

Phylogeny, phylogeography and conservation genetics of the *brachyotis* group of rock-wallabies.



Petrogale brachyotis (short-eared rock-wallaby)

Submitted by

Sally Potter

B. Science – honours

A thesis submitted in total fulfilment of the requirements for the degree of
Doctor of Philosophy

School of Earth and Environmental Sciences

Faculty of Science

The University of Adelaide

Adelaide, South Australia 5005

Australia

January 2011

TABLE OF CONTENTS

List of Figures	6
List of Tables	8
Acknowledgements	10
Thesis Summary	13
Statement of Authorship	15

CHAPTER 1: Introduction

Macroevolution	17
Microevolution	20
Molecular genetics and conservation	22
Biogeographical and evolutionary processes in northern Australia	24
Rock-wallabies: taxonomic history	26
The <i>brachyotis</i> group of rock-wallabies	32
Study objectives	36

CHAPTER 2: Phylogenetic relationships of *Petrogale* and their biogeographic history within Australia

Abstract	39
Introduction	40
Materials and methods	
Taxon sampling	43
DNA extraction, PCR-amplification and sequencing	44
Sequence analysis	48
Phylogenetic analyses	48
Molecular dating analyses	51
Biogeographic analyses	53

Results	
Sequence analyses: mtDNA & nDNA	54
Phylogenetic analyses: mtDNA, nDNA & combined dataset	55
Molecular clock analyses	60
Ancestral area reconstructions	63
Discussion	65
Conclusion	71

CHAPTER 3: Phylogeography of the *brachyotis* group across northern Australia identifies multiple biogeographic barriers

Abstract	74
Introduction	75
Materials and methods	
Sampling and DNA extraction	79
DNA amplification, cloning and sequencing	83
Sequence analyses	84
Phylogenetic analyses	85
Population genetic and historical demographic analyses	87
Results	
Sequence variability	89
Mitochondrial phylogeography	90
Lineage relationships inferred from nuclear regions	93
Population structure	94
Historical demographic analyses	97
Discussion	105
Conclusion	112

CHAPTER 4: Habitat connectivity among populations of the Short-eared Rock-wallaby (*Petrogale brachyotis*) in the Kimberley, Australia

Abstract	116
Introduction	117
Material and methods	
Sampling and DNA extraction	120
DNA amplification, microsatellite genotyping and mtDNA sequencing	123
Population genetic and phylogenetic analyses	125
Genetic structure and phylogeography	126
Estimation of dispersal patterns using assignment tests	128
Relatedness within populations	128
Results	
Genetic diversity	129
Genetic structure	132
MtDNA phylogeography	135
Estimation of dispersal patterns using assignment tests	135
Relatedness within populations	137
Discussion	139

CHAPTER 5: Genetic differentiation within the *brachyotis* group outlines new taxonomic classifications and conservation units

Abstract	150
Introduction	151
Materials and methods	
Samples and sequencing analyses	155
Morphological assessment	157
Results	
Sequence results	157

Morphological assessment	164
Discussion	167
Conclusion	172
CHAPTER 6: General Discussion	
Synthesis	173
Future research directions for northern Australia	175
Future research within the <i>brachyotis</i> group of rock-wallabies	177
Research priorities for <i>Petrogale</i>	179
Conclusion	181
APPENDICES	
Appendix 1	182
Appendix 2	183
REFERENCES	
	184

NOTE:

All files on the CD are in FAS file format.
An appropriate program will need to be used to view these files.
CD accompanies the print copy of the thesis held in the
University of Adelaide Library.

LIST OF FIGURES

Figure 1.1

Map of *Petrogale* species distributions in Australia.

Figure 2.1

Map of *Petrogale* species distributions in Australia together with ancestral area states.

Figure 2.2

BEST species tree of *Petrogale* taxa inferred using both nuclear and mitochondrial data, including posterior probabilities.

