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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

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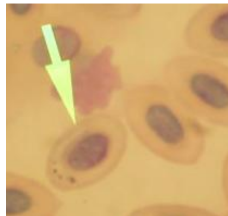
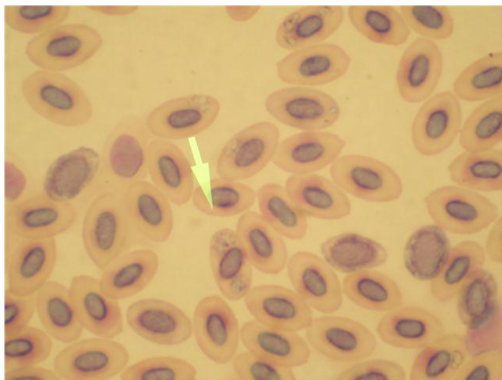
Adult female New Zealand bellbird (Photo: Jordi Segers)

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 lead 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).

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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.

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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 supervisorial 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.

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 *Plasmodium* lineages within New Zealand 138
5.5.2 Biogeographical origins of bellbird *Plasmodium* parasites 139
5.5.3 Lack of *Haemoproteus* 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

Appendix A: *Anthornis melanura* microsatellite DNA data 186

Appendix B: *Anthornis melanura* mitochondrial DNA data 196

Appendix C: *Plasmodium* spp. mitochondrial DNA data 203

Appendix D: Bellbird morphological analyses 207

Appendix E: Bellbird reproductive success and chick growth 213

Appendix F: Statement of author contributions DRC16 forms 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 (ϕ_{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 *Plasmodium* parasites 136
5.2 Worldwide biogeographical distribution of *Plasmodium* 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 *Plasmodium* infections identified in bellbird samples collected at three Hauraki Gulf subpopulations between 2007 and 2010 154
6.3 Significant influences on seasonal variation in *Plasmodium* 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 *Plasmodium* 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 *Plasmodium* lineages from bellbirds 134
5.2 Location map of host population sampling locations and *Plasmodium* prevalence 137

6.1 Location map and bellbird distribution within the Hauraki Gulf, New Zealand 152
6.2 Seasonal variation in prevalence of bellbird *Plasmodium* infections 157

6.3 Seasonal variation in host factors that influence *Plasmodium* prevalence 160
6.4 Retained backward logistic regression terms that influence *Plasmodium* prevalence 162
6.5 *Plasmodium* infections in individual bellbirds that were sampled more than once 164

A.1 Determining *K* using the Evanno et al. (2005) method and STRUCTURE bar plot 195

B.1 Bellbird partial mitochondrial cytochrome *b* sequences for 6 haplotypes 196
B.2 Bellbird mitochondrial control region Domain I sequences for 26 haplotypes 198

C.1 Mitochondrial cytochrome *b* sequences for four *Plasmodium* 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

CHAPTER ONE

Metapopulations, biogeographic theory and evolution of immune phenotype

1.1 Overview

The central theme underpinning this thesis is how the movement of animals through a changing environment shapes 1) the evolution of their genomes via genetic connectivity, 2) their diversity and distribution and 3) their interactions with the parasites that evolve in space and time alongside their hosts. This thesis is a spatiotemporal examination of a highly mobile passerine host and its malaria parasites that merges concepts in population genetics, biogeography and evolution of immune competence of wild populations. I profile bellbird microsatellite DNA, control region mitochondrial DNA, phenotype characteristics and their malaria parasite cytochrome *b* mitochondrial DNA from populations throughout the New Zealand island archipelago, including a comparatively isolated Sub-Antarctic population. Additionally, I had the propitious and rare opportunity to study genetic aspects and host-parasite interactions of a natural founding event, or re-colonization. Another important component of my thesis is environmental change, an inherent quality of biological systems without which evolution might not have occurred at all. In this thesis, I deal with environmental change at the hand of both contemporary human-mediated habitat loss and historical non-anthropogenic oscillations in global climate through time. Furthermore, most bird research in New Zealand, where my PhD is based, has focused on endangered species and

there have been fewer studies on less threatened species. Therefore, a novel feature of this thesis, especially considering the cross-section of studies on endemic insular species, is that my study species is common and abundant according to International Union for Conservation of Nature (IUCN) standards. In endangered or rare species work sample size and study sites are restricting, whereas working with common species offers a greater range of ecological and evolutionary questions (Gaston 2010). The knowledge gained from novel scientific study on large and possibly interconnected populations in the fragmented landscapes of today can provide a fertile foundation for future examinations on how plants and animals adapt to future habitat loss and climate change.

In this introductory chapter, I outline the key theoretical concepts of this thesis on genetic and ecological processes underlying how animals and their parasites move through changing environments. Specifically, I discuss 1) aspects of population genetics as they pertain to genetic connectivity and metapopulations, 2) biogeographic theory on the diversity and distribution of species and 3) evolution of immune phenotype in birds with particular emphasis on immune defense trade-offs and sex dimorphism in parasite susceptibility. Additionally, the host-parasite system upon which this thesis is based is described, followed by a brief summary of the specific aims and outline of this thesis.

1.2 Metapopulation dynamics and genetic connectivity

Loss of genetic diversity has serious implications for species survival on ecological and evolutionary scales (Frankel 1974; Ciofi et al. 1999; Frankham 2010). Human-induced habitat loss and fragmentation alter the distribution and abundance of species, which in many instances leads to genetic differentiation and inbreeding among isolated subpopulations (Pereira et al. 2010). In the 1960s, Robert H MacArthur and Edward O Wilson first made the link between principles of immigration and extinction to the processes of population ecology and genetic evolution (Wilson 2010). These ideas are integral to metapopulation dynamic

theory (Hanski 1999) and our understanding of how extinction-colonization processes are accelerated by reductions in population size due to human-mediated habitat loss and re-distribution of predators and disease.

1.2.1 *Population extinction-colonization model and habitat loss*

A grasp of the extinction-colonization model originally proposed by Gilpin (1991) is conceptually important when reading this thesis. First, it explains that large panmictic populations (etymology: from 'panmixia' meaning state of being genetically homogeneous) are capable of maintaining large amounts of genetic diversity (Figure 1.1). Due to habitat fragmentation, for

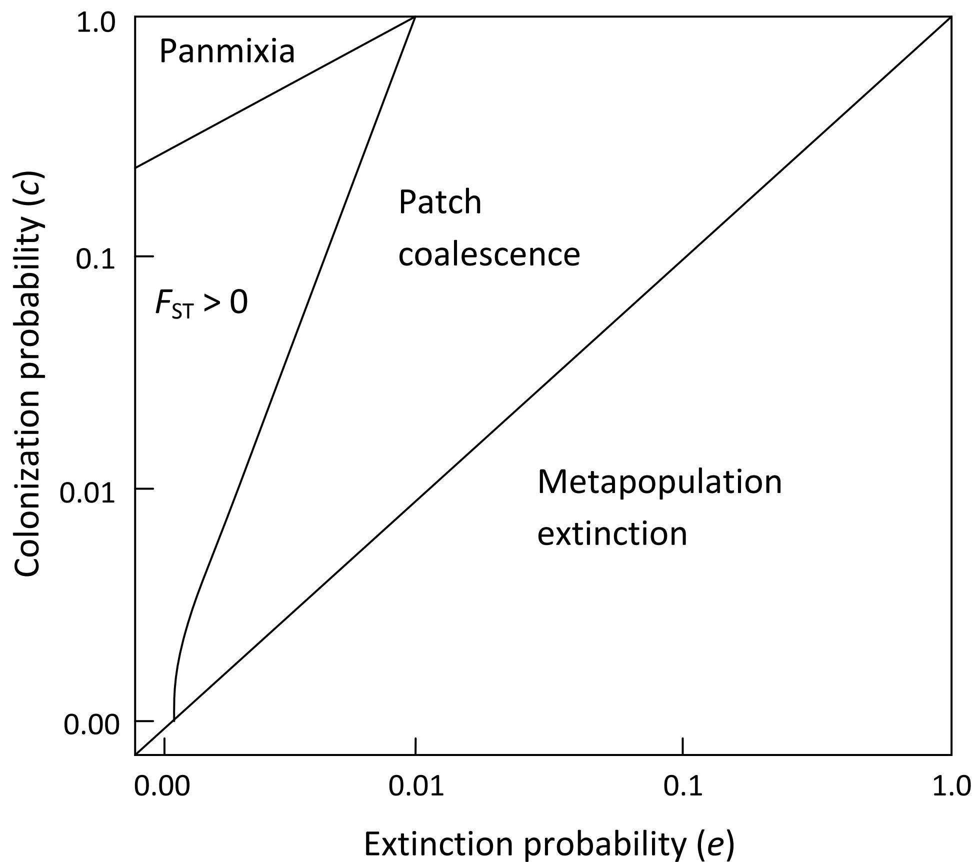

Figure 1.1 The Gilpin (1991) extinction and colonization probability model. Diagram redrawn from Gilpin (1991).

example, a population can take on a metapopulation structure. This is an extreme form of spatial structure in which smaller loosely coupled population fragments 'blink on' and 'blink off', i.e., suffer extinction followed by re-colonization from elsewhere within the metapopulation. These blinking population fragments, however, have smaller effective population sizes (N_E: number of breeding individuals that contribute to the gene pool) than the homogeneous population. Genetic drift, an evolutionary process ubiquitous to all populations, is especially rapacious when N_E is small. Genetic drift is a process of random change in allele frequencies from generation to generation leading to fixation of alleles (lack of allele variants or polymorphisms) and loss of diversity. Genetic drift is common in founder events associated with re-colonization, especially when the founder population has a small N_E (see Figure 1.2). A genetic bottleneck occurs when a population drops to a very small size. If the bottleneck lasts for an extended period of time then genetic drift (which acts most strongly in small populations) will tend to have a much stronger influence than mutation and population heterozygosity will decrease. Because this random process will select alleles differentially among subpopulations they become genetically divergent, a standard measure of genetic distances among subpopulations is F_{ST} (see section 1.2.3 for an explanation of F_{ST}).

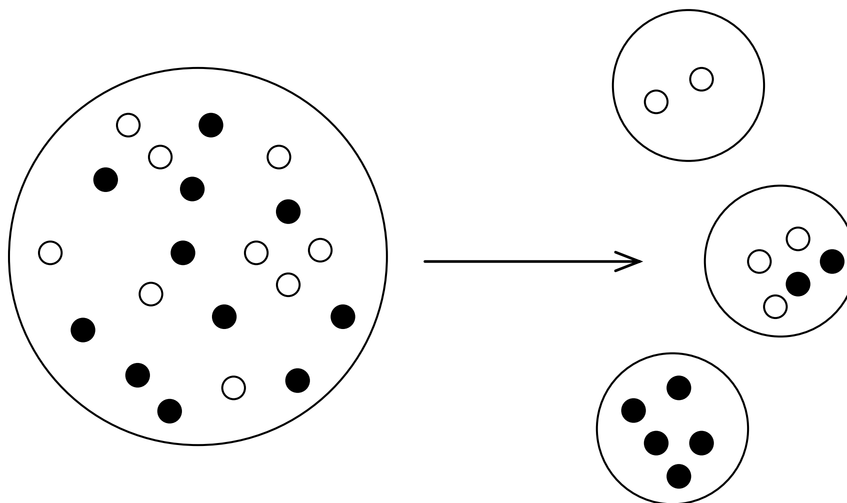

Figure 1.2 A simple illustration of a founder event showing the original population (left) and three possible relatively genetically depauperate founder populations (right).

Re-colonization within small contemporary metapopulations is often inhibited posing increased extinction risk and carries additional ecological risks, e.g., introduced predators or disease (Evans and Sheldon 2008; Frankham 2010). Extinction risk is increased when habitat loss only allows a smaller number of subpopulations to survive and 'blink on' causing genetic coalescence of population patches (see Fig. 1.1). In patch coalescence, all surviving subpopulations are derived from a single but persistent subpopulation that only represents a small proportion of original diversity. Genetic drift slowly leads the genetically coalesced subpopulation(s) to homozygosity, which in turn might contribute to extinction in the face of environmental challenges that require variability to respond. In some cases, genetically homogeneous populations can survive happily for long periods of time (e.g., a large number of inbred plants). In other cases, metapopulation extinction occurs where the populations cannot adapt to changing environmental conditions and therefore cannot persist long enough for mutation to happen.

1.2.2 *Dispersal versus gene flow in migration-drift equilibrium*

Another force that counteracts genetic divergence due to drift is migration. In fact, the ability of a species to disperse over a gradually changing environment could enable a population to survive extreme conditions, such as climate change and habitat fragmentation. Two quite distinct processes must be distinguished when thinking about genetic connectivity through migration. The first is dispersal, which is a transfer of *individuals* among subpopulations. The second is gene flow, or a transfer of *genes* among subpopulations. The term gene flow describes *effective dispersal*, where dispersers mate with individuals in their new non-natal location. Under migration-drift equilibrium, genetic divergence as measured by F_{ST}-values is inversely proportional to the number of dispersers (Figure 1.3a). However, when dispersers do not contribute to the gene pool a mismatch between gene flow and dispersal occurs and genetic divergence is much higher than expected based on the number of dispersers observed (Figure 1.3b). Identification of dispersers and gene flow is crucial in genetic conservation of wild populations because 1) dispersal estimates are a good indicator of future patch re-

colonization potential and 2) where dispersers do not effectively contribute to the local gene pool, genetic divergence may be higher than expected under conditions of migration-drift equilibrium (Palsboll et al. 2007). In natural populations effective dispersal can be resource-limited by habitat loss and poor quality habitats (Gilpin 1991; Ford et al. 2001; Atkinson and LaPointe 2009; Escalona et al. 2009; Baker et al. 2010; Reding et al. 2010).

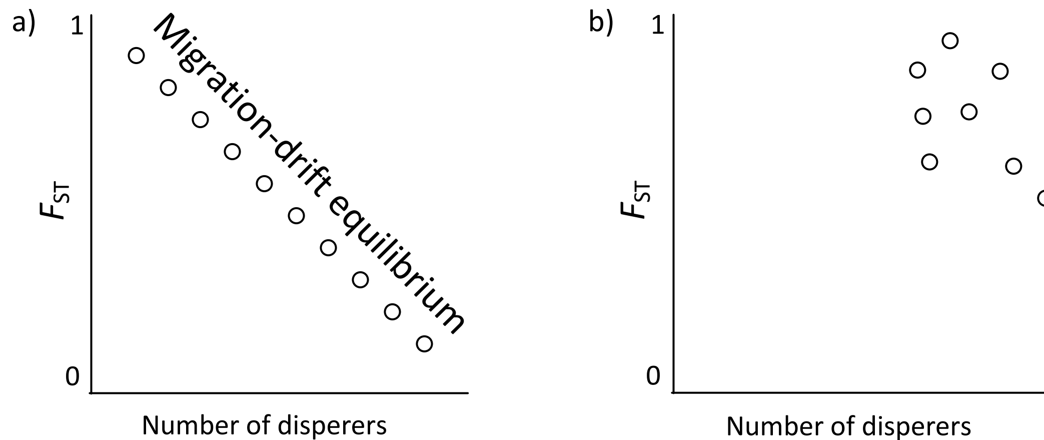

Figure 1.3 Relationships between gene flow, genetic divergence and dispersal in wild populations a) under migration-drift equilibrium and b) when dispersers cannot contribute effectively to the gene pool where genetic divergence is too high to be explained by the amount of dispersal. Dots indicate geographic sampling locations within a species being studied.

1.2.3 *Common statistical measures in population genetics*

Population genetics can be defined as the study of allele frequency distribution and change under the influence of four main evolutionary processes: natural selection, genetic drift, mutation and gene flow (Frankham et al. 2002). It also takes into account the factors of recombination, population subdivision and population structure. The primary founders of population genetics as a field were Sewall Wright, J. B. S. Haldane and R. A. Fisher, who also laid the foundations for the related discipline of quantitative genetics. Over the past couple of decades, technological advances in computing power have allowed statistical genetics to proliferate. In this section, by no means do I attempt to provide an encyclopedic overview of

modern statistical genetics. Instead, the aim of this section is to address some basic statistical concepts behind some of the software programs that I use in this thesis that are not explained elsewhere in my thesis, e.g., program BOTTLENECK, measures of genetic differentiation and distance (G_{ST}, Jost's D and Cavalli-Sforza-Edwards distances), interpretation of STRUCTURE plots, identification of migrants using GENECLASS, and the use of Bonferroni corrections. For simplicity, I have addressed the programs and statistical measures described here in as they apply to analyses of microsatellite DNA data. The statistical methods that I used, which were applied to mitochondrial DNA data are described in the following section on biogeography (section 1.3).

1.2.3.1 *Hardy-Weinberg equilibrium and Wright's inbreeding coefficient (*F_{IS}*)*

In an infinitely large population, allele and genotype frequencies under random mating attain an equilibrium referred to as Hardy-Weinberg equilibrium (HWE) (Frankham et al. 2002). For a bi-allelic locus, such as in microsatellite loci, where the gene frequencies are p and q:

$$p^2+2pq+q^2 = 1$$ (Frankham et al. 2002)

Any deviation from HWE should be a good alert for genotyping errors unless there are biological reasons for deviations, such as selection, mutation or migration. Also, microsatellite loci can be out of HWE when the effective number of breeders in a population is small, the allele frequencies will (by chance) be different in males and females, which causes an excess of heterozygotes in the progeny with respect to Hardy-Weinberg equilibrium expectations (Luikhart and Cornuet 1999).

The Wright's inbreeding coefficient, F_{IS}, is a measure of departure from HWE within subpopulations:

$$F_{IS} = 1 - (H_O / H_S)$$ (Wright 1969)

where H_O is observed heterozygosity averaged across all population fragments and H_S is expected heterozygosity if subpopulations were panmictic (i.e., the average, He, expected

heterozygosity across all subpopulations). F_{IS} is the probability that two alleles in an individual are identical by descent and can be a measure of assortative mating, inbreeding or Wahlund effects in which individuals from multiple populations are sampled in one location.

1.2.3.2 *Measures of genetic differentiation*

In this thesis I used ARLEQUIN to compare pair-wise F_{ST} and R_{ST} between sites, and SMOGD to generate Jost's D estimates and its associated confidence intervals. F_{ST}, the fixation index (sometimes referred to as G_{ST}), is the effect of the population subdivision on inbreeding (Frankham et al. 2002):

$F_{ST} = 1 – (H_S / H_T)$ (Wright 1969)

where H_S is expected heterozygosity averaged across all population fragments and H_T is expected heterozygosity for the total population. F_{ST} is the probability that two alleles drawn randomly from a population fragment are identical by descent. F_{ST} ranges from 0 to 1, and with high rates of gene flow this probability, or F_{ST} value, is low. The F_{ST} value increases as gene flow decreases and populations diverge and become inbred (Frankham et al. 2002). It is important to note that F_{ST} can be equal to 1 only if subpopulations are fixed for different alleles (fixation index).

F_{ST} is a widely used measure of genetic divergence and is almost always reported in studies using microsatellite loci. However, F_{ST} is basically based on an infinite allele model (IAM), therefore not entirely suited for analyses of multiple polymorphic loci such as microsatellites. In many cases F_{ST} values are either over or under significant (explained below). In this study F_{ST} values are very high. Though this significance level is 'real' and not a statistical error, it may not reflect the biological significance or true divergence due to drift in populations (Jost 2009). That is why I also use R_{ST}, which is a more microsatellite-specific mode of mutation (Slatkin 1995). Unfortunately, R_{ST} has lower statistical power partly due to the relatively higher variance of its estimator (Slatkin 1995). F_{ST}, though based on the infinite allele model outperforms R_{ST} when sample sizes and the number of loci are moderate (Gaggiotti et al. 1999).

The problem is that F_{ST} necessarily approaches zero when gene diversity is high, even if subpopulations are completely differentiated (Jost 2008). Thus, when diversity and differentiation are high, F_{ST} can give nonsensical results (Jost 2008). *D* measures the actual relative degree of differentiation of allele frequencies among the demes of a population. Therefore, a rejection of a hypothesis of panmixia resulting from Jost's *D* estimates should indicate more biologically significant conclusion of genetic differentiation among sampling locations (Jost 2009). At present, there is much consensus among the population genetics community that Jost's *D* should be used to measure population differentiation.

1.2.3.3 *Significance tests for genetic bottlenecks*

A genetic bottleneck occurs when a population drops to a very small size. During a bottleneck, alleles will be lost from the population and levels of heterozygosity will temporarily be higher than expected under mutation-drift equilibrium. Heterozygosity (*H*) is the sum of the proportions of heterozygotes at all loci divided by the total number of loci sampled (Frankham et al. 2002). For example, if the proportions of individuals heterozygous at three loci in a population are 0.1, 0.2 and 0.3, then $H = (0.1 + 0.2 + 0.3)/3 = 0.2$. When testing for genetic bottlenecks, we are testing for a statistically significant excess of heterozygosity relative to allelic diversity. In population genetics, the concept of heterozygosity is commonly extended to refer to the population as a whole, i.e., the fraction of individuals in a population that are heterozygous for a particular locus. Population geneticists have shown that even if the number of founders is only 2 individuals, 75% of the heterozygosity will be retained initially. The impact on allelic diversity is often greater in comparison to that on heterozygosity, such that heterozygosity is often far higher when compared to allelic diversity just after a population bottleneck. If this difference between allelic diversity and heterozygosity excess is large enough then it is said to have undergone a population bottleneck.

In this thesis, I used the program BOTTLENECK 1.2.02 (Cornuet and Luikart 1997, Piry et al. 1999) to test whether the level of heterozygosity derived from the observed allele frequencies per sampling location differed from the heterozygosity expected under mutation–drift equilibrium and to determine which populations underwent a recent bottleneck within the last

$2N_e$-$4N_e$ generations. The two-tailed Wilcoxon signed-rank test is the most powerful and robust test of three in BOTTLENECK 1.2.02 for studies using less than 20 loci (Piry et al. 1999). As the mutation model underlying the microsatellite markers was unknown, we analysed the data under three different model assumptions: the IAM, the stepwise mutation model (SMM) and the two-phase model (TPM) with 95% stepwise mutations and a variance of 12 and 1000 iterations, as recommended by Piry et al. (1999). According to Piry et al. (1999), the IAM is recommended for allozyme data and the SMM for microsatellite loci with dinucleotide repeats. However, it is the TPM that should be used for most microsatellites (Piry et al. 1999). Though it may be inappropriate for microsatellite loci, many researchers report results for the IAM model. In some cases the IAM shows a false positive significant bottleneck using microsatellite loci, while the SMM and TPM will not be significant. I show my results for all three models in this thesis both for comparison with other studies and to display the robustness of my findings.

1.2.3.4 *Genetic distance and population clustering*

Common analyses that should always be used in any population genetic study are principal component analysis (PCA) to measure the genetic distance among populations and Bayesian determination of the number populations (e.g., in STRUCTURE). In this thesis, I also used chord distance trees to provide a good visual of clustering and genetic distance relationships among the bellbird locations (‘populations’). This section applies to analysis of microsatellite DNA datasets.

A locus-specific population genetic data set, such as the bellbird dataset in this thesis, consists of a set of individual- and population-indexed gene frequencies at one or more loci. These data can be analyzed as a set of numbers without making any biological assumptions using principal components analyses (PCA), Euclidean distances or somewhat more complex geometric distances. Many of these will allow us to create a sort of abstract "map" of the populations in one, two, three or more dimensions. Paetkau et al. (1997) provide an evaluation of the various distance measures that apply to distance measures potentially useful for microsatellite analysis of wild populations. The Cavalli-Sforza-Edwards (Cavalli-Sforza and Edwards 1967) chord distance is an early measure of genetic distance that is gaining ground for

use with microsatellites today because it was specifically evaluated (and performed well) in simulations of tree-building algorithms by Takezaki and Nei (1996).

Bayesian analysis of the number of population clusters is an inference method radically different from classical frequentist approaches, which takes into account the prior probability for an event. STRUCTURE uses a model-based clustering method based on Markov Chain Monte Carlo (MCMC) to detect the underlying genetic population among a set of individuals genotyped at multiple markers (Pritchard et al. 2000). Basically, it computes the proportion of the genome of an individual originating from each inferred population (quantitative clustering method). When interpreting a STRUCTURE bar chart plot (see Appendix A; Figure A.1) each vertical line represents a single individual and the different colours represent an inferred population. To infer the number of populations, K, I calculated an ad hoc quantity (ΔK) based on the second order rate of change of the likelihood (ΔK) (Evanno et al. 2005). The ΔK shows a clear peak at the true value of K (see Appendix A; Figure A.1: in this example based on the bellbird dataset in this study, $K = 4$). In all cases, when trying to determine the number of populations, one should never set location priors because that will artificially force an individual into a population where it does not belong.

1.2.3.5 *Use of Bonferroni corrections in population genetics*

A Bonferroni correction is always (not just commonly) used to adjust the P-values for frequency-based analyses of highly polymorphic loci in population genetics, e.g., null allele analysis in MICROCHECKER, F_{ST} analysis in ARLEQUIN. This is because there is tendency for over-significant P-values. The problem is that the use of multiple highly polymorphic loci is like using multiple tests of significance in regular statistical analyses. With certain statistical methods, e.g., multiple regression, there are frequently many statistical hypotheses being tested. In such studies, setting $\alpha = 0.05$ does not provide sufficient protection against the Type I Error. What happens is that as the number of separate hypothesis tests increase within a single study, the true α-level for the entire study will be inflated. Therefore, as is standard in the population genetics literature today, I applied a well used sequential Bonferroni correction to, e.g., F_{ST}, R_{ST}, HWE and linkage disequilibrium (LDE) test analyses. The MICROCHECKER program has a built-in

Bonferroni correction when testing for null alleles. For sequential Bonferroni correction in this thesis, I used the formula:

$p_i = \alpha / (1 + k - i)$ at $\alpha = 0.05$ (Peres-Neto 1999)

where k is the total number of tests, and i is the test level.

1.3 Biogeography of birds and their parasites

Biogeography is the study of the distribution of biodiversity over space and time. It aims to reveal where organisms live, and at what abundance and why they may or may not be present. This dynamic amalgamation of disciplines weaves together ecology and evolutionary biology and in recent years has been an epicentre of advances in theoretical and mathematical ecology. Nested within biogeography, phylogeography is an explicit synthesis of phylogenetics and population genetics with geographic distribution. In this thesis, I approach phylogeography of the host species with a wide range of methodological techniques developed to infer a continuum of signals of genetic structure between species, among populations and within populations (Carstens et al. 2004) (see Figure 1.4). In this thesis, I used Tajima's *D* (Tajima 1989), mismatch distribution (Avise 2002), analysis of molecular variance (AMOVA) and an analysis of isolation-with-migration (IM) to assess genetic structure with and among bellbird populations. Likewise, I demonstrate how phylogenetic and spatial ecology (ecographic) considerations can be combined to assess the biogeographical origins and relationships between that host and its parasites.

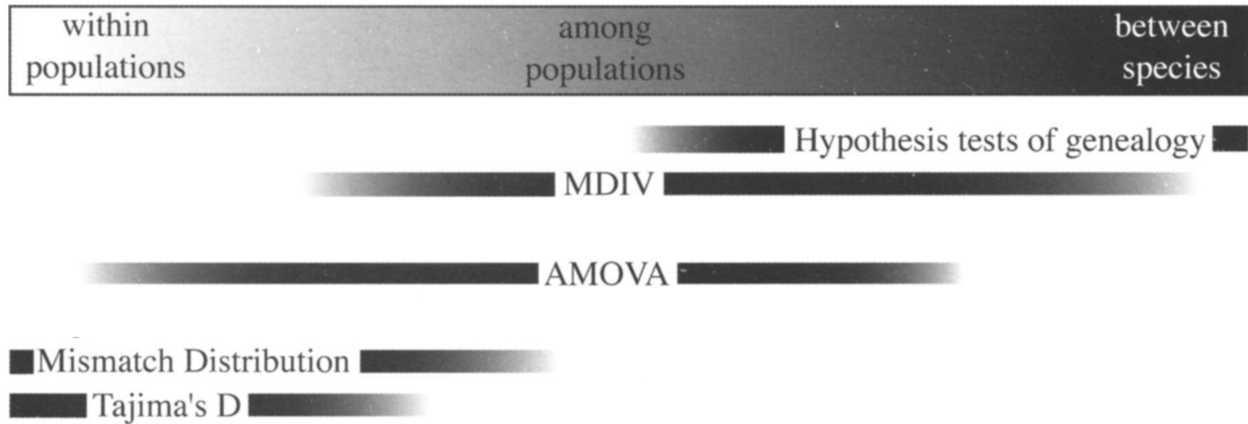

Figure 1.4 The continuum of genetic variation (top) and analytical methods used to interpret the genetic signal (bottom). The bars on either side of the method name show the approximate range to which population geneticists have applied these tests. Diagram modified from Carstens et al. (2004)

1.3.1 *Phylogeographic hypotheses*

Along a continuum of theoretical outcomes, five main hypotheses of intraspecific phylogeographic patterns allow us to discern the mode or pattern of population separation (see Figure 1.5). First, hypothesis I, there is a deep split in the gene tree and major lineages are allopatric (etymology: from ancient Greek *allos* meaning 'other' + Greek *patrā* meaning 'fatherland'). It must be explained here that allopatry occurs where a population splits into two geographically isolated populations, e.g., due to habitat fragmentation. This pattern occurs when long term barriers cause geographic isolation. Second, hypothesis II, there is a similarly deep gene tree as in the first hypothesis, but the major lineages are broadly sympatric. This pattern results from secondary admixture and hybridization of two previously diverged populations. Because this pattern can also be explained by evolutionary processes of lineage sorting and balancing selection, independent evidence for actual barriers to gene flow, gene tree concordance among sympatric species and among multiple loci must corroborate any conclusions made. Before I can present an effective discussion on another type of supportive

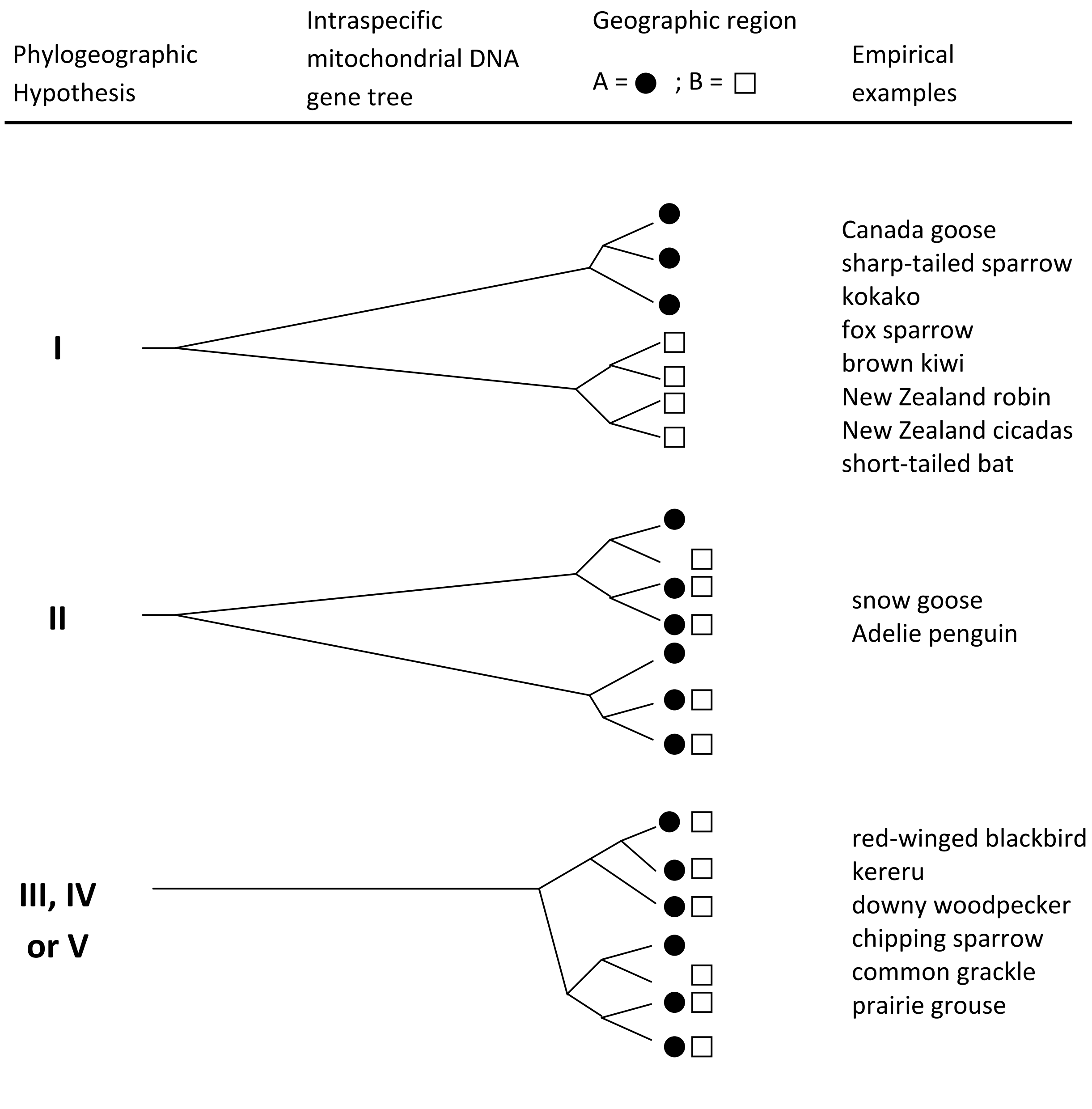

Figure 1.5 A schematic of five common phylogeographic hypotheses. Diagram modified from Avise (2000).

evidence for hypothesis II, I must briefly explain the coalescent as it applies to phylogenetics. Coalescent theory is a retrospective model of population genetics, which uses a sample of individuals from a population to trace backward in time all alleles of a gene shared by all

members of the population to a single ancestral copy, known as the most recent common ancestor (MRCA) (Nordborg 1997). The coalescent runs models of genetic drift and allows estimation of the time to most recent common ancestor (T_{MRCA}). Based on the coalescent, a mitochondrial DNA mismatch distribution is simply the pair-wise frequency distribution of co-ancestry. Finally, hypotheses IV-V all involve shallow gene trees in which haplotype lineages are either IV) closely related and localized geographically, V) sympatric or VI) of variable distribution.

A bimodal mismatch distribution can provide support for a hypothesis of allopatry. Since the amount of sequence divergence between any two mitochondrial DNA sequences depends on the length of time since they diverged, it suggests that we might be able to learn more about the recent demographic history of populations by looking at the whole distribution of sequence differences (Avise 2002). If you count the number of site differences (the mismatches in a sequence alignment) between each pair of DNA sequences in a sample, and use the resulting counts to build a histogram, you end up with a "mismatch distribution" which can be ragged, unimodal or bi-modal (see Figure 1.6a-c). Mutations that happened far in the past, near the base of a gene tree, can be observed at high frequencies in the sample and produce a ragged histogram (Figure 1.6a). Recent mutations, on the other hand, always exist in one or a few copies. If a recent population expansion has occurred, so that the gene tree is star-like, many mutations occur in the long recent branches and there are more singleton sites and sites with low frequency variants than are expected in a stationary population. In recent a population expansion, the histogram produced in the mismatch distribution will be unimodal (Figure 1.6b). Bimodal mismatch distributions typically result when populations are allopatric, i.e., have been separated geographically with no overlap (Figure 1.6c). In these cases there is typically a deep split in a reciprocally monophyletic phylogenetic tree. The pairwise mutation distances are more similar within each main branch of the tree than among the two main branches (Avise 2002). Additionally, with the mismatch distribution analysis output in ARLEQUIN, you get several population point estimates which can in turn be used to calculate estimated time. A comprehensive example of the information that can be acquired from point estimates is better illustrated in Chapter Four of this thesis (see Table 4.5).

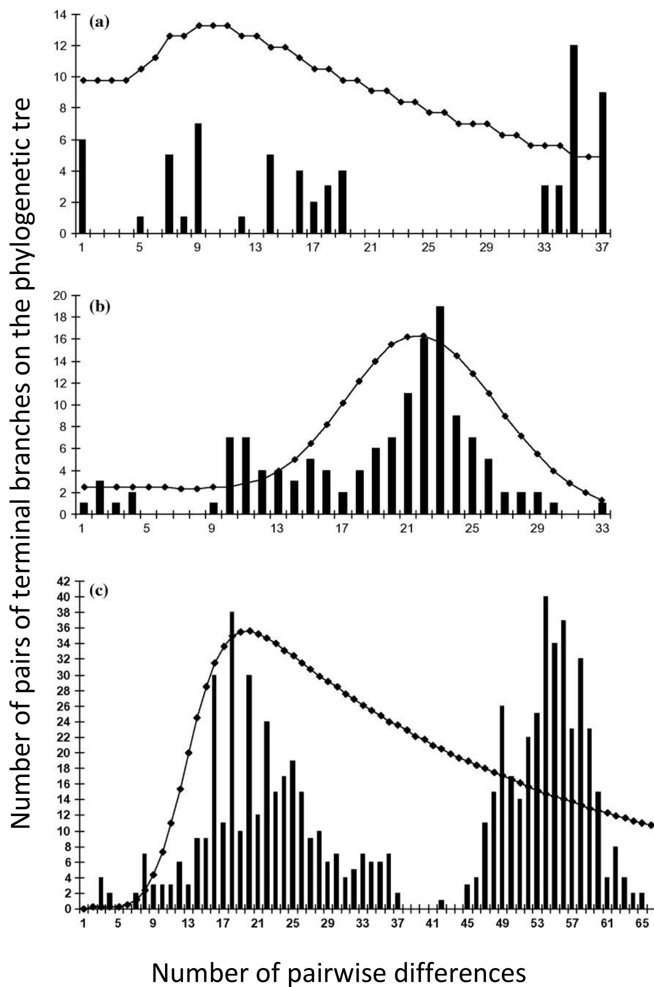

Figure 1.6 Three intraspecific patterns from mitochondrial mismatch analyses of natural populations: a) ragged distribution frequency indicative of stable population, b) unimodal distribution consistent with demographic expansion and c) a bimodal mismatch distribution often resulting from allopatric fragmentation of populations. Diagram taken from Sole et al. (2008)

Another measure of within population genetic variation is Tajima's *D*. Fumio Tajima introduced a statistic that is widely used to test the null hypothesis of mutation-drift equilibrium and constant population size. In this thesis I used it as corroborative tool to test for ancient bottlenecks (deviations from mutation-drift equilibrium). Demographic fluctuations and other violations of the neutral model (including rate heterogeneity and introgression) will change the expected values of segregating sites, *S*, and nucleotide diversity, π, so that they are no longer expected to be equal (Tajima 1989). The difference in the expectations for these two variables (which can be positive or negative) is the crux of Tajima's *D* test statistic (Tajima 1989). A negative Tajima's *D* signifies an excess of low frequency polymorphisms, indicating population size expansion (e.g., after a bottleneck or a selective sweep) and/or purifying

selection (Tajima 1989). A positive Tajima's *D* signifies low levels of both low and high frequency polymorphisms, indicating a decrease in population size and/or balancing selection (Tajima 1989).

