

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.

---

**Population Genetics, Biogeography and Ecological  
Interactions of the New Zealand Bellbird (*Anthornis  
melanura*) and their Avian Malaria Parasites**

---

A thesis presented in partial fulfilment of the requirements for the degree of  
Doctor of Philosophy in Zoology  
at Massey University, Albany, New Zealand

Shauna Maureen Baillie

September 2011

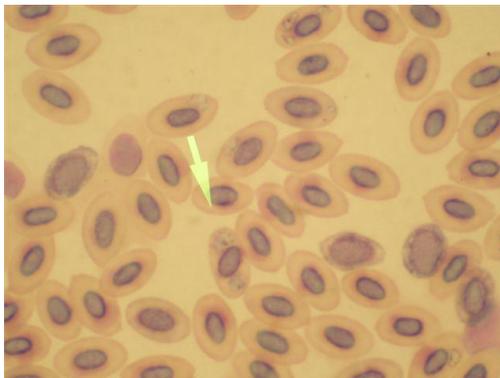
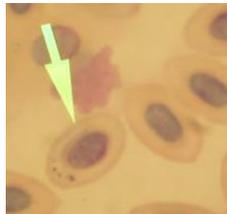




Adult female New Zealand bellbird  
(Photo: Jordi Segers)



Singing adult male New Zealand bellbird  
(Photo: Rob Cross)



Giemsa-stained blood smears from a bellbird at Tawharanui Regional Park infected with intracellular *Plasmodium* sp. (arrows in upper and lower photos)  
(Photos: Gribbles Veterinary)



Three adult male bellbirds at a sugar water feeder on Tiritiri Matangi (Photo: Jordi Segers)

---

# Abstract

---

Habitat loss and redistribution of species has led to population declines and loss of genetic diversity with serious implications to species survival on ecological and evolutionary scales. While there is no doubt that rapidly dwindling endangered populations require our immediate attention, studies on common species are equally important. The purpose of this thesis is to investigate the genetic connectivity, biogeographical relationships and host-parasite interactions of a common and widely distributed bird species, mainly because we want common species to remain common. Furthermore, I illustrate how studies such as this provide invaluable comparisons for sympatric endangered species.

In this thesis, patterns of genetic variation of the New Zealand bellbird (*Anthornis melanura*) are delineated to assess their re-colonization potential among fragmented landscapes. Using a phylogeographic perspective I show how dispersal ability and secondary contact among isolated population fragments shape the evolutionary trajectory of a species. I also determine the biogeographical relationships between the bellbird host and its malaria parasites with key emphasis on host-parasite specificity. Finally, immunological trade-offs are investigated in disease epidemiology by examining host factors that influence malaria prevalence.

I show that an immense capacity for dispersal has prevented divergence and shaped the high levels of genetic diversity and connectivity in bellbirds today. However, substantial genetic differentiation among subpopulations reflects recent habitat fragmentation. Based on these findings I conclude that continued habitat loss can lead to further reductions in gene flow, despite dispersal. Though restricted to northern populations, I provide evidence that the most abundant avian malaria lineage infecting bellbirds is likely an endemic *Plasmodium* (*Novyella*). This parasite exhibits bimodal seasonality and male-biased infections, but these relationships vary among subpopulations. Malaria prevalence appears to be governed by food availability and territory stability, thus habitat disturbance has repercussions to immune phenotype.

With this thesis I advocate a re-thinking of conservation strategies toward spatial planning that enables 'natural' secondary contact among habitat fragments. Translocation is not necessary for all species. In addition to being the first study on seasonal and host factors affecting malaria patterns in the Southern Hemisphere, this thesis makes major contributions to science by elucidating some ecological relationships that underpin the evolution of immunity.

# Acknowledgments

---

First and foremost, I would like to dedicate this thesis to my mother, Diane Sylvester, in memoriam. Her intelligence and free-spirit are the true inspiration behind the undertaking of this thesis.

I could not have done this thesis without the never failing support of my main supervisor Dr. Dianne Brunton (Director of the Ecology and Conservation Group, Institute of Natural Sciences, Albany Campus, Massey University, New Zealand). Dianne is an extraordinary person able to field various students, and somehow keep us happy while demanding the best from us. These traits are a rare combination in an individual, let alone a PhD supervisor (as a quick perusal of PhD Comics.com will illustrate) and I feel very lucky and privileged to have worked under her.

