

GENETIC LINKAGE MAPS AND POPULATION GENETICS OF MACROPODS

KYALL RICHARD ZENGER

B.App.Sc. 1993 (UWS)
M.Sc. (Hons) 1996 (UWS)

Department of Biological Sciences,
Division of Environmental and Life Sciences.

November 2001

A thesis submitted to Macquarie University, in fulfilment of the regulations for the degree of Doctor of Philosophy.

Copyright in relation to this Thesis

Under the Copyright Act 1968 (several provision of which are referred to below), this material must be used only under the normal conditions of scholarly fair dealing for the purposes of research, criticism or review. In particular no results or conclusions should be extracted from it, nor should it be copied or closely paraphrased in whole or in part without the written consent of the author. Proper written acknowledgement should be made for any assistance obtained from this material.

Under Section 35 (2) of the Copyright Act 1968 'the author of a literary, dramatic, musical or artistic work is the owner of any copyright subsisting in the work'. By virtue of Section 32 (1) copyright 'subsists in an original literary, dramatic, musical or artistic work that is unpublished' and of which the author was an Australian citizen, an Australian protected person or a person resident in Australia.

The Act, by Section 36 (1) provides: 'Subject to this Act, the copyright in a literary, dramatic, musical or artistic work is infringed by a person who, not being the owner of the copyright and without the licence of the owner of the copyright, does in Australia, or authorises the doing in Australia of, any act comprised in the copyright'.

Section 31 (1) (a) (i) provides that copyright includes the exclusive right to 'reproduce the work in a material form'. Thus, copyright is infringed by a person who, not being the owner of the copyright, reproduces or authorises the reproduction of a work, or of more than a reasonable part of the work, in a material form, unless the reproduction is a 'fair dealing' with the work 'for the purpose of research or study' as further defined in Sections 40 and 41 of the Act.

Section 51 (2) provides that "Where a manuscript, or a copy, of material of other similar literary work that has not been published is kept in a library of a university or other similar institution or in an archives, the copyright in the material or other work is not infringed by the making of a copy of the material or other work by or on behalf of the officer in charge of the library or archives if the copy is supplied to a person who satisfies an authorized officer of the library or archives that he requires the copy for the purpose of research or study'.

*** Thesis' includes 'treatise', 'dissertation' and other similar productions.**

ACKNOWLEDGMENTS

This project would not have been possible without the contribution of many people. I would like to thank those who provided financial support, samples and the like, and also the wonderful group of people who were my guides, colleges and companions:

- to my supervisors Professor Desmond Cooper for his constant support, enthusiasm, and valued advice, and Dr Jenny Donald for her encouragement and constructive criticism;
- to Professor Trevor Tansley, Dean of Graduate Students, Macquarie University and Professor D. Yerbury, Vice Chancellor, Macquarie University for financial support;
- to the many people who helped collect samples, especially Tony English, Graeme Coulson, Peter Johnson and Steve Cronin;
- to Dr Mark Eldridge and Dr Peter Johnston for reviewing many drafts of my thesis and providing much valued advice and support;
- to my many friends and colleges who have not only contributed immensely to this project through innovative ideas and problem solving, but who have also kept a constant smile on my face for the past few years – Adam Stow, Anne-Marie Vachot, Andrea Taylor, Cathy Adderton, Cushla Metcalfe, Julia Harlfinger, Kathy Belov, Lee Webley, Luciano Beheregaray, Mary Lam, Mathew Sloane, Paul Sunnucks and Valma Cahill;
- to Dr Bill Poole for locating the original tammar wallaby hybrid pedigree data buried within archives for more than ten years;
- to Michael Baxter for compiling and debugging many of the programs used throughout this thesis;
- to Dr Lisa Pope and Professor Craig Moritz for providing many of the microsatellite loci used in this study;
- to Dr Peter Sharp for providing a copy of his PhD thesis;
- and last but not least my family and loved ones, especially Sandra and Buddy for the constant support and encouragement, and putting up with me over the last few years through these tenuous times.