1.3.2 *Why sometimes does speciation not occur given allopatry?*

Given enough time, isolated populations will diverge genetically to the point at which they are incapable of exchanging genes with each other (Grant and Grant 2010). In their theory on island biogeography, MacArthur and Wilson (1963, 1967) made the insightful point that a balance would be struck between the opposing processes of immigration and isolation. As Grant and Grant (2010) put it “if islands are reached once they can be reached again”. In other words, sometimes speciation does not occur because repeated immigration, or gene flow, retards divergence. In 2001, Rasmus Nielsen and John Wakeley published a mathematical method using the coalescent that allowed researchers to quantify the role of gene flow during the divergence of closely related species or even among populations within species. Their algorithms are based on Markov Chain Monte Carlo methods for estimating the relative effects of migration and isolation on genetic diversity among pairs of populations (Nielsen and Wakeley 2001). This model elegantly tests two very different historical trajectories among populations that 1) populations have been exchanging migrants for an infinitely long time and 2) populations are descended from a common ancestral population at some point in time in the past and have since diverged without gene flow (Nielsen and Wakeley 2001). Using an isolation-with-migration approach it is possible to determine the time of divergence and level of migration at that divergence. Thus, we can test hypotheses on whether a population split under strict allopatry has occurred, or whether gene flow was also involved and subsequently estimate the migration rates associated with that gene flow (see Figure 1.7). Isolation with migration analyses can now handle a multitude of genetic markers, e.g., autosomal loci under selection, sex-linked loci, which are not typically used in studies on population structure. MDIV is a program that simultaneously estimates divergence times and migration rates between two populations under the infinite sites model (e.g., mitochondrial DNA) and under a finite sites model (HKY) (e.g., microsatellite DNA) (Nielsen and Wakely 2001). Additionally, MDIV provides

maximum likelihood estimates of the demographic parameters that can be evaluated by plotting them (see Chapter Four). The output of the program are integrated likelihood surfaces for the three parameters: θ (two times the effective population size times the mutation rate), *M* (2 times the migration rate) and *T* (the divergence time divided by the effective population size (Nielsen and Wakely 2001).

Such isolation-with-migration patterns of gene flow, as opposed to complete cessation of gene flow resulting from a vicariant event, are becoming more common in explanations of speciation patterns (Hey and Nielsen 2007; Carling et al. 2010). In fact, today, divergence-with-gene-flow ideas in conjunction with coalescent theory (Nordborg 1997; see Nielsen and Wakeley 2001) are the foundation for contemporary research on how speciation occurs with respect to gene flow and selection dynamics (e.g., Carstens et al. 2004; Weir 2006; Carling et al. 2010; Rovito 2010; Paradis 2010; Turmelle et al. 2011).This area of science will likely develop rapidly over the next few years.

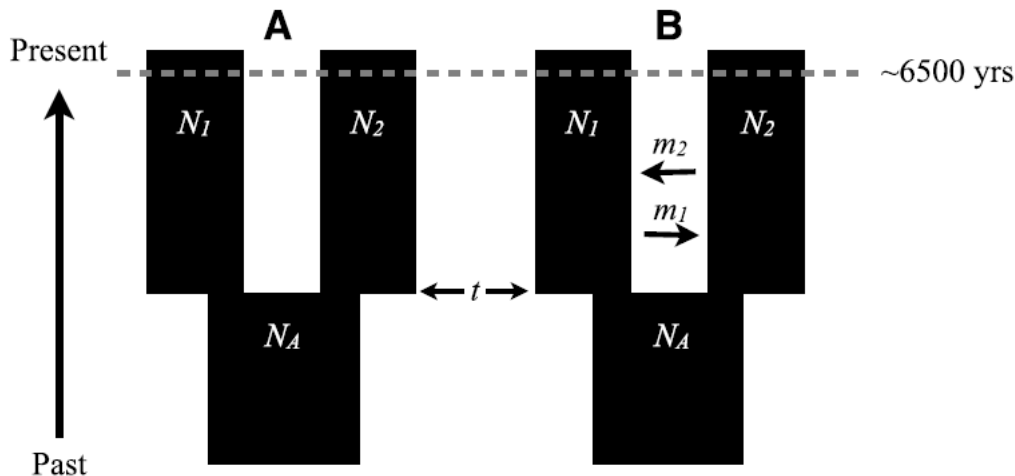

Figure 1.7 Schematic of strict allopatric speciation model (A) versus isolation-with-migration speciation model (B). The dashed line represents the approximate timing of secondary contact that has resulted in current hybridization. Diagram taken from Carling et al. (2010)

In Chapter Four on bellbird phylogeography, in addition to my MDIV analyses, I used an analysis of molecular variance (AMOVA) to assess genetic variation among bellbird populations in programs ARLEQUIN or GENALEX. AMOVA can be used for partitioning diversity within populations (in addition to among populations) by producing estimates of variance components and *F*-statistic analogs (designated as ϕ-statistics). Φ-statistics summarize the degree of differentiation between population divisions and are analogous to *F*-statistics. This was helpful in teasing out nuances in population structure and identifying barriers to gene flow by comparing the significance levels of different types of population-grouping scenarios. For example, among populations within the same geographical region (ϕ_{SC}), among regions (ϕ_{CT}) and among all populations (ϕ_{ST}) (see Chapter Four). A variety of molecular marker data (e.g., microsatellite, mitochondrial sequence data) may be analyzed using this method (Excoffier et al. 1992). The significance of the variance components and ϕ-statistics is tested using a permutational approach, therefore lends statistical power to *P*-values by eliminating the normality assumption that is inappropriate for molecular data (Excoffier et al. 1992).

1.3.3 *Biogeographical relationships between hosts and their parasites*

It may be possible to predict the degree of phylogenetic congruence between two groups from information about their ecology, e.g., co-evolution of host and parasite, host switching and failure to speciate (Clayton et al. 2004). Studies that examine the phylogenetic congruence of a host and its parasites can infer endemism and host-specificity as well as barriers to dispersal among sympatric hosts. Moreover, measures of endemism play a central role in characterizing and mapping the distribution of biological diversity (Myers et al. 2000). In general, parasite lineages with wide host and geographical distributions are considered to be generalist parasites, whereas lineages restricted to particular regions or endemic hosts are considered specialist lineages (Ishtiaq et al. 2010). Often, however, population connectivity of a parasite is not as continuous as that of the host and their respective geographic ranges can vary greatly, especially between and among islands in archipelagos (Ishtiaq et al. 2010). If the prevalence and distribution of a host-parasite interaction is heterogeneous, i.e., there is a spatial mismatch

between the distribution of the host and distribution of the parasite, then constraints on the parasite may be related to limits associated with the invertebrate vector (Valkiunas 2005). 'Ecological transmission barriers' have been shown to cause differentiation and isolation of parasite lineages between different host groups (Hellgren et al. 2008). In Vanuatu and New Caledonia for example, close associations between potentially specialist parasite lineages and endemic mosquito species have been described (Ishtiaq et al. 2008). Furthermore, parasites of the *Plasmodium* genus are known to be less constrained than *Haemoproteus* by the phylogenetic relationships of their avian hosts (Ricklefs and Fallon 2002; Beadell et al. 2004), thus *Plasmodium* species tend to be more geographically mobile than *Haemoproteus*. In an elegant study, Hellgren et al. (2008) show how parasites that can carry out their life cycle in a wide array of hosts, actually have the ability to be the most prevalent in a single host species. This is contrary to conventional wisdom that high malaria prevalence and reduced virulence are the result of a specific and long-term host-parasite co-evolution, i.e., the quintessential evolutionary arms race (Dawkins and Krebs 1979). It is important to assess the biogeographic relationships between hosts and parasites with these ideas in mind.

1.4 Evolution of immune phenotype in birds

1.4.1 *Seasonal variation in immunity: the host perspective*

Rarely are seasonal drivers of parasite prevalence, e.g., *Plasmodium* spp. (avian malaria), well understood due to the complexity of host and vector variables on seasonality (Pascual and Dobson 2005). Furthermore, there are huge gaps in fundamental knowledge on seasonal patterns of avian blood parasites in most wild systems, including in the Australopacific (Steadman et al. 1990; Jarvi et al. 2002, 2003; Beadell et al. 2004; Atkinson and LaPointe 2009). According to the long standing Beaudoin et al. (1971) theoretical model of bimodal malaria seasonality, which is based on temperate climates in the northern hemisphere, a peak in avian malaria prevalence occurs in late summer and autumn during maximum recruitment of disease

naïve young, then prevalence drops in winter when invertebrate vector activity decreases followed by an increase called the 'spring relapse'. The 'spring relapse' is due to a latent rise in infection of previously infected hosts due to the stress of initiating breeding, and independent of mosquito vector activity (Beaudoin et al. 1971). Therefore, Beaudoin patterns attribute parasite seasonality to facets of both host and vector ecology.

In many host animal systems, the photoperiod plays a pivotal role in seasonality of immune phenotype. This is thought to be related to energy savings adaptations (Nelson and Demas 1996). For example, during winter, it has been experimentally shown that animals adjust their immune status to balance the negative effects of stress-induced adrenal steroids that act to suppress the immune system (Nelson and Demas 1996). This adjustment actually occurs before the onset of winter, indicating a highly evolved link with photoperiod cues. Furthermore, stressors on immune phenotype, e.g., scarcity of food and low ambient temperature, raise glucocorticoid levels, which in turn reduce immune function (Nelson and Demas 1996). Recent studies corroborate that host endocrinological stress due to breeding and various other activities is often associated with changes in ecological constraints (Wingfield et al. 1997), humoral immune cell distribution (Dhabhar et al. 1995) and susceptibility to malarial infection (Atkinson et al. 1995; Valkiunas 2005; Wada et al. 2007). Finally, for some host species, the most stressful time of year might not be winter and instead coincide with the establishment or defense of breeding territories and mates (Nelson and Demas 1996).

1.4.2 *Immune defense trade-offs*

A central assumption is that immune defense is traded-off with other functions or activities that share common resources or contribute to an animal's fitness (Sheldon and Verhulst 1996; Zuk and Stoehr 2002; Lee 2006). Recent studies provide evidence that host factors are important in the prevalence and parasitemia of avian malaria (Wood et al. 2007; Cosgrove et al. 2008; Knowles et al. 2011). Reproductive effort (Norris et al. 1994; Merila and Andersson 1999; Stjernman et al. 2004; Nelson and Demas 1996), seasonal variation in stress and sex hormone levels (Deviche et al. 2001) and individual health status are well established characters that influence differences in individual host susceptibility to disease (Norris et al. 1994; Merila and

Andersson 1999). In the following paragraph I will briefly outline three main trade-off mechanisms in immune defense variation: 1) resource limitation, 2) life-history and 3) fitness.

The currency of immune defense trade-offs is often energy, in the form of resource limitation (Raberg et al. 1998). This means that when energy is at a premium, energy that would otherwise be invested in immune phenotype is allocated to other aspects of an animal's ecology and a host is more susceptible to disease. The immunocompetence limiting aspects of food resources such as carbohydrates and protein are thought to be important (see Cook 1980). Saino et al. (1997) show that different nutrients (e.g., protein) mediate specific aspects of immune defense (e.g., cell-mediated immune response) that may in turn be responsible for adaptive immune response to pathogens such as malaria. Trade-offs between life-history traits such as 1) reproductive effort and 2) secondary sex characteristics have been shown to directly affect the risk of parasitism (Moller 1997; Sheldon and Verhulst 1996; Nelson and Demas 1996). Increased reproductive effort is thought to reduce immune competence (Deerenberg et al. 1997) (Fig 8). However, other studies have shown that reproductive effort can actually increase with decreased resistance to parasites, and this is attributed to a trade-off in reproductive effort for longevity (Marzal et al. 2008) (Figure 1.8). In other words, an individual

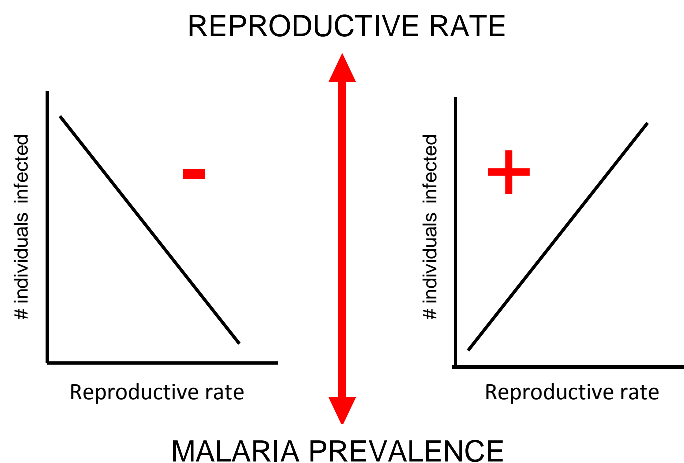

Figure 1.8 The 'sign' of the relationships between life-history output (reproduction, territory defense, body condition) and parasite infection has been shown to vary among different studies.

that invests in immunity just might live longer than an individual who invests in reproduction. Sex ornamentation investment increases the chances of passing genes on to the next

generation but at an energetic cost to immune phenotype. Folstad and Karter (1992) applied the immunocompetence handicap hypotheses (ICHH) to the Hamilton and Zuk (1982) idea that sexually selected ornaments are dependent on honest signals which indicate good genes. The ICHH parasite-mediated sexual selection hypothesis is based on the double function of testosterone, which allows males to fully express their ornaments and at the same time acts as an immunosuppressant.

Host demography at the population level will have important repercussions to individual-based immune defense trade-offs. For example, population density has been shown to be an important influence on parasite prevalence, depending on how parasite transmission relates to host population density (Keymer and Anderson 1979). In epidemics, host density and transmission efficiency strongly influence the evolution of virulence. By contrast, endemic infections place a premium on duration of infection, and virulence is not influenced by opportunities for transmission (Frank 1996). Furthermore, density and growth stage of a host population are often influenced by demographic processes such as population expansion, stability and recent population bottlenecks, which in turn may influence resistance to disease and parasite prevalence (see Sommer 2003; Miller and Lambert 2004; Kilpatrick 2006). In birds, dispersal and range expansion (re-colonizations) are often associated with increases in malaria infections (see Sommer 2003; Perez-Tris and Bensch 2005). It is clear that the overlying energetic stressors of the population translate to each individual regarding the cost of immunity.

1.4.3 *Sexual dimorphism in immune phenotype*

Sex dimorphism in animals is common and it is females that are usually more immunocompetent. Sex differences in parasite infection rates are explained by two leading theoretical approaches (Rolff 2002). First, the immunocompetence handicap hypothesis, which suggests that the immune system competes for resources with sexually selected ornaments, involves different endocrinological mechanisms that alter the immune system in different directions, i.e., parasite-mediated sexual selection (Hamilton and Zuk 1982; Folstad and Karter 1992). Second, an approach seated in natural selection, as opposed to sexual selection, of the

Bateman's Principle. The basic premise of the Bateman's Principle is that sex-specific life-histories result from differences in the trade-off between investment in immunity and other aspects of reproduction (e.g., males gain fitness by increasing their mating success while females increase fitness through longevity because their reproductive effort is much higher) (Rolff 2002). This approach is an alternative to testosterone-mediated immune defense of ICHH, and explains sex dimorphism in immune phenotype without relying on testosterone or other biochemical feedback loops.

1.5 A model host-parasite study system in New Zealand

1.5.1 *A changing environment*

In New Zealand, in a story similar to those of most oceanic islands throughout the globe, the introduction of novel mammalian predators and reduction of the indigenous forest has lead to the extinction of 50% of endemic bird species (Innes et al. 2010). For most of the species that remain, serious declines in bird numbers have culminated in large extirpations, including extinction from northern third of the North Island, from latitudes 37°45 S to 34°25 S (Craig and Douglas 1984). After implementation of a series of protection measures under the 1953 New Zealand Wildlife Act, some bird species with relatively abundant numbers such as the bellbird (*Anthornis melanura*), tui (*Prosthemadera novaeseelandiae*), grey warbler (*Gerygone igata*), whitehead (*Mohoua albicilla*), tomtit (*Petroica macrocephala*), South Island robin (*Petroica australis*) and rifleman (*Acanthisitta chloris*) began to recover. In the remnant island populations of the North Island regions such as the Hauraki Gulf and Poor Knights Islands some endangered bird species are locally dense (Robertson et al. 2007). Many species have been extirpated from the mainland adjacent the Hauraki Gulf and northward in New Zealand since *c.* 1860. The two most populous and species-rich Hauraki Gulf remnant bird populations are Hauturu and Tiritiri Matangi located 20 and 5 km offshore, respectively.

1.5.2 *The host*

The host is the endemic New Zealand bellbird of the Meliphagidae family. Bellbirds are highly mobile but non-migratory forest passerines and able to disperse long distances (Robertson et al. 2007). In contrast to most avifauna endemic to New Zealand, the bellbird along with its sister species the tui have not divided into North and South Island species or sub-species perhaps due to the homogenizing effects of their relatively greater dispersal ability than most New Zealand fauna (see Agudo 2011). Additionally, the two single-species meliphagid genera of New Zealand are relatively young and derive from a common ancestor in the late Pliocene *c.* 2.9 million years, which is now extinct (Driskell et al. 2007). Therefore, the vast majority of their phylogeographic history is confined to and likely influenced by the Pleistocene Epoch.

Historically, the bellbird occupied the once forest-dominated New Zealand (Ewers et al. 2006) up to the alpine tree line (Craig and Douglas 1984; Sagar and Scofield 2006). Today, the spatial distribution of bellbirds (Robertson et al. 2007) closely reflects patterns of remaining indigenous forest (Ewers et al. 2006; Walker et al. 2006). Though locally abundant, bellbirds have never re-established successfully in the large extirpation areas where forest has been removed, thus their distribution and abundance have been irrevocably changed (Craig and Douglas 1984; Robertson et al. 2007). Hauturu supports a long-term stable subpopulation of bellbirds (Gravatt 1980). At Tiritiri Matangi bellbirds experienced a severe population bottleneck and have been supplementary fed with sugar water since the early 1970s, especially during winter as part of an ongoing conservation program involving vegetation re-planting and predator eradication (Craig and Douglas 1984). A third Hauraki Gulf subpopulation, Tawharanui Regional Park (Tawharanui), has newly re-colonized in 2005 and is located on a mainland peninsula within a 35km radius of both islands. The Tawharanui bellbird population has been increasing since re-colonization after implementation of a predator-proof fence in 2004 and predator eradication at both Tawharanui and Hauturu by 2005 (Ornithological Society of New Zealand OSNZ, unpublished data). The source population for Tawharanui bellbird subpopulation is thought to be mainly Hauturu (Brunton et al. 2008).

A strong component of the bellbird social ecology is that they are highly social and aggregate in high numbers to feed and often nest within 10 metres of each other (personal

observation). These features, coupled with a strongly expressed sex-age class dominance hierarchy and considerable sex dimorphism, mean that individual bellbirds can be subject to intense physiological pressures of resource competition, mate selection and territory defense (Brunton et al. 2008). Larger males often exclude females from feeding locations, and male-male competition is physical and fierce (Craig et al. 1982; Craig and Douglas 1986). Natal dispersal intensifies during autumn, and during winter bellbirds often disperse widely in search of food (Craig 1980).

1.5.3 *The parasite and its vectors*

The parasites in this study are pigmented haematozoan blood parasites, namely *Plasmodium* spp., commonly referred to as avian malaria that complete their life cycle in an avian host and an invertebrate vector, e.g., the mosquito. *Plasmodium* is a diverse (over 130 species) and globally distributed genus of haematozoan transmitted by insect vectors, i.e., mosquito, and exhibits a full range of host-specificity from generalists able to infect many clades of birds to specialists able to infect only a host single species (Valkiunas 2005). Little has been documented about the ecology, diversity, distribution and abundance of endemic or exotic avian malaria species in New Zealand. However, Tompkins and Gleeson (2006) report 10 lineages (different mitochondrial DNA sequences) of the *P. relictum* species alone in one survey. Data depositions made to the international MalAvi database (Ruth Brown unpublished data; Bensch et al. 2009) show that at least four exotic, e.g., house sparrow (*Passer domesticus*), European thrushes (*Turdus* spp.), and five endemic, e.g., South Island robin (*Petroica australis*), saddleback (*Philestrunus carnuculatus*), hihi (*Notiomystis cincta*), bird species have been infected by a variety of avian malaria including such well known exotic cosmopolitan parasites as SGS1 (*P. elongatum*) and GRW4 (*P. relictum*) in New Zealand. Furthermore, putatively non-endemic avian malaria (*Plasmodium* spp.) outbreaks have been known to occur in bird species endemic to New Zealand (Alley et al. 2008; Derraik et al. 2008; Sturrock and Tompkins 2008; Alley et al. 2011).

The ecology of malaria transmission can depend largely on climatic conditions, primarily seasonal changes in temperature and rainfall that drive vector populations (Atkinson and

LaPointe 2009). Mosquitoes are most common vector for *Plasmodium*, whereas Hippoboscid flies transmit *Haemoproteus* (Valkiunas 2005). Mosquitoes are endemic to New Zealand (16 species; Derraik et al. 2004), unlike some Pacific Ocean islands, i.e., Hawaii (Atkinson and LaPointe 2009). Depending on the vector, vector activity often peaks in New Zealand during autumn (February-May) (Jose Derraik, personal communication). Of the many exotic mosquito species that have landed in New Zealand, successful invasions have been made by only four species (Derraik et al. 2004).

1.6 Thesis aims and outline

The central aim of this thesis is to identify genetic and ecological aspects of the processes underlying movements and community structure of animals and their parasites in changing environments. I use three approaches to address this central aim:

1) assess metapopulation dynamics and population genetic connectivity;

2) identify origins, diversity and distribution of host and parasite;

3) determine factors associated with spatiotemporal variation in host-parasite interactions.

Concepts in population genetics, biogeography and evolution of immune competence of wild populations are tied together in five complementary data chapters, which feature a common highly mobile passerine host and its malaria parasites. **Chapter Two** is a broad overview of bellbird genetic diversity and population structure in fragmented landscapes. Genetic characterization of a natural (unassisted) re-colonization event of bellbirds to a mainland site from which they had been extirpated for 150 years is the topic of **Chapter Three**. **Chapter Four** is a phylogeographic perspective on how speciation was prevented in a highly mobile species by testing a hypothesis of allopatric separation followed by secondary contact. In **Chapter Five**, I use a phylogenetic analysis and international avian malaria databases to identify the origins and global distribution of bellbird avian malaria parasites with some discussion on endemism and host-specificity. Spatial and seasonal variation in avian malaria parasites among an active bellbird metapopulation is the topic of **Chapter Six**, within which I model the influences of host sex, age and body condition on avian malaria parasite prevalence. Finally, **Chapter Seven** is a short synthesis of ideas and findings in the previous chapters. Here, I discuss potential future work that may fill any gaps in my work, outline my focal research interests in host-parasite ecology and evolution, and highlight emerging research areas enabled by novel molecular and post-genomic approaches to natural systems.

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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

Habitat loss, novel predators and exotic disease have caused dramatic changes in the distribution and abundance of endemic island species such that intensive conservation initiatives, e.g., translocations, are required to maintain genetic diversity and gene flow. Most research has focused on endangered species and there have been fewer studies on less threatened species. This is the first population genetics study to investigate the genetic diversity and population structure of a relatively abundant and vagile passerine species endemic to New Zealand. We used the New Zealand bellbird (*Anthornis melanura*) as a model species and sampled from nine locations throughout its geographic range. Overall, we found mean heterozygosity (H_E) = 0.69 and allelic richness (A_R) = 5.2 in bellbirds and most sampling locations were in mutation–drift equilibrium. Significant genetic divergence (F_{ST}) among sampling locations was evident. A continuous distribution migration model of isolation-by-distance best characterized large-scale genetic differentiation in bellbirds, at least historically. Identification of first generation migrants provided evidence of moderate ongoing dispersal. However, departures from migration-drift equilibrium and high inbreeding levels despite the presence of migrants in urban areas and small previously deforested remnant islands suggested

habitat-limited gene flow. In this study we provide insight into the demographic and genetic connectivity of a vagile passerine across fragmented landscapes and discuss the conservation implications.

Keywords New Zealand bellbird, Extinction potential, Genetic divergence, Genetic diversity, Habitat loss, Dispersal

2.2 Introduction

Loss of genetic diversity has serious implications for species survival on ecological and evolutionary scales (Frankel 1974; Ciofi et al. 1999; Frankham 2010). Human-induced habitat loss and fragmentation alter the distribution and abundance of species, which in turn leads to genetic differentiation and inbreeding among isolated subpopulations (Pereira et al. 2010). Translocation of individuals from remnant or captive-reared populations is a form of genetic rescue that includes re-introduction of species to their former ranges and enhancement of genetic diversity in wild populations (Haig et al. 1990; Hedrick and Fredrickson 2010). There is considerable concern regarding inadvertent negative consequences of translocation (Frankham 2010), especially when migration among population patches is not promoted (Ingvarsson 2001). Additionally, hybridization of inbred adaptively divergent subpopulations can lead to outbreeding depression, which is a loss of alleles through reduced fitness of offspring (Allendorf and Luikart 2007). At present it is not possible to predict whether outbreeding depression will occur using neutral genetic markers because it results from introgressive hybridization of adaptively differentiated regions of the genome that are under selection (Frankham 2010). Meanwhile, population genetic analyses using microsatellite DNA can provide invaluable information to help assess risks associated with translocations by 1) identifying the degree of

genetic differentiation and inbreeding among populations, and 2) promoting our understanding of extant genetic structure to elucidate how population connectivity can be maintained.

Vagile dispersers typically function under a continuous distribution population model, in which genetic differentiation is positively related to geographic distance, i.e., isolation-by-distance (Slatkin 1993). In these cases, genetic connectivity occurs through demographic dispersal (movement of individuals) and genetic migration (gene flow) but populations are not necessarily panmictic, or void of genetic differentiation. In genetic conservation of wild populations identification of dispersers, in addition to assessing genetic divergence, is crucial because 1) dispersal estimates are a good indicator of patch re-colonization potential and 2) where migrants cannot contribute to the local gene pool, genetic divergence may be higher than expected under conditions of migration-drift equilibrium (Palsboll et al. 2007). In the latter case, there is a mismatch between gene flow and dispersal, i.e., dispersal is not effective. In natural populations effective dispersal can be resource-limited by habitat loss and poor quality habitats (Gilpin 1991; Ford et al. 2001; Atkinson and LaPointe 2009; Escalona et al. 2009; Baker et al. 2010; Reding et al. 2010).

Since the arrival of humans to New Zealand, the introduction of novel mammalian predators and reduction of the indigenous forest by 70% (Ewers et al. 2006) has lead to the extinction of 50% of endemic bird species from the two main islands and many other species have become rare or uncommon (Innes et al. 2010). The endemic New Zealand bellbird (*Anthornis melanura*), a relatively abundant and vagile disperser (Robertson et al. 2007) of the Meliphagidae family, historically occupied the once forest-dominated New Zealand (Ewers et al. 2006) up to the alpine tree line (Craig and Douglas 1984; Sagar and Scofield 2006). Serious declines in bird numbers have culminated in the extirpation of the bellbird from the northern third of the North Island from latitudes $37^{o}45$ S to $34^{o}25$ S (Craig and Douglas 1984). After implementation of a series of protection measures under the 1953 New Zealand Wildlife Act, some bird species with relatively abundant numbers such as the bellbird, tui (*Prosthemadera novaeseelandiae*), grey warbler (*Gerygone igata*), whitehead (*Mohoua albicilla*), tomtit (*Petroica macrocephala*), South Island robin (*Petroica australis*) and rifleman (*Acanthisitta

chloris) began to recover. Today, the spatial distribution of bellbirds (Robertson et al. 2007) closely reflects patterns of remaining indigenous forest (Ewers et al. 2006; Walker et al. 2006). Though locally abundant, bellbirds have never re-established successfully in the large extirpation areas where forest has been removed, thus their distribution and abundance have been dramatically altered (Craig and Douglas 1984; Robertson et al. 2007).

This is the first population genetic study of a relatively abundant and well-flighted passerine endemic to New Zealand. Our main objective was to delineate broad-scale patterns of genetic diversity and population structure among bellbirds in their fragmented landscapes. We addressed that aim with four main questions: 1) How genetically diverse are bellbird populations and have they undergone strong genetic drift? 2) What population migration model best characterizes bellbird genetic differentiation? 3) What are bellbird dispersal estimates, i.e., proportions of non-natal migrants among populations? 4) Is there a mismatch between bellbird dispersal estimates and gene flow, i.e., are populations in migration-drift equilibrium? To address this last question, we tested the hypotheses that dispersal estimates were negatively correlated with a) genetic divergence (F_{ST}) and b) inbreeding levels (F_{IS}). Our study will provide insight into the demographic and genetic connectivity of a vagile passerine across fragmented landscapes, as well as an opportunity to evaluate populations for conservation management practices, such as conservation translocations.

2.3 Methods

2.3.1 *Blood sampling*

During 2008–2010, we collected blood samples from 291 adult New Zealand bellbirds at nine locations that represented the geographic range of this species (Figure 2.1). The Poor Knights Islands, Hauturu and Tiritiri Matangi are three remnant island populations on the North Island

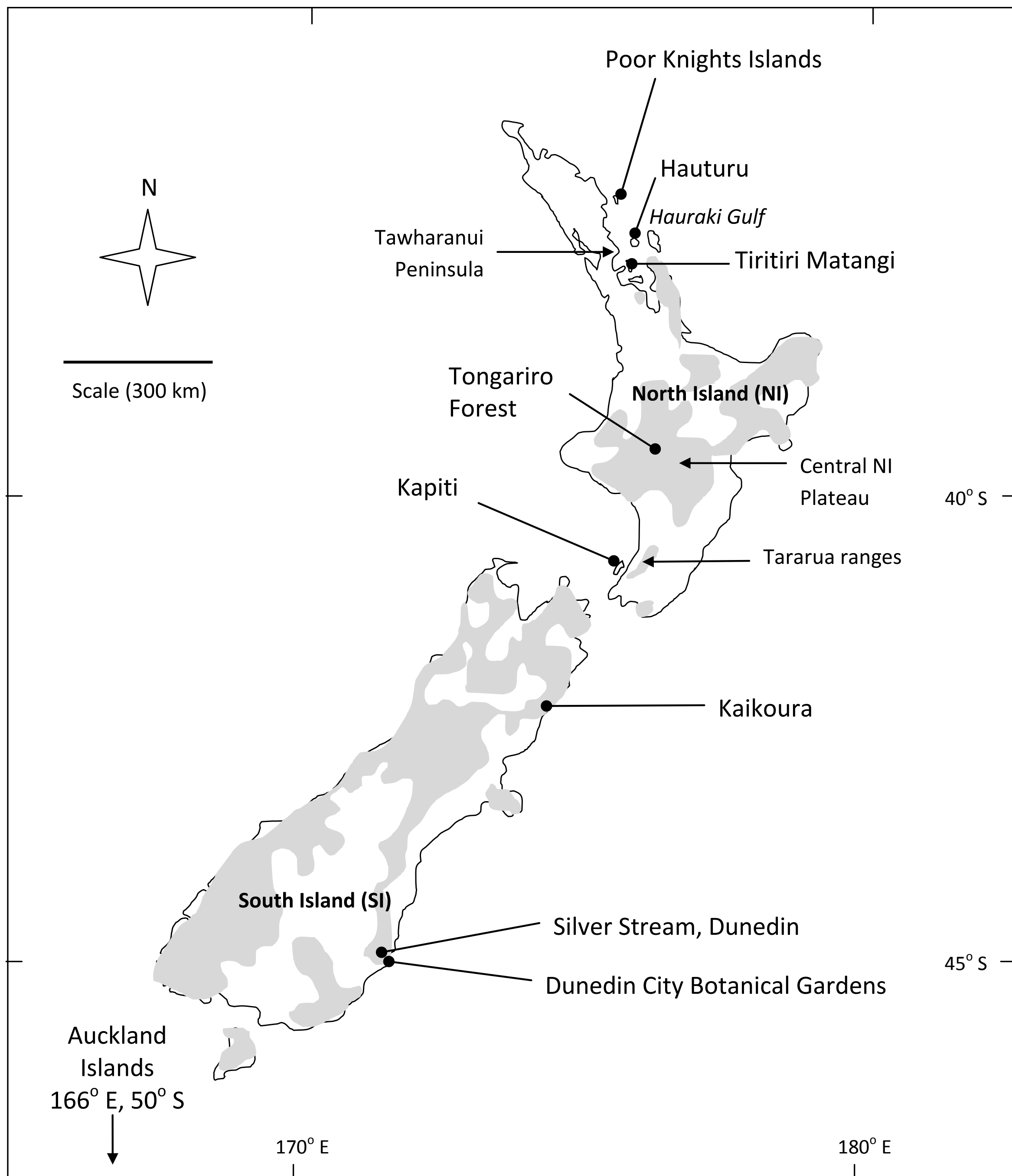

Figure 2.1 Locations of nine New Zealand bellbird sampling sites in this study (lines with terminal dot). Adams Island in the Sub-Antarctic Auckland Islands archipelago (not shown) was located approximately 480 km south of the main New Zealand landmass. Shading indicates current year-round distribution of bellbirds. Arrows indicate other place names mentioned in the text.

(NI) of New Zealand where bellbirds have survived extirpation from the mainland (from latitudes $37^{o}45$ S to $34^{o}25$ S; Craig and Douglas 1984) since *c*. 1860. South of this extirpation area, NI samples were collected from Tongariro Forest on the central NI plateau and Kapiti, an island 5 km off the Kapiti Coast. Bellbirds have been extirpated from the Kapiti Coast plains, though a breeding population of bellbirds has persisted in the mountainous Tararua Ranges. On the South Island (SI), samples were collected from Kowhai Bush, Kaikoura and the Dunedin region. Sampling in the Dunedin region was split into two sites, an exotic treed urban site at the Dunedin City Botanical Gardens and Silver Stream an indigenous forest site 20 km away. Finally, Sub-Antarctic archipelago samples were collected from Adams Island, part of the Auckland Islands archipelago. Forest habitat generally comprised patchily distributed, regenerating, exotic and old growth forest, except at the urban site where no forest existed. Bellbirds were captured using mist nets and bled via brachial venipuncture. Blood was stored in either Queen's buffer (Seutin et al. 1991) or 95% ethanol.

2.3.2 *Microsatellite genotyping*

DNA was extracted from blood samples using a DNeasy extraction kit (Qiagen, Valencia USA). Eight microsatellite loci (Paterson et al. 2010) were amplified by PCR in 15 µL volumes containing 10x Thermopol buffer: 670 mM Tris pH 8.0, 15 mM $MgCl_2$, 160 mM $(NH_4)2SO_4$ (New England Biolabs, NEB), 0.15 µM of each dNTP, 1.5 mM $MgCl_2$, 2.5 units of taq (NEB), 0.6 µM reverse primer, 0.6 µM of M13 primer and 0.2 µM of fluorescent dye-labelled M13 forward primer (either HEX or FAM was used). The PCR profile for all loci used an initial denaturation step for 3 min at $94^{o}C$, followed by 35 cycles of 40 s at $94^{o}C$, 40 s at $60^{o}C$ and 40 s at $72^{o}C$, and finally 8 cycles of 40 s at $94^{o}C$, 40 s at $53^{o}C$ and 40 s at $72^{o}C$. PCR products were then size separated on an ABI 3730 DNA Analyzer (Applied Biosystems, Inc.), and fragment sizes were scored manually using Peak Scanner Software 1.0 (Applied Biosystems, Inc.).

2.3.3 *Genetic diversity, inbreeding levels and bottlenecks*

Microsatellite loci were tested for departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium using ARLEQUIN 3.5 (Excoffier and Lischer 2010) and GENEPOP 4.0.10 (Raymond and Rousset 1995; Rousset 2008). Microsatellite loci were also tested for null alleles, large allele dropout and scoring errors using MICROCHECKER 2.2.3 (van Oosterhout et al. 2004). Observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness (A_R), total number of alleles, private alleles and allele frequencies were calculated using FSTAT 2.9.3 (Goudet 1995) and ARLEQUIN 3.5. A_R was calculated using rarefaction to 50 gene copies, the minimum sample size. Statistical significance for differences in A_R between sampling locations was determined using paired *t*-tests in PASW 18.0 (2010 SPSS Inc.). Inbreeding coefficients (F_{IS}) were calculated using FSTAT, and significance of F_{IS} departure from HWE was tested using 10,000 permutations at $\alpha = 0.05$.