The first 'field ecology' phase of my PhD thesis was spent at Massey University where in addition to conversations with Dr. Dianne Brunton. I am grateful to Dr. Rosemary Barraclough, Dr. Weihong Ji and Dr. Kevin Parker for helpful and incredibly knowledgeable discussions in the earlier phase of my thesis. For the second 'genetics laboratory' phase, Dr. Pete Ritchie welcomed me into his genetics lab at the School of Biological Sciences (SBS), Victoria University of Wellington, Kelburn Campus, Wellington, New Zealand. Dr. Ritchie was well equipped with the generosity, grace and patience required to accept a completely green-to-population-genetics PhD student into his lab. I am forever indebted that he took me in. Pete was an integral part of this thesis, I was very inspired by the work done and expertise within Pete's lab group and learned so much from our weekly Population Genetics Lab Group Meetings.

Gracious thanks especially to Jordi Segers, Morag Fordham and Simon Fordham who were my most dedicated field technicians. Many long days were spent wandering around in the forest on the North Island as well as offshore islands setting up and taking down mistnets. These three wonderful people extracted hundreds of birds of several difference species from the nets while I took blood and measured them at the banding table. They were always up for the long haul with not one complaint. I also want to thank my lovely friends Birgit Ziesemann and Barbara Egli who helped catch bellbirds at Hauturu during my last trip, and also to Miriam

Ludbrook and Eva Krause who helped capture bellbirds at Tawharanui. At the Poor Knights Islands Michael Anderson and Yuri Shanas were crucial to fieldwork and in making that trip an incredible adventure. Additionally, I want to give special thanks to Ngati Wai Iwi Trust Board and particularly to Clive Stone for meeting with me and giving us the permission to visit and conduct my research within their *wāhi tapu*. Dr. Graeme Elliott of the New Zealand Department of Conservation (DoC) generously collected blood samples from the Auckland Islands. All blood collection from birds was performed under DoC permit, Auckland Regional Council (ARC) and Massey University Animal Ethics Committee (MUAEC).

I extend many gracious thanks especially to my officemates at VUW including Sebastien Rioux-Paquette, Monica Gruber and Elizabeth Heeg for their energetic and inspired conversation and for sharing of their easy guru-like knowledge with regard to population genetics. Access to post-doctorate researchers such as Sebastien Rioux-Paquette, Hilary Miller, Kristina Ramstad and Christian Bödeker was important. Importantly, I also want to extend warm gratitude to the proverbial ‘cogs’ of SBS who include Mary Murray, Sandra Taylor, Patricia Stein, Paul Marsden and Delwyn Carter-Jarratt who were supportive and fun, and the latter two were stalwart soccer mates for the short while I overlapped with them on the field.

My father, Dr. Robert Baillie, his wife Michelle and my two brothers Mark and Michael were always there for me in their respective quiet ways on the far side of the globe. I love the four of them very much. A special thanks to all the musos, especially Dr. Weihong Ji, for the celtic jam sessions that were so enjoyable over the past few years. Also, thanks to friends who were there to provide much needed diversions such as my rock climbing buddies who also doubled as Friday Happy Hour buddies from time to time.

This study was funded by a Canadian Natural Science and Engineering Research Council (NSERC) PGS scholarship to SMB and the New Zealand Institute of Natural Sciences (INS) Massey University. Dr. Pete Ritchie covered a generous amount of consumables and primer costs while I worked in his genetics lab. Invaluable contributions from the New Zealand Ecological Society Hutton Fund, Tiritiri Supporters and Little Barrier Trust Fund were greatly appreciated over the course of this thesis.

# Thesis Structure and Format

---

I have written this thesis as a collection of five scientific papers for publication in peer reviewed journals. An introductory chapter (Chapter One) precedes the five data chapters and is written for a general science audience that provides some background on the theory and analytical components of my thesis. This format has meant that some of the Introduction is repeated in the various Introductions throughout the thesis, but this is necessary for the reader to have a complete overview of the thesis. Chapter Seven is a synthesis that summarizes my findings and outlines my contributions to science and future research.

