part of

the mainland. Hence the populations of *M. f. fuliginosus* which became isolated were established prior to isolation, rather than a small population invading via a short-term land-bridge which results in the type of founding event leading to genetic bottlenecks. It is, therefore, unusual that this population exhibits evidence of a recent bottleneck. Several potential sources for the observed bottleneck may exist including disease or drought. However, historic records show this population potentially suffered a dramatic reduction in population size in the 1800s prior to settlement (in 1836) due to hunting by the periodic European inhabitants of the island who reportedly killed large numbers of kangaroos for meat and skin (Clumpston, 1986; Flinders, 1814). The intense hunting during this period has been linked to the extinction of a dwarf species of emu, *Dromaius baudinianus* which disappeared from Kangaroo Island during this time (Garnett, 1993).

Migration and Gene flow

Prior to this study, information on dispersal and potential gene flow in *M. fuliginosus* was limited and varied across the range. Ecological investigations indicate that 90% of the population is largely sedentary moving less than 6 km, on average (Priddel *et al.*, 1988a; Priddel *et al.*, 1988b). Contrary to these finding, only weak genetic structuring is evident at a local level, with few sites displaying significant differences between populations (Table II-3). This discrepancy is not surprising since ecological estimates are typically acquired from adult animals and unlikely to encapsulate natal dispersal. One male, for instance was recorded 85km from its point of capture, whilst several others travelled more than 10km (Priddel *et al.*, 1988b). Interestingly, when comparisons of relatedness were made at a smaller scale, <50km (2 comparisons), although considerably reduced, differences were apparent ($p=0.03$) indicating the presence of some very fine scale weak structuring.

Some *M. fuliginosus* populations are more highly differentiated than would be expected based on geographical proximity. ParaWirra National Park, located within the Flinders Ranges area displays high levels of within population relatedness and differentiation even to populations which are geographically close. These low immigration rates may be attributed to a lack of successful dispersal through the mountainous habitat as observed in *M. rufus* (Clegg *et al.*, 1998). Similarly, the

Shark Bay population also possesses high levels of internal relatedness and low levels of immigration/emigration (Table II-3). This may be site specific or associated with its location at the edge of the northern range. *M. fuliginosus* exhibits a ramp function towards the edges of its range, whereby densities gradually decrease towards zero (Caughley *et al.*, 1988), rather than a sudden absence. Little successful migration may arise from the reduced productivity and low suitability of these regions of the range potentially isolating the population and allowing drift to occur. The impact of some features on sub-structuring is evident even within sampled populations. The Madura site, for instance, which is divided by an animal proof fence displays evidence of sub-structuring, with highly significant departures from random mating. Typically animal proof fencing is not found to present a substantial barrier to kangaroo movements, with animals observed to cross fences (Priddel *et al.*, 1988a; Priddel *et al.*, 1988b). However, at this site at least the presence of the fence appears to reduce gene flow somewhat, resulting in sub-structuring within the site.

Overall in *M. fuliginosus* weak genetic structuring is also evident at a regional scale, with θ_{ST} averaging 0.055, indicating high levels of gene flow. This is supported by the lack of further structuring identified by STRUCTURE and evidence of panmixia in each of the continental groupings. Although genetic structuring is evident throughout the range, the effects of isolation by distance are only evident in the western half of the range where a correlation with geographical distance is apparent. This may be associated with sampling density in this region is reduced compared to the west reducing the ability to detect IBD. Furthermore, three pairwise comparisons in particular appear influence this pattern and hence greater sampling would be required to further elucidate the patterns of genetic differentiation within this region of the range. However several other potential explanations for this difference also exist.

A relatively recent expansion into the eastern areas of the range would result in the observed pattern. This eastward expansion is supported by the reduction in genetic diversity across the range (this study) and resistance to fluoroacetate (Mead, 1985; Oliver *et al.*, 1979). Human induced habitat changes, such as increased water availability, are known to have increased the population size of the large macropods

but may also have facilitated range expansions in the last 100 years. Similarly the habitat changes associated with the climatic oscillations of the Pleistocene may have equally facilitated earlier range changes.

The absence of a correlation of the observed genetic structuring with geographic distance may also have resulted from an adaptation to or may reflect the influence of changing environmental conditions on dispersal tendencies. Temporal variation in resource availability has been observed to increase levels of dispersal, as individuals attempt to track moving resources (Denno *et al.*, 2001; Denno *et al.*, 1991; Johst and Brandl, 1997; McPeek and Holt, 1992; Travis and Dytham, 1999). Typically harsher and more variable conditions prevail in the east of the range, with cold winters and hot, dry summers (Bureau of Meteorology; www.bom.gov.au) and larger movements may be required to attain sufficient resources. The effects of isolation by distance may be further complicated when movements follow other geographical patterns. The dry conditions for instance, may result in individuals following permanent creek lines, rather than straight geographic distances (Newsome *et al.*, 1996; Norbury *et al.*, 1994). Similarly, spatial variation is capable of influencing dispersal rates, reducing rates in complex habitats, due to the increased chances of encountering only unsuitable habitat (McPeek and Holt, 1992; Travis and Dytham, 1999). Once again eastern sites are associated with variables typically related to increasing dispersal tendencies, as much of eastern range of *M. fuliginosus* displays relatively little habitat or topographic complexity, particularly in comparison to Western Australia. The potential impacts of habitat/topographic complexities have previously been discussed where they are apparent in *M. fuliginosus* on a local scale.