Figure 2.3

Mitochondrial tree of combined data (*COI*, *Cytb* and *ND2*) for *Petrogale* taxa inferred using MrBayes version 1.3.2 using codon-partitions.

Figure 2.4

Concatenated nuclear tree of *Petrogale* taxa based on a partitioned Bayesian inference inferred using MrBayes version 1.3.2.

Figure 2.5

Chronogram of *Petrogale* taxa inferred from a relaxed molecular clock in BEAST based on the normal prior distribution of fossil calibrations.

Figure 2.6

Reconstruction of ancestral area states for *Petrogale* using DIVA and BayesTraits.

Figure 3.1

Distribution and sampling localities of *P. brachyotis*, *P. burbidgei* and *P. concinna* across northern Australia.

Figure 3.2

Mitochondrial tree of *P. brachyotis*, *P. burbidgei* and *P. concinna* including Bayesian posterior probabilities and maximum likelihood bootstrap values.

Figure 3.3

Nuclear haplotype networks for *BRCA1*, A1, A2 and ω -globin for the *brachyotis* group.

Figure 3.4

Marginal posterior density distributions of migration and population sizes estimated from the isolation with migration program based on nDNA and mtDNA for four divergences within the *brachyotis* group.

Figure 3.5

Chronogram of *brachyotis* group taxa inferred from a relaxed molecular clock in BEAST based on the normal prior distribution of fossil calibrations.

Figure 4.1

The distribution and sampling localities of *P. brachyotis* along with sample sizes at each site in the Kimberley, Western Australia.

Figure 4.2

Proportional membership (Q) of *P. brachyotis* individuals into East and West Kimberley clusters identified by STRUCTURE.

Figure 4.3

Unrooted neighbour-joining tree displaying Nei's genetic distance (Nei's D from microsatellite genotypes) between sampled populations of *P. brachyotis*.

Figure 4.4

Mitochondrial tree of *P. brachyotis* haplotypes from the East and West Kimberley with maximum likelihood bootstrap values and Bayesian posterior probabilities.

Figure 4.5

Mean pairwise relatedness within populations of *P. brachyotis* from the East and West Kimberley for males and females, including error bars associate with 95% confidence bounds.

Figure 5.1

Sampling localities and locality names of *P. brachyotis*, *P. burbidgei* and *P. concinna* in northern Australia.

Figure 5.2

Mitochondrial tree of *P. brachyotis*, *P. burbidgei* and *P. concinna* taxa, including Bayesian posterior probabilities and maximum likelihood bootstrap values.

Figure 5.3

Nuclear haplotype networks of *BRCA1*, *A1*, *A2* and ω -globin for the *brachyotis* group of rock-wallabies.

Figure 5.4

Photographs representing the variation in morphology of *P. brachyotis* from the Northern Territory, Victoria River region and Kimberley.

LIST OF TABLES

Table 1.1

Summary of chromosome data for *Petrogale* taxa.

Table 1.2

Chromosomal and morphological characteristics which separate the *brachyotis* species.

Table 2.1

Collection localities and sources of *Petrogale*, *Thylogale* and *Dendrolagus* taxa.

Table 2.2

Primers used to amplify segments of *Cytb*, *ND2*, *COI*, *ω -globin* and *BRCAL* within *Petrogale* taxa.

Table 2.3

Summary of the nucleotide substitution models implemented in MrBayes and RAxML, selected for data partitioning using the Akaike Information Criterion in Modeltest 3.06.

Table 3.1

Collection localities of *P. brachyotis*, *P. burbidgei* and *P. concinna*.

Table 3.2

Primers used to amplify segments of *CR*, *ND2*, *BRCAL*, *ω -globin*, A1 and A2.

Table 3.3

Descriptive statistics for mtDNA, *BRCAL*, A1, A2 and *ω -globin*, includes: sequence length (*L*), number of samples (*n*), number of haplotypes (*H*), number of segregating sites (*S*), haplotype diversity (*h*) and nucleotide diversity (π).

