

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

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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.

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Publications

Mitchell, K.J., Pratt, R.C., Watson, L.N., Gibb, G.C., Llamas, B., Kasper, M., Edson, J., Hopwood, B., Male, D., Armstrong, K.N., Meyer, M., Hofreiter, M., Austin, J., Donnellan, S.C., Lee, M.S.Y., Phillips, M.J., Cooper, A., 2014. Molecular phylogeny, biogeography, and habitat preference evolution of marsupials. *Molecular Biology and Evolution* 31 (9), 2322-2330

Mitchell, K.J., Llamas, B., Soubrier, J., Rawlence, N.J., Worthy, T.H., Wood, J., Lee, M.S.Y., Cooper, A., 2014. Ancient DNA reveals elephant birds and kiwi are sister taxa and clarifies ratite bird evolution. *Science* 344, 898-900.

Mitchell, K.J., Wood, J.R., Scofield, R.P., Llamas, B., Cooper, A., 2014. 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. *Molecular Phylogenetics and Evolution* 70, 420-428.

Wood, J.R., **Mitchell, K.J.**, Scofield, R.P., Tennyson, A.J.D., Fidler, A.E., Wilmshurst, J.M., Llamas, B., Cooper, A., 2014. An extinct nestorid parrot (Aves, Psittaciformes, Nestoridae) from the Chatham Islands, New Zealand. *Zoological Journal of the Linnean Society* 172, 185-199.

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