Variation in climate, rainfall, vegetation and topography may influence the frequency and extent of dispersal. In eastern populations of *M. fuliginosus,* dispersal may be promoted by the increased climatic and rainfall variation coupled with reduced habitat and topographic complexity compared to the western regions of the may range. Increased mobility in response to environmental conditions, such as increased aridity and homogeneous habitat have been noted in *M. rufus* (Croft, 1991; Norbury *et al.*, 1994). Furthermore this pattern accords with that observed in ecological studies of *M. fuliginosus* in which home ranges in Western Australia

were approximately a sixth the size of those reported in eastern Australia (Arnold *et al.*, 1992; Priddel *et al.*, 1988a). While the two halves of the range roughly correspond to climatic zones, from which a potential response to environmental conditions may be inferred, further investigation would be required to elucidate the patterns of dispersal and the variables which influence them as more extensive sampling would allow for both correlation with landscape features, such as creek lines as well as stratification into rainfall and climatic classes.

Population Structure Across the Continent

At a continental scale, five distinct units were resolved by the Bayesian clustering method of Pritchard *et al.* (2000), comprising of an eastern, central, and western, which is further separated into south-western and north-western mainland populations and Kangaroo Island. *M .f. fuliginosus* on Kangaroo Island was shown to represent a distinct cluster, which is not surprising given its isolation. Populations isolated on islands may develop unique characteristics, in terms of morphology and/or differences in allele frequency and distribution at microsatellite loci, which distinguish them from mainland populations of the species. However, these differences are not indicative of subspecific separation and while Kangaroo Island appears more differentiated than other mainland populations, no unique alleles were present. Hence this population is most likely to represent a management unit (Moritz, 1994). Further examination of phylogenetic relationships of *M. fuliginosus* via DNA sequence data will further elucidate this relationship, as insular populations which display differences in other traits may lack the necessary levels of differentiation (eg. reciprocal monophyly) necessary to warrant subspecific status (Moritz, 1994; Moro *et al.*, 1998; Pruett *et al.*, 2004; Zenger *et al.*, 2003).

Despite previous suggestions that barriers capable of restricting the movement of *M. fuliginosus* were unlikely (Kirsch and Poole, 1972), distinct genetic units are evident, indicating such barriers occur within mainland populations of *M. fuliginosus*. Lying at the geographic boundary between the eastern and central units of *M. fuliginosus* are the Flinders Ranges and a series of large salt lakes (Figure II-1). The mountainous habitat of the Flinders Ranges is largely unsuitable for *M. fuliginosus* and incapable of sustaining large populations, as evident by the reduced

densities observed within this region (Cairns *et al.*, 2000). Furthermore, the apparent impact of mountainous habitat on large macropods has previously been noted in *M. rufus* (Clegg *et al.*, 1998) . The extensive salt lakes which occur to the west of the Flinders Ranges would also present a substantial barrier, with Lake Torrens extending further north than the distributional limit of *M. fuliginosus* (Figure II-1). The barrier to free movement and dispersal presented by this region is also evident in several other taxa (Degnan and Moritz, 1992; Fairbairn *et al.*, 1998). These features appear to represent the most substantial barrier within the range of *M. fuliginosus* with relatively little admixture evident compared with other boundaries between genetic units found in the western part of the distribution (see below).

The barrier between the western and central genetic units does not appear as great as that observed in the east, with admixture evident across a relatively broad area. This barrier appears to centre on the Nullarbor Plain, a dry and treeless expanse largely lacking the preferred habitat of *M. fuliginosus.* Kangaroos inhabiting the Nullarbor Plain were assigned to the central cluster and those with more coastal habitat represent the south-western cluster. Esperance, located on the coast near the edge of the Nullarbor Plain, appears to be within the contact zone and displays high levels of admixture. Whilst the Nullarbor Plain represents a less suitable habitat type (Caughley, 1964), it still currently supports densities comparable to other regions (Caughley *et al.*, 1987). However, during the last glacial maxima approximately 18,000 years ago the Nullarbor Plain reached maximum aridity and Aeolian activity with large mobile dunes formed across parts of southern Australia (Bowler, 1976). At this time it is likely to have presented a more substantial barrier to dispersal allowing differentiation to occur. The observed contemporary structure may, therefore, be an artefact of a past barrier and the current levels of admixture occurring between the genetic units reflects a reduction in the filtering effect of the Nullarbor Plain on gene flow.

