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This thesis represents a major part of the prescribed program of study.

THE POPULATION GENETICS OF THE WESTERN GREY KANGAROO, *MACROPUS FULIGINOSUS.*

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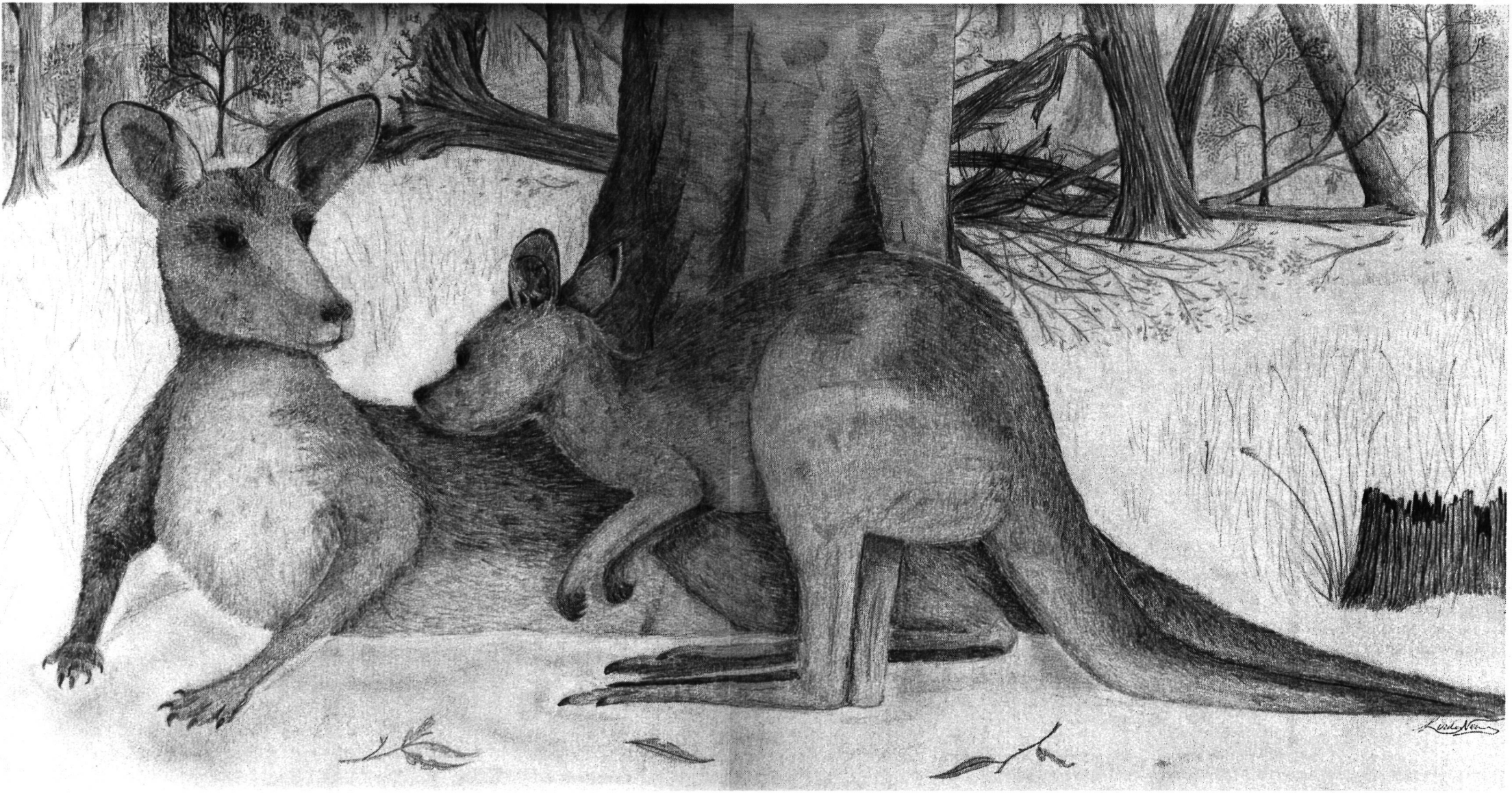
2nd May 2007

'This thesis is presented in fulfillment of the degree of Doctor of Philosophy.'



