

*Ancient DNA in the Australian context: Investigating
evolutionary and ecological responses to
environmental change.*

Vicki Ann Thomson

Australian Centre for Ancient DNA
School of Earth and Environmental Sciences
University of Adelaide

A thesis submitted for the degree of Doctor of Philosophy at the University of Adelaide

November 2012

Table of Contents

Table of Contents	iii
Abstract	ix
Thesis declaration	xi
Acknowledgements	xii
Chapter 1: Introduction	1
Phylogeography	1
Definitions and background	1
Spatial arrangement of genetic diversity.....	1
Incorporating other fields into phylogeography.....	2
Ancient DNA	3
Definitions and background.....	3
The history of ancient DNA.....	3
Polymerase Chain Reaction (PCR).....	4
Contamination	4
Degradation processes	5
DNA preservation.....	5
Natural History Collections.....	7
Ancient DNA in the Australasian region.....	8
The Australasian region	8
Australia’s arid zone	9
Tropical sites in the Pacific	10
Caves as repositories of faunal remains.....	12
Applications of ancient DNA	12
Taxonomy, Natural History and Evolutionary biologists	13
The Emu	13
The Emu: morphology.....	13
The conservation of vulnerable species.....	14
The Ghost Bat: extinction hypotheses.....	14
Anthropology and Archaeology	15
Colonisation of the Pacific	15
The Commensal Model	15
Haplogroups D and E: the Pacific	16
Polynesian chickens in South America	16
Thesis outline	18
References	19
Chapter 2	35
Methodology	35
Ancient DNA laboratory methods.....	35

Extraction method.....	36
Population genetic statistics	37
The coalescent.....	39
Bayesian Evolutionary Analysis of Sampling Trees (BEAST).....	40
Approximate Bayesian Computation (ABC).....	44
Bayesian Serial SimCoal (BayeSSC)	45
Demographic and Range expansion tests in Arlequin.....	47
Transforming expansion time estimates to a demographic scale	50
Ecological Niche Modelling.....	51
Theory.....	51
Reducing complexity using Ecological Niche Factor Analysis (ENFA).....	52
Maximum Entropy (MaxEnt).....	54
Statistical tests of significance.....	55
Conclusion.....	57
References	58
Chapter 3: The Emu	65
Postglacial expansions from a south-western refugium: reconstructing the recent evolutionary history of Emus (<i>Aves: Dromaius</i>) using ancient DNA.....	67
Abstract:	67
Introduction	68
Materials and Methods	76
Samples.....	76
aDNA Extraction, Amplification, and Sequencing.....	76
Morphometric analyses.....	76
Population Genetic Analyses.....	77
Phylogenetic and phylogeographic analyses.....	77
Population demographic structure.....	78
Results.....	78
Phylogenetic analyses of island taxa vs. the mainland Emu.....	79
Morphometrics of dwarfed island vs. mainland Emu populations	83
Phylogenetic and phylogeographic analyses of the mainland Emu	84
Reconstructing Emu demographic history	87
Discussion	96
The evolutionary origin of dwarf island Emus.....	96
Demography - west to east spatial expansion at 350 kya	98
Demography – western expansion at 100 kya	100
Conclusion.....	101
Acknowledgements.....	102
Supplementary Materials and Methods.....	103

DNA extraction and sequencing	103
Morphometric analyses.....	104
Population Genetic Analyses.....	105
Phylogenetic and phylogeographic analyses	106
Population demographic structure	107
Supplementary Information.....	109
Supplementary Tables and Figures	109
References	131
Chapter 4: the Ghost Bat.....	141
A Ghost of a chance? Evolutionary history of the Ghost Bat (<i>Macroderma gigas</i>) and its chances of surviving future climate change	145
Abstract	146
Introduction.....	148
Methods	152
Dataset collection (M. gigas presence localities).....	152
DNA extraction and sequencing	153
Phylogenetic and phylogeographic analyses	154
Population genetic and demographic analyses.....	154
Climatic Envelope reconstruction	155
Presence and pseudo-absence dataset generation	157
Results.....	157
Population genetic and demographic analyses.....	157
Intra-specific Diversity	157
Environmental variables involved in extinctions	161
Climatic modelling.....	165
Last Glacial Maximum (LGM).....	165
Last Inter-Glacial (LIG)	166
Future.....	166
Discussion.....	168
Genetic substructuring	168
Northern colonies	168
Central West.....	168
Central East.....	169
Temporal signatures	170
Climate modelling signatures	174
Assumptions inherent in the ecological niche modelling used	175
Future distributions.....	175
Conclusion	176
Supplementary Information.....	178

Chapter 5: The Polynesian chicken	193
Don't count your chickens: Resolving migration patterns of humans and commensals across the Pacific.	197
Abstract.....	198
Introduction	199
Using chickens to investigate Polynesian contact with South America	202
Results.....	203
Damaged nature of ancient Pacific chicken samples	204
Phylogeography	206
Discussion	210
Phylogeography of D_{AP} and D_{WP}	210
South American contact	212
Materials and Methods	213
Supplementary Information - Materials and Methods.....	215
Replication.	217
Sequence analysis	218
Supplementary Information	220
Ancient Pacific samples	220
Independent replication.....	220
Cloning	220
Shrimp DNase experiment to test ACAD3890 and ACAD9060 samples	221
Phylogenetic analyses including Storey <i>et al.</i> (2007) data	222
References	271
Chapter 6	279
Discussion and Conclusion.....	279
Overall summary of this thesis	279
Chapter 2: Methodology of this thesis	280
Chapter 3: the Emu (<i>Dromaius spp.</i>).....	281
Issues encountered.....	281
Sample size	281
Sample preservation.....	282
Independence of sampling.....	283
Island formation as calibration dates	284
Expansion tests in Arlequin.....	286
Linking dates to climate events	291
Other demographic events	292
Significance of the work	294
Future directions.....	295