TABLE OF CONTENTS

	<i>Page</i>
<i>Acknowledgments</i>	<i>i</i>
<i>Table of Contents</i>	<i>ii</i>
<i>List of Figures</i>	<i>v</i>
<i>List of Tables</i>	<i>ix</i>
<i>Glossary</i>	<i>xi</i>
<i>Abbreviations</i>	<i>xii</i>
<i>Declaration</i>	<i>xiii</i>
<i>Summary</i>	<i>xiv</i>

CHAPTER 1. GENERAL INTRODUCTION

1.1.1 Marsupial Genetics	1
1.1.2 Molecular Markers as Analytical Tools	2
1.1.3 Thesis Overview	3
1.1.4 Publications	4

CHAPTER 2. MOLECULAR MARKERS FOR COMPARATIVE AND QUANTITATIVE STUDIES IN MACROPODS

2.1 Introduction	6
2.1.1 General Introduction	6
2.1.2 Genetic Markers Already Typed in Tammar Backcrosses	8
2.1.3 Cross-Species Amplification of Microsatellite Loci	9
2.1.4 Microsatellite Homoplasmy and SSCP	11
2.1.5 Aims	12
2.2 Materials & Methods	13
2.2.1 Evaluation of Existing Microsatellite Genetic Markers	13
2.2.2 Library Construction for Novel Microsatellite Loci	14
2.2.3 Cross-Species Amplification Artifacts	15
2.2.4 Isolation and Characterisation of <i>HPRT</i> and <i>PGK1</i> as Type I Genetic Markers	15
2.3 Results	21
2.3.1 Evaluation of Existing Microsatellite Genetic Markers	21
2.3.2 Library Construction for Novel Microsatellite Loci	23
2.3.3 Cross-Species Amplification Artifacts	25
2.3.4 Isolation and Characterisation of <i>HPRT</i> and <i>PGK1</i> as Type I Genetic Markers	33

2.4 Discussion	39
2.4.1 Evaluation of Type II Microsatellite Genetic Markers	39
2.4.2 Cross-Species Amplification Artifacts	41
2.4.3 Isolation and Characterisation of <i>HPRT</i> and <i>PGK1</i> as Type I Genetic Markers	43
2.4.4 Conclusions	44

CHAPTER 3. GENETIC LINKAGE MAP CONSTRUCTION IN THE TAMMAR WALLABY (*M. eugenii*)

3.1 Introduction	45
3.1.1 General Introduction	45
3.1.2 Marsupials in the Mammalian Scheme	46
3.1.3 Marsupial Gene Maps	47
3.1.4 Tamar Wallaby (<i>M. eugenii</i>) Gene Mapping	48
3.1.5 Genetic Linkage	49
3.1.6 Marsupial Recombination Heterogeneity	50
3.1.7 Marsupial Crossover Interference	52
3.1.8 Marsupial X-Chromosome	53
3.1.9 Aims	54
3.2 Materials & Methods	56
3.2.1 Reference Pedigrees	56
3.2.2 Genetic Markers and Data Integrity	56
3.2.3 Map Construction	59
3.2.4 Heterogeneity	60
3.2.5 Interference	61
3.3 Results	63
3.3.1 Genotyping	63
3.3.2 Segregation Distortion	64
3.3.3 Map Construction	66
3.3.4 Validation of the Multipoint Maps	67
3.3.5 Recombination Heterogeneity	82
3.3.6 Interference	83
3.4 Discussion	87
3.4.1 Genotyping	87
3.4.2 Segregation Distortion	89
3.4.3 Map Construction	91
3.4.4 Assignment of Linkage Groups to Chromosomes	92
3.4.5 Recombination Heterogeneity	94
3.4.6 Interference	97
3.4.7 X-Chromosome	98
3.4.8 Conclusions	101

CHAPTER 4. INTRASPECIFIC VARIATION, SEX-BIASED DISPERSAL AND PHYLOGEOGRAPHY OF THE EASTERN GREY KANGAROO (*M. giganteus*)

4.1 Introduction	102
4.1.1 Wildlife Management	102
4.1.2 Eastern Grey Kangaroo	103
4.1.3 Aims	106
4.2 Materials & Methods	107
4.2.1 Sample Collection and DNA Extraction	107
4.2.2 Microsatellite Amplification and Screening	108
4.2.3 Mitochondrial DNA Amplification and Screening	108
4.2.4 Diversity Indices	109
4.2.5 Population Structure	109
4.2.6 Geneflow and Geographic Distance	111
4.2.7 Phylogenetic Analysis	111
4.3 Results	112
4.3.1 Microsatellite Genetic Variability	112
4.3.2 Mitochondrial DNA Variability	116
4.3.3 Population Structure	116
4.3.4 Geneflow and Geographical Distance	121
4.3.5 Phylogeographical Analysis	123
4.4 Discussion	126
4.4.1 Genetic Diversity	126
4.4.2 Population Structure and Dispersal	127
4.4.3 Phylogeography	128
4.4.4 Implications for Management	129
4.4.5 Conclusions	130

