Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

## Ancient DNA studies of the New Zealand kiwi and wattlebirds: evolution, conservation and culture.

Lara Dawn Shepherd

A thesis presented in fulfilment of the requirements of

Doctor of Philosophy in Molecular BioSciences

at Massey University, Albany, New Zealand. 2006

## Abstract

Ancient DNA was used to provide a temporal perspective for examining a number of evolutionary, conservation and cultural questions involving members of the New Zealand avifauna. Ancient mitochondrial DNA (mtDNA) sequences were used to examine the past levels and patterns of genetic diversity in the five species of New Zealand kiwi (Apterygidae). Brown kiwi, particularly in the South Island, exhibited high levels of genetic structuring with nearly every population exhibiting private mitochondrial haplotypes. The extinction of a large number of brown kiwi populations has, therefore, led to the loss of a large amount of genetic variation in these species. The past ranges of great spotted kiwi and the three brown kiwi species, whose bones are morphologically indistinguishable, were determined. This information can aid conservation programmes aiming to re-introduce kiwi to regions where they are now extinct.

In contrast to the high level of genetic structuring in South Island brown kiwi, the majority of little spotted kiwi samples from the South Island shared a common haplotype. The difference in phylogeography between brown kiwi and little spotted kiwi is hypothesised to relate to differences in their dispersal behaviour and/or their population histories. The addition of ancient samples of little spotted kiwi from the North Island indicated a complex relationship with great spotted kiwi.

Nuclear microsatellite DNA markers were isolated from North Island brown kiwi and tested for cross amplification in the other kiwi species. Five loci were polymorphic in all kiwi species. Preliminary analyses of genotyping results indicated that the kiwi species were distinguished by assignment tests and that subdivision may occur within several of the species.

An extensive reference database of modern and ancient mtDNA sequences was used to determine species and provenance of a number of unlabelled museum subfossil bones and skins. This method was also used to examine provenance of brown kiwi feathers from Maori artefacts (cloaks and baskets).

Ancient DNA methodology was also used in a molecular examination of the relationships of a second endemic avian family, the New Zealand wattlebirds (Callaeatidae). Analyses of nuclear gene sequences, *c-mos* and RAG-1, revealed kokako, saddleback and huia comprised a strongly supported monophyletic group. A divergence time estimate for the New Zealand wattlebirds indicated that they are more likely to have arrived by transoceanic dispersal than have a Gondwanan origin. Sequences from three mtDNA genes, 12S, ND2 and cytochrome b, were also analysed but could not resolve the relationships between the three genera.

Microsatellite DNA from the extinct New Zealand huia exhibited considerable genetic variation, exceeding that found in extant North Island saddleback, from which the loci were isolated. Assignment tests indicated no genetic structuring within huia, although interpretation was complicated by a lack of provenance details for many of the skins.

The results presented here suggest that ancient DNA can not only provide information about the relationships of extinct taxa but also demonstrates the importance of placing the present day genetic diversity found in endangered taxa within the context of past patterns and levels of genetic variation.

## Acknowledgements

Firstly I would like to thank my supervisors Professor Dave Lambert and Professor Mike Hendy, without whom this research would not have been possible. Dave obtained funding for this research, generously provided a space in his laboratory and was extremely enthusiastic about the cloak research. Thank you also to Mike for providing comments on Chapter Seven.

Thanks must also go to the all the past and present members of the Molecular Ecology lab that I have worked with: Jennie, Jenn, Hayley, Hilary, Pete, Anthony, Ines, Barbara, Iman, Esther, Justin, Gillian, Olly, Gwilym, Gabi, Justin and Tania. I really appreciate all the advice I received, as well as the sanity-preserving coffee breaks.