Recent population decline was examined using the program BOTTLENECK 1.2.02 (Cornuet and Luikart 1997, Piry et al. 1999). We used the two-tailed Wilcoxon signed-rank test that accounts for both heterozygosity excess and deficiency in studies with less than 20 loci, and where effective population sizes may have been constant for long periods of time (Piry et al. 1999). For transparency of results, we presented the data under three mutation models: two mutation model extremes, the infinite allele model (IAM) and the stepwise mutation model (SMM), and an intermediate two-phase model (TPM) with 95% stepwise mutations with a variance of 12 and 10,000 iterations as recommended by Piry et al. (1999).

2.3.4 *Population migration model and structure*

Population differentiation was explored to determine the level of population divergence and broad-scale population migration model (discrete or continuous distribution) that best described bellbird population structure. We used ARLEQUIN to compare pair-wise F_{ST} and R_{ST} between sites and SMOGD to generate Jost's *D* estimates and the associated confidence intervals. R_{ST} incorporates microsatellite-specific modes of mutation (Slatkin 1995), yet has

lower statistical power partly due to the relatively higher variance of its estimator. On the other hand, F_{ST}, though based on the infinite allele model outperforms R_{ST} when sample sizes and the number of loci are moderate (Gaggiotti et al. 1999). Jost's *D* measures the actual relative degree of differentiation of allele frequencies among the demes of a population (Jost 2008, 2009). Finally, a hypothesis of isolation-by-distance was tested by comparing the genetic [$F_{ST}/(1 - F_{ST})$] and geographical distances (km) using a Mantel test in GENALEX 6.3 (Peakall and Smouse 2006) and significance was tested with 10,000 permutations.

Bayesian clustering analysis using the admixture model in STRUCTURE 2.3.3 (Pritchard et al. 2000) with no *a priori* location assumptions and the Evanno et al. (2005) method was used to estimate the most probable number of populations (*K*). The burnin length was set at 10^5, followed by 10^6 randomisation steps and five independent runs were conducted for each value of *K*. These results were then corroborated using principal components analysis (PCA) performed in GENALEX. Cavalli-Sforza and Edwards (1967) genetic distances (CSE) were calculated using PHYLIP 3.66 (distributed by Felsenstein J, University of Washington, Seattle). A consensus unrooted neighbor-joining (NJ) tree (Saitou and Nei 1987) was constructed in NEIGHBOUR from the genetic distances in CONSENSE (Margush and McMorris 1981) with 1000 bootstrap iterations computed using SEQBOOT (Felsenstein 1985) as implemented in PHYLIP, and then visualized using TREE-VIEW 1.6.1 (Page 1996).

2.3.5 *Dispersal estimates and mismatch with gene flow*

We identified first-generation migrants (M_0), non-natal migrants, at each sampling location in GENECLASS 2 (Piry et al. 2004) using the L_h test statistic, which assumes that not all source populations were sampled. Computations were performed using the frequency method (Paetkau et al. 1995) implemented in GENECLASS with significance testing at a likelihood threshold of $P = 0.01$ for 10,000 Monte Carlo Markov Chain (MCMC) re-sampled individuals (Paetkau et al. 2004). We tested for migration-drift equilibrium, where F_{ST} is a function of M_0, by linear regression on the base 10 logarithm of mean $F2_{ST}$ against that of M_0, as a proportion

of total individuals sampled ($N_{sampled}$) for each location. Finally, to test whether dispersal counteracted inbreeding we performed a linear regression of $M_0/N_{sampled}$ and F_{IS}. All statistical regressions were performed in PASW 18.0 and proportion data were square-root transformed to fit assumptions of homoscedasticity.

2.4 Results

2.4.1 *Genetic diversity, inbreeding levels and bottlenecks*

All eight microsatellite loci were highly polymorphic at each location (Table 2.1). All loci were in HWE after Bonferroni correction, except for the following: Ame7 in sampling locations Hauturu, Kapiti and Auckland Islands; Ame10 in Tiritiri Matangi; Ame14 in Tiritiri Matangi, Hauturu, Dunedin and Auckland Islands; Ame20 in Dunedin (Appendix A: Tables A.1 and A.2). Missing data for each locus was less than 5% and no linkage disequilibrium was detected between any pair of loci (Appendix A: Tables A.3 and A.4, respectively). Cases of null alleles were apparent in MICROCHECKER for loci Ame7 and Ame14, but null alleles were not present across all sampling locations (loci with null alleles by location: Ame7 in Tiritiri Matangi and Hauturu; Ame14 in Tiritiri Matangi, Dunedin and Auckland Islands) (Appendix A: Table A.2). As a precautionary measure, we compared results of all analyses run with and without loci having putative null alleles (Ame7 and Ame14) and found no differences in most analyses, e.g., in ARLEQUIN, GENECLASS, STRUCTURE. Inbreeding estimates and bottleneck tests were sensitive to null alleles (van Oosterhout et al. 2004), thus we report those results without loci Ame7 and Ame14.

Total mean H_E and A_R for all sampling locations was 0.69 ± 0.02 95% *CI* and 5.2 ± 0.2 95% *CI*, respectively (Table 2.1). H_E and A_R were significantly lower at Poor Knights, Tiritiri Matangi and Auckland Islands (P = 0.001–0.03), yet there were no differences among Hauturu,

Tongariro, Kapiti, Kaikoura, Dunedin City and Silver Stream (P = 0.1–0.9). Among all samples there were 77 alleles and the mean number of alleles per locus (k) was 5.6 ± 0.2 95% *CI*. Again, Poor Knights, Tiritiri Matangi and Auckland Islands had lower k values (3.5–4.6) than all other

Table 2.1 Allelic and genetic diversity statistics for 291 adult New Zealand bellbirds sampled at nine locations: N, number of samples; k, mean number of alleles; A_R, allelic richness; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient (null alleles excluded) and private alleles (PA). Asterisks demark significant F_{IS} deviation from HWE within sampling locations.

	N	k	A_R	H_O	H_E	F_{IS}	PA
Poor Knights Islands	32	3.5	3.16	0.369	0.412	0.080	0
Tiritiri Matangi	55	4.6	4.05	0.496	0.624	0.134*	2
Hauturu	40	7.0	6.38	0.592	0.693	0.049	4
Tongariro	30	6.6	6.24	0.640	0.676	-0.019	2
Kapiti	31	6.1	5.70	0.581	0.642	0.097	0
Kaikoura	32	6.1	5.68	0.622	0.656	0.014	5
Dunedin City	25	5.8	5.56	0.528	0.633	0.190*	2
Silver Stream	21	5.6	5.61	0.596	0.680	0.045	3
Auckland Islands	25	4.1	4.03	0.505	0.557	-0.096	0

locations (6.1–7.0). Out of the 77 total alleles, 18 were private alleles found at very low frequency (mostly < 0.04; mean = 0.03). Poor Knights and Auckland Islands had no private alleles, and SI locations (Kaikoura and Dunedin) had the most private alleles (Table 2.1) with frequencies ranging from 0.011 to 0.056. Inbreeding coefficients (F_{IS}) were highest at Dunedin City and Tiritiri Matangi (> 0.13), lowest at Hauturu, Tongariro, Kaikoura, Silver Stream and Auckland Islands (< 0.05), and the islands of Kapiti and Poor Knights had intermediate values

(Table 2.1). Additionally, permutation tests revealed that F_{IS} at Tiritiri Matangi ($P < 0.001$) and Dunedin City ($P < 0.001$) were significant. Mean A_R values were highly correlated with mean H_E ($R^2 = 0.77$, $P = 0.004$) and we found no relationship between sample size and number of alleles ($R^2 = 0.009$, $P = 0.8$), thus it is reasonable to assume that allelic diversity has been accurately represented. Finally, mean A_R values for each sampling location were significantly correlated with mean pair-wise F_{ST} values ($R^2 = 0.95$, $P < 0.001$) indicating evidence of strong genetic drift. When this linear regression was repeated with Poor Knights, Tiritiri Matangi and Auckland Islands excluded from the analysis, the relationship was not significant ($R^2 = 0.44$, $P = 0.2$) thus populations at most sampling locations, except for Poor Knights, Tiritiri Matangi and Auckland Islands, were not subject to strong genetic drift. Under the TPM, SMM and IAM models as implemented in BOTTLENECK, Tiritiri Matangi was the only location with a recent bottleneck footprint (Table 2.2).

Table 2.2 Results for tests on population size reduction using IAM, TPM and SSM mutation models (see Methods). Asterisks indicate *P* values *P < 0.05, **P < 0.01, ***P < 0.001 for significance of two-tailed Wilcoxon test for H_E excess and deficiency in BOTTLENECK, and † indicates significant results of the one-tailed test for H_E excess.

Sampling location	IAM	TPM	SSM
	P-value	*P-value*	*P-value*
Poor Knights Islands	0.563	0.156	0.156
Tiritiri Matangi	0.016*	0.016*	0.031*
Hauturu	0.109	1.00	0.843
Tongariro	0.438	0.688	0.844
Kapiti	0.563	0.688	0.563
Kaikoura	0.078†	0.844	0.844
Dunedin City	0.250	0.945	0.641
Silver Stream	0.039*	0.547	0.250
Auckland Islands	0.016*	0.688	0.688

2.4.2 *Population migration model and structure*

Mean overall F_{ST} was 0.11 ± 0.02 95% *CI* ($P < 0.001$), R_{ST} was 0.05 ± 0.003 95% *CI* ($P < 0.001$) and Jost *D* was 0.13 indicating significant genetic differentiation among sampling locations. Correspondingly, most pair-wise comparisons of F_{ST} among sampling locations were significantly genetically divergent, with three exceptions: Hauturu and Tongariro, Dunedin City and Silver Stream, Dunedin City and Kapiti (Table 2.3). Additionally, F_{ST}–values for Poor Knights, Tiritiri Matangi and Auckland Islands (range 0.08–0.3; mean 0.2 ± 0.001 95% *CI*) were an order of magnitude higher than for Hauturu, Tongariro, Kapiti, Kaikoura, Dunedin City and Silver Stream (range 0.004–0.03; mean 0.02 ± 0.001 95% *CI*). This magnitude of difference was shown

Table 2.3 Pair-wise F_{ST} (below diagonal) and R_{ST} (above diagonal) values among bellbird sampling locations calculated in ARLEQUIN. Asterisks indicate significant divergence among pair-wise population comparisons: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

	Poor Knights	Tiritiri Matangi	Hauturu	Tongariro	Kapiti	Kaikoura	Dunedin City	Silver Stream	Auckland Islands
Poor Knights		0.073***	0.048*	0.041*	0.135***	0.056**	0.052	0.073***	0.219***
Tiritiri Matangi	0.314***		0.013	0.074***	0.078**	0.075**	0.044*	0.040	0.067*
Hauturu	0.161***	0.101***		0.042	0.044*	0.027	0.009	0.005	0.030
Tongariro	0.157***	0.138***	0.007		0.004	0.043	0.020	0.023	0.078***
Kapiti	0.202***	0.141***	0.031***	0.022***		0.050	0.028	0.028	0.094***
Kaikoura	0.174***	0.128***	0.014**	0.016**	0.023***		0.034	0.032	0.071
Dunedin City	0.220***	0.164***	0.037***	0.027***	0.012	0.032***		0.002	0.019
Silver Stream	0.200***	0.151***	0.028***	0.024**	0.024**	0.020**	0.011		0.018
Auckland Islands	0.310***	0.228***	0.106***	0.113***	0.150***	0.089***	0.141***	0.082***	

more clearly in significance tests of pair-wise R_{ST} comparisons, which additionally showed less differentiation at Auckland Islands than F_{ST}–values (Table 2.3). Jost D, however, appears to agree with F_{ST} indicating that panmixia must be rejected even among the New Zealand mainland locations as confidence intervals do not overlap with zero (Table 2.4; Appendix A: Table A.6).

Table 2.4 Jost's *D* values among bellbird sampling locations calculated in SMOGD. See Appendix A; Table A.6 for confidence intervals showing a lack of panmixia among mainland locations.

	Poor Knights	Tiritiri Matangi	Hauturu	Tongariro	Kapiti	Kaikoura	Dunedin City	Silver Stream	Auckland Islands
Poor Knights	–	0.429	0.129	0.125	0.162	0.152	0.173	0.162	0.233
Tiritiri Matangi		_	0.178	0.234	0.264	0.231	0.315	0.258	0.329
Hauturu			_	0.006	0.048	0.018	0.043	0.045	0.127
Tongariro				_	0.025	0.025	0.031	0.038	0.120
Kapiti					_	0.033	0.014	0.030	0.191
Kaikoura						_	0.035	0.010	0.115
Dunedin City							_	0.007	0.177
Silver Stream								_	0.088
Auckland Islands									_

Due to high genetic differentiation, the islands of Poor Knights, Tiritiri Matangi and Auckland had to be excluded from tests on isolation-by-distance. In the Mantel test, we found significant isolation-by-distance (r_M = 0.55, P = 0.02) and near-shore islands of Hauturu and Kapiti did not deviate from this pattern (Figure 2.2).

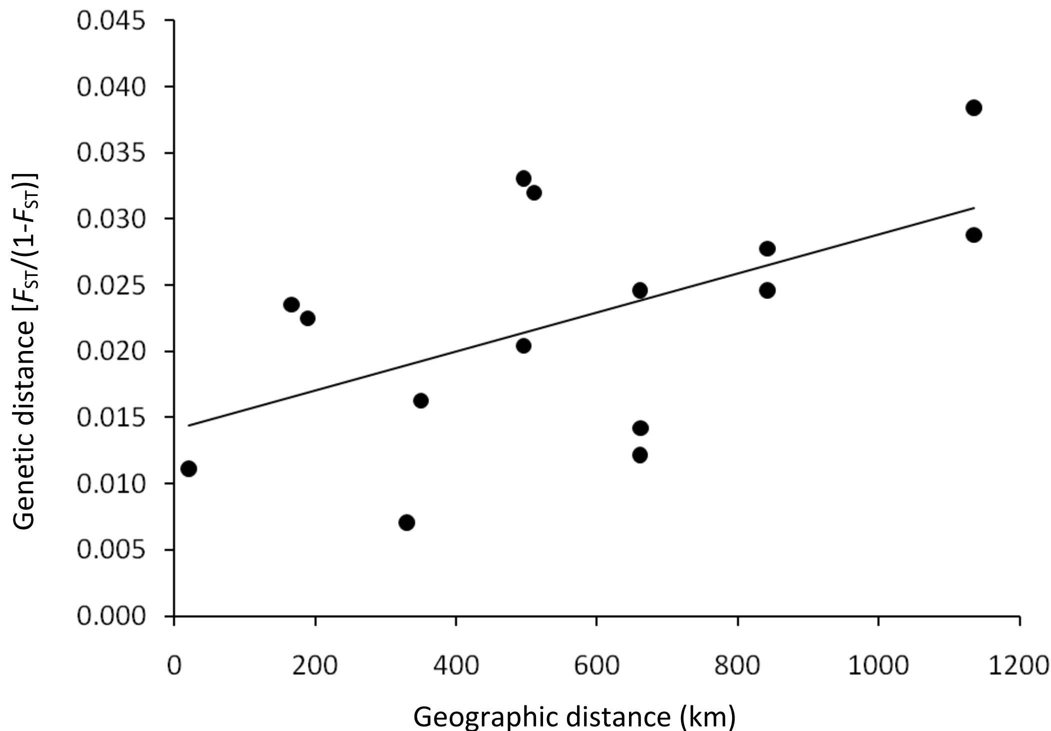

Figure 2.2 Bellbirds on the two main islands of New Zealand function under a continuous distribution population model as evidenced by significant isolation-by-distance (Mantel test: r_M = 0.55, P = 0.02).

Results from Bayesian clustering analysis implemented in STRUCTURE indicated that the estimated number of subpopulations was K = 4 (see Appendix A; Figure A.1 for STRUCTURE bar plot). This was evidenced by both the a) smallest ΔK, and b) asymptote of K plotted against $L(K)$, occurring between K = 4 and K = 5 (Evanno et al. 2005) (Appendix A; Figure A.1). This broad clustering pattern, which clearly separated Tiritiri Matangi, Poor Knights and Auckland Islands from each other as well as from the six other locations, was corroborated by PCA. The first two PCA axes accounted for 75% of the total variance (first axis, PC1 = 44% and second axis, PC2 = 31%) (Figure 2.3). The genetic and geographic spatial arrangement of sampling locations on the NJ tree concurred with our F_{ST} and isolation-by-distance results (Figure 2.4). There was no strong bootstrap support for a separation between the NI and SI. Among the NI sampling locations, there was moderate bootstrap support for a common origin between Tiritiri Matangi and Hauturu, but the pattern of branching shows that Poor Knights was more closely connected to Tongariro in the NI central plateau than with the Hauraki Gulf island populations.

Among the SI locations, there was weak to moderate bootstrap support for a common origin between the two Dunedin sites, and also between Kaikoura and Auckland Islands.

Figure 2.3 Principal component analysis of genetic distances among New Zealand bellbird sampling locations based on eight microsatellite markers.

Figure 2.4 Unrooted NJ tree for eight microsatellite DNA data based on Cavalli-Sforza distances among all nine bellbird sampling locations. Nodes with bootstrap support of > 50% are shown.

2.4.3 *Dispersal estimates and mismatch with gene flow*

The detected $M_0/N_{sampled}$ at each location revealed that bellbird dispersal was ongoing at most locations (Table 2.5). Where first generation migrants were detected, dispersal estimates ranged from 3.1-9.5% with a mean of 5.5% (12/219). The highest dispersal estimates (8.0-9.5%) were found at the two Dunedin sites and Auckland Islands. Dispersal estimates were moderate at the remaining islands of Tiritiri Matangi and Kapiti, 3.6 and 6.5%, respectively, although no

migrants were detected at Poor Knights or Hauturu. In a linear regression on the relationship between dispersal estimates and gene flow, dispersal estimates were not significantly correlated with mean F_{ST} (R^2 = 0.09, P = 0.4) among locations (Figure 2.5a). Similarly, dispersal estimates were not significantly correlated with F_{IS} (R^2 = 0.01, P = 0.8) as Dunedin City and Tiritiri Matangi had F_{IS} too high to be explained given the observed dispersal estimates, and there were no instances where low dispersal corresponded to high F_{IS} (Figure 2.5b).

Table 2.5 Proportion and number of first-generation migrants (M_0) detected using the L_h test statistic, in which no source populations were assumed, using GENECLASS.

		M_0
Sampled population	*N*	% (count)
Poor Knights	32	0
Tiritiri Matangi	55	3.6 (2)
Hauturu	40	0
Tongariro	30	3.3 (1)
Kapiti	31	6.5 (2)
Kaikoura	32	3.1 (1)
Dunedin City	25	8.0 (2)
Silver Stream	21	9.5 (2)
Auckland Islands	25	8.0 (2)

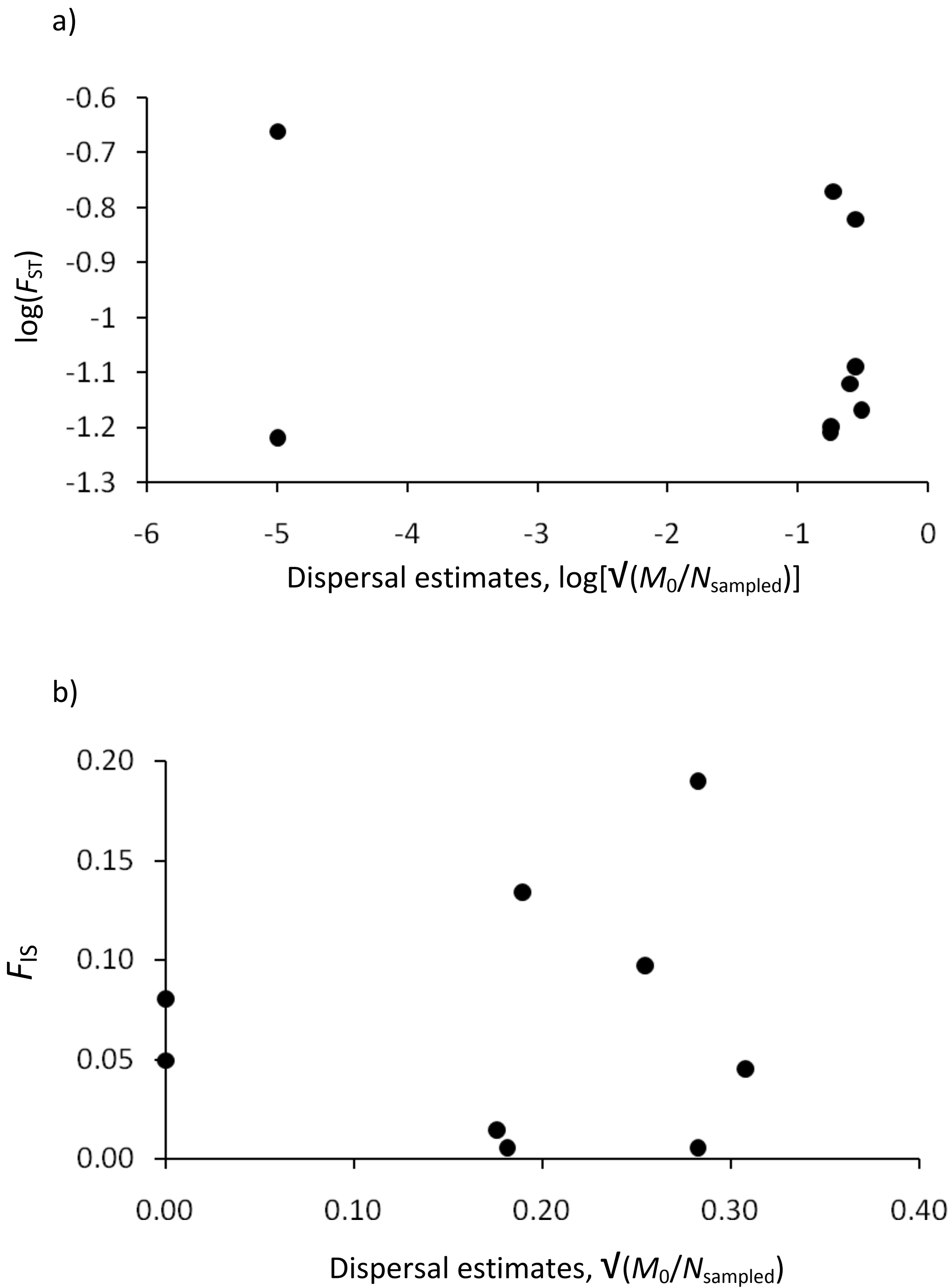

Figure 2.5 Linear regressions of the relationships between dispersal estimates of first-generation migrants and a) genetic divergence [log(F_{ST})] (R^2 = 0.09, P = 0.4) and b) inbreeding coefficient (F_{IS}) (R^2 = 0.01, P = 0.8) as tests of effective dispersal in bellbirds.

2.5 Discussion

2.5.1 *Genetic diversity and inbreeding levels*

We found that genetic diversity estimates for bellbirds were high despite striking changes in their spatial distribution including large local extirpations (Craig and Douglas 1984). In fact, diversity was higher than that for any other endemic New Zealand passerine examined using microsatellite DNA to date and were at the high end of estimates documented for passerine studies in the Australopacific, Europe, Africa and North America (Table 2.6). Our heterozygosity estimates (0.4–0.7) overlapped with those reported for Australian meliphagids (0.6–0.9) (Painter et al. 1997; Abbott et al. 2002) despite the smaller New Zealand landmass, which may support smaller avian population sizes than Australia. In a comparison of historical and contemporary levels of genetic diversity in the SI robin and NI saddleback (*Philesturnus carunculatus*), Taylor et al. (2007) showed that heterozygosity in robins declined from 0.4 to 0.3 and in the comparably less vagile saddlebacks from 0.4 to 0.1 since the mid- to late-1800s. High genetic diversity in passerines at both continental and island endemism hotspots [Hawaiian honey creeper (*Loxops coccineus coccineus*), Reding et al. 2010; silver eye (*Zosterops lateralis*), Clegg et al. 2002; Stellar's jay (*Cyanocitta stelleri*), Burg et al. 2005] is often associated with large population sizes and dispersal ability (Watanabe et al. 2010), or re-colonization potential (Gilpin 1991). Members of the Meliphagidae family in Australia are known to be excellent re-colonizers with large historical effective population sizes (Toon et al. 2010), and historical accounts demonstrate that the New Zealand bellbird was no exception with regard to population size (Sagar 1985; Craig 1980; Craig and Douglas 1984). Even the least diverse bellbird populations in this study likely harbour sufficient genetic diversity and numbers to offset genetic drift in the long term provided that numbers do not decrease (see Ruarus et al. 2011). The highest inbreeding coefficient found in this study was from the urban site Dunedin City, which was four times higher than at Silver Stream (a forest patch 20 km away) and more than 10 times higher than most other locations, including islands. High inbreeding coefficients represent a departure from HWE that can indicate inbreeding and population subdivision

Table 2.6 Comparison of microsatellite DNA genetic diversity estimates from studies on passerines world-wide. *N*, number of samples; H_E, expected heterozygosity; A_R, allelic richness; asterisks denote where A_R was not standardized for sample size.

Species or subspecies	Country	*N*	No. of loci	Diversity		Reference
				H_E	A_R	
Passerines endemic to New Zealand						
New Zealand bellbird *Anthornis melanura*	New Zealand	291	8	0.69	5.4	This study
Hihi (Stitchbird) *Notiomystis cincta*	New Zealand	291	19	0.65	4.6	Brekke et al. 2011
South Island robin *Petroica australis*	New Zealand	283	6-8	0.36	2.5	Taylor et al. 2007
North Island saddleback *Philesturnus carunculatus*	New Zealand	51	5-6	0.11	1.4	Taylor et al. 2007
Passerines of other countries and continents						
Basra warbler *Acrocephalus griseldis*	Iraq/Kuwait	22	10	0.76	10.5*	Hansson and Richardson 2005
Golden-cheeked warbler *Dendroica chysoparia*	United States	109	9	0.75	7.7	Lindsay et al. 2008
Stellar's jay *Cyanocitta stelleri annectens*	United States	20	5	0.74	5.5	Burg et al. 2005
Stellar's jay *C. s. stelleri*	United States	107	5	0.69	4.5	Burg et al. 2005
Florida scrub jay *Aphelocoma coerulescens*	United States	1024	20	0.67	9.6*	Coulon et al. 2008
White-starred robin *Pogonocichla stellata*	Africa	320	7	0.66	5.7	Galbusera et al. (2004)
Stellar's jay *C. s. carlottae*	United States	23	5	0.58	4.1	Burg et al. 2005
Great reed warbler *Acrocephalus arundinaceus*	Eurasia	22	10	0.54	9.2*	Hansson and Richardson 2005
White-throated dipper *Cinclus cinclus*	Norway	108	8	0.52	4.6*	Øigarden et al. 2010
Yellow-fronted white-eye *Zosterops flavifrons*	Vanuatu	438	8	0.50	3.2	Clegg and Phillimore 2010
Silver eye *Z. lateralis*	Vanuatu	229	11	0.47	2.8	Clegg and Phillimore 2010
Seychelles warbler *Acrocephalus sechellensis*	Seychelles	22	10	0.37	2.5*	Hansson and Richardson 2005

(Allendorf and Luikart 2007). Since we have separated the Dunedin sites for analyses, we feel confident that significant F_{IS} explains inbreeding levels at both Tiritiri Matangi and Dunedin City. These results suggest that inbreeding levels for vagile avian dispersers may be high at urban and previously deforested remnant island populations.

2.5.2 *Some bellbird populations subject to strong genetic drift on islands*

Widespread increases in bellbird numbers since the mid-1900s (Robertson et al. 2007) should preclude recent genetic bottlenecks, and indeed we found that bellbirds at most sampling locations at the time of our study were in mutation-drift equilibrium. A recent bottleneck was detected at Tiritiri Matangi and that finding was corroborated by population census data. The Tiritiri Matangi bellbird population was reduced to between 12 and 24 individuals (Diamond and Veitch 1981) after forest removal for agriculture by the early 1970s (Craig and Douglas 1984). The resident bellbird population at Tiritiri Matangi has grown to a carrying capacity of approximately 1200-2000 individuals (Kevin Parker, unpublished data) largely due to supplementary feeding with sugar-water and forest re-growth over the past 30 years. Bellbirds are socially monogamous, physically aggressive and territorial, thus even if levels of bellbird migration within the Hauraki Gulf were sufficient to obscure the bottleneck (Cornuet and Luikart 1997; Keller et al. 2001; Allendorf and Luikart 2007), any immigrants to Tiritiri Matangi would find it difficult to establish the territory and mates required for effective dispersal (contribution of migrants to the non-natal gene pool). We also found evidence of strong genetic drift at Poor Knights and Auckland Islands, two highly genetically differentiated island bellbird populations. Not much is known about the population history of Poor Knights and Auckland Islands, thus we do not have a firm explanation for the lower genetic diversity and lack of equilibria observed. At Poor Knights, a productive (Sagar 1985; Massaro et al. 2008) dense subpopulation of several thousand bellbirds (SMB, personal observation) exists today and the subpopulation at Auckland Islands with a land area of 625 km^2 could possibly be home to more than 10,000 bellbirds (Colin Miskelly, personal communication). Parsimonious explanations for the observed strong genetic drift at those two locations may involve, at Poor

Knights, an historical bottleneck (or founder event) followed by relative isolation due to extirpation of the adjacent mainland, and at Auckland Islands, founder events by small vagrant groups of bellbirds that were subsequently isolated from source populations by 480km of ocean. In both cases, the island populations must have remained small for a considerable period of time in order to experience strong genetic drift. Subsequently, bellbird populations at Poor Knights and Auckland Islands have reached large population sizes yet the low levels of gene flow did not restore genetic variation.

2.5.3 *Population migration model and structure*

We found that a continuous distribution migration model of isolation-by-distance best characterized genetic differentiation in bellbirds, where adjacent populations are more similar to each other than geographically distant populations (Slatkin 1993). This result means that bellbirds would have dispersed continuously through the New Zealand landscape, at least in recent history. Furthermore, the ocean strait between NI and SI, which is 23 km wide at its narrowest point, does not appear to have posed a sharp barrier to gene flow as has been reported for other species, such as the New Zealand robin (Miller and Lambert 2006). Our results suggest that distances of approximately 5 to 35km, which represent the distance between any of our island sites and the mainland, over open water were not strong barriers to bellbird dispersal. This capacity to traverse open water has been shown in other non-migratory but well-flighted species such as the silver eye (Clegg et al. 2002). Furthermore, this observation was substantiated by a recent unassisted bellbird re-colonization at the Tawharanui peninsula (Brunton et al. 2008) (see Figure 2.1) and regular sightings of bellbirds along coastal areas adjacent to remnant island populations (Sagar and Scofield 2006; Robertson et al. 2007).

Habitat fragmentation has been empirically shown to affect divergence rates among intraspecific passerine populations (Taylor et al. 2007; Coulon et al. 2008, 2010). Clearly, the highly differentiated island populations of Poor Knights, Tiritiri Matangi and Auckland Islands will be more susceptible to drift than the other bellbirds. It remains unclear, however, as to

whether significant genetic differentiation among bellbird mainland locations reflects historical population structure or divergence exacerbated by recent population fragmentation. The non-significant F_{ST} between the two Dunedin sites, and also between Tongariro and Hauturu, may indicate 1) recent population separation not yet detectable using F_{ST} methods, or 2) that migration is ongoing. The forest between Tongariro and Hauturu was the last to be highly fragmented (Ewers et al. 2006), therefore, we suggest that gene flow would have occurred through step-wise short-distance dispersal across the NI central plateau to the Hauraki Gulf, but that this population continuity may no longer exist (see Figure 2.1).

2.5.4 *Mismatch between dispersal estimates and gene flow*

At the time of our study it was evident that bellbird dispersal was ongoing. At any given location, 3 to 10% of the birds we sampled were first generation migrants that were born elsewhere, except at Hauturu and Poor Knights where no migrants were detected. It is common that allele frequency based methods that assess number of migrants underestimate actual dispersal levels, especially in large populations with high genetic diversity, e.g., Hauturu, because gene frequencies are often too similar to discern migrants from natives (Piry et al. 2004). Thus, bellbird dispersal estimates are likely higher than we estimated, especially at Hauturu and Tongariro. Mean bellbird first-generation migrant estimates (5.5%) in our study were higher than those reported by Galbusera et al. (2004) (mean 3.2%; 10/320) in a microsatellite marker study of passerines in fragmented Kenyan forests. Using re-sightings of colour-marked individuals, Coulon et al. (2010) found that 1 to 10% of Florida scrub jays (*Aphelocoma coerulescens*) dispersed more than 10km from natal sites across a highly fragmented habitat to breed elsewhere. Taken together with results from other studies on bird dispersal estimates (e.g., Hansson et al. 2003; Jønsson et al. 2010), we conclude that bellbird dispersal estimates are moderate to high despite habitat fragmentation and genetic differentiation among populations. Knowledge of a species' dispersal activity has important conservation implications with regard to genetic and demographic connectivity of metapopulations (Waples and Gaggiotti 2006; Frankham 2010), because it aides evaluation of

re-colonization potential and any mismatch between gene flow and dispersal of individuals (Palsboll et al. 2007). In this study, we rejected the hypothesis that bellbird dispersal estimates correlate with genetic divergence. Dispersal estimates were fairly similar regardless of genetic divergence, thus bellbirds appear to never stop dispersing despite apparent limited breeding success in their new homes. Moreover, we found high inbreeding levels in urban areas and rejected the hypothesis that dispersal counteracts inbreeding in highly modified habitats.

2.5.5 *Conclusions and conservation implications*

The most important finding in our study was that effective dispersal appeared to be inhibited in urban areas and small deforested islands suggesting that habitat quality limits the genetic contribution of migrants. We also found that bellbirds had high genetic diversity and dispersal estimates despite significant genetic differentiation among sampling locations. In the Hauraki Gulf, an active metapopulation was evident such that in the event of a subpopulation extinction, e.g., at Tiritiri Matangi, re-colonization by other Hauraki Gulf bellbirds should occur quickly. Lower re-colonization potential at Poor Knights and Auckland Islands, however, suggests their separate protection even though the bellbirds are not genetically unique (Palsboll et al. 2007). Moreover, Kapiti and the Tararua forest on the adjacent mainland should be considered together for conservation initiatives, i.e. habitat restoration and predator eradication, as Tararua is the only plausible origin of migrants to Kapiti.

While translocation has successfully enhanced gene flow in threatened species (Frankham 2010), we suggest that translocation is not necessary for well-flighted species that have the proven ability to re-colonize without human assistance, provided sufficient protected native habitat exists. Translocation can cause adverse genetic change to wild populations including loss of genetic variation and change of population structure (Allendorf 2001; Laikre et al. 2010). We recommend that translocation of bellbirds from the recently bottlenecked Tiritiri Matangi population to genetically depauperate and divergent small populations should be avoided due to an elevated risk of outbreeding depression (Frankham 2010). Importantly,

previous translocations of bellbirds have had little success in establishing viable populations, whereas instances of natural bellbird re-colonization and rapid population growth are known to occur after predator control and habitat protection efforts (Brunton et al. 2008; SMB, unpubl data). Focussing conservation efforts on restoration, preservation or expansion of high quality habitat that is distant from expanding urban centres is likely to have positive and cost effective long-term conservation outcomes (see Keller et al. 2001). A stepping-stone model of protected habitat patches that radiate from pre-existing genetically diverse populations could be adopted and empirically evaluated. Spatial genetic planning that encourages secondary contact among re-colonizing patches and leads to admixture among adjacent subpopulations will help ensure the future of well-flighted endemic avifauna (Ingvarsson 2001; Parisod and Bonvin 2008).

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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

Many endemic bird populations have been extirpated from a vast region of northern New Zealand since the mid-1800s. Some species, however, survive in remnant populations on offshore islands and in forest patches further south. New Zealand bellbird (*Anthornis melanura*) numbers have undergone dramatic declines and are currently increasing due to conservation efforts. In 2004, bellbirds started to re-colonize the mainland peninsula at Tawharanui immediately after implementation of a predator-proof fence. We took the opportunity to characterize the re-colonization process at Tawharanui using eight microsatellite loci and a 436bp segment of the mitochondrial DNA control region. Four years after the onset of colonization, bellbird blood samples were collected in the Tawharanui peninsula (the colony) and in the Hauraki Gulf (Hauturu, Tiritiri Matangi and Hunua Ranges) as well as the Poor Knights Islands and central North Island plateau (the putative source populations). Our aims are to assess 1) the strength of the bottleneck and founder effects at Tawharanui; 2) the number of source populations (one or more than one), 3) the rates of gene flow and dispersal between source(s) and colony; 4) the minimum number of colonizers and population growth rate required to explain the genetic variability observed in the colony. We find that bellbirds at Tawharanui 1) do not have significantly lower genetic diversity than bellbirds in any given putative source population; 2) are only slightly genetically differentiated; 3) are in genetic equilibrium (non-significant bottleneck test). First-generation migrants analysis indicates that

migration is bidirectional and female-biased. Simulations illustrate that at least *c.* 50-80 effective founders from multiple- and ≥100 from single-source populations are needed to explain the genetic diversity observed at Tawharanui and that the genetically effective population is growing at a rate of ~25% per year. These results suggest that: 1) Tawharanui was colonized by many short-range migrating bellbirds originating mainly, but not entirely, from Hauturu; 2) during the colonization process there was no population bottleneck; 3) colonization was succeeded by rapid population growth. The success of mainland re-colonization for passerines of high vagility will rely on the integrity of natural metapopulation dynamics, future rates of gene flow and population growth of bellbird subpopulations among high quality and predator-controlled habitat fragments.