"The beauty of nature is in the detail" Stephen Jay Gould

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SYNOPSIS

The western grey kangaroo, *Macropus fuliginosus* is one of the largest species of extant macropod and is distributed across most of southern Australia. However, existing research is limited to ecological and captive studies and major gaps in our knowledge remain. In this study, variation at autosomal and Y-chromosome microsatellite loci, as well as the mitochondrial DNA (mtDNA) control region was assessed in *M. fuliginosus* populations throughout the transcontinental distribution to elucidate aspects of contemporary structure, phylogeography and hybridisation. Overall diversity in *M. fuliginosus* was among the highest recorded in marsupials at both autosomal microsatellites and mtDNA, although Kangaroo Island was a notable exception. In contrast, Y-chromosome microsatellites showed less diversity, potentially associated with the skewed sex ratio and polygynous mating system. Five genetically distinct units, some associated with landscape discontinuities, were apparent within *M. fuliginosus.* Of these, the Lake Torrens/Flinders Ranges area represents the most substantial filter, with populations on either side displaying little admixture and reduced male gene flow. Overall high levels of dispersal were evident, with a strong male bias in effective migration rates. The absence of isolation by distance in the east of the range appears associated with the recent eastward expansion or the influence of environmental/ landscape variables on dispersal. Climatic fluctuations during the Pleistocene have influenced the genetic structure of *M. fuliginosus*, with the temporary historic isolation of north and south-western populations and subsequent eastward expansion into the current distribution, from south-western Australian populations, prior to the last glacial maxima. Colonised during the easterly expansion, the Kangaroo Island population appears to display differing maternal and paternal lineages. *M. fuliginosus* represents a single evolutionary significant unit comprised of several management units some of which relate to morphological and reproductive variants. Kangaroo Island represents a reproductive and morphological variant resulting from 9,500 years of isolation and drift, rather than a major component of the genetic diversity. Where *M. fuliginosus* occurs sympatrically with *Macropus giganteus* two-way introgression was apparent but no F1 hybrids were detected. This level of hybridisation is indicative of occasional breakdowns in reproductive isolation rather than an active contact zone.

DECLARATION

I declare that this submission is my own work and that, to the best of my knowledge it contains no material written by another person, nor material which has been submitted for a higher degree to Macquarie University or any other institution, except where due acknowledgement has been made in the text.

Linda Neaves
2nd May 2007

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THESIS OVERVIEW

This thesis examines the population genetics of the one of Australia's large macropods, the western grey kangaroo, *Macropus fuliginosus*. The research covers several aspects of the species biology via an examination of autosomal and Y-chromosome microsatellites as well as mitochondrial DNA. In brief, it investigates pattern of dispersal and contemporary population structure, the phylogeography and its relationship to the environmental changes of the Pleistocene. Finally the relationship between the western grey kangaroo and its sister species the eastern grey kangaroo, *Macropus giganteus* and the occurrence of hybridisation where the two species occur sympatrically is explored. Each aspect is presented as independent scientific papers, each with it own Introduction, Methods, Results, Discussion and References. The chapters are written chronologically, with references only made to preceding chapters. The respective aims and rationale of each chapter are outlined below.

CHAPTER I introduces and reviews molecular techniques and examines their current and potential applications to macropods including parentage, population structure and dispersal patterns, as well as the implications for conservation and management.

CHAPTER II examines the population dynamics of *M. fuliginosus* through the application of high resolution autosomal microsatellite markers. Analysis of genetic information provides an opportunity to address the current gaps in the knowledge of demic structure and dispersal which also have management implications. This chapter aims to investigate the extent of genetic diversity throughout the trans-continental distribution of *M. fuliginosus* and how this diversity is partitioned, examining genetic structuring on local, regional and continental scales using both genic and genotypic approaches. Furthermore, the patterns of dispersal in males and females are examined as well as the relationship between genetic and geographic distance.

CHAPTER III investigates the influence of the climatic fluctuations associated with the Pleistocene on *M. fuliginosus* via analysis of mitochondrial DNA control region sequence data. In this chapter the phylogeography and historical expansion events are examined and based on dating estimates related to the environmental fluctuations of the Pleistocene. Additionally, female dispersal was investigated, and sex biases in dispersal were inferred from comparisons with overall dispersal.

CHAPTER IV addresses the occurrence of hybridisation between sympatric species of macropod, *M. fuliginosus* and *M. giganteus*. Despite several macropod species occurring sympatrically and displaying the ability to hybridise in captivity, no species have been examined using techniques capable of detecting low levels of hybridisation. The extent of introgression between the two species as well as the patterns and directions of hybridisation was assessed at both autosomal microsatellite loci and mitochondrial DNA.

CHAPTER V assesses the variation in Y-chromosome microsatellites to examine male specific attributes of population biology in grey kangaroos. This chapter investigates genetic variation in *M. fuliginosus* across the range in order to examine population structure and directly assess patterns of male dispersal as well as population histories. This chapter also investigates the potential introgression of Y-chromosome haplotypes where the two species of grey kangaroo occur sympatrically.

CHAPTER VI reviews the significance of the findings from each of these chapters and how they combine to produce a detailed account of contemporary structure and population history in *M. fuliginosus*. The implications of this research to the continued management of *M. fuliginosus* as well as aspects of future research are also discussed.

Publications

The publications emanating form the period of PhD candidature, that are been published, submitted or are currently in preparation include:

Neaves L, Wapstra E, Birch D, Girling JE, Joss, JMP (2006) Embryonic Gonadal and Sexual Organ Development in a Small Viviparous Skink, *Niveoscincus ocellatus. Journal of Experimental Zoology*: **305A**, 74–82.