Essential to this research was the generosity of those who provided the ancient and modern samples. Professor Charles Daugherty and Dr Murray Potter made kiwi blood samples available. Thank you also to Alan Tennyson and Gillian White (Te Papa Tongarewa), Paul Scofield (Canterbury Museum), the South Taranaki District Museum, Brian Gill (Auckland Museum), Kaaren Mitcalfe (Napier Museum), Kevan Wilde (Waitomo Museum) and Neville Hudson (Geology Dept. Auckland University) for help with and/or permission to sample bones, skins and /or feathers from their collections. Trevor Worthy engaged in many helpful discussions and provided great advice regarding which bone samples were likely to yield DNA, thus limiting the amount of destructive sampling required. Thank you also to Alan Tennyson and Trevor Worthy for proposing the research that led to Chapter Four.

I am grateful for to the financial support of the Allan Wilson Centre, the New Zealand Marsden Fund, Massey University PhD Scholarship, IMBS travel fund and the Federation of University Women (North Shore Branch).

In addition to the Albany members of the Molecular Ecology lab, a number of other people also made life in Auckland more bearable: Wendy, Su Ling, Ang, Al, Nat, Gus and the Three Streams and Kauri Grove Volunteers, especially John and Alan. Whenever I think about my time in Auckland I will always remember fondly the fantastic time I had with my flatmates Robine, Cameron, Alex (baby Bruce), Kerrie, Tash, Chloe and Saffy (there was never a dull moment living with you guys!).

I am very grateful to my family both in New Zealand and overseas for their continued support of everything I do.

I would like to acknowledge my unofficial supervisors: the two Leons. Leon Huynen (Dr Huynie) initially started the brown kiwi research and sampled the huia skinsthanks for letting me take over! Thanks also for your encouragement and for always being willing to give me a hand in the lab. In particular, thank you for performing the ancient DNA extraction verifications at Auckland University.

Finally, I would like to thank my partner Leon Perrie. I can't thank you enough for your support and encouragement, and for talking me out of running away from Auckland more times than I care to remember! Thank you also for putting up with me constantly talking about science every time you came to visit or phoned, and for helpful discussions regarding tree building and various other analyses. Thank you also for proof reading my entire thesis (without complaining!).

Abstract		ii
Acknowledgements		iv
Table of Contents		vi
CHAPTER ONE:	General Introduction	1
	References	8
CHAPTER TWO:	Ancient DNA and conservation: lessons from	
	the kiwi of New Zealand.	
	Introduction	14
	Methods	18
	Results	22
	Discussion	29
	References	32
	Appendix 1	35
	Appendix 2	36
CHAPTER THREE:	Ancient DNA analyses reveal contrasting	
	phylogeography in the New Zealand kiwi	
	(Apteryx spp.).	
	Introduction	38
	Methods	41
	Results	46
	Discussion	51
	References	57
	Appendix 1	62
CHAPTER FOUR:	Identification of kiwi ( <i>Apteryx</i> spp.) museum	

specimens using ancient DNA analysis.

Introduction	63
Methods	65
Results	70
Discussion	70
References	77
Appendix 1	80

CHAPTER FIVE:The provenance of brown kiwi feathers in Maori<br/>cloaks (Kahu Kiwi) and kete from DNA analysis.Introduction81Methods83Results87Discussion94References96

CHAPTER SIX:	Nuclear microsatellite DNA markers for New	
	Zealand kiwi ( <i>Apteryx</i> spp.).	
	Introduction	99
	Methods	100
	Results	105
	Discussion	109
	References	113

CHAPTER SEVEN: The relationships and origins of the New Zealand wattlebirds (Passeriformes, Callaeatidae) from DNA sequence analyses. Introduction 116

Introduction	116
Methods	119
Results	126
Discussion	136
References	140
Appendix 1	145

CHAPTER EIGHT:	Genetic homogeneity of nuclear microsatellite	
	DNA markers in the extinct New Zealand huia.	
	Introduction	146
	Methods	149
	Results	154
	Discussion	157
	References	160
	Appendix 1	164
CHAPTER NINE:	General discussion and future work.	
	Summary of major findings	170
	Proposed future directions	172
	References	177
APPENDIX A:	Details of kiwi samples used in this study.	180
APPENDIX B:	Published manuscripts.	187