CHAPTER 5. GENERAL DISCUSSION

5.1.1 Assessment and Implications	131
5.1.2 Areas of Future Research	132

<i>References</i>	136
<i>Appendix 1</i>	158
<i>Appendix 2</i>	167
<i>Appendix 3</i>	182

LIST OF FIGURES

	<u>Page</u>
Figure 2-1. Size range of <i>M. eugenii</i> and source species alleles. <i>M. eugenii</i> represented in black, <i>M. giganteus</i> represented in red, <i>P. xanthopus</i> represented in blue, <i>P. assimilis</i> represented in yellow and <i>O. fraenata</i> represented in green.....	26
Figure 2-2. (A) Alignment of flanking and repeat region sequence for three monomorphic microsatellite loci in <i>M. eugenii</i> , which are polymorphic within source species. (B) Alignment of flanking and repeat region sequence for two polymorphic loci in <i>M. eugenii</i> , which were also polymorphic within source species. Within all sequences a dot “.” indicates identity to the source sequence, a dash “-“ indicates missing sequence and underlined sequence denotes primer site.	27
Figure 2-3. Representation of quality and distribution of alleles across fourteen <i>M. eugenii</i> loci in eight <i>M. eugenii</i> individuals. Individuals (from left to right) 1, 2, 5 and 8 are females while 3, 4, 6 and 7 are males. Size of alleles shown in base pairs.	29
Figure 2-4. Representation of quality and distribution of alleles across fourteen <i>M. eugenii</i> loci in eight <i>M. giganteus</i> individuals. Individuals (from left to right) 1, 2, 3 and 4 are females while 5, 6, 7 and 8 are males. Size of alleles shown in base pairs.	30
Figure 2-5. Representation of quality and distribution of alleles across nine <i>M. giganteus</i> loci in eight <i>M. eugenii</i> individuals. Individuals (from left to right) 1, 2, 5 and 8 are females while 3, 4, 6 and 7 are males. Size of alleles shown in base pairs.	31
Figure 2-6. Representation of quality and distribution of alleles across nine <i>M. giganteus</i> loci in eight <i>M. giganteus</i> individuals. Individuals (from left to right) 1, 2, 3 and 4 are females while 5, 6, 7 and 8 are males. Size of alleles shown in base pairs.	32
Figure 2-7. Representation of <i>SSCP</i> gel incorporating <i>M. eugenii</i> individuals screened with anonymous DNA markers <i>pHPRT1</i> and <i>pHPRT2</i> . F represents female while M indicates male. Allele classification and identification indicated with arrows.....	35
Figure 2-8. Alignment of <i>HPRT</i> sequences between <i>M. eugenii</i> KI and GI individuals, incorporating regions between exon 6 and exon 9. A dot “.” indicates homology to the GI sequence, bold sequence represents coding regions (exons), and underlined sequence signifies primer site. Restriction enzyme sequence recognition site and cleaving position “▼” also indicated.	36

Figure 2-9. (A) Representation of resolution and X-linked inheritance of *HPRT* within *M. eugenii* backcross progeny. F represents female while M indicates male. (B) Restriction map of *M. eugenii HPRT* gene incorporating regions exon 6 to exon 9. Primer and restriction sites indicated with arrows. Relative fragment sizes of GI and KI alleles also shown..... 38

Figure 2-10. Allelic diversity ratio between source species and *M. eugenii*. Species are positioned in increasing evolutionary distance away from *M. eugenii* based on data from Kirsch *et al.* (1997), while the relative position of *O. fraenata* is based on Colgan (1999). “*” denotes significant ($P < 0.05$) variability differences from *M. eugenii*. 40

Figure 2-11. A consensus cladogram of 18 macropodids obtained from Kirsch & Lapointe (2001). Arrows indicate relative positions where source microsatellite loci are derived. Dotted lines indicate uncertain topologies. 41