Keywords: *Anthornis melanura*, founder event, conservation genetics, extinction, first-generation migrants, mixed stock analysis, Bottlesim, population growth

3.2 Introduction

In the 1960s, Robert H MacArthur and Edward O Wilson first made the link between principles of immigration and extinction to the processes of population ecology and genetic evolution (Wilson 2010). These ideas are integral to metapopulation dynamic theory (Hanski 1999) and our understanding of how extinction-colonization processes are accelerated by reductions in population size due to human-mediated habitat loss and fragmentation. The re-colonization of small contemporary metapopulations is often inhibited, posing increased extinction threat, and carries additional ecological risks, e.g., introduced predators or disease (Evans and Sheldon 2008; Frankham 2010). Where genetic diversity, dispersal levels, sex-biased dispersal and founding population size are assessed within and among population patches, genetic patterns

of ongoing population expansion and re-colonization can be elucidated (Fabbri et al. 2007; Coulon et al. 2008, 2010; Rioux-Paquette et al. 2010). For instance, it has been shown that founder events with a small number of effective founding individuals frequently incur reduced allelic diversity and heterozygosity (Clegg et al. 2002a; Pruett and Winker 2005; Hundermark and Van Daele 2010). The re-colonization of species to former but now fragmented habitats provides an opportunity for empirical evaluation of the underlying genetic and demographic processes (Fabbri et al. 2007). In this study, we explore whether natural re-colonization processes of endemic species can provide practical information toward the success of future natural and human-assisted re-introductions.

Endemic passerines have declined throughout New Zealand in the 19th century (Craig and Douglas 1984) and some species survived in fragmented populations in the North and South Islands as well as smaller coastal and offshore islands (Robertson et al. 2007). New Zealand bellbirds (*Anthornis melanura*) on the North Island have been extirpated from most mainland locations north and south of central North Island since 1860 (Craig and Douglas 1984). Due to more effective legal habitat and wildlife protection, and substantial changes in exotic mammalian predator control, this declining demographic trend began to reverse in the 1970s. Consequently, bellbird numbers have increased on the Hauraki Gulf islands and Coromandel peninsula, and a remnant population in the Hunua Ranges was ensured (Robertson et al. 2007). After the recent implementation of a predator-proof fence across the Tawharanui peninsula in 2004, more than *c*. 100 individuals at a time were observed (personal communication Tim Lovegrove) landing in the forest fragments of the Tawharanui peninsula (Brunton et al. 2008). It is thought that Hauturu, an island 35 km away in the Hauraki Gulf, is the source population of the colony at Tawharanui based on song dialect (Brunton et al. 2008). Reappearance of breeding bellbirds on the mainland speaks to the success of habitat reclamation and decimation of predators, nevertheless success of manual translocations for bellbirds, and other species, e.g., hihi (*Notiomystis cincta*) and kakariki (*Cyanoramphus novaezelandiae*), have been largely unsuccessful in the northern North Island extirpation region. Thus, any population expansion and colonization, whether natural or human-assisted, needs to be carefully evaluated. Studies in Europe and North America revealed that colonization may be sustained by long-distance

dispersers, i.e., in cuckoo-shrikes (*Coracina* spp., Jonsson et al. 2010), and simulations on founder events in saddlebacks (*Philesturnus carunculatus*) predict that genetically depauperate birds may not lose significant fractions of their original genetic variability (Taylor and Jamieson 2008; Ruarus et al. 2011). Sporadic, but recurrent migration is known to increase the heterozygosity and sustain population growth in even small founder populations (Fabbri et al. 2007). Thus, studying the natural re-colonization process will provide practical information toward the success of future natural and human-assisted range expansions.

Our aims in this study are 1) to assess the strength of any founder effects four years after the onset of bellbird re-colonization at Tawharanui, including the extent of genetic differentiation between the putative source and founder populations; 2) to estimate the number of source populations (one or more than one); 3) to estimate the rates of gene flow and natal dispersal between source(s) and colony; and 4) to infer by simulation the minimum numbers of colonizers and population growth rate that could explain the observed population genetic parameters. We used eight microsatellite loci and a 436bp segment of mitochondrial DNA control region to genotype and sequence, respectively, DNA samples taken from bellbirds in the Hauraki Gulf Islands, Poor Knights Islands, Hunua Ranges and central North Island plateau (the putative source populations) and in the Tawharanui peninsula (the colony) four years after the onset of colonization.

3.3 Methods

3.3.1 *Blood sample collection and DNA analysis*

Between 2008 and 2010, bellbird blood samples were collected from 237 adult birds at six locations in northern New Zealand hereafter referred to as six ‘populations’: two islands (Hauturu and Tiritiri Matangi); two mainland locations (Hunua ranges and the newly re-

colonized Tawharanui peninsula) in the Hauraki Gulf region; Poor Knights Islands; Tongariro Forest in the central North Island plateau (central NI) (Figure 3.1). The sex ratio of bellbirds sampled was 1:1 at Tawharanui, Tiritiri and Hauturu, 2:1 male-female ratio at Poor Knights and central NI, and the sex of the nine Hunua bellbirds was unknown.

DNA was extracted using DNeasy Blood and Tissue Kit (QIAGEN) and standard phenol-chloroform protocol (Sambrook et al. 1989). Polymerase chain reaction (PCR) was used to amplify eight microsatellites (Ame6, Ame7, Ame10, Ame11, Ame14, Ame18, Ame20, Ame25; Paterson et al. 2010) and a 436bp segment of mitochondrial DNA control region Domain I. Microsatellite loci PCR and genotyping protocols are as described in Baillie et al. (*in revision*) (Chapter Two). Microsatellite loci were tested for null alleles, large allele dropout and scoring errors using MICROCHECKER 2.2.3 (van Oosterhout et al. 2004). For mitochondrial DNA sequencing, the forward and reverse primers were bellbird-cytb2 (5'-GCCGGTAGAACACCCATTTA-3') and CRDR1-short (5'-GTGGCGCAAAAGAGCAAGTT-3'; modified version of CRDR1, Miller 2003) (developed in this study by SMB; these sequences will be submitted to GenBank; Appendix B). Each 15 uL reaction included 2 µL of genomic DNA, 1 µL of BSA, 0.75 mM of each dNTP, 0.6 uM of each primer, 1.5 mM of $MgCl_2$, and 2.5 units of *Taq* DNA polymerase (Invitrogen). The thermal profile consisted of a 3-min, 94°C activation step, followed by 35 cycles of 94 ^{o}C for 1 min, 58.5 ^{o}C for 2 mins and 72 ^{o}C for 1 min, ending with an elongation step of 72 ^{o}C for 10 min. 3 µL of PCR products were run on 1.5% agarose then stained with ethidium bromide and viewed under UV. Mitochondrial DNA PCR products were then sequenced using the reverse primer CRCR1-short on an ABI 3730 DNA Analyzer (Applied Biosystems, Inc.). Sequences were aligned and edited manually in MEGA 4.1 (Tamura et al. 2007). Negative (no DNA in PCR) and positive (samples with known genotypes or sequences) controls were always used. PCR products were analysed using an ABI 3730 DNA Analyzer (Applied Biosystems, Inc.).

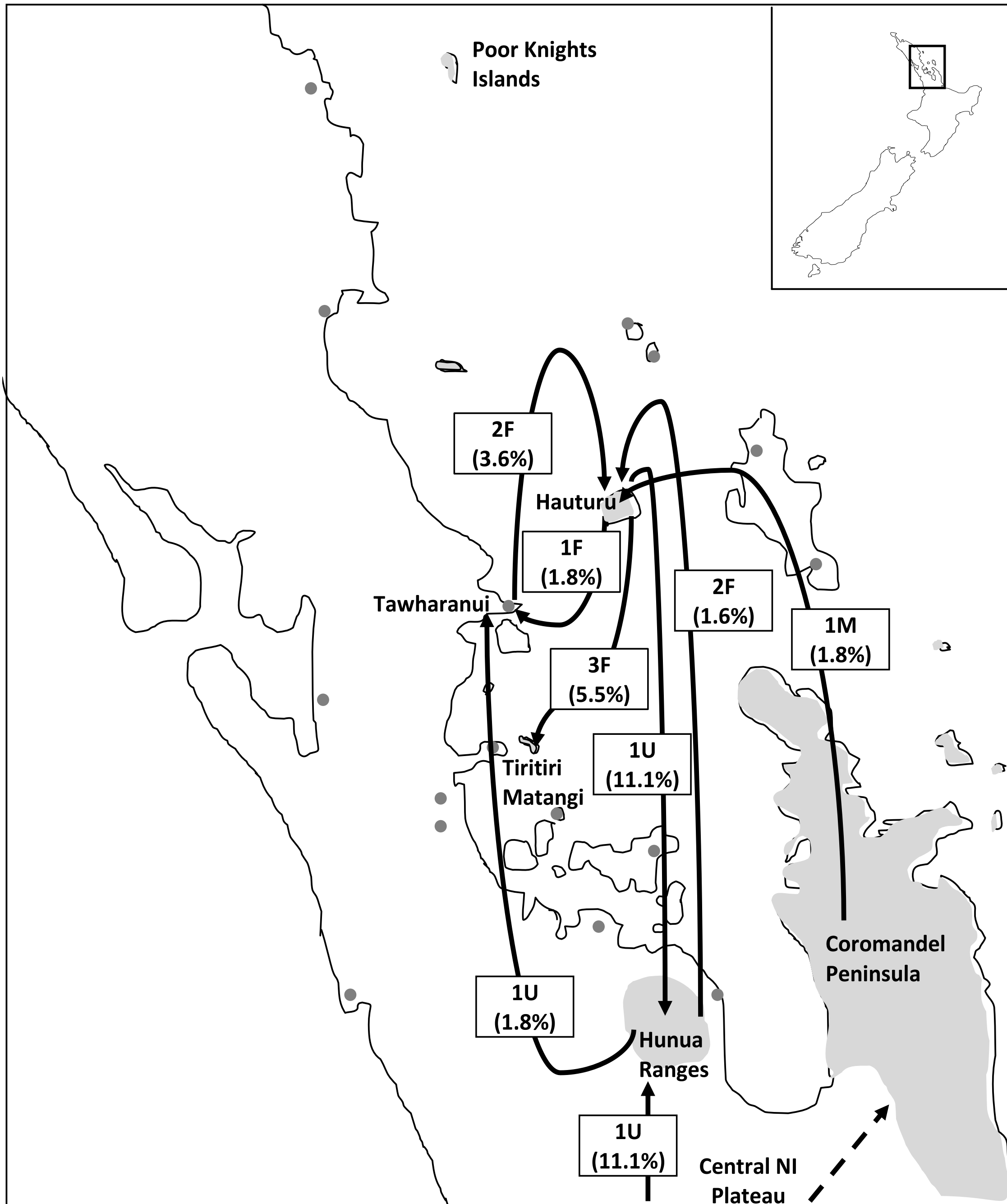

Figure 3.1 Approximate New Zealand bellbird (*Anthornis melanura*) distribution in the North Island (NI) of New Zealand (in light grey). Dark grey dotted areas indicate regions of bellbird sightings during winter. The sites of putative origin of the bellbirds sampled at Tawharanui, Poor Knights, Hauraki Gulf Islands (Hauturu and Tiritiri), Hunua Ranges and Coromandel Peninsula (as a proxy for central NI plateau genotypes) are indicated. The arrows indicate the directions (with the number, sex and percent) of first generation migrant bellbirds that were detected using Rannala and Mountain's (1997) method as implemented in GENECLASS (Piry et al. 2004).

3.3.2 *Genetic variability and bottleneck detection*

For microsatellite DNA data, we used the software ARLEQUIN 3.5 (Excoffier and Lischer 2010) and FSTAT 2.9.3.2 (Goudet 1995) to estimate allele frequencies by locus and population, observed (H_O) and expected unbiased (H_E) heterozygosity, mean number of alleles per locus (k), number of private alleles per population (i.e., the number of alleles unique to a single population in the data set), allelic richness (A_R) and Wright's F_{IS} (Weir and Cockerham 1984). Mitochondrial DNA nucleotide (π) and haplotype (h) diversities were calculated using DNASP 5.10.0.1 (Librado and Rozas 2009). We used Friedman and Wilcoxon signed-rank test in PASW 18.0 (SPSS Inc.) for pairwise comparisons of genetic diversity estimates between populations. With the microsatellite DNA dataset, we tested the hypothesis of a significant genetic bottleneck at Tawharanui using 10^5 iterations of the two-phase model (TPM) Wilcoxon sign-rank test in the program BOTTLENECK 1.2.02 (Piry et al. 1999) with 95% stepwise mutations and variance set to 12. Additionally, with the mitochondrial DNA dataset we tested for significant negative Tajima's D as implemented in ARLEQUIN to detect bottlenecks.

3.3.3 *Population structure and gene flow*

For the microsatellite DNA dataset, ARLEQUIN was used to calculate F_{ST} and R_{ST} pairwise comparisons, and AMOVA (analysis of molecular variance; Excoffier et al. 1992; Michalakis and Excoffier 1996) was performed in GENALEX 6.3 (Peakall and Smouse 2006) to estimate the proportion of variance among populations relative to the total variance. Pairwise estimates of gene flow (Nm) among populations were computed using ϕ_{PT} (an analogue of F_{ST}) in GENALEX. Population structure was assessed as implemented in the program STRUCTURE 2.1 (Falush et al. 2003) using the admixture model with correlated allele frequencies and no *a priori* locations defined in five runs of 10^5 burnins followed by 10^6 iterations. Optimal K value was identified using a procedure described by Garnier et al. (2004). Population structure was also investigated using principal components analysis (PCA) in GENALEX. For the mitochondrial DNA dataset, we assessed F_{ST} pairwise comparisons among sampling locations in ARLEQUIN and

relationships among mitochondrial haplotype sequences by creating a haplotype network using statistical parsimony as implemented in TCS 1.21 (Clement et al. 2000).

3.3.4 *Identification of source populations and non-natal dispersers*

To determine what fraction of individuals at Tawharanui, the 'mixed stock', were derived from each putative source population, we performed a Bayesian mixed stock analysis using mitochondrial DNA data in the program R 2.11.0 (R Foundation for Statistical Computing, Vienna, Austria) with the MIXSTOCK library (GNU General Public License © 2008 Ben Bolker and Toshinori Okuyama). The Markov Chain Monte Carlo (MCMC) algorithm (Bolker et al. 2007) offers improved estimation of confidence intervals over parametric maximum likelihood (ML) bootstrapping (see Bolker et al. 2003). To test whether Markov chains converged to a stationary distribution, two MCMC chains were run from over-dispersed starting points using the Gelman-Rubin diagnostic test as implemented in MIXSTOCK. For mixed stock analysis using microsatellite DNA data, we used the program ONCOR (Anderson et al. 2008), which uses a conditional maximum likelihood (CML) to estimate mixture proportions. Bootstrap re-sampling of 1000 individuals was used to calculate genotype probabilities at a likelihood threshold of P = 0.01 (Rannala and Mountain 1997).

Detection of first-generation migrants (Paetkau et al. 2004) was performed using the software GENECLASS 2 (Piry et al. 2004) using the L_{HOME}/L_{MAX} test statistic, which assumes that all source populations were sampled. Computations were performed using the frequency method (Paetkau et al. 1995) as implemented in GENECLASS with significance testing at a likelihood threshold of $P = 0.01$ and $P = 0.05$ for 10^5 MCMC resampled individuals (Paetkau et al. 2004). In addition to tallying the ratio of male and female first-generations migrants identified by GENECLASS, sex bias in dispersal was assessed in FSTAT using statistical comparisons between males and females of three types of analyses (i) mean of corrected assignment indices (*mAIc*) in FSTAT that use one-tailed tests (alternate hypothesis = female-biased dispersal), (ii) sex-specific F_{ST} values and (iii) mean pairwise relatedness (R) values (Goudet et al. 2002).

3.3.5 *Simulations of minimum number of colonizers*

The effects i) founder numbers and ii) number of source populations on genetic variability were simulated using BOTTLESIM (Kuo and Janzen 2003). We specified the following parameters: diploid multilocus, variable population size, completely overlapping generations, random mating system, 5 years of expected organism longevity, sexual maturity of 1 year, sex ratio 1:1. We ran simulations of a minimum of 20 and up to 200 founders and allowed the newly founded population to grow for four years at an exponential rate, i.e., population size doubled each year. We use two source population scenarios (i) a single source population (Hauturu), and (ii) multiple source populations (weighted by contributions according to mixed stock analyses for Hauturu, Hunua and central NI). To test the relationship between population growth rate and retention of allelic diversity (influence of genetic drift), we ran simulations for 100, 25 and 5% population growth rates for the first four years after colonization. The central NI population was used as a proxy for the Coromandel peninsula bellbirds, which represents the largest bellbird population proximal to the Hauraki Gulf Islands remnant populations, but for which we were unable to obtain samples. In a previous study, we showed that F_{ST} values between central NI and Hauturu were low and not significant, thus birds likely had been connected in a genetic corridor that includes the Coromandel peninsula (Baillie et al. *in revision*; Chapter Two). Using 100 iterations, simulated values of mean k and H_E were plotted and compared with actual values observed in the Tawharanui bellbird population. Finally, to evaluate our simulations, we compared the simulated and real F_{ST} values computed between the Tawharanui and the Hauraki Gulf populations.

3.4 Results

3.4.1 *Genetic variability and strength of bottleneck at Tawharanui*

All loci were polymorphic in all populations, with heterozygosity (H_O = 0.37-0.64; H_E = 0.42-0.69), and mean number of alleles per locus ranging from 3.5 (in the Poor Knights) to 7.1 (at Hauturu; Table 3.1a). Differences between H_E values of Tawharanui versus the Hauraki Gulf islands and Hunua were not significant (*P*-value range: 0.09-0.9; Wilcoxon signed-rank test). Generally, H_E values were not significantly different among the six populations (P = 0.047; Friedman test), except that H_E was significantly lower at the Poor Knights (*P*-value range: 0.02-0.03; Wilcoxon signed-rank test). Bellbirds sampled at Tawharanui (k = 6.9) have approximately three more alleles per locus than at Tiritiri and Poor Knights. On the other hand, there was less than a one allele per locus difference between Tawharanui bellbirds and the remaining putative source populations. Allelic richness at Tawharanui, estimated by rarefaction for a sample size N = 9 (corresponding to the number of bellbirds sampled at Hunua), was not significantly lower than any other population in this study (*P*-value range: 0.1-0.9; Wilcoxon signed-rank test).

Bellbirds at Tawharanui had four private alleles ranging from 0.01 to 0.05 in frequency, while there were one to four private alleles at lower frequencies (0.009 to 0.05) in the Hauraki Gulf Islands (Hauturu and Tiritiri) and the two mainland populations. Results of single-locus Hardy-Weinberg equilibrium tests showed that significant heterozygote deficits were strongly contributed only by loci Ame7 and Ame14, which were then removed from the F_{IS} and BOTTLENECK analyses. Null alleles were apparent for loci Ame7 and Ame14 in MICROCHECKER, but null alleles were not present across all sampling locations (loci with possible null alleles by location: Ame7 in Tiritiri Matangi and Hauturu; Ame14 in Tiritiri Matangi). We found that bellbirds in the putative source populations (except at Tiritiri) were in Hardy-Weinberg equilibrium (multilocus test). On the contrary, bellbirds had a deficit of heterozygosity (significantly positive F_{IS} values) at the colony, Tawharanui. Mitochondrial DNA analyses of 127 bellbird control region sequences revealed a total of 13 haplotypes, 12 polymorphic sites, 5 singletons and mean h = 0.822 ± 0.02 *SD* and mean π = 0.0101 ± 0.0004 *SD* (Table 3.1b).

Table 3.1 Genetic diversity in bellbirds sampled at six sites in northern New Zealand: *N*, numbers analysed. (a) Microsatellite diversity statistics based on eight loci: N_A, total number of alleles, *k*, mean number of alleles; H_O, expected heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient. (b) Mitochondrial DNA diversity statistics based on a 436bp segment of the control region: *SS*, number of segregating sites; π, nucleotide diversity; *h*, haplotype diversity; *Hap*, number of haplotypes; Tajima's *D* (*P*-value in parentheses).

(a)	*N*	N_A	*k*	Allele richness	H_O	H_E	F_{IS}	Private alleles
Poor Knights	32	28	3.6	2.5	0.3672	0.4162	0.079	0
Tiritiri Matangi	55	36	4.6	3.4	0.4750	0.6532	0.133*	1
Tawharanui	56	54	6.9	4.5	0.4665	0.6342	0.121*	4
Hauturu	55	57	7.1	4.8	0.5795	0.6905	0.022	4
Hunua Ranges	9	38	4.8	4.4	0.5833	0.6740	-0.027	2
Central North Island	30	53	6.6	4.8	0.6375	0.6772	-0.019	4
Totals	237	232	9.0	4.8	0.5182	0.6986	0.052	15

* Significant *P*-value for F_{IS}

(b)	*N*	*SS*	π	*Hap*	*h*	Private haplotypes	Tajima's *D*
Poor Knights	24	8	0.0019	3	0.301	1	-1.99 (0.007)**
Tiritiri Matangi	21	2	0.0023	3	0.667	1	1.88 (1.0)
Tawharanui	25	8	0.0080	5	0.807	0	2.04 (1.0)
Hauturu	28	9	0.0096	6	0.836	1	2.54 (1.0)
Hunua Ranges	6	10	0.0083	4	0.800	1	-0.91 (0.2)
Central North Island	22	11	0.0064	7	0.684	3	-0.28 (0.4)
Totals	127	12	0.0101	13	0.822	7	1.07 (1.0)

** Significant *P*-value for Tajima's *D*

In agreement with microsatellite DNA analyses, pairwise comparisons for *h* and π among the colony and putative source populations revealed that diversity estimates at Tawharanui were similar to Hauturu, Hunua and central NI, and much higher than at Poor Knights and Tiritiri. There were no private haplotypes among the Tawharanui bellbirds, the greatest number of which were recorded for Hauturu and the central NI population. Finally, results of the BOTTLENECK test on microsatellite DNA data at Tawharanui revealed no significant heterozygosity excess at the colony (P = 0.9; Wilcoxon signed-rank test). Tajima's D was negative and significant only for Poor Knights bellbirds (Table 3.1b).

3.4.2 *Genetic differentiation and structure among bellbird populations*

For microsatellite DNA data, the mean F_{ST} value between Tawharanui versus Hauturu, Hunua and central NI was 0.02 ± 0.005 *CI* and these pairwise comparisons were statistically significant, including the comparison between Tawharanui versus Hauturu (F_{ST} = 0.02, P < 0.001) (Table 3.2). In contrast, mean F_{ST} values between Tawharanui versus Tiritiri and Poor Knights was 0.17 ± 0.02 *CI* (P < 0.001), an order of magnitude higher than Tawharanui versus all other populations. Values for genetic differentiation as shown by R_{ST} were typically lower than F_{ST} in this study, and no significant pairwise comparisons were evident among Tawharanui, Hauturu and Hunua (Table 3.2). For mitochondrial DNA data, the most important findings with regard to the Tawharanui founder event were that genetic divergence was not significant between Tawharanui versus Hauturu (F_{ST} = 0). This finding contradicts genetic differentiation apparent for F_{ST} but not R_{ST} using microsatellite DNA data.

Likelihood values from STRUCTURE revealed that out of the six sampling locations in this study, there are three populations (K = 3) (Table 3.3). The first population split, at K = 2, separated the Tiritiri bellbirds from all other bellbirds. At K = 3, the Tawharanui bellbirds were assigned to cluster II with probability QII = 0.86 along with Hauturu, Hunua and central NI bellbirds. Bellbirds sampled at Poor Knights and Tiritiri were assigned to single-population clusters, QI = 0.96 and QIII = 0.95, respectively (Table 3.3). The lack of subsequent population

subdivision, obtained with $K = 4$ to $K = 6$ (data not shown) confirmed that there was no strong genetic distinction of the Tawharanui bellbirds versus the Hauturu or mainland bellbirds. PCA (not shown) provided similar results, as *PC*1 (65%) divided populations into three main clusters: i) Tiritiri, ii) Poor Knights and iii) all other populations together. Finally, the mitochondrial DNA haplotype network shows that only Hauturu haplotypes consistently co-occurred with the five Tawharanui mitochondrial DNA haplotypes (Figure 3.2).

Table 3.2 Pairwise *F*-statistics for bellbirds between sampling locations within the Hauraki Gulf and Central North Island (NI), New Zealand bellbird metapopulation comprising (a) F_{ST} (below diagonal) and R_{ST} (above diagonal) values for eight microsatellite loci and (b) F_{ST} values (below diagonal) for a 436 bp fragment of the mitochondrial DNA control region. Asterisks indicate significant values: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

(a)	Poor Knights	Tiritiri Matangi	Tawharanui	Hauturu	Hunua Ranges	central NI
Poor Knights	_	0.073**	0.021	0.037*	0.200*	0.041*
Tiritiri Matangi	0.314***	_	0.034*	0.024	0.095	0.074**
Tawharanui	0.178***	0.158***	_	0.015	0.062	0.035
Hauturu	0.163***	0.107***	0.017***	_	0.070	0.025
Hunua Ranges	0.233***	0.155***	0.013	0.019	_	0.200**
Central NI	0.157***	0.138***	0.021***	0.009*	0.021	_

(b)	Poor Knights	Tiritiri Matangi	Tawharanui	Hauturu	Hunua Ranges	central NI
Poor Knights	_					
Tiritiri Matangi	0.867***	_				
Tawharanui	0.569***	0.230***	_			
Hauturu	0.425***	0.292***	0.000	_		
Hunua Ranges	0.449***	0.181**	0.155*	0.174*	_	
Central NI	0.695***	0.114*	0.033	0.080	0.130	_

Table 3.3 Average proportion of membership (QI; computed using STRUCTURE; Falush et al. 2003) of individual bellbirds assigned to two (*K* = 2) or three (*K* = 3) inferred clusters. Likelihood of membership in a cluster is indicated by the Q scores. The Q class with the highest proportion of membership is shaded in grey.

	K = 2		K = 3		
Given population	QI	QII	QI	QII	QIII
Poor Knights	0.988	0.012	0.963	0.024	0.013
Tiritiri Matangi	0.087	0.913	0.011	0.037	0.953
Tawharanui	0.914	0.086	0.010	0.855	0.046
Hauturu	0.884	0.116	0.040	0.908	0.053
Hunua Ranges	0.955	0.045	0.041	0.914	0.045
central NI	0.945	0.055	0.088	0.881	0.032

3.4.3 *Identification of source populations using mixed stock analyses*

Point estimates (which are the mean values given in the MCMC chain) of running the 'mixstock' MCMC algorithm on data from bellbird mitochondrial DNA shows a 90% contribution of individuals to the colony at Tawharanui from Hauturu, and 2-3% from each of Poor Knights, Tiritiri, Hunua and central NI (Figure 3.3a). There is a general tendency, however, for MCMC to predict nonzero contributions from more different source populations (Bolker et al. 2003). Examination of confidence intervals reveal i) no overlap between Hauturu and the other populations and ii) that there is a high probability that contributions from Poor Knights, Tiritiri, Hunua and central NI are equal to zero. Thus our MCMC analysis on mitochondrial DNA data indicates that Hauturu is most likely the single source population of the Tawharanui bellbirds. We also performed a conditional maximum likelihood algorithm (CML) on the same mitochondrial DNA dataset. CML with 1000 bootstrapping showed a similar range of

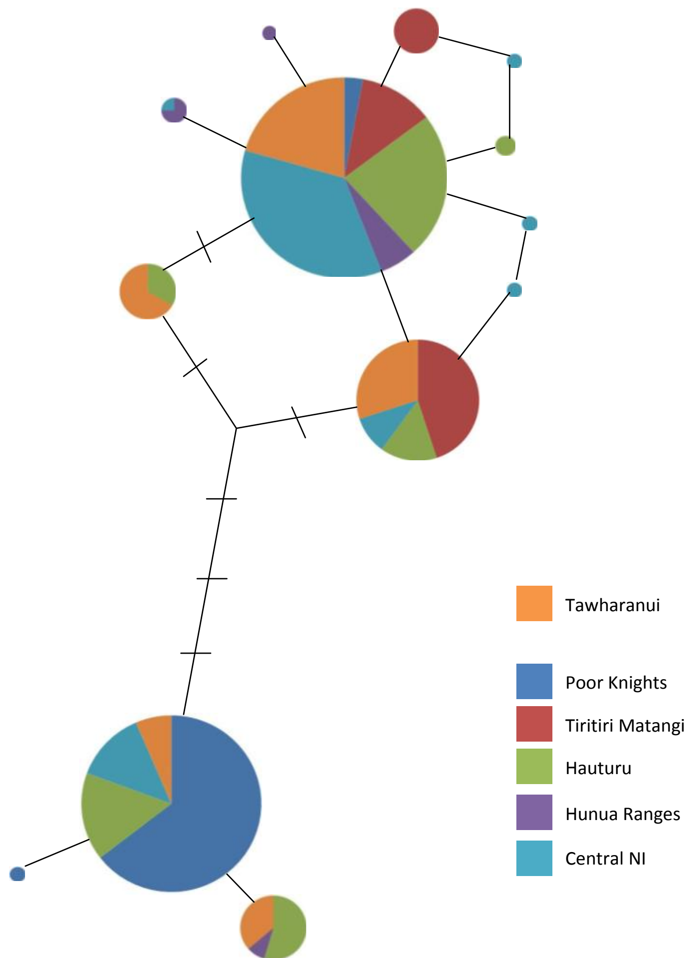

Figure 3.2 Parsimony network of bellbird mitochondrial DNA control region haplotypes from Tawharanui (the colony) and five putative source populations in northern New Zealand. Diameters of circles (nodes) are proportional to the frequency of the haplotype. Hash marks and nodes represent mutational steps separating haplotypes. All individuals connected within ≥ 95% confidence level equivalent to eight mutational steps.

confidence intervals as from MCMC, except that the point estimates were 94% (*CI* limits: 47-100%) for Hauturu, 6% (*CI* limits: 0.01-40%) for Tiritiri and 0% (*CI* limits: 0.0-38%) for all other populations (data not shown). Finally, point estimates of running the ONCOR CML algorithm (there is no MCMC option in ONCOR) on data from bellbird microsatellite DNA shows a 65% contribution of individuals to Tawharanui from Hauturu, 19% from Hunua, 16% from central NI

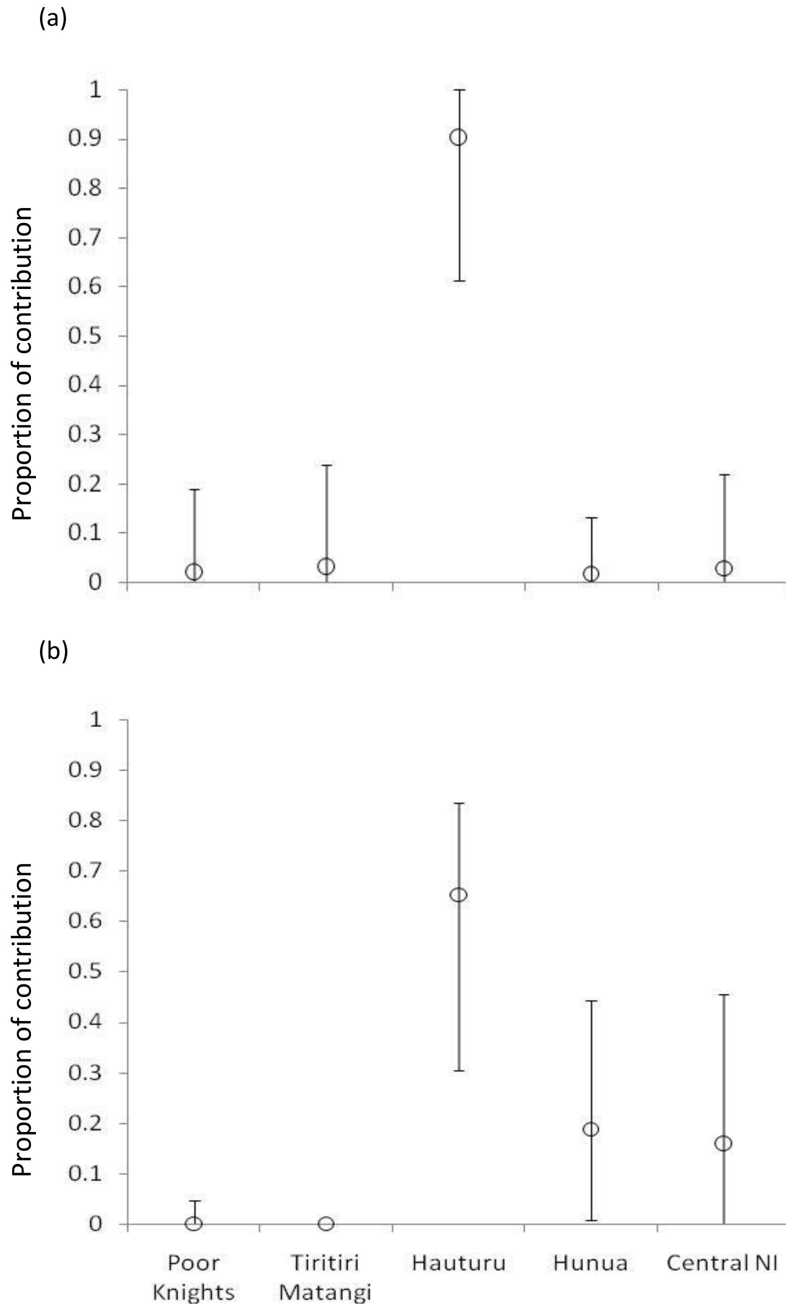

Figure 3.3 Mixed stock analysis for (a) mitochondrial and (b) microsatellite DNA data showing proportion of genetic contribution from putative source populations to the Tawharanui (mixed-stock). Error bars represent upper and lower 95% confidence limits.

and 0% from both Poor Knights and Tiritiri (Figure 3.3b). Similar to the mitochondrial DNA results, the *CI* overlaps with zero at Poor Knights, Tiritiri and central NI (Figure 3.3b). The *CI* of Hunua did not overlap with zero, but this may be an artifact of low sample size at that location (N = 9). Thus both our microsatellite and mitochondrial DNA datasets indicate that Hauturu is the single source population for Tawharanui. Finally, all analyses indicate that Poor Knights and

Tiritiri definitively can be eliminated as possible source populations for the re-colonizing bellbird population at Tawharanui.

3.4.4 *Rates of gene flow and detecting natal dispersal of individuals*

A significant mean multilocus ϕ_{PT} = 0.18 (P = 0.01; computed from AMOVA using microsatellite DNA data) indicates that gene flow was significantly limited among the six bellbird groups. However, an order of magnitude lower ϕ_{PT} = 0.02 (P = 0.02) when Poor Knights and Tiritiri were excluded from this analysis indicates higher gene flow among Tawharanui, Hunua and central NI. Assuming migration-drift equilibrium, gene flow was highest between Tawharanui and Hunua (*Nm* = 34.8), Hauturu (*Nm* = 10.0) and central NI (*Nm* = 7.7), and lowest between Poor Knights (*Nm* = 0.73) and Tiritiri (*Nm* = 1.3). Excluding Tiritiri and Poor Knights, bellbirds at Tawharanui should exchange mean $Nm = [1/(F_{ST} - 1)]/4$ = 17.5 effective individuals per generation with the Hauraki Gulf populations (data not shown). Rates of gene flow associated with the Hunua population, however, likely inflate the mean Nm estimate due to low sample size. Thus, a more conservative mean *Nm* for Tawharanui is 8.9 effective non-natal dispersers per generation.

The numbers (and proportions) of inferred first-generation migrants (males and females), as identified by GENECLASS using microsatellite DNA data, are reported in Figure 3.1. These data are evidence of sustained dispersal to the recently colonized Tawharanui from Hauturu and Hunua, but bellbirds from Tiritiri and the Poor Knights do not appear to contribute to the ongoing re-colonization of Tawharanui. Migration appears to be female-biased because the number of female first-generation migrants identified outnumbered males by 9:1. Additionally, statistically significant female-bias in dispersal was detected in FSTAT when Tawharanui, Hauturu and Tiritiri were considered. Results of (i) the difference between mean assignment index for males (*mAIc* = 0.58) and females (*mAIc* = -0.65) was significant (P = 0.02), (ii) sex-specific F_{ST} values were significantly (P = 0.01) twice as high for males (F_{ST} = 0.14) as for

females (F_{ST} = 0.07) and (iii) the relatedness index for any given location was two times higher for males then females (R_{MALES} = 0.23, $R_{FEMALES}$ = 0.10, P = 0.01).