Chapter 4: the Ghost Bat (<i>Macroderma gigas</i>)	297
Issues Encountered.....	297
Sample Type	297
Calibrations.....	297
BEAST vs. *BEAST: the Ghost Bat.....	298
Assumptions.....	302
Sample size	304
Expansion tests from Arlequin.....	304
Significance.....	305
Future work.....	306
In the short-term	306
In the long-term	306
Chapter 5: the Polynesian Chicken (<i>Gallus gallus</i>)	307
Issues Encountered	307
Sample size	308
Replication	308
Independence of sampling	308
Significance.....	309
Future work.....	310
In the short-term	310
In the long-term	311
Future work in general.....	312
Concluding remarks	313
References	315

Ancient DNA in the Australian context: Investigating evolutionary and ecological responses to environmental change.

Abstract

This thesis investigates how ancient DNA (aDNA) can provide insight into past (and potentially lost) genetic diversity, and how this information can be used to reconstruct evolutionary history. Specifically, it examines how the combination of aDNA data with other analytical techniques can reveal information unavailable from modern genetic studies of mitochondrial DNA, and help to refine models and test hypotheses about the history of individual species through time. Understanding ‘patterns’ of past genetic diversity and the ‘processes’ involved in forming these patterns at different temporal and spatial scales is of critical importance in the management of biodiversity, and for predicting future impacts of on-going environmental changes.

This thesis has particular significance for the ancient DNA community in Australia as it demonstrates how DNA may be preserved even in sub-optimal environmental conditions, such as tropical, sand-blown, and arid environments. The genetic information successfully extracted from these sub-optimal samples has allowed a range of phylogeographic questions to be addressed. The overarching question I ask in these studies is: what insights can aDNA provide into evolutionary history and, where these insights contrast with existing models, which additional analytical techniques help clarify or refine these models?

Chapter 2 (Methods) details the optimisation of extraction methods for the isolation of ancient DNA from sub-optimal samples (where often only minimal sample volumes are available for study). I then review the analytical techniques used to investigate the evolutionary history of the three species described in the following chapters.

The first species examined (chapter 3), is the Australian Emu species complex (*Dromaius novaehollandiae*, *D. baudinianus*, *D. ater*, and *D. novaehollandiae diemenensis*). I use both morphological methods and coalescent analysis to investigate the evolutionary history of emu, and the basis for separate taxonomic designations of the Tasmanian Emu, and the dwarf Kangaroo Island and King Island Emus. The morphological differences of the latter two species are first used to investigate potential causes of their dwarfism. Based on this assessment, I use genetic data to examine the demographic/dispersal history and phylogeographic structuring of the mainland Emu across its range.

In chapter 4, I investigate the range restricted and vulnerable Australian Ghost Bat (*Macroderma gigas*). Ancient DNA from subfossil cave deposits is used to describe the genetic relationship between extinct southern and extant northern populations and highlight how ancient DNA is essential in examining ancient patterns of gene flow. I then use species distribution modelling to test whether changing climates are responsible for the isolation and extirpations of these southern populations. I approximated the ecological niche currently occupied by Ghost Bats and then hindcast to the last glacial maximum (LGM) and last interglacial (LIG) to examine potential dispersal opportunities in the past, and forecast using models of projected future climates to investigate likely responses to anthropogenic climate change.

In chapter 5, I explore the use of chicken (*Gallus gallus*) genetic diversity as a record of the migration of early Polynesians across the Pacific. The genetic analysis of commensals allows identification of population bottlenecks and examination of potential source populations, and also a separate means to establish the route that their human-counterparts travelled. I incorporate archaeological models of Polynesian history into patterns of chicken diversity across the Pacific, and test hypotheses of potential dispersal routes of this human-commensal dyad using statistical significance testing.

Thesis declaration

I, Vicki Thomson certify that 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 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.

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

Vicki Thomson

16th November 2012

Acknowledgements

Firstly, I would like to thank my supervisors, Professor Alan Cooper, Professor Steve Donnellan, and Dr. Ken Aplin. Their assistance and encouragement during my PhD was greatly appreciated. Also I would like to thank the School of Earth and Environmental Sciences at the University of Adelaide for supporting my candidature.

I would like to greatly thank all the members of the Australian Centre for Ancient DNA (ACAD) lab for their help, support, provision of alcohol, and wide range of knowledge, which was freely shared. In particular, I would like to thank my two thesis mates, Clio Der Sarkissian for help with BayeSSC scripts and late night chats, and Julien Soubrier for general discussions about BEAST, manuscript and thesis editing, and late night chats. Going through the final stages of a PhD is helped enormously when you have others to share it with. Also I'd like to thank those who've provided great support in the lab and/or over lunches and after-work drinks, including Maria Lekis (our unofficial social director), Bastien Llamas, Wolfgang Haak, Marta Kasper (Nanna Kasper), Jessica Metcalfe, and Janette Edson. In addition, I'd like to thank the ACAD thesis writing group for reading and providing feedback on various parts of my thesis.

My final thank you is to family and friends, especially those that have put up with missed birthdays, and emails with no response, during the final stages of the PhD.