Figure 3-1. Systematic representation of crosses derived from Kangaroo Island (KI) and Garden Island (GI) animals. Females are presented first in all parental genotypes, e.g., (♀GI-♂KI) and (♀KI-(♀KI-♂GI)). Pedigree 4 and 5 mothers are the progeny of pedigrees 2 and 3 respectively. Filled symbols represent multiple individuals..... 58

Figure 3-2. Systematic representation of the sex-pooled multipoint linkage maps for groups one and two. Confidence limits ($Z_{\max}-1$) of maximum likelihood locations for each locus are indicated in shaded bars. Distance (cM) of loci relative to first locus indicated within parentheses..... 70

Figure 3-3. Systematic representation of the sex-pooled multipoint linkage maps for groups three and four. Confidence limits ($Z_{\max}-1$) of maximum likelihood locations for each locus are indicated in shaded bars. Distance (cM) of loci relative to first locus indicated within parentheses..... 71

Figure 3-4. Systematic representation of the sex-pooled multipoint linkage maps for groups five and six. Confidence limits ($Z_{\max}-1$) of maximum likelihood locations for each locus are indicated in shaded bars. Distance (cM) of loci relative to first locus indicated within parentheses..... 72

Figure 3-5. Systematic representation of the sex-pooled multipoint linkage maps for groups seven, eight and nine. Confidence limits ($Z_{\max}-1$) of maximum likelihood locations for each locus are indicated in shaded bars. Distance (cM) of loci relative to first locus indicated within parentheses. 73

Figure 3-6. Maximum likelihood gene order (a), as well as the next most likely order (b) greater than 100:1 odds against, for linkage group one. Distance (cM) of loci relative to first locus also indicated..... 74

Figure 3-7. Maximum likelihood gene order (a), and gene orders under 100:1 odds against (b and c), as well as the next most likely order (d) greater than 100:1 odds against for linkage group two. Distance (cM) of loci relative to first locus also indicated. 75

Figure 3-8. Maximum likelihood gene order (a), and gene orders under 100:1 odds against (b, c and d), as well as the next most likely order (e) greater than 100:1 odds against for linkage group three. Distance (cM) of loci relative to first locus also indicated..... 76

Figure 3-9. Maximum likelihood gene order (a), as well as the next most likely order (b) greater than 100:1 odds against for linkage group four. Distance (cM) of loci relative to first locus also indicated..... 77

Figure 3-10. Maximum likelihood gene orders (a), as well as the next most likely order (b) greater than 100:1 odds against for linkage group five. Distance (cM) of loci relative to first locus also indicated..... 78

Figure 3-11. Maximum likelihood gene orders (a), as well as the next most likely order (b) greater than 100:1 odds against for linkage group six. Distance (cM) of loci relative to first locus also indicated..... 79

Figure 3-12. Maximum likelihood gene orders (a), as well as the next most likely order (b) greater than 100:1 odds against for linkage group seven. Distance (cM) of loci relative to first locus also indicated..... 80

Figure 3-13. Maximum likelihood gene order (a), and gene orders under 100:1 odds against (b), as well as the next most likely order (c) greater than 100 times worse than the best order for linkage group eight. Distance (cM) of loci relative to first locus also indicated. 81

Figure 3-14. Karyotype of male tammar wallaby. Chromosomes numbered in decreasing order of size with percent total length indicated for each chromosome. Karyotype provided by Mark Eldridge. Total length percentage values obtained from Hayman & Martin (1974). Location of physically mapped genes within linkage groups indicated with arrows. 100

Figure 4-1. Map of Australia showing geographical distribution (from Poole 1995) and sampling locations of *M. giganteus*. Sample locations identified by a “closed circle” constitute populations used to determine continental population genetic differentiation. Populations within “box” signify samples used to determine fine-scale population structure and dispersal. Sample localities represented by “letters” constitute additional individuals used in mtDNA variability and phylogeography analysis. Sample sizes and geographical location identified in Table 4-1 104

Figure 4-2. Variable sites of a 648-bp segment of mtDNA control region from 202 eastern grey kangaroos. Variable nucleotide positions are relative to the beginning of the sequence. Dots “.” represent identical bases to the first haplotype. Haplotypes are labelled relative to their geographical region i.e. ‘northern’ (e.g., N1), ‘southern’ (e.g., S1) or ‘Tasmanian’ (e.g., T1). 117