Table 3.4

Results of analyses of molecular variance for mtDNA, *BRCAL*, *ω -globin*, A1 and A2 for: *P. brachyotis* (Northern Territory), *P. burbidgei*, *P. brachyotis* (Victoria River), *P. concinna* and three *P. brachyotis* (Kimberley) groups; EK1, EK2 and WK.

Table 3.5

Demographic results of each independent locus for all geographic subdivisions of *P. brachyotis* (NT – Northern Territory, VR – Victoria River, WK – West Kimberley and two East Kimberley – EK1 and EK2) including results for *P. burbidgei*.

Table 3.6

Results of isolation with migration analysis of mtDNA subdivisions within the *brachyotis* group, including: θ_1 and θ_2 (population sizes); θ_A (ancestral population size); m_1 and m_2

(migration rate at which genes come into the two populations); and t indicates the divergence time between the two populations.

Table 3.7

Population migration rates (m_1, m_2) for the *brachyotis* group populations, which represent the migration rate at which genes come into the populations.

Table 4.1

Location of sampled *P. brachyotis* populations and the number of identified mtDNA haplotypes.

Table 4.2

Summary of genetic diversity indices for sampled populations of *P. brachyotis*, including: haplotype diversity (h), nucleotide diversity (π), allelic diversity (AD), allelic richness (AR), mean expected heterozygosity (H_E), unique alleles (A_U) and inbreeding levels (F_{IS}).

Table 4.3

Genetic differentiation (pairwise Φ_{ST} of mtDNA and F_{ST} for microsatellites) between sampled *P. brachyotis* populations (Couchman – C; Cyprus Valley – CV; Monsmont Island – M Is; Bullanyin Island – B Is; King Edward River – KER; and Pump Hill – PH).

Table 4.4

Reported allelic diversity (AD) and average heterozygosity (H_E) at polymorphic microsatellite loci in 25 populations of *Petrogale* which were assessed using an assortment of the markers used in this study.

Table 5.1

Pairwise genetic sequence divergence between mtDNA lineages of the *brachyotis* group using a Tamura-Nei distance model.

Table 5.2

Results of analyses of molecular variance for mtDNA, *BRCA1*, ω -globin, A1 and A2 for the *brachyotis* group of rock-wallabies.

Table 5.3

Population migration rates (m_1, m_2) estimated from the isolation with migration program.

Table 5.4

Pelage characteristics of *P. brachyotis* populations from four geographic regions.

ACKNOWLEDEMENTS

First of all I would like to thank my supervisors Dr David Taggart and Dr Mark Eldridge, who without their vision, this project would not have commenced. Their collaboration and insight allowed me to explore this amazing Australian marsupial and visit incredible places in the Kimberley. Each of my supervisors has contributed differently to my development as a professional researcher and I am thankful for various reasons. Thank you to Dr David Taggart who has included me in any and every opportunity he could, to experience as much of the marsupial world as possible. His friendship, guidance, encouragement and support have helped me through some tough times throughout my PhD and kept me in a positive state of mind. To Dr Mark Eldridge, thank you for being available for weekly updates and keeping my stress levels minimal. You have inspired me in marsupial genetics and helped me to develop a critical thought process and gotten me hooked on this intriguing genus. Your friendship and encouragement has been invaluable throughout my study. I would also like to thank Dr Steve Cooper, who came on board to support me in my genetic analyses. You have helped me to develop my skills in this field and think about evolution in a clear and concise manner. I want to thank you for your support, time and efforts throughout my candidature to produce the best research out of me.

