Using high-throughput DNA sequencing and molecular phylogenies to investigate the evolution and biogeography of the southern hemisphere fauna

Kieren James Mitchell

Australian Centre for Ancient DNA
School of Biological Sciences
Faculty of Sciences
University of Adelaide

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

June 2015
# TABLE OF CONTENTS

**THESIS ABSTRACT** ............................................................................................................................................. v  
**THESIS DECLARATION** ................................................................................................................................. vii  
Publications......................................................................................................................................................... ix  
**ACKNOWLEDGEMENTS** ................................................................................................................................... xi  

**CHAPTER 1: General Introduction** .................................................................................................................. 1  
Background .......................................................................................................................................................... 3  
Southern hemisphere biogeography ..................................................................................................................... 5  
Phylogenetics for biogeographical hypothesis testing ......................................................................................... 8  
Ancient DNA and next-generation sequencing ................................................................................................. 13  
Pros and cons of mitochondrial DNA .................................................................................................................. 18  
Summary .............................................................................................................................................................. 23  
Thesis Overview .................................................................................................................................................. 24  
References ........................................................................................................................................................... 29  

**CHAPTER 2: Molecular phylogeny, biogeography, and habitat preference**  
evolution of marsupials ........................................................................................................................................ 41  
Supplementary Information ................................................................................................................................. 57  

**CHAPTER 3: Ancient DNA reveals elephant birds and kiwi are sister taxa and clarifies ratite bird evolution** ........................................................................................................................................... 77  
Supplementary Information ................................................................................................................................. 85  

**CHAPTER 4: Origin and evolution of the New Zealand wrens (Acanthisittidae)**  
........................................................................................................................................................................ 127  
Abstract ............................................................................................................................................................... 131  
Introduction .......................................................................................................................................................... 132  
Results ................................................................................................................................................................. 136  
Discussion ............................................................................................................................................................ 145  
Methods .............................................................................................................................................................. 151  
Acknowledgements ............................................................................................................................................ 164  
References .......................................................................................................................................................... 165  
Supplementary Information ................................................................................................................................. 173
CHAPTER 5: Ancient mitochondrial genome reveals unsuspected taxonomic affinity of the extinct Chatham duck (*Pachyanas chathamica*) and resolves divergence times for New Zealand and sub-Antarctic brown teals

Supplementary Information

CHAPTER 6: An extinct nestorid parrot (Aves, Psittaciformes, Nestoridae) from the Chatham Islands, New Zealand

Supplementary Information

CHAPTER 7: Ancient DNA analyses of mammalian megafauna from La Chumbiada (Argentina; South America)

Abstract
Introduction
Methods
Results
Discussion
References
Supplementary Information

CHAPTER 8: General discussion and concluding remarks

Summary, synthesis and significance
Limitations of single loci
Building better datasets
Inferring accurate evolutionary timescales
Future directions
Conclusion
References

APPENDIX: Molecular dating, genomic data, and the temporal origin of modern birds

Abstract
Introduction
Methods
Results
Discussion
Acknowledgements
References
Supplementary Information
THESIS ABSTRACT

Biogeography is the study of how and why organisms are distributed the way they are, and is consequently intimately tied to evolution. By investigating biogeographic patterns we can learn more about fundamental evolutionary processes and the history of life on Earth. Molecular phylogenies are an invaluable tool for biogeographical hypothesis testing, allowing the relationships among taxa to be confidently reconstructed and the timescale of their evolution to be estimated. However, many biogeographic hypotheses have not been extensively evaluated in a phylogenetic context due to difficulties associated with obtaining sufficient nucleotide sequence data to construct adequately resolved phylogenies. In the past, a major obstacle to this process was the amount of labour and expense involved in generating large quantities of sequence data. However, the recent advent of high-throughput sequencing has revolutionised the collection of nucleotide sequence data, greatly decreasing the costs associated with generating large nucleotide sequence datasets.

A second problem for building molecular phylogenies is obtaining sequence data from degraded sub-fossil remains of extinct species. A large proportion of the world’s terrestrial megafauna became extinct within the last fifty thousand years, and understanding the relationships of these species to their extant relatives is crucial for testing many biogeographical and evolutionary hypotheses. While high-throughput sequencing provides many benefits for the sequencing of ancient DNA, methods are still required to increase the concentration of target endogenous molecules in order to make sequencing cost-effective. One solution to this problem is hybridisation enrichment.
In this thesis I use both hybridisation enrichment and high-throughput sequencing to gather nucleotide sequence data from a range of extant and extinct southern hemisphere species in order to construct well resolved phylogenies. I sequence near-complete mitochondrial genomes from extinct elephant birds from Madagascar (*Aepyornis* and *Mullerornis*), acanthisittid wrens from New Zealand (*Pachyplichas, Traversia* and *Xenicus*), the Chatham Island duck (*Pachyanas*), and South American horses (*Hippidion*) and glyptodontids (*Glyptodon*). I am also able to retrieve fragments of mitochondrial DNA from the previously undescribed (extinct) Chatham Islands parrot. In addition to data from these extinct species, I obtain mitochondrial genomes from 69 extant marsupial species, tripling the number of marsupials for which mitochondrial genomes are available. Using these new data I investigate how patterns of bird and mammal distribution have been influenced by important geological events that shaped the southern hemisphere over the past 100 million years: the breakup of Gondwana during the Cretaceous, Palaeocene and Eocene; the submergence of Zealandia in the Oligocene; the emergence of New Guinea and Wallacea beginning in the Miocene; and formation of the Isthmus of Panama and emergence of the Chatham Islands archipelago in the Pliocene. Ultimately, I resolve several long-standing evolutionary mysteries, most prominently the geographical origin of the flightless ratite birds: I demonstrate that their modern distribution is the result of overwater dispersal by flighted ancestors rather than Gondwanan vicariance as traditionally thought. I also highlight how taxon sampling, model choice, and calibration of the molecular clock can impact our evaluation of different biogeographical and evolutionary scenarios.
THESIS DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works.

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 Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

.................................................. ..................................................
Kieren James Mitchell Date
Publications


ACKNOWLEDGEMENTS

Firstly I would like to thank my principal supervisor, Alan Cooper, for giving me the opportunity to work at ACAD, and for his great advice and tuition during my candidature. I’d also like to thank my other supervisors Mike Lee and Matt Phillips for always being available to discuss theoretical and practical issues, and providing valuable feedback on my work.

Thanks to everyone at ACAD (past and present) for maintaining a supporting and collaborative work environment, providing guidance, and partaking in constructive and informative discussions. I would particularly like to acknowledge: Bastien Llamas, Jess Metcalf, Kyle Armstrong, Steve Richards, Janette Edson and Jeremy Austin for their direct tutelage in the wet lab; Bastien Llamas (again), Julien Soubrier and Jimmy Breen for their help with bioinformatics and computer analyses; and Maria Lekis for her tireless administrative efforts. Thanks also to all my collaborators outside of ACAD, both local and international – your assistance has been invaluable.

For providing the financial resources necessary to complete the work I have undertaken I would like to thank the Australian Research Council and the New Zealand Marsden Fund Council, and therefore ultimately the tax-paying public (both of Australia and New Zealand).
I would also like to thank all of my amazing friends, fellow PhD students, and partner Laurati, for their patience, camaraderie, sympathy, understanding and moral support over the last few years.

Finally I would like to thank my biggest fans and number one supporters, my parents Jim and Sally, for their moral and financial support not only throughout my candidature but also in getting me to the point where I could undertake a PhD in the first place. None of my personal achievements could have been possible without their effort, and they have my eternal love and gratitude.