Figure 4-3. Genetic relationships between the eight populations sampled, as inferred from hierarchical analysis of nucleotide diversity. Values at nodes represent the proportion of total genetic diversity partitioned between the sister locations. Numbers in parentheses indicate the significance of these values..... 120

Figure 4-4. Relationship between pairwise *R* and corresponding distance values for male (A) and female (B) eastern grey kangaroos. Mantel test results for males $r = -0.000021$, $P = 0.75$ and females $r = -0.000017$, $P = 0.74$ 122

Figure 4-5. Neighbour-joining tree displaying relationships between eastern grey kangaroo mtDNA haplotypes and geographic region. *M. g. tasmaniensis* haplotypes identified by the arrow, while all other haplotypes are *M. g. giganteus* except for the outgroup which are *M. fuliginosus* (western grey e.g., WG1) individuals. Values at nodes indicate the statistical support as obtained from 1000 bootstrap replicates. Numbers in plain text are from NJ analysis while values in bold are derived from the MP method. 124

LIST OF TABLES

	<u>Page</u>
Table 2-1. Type I reference markers that have been used for linkage mapping in the tammar wallaby. Comparative marker locations based on physical and linkage mapping also indicated including human and mouse data.	8
Table 2-2. Linkage data from hybrid backcross progeny of <i>M. eugenii</i>	9
Table 2-3. Amplification details of existing microsatellite loci within the Macropodidae trialed on <i>M. eugenii</i> individuals. All annealing temperatures are in decrements of 2°C unless otherwise stated.	18
Table 2-4. Amplification details of <i>M. eugenii</i> and <i>M. giganteus</i> microsatellite loci trialed on both species. All annealing temperatures are in decrements of 2°C unless otherwise stated.	20
Table 2-5. Locus and diversity characteristics for microsatellites polymorphic within <i>M. eugenii</i> and / or source species. <i>M. eugenii</i> characteristics are indicated in bold, while source species is below in parentheses.	22
Table 2-6. Mean diversity indices of microsatellite loci amplified within <i>M. eugenii</i> . Microsatellite source species are ordered in increasing genetic distance down the column.	23
Table 2-7. Locus and diversity characteristics for microsatellites polymorphic within <i>M. eugenii</i> and / or <i>M. giganteus</i> . <i>M. eugenii</i> characteristics are indicated in bold, while <i>M. giganteus</i> is below.	24
Table 2-8. Mean diversity indices of <i>M. eugenii</i> and <i>M. giganteus</i> microsatellite loci amplified within both species. <i>M. eugenii</i> characteristics are indicated in bold, while <i>M. giganteus</i> is below.	25
Table 2-9. Degree of cross-species amplification within <i>M. eugenii</i> relative to evolutionary distance from source species.	41
Table 3-1. Mutation rates per locus within female backcross progeny.	63
Table 3-2. Total number of male and female informative meioses and segregation distortion / inheritance of F1 (KI-GI) × KI type crosses for each locus used in the genetic linkage map.	65
Table 3-3. Significant two-point linkage analysis results arranged into linkage groups. Group numbering is related to linkage group length. Map order corresponds to multipoint analysis.	68

Table 3-4. Multipoint linkage analysis results, including distance and standard error of each linkage group and overall total length.	69
Table 3-5. Likelihood ratio tests for sex-recombination heterogeneity using two-point LOD scores across autosomes. Significant results for intervals, linkage groups and overall is shown. Genetic distances and female / male ratios are also shown.	85
Table 3-6. Multipoint map function tests on the sex-average linkage groups one, two, four and five. Significant values indicated for each map function with map distances. Most likely map functions are in decreasing order for each group.	86
Table 3-7. Tentative assignment of linkage groups to chromosomes. Based on linkage groups sizes and physical assignment of genes.....	94
Table 4-1. Sample location and sample size for <i>M. giganteus</i> localities within Australia....	107
Table 4-2. Microsatellite locus characteristics including total number of alleles, allele size (bp) and F_{IS} calculated across all individuals.	112
Table 4-3. Allele frequencies for 10 microsatellite loci examined at eight populations. Numbers of individuals examined per locus per site are given in parentheses.....	113
Table 4-4. Microsatellite and mtDNA diversity indices for each population. Sample size shown in parenthesis next to sample location. Average number of alleles (A), average observed and expected heterozygosity (H_O and H_E) and F_{IS} shown for microsatellite data. Number of unique haplotypes, haplotypic diversity (h) and nucleotide diversity (π) shown for mtDNA data.....	115
Table 4-5. Differentiation between populations. (A) Pairwise Φ_{ST} values for mtDNA data below diagonal and pairwise θ_{ST} for microsatellite data above diagonal. (B) Pairwise number of migrants per generation between populations for mtDNA data (N_m) below diagonal and for microsatellite data (N_{em}) above diagonal.....	118
Table 4-6. Results of the assignment test. Sample origin is shown with number of individuals indicated in parenthesis. The percentage of individuals assigned correctly is shown in bold.	119
Table 4-7. Mean relatedness ($R \pm SD$) between pairs of females, males and total individuals within and among groups. Total number of pairwise comparisons (N) and significance values indicated for each category.	119
Table 4-8. Analysis of molecular variance for subdivisions of <i>M. giganteus</i> populations. ...	121
Table 4-9. Total number of <i>M. giganteus</i> mtDNA haplotypes sampled in each locality.....	125