Chapters Two to Six are data chapters that each stand as independent scientific papers. Within the text of these five data chapters, I used the plural first person because my supervisors are on these papers as co-authors. However, the study design, fieldwork, laboratory work and all statistical analyses and writing were performed by me with only supervisory input from co-authors. During the process of my first population genetics chapter, Chapter Two, I presented my work at several stages to Dr. Pete Ritchie's Population Genetics Discussion Group at Victoria University of Wellington (VUW) in order to receive feedback. After this first chapter, I had amassed a foundation of programs, analyses and genetic theory required to perform a thesis in population genetics. I submitted Chapter Two for publication in *Conservation Genetics* and it is currently in review. I invited Dr. Sebastien Rioux-Paquette, a post-doctorate researcher in Dr. Pete Ritchie's lab at VUW the time to be a co-author on the journal article for Chapter Three, which will be submitted to *Molecular Ecology*. Dr. Rioux-Paquette specifically suggested the 'mixed-stock' analysis and I was introduced to population computer simulations through exposure to his concurrent work. However, I independently researched, learned and decided upon the programs and analyses used in this thesis. Once this manuscript (Chapter Three) was completely written to a high standard, I circulated it to my supervisors and Sebastien, my soon to be co-authors. The remaining chapters are independent works, which have received commentary from my supervisors and other PhD students. Chapter Four will be submitted to *Journal of Biogeography* within the coming months. Chapter Five has

been accepted by *Parasitology* (please refer to Appendix F for a copy of that submission) and Chapter Six has been submitted to *Journal of Animal Ecology* recently. There are two papers sketched out in the Appendix D and E on bellbird morphological congruence with genotype and bellbird breeding biology, respectively. These papers will be written and submitted to peer-reviewed journals after thesis submission.

I have retained the Abstract, Keywords and Acknowledgments sections within each of the five data chapters so that the 'collection of scientific papers' format is consistent throughout. Additionally, I feel that each individual Abstract facilitates the reader of this thesis in that they provide specific summaries of the material about to be read. Similarly, the Acknowledgments sections, though repetitive at times, allow for each data chapter to exist as a separate paper without the reader having to search back to the beginning for contributions.

The purpose of the Appendices section in this thesis is twofold. First, the Appendices provide specific information that supplement analyses within each data chapter. In these cases, the appropriate Table or Figure in the Appendix is specified, e.g., Table D.3 means the third table in Appendix D. In this manner, tables and figures that might disrupt the flow of the text but provide supportive information are accessible. Second, I have used the Appendices to disseminate information or data that is not directly referred to in my thesis. In these cases, the items within each Appendix is thought to be informative for future researchers, e.g., DNA sequences, observations in the field and breeding biology and habitat information. All Literature Cited sections in this thesis are consistent with the format used for *Conservation Genetics* and journal title abbreviations follow the International Standard Serial Number (ISSN) list of title word abbreviations, see [/www.csa.com/ids70/serials\\_source\\_list.php?db=biolclust-set-c](http://www.csa.com/ids70/serials_source_list.php?db=biolclust-set-c).

# Table of Contents

---

Abstract	i
Acknowledgments	ii
Thesis Structure and Format	iv
Table of Contents	vi
List of Tables	xi
List of Figures	xii

## Chapter One: Metapopulations, biogeographic theory and evolution of immune phenotype

1.1 Overview	1
1.2 Metapopulation dynamics and genetic connectivity	2
1.2.1 Population extinction-colonization model and habitat loss	3
1.2.2 Dispersal versus gene flow in migration-drift equilibrium	5
1.2.3 Common statistical measures in population genetics	6
1.2.3.1 Hardy-Weinberg equilibrium and Wright's inbreeding coefficient ( $F_{IS}$ )	7
1.2.3.2 Measures of genetic differentiation	8
1.2.3.3 Significance tests for genetic bottlenecks	9
1.2.3.4 Genetic distance and population clustering	10
1.2.3.5 Use of Bonferroni corrections in population genetics	11
1.3 Biogeography of birds and their parasites	12
1.3.1 Phylogeographic hypotheses	13
1.3.2 Why sometimes does speciation not occur given allopatry?	17
1.3.3 Biogeographical relationships between hosts and their parasites	19
1.4 Evolution of immune phenotype in birds	20
1.4.1 Seasonal variation in immunity: the host perspective	20
1.4.2 Immune defense trade-offs	21
1.4.3 Sexual dimorphism in immune phenotype	23
1.5 A model host-parasite study system in New Zealand	24
1.5.1 A changing environment	24