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Roberts MW, Herbert CA, **Neaves L**, Claassens R (2007) Darting eastern grey kangaroos (*Macropus giganteus*): A protocol for free ranging populations. *Kangaroos, Wallabies and Rat Kangaroos. Volume* 3. Submitted

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Neaves L, Zenger K, Prince R, Eldridge MDB, Cooper DW (2007) Landscape discontinuities influence gene flow and genetic structure in the western grey kangaroo, *Macropus fuliginosus*. In prep

Neaves L, Zenger K, Prince R, Eldridge MDB, Cooper DW (2007) Pleistocene divergence and range expansions: phylogeography of the western grey kangaroo, *Macropus fuliginosus*. In prep

Neaves L, Roberts, M (2007) An examination of the mating system in free ranging eastern grey kangaroo, *Macropus giganteus*. In prep

Neaves L, Roberts M (2007) Relatedness and group formation in the eastern grey kangaroo, *Macropus giganteus*. In prep

Neaves L, Wapstra E, Birch D, Girling JE, Joss JMP (2007) Timing of gonadal differentiation in *Niveoscincus microlepidotus:* a biennially reproducing viviparous skink. In prep

Neaves L, Zenger K, Prince R, Eldridge MDB, Cooper, DW (2007) Genetic structure in *Macropus fuliginosus* and occurrence of hybridisation with *Macropus giganteus* as revealed by Y-chromosome microsatellites. In prep

Neaves L, Zenger K, Cooper DW (2007) The influence of Pleistocene climatic fluctuations on the eastern grey kangaroo, *Macropus giganteus*. In prep

Roberts MW, **Neaves L**. Dispersal in the eastern grey kangaroo, *Macropus giganteus* in topographically complex habitat. In prep

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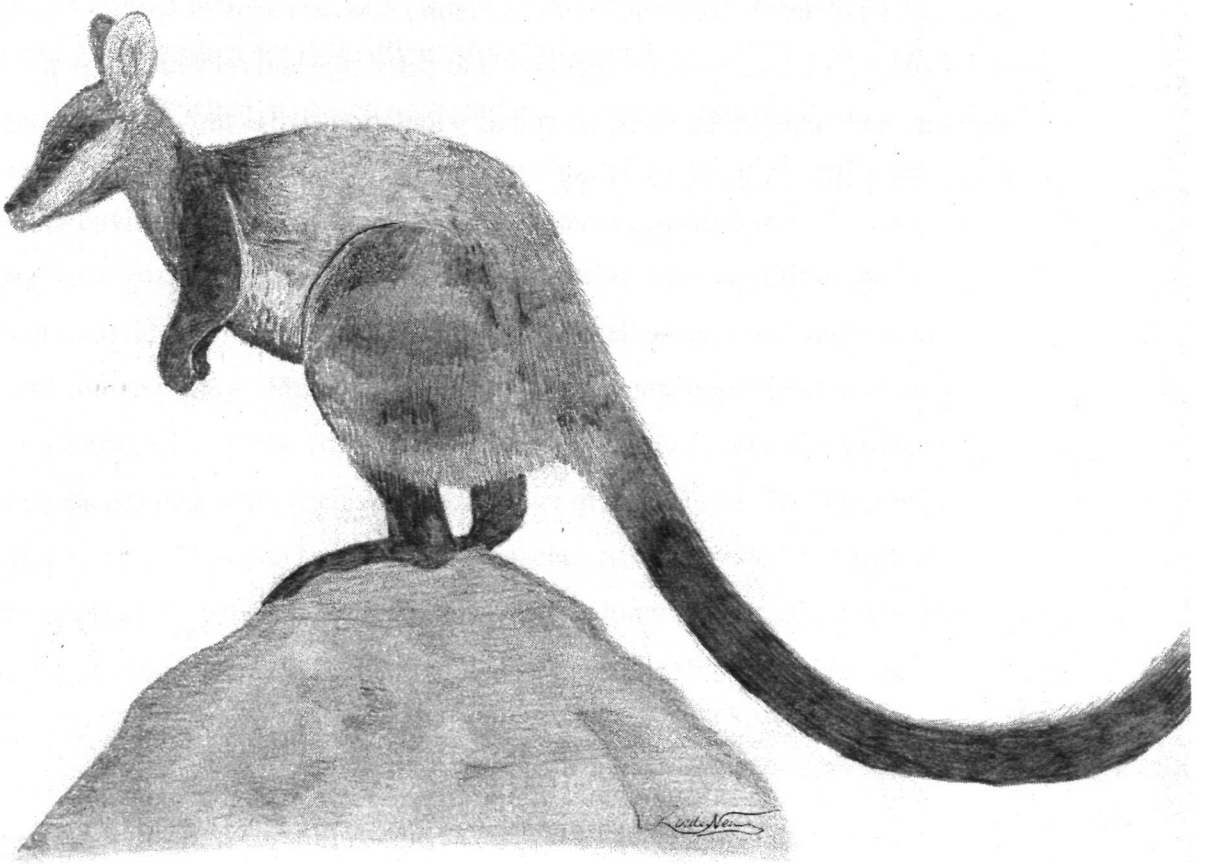
CHAPTER I:

Molecular Ecology: Its Use and Application in Macropods.