G L O S S A R Y

- Ascertainment bias**¹ – The hypothesis that a microsatellite selected in a focal species will differ systematically from its orthologues in related species due to the criteria used to isolate it in the focal species.
- Cross species amplification** – Primer binding sites sufficiently conserved in a related species allows amplification using primers designed in a different species.
- Effective population size** – The number of individuals that actually reproduce and contribute genes to the next generations.
- Genic analysis** – allele and / or haplotype frequencies are population statistics that can be changed by genetic drift, founder effect, gene flow and selection. They are estimated most accurately from multiple, separate, nuclear loci and mtDNA.
- Genotypic analysis** – consist of composite genotypes of multiple loci. Individual genotypes are labile – a single round of sexual recombination usually destroys a genotype. They are quantified most rigorously using multiple single-locus nuclear markers (typically microsatellites).
- Gene synten** – Where loci are located on the same chromosome.
- Homoplasy** – Similarities in character states for reasons other than inheritance from a common ancestor. At a microsatellite locus, homoplasy occurs when two allelic lineages converge to the same size but have different histories of mutations. Thus, identity by state does not always entail identity by descent.
- Likelihood ratio** – The ratio of probabilities of obtaining the observed data under different hypotheses concerning the assumed model used to generate the expected values.
- Linkage disequilibrium** – Departure from the predicted frequencies of multiple locus gamete types assuming alleles of different loci are randomly associated.
- Meiotic drive** – Aberrant segregation ratios among the gametes of heterozygotes.
- Monophyletic group** – Set of species containing common ancestor and all its descendants.
- Nucleolar organiser region** – A region on a chromosome that contains the ribosomal RNA genes and associated spacers.
- Null allele** – An allele that fails to be expressed under the conditions analysed.
- Orthologous loci** – Loci in two or more species where sequences are similar because of their common derivation from a common ancestor.
- Paraphyletic group** – Set of species containing an ancestral species together with some, but not all, of its descendants. The species included in the group are those that have continued to resemble the ancestor; the excluded species have evolved rapidly and no longer resemble their ancestor.
- Parsimony** – Principle of phylogenetic reconstruction in which the phylogeny of a group of species is inferred to be the branching pattern requiring the smallest number of evolutionary changes.
- Polyphyletic group** – Set of species descended from more than one common ancestor. The ultimate common ancestor of all the species in the group is not a member of the polyphyletic group.
- $\Delta T_m H$** – The difference between the median melting temperature of homoduplex DNA and heteroduplex DNA formed in a DNA-DNA hybridisation reaction.

¹ In the context of this thesis.