1.5.2	The host	25
1.5.3	The parasite and its vectors	26
1.6	Thesis aims and outline	28
1.7	Literature cited	29

## **Chapter Two: They never stop trying: high levels of genetic differentiation despite ongoing dispersal indicates habitat-limited gene flow in an abundant and vagile passerine endemic to New Zealand**

2.1	Abstract	36
2.2	Introduction	37
2.3	Methods	39
2.3.1	Blood sampling	39
2.3.2	Microsatellite genotyping	41
2.3.3	Genetic diversity, inbreeding levels and bottlenecks	42
2.3.4	Population migration model and structure	42
2.3.5	Dispersal estimates and mismatch with gene flow	43
2.4	Results	44
2.4.1	Genetic diversity, inbreeding levels and bottlenecks	44
2.4.2	Population migration model and structure	47
2.4.3	Dispersal estimates and mismatch with gene flow	51
2.5	Discussion	54
2.5.1	Genetic diversity and inbreeding levels	54
2.5.2	Bellbird populations subject to strong genetic drift	56
2.5.3	Population migration model and structure	57
2.5.4	Mismatch between dispersal estimates and gene flow	58
2.5.5	Conclusions and conservation implications	59
2.6	Literature cited	60

## **Chapter Three: Back to the mainland 150 years after extirpation: genetic analysis of natural re-colonization by an endemic New Zealand passerine**

3.1	Abstract	66
3.2	Introduction	67
3.3	Methods	69
3.3.1	Blood sample collection and DNA analysis	69
3.3.2	Genetic variability and bottleneck detection	72
3.3.3	Population structure and gene flow	72
3.3.4	Identification of source populations and non-natal dispersers	73
3.3.5	Simulations of minimum number of colonizers	74

3.4 Results	75
3.4.1 Genetic variability and strength of bottleneck at Tawharanui	75
3.4.2 Genetic differentiation and structure among bellbird populations	77
3.4.3 Identification of source populations using mixed stock analyses	79
3.4.4 Rates of gene flow and detecting natal dispersal of individuals	82
3.4.5 Simulations of colonization via single versus multiple populations	83
3.5 Discussion	86
3.5.1 Origin of the Tawharanui bellbird population	86
3.5.2 Absence of founder effect during the re-colonization process	87
3.5.3 Population genetics of natural versus human-assisted bird re-introductions	88
3.5.4 Conclusions	90
3.6 Literature cited	91

## **Chapter Four: An almost speciation of *Anthornis melanura* hypothesis: allopatry and postdivergence hybridization in a vagile disperser**

4.1 Abstract	96
4.2 Introduction	97
4.3 Methods	99
4.3.1 Sample collection	99
4.3.2 DNA sequencing and genotyping	100
4.3.3 Diversity and population structure	100
4.3.4 Phylogenetic analyses	101
4.3.5 Coalescent analyses of historical divergence, migration and population sizes	102
4.4 Results	105
4.4.1 Genetic diversity and population structure	105
4.4.2 Phylogenetic analyses	109
4.4.3 Coalescent analyses of historical divergence, migration and population sizes	111
4.5 Discussion	116
4.5.1 Allopatric population separation hypothesis	116
4.5.2 Post-divergence population expansion and secondary contact	118
4.5.3 Post-glacial colonization of the Sub-Antarctic Islands and evolutionary rate calibration	119
4.5.4 Discordance between gene trees and species trees and other issues	120
4.5.5 Conclusions	121
4.6 Literature cited	123

## **Chapter Five: Diversity, distribution and biogeographical origins of *Plasmodium* parasites from the New Zealand bellbird *Anthornis melanura***

5.1 Abstract	129
5.2 Introduction	130
5.3 Methods	132
5.3.1 Blood sampling	132
5.3.2 PCR detection of the parasite	133
5.3.3 Phylogenetic analyses of the haemosporidian parasite	133
5.4 Results	135
5.4.1 Phylogenetic identification and prevalence of avian malaria lineages	135
5.5 Discussion	138
5.5.1 Diversity and distribution of bellbird <i>Plasmodium</i> lineages within New Zealand	138
5.5.2 Biogeographical origins of bellbird <i>Plasmodium</i> parasites	139
5.5.3 Lack of <i>Haemoproteus</i> infections and underestimation of parasite prevalence	141
5.5.4 Conclusions	142
5.6 Literature cited	143

## **Chapter Six: Sex, body condition and seasons: factors that influence haematozoan blood parasite prevalence within and among three different subpopulations of a single host**