I would like to thank and make mention of all of the people who I have worked with in the field, who have taught me a variety of skills which I will hold onto forever. First of all I would like to thank the Myers family, whom without their financial support and inclusion of me on field surveys on their properties in the Kimberley, my research would not have happened. Thanks to Tags, Jim, Cecelia, George, Alex, Henry, Raz and Maria who all helped me in my trapping efforts on Doongan and Theda Stations. A special thanks to Liberty, who has provided me friendship and support throughout my PhD and provided assistance whenever she possibly could. I would also like to thank Susan Bradley and the Hallen family who looked after us whilst in the field and to all others involved in the smooth running of these fieldtrips. Thanks to David Pearson for allowing me to volunteer in the field with his staff and trap on the islands in Lake Argyle, at Mitchell Plateau and in Mirima National Park. Thanks to Emily and Bill his work colleagues who helped me on these trips and to all the other DEC employees on this trip who helped in my trapping success.

The rest of my time throughout my PhD was spent in the evolutionary biology unit (EBU) at the University of Adelaide and there are numerous people within the lab whom I'd like to thank for their support and guidance throughout my study. In particular, Kathy Saint who provided amazing assistance and knowledge in microsatellite genotyping, Dr Gaynor Dolman, Dr Lizzie Perkins and Mark Siström, who provided valuable support and friendship, as well as valuable advice about my work when we had long discussions over coffees and drinks. To Kym and Jo for their friendship in and out of the lab, and to Dr Terry Bertozzi, for all of his assistance and friendship throughout my years at the University. Thanks to Dr Steve Donnellan who also provided assistance when I required it and for taking an interest in my work, and Dr Jeremy Austin for also helping me throughout my candidature.

I would also like to mention special thanks to the Taggart and Carthew lab groups. It was a real privilege to attend Carthew lab meetings and expand my knowledge through interactions with all of Sue's students. Also being able to discuss my work amongst other students allowed me to better focus my energies and gain the most from this experience. I would like to make special mention to Dr Melanie Lancaster, whom I have turned into a sponge around and absorbed as much as I possibly could from. Her interest, support and encouragement have made an immense difference to my approach over the last year in particular and opened up my eyes to a broader field of research. Her friendship has been invaluable and aided in my personal wellbeing. I'd also like to make a special mention to Elisa, Bec and Nik, whose friendships have been important to me throughout the last few years, but to all members of these groups as I have formed a friendship with all of you and look forward to continuing updates about your work and life. Also a special thanks to Skye, Imogen, Rupert and Matt in the office who have provided comic relief and support throughout the last couple of years.

Thank you to my co-authors for their contributions to my manuscripts and for putting in the time and effort, especially when they've had their hands full. Thank you to Justyna Paplinska for making available the time to go through non-invasive techniques with me and providing expertise in this field. Thank you to Cushla Metcalfe for allowing me to include her DNA sequences in my analysis of *Petrogale*.

In particular I would like to thank my friends and family, most of whom have had very little understanding of what I have been doing over the last few years but have supported me none

the less. Their ability to help take the pressure off was much appreciated and needed. To Missy, Han and Laursh, who've always supported my interests and kept me sane (as best they could). Thanks to Alana, Meg, Presh, Shez and all of the basketball girls for supporting me and providing me an outlet from my PhD world and keeping me in tune with „real“ life. Thanks to my sister Amy and Candace, who provided friendship and support and gave me a sanctuary in a time of need. Thanks to Mark, Shane, Kristy, Bradley, Tyson and Mikayla for bringing light to work which at times consumed me. Thanks to mum and dad for supporting me through all of my endeavours and providing a relaxing space for me to visit. Your encouragement and love has helped me through the tough times. I would like to thank Justin, who has been my rock throughout my PhD. He's provided support, encouragement and challenged me to further myself in every possible way and helped me to achieve the most from my studies. He's listened to all of the day to day issues that arise throughout a PhD and taken it on board, to provide me the strength that I needed. I would also like to thank his family for their support throughout the last three years.

Lastly I would like to thank the funding bodies, who without their financial support this work could not have been achieved. Thanks to the University of Adelaide, Holsworth Wildlife Research Endowment, Dunkeld Pastoral Company, the Australian Museum and the Schultz Foundation.