ABBREVIATIONS

AR	androgen receptor
CASA	alpha - casein
cDNA	complementary DNA
cM	centimorgan
DBB	MHC class II B-chain
ESUs	evolutionary significant units
ETL	economic trait locus
FISH	fluorescence <i>in situ</i> hybridisation
G6PD	glucose-6-phosphate dehydrogenase
GI	'Garden Island'
HBB	haemoglobin beta chain
H_E	expected heterozygosity
H_O	observed heterozygosity
HPRT	hypoxanthine phosphoribosyltransferase
ISHR	<i>in situ</i> hybridisation radiolabeled probes
KI	'Kangaroo Island'
LALBA	alpha - lactalbumin
LD	linkage disequilibrium
LLP	late lactation protein
LPL	lipoprotein lipase
MFL	mean fragment length
MHC	multiple histocompatibility complex
MLE	maximum likelihood estimation
MP	maximum parsimony
MPI	mannose phosphate isomerase
mtDNA	mitochondrial DNA
MUs	management units
Mya	million years ago
N_e	effective population size
NJ	neighbour joining
NOR	nucleolar organiser region
NP	nucleoside phosphorylase
NSW	New South Wales
PGK1	phosphoglycerate kinase
PE	probability of exclusion
PIC	polymorphic information content
PCR	polymerase chain reaction
QLD	Queensland
QTL	quantitative trait locus
r	recombination fraction
RFLP	restriction fragment length polymorphism
RNR	ribosomal RNA
SDL	segregation distorting locus
SNPs	single nucleotide polymorphisms
SNuPE	single nucleotide primer extension analysis
SSCP	single strand conformation polymorphism
STRs	short tandem repeats
STRPs	short tandem repeat polymorphisms
SA	South Australia
TAS	Tasmania
TF	transferrin
tRNA	transfer RNA
VIC	Victoria

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 persons nor material which has been submitted for a higher degree to this or any other institution, except where due acknowledgement has been made in the text.



Kyall R. Zenger

November 2001

S U M M A R Y

The analysis of DNA using molecular techniques is an important tool for studies of evolutionary relationships, population genetics and genome organisation. The use of molecular markers within marsupials is primarily limited by their availability and success of amplification. Within this study, 77 macropodid type II microsatellite loci and two type I genetic markers were characterised within *M. eugenii* to evaluate polymorphic levels and cross-species amplification artifacts. Results indicated that 65 microsatellite loci amplified a single locus in *M. eugenii* with 44 exhibiting high levels of variability. The success of cross-species amplification of microsatellite loci was inversely proportional to the evolutionary distance between the macropod species. It is revealed that the majority of species within the Macropodidae are capable of using many of the available heterologous microsatellites. When comparing the degree of variability between source-species and *M. eugenii*, most were significantly higher within source species ($P < 0.05$). These differences were most likely caused by ascertainment bias in microsatellite selection for both length and purity.

The production of a marsupial genetic linkage map is perhaps one of the most important objectives in marsupial research. This study used a total of 353 informative meioses and 64 genetic markers to construct a framework genetic linkage map for *M. eugenii*. Nearly all markers (93.7%) formed a significant linkage ($\text{LOD} > 3.0$) with at least one other marker. More than 70% (828 cM) of the genome had been mapped when compared with chiasmata data. Nine linkage groups were identified, with all but one (LG7; X-linked) allocated to the autosomes. These groups ranged in size from 15.7 cM to 176.5 cM, and have an average distance of 16.2 cM between adjacent markers. Of the autosomal linkage groups, LG2 and LG3 were assigned to chromosome 1 and LG4 localised to chromosome 3 based on physical localisation of genes. Significant sex-specific distortions towards reduced female recombination rates were revealed in 22% of comparisons. Positive interference was observed within all the linkage groups analysed. When comparing the X-chromosome data to closely related species it is apparent that it is conserved both in synteny and gene order.

The investigation of population dynamics of eastern grey kangaroos has been limited to a few ecological studies. The present investigation provides analysis of mtDNA and microsatellite data to infer both historical and contemporary patterns of population structuring and dispersal. The average level of genetic variation across sample locations was exceedingly

high ($h = 0.95$, $H_E = 0.82$), and is one of the highest observed for marsupials. Contrary to ecological studies, both genic and genotypic analyses reveal weak genetic structure of populations where high levels of dispersal may be inferred up to 230 km. The movement of individuals was predominantly male-biased (average $N_e m = 22.61$, average $N_f m = 2.73$). However, neither sex showed significant isolation by distance. On a continental scale, there was strong genetic differentiation and phylogeographic distinction between southern (TAS, VIC and NSW) and northern (QLD) Australian populations, indicating a current and / or historical restriction of gene flow. In addition, it is evident that northern populations are historically more recent, and were derived from a small number of southern eastern grey kangaroo founders. Phylogenetic comparisons between *M. g. giganteus* and *M. g. tasmaniensis*, indicated that the current taxonomic status of these subspecies should be revised as there was a lack of genetic differentiation between the populations sampled.