6.1 Abstract	147
6.2 Introduction	148
6.3 Methods	150
6.3.1 Host-parasite system	150
6.3.2 Blood sampling and PCR detection of the parasite	153
6.3.3 Sex and age determination of the host	155
6.3.4 Calculation of host body condition index	155
6.3.5 Statistical modelling of factors that influence seasonal variation in parasite prevalence	156
6.4 Results	156
6.4.1 Spatial and temporal malaria patterns within a host metapopulation	156
6.4.2 Factors affecting LIN1 prevalence	158
6.4.3 Gain and loss of infection	163
6.5 Discussion	164
6.5.1 Spatial and temporal malaria patterns within a host metapopulation	164
6.5.2 Sex-biased infection varies among different host subpopulations	166

6.5.3	Paradoxical relationships between parasite prevalence and body condition in bellbirds	167
6.5.4	Conclusions	168
6.6	Literature cited	170

## **Chapter Seven: Chapter synthesis and future research directions**

7.1	Introduction	175
7.2	Main findings	176
7.2.1	Metapopulation dynamics and genetic connectivity	176
7.2.2	Biogeography of birds and their parasites	177
7.2.3	Evolution of immune phenotype	178
7.2.4	Filling in the gaps	179
7.3	Conclusion	181
7.4	Future research directions	182
7.5	Literature cited	183

<b>Appendix A:</b>	<b><i>Anthornis melanura</i> microsatellite DNA data</b>	186
--------------------	--	-----

<b>Appendix B:</b>	<b><i>Anthornis melanura</i> mitochondrial DNA data</b>	196
--------------------	---	-----

<b>Appendix C:</b>	<b><i>Plasmodium</i> spp. mitochondrial DNA data</b>	203
--------------------	--	-----

<b>Appendix D:</b>	<b>Bellbird morphological analyses</b>	207
--------------------	--	-----

<b>Appendix E:</b>	<b>Bellbird reproductive success and chick growth</b>	213
--------------------	---	-----

<b>Appendix F:</b>	<b>Statement of author contributions DRC16 forms</b>	217
--------------------	--	-----

# List of Tables

---

2.1	Allelic and genetic diversity statistics of bellbirds using eight microsatellite loci	45
2.2	Results for tests on population size reduction	46
2.3	Pair-wise $F_{ST}$ and $R_{ST}$ values among microsatellite loci bellbird sampling locations	47
2.4	Jost's $D$ values among bellbird sampling locations calculated in SMOGD	48
2.5	Proportion and number of first-generation migrants detected	52
2.6	Genetic diversity estimates from studies on passerines world-wide	55
3.1	Microsatellite and mitochondrial genetic diversity in bellbirds	76
3.2	Pairwise $F$ -statistics for bellbirds within the Hauraki Gulf	78
3.3	Average proportion of membership of individual bellbirds assigned to inferred population clusters	79
4.1	Control region mitochondrial DNA variability of bellbirds	105
4.2	Pair-wise $F_{ST}$ values for bellbirds at the mitochondrial DNA control region	106
4.3	AMOVA results for a) mitochondrial DNA using haplotype frequency differences and sequence divergence ( $\phi_{ST}$ ) estimates and b) eight microsatellite markers using $F_{ST}$	107
4.4	Demographic parameters results and 95% credible intervals from posterior probability distribution as implemented in MDIV	111
4.5	Hypotheses tests of recent demographic expansion for bellbirds using mismatch analysis	114
5.1	Sequence divergence estimates of the bellbird <i>Plasmodium</i> parasites	136
5.2	Worldwide biogeographical distribution of <i>Plasmodium</i> lineages that most closely match parasite sequences found in New Zealand bellbirds	140
6.1	Phenology and biological significance of the five sampling seasons used in this study	151
6.2	Number and proportion of positive <i>Plasmodium</i> infections identified in bellbird samples collected at three Hauraki Gulf subpopulations between 2007 and 2010	154
6.3	Significant influences on seasonal variation in <i>Plasmodium</i> infections	159
A.1	Characterisation of eight microsatellite loci in the New Zealand bellbird populations	186
A.2	Results of tests for departures from Hardy-Weinberg equilibrium for each sampling location	187
A.3	Percent missing data and number of alleles for each of eight bellbird microsatellite loci	187
A.4	Results of linkage disequilibrium tests before Bonferroni correction	188
A.5	Tables of allelic frequencies for eight microsatellite loci at each of eleven sampling locations	191
A.6	Confidence intervals (CI) for Jost's $D$ estimates from program SMOGD	194
C.1	Sample sizes for number of bellbirds screened for and infected by <i>Plasmodium</i>	204
C.2	AIC model selection results from tests of factors affecting bellbird malaria	205
D.1	Standardized discriminant function analysis (DFA) coefficients for phenotypic traits	209
D.2	Assignment tests of individuals each sampling location for DNA and morphology	211