3.4.5 *Simulations of colonization via single versus multiple source populations*

Simulated results for genetic diversity and number of founders at Tawharanui using a single source population (Figure 3.4) and multiple source populations (Figure 3.5) are presented for a population growth rate of 25% per year. A 25% population increase per year best describes the Tawharanui colonization because pair-wise F_{ST} values between source and simulated colony populations was not significantly different (data not shown). On the other hand, when growth rates were set to 5 and 100% at the simulated colony, F_{ST} values were significantly different with source populations and did not accurately represent growth rates at Tawharanui. Additionally, we found that simulated H_E four years after colonization was ≥ 0.63 (the actual H_E for the Tawharanui dataset) at 20 founders for all simulations (Figs. 4b and 5b). Because H_E is typically inflated after a founder event, we focus on number of alleles henceforth. In the first set of simulations for a founder event involving a single source population (Figure 3.4a), we used the data from the 55 individuals sampled at Hauturu and found that ≥ 100 effective founders (and a minimum of 30 founders when *SE* bars are considered) are required to explain mean *k* after four years at Tawharanui. Additionally, *k* reaches asymptote (optimal *k* retention from source population) after 100 founders. In the second set of simulations for a founder event involving multiple source populations, we used the 55 individuals sampled at Hauturu, and an additional 16 from Hunua (7 of which were randomly generated based on allele frequencies derived from the 9 individuals genotyped) and 14 individuals (randomly selected from 30 genotyped individuals) from central NI. These simulations reveal that 50 effective founders explain observed *k* at Tawharanui (and a minimum of 20 founders when *SE* bars are considered), and the asymptote for optimal *k* was reached at 80 founders (Figure 3.5a). Furthermore, F_{ST} values were too high in pair-wise comparisons of actual versus simulated colony populations when fewer than 30 individuals are used, thus more than 30 founding individuals are required to accurately represent the genetic differentiation

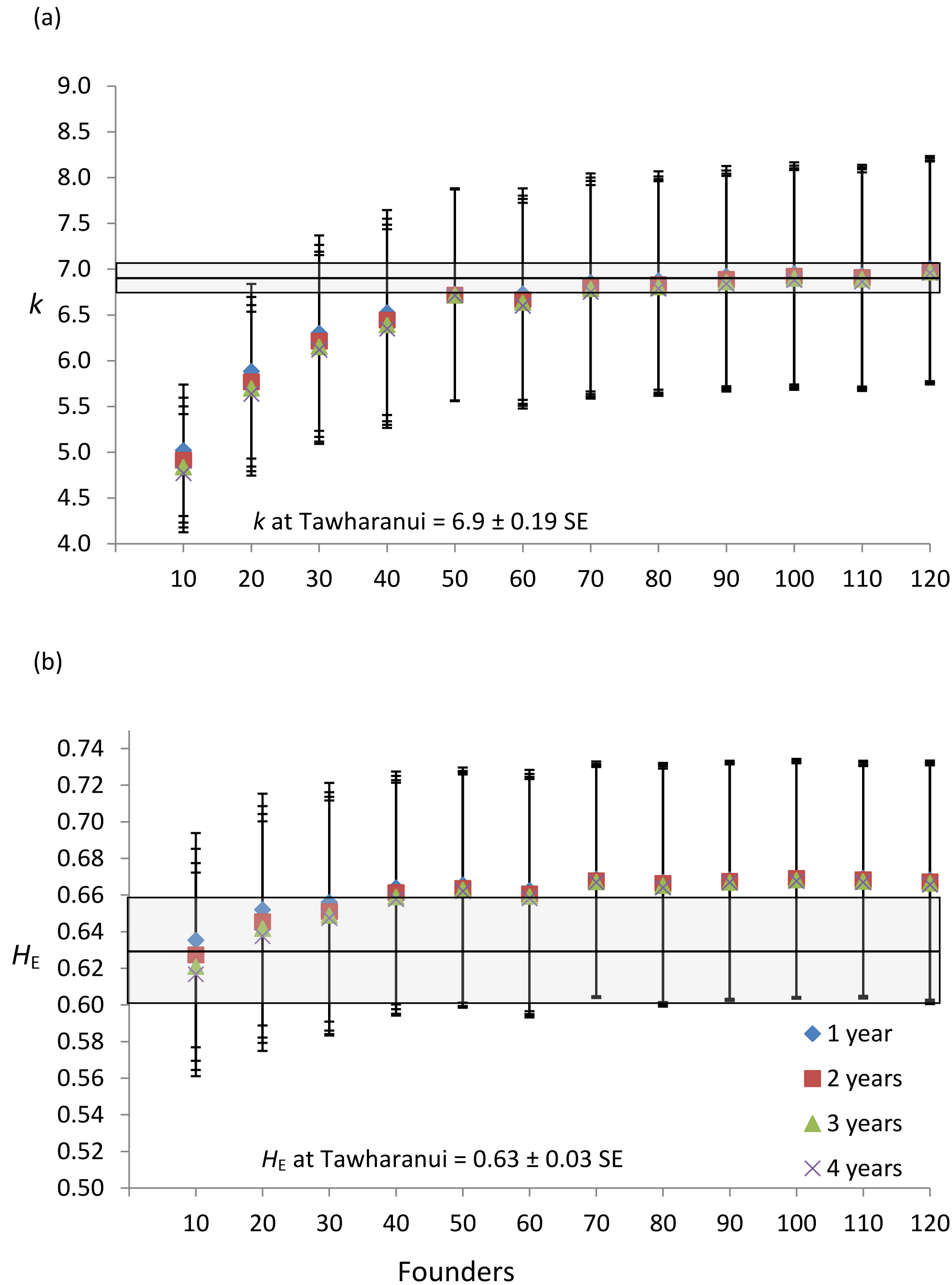

Figure 3.4 Bottleneck simulations obtained using BOTTLESIM assuming a single source population (Hauturu). Effects of founder events of variable size (from a minimum of 20 up to 120 founders) on (a) the mean number of alleles per locus (k), and (b) the expected heterozygosity (H_E) in newly founded populations that grow exponentially for 1–4 years. Vertical error bars represent the standard error of the mean (*SE*) and dark bands indicate *SE* of the observed values in the Tawharanui bellbirds.

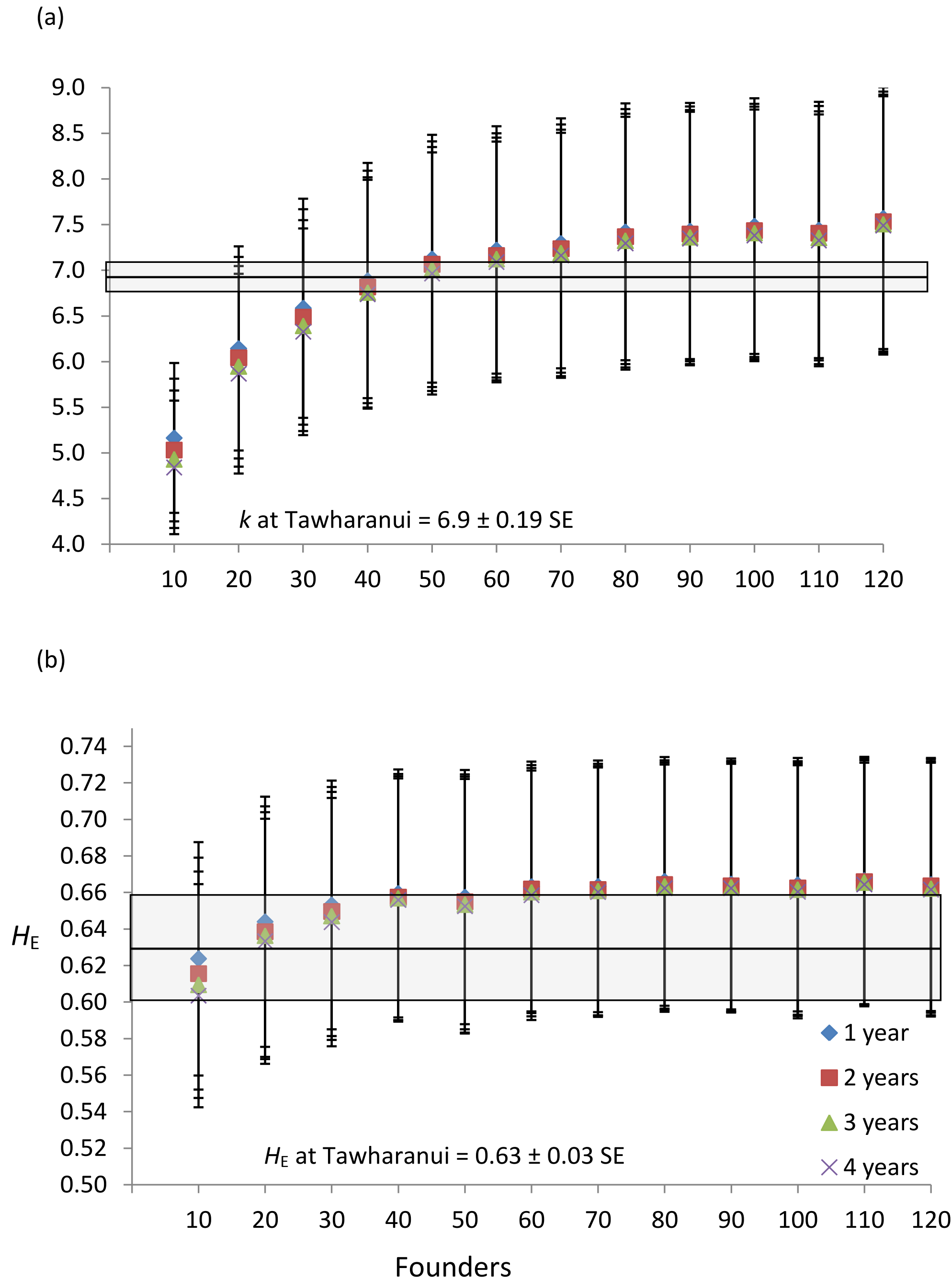

Figure 3.5 Bottleneck simulations obtained using BOTTLESIM assuming multiple source populations (Hauturu, Hunua and central NI weighted by contributions determined through 'mixed stock' analysis). Effects of founder events of variable size (from a minimum of 20 up to 120 founders) on (a) the mean number of alleles per locus (*k*), and (b) the expected heterozygosity (H_E) in newly founded populations that grow exponentially for 1–4 years. Vertical error bars represent the standard error of the mean (*SE*) and dark bands indicate *SE* of the observed values in the Tawharanui bellbirds.

between source and colony (Table 3.4). Observed allele retention four years after the onset of re-colonization at Tawharanui was 95% (54/57), whereas simulated allele retention was 88, 89 and 89% based on 40, 50 and 60 effective founders, respectively. Thus, it is apparent that more than 60 effective founders colonized Tawharanui. We found that retention of *k* varied significantly depending on population growth rate in simulations, e.g., involving 50 effective founders: 84% of alleles were retained at a population increase of 25% effective breeders per year over four years; 99 and 67% of alleles were retained when growth rate was set at 100 and 5%, respectively. Thus, a combination of high founding numbers and rapid population growth likely characterizes the Tawharanui bellbird colonization.

3.5 Discussion

3.5.1 *Origin of the Tawharanui bellbird population*

Our findings using microsatellite and mitochondrial DNA reveal that bellbirds re-colonizing the mainland peninsula at Tawharanui mainly originated from Hauturu, an Hauraki Gulf island approximately 25 km from the Tawharanui peninsula headland. These findings are concordant with those of a previous study on bellbird song dialect implicating Hauturu as the source population (Brunton et al. 2008). Furthermore, bellbirds at Tawharanui share one diagnostic mitochondrial DNA control-region haplotype that is unique to the Hauturu population. Mixed stock analyses of both mitochondrial and microsatellite DNA in this study strongly suggest Hauturu as the single source population for the newly established Tawharanui bellbird population. Furthermore, we found four private alleles at Tawharanui. Though our sample sizes are reasonably high (except at Hunua), it is likely we would discover these four putatively private alleles by increasing the sampling effort at Hauturu.

Our first-generation migrant analysis indicates that bellbirds were actively flying to Tawharanui from Hauturu, and also Hunua, four years after onset of re-colonization. Despite geographic proximity, the contribution of effective dispersers from Tiritiri appears to be minimal, or non-existent, within the first four years of colonization. Furthermore, we found different patterns of population structure for males and females where males were significantly more likely to be assigned to their location of sampling, relatedness among males was significantly higher than that for females and genetic differentiation among males was significantly higher than that for females. Thus, female sex-biased dispersal is evident. It is thought that in avian systems only the resource-competition model (Greenwood 1980) predicts female-biased dispersal, but there is a lack of empirical data to test well-developed theoretical predictions (Prugnolle and Meeus 2002). Recent study on Seychelles warblers (*Acrocephalus sechellensis*; Eikenaar et al. 2010) suggests that where habitat quality is lower sex-bias in dispersal will be higher. However, a study on female-biased natal dispersal in an endemic passerine sympatric with the bellbirds explains that causal relationships are complex (Richardson et al. 2010). Wahlund effects likely influenced the Tawharanui colonization in that F_{IS} was significantly positive and this is the expected result of genetic sampling of multiple distinct source populations (Wahlund 1928). Although migrant contributions from Hunua and central NI would be small in the context of the overall population dynamics of the founder population (Bolker et al. 2003), they have important implications for understanding how metapopulation dynamics relate to re-colonization success.

3.5.2 *Absence of founder effect during the re-colonization of Tawharanui*

We provide strong evidence that this first re-colonization of a northern New Zealand mainland site since extirpation 150 years ago is characterized by high founder numbers (≥ 100 individuals) and rapid population growth (at least 25% per year). Thus, the newly establish bellbird subpopulation at Tawharanui did not experience a genetic bottleneck or strong founder effects. As a consequence of the lack of genetic bottleneck, genetic diversity (in terms of microsatellite heterozygosity, and allelic richness, as well as estimates from mitochondrial DNA

analyses) of bellbirds at Tawharanui was not significantly lower than that at the Hauturu, Hunua, Tiritiri, Poor Knights Islands and central NI populations sampled in this study. Bellbirds at Tawharanui maintained 97% of the allelic richness, 92% of the expected heterozygosity and 96% of the mitochondrial DNA haplotype diversity of Hauturu, which is the most geographically proximate putative source population and consequently the most genetically diverse population sampled in this study. Additionally, there is the possibility of ongoing colonization during the four years since the initial founder event. Other researchers have found that single-step re-colonization events of passerines do not necessarily produce strong founder effects (Clegg et al. 2002a) and are often similar to successful repeated invasions by exotic species (Hawley et al. 2006). Furthermore, greater allelic diversity is found where population growth rates are high (Brekke et al. 2011). The ecological conditions that promoted this growth and a successful colonization of bellbirds at Tawharanui directly involve the eradication of mammalian predators at both Hauturu and Tawharanui during *c*. 2004-2005. Furthermore, bellbirds at Tawharanui are not genetically differentiated from those at Hauturu according to most microsatellite and mitochondrial DNA as measured by pair-wise *F*-statistics. The slightly significant F_{ST} for microsatellite DNA distances between Tawharanui and Hauturu may 1) be an artefact of the infinite allele model assumed, whereas R_{ST} may more accurate reflect microsatellite DNA mutation models (Slatkin 1995) or 2) indicate ongoing bellbird dispersal within and among these regions. Taken together these results suggest that the colonization at Tawharanui is ongoing and is a natural extension of a functional metapopulation that has been rehabilitated by predator control, habitat protection, successional growth of vegetation causing a general increase in bellbird population numbers.

3.5.3 *Population genetics of natural versus human-assisted bird re-introductions*

We put forward that an understanding of the genetic and demographic processes of naturally founded populations in fragmented landscapes has value as genuine models for human-assisted re-introductions. It has been suggested previously to use introduced invasive species as models for re-introduction of threatened species (Kolbe et al. 2004; Hawley et al. 2006). This

is likely because natural endemic founding events are rarely detected (see Parker et al. 2010). Clegg et al. (2002b) documented that self-introducing silvereye (*Zosterops lateralis*) populations from Australia were large in number, prolific and highly diverse genetically. Our study is the first, however, to characterize the genetic effects of a natural re-introduction of an endemic New Zealand passerine. Fortunately, there are a number of published studies that examine the pre- and/or post-translocation genetic conditions of relatively poor flighted birds in New Zealand: kokako (*Callaeas cinerea*), Hudson et al. 2000; saddleback and robin (*Petroica* spp.), Lambert et al. 2005; also see Taylor et al. 2007; South Island robin (*Petroica australis*), Boessenkool et al. 2007); saddleback, Taylor and Jamieson 2008; takahe (*Porphyrio hochstetteri*), Jamieson 2010; North Island saddleback, Ruarus et al. (2011)*;* hihi, Brekke et al. 2011. The remnant hihi population at Hauturu has similar levels of genetic diversity to bellbirds, neither species has experienced recent genetic bottlenecks at Hauturu and these species co-occupy similar ecological niches as non-obligate nectivores (Brekke et al. 2011; Baillie et al. *in revision*; Chapter Two). Unlike the bellbird, however, the hihi is completely extirpated from New Zealand except at Hauturu. Reasons for this difference between bellbirds and hihi are not entirely clear and may involve flight ability and mating system. During some single-step founder events, both the hihi (Hauturu to Tiritiri; Brekke et al. 2011) and bellbird (Hauturu to Tawharanui; this study) experienced little founder effects. A transfer of hihi from Hauturu to Kapiti resulted in low genetic diversity and was attributed to slow re-introduction population growth rate (Brekke et al. 2011). Furthermore, inbreeding hotspots characterized by reduced genetic diversity and serial bottlenecks have been confirmed to result from two-step human-assisted translocations (hihi, Brekke et al. 2011; saddleback, Lambert et al. 2005). Thus, it is a concern that bellbirds dispersing out from Tawharanui will form inbreeding hotspots as has occurred in other natural movements of populations in fragmented landscapes (Fabbri et al. 2007). Genetic erosion can be prevented through admixture from elsewhere or even a genetic cascade from Tawharanui, which is continually being “topped-up” (Fabbri et al. 2007). Sutherland et al. (2010) explain that 95% of genetic diversity represented is a goal in good transfers. Based on empirical study of hihi transfers, Brekke et al. (2011) conclude that only 30 birds are needed to establish a population with 95% genetic diversity of the source

population. Whereas, data from a natural re-introduction in this study show that two to three times those numbers represented 97% of allelic diversity. Moreover, even when sufficient numbers of bellbirds to represent genetic diversity were selected for translocation in the past, none of these translocations were successful. Observably, the ecological and social factors that limit the success of these translocations are not well understood. The results of this and numerous studies on plants and animals demonstrate the importance of empirical study of the life history, mating system, ecology and population genetics both before and after translocation [Guam rail (*Gallirallus owstoni*), Haig et al. 1990; *Corregin grevillea*, Krauss et al. 2002; Mauna Kea silversword (*Argyroxiphium sandwicense*), Robichaux et al. 1998]. We conclude that founder events are likely to have higher success when natural dispersal is an option.

3.5.4 *Conclusions*

Here we show that a natural bellbird re-colonization is characterized by: (i) only a slight founder effect; (ii) an absence of a genetic bottleneck; (iii) gene flow that is maintained by frequent bidirectional and sex biased dispersal events, that is female bellbirds preferentially disperse to non-natal sites among the Hauraki Gulf islands and coastline. Our bottleneck simulations show that a greater amount of genetic diversity in the founding colony can be accomplished with fewer individuals when source populations are many, as opposed to a single source. Founder individuals, population growth rate and patterns of gene flow subsequent to an initial colonization event are the main variables influencing how the new population is differentiated (Fabbri et al. 2007). As a consequence, metapopulation dynamics associated with colonizing events may have important influences on the evolutionary path of newly established populations in that they can enhance secondary contact and increase genetic variability. We suggest that, though the bellbird is not an endangered species, a monitoring program of the Tawharanui colonization and surrounding environs be initiated to assess how species of high vagility will spread through a fragmented landscape and whether secondary contact from other restored habitat fragments will occur.

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CHAPTER FOUR

An almost speciation of *Anthornis melanura* hypothesis: allopatry and post-divergence hybridization in a vagile disperser

4.1 Abstract

Glacial cycling during the Pleistocene *c.* 2,400 – 10 kyr is known to have induced genetic diversity through allopatric fragmentation of populations. Phylogeographic studies focused on sedentary forest taxa in New Zealand report that repeated submersion of a land bridge between the North and South Islands of modern day New Zealand caused allopatric separation, even speciation, into North Island and South Island forms during the Pleistocene (e.g., passerines, cicadas, skinks). This study aims to determine the historical processes that explain patterns of genetic variation of a relatively vagile forest bird species *Anthornis melanura*. We sequenced one mitochondrial control region Domain I locus and genotyped eight microsatellite loci from 212 and 371 individuals, respectively, located throughout the range of *A. melanura* in New Zealand. Our results suggest the existence of two distinct haplogroups: haplogroup A is present in all locations and dominant on the North Island and a less diverse haplogroup B is dominant on the South Island. AMOVA results revealed a significant historical barrier to gene flow between North and South Islands. Phylogenetic and coalescent modelling approaches confirm that divergence of the northern and southern haplogroups occurred during the mid-Pleistocene at *c.* 199 kyr. Our findings support the hypothesis that a vagile forest species had separated into two or more populations during the Pleistocene before the Last Glacial

Maximum (LGM) ~ 22 kyr. Furthermore, subsequent population expansion and high migration rates allowed secondary contact and co-distribution of *A. melanura* lineages. The lack of speciation or division into sub-species in the *Anthornis* genus may be partially due to dispersal ability and high levels of gene flow.

Keywords Coalescence, Divergence, Phylogeography, Population, Secondary contact, Pleistocene glaciations, New Zealand

4.2 Introduction

Why sometimes does speciation occur and sometimes it does not? Given enough time, isolated populations will diverge genetically to the point of being unable to exchange genes with each other (Grant and Grant 2010). In their theory on island biogeography, MacArthur and Wilson (1963, 1967) made the insightful point that a balance would be struck between the opposing processes of immigration and isolation. In simpler terms, if migrants could reach islands once they would be reached again by subsequent migrants and that repeated immigration (and breeding) would retard divergence thus preventing speciation (Grant and Grant 2010). Today, divergence-with-gene-flow ideas in conjunction with coalescent theory (Nordborg 1997; see Nielsen and Wakely 2001) are the foundation for contemporary research on how speciation occurs with respect to gene flow and selection dynamics (e.g., Carstens et al. 2004; Weir 2006; Carling et al. 2010; Rovito 2010; Paradis 2010; Turmelle et al. 2011). It has become obvious, however, that discordance between gene trees and species trees often reflect real population genetic processes (Grant et al. 2004; Zachos 2009). Consequently, a common problem among all phylogeographic studies is that conclusions can often be erroneous due to 1) incomplete lineage sorting, 2) introgressive hybridization and 3) gene duplication and paralogous sampling (Zachos 2009). Fortunately, the occurrence and probability of discordance between gene and

species trees can be circumvented in several ways (Zachos 2009). Ultimately, it is imperative that phylogeographic conclusions are corroborated with evidence based on geography, palaeontology, geology, climatology and comparisons with findings from sympatric species (Avise 2000).

Pleistocene glacial cycles have been shown to be responsible for allopatric separation of taxa into refugia eventually leading to speciation (see Lovette 2005). There is growing evidence from phylogeographic studies on plants and animals in New Zealand that widespread population extinction and bottlenecking is linked with Pleistocene glacial cycling (Goldberg et al. 2008; Marshall et al. 2009; Wallis and Trewick 2009). In present day New Zealand, the Cook Strait divides the country into two main islands, North Island and South Island. The last time global sea level was as high as it is today was during a very warm interglacial period *c.* 120 kyr. These warm periods occur approximately every 100,000 years, e.g., *c.* 250, 320 and 410 kyr (Petit et al. 1999; Carter and Gammon 2004; Campbell and Hutching 2007). Between these interglacial periods there are typically at least five cooling phases (glacial maxima) at a periodicity of ~21,000 years (Campbell and Hutching 2007). During these cold glacial maxima, the landscape was much different than from today and the North and South Islands physically formed a single landmass (Alloway et al. 2007). Continuous forest was mainly restricted to the northern North Island (Campbell and Hutching 2007), though Alloway et al. (2007) show evidence of extensive coastal forest throughout the landmass. During the warm interglacial periods, the flooded Cook Strait constituted a barrier to gene flow for terrestrial fauna, including bird species. In fact, many terrestrial species in New Zealand have separated into North Island and South Island species or subspecies forms, e.g., short-tailed bat, Lloyd 2003; cicada, Hill et al. 2009; cicada, Marshal et al. 2009; kokako, Murphy et al. 2006; robin, Miller and Lambert 2006; brown kiwi, Baker et al. 1995, Burbidge et al. 2003.

In contrast, the two endemic Meliphagidae species (New Zealand bellbird *Anthornis melanura* and tui *Prosthemadera novaeseelandiae*) are not considered to have separated into North and South Island forms (Robertson et al. 2007), perhaps due to the homogenizing effects of their high dispersal ability compared to most New Zealand fauna (Agudo et al. 2011).

Additionally, the New Zealand meliphagid genera are relatively young and diverged from a common ancestor in the late Pliocene *c.* 2.9 million years (Driskell et al. 2007). Therefore, the vast majority of their phylogeographic history is confined to and likely influenced by the Pleistocene Epoch. In a previous study, our initial inspection of the *A. melanura* mitochondrial control region (see Chapter Three) revealed a deeply diverged dumbbell-shaped haplotype network leading us to postulate that population separation had occurred at some point in the past. Furthermore, using only microsatellite DNA data (Baillie et al. *in revision*; see Chapter Two) we provided evidence of high genetic admixture and hybridization among populations throughout the two main islands of New Zealand. In this study, we test two hypotheses to explain the co-distribution of the two deeply diverged mitochondrial lineages observed in *A. melanura*. First, *A. melanura* experienced allopatric population separation into at least two refugia at some time during the Pleistocene glacial cycles. If this is the case, we expect migration rates to be low between geographic regions at the time of divergence. Second, a sharp increase in migration rates and population size occurred at some point during or after the LGM as the climate stabilized. Under this scenario, separation followed by secondary contact could explain the genetic patterns we see today in *A. melanura*.

4.3 Methods

4.3.1 *Sample collection*

Blood samples were collected from 10 locations throughout New Zealand (NZ) between 2008 and 2010 (Figure 4.1). To avoid sampling an individual more than once, individuals were marked under permit either with Department of Conservation (DoC) Banding Office stainless-steel leg bands or by clipping a tail feather. These locations represented five main geographical regions 1) Poor Knights Islands, 2) the Hauraki Gulf (Hauturu, Tawharanui and Tiritiri Matangi), 3) North Island NZ (Hunua, Tongariro and Kapiti), 4) South Island NZ (Kaikoura and Dunedin) and 5) the Sub-Antarctic (Auckland Islands). *A. melanura* populations north of Tongariro are

remnant populations from a large-scale anthropogenic extirpation. Additionally, Tiritiri Matangi is an island which had undergone a recent bottleneck *c.* 150 years due to anthropogenic causes. The population at Tawharanui was recently founded *c.* 5 years after intensive conservation efforts. Therefore, much of the analyses on genetic population structure in this paper focus on the mainland populations only, including Kapiti.

4.3.2 *DNA sequencing and genotyping*

DNA was extracted from blood samples using a DNeasy extraction kit (Qiagen, Valencia, CA, USA) as well as the phenol-chloroform method. Polymerase chain reaction (PCR) was used to amplify a 436bp segment of mitochondrial DNA control region Domain I for 212 individuals (see the PCR protocol described in Chapter Three). Eight microsatellite loci (Paterson et al. 2010) were amplified by PCR (as described in Baillie et al. *in revision*; see Chapter Two) for N = 371 individuals and tested for null alleles using MICROCHECKER 2.2.3 (van Oosterhout et al. 2004). Departures from Hardy-Weinberg equilibrium and linkage disequilibrium were assessed (Table A.3) using ARLEQUIN 3.5 (Excoffier and Lischer 2010). PCR products were separated on an ABI 3730 DNA Analyzer (Applied Biosystems, Inc.). Mitochondrial DNA sequences were aligned and edited manually in MEGA 4.1 (Tamura *et al.* 2007) and microsatellite marker fragment sizes were scored manually using Peak Scanner Software 1.0, Applied Biosystems, Inc.).

4.3.3 *Diversity and population structure*

We calculated several estimates of genetic diversity for the mitochondrial control region in DNASP 5.10.0.1 (Librado and Rozas 2009): number of haplotypes (Hap), haplotype diversity (H), average number of nucleotide differences (k), number of segregating sites (S), nucleotide diversity (π). Pair-wise comparisons of populations were calculated using the mitochondrial DNA dataset (F_{ST} comparisons of microsatellite dataset reported in Baillie et al. *in revision*, see Chapter Two). We performed analysis of molecular variance (AMOVA) in ARLEQUIN on both mitochondrial (Φ_{ST}) and microsatellite DNA data (F_{ST}) using the different geographical regions to test the locations of hypothetical barriers to gene flow in an attempt to maximize differentiation among groups (F_{CT}) (Toon et al. 2007) and identify refuge areas. Hauraki Gulf

Islands and Kapiti were excluded from this analysis to avoid influence of bottleneck and human-induced isolation (*A. melanura* has been extirpated from the mainland adjacent these islands likely due to introduced predators and habitat loss), while Poor Knights and Auckland Islands were used as controls. Sampling locations are abbreviated as follows: Poor Knights, Po; Tiritiri Matangi, Ti; Tawharanui, Tw; Hauturu, Ha; Hunua, Hu; Tongariro, To; Kapiti, Kp; Kaikoura, Kk; Dunedin, Du; Auckland Islands, Au. As an initial investigation into the phylogenetic relationships among mitochondrial DNA haplotypes and their geographic locations, we generated a parsimony network using TCS 1.21 (Clement *et al.* 2000) with a 95% connection limit and found two apparently divergent haplogroups (haplogroup A and B; Figure 4.2). Bayesian clustering analysis using the admixture model in STRUCTURE 2.3.3 (Pritchard et al. 2000) with *a priori* location assumptions was used to determine the levels of admixture among contemporary *A. melanura* populations. We know from a previous study that is $K = 4$, where K is the number of population clusters (Baillie et al. *in revision*, see Chapter Two). The burnin length was set at 10^5, followed by 10^6 randomisation steps.

4.3.4 *Phylogenetic analyses*

We tested the phylogenetic hypothesis of ancient allopatric divergence of haplogroup A and B, which requires statistically significant reciprocal monophyly among clades in order to reject the null hypothesis of panmixia. The TrN+I model (Tamura and Nei 1993) was chosen using maximum likelihood analyses and inferred by the Akaike Information Criterion (AIC) as the model of sequence evolution that best fitted our 26 mitochondrial DNA haplotype dataset (212 individuals) in JMODELTEST 0.1.1 (Guindon and Gascuel 2003; Posada 2008). This TrN+I model has assumed base frequencies (A = 0.2612, C = 0.1377, G = 0.3034, T = 0.2977) with proportion of invariable sites (I) = 0.935 and a substitution rate matrix A-C = 1.0, A-G = 11.3325, A-T = 1.0, C-G = 1.0, C-T = 53.4172 and G-T = 1.0. Using the TrN+I model, we estimated the gene genealogy using the posterior probabilities from Bayesian inference in MRBAYES 3.1 (Ronquist and Huelsenbeck 2005). In MRBAYES, we ran four chains (one hot, three cold) for 50 million generations sampling trees every 100 generations with a 25% burnin period. Convergence was ascertained when average standard deviation of split frequencies between chains were below

0.01 and the potential scale reduction factors (PSRF) were within 0.997 < PSRF < 1.003. Additionally, we ran 5,000 bootstrap replicates of the maximum likelihood (ML) analysis in MEGA. For both the Bayesian and ML methods, control region sequences from two Australian meliphagid species with the best sequence similarity results in a GenBank BLAST search [*Menura novaehollandiae* and *Pomatostomus halli* (Driskell and Christidis 2004); GenBank Accessions AY542313.1 and AF210150.1, respectively] were chosen as outgroups.

4.3.5 *Coalescent analyses of historical divergence, migration and population sizes*

The program MDIV (see Nielsen and Wakeley 2001; implemented at http://cbsuapps.tc.cornell.edu/mdiv.aspx) was used to test that 1) this species diverged into two separate populations long before the LGM, and 2) secondary contact of isolated populations occurred prior to or during the LGM. We ran three classes of hypothesis tests: 1) allopatric divergence among phylogenetic clades (haplogroup A and B), 2) allopatric divergence among northern and southern populations, 3) migration levels increased among populations after divergence during or after the LGM. MDIV estimates the joint distributions of the population parameter theta (ϑ), migration rate (M), and time since population divergence (T_{MRCA}) (e.g., see Carstens et al. 2004; Smith and Farrell 2005). We conducted several runs of MDIV using a finite sites model all with a chain length of 10 million generations with a burnin of 2.5 million generations. Starting with T_{MAX} = 5 and M_{MAX} = 10, we scaled down the prior maximum T to refine our estimates in each subsequent run, and increased M_{MAX}, where applicable.

To further test phylogenetic divergence times at several nodes of the ML phylogenetic tree, we used the expansion growth method of Drummond et al. (2005) as implemented in BEAST 1.6.0 (Drummond and Rambaut 2007). Additionally, Bayesian Skyline Plot (BSP) methods were used to estimate the timing and magnitude of population size changes for 1) entire dataset, 2) haplogroup A and 3) haplogroup B. Preliminary runs were carried out to fine-tune the parameters of the Markov Chain Monte Carlo (MCMC) analysis, using the auto-optimization option. The final analyses were performed were run for 50 million steps and sampled every 1000 steps, with the first 25% discarded as burnin. We ran both methods of analyses using a

relaxed molecular clock model, with uncorrelated rates drawn from a log-normal distribution (Drummond and Rambaut 2007). These conditions yielded large effective sample sizes (*ESS* > 200) for estimates of T_{MRCA}. TRACER 1.5 (Drummond and Rambaut 2007) was used to analyse the results.

Finally, we examined intra-population historical processes with mitochondrial mismatch distributions (Rogers and Harpending 1992; Avise 2000). The distribution of pair-wise mutational distances was fitted to a model of instantaneous population expansion by a generalized nonlinear least-square procedure as implemented in ARLEQUIN. The validity of this model was tested by a parametric bootstrap approach running 10,000 bootstrap replicates. Time of population expansion (scaled by mutation rate), population size before and population size after expansion was estimated directly from the mismatch distribution. We used Saunders et al. (1984) equation (N-1/N+1) to calculate the probability of the deepest coalescent convergence (see also Carstens et al. 2004). Additionally, Fu's FS (Fu 1997) was calculated to test for a signature of recent rapid demographic expansion. Estimates and tests for significance involving were calculated with ARLEQUIN using 10,000 sampling replicates.

For all four coalescent approaches, to translate estimates of T_{DIV} (MDIV), T_{MRCA} (multi-node divergence time analysis in BEAST), T_{MRCA} (BSP in BEAST) and t_E (mismatch analysis in ARLEQUIN) to years, we calculated a species-specific divergence rate for our fragment of control region using the divergence between clades for cytochrome *b* (cyt *b*). Three individuals from each of the two major haplogroups were sequenced for cyt *b* using primers bellbird-cytb2 (5'-GCCGGTAGAACACCCATTTA-3') and CRDR1-short (5'-GTGGCGCAAAAGAGCAAGTT-3'; modified version of CRDR1, Miller 2003) (developed in this study by SMB; these sequences will be submitted to GenBank) (Appendix B: Figure B.1). Similarly, the mean net divergence calculated for the control region (0.0138) was 2.85 times greater than for cytochrome *b* (0.00484). Assuming a 2%/Myr sequence divergence rate for cytochrome *b*, which was estimated for avian mtDNA coding region (Weir 2006), we calculated a sequence divergence rate for the control region of 5.7%/Myr. We also evaluated mutation rates of 7.6 and 30%/Myr based on estimates from the evolutionary rate of the control region domain I in finches (Baker and Marshall 1997;

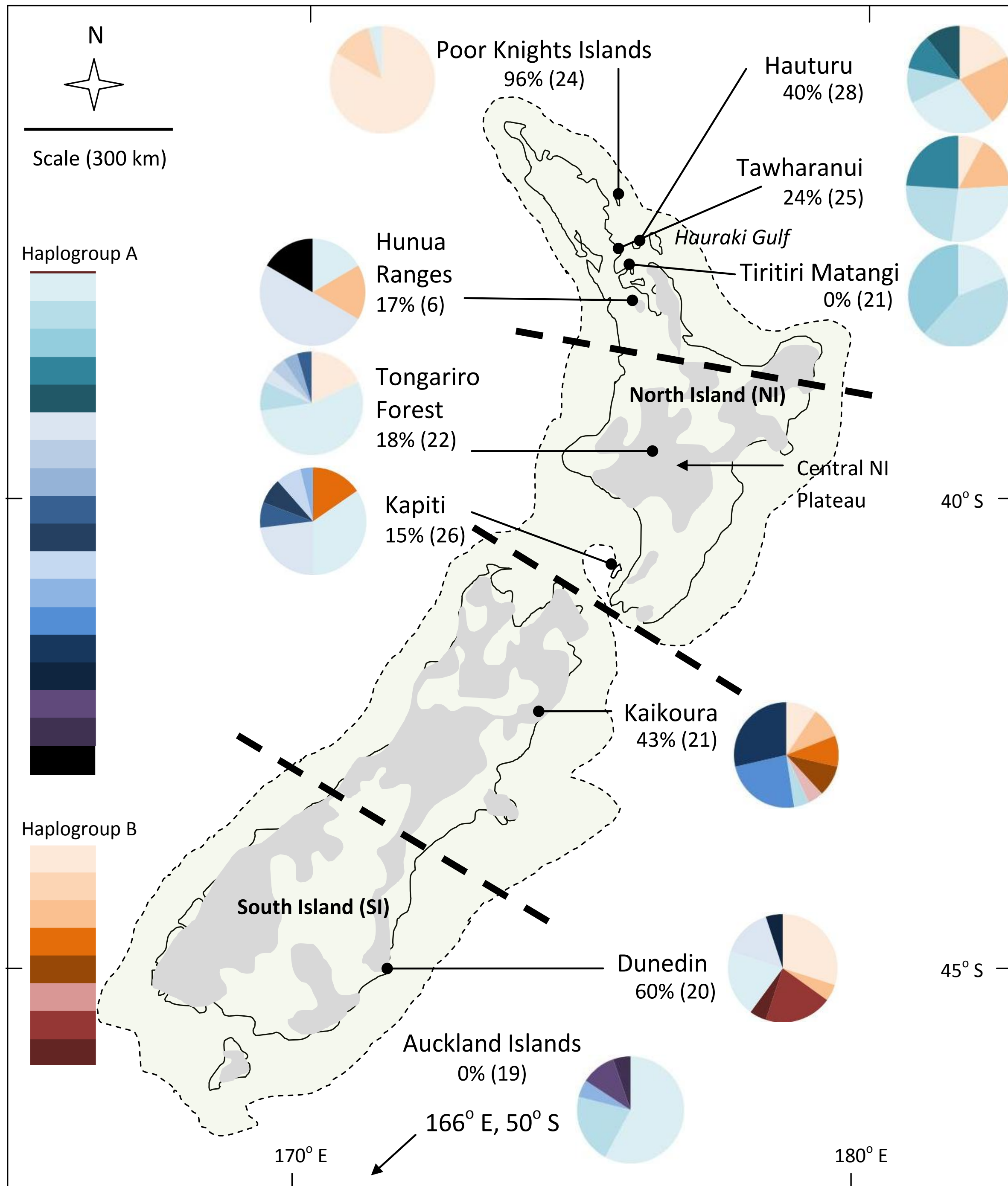

Figure 4.1 Geographical distribution of 26 *A. melanura* haplotypes. Slices of the pie represent the proportion of individuals that belong to each haplotype. Percentage of haplotype B are shown with number of individuals in parentheses. The translucent green area represents the New Zealand landmass at *c.* 22 kyr during the Last Glacial Maximum. The grey shaded areas represent the modern day distribution of *A. melanura*. Hypothetical barriers to gene flow are demarcated with thick dashed lines.

see also Bensch et al. 1999; Griswold and Baker 2002) and a higher mutation rate from other passerine taxa (Perez-Tris et al. 2004), respectively.