Finally the sub-division occurring within the western genetic unit appears to divide populations north of, and including Perth, from those further south. However, admixture is extensive with most northern sites displaying some level of admixture. No obvious barriers explaining this boundary currently exist. Furthermore, high

levels of admixture indicate secondary contact may have occurred some time ago. Hence the two clusters may represent an historic vicariance event, although further analysis at the sequence level would be required to determine if this is the case. Low dispersal into the most northerly of sites, Shark Bay may have aided in the preservation of the genetic group at this site, which displays relatively little evidence of admixture with the southern genetic group, resulting in the high levels of differentiation from other sites. Conversely, this genetic unit may represent a ghost population due to increased drift resulting from low dispersal at the edge of the range (Caughley *et al.*, 1988). However, this is the only sampling region where a population at the edge of the range is surveyed and further sampling would be required to establish the reasons for the observed genetic unit.

Sex Biased Dispersal

The typical pattern of dispersal for large mammals, particularly those which display polygynous mating systems (Dobson, 1982; Wolff, 1994), including macropods is male biased (Johnson, 1989). However, no clear evidence of the strong male biased dispersal expected in *M. fuliginosus* is apparent in this study. Some biases are evident in dispersal tendencies, with females correctly assigned to their population more frequently than males and the lowest likelihood of originating locally was also predominately associated with males. These results are similar to ecological based studies of *M. fuliginosus* in which no significant differences in the movement from the point of capture were observed (Priddel *et al.*, 1988a). Furthermore, juveniles of both sexes have been noted to remain within or nearby the maternal home range (Arnold *et al.*, 1992). However, documentation of dispersal by field studies is often difficult in species such as kangaroos which may disperse large distances often ranging outside the study area (Priddel *et al.*, 1988b). A similar pattern of dispersal has been observed in *M. giganteus*, sister species to *M. fuliginosus,* with no significant differences apparent between male and female dispersal in terms of relatedness (Zenger *et al.*, 2003). However, in contrast the levels of effective female migration per generation inferred from mitochondrial DNA (mtDNA) in this study the authors indicated a much lower level of effective female migration, indicating a male bias in successful dispersal. Rarely are studies capable of determining the reproductive success of dispersing individuals, particularly in

highly mobile species, such as kangaroos. The lack of differences in terms of relatedness may indicate a differential in the relative reproductive success of dispersing males versus females. Naturally, an examination of mtDNA and effective female migration rates in *M. fuliginosus* would be required to ascertain any differences in the patterns of effective dispersal between the sexes.

Management Implications

The success of any management strategy is often largely dependent on the extent of knowledge on the biology and ecology of the species concerned. The scale at which both monitoring and management should take place is crucial to the efficacy of any strategy and requires an understanding of the spatial and demic structure of the species at various levels (Moritz, 1999). Kangaroos, including *M. fuliginosus* range over large distances and may present a dichotomy of potential management options. In many areas the impact of habitat modification has largely been beneficial, with populations increasing in size, often becoming locally overabundant and requiring control (Coulson, 1998; Coulson, 2001; Viggers and Hearn, 2005; Wilson, 1991). *M. fuliginosus* is also commercially harvested through most of its range for meat and skin. The results of this study are relevant to the continued management of this species, with the identification of several distinct genetic units within the distribution..

The distribution of *M. fuliginosus* encompasses a range of environmental and climatic conditions, and subsequent morphological variations appear to have arisen. As a result there has been uncertainty over where population boundaries may lie. Analysis at the genetic level has revealed that at a continental scale, there is evidence for several grouping across the range which should be incorporated into management regimes, particularly in Western Australia, where three units exist. Mitochondrial DNA analysis would also be required in order to further elucidate the population structure and subspecific taxonomy of this species. The genetic units identified herein, including Kangaroo Island, represent management units, with some incorporating morphological variants, despite the presence of some admixture at the boundaries (Moritz, 1994). Furthermore, the management regimes should, as much as possible reflect the knowledge acquired from local populations as

differences in structure and dispersal (this study) as well as home range size (Arnold *et al.*, 1992; Priddel *et al.*, 1988a) exist across the distribution. In general within each unit, these results indicate that management would be most effective at a regional scale. Hence, current management practices, in Western (CALM, 2002) and South Australia (DEH, 2002) as well as New South Wales (DEC, 2007) which manage populations at this scale, appear appropriate. However, based on these results we would recommend a revision of the arrangement of current management areas to incorporate the findings of this study, particularly the distinct genetic units identified within the distribution of *M. fuliginosus*.

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