E.1. Description of 21 New Zealand bellbird nests at Tiritiri Matangi	214
E.2. Nest success for 21 New Zealand bellbird nests at Tiritiri Matangi, Hauraki Gulf during 2007	215

## List of Figures

---

1.1 The Gilpin (1991) extinction and colonization probability model	3
1.2 A simple illustration of a founder event	4
1.3 Relationships between gene flow, genetic divergence and dispersal in wild populations	6
1.4 The continuum of genetic variation and analytical methods used to interpret genetic signals	13
1.5 A schematic of five common phylogeographic hypotheses	14
1.6 Three intraspecific patterns from mitochondrial mismatch analyses	16
1.7 Schematic of strict allopatric speciation model	18
1.8 The 'sign' of the relationships between life-history output and parasite prevalence	22
2.1 Locations of nine New Zealand bellbird sampling sites in this study	40
2.2 Bellbird population model	49
2.3 Principal component analysis among bellbird sampling locations	50
2.4 Unrooted NJ tree based on Cavalli-Sforza distances	51
2.5 Relationships between dispersal and genetic divergence and inbreeding coefficient	53
3.1 New Zealand bellbird distribution in the North Island and sample sites	71
3.2 Parsimony network of bellbird mitochondrial DNA control region	80
3.3 Mixed stock analysis for microsatellite and mitochondrial DNA data	81
3.4 Single source population bottleneck simulations of Tawharanui bellbird founder event	84
3.5 Multiple source populations bottleneck simulations of Tawharanui bellbird founder event	85
4.1 Geographical distribution of 26 bellbird haplotypes	104
4.2 Parsimony network of mitochondrial control region haplotypes from ten populations	108
4.3 Bellbird populations from throughout their geographical range cluster into four populations	108
4.4 Phylogenetic tree for bellbird mitochondrial control region haplotypes	110
4.5 Marginal distributions for IM migration results for each of two divergent haplogroups	112
4.6 Mismatch distribution of mitochondrial control region sequences for the full dataset	113
4.7 Bayesian skyline plot of bellbird historical population size changes	115
4.8 Timeline of glacial cycling between c. 420 kyr and the present in the South Hemisphere	117
4.9 Biogeographic reconstruction of bellbird c. 2.9 million years to present	122
5.1 Phylogenetic identification of four <i>Plasmodium</i> lineages from bellbirds	134
5.2 Location map of host population sampling locations and <i>Plasmodium</i> prevalence	137
6.1 Location map and bellbird distribution within the Hauraki Gulf, New Zealand	152
6.2 Seasonal variation in prevalence of bellbird <i>Plasmodium</i> infections	157

6.3	Seasonal variation in host factors that influence <i>Plasmodium</i> prevalence	160
6.4	Retained backward logistic regression terms that influence <i>Plasmodium</i> prevalence	162
6.5	<i>Plasmodium</i> infections in individual bellbirds that were sampled more than once	164
A.1	Determining <i>K</i> using the Evanno et al. (2005) method and STRUCTURE bar plot	195
B.1	Bellbird partial mitochondrial cytochrome <i>b</i> sequences for 6 haplotypes	196
B.2	Bellbird mitochondrial control region Domain I sequences for 26 haplotypes	198
C.1	Mitochondrial cytochrome <i>b</i> sequences for four <i>Plasmodium</i> haplotypes sampled	203
C.2	LogDet (Tamura-Kumar) pair-wise sequence divergence estimates	206
D.1	Validation of residual body mass index using regressions against body fat	207
D.2	Histograms of bellbird morphology showing sex dimorphism	208
D.3	Discriminant function analysis (DFA) plot of bellbird body morphometrics for both sexes	210
D.4	Significant increase in body size with increasing latitude	212
E.1	Bellbird nest locations that were regularly visited at Tiritiri Matangi during 2007	223
E.2	New Zealand bellbird chick growth curves	216