4.4 Results

4.4.1 *Genetic diversity and population structure*

Mitochondrial DNA analyses of 212 *A. melanura* control region sequences revealed a total of 26 haplotypes, 19 variable sites and 4 singletons with an overall haplotype diversity (*H*) of 0.87 ± 0.01 *SD* and nucleotide diversity (π) of 0.009 ± 0.0003 *SD* (Table 4.1) (Appendix B: Figure B.2) Genetic diversity was similar among sampling locations (except at the Poor Knights, Tiritiri Matangi and Auckland Islands where diversity was considerably lower) with 4-8 haplotypes, 2.7-4.9 nucleotide differences and 8-12 variable sites at each location. Microsatellite diversity reflected patterns of mitochondrial control region diversity (see Baillie et al. *in revision*; Chapter

Table 4.1 Control region mitochondrial DNA variability of *Anthornis melanura*: sample size (*N*), number of haplotypes (Hap), haplotype diversity (*H*), average number of nucleotide differences (*k*), number of segregating sites (*S*), nucleotide diversity (π) and their standard deviation (*SD*).

Locality	*N*	Hap	*k*	*H* ± *SD*	*S*	π (x10^3) ± *SD*
Poor Knights I.*	24	3	0.81	0.30 ± 0.11	8	1.86 ± 1.21
Hauturu*	28	6	4.17	0.84 ± 0.03	9	9.56 ± 0.64
Tawharanui*	25	5	3.48	0.81 ± 0.03	8	7.98 ± 1.15
Tiritiri Matangi*	21	3	1.01	0.67 ± 0.05	2	2.32 ± 0.16
Hunua Ranges*	6	4	3.60	0.80 ± 0.17	10	8.26 ± 3.93
Tongariro Forest*	22	7	2.77	0.68 ± 0.10	11	6.35 ± 1.56
Kapiti	26	7	2.74	0.82 ± 0.05	11	6.27 ± 1.24
Kaikoura	21	8	4.93	0.86 ± 0.05	12	11.31 ± 0.85
Dunedin	20	7	4.89	0.84 ± 0.05	11	10.30 ± 0.93
Auckland Islands	19	5	0.84	0.64 ± 0.11	4	1.93 ± 0.46
Haplogroup A	142	18	1.34	0.79 ± 0.03	14	3.07 ± 0.23
Haplogroup B	70	8	0.89	0.65 ± 0.05	6	2.05 ± 0.23
Total	212	26	3.95	0.87 ± 0.01	19	9.06 ± 0.32

*Diversity statistics of data re-analyzed from Baillie *et al. in revision*; see Chapter Two

Table 4.2 Pair-wise F_{ST} values for *A. melanura* among sampling locations in New Zealand for a 436bp fragment of mitochondrial DNA control region. Symbols indicate significant values: *P < 0.05, †P < 0.01, ŦP < 0.001.

	Poor Knights	Tiritiri Matangi	Tawharanui	Hauturu	Hunua	Tongariro	Kapiti	Kaikoura	Dunedin	Auckland Islands
Poor Knights										
Tiritiri Matangi	0.867Ŧ									
Tawharanui	0.569Ŧ	0.230Ŧ								
Hauturu	0.425Ŧ	0.292Ŧ	0							
Hunua	0.777Ŧ	0.255†	0.058	0.088						
Tongariro	0.695Ŧ	0.114*	0.033	0.080*	0					
Kapiti	0.713Ŧ	0.135Ŧ	0.091†	0.136*	0	0				
Kaikoura	0.417Ŧ	0.308Ŧ	0.055*	0.009	0.073	0.115	0.151Ŧ			
Dunedin	0.305Ŧ	0.444Ŧ	0.121*	0.026	0.173	0.217†	0.255Ŧ	0.006		
Auckland Islands	0.878Ŧ	0.159Ŧ	0.211Ŧ	0.276Ŧ	0.186†	0.071	0.063	0.307Ŧ	0.438Ŧ	

Two). Pair-wise F_{ST} comparisons between populations revealed that mainland populations within both the North and the South Island are genetically similar (non-significant F_{ST} values; (Table 4.2). Among the hierarchical groupings for the AMOVA, Φ_{CT} was maximized significantly (mitochondrial DNA: 0.402, P = 0.048; microsatellite DNA: 0.099, P = 0.03) when populations were divided into North and South Island compared to when all populations were combined for analysis (Po and Au used as controls) (Table 4.3). For mitochondrial DNA, higher differentiation was evident between the north and south groups (40%) than among populations within groups (1.8%), however, most variation was within populations (53-58%) (Table 4.3a). This pattern was reflected in microsatellite DNA except that there was less differentiation among groups (9-13%) and among populations within groups (2-3%) (Table 4.3b). These results indicate a possible

Table 4.3 AMOVA results for a) mitochondrial DNA using haplotype frequency differences and sequence divergence (ϕ_{ST}) estimates (ϕ_{CT}, differentiation among groups; ϕ_{SC}, differentiation among populations within groups) and b) eight microsatellite markers using F_{ST}. The groups are as follows: north (Hu, To), south (Kk, Du); Po and Au are used as controls. Asterisks mark significant *P*-values to α = 0.05. Abbreviations for sites can be found in Figure 4.3. Groupings represent the following hypothetical geographical barriers to dispersal: 1. central South Island boundary (location of the Biotic Gap), 2. northern North Island boundary, 3. Cook Strait boundary and 4. no boundary (also see Figure 4.1). The strongest (highest significance) F_{CT} results are demarked in bold font.

a)					Percentage variation		
	ϕ_{CT}	*P*	ϕ_{SC}	*P*	Among groups	Among populations within groups	Within populations
1. (Po)(north+Kk)(south)(Au)	0.328	0.2	0.140	0.05*	32.80%	9.39%	57.81%
2. (Po)(north)(south+To)(Au)	0.331	0.1	0.171	0.01*	33.12%	11.45%	55.43%
3. (Po)(north)(south)(Au)	**0.402**	**0.05***	**0.030**	**0.3**	**40.22%**	**1.80%**	**57.98%**
4. (Po)(north+south)(Au)	0.357	0.1	0.169	0.01*	35.73%	10.85%	53.42%

b)					Percentage variation		
	F_{CT}	*P*	F_{SC}	*P*	Among groups	Among populations within groups	Within populations
1. (Po)(north+Kk)(south)(Au)	0.096	0.5	0.029	< 0.001*	9.62%	2.59%	87.79%
2. (Po)(north)(south+To)(Au)	0.124	0.05*	0.025	< 0.001*	12.37%	2.20%	85.43%
3. (Po)(north)(south)(Au)	**0.099**	**0.03***	**0.027**	**< 0.001***	**9.95%**	**2.46%**	**87.60%**
4. (Po)(north+south)(Au)	0.127	0.07	0.033	< 0.001*	12.72%	2.84%	84.44%

historical barrier between northern and southern populations aligned with the modern day Cook Strait (see Figure 4.1).

The *A. melanura* haplotype parsimony network was characterized by a dumbbell pattern with haplotypes of the opposing ‘bells’ (haplogroups) separated by a mean of 7.4 (4-12) mutational steps (Figure 4.2). Haplogroup A and B comprised 67% (142/212) and 33% (70/212) of haplotypes in the dataset, respectively (Figure 4.2; haplogroup A and B shown in the upper and lower part of the network, respectively). No geographic structure was evident as both haplogroups were represented at all locations with the exception of Tiritiri Matangi and the

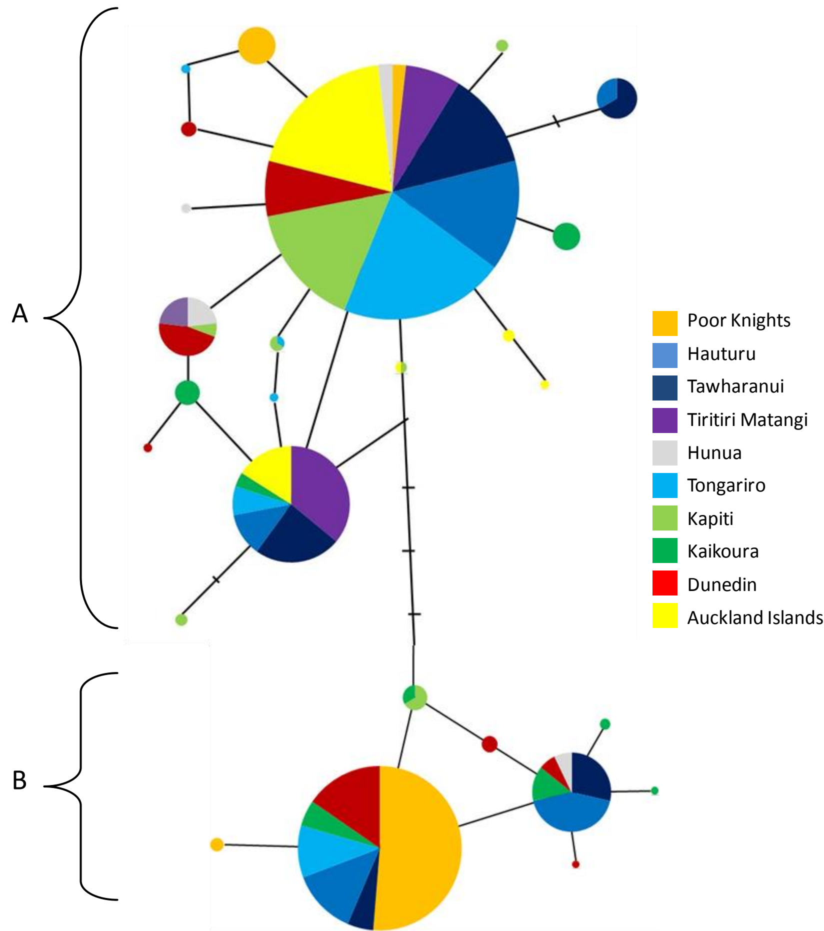

Figure 4.2 Parsimony network of mitochondrial control region haplotypes from 10 different *A. melanura* populations showing two distinct haplogroups, A and B. Diameters of circles are proportional to the frequency of the haplotype. Hash marks and circles represent unknown and known mutational steps separating haplotypes, respectively. All individuals connected within ≥ 95% confidence level equivalent to eight mutational steps.

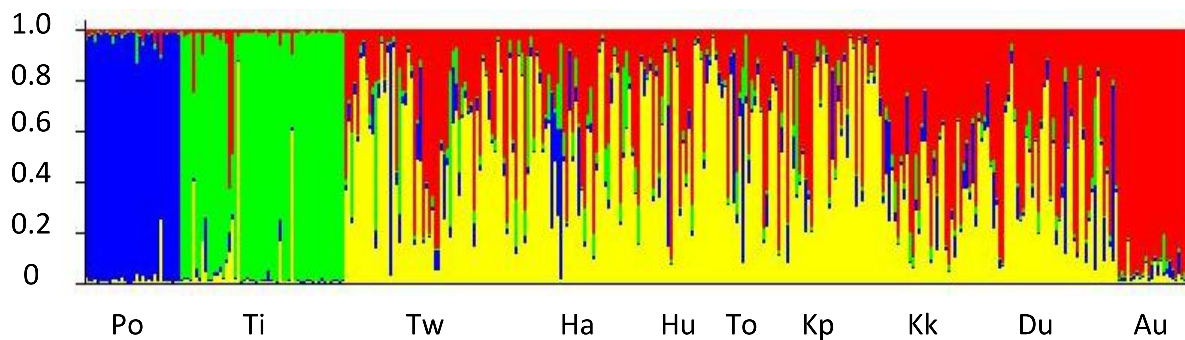

Figure 4.3 *A. melanura* populations from throughout their geographical range cluster into four populations (K = 4) as implemented in STRUCTURE using eight microsatellite markers. Sampling locations are abbreviated as follows: Poor Knights, Po; Tiritiri Matangi, Ti; Tawharanui, Tw; Hauturu, Ha; Hunua, Hu; Tongariro, To; Kapiti, Kp; Kaikoura, Kk; Dunedin, Du; Auckland Islands, Au.

Auckland Islands (Figure 4.2). All Auckland Island and Tiritiri Matangi individuals belonged to haplogroup A, whereas most of the Poor Knights samples belong to haplogroup B. Geographic distribution of the 26 *A. melanura* haplotypes revealed that haplogroup A occurred in higher frequency on the North Island (82-83%) than B, and haplogroup B dominated the South Island (43-60%) (see Figure 4.1). STRUCTURE analysis revealed high levels of admixture among the microsatellite markers of the 10 populations (Figure 4.3).

4.4.2 *Phylogenetic analyses*

Similar to the haplotype network, the phylogenetic tree supported the placement of haplotypes in two well defined clades (haplogroups A and B; Figure 4.4). Branch lengths dividing clades were long and this is reflected in the high Bayesian posterior probabilities (0.99) and 91% ML bootstrap support that B is different from A and any ancestor. This finding indicates that populations indeed became genetically separated at some point in the past. The mean sequence divergence between the two clades A and B (counting each haplotype once) was 1.9%. The mean mitochondrial control region sequence divergences between *P. halli* and the two *A. melanura* clades A and B were 42.6 and 41.3%, respectively. Similarly, mean sequence divergences between *M. novaehollandiae* and clades A and B were 53.7% and 53.2%, respectively. The mean sequence divergence between *P. halli* and *M. novaehollandiae* was 43.6%. Taken together, both outgroups were similarly distant to haplogroup A and B. Within each haplogroup, there were instances of high bootstrap support, but most haplotypes differed from one another by only a small number of base pair changes (Figure 4.4). The mean divergence time for each of the three major nodes (see Figure 4.4) as implemented in BEAST indicated a common ancestor for both A and B haplogroups that was no more ancient than the mid-Pleistocene: Node 1 *c.* 199 kyr (95% credible interval 82-318 kyr; *ESS* = 403); Node 2 *c.* 98 kyr (95% credible interval 41-170 kyr; *ESS* = 386); Node 3 *c.* 87 kyr (95% credible interval 20-176 kyr; *ESS* = 658) with the species-specific mutation rate of 0.057 (the slowest of three evolutionary rates used in this study).

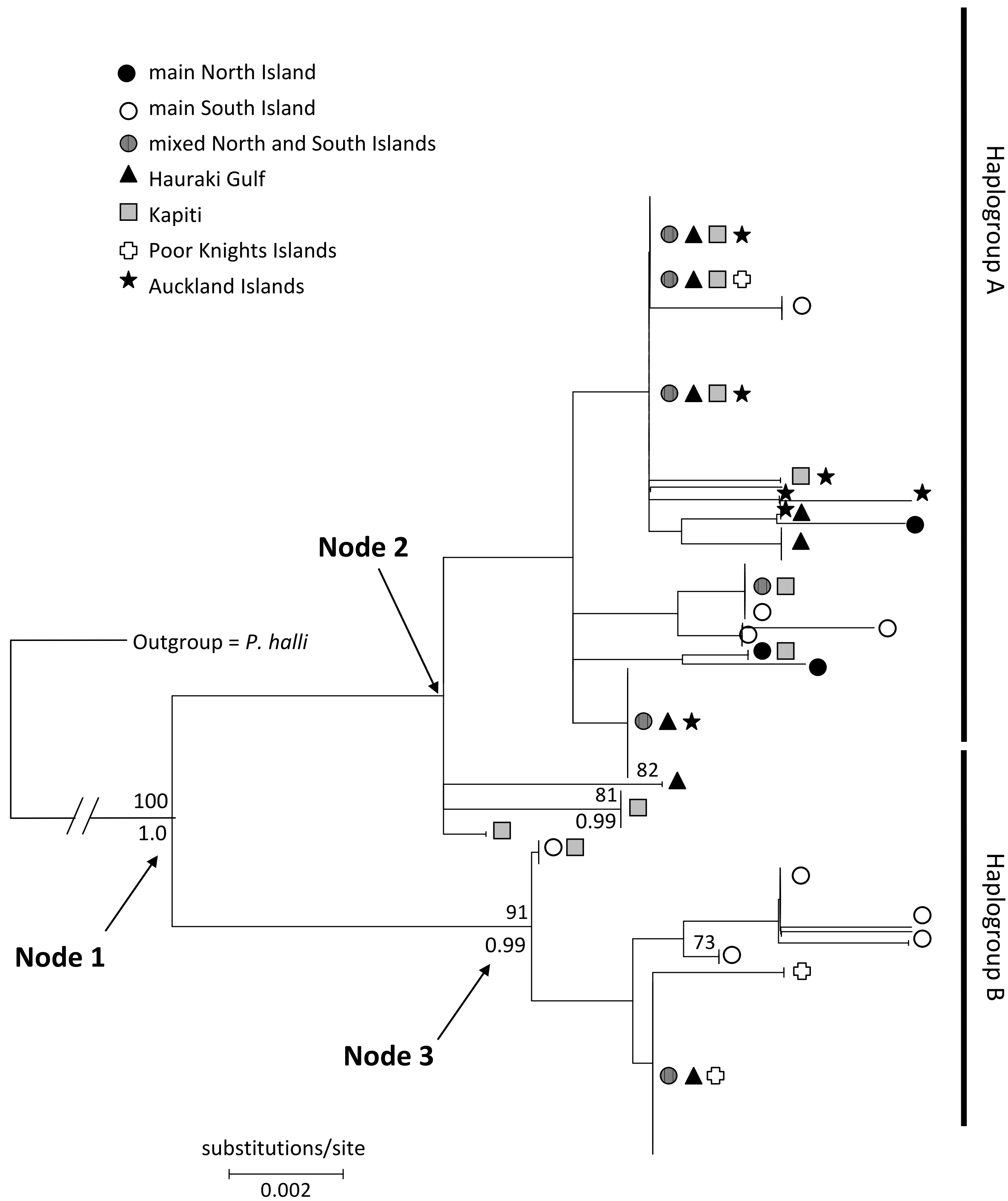

Figure 4.4 Maximum likelihood (ML) phylogenetic tree for *A. melanura* mitochondrial control region haplotypes showing ML bootstrap scores and MRBAYES posterior probabilities above and below the horizontal branches, respectively. Only nodes with either bootstrap scores and/or posterior probabilities greater than 70% and 0.7, respectively, are shown. Symbols at terminal branches represent geographic region in which each haplotype was present.

Table 4.4 Demographic parameters (ϑ, population size estimator; M, migration rate; T_{MRCA}, time since most recent ancestor; μ, mutation rate) and 95% credible intervals from posterior probability distribution as implemented in MDIV. Populations included in the analysis are North Island (Hunua and Tongariro) and South Island (Kaikoura and Dunedin). Three different evolutionary mutation rates were used. From left to right: an *A. melanura*-specific rate (this study); from finches (Baker and Marshall 1997); a higher mutation rate from other passerine taxa (Perez-Tris et al. 2004).

				Divergence time (kyr)		
Analysis	ϑ	M	T_{MRCA}	μ=0.057	μ=0.076	μ=0.3
Haplotype A vs. B: entire dataset	2.8 (2.6-2.9)	0.02 (0-0.06)	3.36	191 (175-199)	143 (131-149)	36 (33-38)
North Island vs. South Island: entire dataset	2.6 (2.4-2.8)	1.7 (1.4-1.9)	3.18	168 (152-176)	126 (59-170)	32 (29-33)
North Island vs. South Island: Haplogroup A only	1.4 (0.6-1.6)	1.0 (1.0-1.0)	2.18	62 (27-70)	47 (20-52)	12 (5-13)
North Island vs. South Island: Haplogroup B only	1.0 (0.8-1.1)	28.7 (16.2-29.9)	2.43	49 (40-52)	37 (31-39)	9 (8-10)

4.4.3 *Coalescent analyses of historical divergence, migration and population sizes*

Isolation-with-migration (IM) models in MDIV support our hypothesis that *A. melanura* separated into two populations at a time well before the LGM (Table 4.4). Time to most recent common ancestor between haplogroup A and B was estimated to be 191 kyr (95% credible interval 175-199 kyr) and 143 kyr (95% credible interval 131-149 Myr) using mutation rates of 0.057 and 0.076, respectively. For all three mutation rates MDIV placed population divergence time within the Pleistocene and before the LGM (see Table 4.4). Furthermore, estimated levels of migration between the haplogroups was low and not significantly different from 0 (M = 0.02 individuals per generation; 95% credible interval 0-0.06) at the time of population separation.

The result was similar when the analysis was run with North Island versus South Island instead of by phylogenetic clade (haplogroup A versus B). A second class of North Island versus South Island MDIV analyses were run for each haplogroup separately to assess the timing and levels of migration between North and South Islands that may be associated with secondary contact (Table 4.4; Figure 4.5). For haplotype A. we found that at

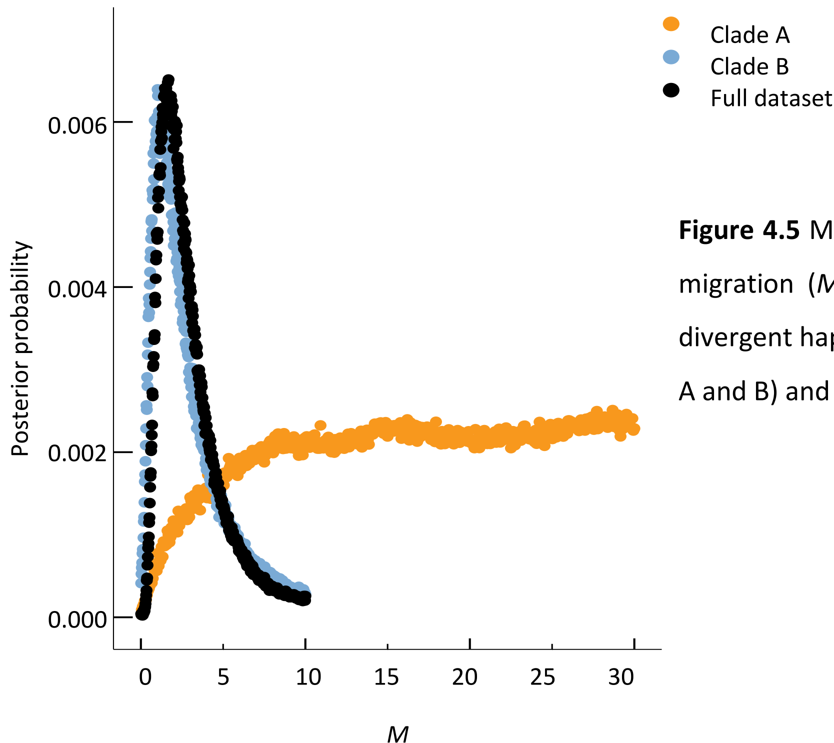

Figure 4.5 Marginal distributions for IM migration (*M*) results for each of two divergent haplogroups separately (clade A and B) and the full dataset.

c. 62 kyr (95% credible interval 27-70 kyr) migration levels were very low (*M* = 1.0), whereas haplotype B had extremely high migration rates *M* = 29 at *c*. 49 kyr (95% credible interval 40-52 kyr) (Figure 4.5). The estimator of effective population size (ϑ) for haplogroup A was 50% larger than haplogroup B (Table 4.4).

The mismatch distribution of control region mitochondrial DNA haplotypes revealed a distinct bi-model pattern (Figure 4.6a; Table 4.5). The bi-modal distribution revealed a mean of 7.4 pair-wise differences between populations and 0.9-1.3 within populations (Table 4.5). Using the 5.7%/Myr mutation rate, the mismatch expansion time (τ) and population size parameters (ϑ_0 and ϑ_1) indicated that a sudden population expansion should have occurred *c.* 169 kyr (95% confidence interval 12–256 kyr) (see Table 4.5). When mismatch distributions

were analyzed separately for haplogroup A (Figure 4.6b), the significant raggedness index implied a stable or large population. Results for haplogroup B (Figure 4.6c), on the other hand, were consistent with sudden demographic expansion (Table 4.5). Significant Fu's F_S indicated that all populations, as well as haplogroups analysed separately, have undergone recent rapid population expansion (Table 4.5).

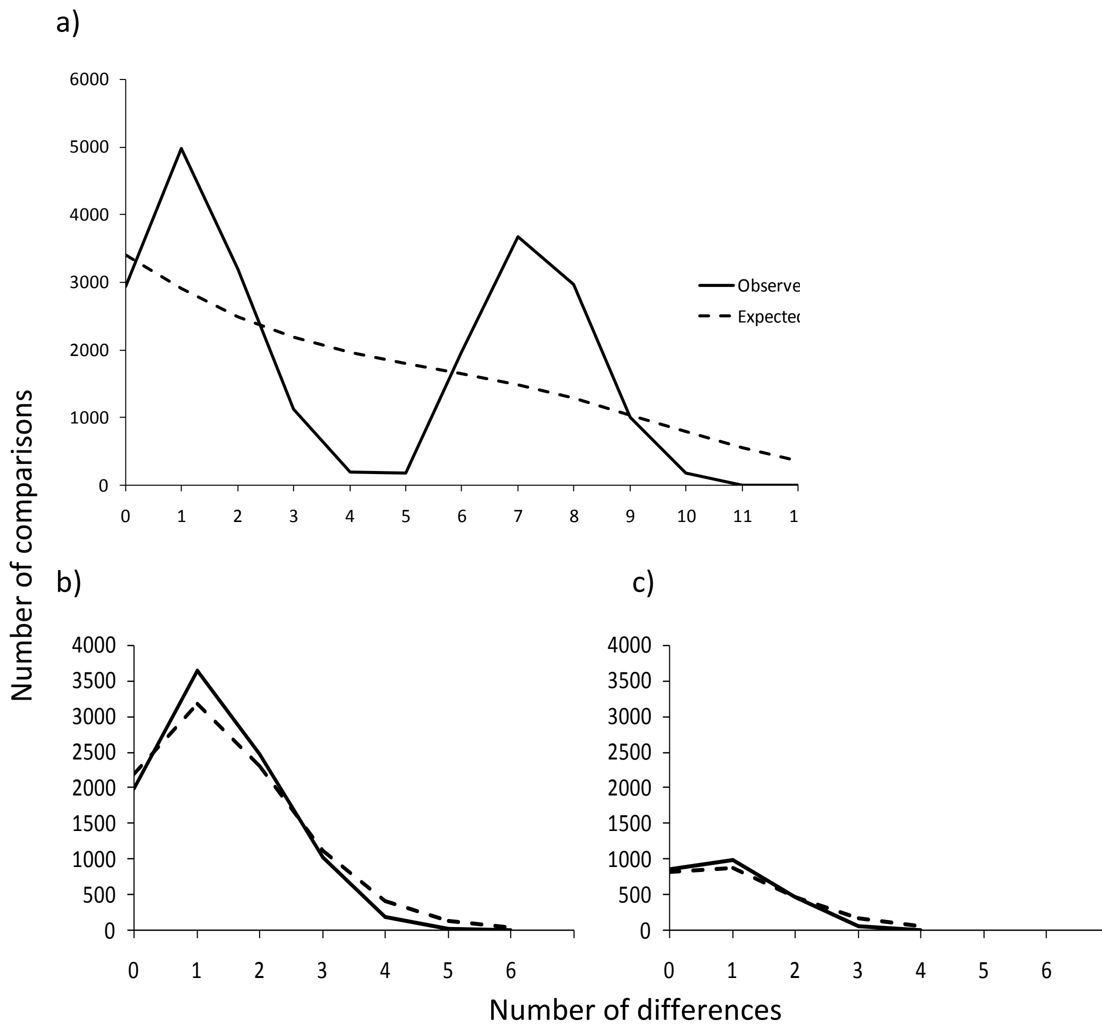

Figure 4.6 Mismatch distribution showing the observed (solid line) and expected (dashed line) frequencies for number of pair-wise differences in mitochondrial control region sequences for the a) full dataset, b) haplogroup A and c) haplogroup B. The expected frequency distribution is based on the assumption of sudden population expansion.

Table 4.5 (Next page) Probability values and results of hypotheses tests of recent demographic expansion for *A. melanura* using mismatch analysis in ARLEQUIN. Probability of Fu's *F*s test [*P*(*F*s)], that samples from each location capturing the deepest coalescent event (*Prob*), raggedness index [*P*(*Ragg*)], sum squared deviation of goodness-of-fit test [*P*(*SSD*), mean pair-wise differences (*k*). Calculations for time since expansion (t_E), and effective female population size before ($N_{F(e)0}$) and after ($N_{F(e)1}$) expansion were performed using mismatch estimates of τ, ϑ_0 and ϑ_1 and the species-specific 5.7%/Myr rate of evolutionary change.

Sampling location	*P*(*F*s)	*Prob*	*P*(*Ragg*)	*P*(*SSD*)	*k*	$\tau=2\mu t_E$	$\vartheta_0=2\mu N_{F(e)0}$	$\vartheta_1=2\mu N_{F(e)1}$	t_E= (kyr)	$N_{F(e)0} \times 10^3$	$N_{F(e)1} \times 10^3$
Poor Knights Islands	< 0.001	0.92	0.6	0.4	0.81	3.0	0	0.40	60.4 (7-70)	0 (0-0.1)	8.0 (0-∞)
Hauturu	< 0.001	0.93	0.4	0.1	4.17	8.7	0.002	6.89	175.0 (3-304)	0.04 (0-79)	108.5 (93-∞)
Tawharanui	< 0.001	0.92	0.6	0.3	3.48	8.5	0	4.95	171.0 (0.7-1,237)	0 (0-72)	99.6 (70-∞)
Tiritiri Matangi	< 0.001	0.91	0.5	0.2	1.01	1.5	0	7.32	30.2 (5-53)	0 (0-5)	147.2 (46-∞)
Hunua Ranges	< 0.001	0.71	0.6	0.1	3.60	1.5	0.005	13.27	30.2 (9-63)	0.1 (0-0.4)	267.0 (43-∞)
Tongariro Forest	< 0.001	0.91	0.6	0.4	2.77	8.4	0.002	2.22	169.0 (3-1,275)	0.04 (0-33)	44.7 (11-∞)
Kapiti	< 0.001	0.95	0.5	0.4	2.74	7.9	0.002	3.52	158.9 (8-1,226)	0.04 (0-73)	70.8 (30-∞)
Kaikoura	< 0.001	0.91	0.5	0.3	4.93	9.6	0	9.39	193.1 (17-336)	0 (0-100)	188.9 (119-∞)
Dunedin	< 0.001	0.90	0.1	0.1	4.49	9.0	0	7.65	181.1 (15-308)	0 (0-0.3)	154.0 (90-∞)
Auckland Islands	< 0.001	0.90	0.4	0.4	0.84	1.0	0	∞	20.1 (9-35)	0 (0-0.24)	∞ (118-∞)
Haplogroup A	< 0.001	0.99	0.03	0.1	1.34	1.4	0.004	∞	28.2 (23-36)	0.07 (0-1)	∞ (173-∞)
Haplogroup B	< 0.001	0.97	0.1	0.2	0.88	1.0	0	∞	20.1 (13-27)	0 (0-0.5)	∞ (284-∞)
All locations pooled	< 0.001	0.99	0.4	0.2	7.4 (1.2-8.6)	8.4	0.002	5.55	169.0 (12-256)	0.04 (0-37)	111.7 (83-2,012)

The Bayesian skyline plot (BSP) analyses dated genetic separation to T_{MRCA} = 238 kyr (95% credible interval 76-463 kyr; *ESS* = 446) (Figure 4.7). Initially stable population sizes of ~1 million declined dramatically at *c.* 200 kyr (after the main split into haplogroups A and B) until the LGM (*c.* 22 kyr) after which populations sharply increased (Figure 4.7). When haplogroups A and B were analysed separately, the Bayesian skyline shows a population size of ~250,000 individuals at the LGM that doubles after the LGM between *c.* 20-5 kyr (data not shown).

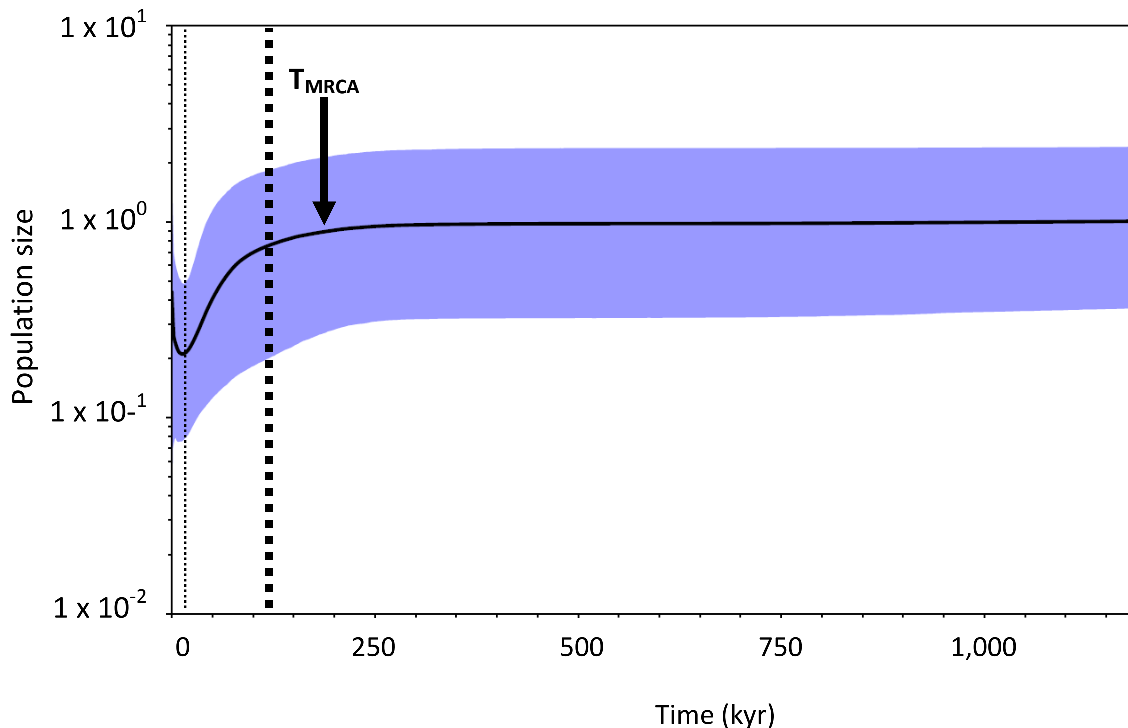

Figure 4.7 Bayesian skyline plot of *A. melanura* historical population size changes. Population size (y-axis) as the product of effective population size per generation length (N_E). The arrow shows the T_{MRCA} for the ancient divergence of *A. melanura* into haplogroups A and B to be *c.* 238 kyr according to the BSP method. The thick and thin dashed line shows the time of the warmest mid-Pleistocene interglacial *c.* 120 kyr and the Last Glacial Maximum *c.* 22 kyr, respectively.

4.5 Discussion

Our data provides evidence that two distinct lineages of *A. melanura*, a northern and a southern, diverged during the Pleistocene (see also Murphy et al. 2006; Hill et al. 2009; Marshall et al. 2009). Our results suggest that mid-Pleistocene events < *c.* 318 kyr (upper 95% credible limit for T_{MRCA}) played an integral role in structuring genetic variation for vagile forest passerines in New Zealand. A ubiquitous problem common in phylogenetics studies concerns the misinterpretation of phylogenetic trees due to lineage sorting, extinction of lineages and introgressive hybridization due to secondary contact (Zachos 2009). To address these potential problems, we outline counter measures to these problems and provide corroborative evidence from other taxa, geological and climatic data to support our findings (Avise 2000).

4.5.1 *Allopatric population separation hypothesis*

In order to accept a hypothesis of allopatric separation, reciprocal monophyly with two supported clades must be confirmed (Carling et al. 2010). Our data reveal a genetically distinct well supported clade (haplogroup B) within *A. melanura*. Thus, we cannot reject our hypothesis of reciprocal monophyly. Average nucleotide divergence between *A. melanura* clades was 2%, which is relatively high compared to other passerine studies (Bensch and Hasselquist 1999). Furthermore, the mismatch distribution was strongly bimodal and indicative of allopatric separation (Avise 2000). Precise dating of this kind of separation event is not possible using molecular genetic data alone (see Zachos 2009). However, three widely different evolutionary rates, 0.057 (this study), 0.076 (Baker and Marshall 1997) and 0.3, placed the divergence of haplogroup A and B before the LGM. The BEAST analyses dated genetic separation to 199 kyr (95% credible interval 82-318 kyr) indicating that population separation, or isolation of haplogroup B, predated the LGM. This broad timing of separation was corroborated using the IM method coalescent analyses in MDIV revealing $32 < T_{DIV} < 168$ kyr using three widely different

evolutionary rates. Using the species-specific rate, the T_{MRCA} date closely corresponds with a well-known major warm interglacial period around *c.* 250 kyr (Petit et al. 1999; Carter and Gammon 2004; Ritchie et al. 2004) (Figure 4.8). Additionally, according to our BEAST analysis both haplogroups A and B further divided between *c*. 87-98 kyr (95% credible interval 20-176 kyr) corresponding to the subsequent interglacial period of *c.* 120 kyr (Figure 4.8).