THESIS SUMMARY

This thesis explores phylogenetic relationships amongst rock-wallabies, genus *Petrogale*, throughout Australia and in particular the phylogeographic relationships of three species (*brachyotis* taxonomic group) from north-west Australia. A combination of nuclear and mitochondrial DNA markers have been utilised to assess evolutionary history at several spatial scales. The first data chapter assesses broad scale relationships amongst rock-wallaby species with subsequent chapters examining the impacts of biogeographic processes on genetic diversification within the *brachyotis* group and conservation implications. These studies have increased our understanding of rock-wallaby evolution and provide valuable data to support the recognition of multiple species within *Petrogale brachyotis* (short-eared rock-wallaby). It has also established new hypotheses about the relationships of *P. burbidgei* (monjon) and *P. concinna* (nabarlek) to *P. brachyotis* within the *brachyotis* group. Although *P. brachyotis* is widespread, we have found this species is highly divergent across its range, with future management needing to ensure the survival of multiple highly diverse genetic lineages.

The phylogenetic analysis (chapter two) identified four distinct clades within *Petrogale*, with three comprising taxa with the ancestral karyotype ($2n=22$). The *brachyotis* group was the first to diverge and phylogenetic relationships within this lineage suggest the need for a focused phylogeographic study of this group and the likelihood of taxonomic revisions. There was support for *P. purpureicollis* being reinstated as a full species and *P. concinna* being classified within *Petrogale* rather than the monotypic genus *Peradorcas*. Ancestral habitat reconstructions suggested ancestral *Petrogale* were originally widespread across Australia and have undergone vicariance as a result of isolation caused by environmental/climatic changes during the Plio-Pleistocene.

The third chapter concentrated on north-west Australia, the main focus of this thesis, and its numerous proposed biogeographic barriers which have remained largely untested by phylogeographic studies. This thesis provides the first evidence of how these barriers have profoundly influenced the genetic differentiation of mammals within north-west Australia. Rock-wallabies, with their habitat specificity and naturally low gene flow are a good indicator

species for biogeographic barriers and provide good evidence of how past climatic cycles during the Plio-Pleistocene have influenced genetic differentiation across northern Australia.

The fourth chapter was more localised and focused on the genetic diversity of *P. brachyotis* within the Kimberley. This study provided evidence of greater connectivity of populations than previously recorded for any other rock-wallaby, highlighting how suitably connected habitat can allow dispersal of rock-wallabies across large distances. Large genetic differentiation was detected between the East and West Kimberley *P. brachyotis*, supporting the need for a reassessment of taxonomic classifications and conservation units within *P. brachyotis*.

The final data chapter examined the conservation status of the *brachyotis* group of rock-wallabies and outlined taxonomic reclassifications of *P. brachyotis*. This analysis provided evidence for division of *P. brachyotis* into two species. It is likely that additional taxonomic changes will be necessary in the future as further sampling and analyses are undertaken. Although this thesis has significantly advanced our understanding of the relationships within the *brachyotis* group, it also highlights the need for future work on this group of rock-wallabies.

STATEMENT OF AUTHORSHIP

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Sally Potter and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

This thesis represents an original and independent piece of research. All significant aspects of analysis and interpretation of results were done by myself. The thesis is presented as a series of papers. The nature of the collaborations indicated by the co-authorship of these papers takes two forms: 1) Authors D.A. Taggart, M.D.B. Eldridge and S.J.B. Cooper were included in recognition of the contribution they have made to my training as my supervisors. 2) Author C.J. Metcalfe was included as co-author in recognition of her providing *Cytb* sequence data. 3) Author J.Z. Paplinska was included as co-author in recognition of her training in genotyping microsatellites from rock-wallaby faecal samples and expert advice. I carried out all further analyses of this material. These contributions in no way diminish the originality or my overall contribution to the thesis.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

This study was funded by a University of Adelaide Divisional Scholarship awarded to Sally Potter. All research procedures reported in the thesis were approved by the University of Adelaide Animal Ethics Committee and all permits were issued by the Western Australian Government.

Sally Potter