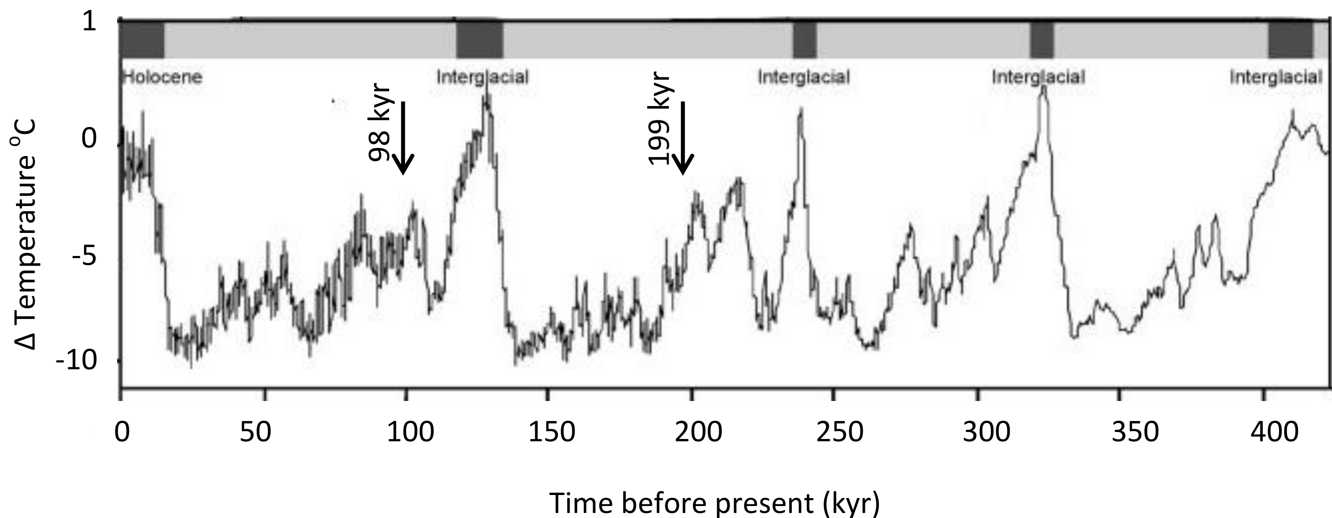

Figure 4.8 Timeline of glacial cycling between *c.* 420 kyr and the present in the South Hemisphere, based on the Vostok Ice Sheet, Antarctica (Petit et al. 1999). Warm interglacial periods that correspond to periods of sea level rise in coastal New Zealand (Carter and Gammon 2004) are denoted by dark shaded bars above the temperature profile. Arrows indicate the mean T_{MRCA} as implemented in BEAST for the origin of haplogroups A and B (right) and a second period of divergence within haplogroup B (left).

The AMOVA analyses identified the presence of an historical barrier to *A. melanura* gene flow at approximately the location of the current day Cook Strait. Haplogroup A is more diverse and prominent on the North Island. The less diverse haplogroup B is more common in the southern South Island. Taken together, haplogroup A appears to be a North Island clade and haplogroup B likely originated on the South Island. As expected under the allopatric divergence hypotheses (Nielsen and Wakeley 2001), we found that migration between clades

was not significantly different from zero. However, when the analysis was repeated based on island 'populations' we could not rule out that low levels of gene flow may have occurred between the North and South Island at the time of ancient divergence.

During the glacial cycles of the Pleistocene, the loss of forest habitat was more pronounced on the South Island (Campbell and Hutching 2007). New Zealand fungus beetle lineages obligatory to the South Island beech *Northofagus* forests were found to coalesce at the same time as *A. melanura* lineages, *c.* 200 kyr (Leschen et al. 2008). Beetle population divergence is thought to be consistent with extirpation during a recent glaciation in a phenomenon known as the formation of the Biotic Gap (Leschen et al. 2008; Hill et al. 2009). Other South Island fauna populations were affected by Pleistocene cold periods, e.g., Otago skink (Berry and Gleeson 2005). In fact, the estimated divergence of *A. melanura* haplogroups B corresponds with that of short-tailed bats at *c.* 100 kyr (Lloyd 2003). For these more vagile species migration at low latitudes is thought to have been facilitated during colder periods (Lloyd 2003). The temporal cadence of the two major *A. melanura* divergence events suggests that gene flow was inhibited by reduction in forest availability during cold glacial maxima and/or by the infilling of the Cook Strait during warm interglacials preceding those maxima.

4.5.2 *Post-divergence population expansion and secondary contact hypothesis*

Under the secondary contact hypothesis populations are expected to be admixed and estimates of migration should be higher than at the time of ancient divergence. We provide strong evidence for genetic admixture in *A. melanura* in the present and a previous study (Baillie et al. *in revision*; Chapter Two). Bayesian clustering analyses demonstrate that all mainland populations, as well as Hauturu and Kapiti islands, belong to one population. Furthermore, the two divergent mitochondrial DNA haplogroups are co-distributed throughout the country today. Estimated migration levels from IM methods show significantly higher levels of migration of haplogroup B at *c.* 49 kyr than at *c.* 168 kyr. Thus, secondary contact associated with population expansions and higher migration levels occurred sometime between a maximum of *c.* 70 kyr (upper 95% credible interval MDIV) and *c.* 27 kyr (lower 95% credible

interval MDIV). This diaspora is likely to have led to the presence of haplogroup B in the modern Poor Knights population.

Migration estimates for haplogroup A, on the other hand, remained low. We know from mismatch analyses that expansion and population increases of haplogroup A may be more recent than IM methods can accurately detect (Carstens et al. 2004). The northern North Island did not undergo periods of extensive deforestation during the Pleistocene Epoch (see Wallis and Trewick 2009) and *A. melanura* population sizes would have been very large. The mismatch raggedness index for haplogroup A indicates that northern populations were stable for long period of time. On the South Island, however, climate oscillations caused extensive fluctuations in forest cover (McGlone et al. 2001; see Leschen et al. 2008). The climate slowly improved after the LGM possibly allowing the more numerous haplogroup A to spread southward ultimately leading to the colonization of the Sub-Antarctic islands. Bayesian skyline plots support such a post-LGM population expansion and also revealed a constant population size throughout much of the Pleistocene followed by a decline in numbers after the T_{MRCA} until the LGM. After the LGM population size doubled but never again reached former large sizes. Seventy percent of the New Zealand forest cover has been removed over the past 1,000 years (Alloway et al. 2007; Ewers et al. 2006), thus contemporary population sizes should be much smaller than in the past. Furthermore, some long branches in the phylogenetic tree indicate that *A. melanura* began to diversify at *c.* 20 kyr, possibly signaling population expansion and radiation into newly available habitats (Avise 2000). Goldberg et al. (2011) also found that the kereru *Hemiphaga novaeseelandiae* experienced population declines throughout the Pleistocene followed by post-LGM population expansions.

4.5.3 *Post-glacial colonization of the Sub-Antarctic Islands and evolutionary rate calibration*

The exact timing of biogeographical events cannot be derived from mitochondrial DNA data alone, though calibration of genetic data with fossils or known geological events can aid phylogeographic inference (Avise 2000; Zachos 2009). In this study, our most reliable time

benchmark was the colonization of the Sub-Antarctic Islands, which is unlikely to have occurred prior to *c.* 22 kyr because the islands were completely covered with glacier and devoid of forest at that time (Campbell and Hutching 2007). Our species-specific mitochondrial control region evolutionary rate is the slowest possible rate to place Sub-Antarctic colonization after the LGM. Moreover, this rate of 5.7% falls with the realm of estimates recently used for avian control region studies range from 5% (Freeland and Boag 1999) to 9.7% (Peters et al. 2005). The fastest rate we used (30%) is not convincing because it would place rapid expansion and high migration too close to the present day when it is more likely that any great expansions occurred before the North-South Island land bridge disappeared > *c.* 5,000 year. Furthermore, the anthropogenic-based deforestation of the past 1,000 years is far greater than by any glacial activity in the past (Alloway et al. 2007).

4.5.4 *Discordance between gene trees and species trees and other issues*

Mitochondrial DNA may not recover a species phylogeny because of incomplete lineage sorting and introgression. However, even in cases with widespread incomplete lineage sorting, a significant historical signal persists (Maddison and Knowles 2006). Moreover, the fact that the signal from earlier extinctions (and colonizations) is obscured by later events should not prevent us from seeking evidence for them (Grant et al. 2004; Goldberg et al. 2008). Two principal conclusions of Maddison and Knowles (2006) were that 1) sufficient signal to reconstruct trees remains even in the face of considerable incomplete lineage sorting and 2) the optimal strategy (multiple loci versus multiple individuals) depends on depth of divergence time. In our dataset, divergence is relatively recent (on the order of hundreds of thousands of years as opposed to millions of years) and we sampled a large number of individuals, thus we can be confident that our phylogeny is reasonably robust. Additionally, we calculated the probability of the deepest coalescent convergence of the full, haplotype A and haplotype B datasets to be 0.99, 0.99 and 0.97, respectively (see Table 4.5). The Hunua Ranges dataset, on the other hand, has a low probability (0.71) and the population estimates from that coalescence are not reliable.

With populations there is a higher risk that shared haplotypes might reflect gene flow and hybridization rather than incomplete lineage sorting (Maddison and Knowles 2006). Buckley et al. (2006) used computer simulations to distinguish between hypotheses of lineage sorting and introgressive hybridization. They conclude that observed discordance among multiple mitochondrial loci was more likely due to ancient introgression rather than lineage sorting (Buckley et al. 2006). Miller and Lambert (2006) also indicate that lineage sorting or introgressive hybridization may have occurred in another New Zealand passerine, *Petroica* spp. The Stewart Island *Petroica traversi* grouped strongly with the North Island *P. macrocephala* instead of the South Island *P. australis* (Miller and Lambert 2006), which is geographically closer. We observed the same phenomenon with *A. melanura*, where the Auckland Island birds are largely characterized by the most common North Island haplotypes. We suggest that our results may reflect real biogeographical processes involving dispersal and introgressive hybridization through low altitude forests during and after the LGM. The sheer number of northern haplotypes (where the climate was consistently better) would have outweighed the South Island haplotype numbers and probability may have dictated that Sub-Antarctic colonization was mostly by North Island haplotypes. A majority-rule consensus approach among phylogenetic patterns using multiple mitochondrial and possibly sex-linked loci, however, would provide a further test of hypotheses on lineage sorting or introgression (Buckley et al. 2006; Carling et al. 2010).

4.5.5 *Conclusions*

In summary, our results are consistent with a scenario of repeated periods of allopatry followed by secondary contact during the Pleistocene Epoch. Importantly, although *A. melanura* do fly over open water, such features can pose barriers to or inhibit gene flow, especially when population sizes are low. We suggest that forest habitat availability and the relatively higher dispersal ability of *A. melanura* have played important roles in allowing sufficient post-divergence immigration to retard genetic divergence and prevent speciation. We present a

biogeographical model of *A. melanura* (Figure 4.9) showing that an ancestral population separated by allopatric fragmentation possibly due to sea level rise *c.* 250 kyr, which was followed by another cold maximum (Petit et al. 1999; Carter and Gammon 2004). Then *c.* 70-27 kyr population expansion and repeated appearance of a land bridge between the North and

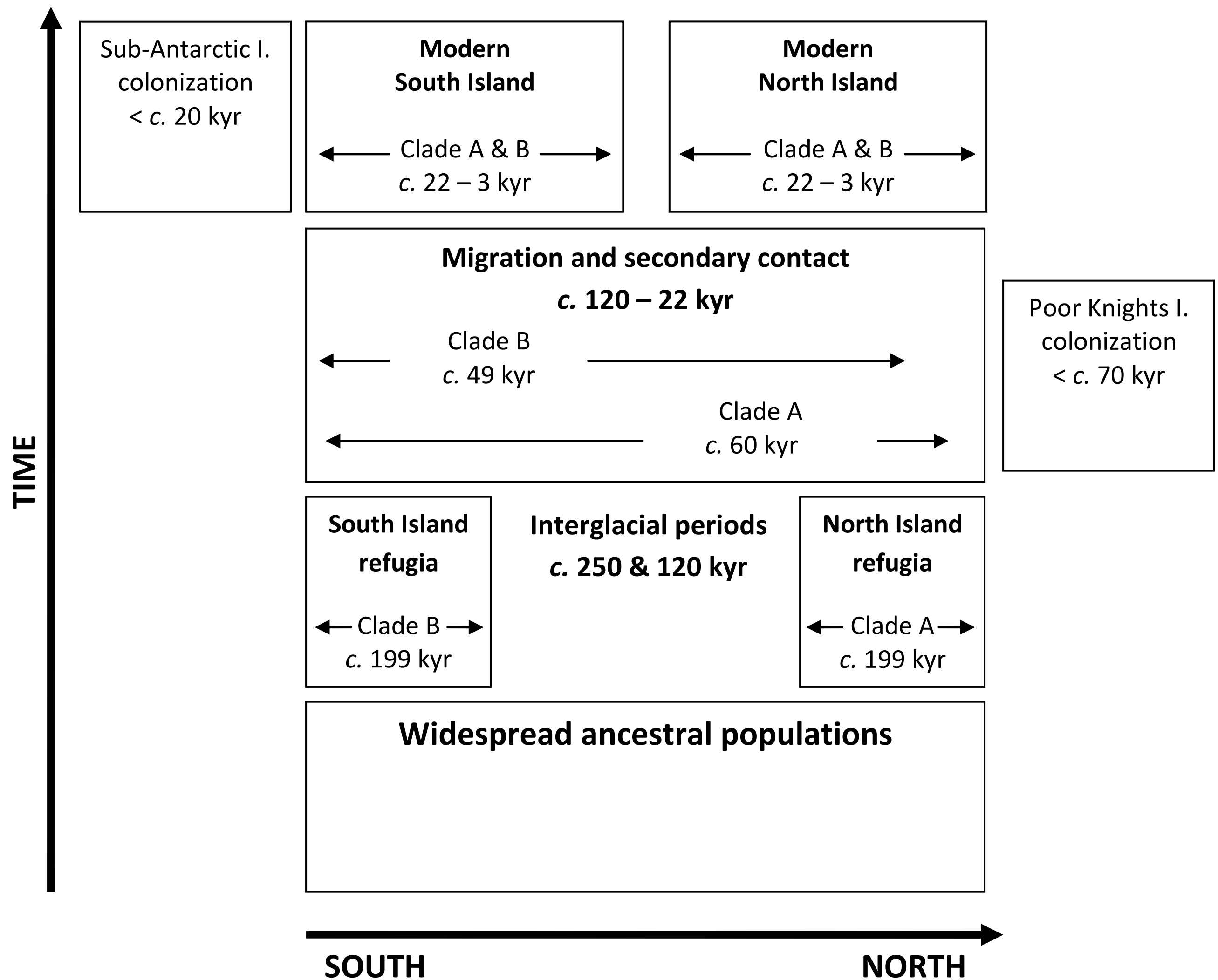

Figure 4.9 Biogeographic reconstruction of *A. melanura* (*c.* 2.9 million years to present). Dates for the oldest genetic separation are based on BEAST T_{MRCA} dating, secondary contact based on IM population separation dating and timing of post-glacial population expansions are based on t_E from mismatch distributions.

South Islands allowed post-divergence secondary contact between the two haplogroups. Colonization of the current Poor Knights Islands population happened no earlier than *c.* 87 kyr. After the LGM (< *c.* 22 kyr) population expansion continued again, leading to the colonization of the Sub-Antarctic islands.

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CHAPTER FIVE

Diversity, distribution and biogeographical origins of *Plasmodium* parasites from the New Zealand bellbird (*Anthornis melanura*)

5.1 Abstract

Knowledge of the biogeographical distribution of a parasite can give insight on whether a parasite is a specialist or a generalist able to complete its life cycle in only a few or many host species, respectively. The distribution of blood parasites, however, can often be underestimated due to low sampling effort. Despite such limitations, phylogenetic approaches that examine sequence divergence among parasite lineages in different parts of the globe can help identity and infer temporal relationships between a parasite and its host. Furthermore, heterogeneity within a host-parasite distribution can be informative about the specificity inherent in a host-parasite-vector system. In this study we examine host-parasite relationships between a single host species, the New Zealand bellbird (*Anthornis melanura*), and its haematozoan blood parasites. Our three main aims are to assess 1) the diversity of *Plasmodium* and *Haemoproteus* parasites infecting a highly mobile passerine endemic to New Zealand, 2) the distribution of these parasites relative to that of their host and 3) the worldwide distribution and phylogenetic relationships of bellbird parasite lineages. Four *Plasmodium*, and no *Haemoproteus*, lineages were identified among the 693 bellbird samples screened by nested PCR. Though confined to the Hauraki Gulf, LIN1 was the most common lineage (11%, *N* = 74/693) and is genetically similar to but not identical to a *Plasmodium* (subgenus *Novyella*) sp. (4.9% sequence divergence) found only in Australian honeyeaters. LIN2 (2%, N = 13/693) on the other hand, is an exact match with a global cosmopolitan (*P. elongatum* GRW06) present in

bellbirds throughout the country (1-4% prevalence). The much less abundant LIN3 and LIN4 parasite sequences closely matched (< 1% sequence divergence) previously deposited GenBank sequences of *P.* (*Novyella*) sp. and *P. relictum* collected from passerines in Africa, respectively. Our phylogenetic analyses provides strong evidence of long-term host-parasite relationship between LIN1 and bellbirds, whereas LIN2, LIN3 and LIN4 are more recent introductions. The mismatch between host and parasite LIN1 spatial patterns suggest this parasite is limited by vector distribution and that a relatively specific host-parasite-vector system exists within the Hauraki Gulf bellbird metapopulation.

Keywords Plasmodium, avian malaria, biogeography, New Zealand, host-parasite distribution

5.2 Introduction

Parasite lineages with wide host and geographical distributions are considered to be generalist parasites, whereas lineages restricted to particular regions or endemic hosts, are considered to be specialist lineages (Ishtiaq et al. 2010). Often, however, population connectivity of a parasite is not as continuous as that of the host, especially in island archipelagos (Ishtiaq et al. 2010). If the prevalence and distribution of a host-parasite interaction is heterogeneous, i.e., there is a spatial mismatch between the distribution of the host and distribution of the parasite, then constraints on the parasite may be related to limits associated with the invertebrate vector (Valkiunas 2005). Furthermore, parasites of the *Plasmodium* genus tend to be less constrained than *Haemoproteus* by the phylogenetic relationships of their avian hosts (Ricklefs and Fallon 2002; Beadell et al. 2004), thus *Plasmodium* species are more geographically mobile than *Haemoproteus*. ‘Ecological transmission barriers’ have been shown to cause differentiation and isolation of parasite lineages between different host groups (Hellgren et al.

2008). In Vanuatu and New Caledonia for example, close associations between potentially specialist parasite lineages and endemic mosquito species have been described (Ishtiaq et al. 2008).

There are 12 species of endemic and/or native mosquito in New Zealand, which can act as potential vectors for malaria (Derraik et al. 2004). One of these species is particularly continuously distributed throughout the country, *Culex pervigilans* (Derraik et al. 2004), however, less is known about the other species. Of the many hundreds of exotic mosquitoes that have been inadvertently imported into New Zealand, only four species (*Culex quinquefasciatus*, *Ochlerotatus notoscriptus*, *Ochlerotatus australis*, *Ochlerotatus camptorhynchus*), have established successfully and their distribution throughout New Zealand still appears to be patchy (Derraik et al. 2004; Derraik et al. 2008). Tompkins and Gleeson (2006) provide evidence for a latitudinal (north to south) decrease in abundance of one exotic lineage of *P. relictum* infecting non-native birds, which correlates with latitudinal trends in its putative mosquito vector, the exotic *Culex quinquefasciatus*. Similarly, haematozoan blood parasites, *Plasmodium* or avian malaria in particular, are equally represented throughout New Zealand. For example, Tompkins and Gleeson (2006) report 10 lineages of *P. relictum* alone in one survey. Data depositions made to the international MalAvi database (Ruth Brown unpublished data; Bensch et al. 2009) show that at least four exotic, e.g., house sparrow (*Passer domesticus*), European thrushes (*Turdus* spp.), and six endemic, e.g., South Island robin (*Petroica australis*), saddleback (*Philestrunus carnuculatus*), hihi (*Notiomystis cincta*), bird species have been infected by a variety of avian malaria including such well known exotic cosmopolitan parasites as SGS1 (*P. elongatum*) and GRW4 (*P. relictum*) in New Zealand. Not much is known about virulence of these parasites, however, recent outbreaks of avian malaria causing mortality of endemic species have been recorded in New Zealand zoos, e.g., Mohua (*Mohoua ochrocephala*, Alley et al. 2008). Studies from the northern hemisphere, e.g., blue tits (*Cyanistes caeruleus*, Cosgrove et al. 2008), house finch (*Carpodacus mexicanus*, Kimura et al. 2006), show that a host population can have up to 12-15 lineages of malaria within a small geographic area (Cosgrove et al. 2008). Ecological study of avian malaria in New Zealand is a young and growing science, and at the onset of this study we expected to find a high diversity

of malarial parasites by studying a single host system involving the New Zealand bellbird (*Anthornis melanura*).

The bellbird is an abundant, genetically diverse endemic passerine with the potential for high levels of dispersal (see Chapters Two and Three). Furthermore, the bellbird is continuously distributed throughout New Zealand, and their numbers generally increase southward in a latitudinal cline consistent with decreases in ambient temperature. These characteristics make the bellbird an ideal candidate for studying parasite distribution, ecological barriers to vectors and host-parasite specificity. The main objectives of this study are to assess the 1) diversity of *Plasmodium* and *Haemoproteus* parasites infecting a highly mobile passerine endemic to New Zealand, 2) distribution of these parasites relative to that of their host and 3) worldwide distribution and phylogenetic relationships of bellbird parasite lineages. This study can be useful for comparison with future studies on host-specificity and parasite biogeography.

5.3 Methods

5.3.1 *Blood sampling*

We collected 770 blood samples from individual bellbirds throughout New Zealand between 2007 and 2010 to assess the diversity and distribution of avian malaria parasites. A subsample of 693 blood samples was selected for parasite PCR screening blindly of individual ID and malaria status. Under permission of the Department of Conservation (DoC) and Massey University Animal Ethics Committee (MUAEC), bellbirds were captured via mist nets, individuals were marked with DOC Banding Office stainless-steel and colour leg bands and blood samples were extracted by venipuncture of brachial vein and samples were stored in either lysis buffer or 95% ethanol. Blood smears were aired-dried on microscope slides, preserved with 100% methanol and then stained using Giemsa stain.

5.3.2 *PCR detection of the parasite*

DNA was extracted from blood samples using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). Samples were screened for *Plasmodium* and *Haemoproteus* infections using a nested polymerase chain reaction (PCR) method that amplifies a 478 base pair (bp) fragment of mitochondrial DNA (mtDNA) cytochrome *b* gene (Hellgren et al. 2004). This nested PCR comprises two rounds of PCR reactions performed in 15 µL volumes using positive and negative controls. The forward and reverse primers used in the first round of reactions were HaemNF (5'-CATATATTAAGAGAATTATGGAG-3') and HaemNF (5'-AGAGGTGTAGCATATCTATCTAC-3'), respectively. Each 15 µL reaction included 2 µL of genomic DNA, 0.75 mM of each dNTP, 0.6 µM of each primer, 1.5 mM of $MgCl_2$ and 0.3 units of *Taq* DNA polymerase (Invitrogen). The thermal profile consisted of a 3-min, 94°C activation step, followed by 20 cycles of 94 ^{o}C for 30 s, 50 ^{o}C for 30 s and 72 ^{o}C for 45 s, ending with an elongation step of 72 ^{o}C for 10 min. In the second round of PCR reactions, primers HaemF (5'-TGGTGCTTTCGATATGCATG-3') and HaemR2 were used (5'-GCATTATCTGGATGTGATAATGGT-3'). The protocol for the second round of PCR was the same as the first, except that 2 µL of PCR product from the first reaction was used as template instead of genomic DNA and the number of cycles in the thermal profile was increased to 35 cycles. 3µL of PCR products were run on 1.5% agarose then stained with ethidium bromide and viewed under UV. PCR products containing bands around 500 bp in size were considered malaria positive and purified using SureClean (Bioline Inc.), then sequenced using the forward primer HaemF on an ABI 3730 DNA Analyzer (Applied Biosystems, Inc.). Sequences were edited and aligned in MEGA 5 (Tamura et al. 2011).

5.3.3 *Phylogenetic analysis of the haemosporidian parasite*

The GTR+I+G model of sequence evolution best fitted our four cytochrome *b* mitochondrial DNA haplotype dataset (N = 93 individuals) as determined using maximum likelihood analyses and the Akaike Information Criterion (AIC) in JMODELTEST 0.1.1 (Guindon and Gascuel 2003; Posada 2008). This GTR+I+G model assumes base frequencies A = 0.2994, C = 0.1352, G = 0.1310, T = 0.4345) with proportion of invariable sites (I) < 0.001 and a substitution rate matrix A-C = 2.6951, A-G = 1.2005, A-T = 5.8779, C-G = 1.2931, C-T = 20.3541 and G-T = 1.0. Using the

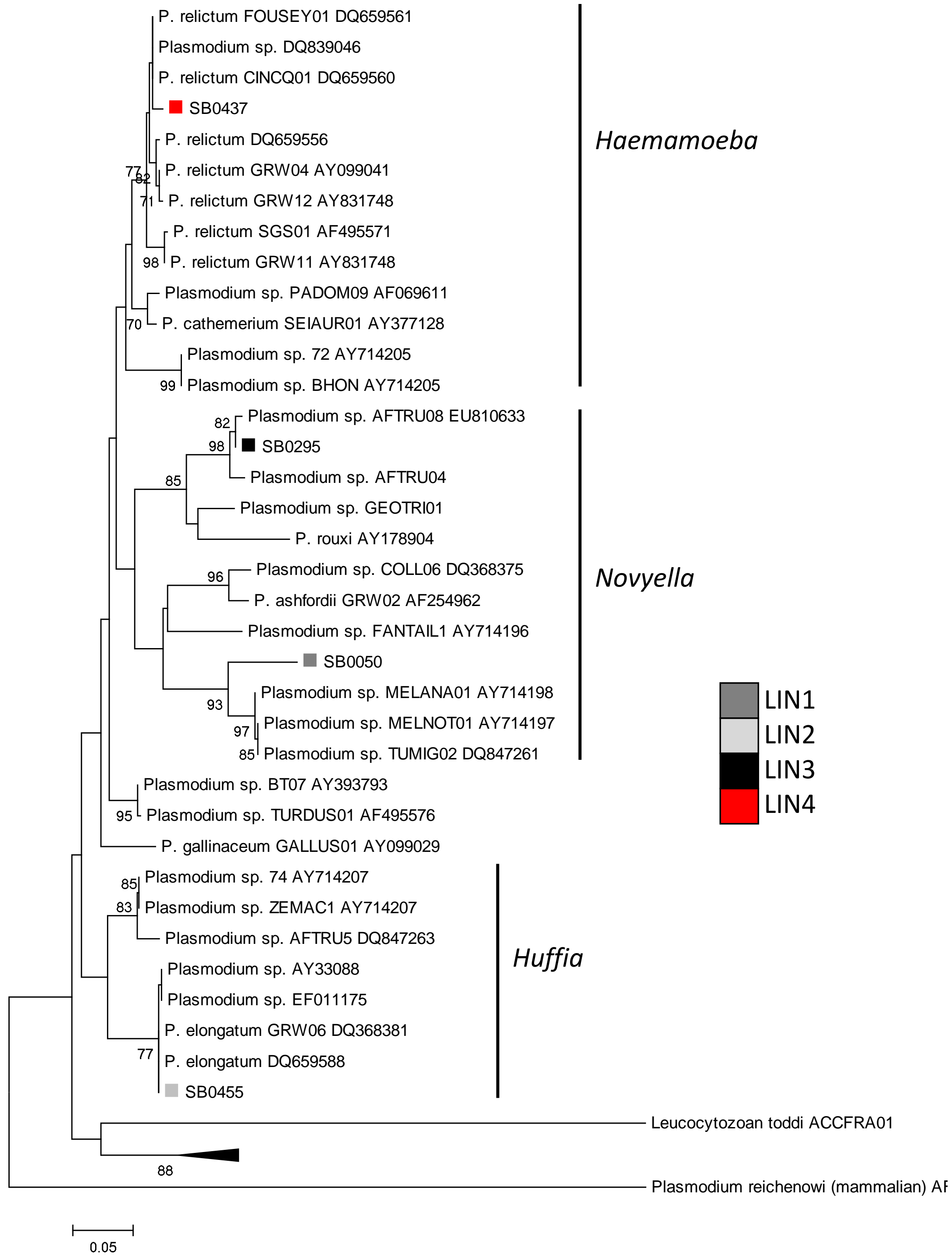

Figure 5.1 The maximum likelihood (ML) tree shows the estimated phylogenetic relationships among parasite lineages using cytochrome *b* sequences (length 478 bp). *Leucoctytozoan* and a mammalian *Plasmodium* were used as outgroups to root the tree. The numbers at the branches represent ML bootstrap support ≥ 70% (5000 replicates). Previously documented sequences have their Latin, MalAvi database and GenBank names at the end of each branch.

GTR+I+G model, we estimated the phylogeny of haplotypes using 5,000 bootstrap replicates of the maximum likelihood (ML) analysis in MEGA. The phylogenetic relationships among parasite lineages were estimated using cytochrome *b* sequences ≥ 400 bp. We used all avian malaria sequences in the MalAvi (http://mbio- serv4.mbioekol.lu.se/avianmalaria/index.html) database and some additional Southern Hemisphere Meliphagidae parasite sequences from GenBank (Beadell et al. 2004; Bensch et al. 2009) to aid in the identification and biogeographical relationships of haemosporidian lineages found in bellbirds. A mammalian *Plasmodium* outgroup sequence to root the tree (see Wood et al. 2007). Pair-wise LogDet sequence divergence estimates (Lockhart et al. 1994) were calculated in MEGA.

5.4 Results

5.4.1 *Phylogenetic identification and prevalence of avian malaria lineages*

Phylogenetic analysis on cytochrome *b* sequences identified four distinct lineages (referred to as lineage LIN1, LIN2, LIN3 and LIN4 for the remainder of the text) of a single genus, *Plasmodium* (Table 1) (Appendix C: Figure C.1; these sequences will be submitted to GenBank). There is strong bootstrap support (91%) that LIN1 is genetically similar but not identical to *Plasmodium* (*Novyella*) sp. infections found only in Australian and Papua New Guinean Meliphagidae species (Beadell et al. 2004) (Figure 5.1). LogDet sequence divergence between LIN1 its two closest phylogenetic matches MELANA01 and MELNOT01 is 4.9% ($22_{\text{BASE-PAIR CHANGES}}/454_{\text{COMMON SITES}}$) and 5.1% ($23_{\text{BASE-PAIR CHANGES}}/454_{\text{COMMON SITES}}$), respectively (Table 1) (Appendix C: Figure C.2 for LogDet pair-wise sequence divergences). LIN1 parasites comprise 80% (N = 74/93) of positive malaria infections in bellbirds and our country-wide prevalence estimate is 11% (N = 74/693) (Figure 5.2). We identified LIN2 to be the prolific cosmopolitan *P.* (*Huffia*) *elongatum* GRW06 (Perez-Tris et al. 2007; see Valkiunas et al. 2008a). The LIN2 and GRW06 cytochrome *b* sequences are an exact match ($0_{\text{BASE-PAIR CHANGES}}/454_{\text{COMMON SITES}}$) (Table 1). LIN2 is the most geographically widespread infection throughout New Zealand and occurs in

14% (*N* = 13/93) of positive infections, but country-wide prevalence is low (2%, *N* = 13/693) (Figure 5.2). LIN3 is phylogenetically similar to infections previously found in African and Seychelles passerines, AFTRU08 *Plasmodium* (*Novyella*) sp. (Beadell et al. 2009) (Figure 5.1). There is only a 0.7% LogDet sequence difference between LIN3 and AFTRU08 (Table 1). Finally, we found that LIN4 only has a 0.7% ($3_{\text{BASE-PAIR CHANGES}}/478_{\text{COMMON SITES}}$) sequence difference from the *P. relictum* LINOLI01 (Beadell et al. 2006) (see Table 1). This lineage is an exact match with both *P. relictum* FOUSEY01 and CINCQ01 sampled in Seychelles passerines (Beadell et al. 2006) (Figure 5.1). We detected LIN3 and LIN4 in 0.7% (*N* = 5/693) and 0.1% (*N* = 1/693) of individuals screened throughout New Zealand, respectively (Figure 5.2).

Table 5.1 Sequence divergence estimates of the bellbird *Plasmodium* parasites detected in this study and the closest sequence matches documented in the MalAvi database and GenBank. LIN1 through LIN4 is the naming convention adopted for this publication until sequences are resolved. Resolved lineages are listed according to previously published host species codes and GenBank accession numbers for sequences with which we found a ≥ 95% match.

	Lineage code	GenBank no.	Genus (subgenus) species	Sequence divergence (%)
LIN1	unresolved	unresolved	*Plasmodium* (*Novyella*) sp.Ŧ	4.9
LIN2	GRW6	DQ368381	*P. (Huffia) elongatum**	0
LIN3	AFTRU08	EU810633	*P. (Novyella)* sp.**	1.2
LIN4	LINOLI01	DQ839046	*P. (Haemamoeba) relictum*†	0.7

Ŧ This sequence could not be resolved to a known malaria lineage, but belongs to the subgenus *Novyella* and a clade of *Plasmodium* found only in Australopacific honeyeaters (Beadell et al. 2004).

* Mitochondrial cytochrome *b* lineages found in bellbirds that were identical to a well-known previously defined cosmopolitan species (Perez-Tris et al. 2007; Valkiunas et al 2008a).

** This sequence matches most closely to AFTRU08 found in a thrush (*Turdus pelios*) host in Cameroon, western Africa (Beadell et al. 2009).

† This sequence matches most closely to LINOLI01 (a *P. relictum* lineage very similar to the cosmopolitan SGS01) collected from a Seychelles sunbird (*Cinnyris dussumieri*) host off the coast of eastern Africa (Beadell et al. 2006).

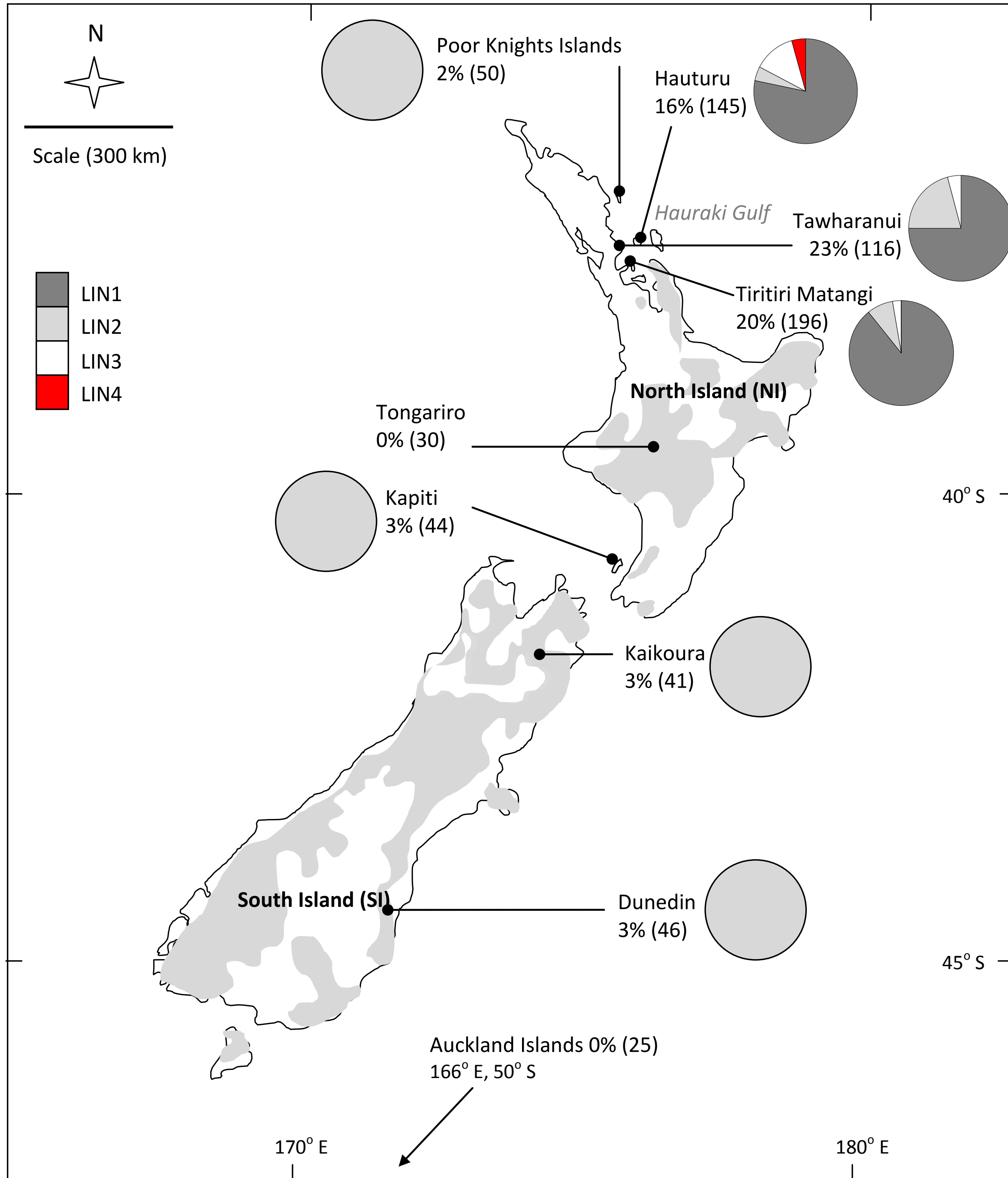

Figure 5.2 Location map of host population sampling locations. The numbers in percents and parentheses represent *Plasmodium* infection prevalence (number of individuals infected/individuals screened) using PCR. The pie charts show relative proportion of positive infections represented by each *Plasmodium* lineage at each location. Approximate New Zealand bellbird (*Anthornis melanura*) distribution range is shown in light grey shading.

5.5 Discussion

5.5.1 *Diversity and distribution of bellbird Plasmodium lineages within New Zealand*

We detected only four lineages of *Plasmodium* parasites from a widely distributed endemic passerine host throughout the island archipelago of New Zealand. No *Haemoproteus* was found. The distribution of *Plasmodium* was heterogeneous with respect to that of the bellbird host in two key ways 1) the most numerous avian malaria parasites, LIN1, were restricted geographically to the Hauraki Gulf and 2) LIN2 (*P. elongatum* GRW06) was the only lineage that reflected the bellbird distribution and was ubiquitous throughout most of New Zealand. Furthermore, LIN3 and LIN4 were only detected within the Hauraki Gulf in northern New Zealand. The lack of any *Plasmodium* detected at Tongariro may be due to low sample size and in the Sub-Antarctic Auckland Islands a complete absence of mosquito vectors precludes transmission at that location. Clearly, the availability of competent vectors or suitable environmental variables in addition to the geographic distribution of the host play an important role in the biogeographic distribution of avian malaria parasites (Hellgren et al. 2007; Ishtiaq et al. 2010).

Our parasite diversity estimates are low compared to studies on continental locations, e.g., typically in Europe three genera and more than ten species are found in a single host population (see Derraik et al. 2004; Fallon et al. 2004; Bensch et al. 2007; Wood et al. 2007; Norte et al. 2009). The low malaria diversity and prevalence (≤ 20%) in New Zealand bellbird is in congruence, however, with findings from other endemic Australopacific island host bird populations (Jarvi et al. 2003; Beadell et al. 2004; Alley et al. 2011). Islands generally have lower parasite diversity owing to isolation and small landmasses that proffer a biogeographic history distinct from that of continents, though species richness will increase with the area of the island (MacArthur and Wilson 1967; see also Ishtiaq et al. 2010). Consequently, islands such as New Zealand provide a good opportunity to discern malaria endemism and the co-evolutionary relationships between blood parasites and their hosts.

5.5.2 *Biogeographical origins of bellbird Plasmodium parasites*

Our detailed phylogenetic analysis using the complete MalAvi database (Bensch et al. 2009) and GenBank provides no mitochondrial DNA sequence match close enough to resolve the LIN1 lineage to the species level, let alone a particular lineage for LIN1. However, we found that the LIN1 lineage belongs to a clade of parasites known to infect only Australopacific meliphagids: the yellow-spotted honeyeater (*Meliphaga notata*) that inhabits Australia; Lewin's honeyeater (*Meliphaga lewinii*) that inhabits northeastern Australia and Papua New Guinea (Beadell et al. 2004). It is plausible that bellbirds retained their parasites during the historical Meliphagidae radiation from Australia to New Zealand (see Beadell et al. 2004; Driskell et al. 2004) and that the lineage has evolved and mutated along with its avian host, locked in a classic evolutionary arms race (Dawkins and Krebs 1979).

LIN2 was an exact match with the *P. elongatum* GRW06 lineage originally described in the great reed warbler (*Acrocephalus arundinaceus*; Perez-Tris et al. 2007; see also Valkiunas et al. 2008a) and is a global cosmopolitan species found in many species of avian hosts throughout Europe, Africa, Australia and North America (Table 2). Despite its relatively low prevalence in bellbirds, the geographic distribution of LIN2 is ubiquitous throughout New Zealand (this study) and this same parasite has been sampled from several endemic and exotic avian species throughout New Zealand (Alley et al. 2011; Ruth Brown unpubl data). Furthermore, it is unlikely that the more rare lineages LIN2-4 maintain their population solely within the bellbird (Bensch et al. 2007). The most probable reservoir for LIN2-4 parasite lineages are the European and Asian passerines, and indeed *Passer domesticus* is the most common host listed in the MalAvi database for LIN2 infections. A recently published paper (Alley et al. 2011) states that this same *P. elongatum* GRW06 (GenBank No. DQ659588) found in New Zealand saddlebacks is believed to be an endemic strain of avian malaria because one individual tested negative several months after testing positive (Castro and Howe, unpublished report). We stress, however, that it is highly improbable that GRW06 originated in New Zealand as 1) the negative test result may be due to failings of PCR detection (see below), 2) exotic birds known to carry

Table 5.2 Worldwide biogeographical distribution of *Plasmodium* lineages most closely matching each parasite sequence we found in New Zealand bellbirds (*Anthornis melanura*) in this study (information for only sequences with < 2% LogDet sequence divergence from LIN1-4).

Bellbird parasite lineage	Host Country	Number of avian host species	Publication
LIN1 *P.* (*Novyella*) sp.			
	New Zealand	1	This study
LIN2 *P. elongatum* GRW06			
	Australia	1	Beadell et al. 2004
	Bulgaria	4	Valkiunas et al. 2008a; Marzal et al. unpubl; Zethindjiev unpubl
	Cameroon	2	Beadell et al. 2009
	Gabon	1	Hellgren et al. 2007
	Galapogos I.	1	Santiago-Alarcon et al. 2008, 2010
	Myanmar	2	Ishtiaq et al. 2007
	New Zealand	9	Ruth Brown unpubl; this study
	Papua New Guinea	1	Beadell et al. 2004
	United States	8	Beadell et al. 2006; Ishak et al. 2008; Outlaw and Ricklefs 2009; Kimura et al. 2006
	Spain	1	Fernandez et al. 2010
	Sweden	2	Bensch et al. 2007; Hellgren et al. 2007
LIN3 *P.* (*Novyella*) sp. AFTRU08			
	Cameroon	1	Beadell et al. 2009
	New Zealand	1	This study
LIN4 *P. relictum* LINOLI01			
	New Zealand	1	This study
	Cameroon	1	Beadell et al. 2006
	Botswana	1	Beadell et al. 2006
	South Africa	1	Beadell et al. 2006
	Zimbabwe	1	Beadell et al. 2006
CINQO01	Mayotte, Fr.	1	Beadell et al. 2006
	Madagascar	1	Beadell et al. 2006
FOUSEY	Fregate	1	Beadell et al. 2006

GRW06 are abundant in New Zealand, e.g., European thrushes, sparrows, Asian mynas, and 3) the lack of nucleotide base pair substitutions suggest that GRW06 has arrived in New Zealand relatively recently. *Plasmodium* is not always highly virulent, and it is apparent that the GRW06 is a host generalist that has adopted a strategy of chronic infections characterized by low virulence in all species it infects (Valkiunas et al. 2008a).

The two remaining lineages grouped within two *Plasmodium* clades representing species reported from African birds only: LIN3, *Plasmodium* (*Novyella*) sp. AFTRU08 (Beadell et al. 2006); and LIN4, *P.* (*Haemoproteus*) *relictum* LINOLI01 (see Table 2). A single bellbird screened positive for *Plasmodium relictum*, LIN4, at Hauturu and it is unknown to date whether this exact lineage has been detected in other New Zealand birds. The *P. relictum* lineages documented by Tompkins and Gleeson (2006) cannot be compared directly to our results because the cytochrome *b* markers they used differed from those in our study and those generally used in the MalAvi database. The *P. relictum* lineage that we detected in bellbirds may be a distance variant of the well-known GRW04 *P. relictum* lineage (LIN4 vs. GRW04: 1.7% sequence divergence; LIN4 vs. LINOLI01: 0.7% sequence divergence). GRW04 is well known to be responsible for the decimation of Hawaiian avifauna (Perkins and Schall 2002). That tragedy has not occurred in New Zealand likely because mosquitos are endemic, unlike in Hawaii, (Derraik et al. 2004). Thus, endemic New Zealand birds should have some acquired resistance to exotic strains of malaria as well as endemic strains (Jarvi et al. 2002).

5.5.3 *Lack of Haemoproteus infections and underestimation of parasite prevalence*

We found no *Haemoproteus* infections in bellbirds despite the fact that their usual hippoboscid vector (Valkiunas 2005) was observed on most individuals captured in this study (SMB, unpubl. data). The New Zealand bellbird host lineage originated from Australian meliphagids (Driskell et al. 2007), a family in which *Haemoproteus* infections are common (Beadell et al. 2006). Thus, we suggest that *Haemoproteus* infections in bellbirds 1) might have been lost through the process of host population bottleneck, 2) are too low in numbers to detect, or 3) have never been part of New Zealand meliphagid system. PCR detection of parasites has been

experimentally shown to underestimate prevalence by 30%, especially 1) in cases of low parasitemia characteristic of chronic infection and 2) if infections temporarily evacuate the peripheral blood stream (Jarvi et al. 2002; Valkiunas 2008b). This large margin of error alone may explain the absence of *Haemoproteus* in our study and means that our *Plasmodium* prevalence estimates are underestimated. Microscopy yields varying results depending on both the skill level of the observer and quality of specimens. For most non-specialized researchers microscopy techniques will underestimate prevalence by 70% (Jarvi et al. 2002). In this study, we found that in a subsample of 97 samples, 39% (16/26) of the malaria detected by nested PCR was not detected by microscopy performed by a professional haematologist experienced in malaria detection. A more sensitive malaria detection technique involves serological assay of antibodies, but parasite lineage identification is not possible (Jarvi et al. 2002). The most promising blood parasite detection approach for future studies is a relatively new quantitative PCR (qPCR) that has been shown to have higher detection sensitivity than nested PCR and it can be used to estimate parasitemia, or concentration of parasites in a given amount of blood (see Knowles et al. 2011).

5.5.4 *Conclusions*

Relative to continental bird populations, we found a low diversity of haematozoan parasites in New Zealand bellbirds. The distribution of this host-parasite relationship is highly heterogeneous despite high levels of population connectivity of the host. Latitudinal trends of increasing or decreasing parasite prevalence were not apparent, though avian malaria parasite diversity and abundance was concentrated in the northern Hauraki Gulf region. Three of the four parasite lineages detected here could be considered to be generalist parasites with wide host and geographical distributions. The only malaria lineage present throughout the country was an exotic *P. elongatum* GRW06 (LIN2 this study) and our phylogenetic analysis provides strong evidence that this lineage (as well as LIN3 and LIN4) has been recently introduced to New Zealand. On the other hand, LIN1 infections are likely endemic to New Zealand because 1) sequence divergence with parasites is large and 2) the closest matches are two parasite lineages that infect closely related taxa to the bellbird. The mismatch we reveal among host-

parasite spatial pattern suggests this parasite is limited by vector distribution and a relatively specific host-parasite-vector system exists within the Hauraki Gulf bellbird metapopulation.

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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

High endemism and variable disease naivety in Australopacific island avifauna in some cases leads to high susceptibility of pigmented haemosporidian blood parasites, such as avian malaria (*Plasmodium* spp.). Knowledge of host factors that influence malaria prevalence patterns can help assess the ecological and immunological trade-offs at play in disease epidemiology and virulence. Our study is the first in New Zealand to investigate host factors that influence seasonal patterns of haematozoan blood parasites using a single host species, the New Zealand bellbird (*Anthornis melanura*). Blood samples were collected year round from three very different subpopulations of the Hauraki Gulf bellbird metapopulation (*N* = 457). Four lineages (LIN1-4) were detected among the 89 samples that tested positive for *Plasmodium*. Our four key findings were that 1) bellbird parasite assemblages are homogeneous among subpopulations, 2) a strict Beaudoin hypothesis of bimodal seasonality with spring and autumn peaks applied to some but not all locations, 3) marked sex differences in infection at Tawharanui but not at Hauturu or Tiritiri Matangi and 4) body condition in bellbirds paradoxically varied both positively or negatively with malaria prevalence depending on location. We suggest that sex-biased immune response is plastic due to heterogeneity in demographic history (stable versus re-colonizing populations) and that prevalence is likely influenced by food supply as a limiting factor that constraints allocation of a host's energy to immune function. Our study on the ecological and environmental processes underlying how

malaria parasites interact with endemic passerines is a critical step toward predicting and understanding the impact of environmental changes on the health and evolution of host-parasite-vector systems.

Keywords Plasmodium, body condition, seasonal variation, avian malaria, population malaria transmission, New Zealand

6.2 Introduction

Rarely are seasonal drivers well understood due to the multiplicity and covarying nature of host and vector variables on seasonality (Pascual and Dobson 2005). Recent studies provide evidence that host factors are important in the prevalence and intensity of infections (Wood et al. 2007; Cosgrove et al. 2008; Knowles et al. 2011). It is well established that a range of characters influence differences in individual host susceptibility to disease, e.g., reproductive effort (Norris et al. 1994; Merila and Andersson 1999; Stjernman et al. 2004), seasonal variation in stress and sex hormone levels (Deviche and Sharp 2001; Deviche et al. 2001) and individual health status, which is often reflected by body condition (Norris et al. 1994; Merila and Andersson 1999). According to the Beaudoin et al. (1971) theoretical model of bimodal malaria seasonality in temperate climates, a peak in avian malaria prevalence occurs in late summer and autumn during maximum recruitment of disease naïve young, then prevalence drops in winter when invertebrate vector activity decreases followed by an increase called the ‘spring relapse’. The ‘spring relapse’ is due to a latent rise in infection of previously infected hosts due to the stress of initiating breeding, and is independent of mosquito vector activity (Beaudoin et al. 1971). Recent studies have corroborated that endocrinological stress is often associated with changes in ecological constraints (Wingfield et al. 1997), humoral immune cell distribution

(Dhabhar et al. 1995) and susceptibility to malarial infection (Atkinson et al. 1995; Valkiunas 2005; Wada et al. 2007).

Sex differences in parasite infection rates are explained by two leading theoretical approaches (Rolff 2002). The first approach is characterized by the immunocompetence handicap hypotheses (ICHH), wherein different endocrinological mechanisms alter the immune system in different directions, i.e., parasite-mediated sexual selection (Hamilton and Zuk 1982; Folstad and Karter 1992). The second approach is seated in natural selection, as opposed to sexual selection, of the Bateman's Principle, which has its basic premise in that sex-specific life-histories result in differences in the trade-off between investment in immunity and other aspects of reproduction (e.g., males trade-off longevity/immunity for reproductive success; Rolff 2002). Most likely, hormone-mediated immunity acts in concert with differential investment in immunity of the sexes (Rolff 2002). An increase in prevalence with age is typical among many passerine host studies (Wood et al. 2007). Age differences in infection are generally explained due to increased duration of exposure to parasites among older individuals and maintenance of chronic infections (Stjernman et al. 2004; Wood et al. 2007). However, it is possible that several non-exclusive processes, including age-specific probabilities of infection gain or loss (e.g., nestling exposure time) and infection-related mortality are involved (Knowles et al. 2011). Population density may also influence parasite prevalence, depending on how parasite transmission relates to host population density (Keymer and Anderson 1979). In epidemics, host density and transmission efficiency strongly influence the evolution of virulence. By contrast, endemic infections place a premium on duration of infection, and virulence is not influenced by opportunities for transmission (Frank 1996). Density and growth stage of a host population are often influenced by demographic processes such as population expansion, stability and recent population bottlenecks, which in turn may influence resistance to disease and parasite prevalence (see Sommer 2003; Miller and Lambert 2004; Kilpatrick 2006). Furthermore, dispersal and range expansion (re-colonizations) can cause an increase in malaria infections (see Sommer 2003; Perez-Tris and Bensch 2005).

Seasonal drivers of avian blood parasites in most wild systems, including the Australopacific, are poorly understood despite the susceptibility of endemic birds to

devastating outbreaks of avian malaria and their centrality to our understanding of disease dynamics and host-parasite evolution (Steadman et al. 1990; Jarvi et al. 2002, 2003; Beadell et al. 2004; Atkinson and LaPointe 2009). Our study is the first to document year-round seasonal patterns of multiple haematozoan blood parasites in a temperate region of the Australopacific using a single host species, the New Zealand bellbird (*Anthornis melanura*). The objectives of this study are to 1) provide new data where little to none is available on spatial and seasonal patterns in avian malaria prevalence in a host metapopulation, 2) determine whether or not malaria infections persist within individuals through time and 3) assess host factors of sex, age, body condition and location that influence patterns in parasite prevalence. The basic biological question of our research is to identify the processes underlying how malaria parasites interact with endemic passerines.

6.3 Methods

6.3.1 *Host-parasite-vector system*

The host is the New Zealand bellbird *Anthornis melanura*, a vagile but non-migratory forest passerine able to disperse long distances (Baillie et al. *in revision*; Chapter Two). Individual bellbirds are subject to the physiological pressures of intense mate selection and resource competition, and the species is sexually dimorphic though both sexes defend territory during the breeding season (Brunton et al. 2008). Larger males often exclude females from feeding locations, and male-male competition is physical and fierce (Craig and Douglas 1986). Natal dispersal intensifies during autumn, and during winter bellbirds often disperse widely in search of food (Craig 1980). In the semi-subtropical North Island regions such as the Hauraki Gulf and Poor Knights Islands, bellbird populations are locally dense, e.g., ~1500 individuals within 130 hectares during 2009 and 2010 (Brunton and Roper, unpublished data). Bellbirds have been extirpated from the mainland adjacent the Hauraki Gulf and northward in New Zealand since

1860. Introduced novel mammalian predators, disease and intensive deforestation are thought to play a role in bellbird extinctions.

During 2007-2009, we collected 457 blood samples from the Hauraki Gulf metapopulation during five biologically meaningful seasons with 01 February as the beginning of the sampling year when newly fledged young would have joined the population (Table 6.1). The two most populous Hauraki Gulf remnant bellbird populations, Hauturu and Tiritiri Matangi, are located 20 and 5 km offshore, respectively (Figure 6.1). Hauturu supports a long-term stable and genetically diverse subpopulation of bellbirds (Baillie et al. *in revision*; Chapter Two). At Tiritiri Matangi bellbirds experienced a severe population bottleneck and are supplementary fed with sugar water, especially during winter as part of an ongoing conservation program involving vegetation re-planting and predator eradication (Baillie et al. *in revision*; Chapter Two). A third Hauraki Gulf subpopulation, Tawharanui Regional Park (Tawharanui), has newly re-colonized in 2005 and is located on a mainland peninsula within a 35km radius of both islands (Figure 6.1). The Tawharanui bellbird population has been increasing since re-colonization after the implementation of a predator-proof fence in 2004 and predator eradication at both Tawharanui and Hauturu by 2005 (Ornithological Society of New Zealand OSNZ, unpublished data).

Table 6.1 Phenology and biological significance of the five sampling seasons used in this study. AHY represents after hatch year age birds, i.e., birds ≥ 1 year of age. HY represents hatch year birds, i.e., birds less than one year of age.

Description	Duration	Biological significance
Early Autumn	01 Feb – 31 Mar	Post-breeding pre-basic adult moult; HY recruitment
Late Autumn	01 Apr – 30 May	Post-breeding pre-basic adult moult; HY recruitment
Winter	01 Jun – 31 Aug	Overwintering; HY pre-alternate moult; courtship initiates by late August
Spring	01 Sep – 15 Nov	Breeding period: egg laying initiates; peak incubation
Summer	16 Nov – 31 Jan	Breeding period: peak chick hatching and rearing; adult pre-basic moult initiates in January

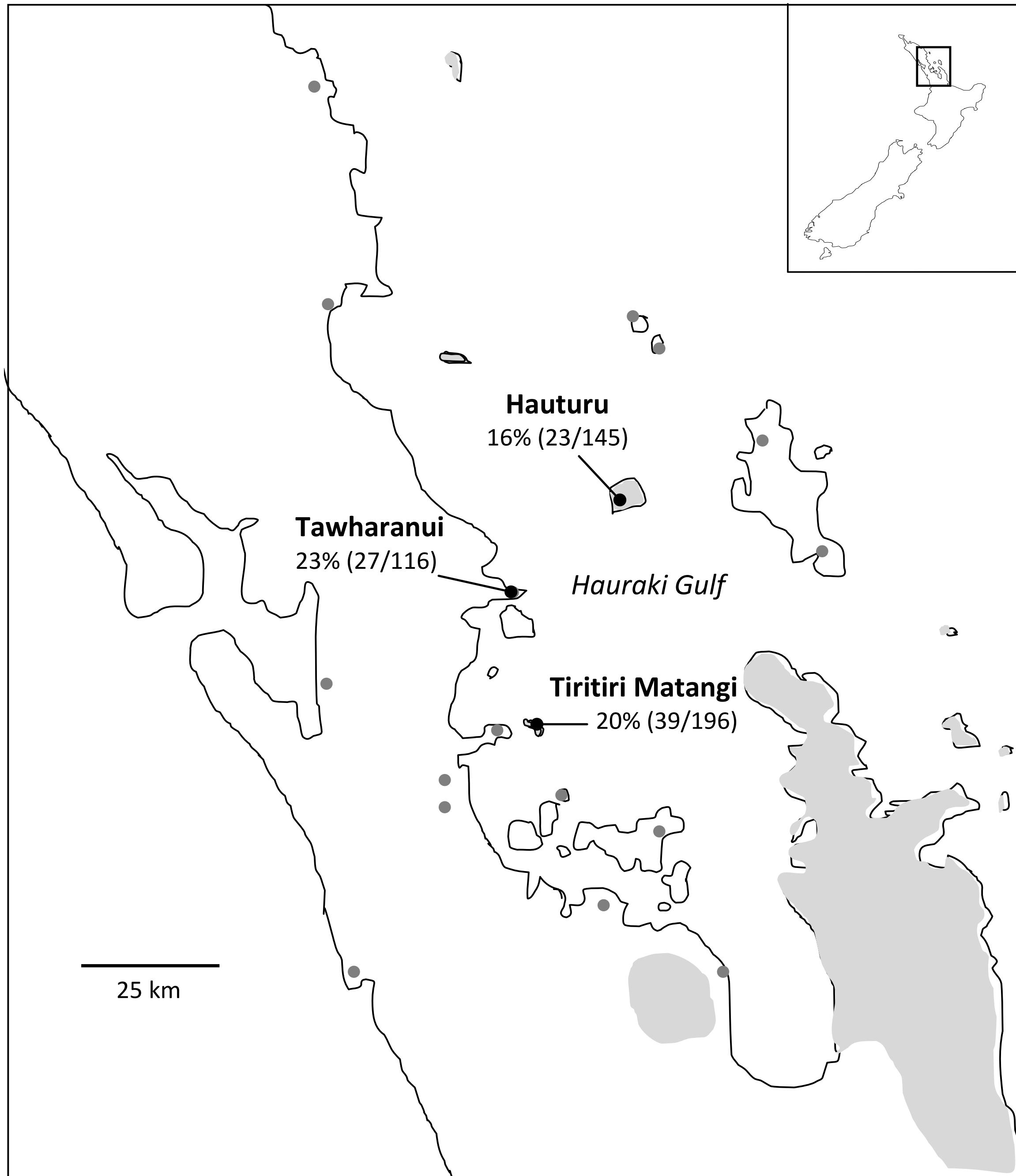


Figure 6.1 Location map and New Zealand bellbird (*Anthornis melanura*) distribution (in light grey) within the Hauraki Gulf, North Island, New Zealand. The bellbird subpopulations included in the avian malaria seasonal variation study at Hauturu, Tawharanui and Tiritiri Matangi, as well as the nearby Poor Knights Islands are indicated. Dark grey dotted areas indicate regions of bellbird sightings during winter.

The parasites in this study are pigmented haematozoan blood parasites, namely *Plasmodium* spp., commonly referred to as avian malaria. *Plasmodium* is a diverse (over 130 species) and globally distributed genus of haematozoan transmitted by insect vectors, i.e., mosquito, and exhibits a full range of host-specificity from generalists able to infect many clades of birds to specialists able to infect only a single species (Valkiunas 2005). Little has been documented about the ecology, diversity, distribution and abundance of endemic or exotic avian malaria species in New Zealand. In a previous study we identified four lineages within the bellbird using a phylogenetic approach (Table 6.2; see also Baillie and Brunton (2011) and Chapter Five). Because some lineages could not be resolved, we refer to them as LIN1, LIN2, LIN3 and LIN4 through this text. Putatively non-endemic avian malaria (*Plasmodium* spp.) outbreaks have been known to occur in bird species endemic to New Zealand (Alley et al. 2008; Derraik et al. 2008; Sturrock and Tompkins 2008). Mosquitos are endemic to New Zealand (16 species; Derraik et al. 2004), unlike some Pacific Ocean islands, i.e., Hawaii (Atkinson and LaPointe 2009). Depending on the vector, vector activity often peaks in New Zealand during autumn (February-May) (Jose Derraik, personal communication). Of the many exotic mosquito species that have landed in New Zealand, successful invasions have been made by only four species (Derraik et al. 2004).

6.3.2 *Blood sampling and PCR detection of the parasite*

Under Department of Conservation (DoC) and Massey University Animal Ethics Committee (MUAEC) permission, 457 bellbirds were captured by mist nets and blood samples were extracted by venipuncture of brachial vein and stored in either Queen's buffer (Seutin et al. 1991) or 95% ethanol. Feeder traps were used to capture bellbirds at Tiritiri Matangi during 2007 only. Individuals were marked with DOC Banding Office stainless-steel and colour leg bands. Effort was made to attain an even number of sample sizes among sex and age classes of individuals for each sampling location and time period. A few Hauraki Gulf birds (18 individuals) were screened more than once (16 individuals twice; 1 individual three times; 1 individual four times) over the period of one year. This proportion of samples is < 4% (N = 18/457) and is not

thought to cause large demonic intrusion effects due to repeated measures in statistical analyses. Thus, each sample was treated as independent and included in all analyses unless otherwise stated. We extracted DNA from blood samples using a highly efficient nested polymerase chain reaction (PCR) method to detect *Plasmodium* and *Haemoproteus* parasites infections by amplification of 478 base pair (bp) fragment of mitochondrial DNA (mtDNA) cytochrome *b* gene (Hellgren et al. 2004; for more detailed PCR methods see Baillie and Brunton (2011); Chapter Five).

Table 6.2 Number and proportion (in parentheses) of positive *Plasmodium* infections (*N* = 89) identified in bellbird (*Anthornis melanura*) samples (*N* = 457) collected at three Hauraki Gulf subpopulations between 2007 and 2010. LIN1 through LIN4 is the naming convention adopted for this publication until sequences are resolved. Resolved lineages are listed according to previously published MalAvi database host species codes and GenBank accession numbers for sequences with which we found a ≥ 95% match. This table is modified from the original Table in Baillie and Brunton (2011).

Lineage	MalAvi Code	GenBank no.	Genus (subgenus) species	N_{INFECTED}
LIN1	unresolved	unresolved	*Plasmodium* (*Novyella*) sp.Ŧ	74 (83%)
LIN2	GRW6	DQ368381	*P. (Huffia) elongatum**	9 (10%)
LIN3	AFTRU08	EU810633	*P. (Novyella)* sp.**	5 (6%)
LIN4	LINOLI01	DQ839046	*P. (Haemamoeba) relictum*†	1 (1%)

Ŧ This sequence could not be resolved to a particular known malaria lineage, but belongs to the subgenus *Novyella* and a clade of *Plasmodium* found only in Australopacific honeyeaters (Beadell et al. 2004).

* Mitochondrial cytochrome *b* lineages found in bellbirds that were identical to a well-known previously defined cosmopolitan species (Perez-Tris et al. 2007; Valkiunas et al 2008).

** This sequence matches most closely to AFTRU08 found in a thrush (*Turdus pelios*) host in Cameroon, western Africa (Beadell et al. 2009).

† This sequence matches most closely to *P. relictum* lineages LINOLI01, CINCQ01 and FOUSEY01) collected from three different species of Seychelles passerines off the coast of eastern Africa (Beadell et al. 2006).

6.3.3 *Sex and age determination of the host*

Male bellbirds are easily distinguishable from female bellbirds even at a distance using body size, shape and plumage characteristics. Males have proportionally longer bills than females, are darker and have a dark purple-blue iridescent sheen on the head and wings. Whereas, females are generally smaller, lighter in colour and have a light blue-green iridescent sheen. The age of each individual bellbird is determined by plumage characteristics. Moult condition, moult score, and feather shape, i.e., primary feather notch emarginations and tips of tail retrices were recorded for most birds captured. Head-bill length, unflattened wing length and short tarsus length were measured to the nearest mm, mass to the nearest gram (g). Body fat measurements were estimated using a relative scale (from 0 = no fat to 4 = fat bulged out from furculum). Birds having no emargination on the ninth primary (p9) and with all retrices sharply pointed were aged as juveniles (HY = hatch year) within their first 12 months after hatching. HY plumage is similar to the colouration of the adult (AHY = after hatch year) female, but is duller and contour feathers are less dense and HY birds lack the aqua-blue-green reflectance sheen of AHY females. HY birds go through a moult to second year (SY) plumage during autumn and winter into their first spring (August-October). All SY birds were designated as AHY age class in this study. AHY bellbirds typically have a slight to deep emargination and the retrices are distinctly more rounded, broad, and blunt than in HY birds. We use the age class designations ‘after hatch year’ (AHY) and ‘hatch year’ (HY) where relevant in this manuscript.

6.3.4 *Calculation of host body condition index*

In order to compare body condition among the sexes we used an index of body condition called the residual body condition index, or RBCI (Freeman and Jackson 1990). RBCI is calculated as the residual from the regression of the cubed root of body mass against the first principle component score of a principle component analysis of skeletal measurements (tarsus length and head-bill length). We validated the utility of the RBCI and the extent to which RBCI can explain variation in external fat content (a relative scale from 0 to 4) using a generalized linear regression (GLZ) of RBCI on ‘sex’ and ‘fat’ variables with ‘date’ as a covariant (Appendix D:

Figure D.1). The RBCI allows for comparisons among males and females, which do not exhibit simple allometric scaling where females are smaller version of males (Appendix D: Figure D.2, Table D.1, Figure D.3).

6.3.5 *Statistical modelling of factors that influence seasonal variation in parasite prevalence*

The factor and linear covariant terms (location, sampling season, host age, host sex and residual body condition index) and their interactions were tested as factors affecting the binomial dependent variable (infected = 1, not infected = 0) using generalized linear regression (GLZ) and backward step-wise logistic regression. Model terms were retained if their removal caused significant change ($P < 0.05$) in model deviance. A GLZ showed no significant year effect on malaria prevalence at Tiritiri Matangi (Likelihood ratio: $X^2_8 = 5.0$, $P = 0.8$; year: Wald $X^2_1 = 3.9$, $P = 0.7$; season: Wald $X^2_4 = 2.2$, $P = 0.7$; year*season: Wald $X^2_2 = 1.4$, $P = 0.5$), thus the samples collected over two years at that location are pooled as one year. To further explore the data and corroborate parametric tests, we use an AIC approach to determine the best fit model describing LIN1 infections using the same model terms described above. All statistical analyses were performed in PASW Statistics 18.0 (SPSS, Inc.).

6.4 Results

6.4.1 *Spatial and temporal malaria patterns within a host metapopulation*

Our GLZ showed that differences in overall parasite prevalence estimates for pooled *Plasmodium* (23, 16 and 20%) and LIN1 prevalence (12, 18 and 18%) between Hauturu, Tawharanui and Tiritiri Matangi, respectively, are not statistically significant (pooled *Plasmodium*: $-2LL = 448$, $R = 0.005$, $X^2_2 = 2.3$, $P = 0.3$; LIN1: $-2LL = 402$, $R = 0.005$, $X^2_2 = 2.3$, $P = 0.3$) (Figure 6.1). Temporal patterns in parasite prevalence, however, are markedly different within each of the three host subpopulations (Figure 6.2). Autumn (46%, $N = 10/22$) and spring

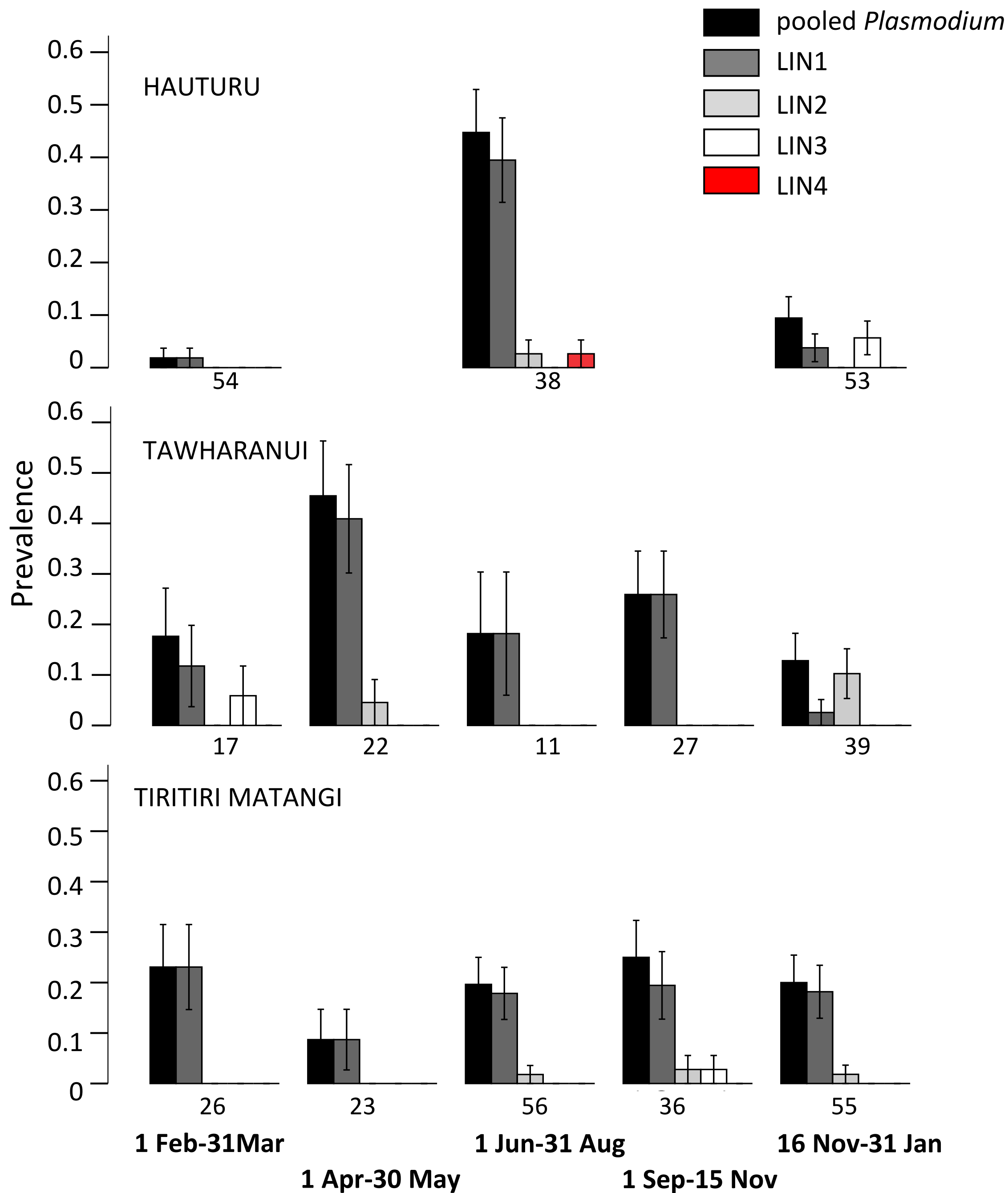


Figure 6.2 Seasonal variation in prevalence of bellbird *Plasmodium* infections in the Hauraki Gulf (*N* = 457). The '1 Apr-30 May' and '1 Sep-15 Nov' seasons were not sampled at Hauturu. The number of individuals screened by PCR during each season and location is denoted below each cluster of bars. Error bars represent ± 1 *SE*.

(26%, N = 7/26) peaks in pooled *Plasmodium* prevalence occurred at Tawharanui. At Hauturu, however, prevalence was highest during winter (45%, N = 17/38). Pooled *Plasmodium* prevalence at Tiritiri Matangi increases during autumn and spring but this pattern is not pronounced (~ 20% year-round) (Figure 6.2). LIN1 infections are largely responsible for these overall spatiotemporal patterns (Figure 6.2). LIN2 and LIN3 infections occurred in ≤ 6-10% of individuals screened in any given season at all three locations. LIN4 was detected in only one individual, an HY female during winter at Hauturu (Figure 6.2). Since LIN2, LIN3 and LIN4 occurred in numbers too low to support statistical modelling and LIN1 closely reflects pooled *Plasmodium* prevalence, regression analyses were performed only for LIN1. We used two-way GLZ analyses to test whether observed seasonal patterns in parasite prevalence (LIN1) varied by location (Hauturu, Tawharanui and Tiritiri Matangi) over five discrete seasons: early autumn, late autumn, winter, spring and summer (see Table 1). We found that LIN1 infections varied significantly among seasons (Wald X^2_4 = 10.3, P = 0.04) with no overall difference among locations (Wald X^2_2 = 0.2, P = 0.9) (Figure 6.2). The interaction term between season and location was statistically significant (Wald X^2_6 = 17.1, P = 0.009), thus analyses on factors affecting prevalence are conducted separately for each location hence forth.

6.4.2 *Factors affecting LIN1 prevalence*

At Hauturu the backward stepwise logistic regression retained three significant interaction terms in the final model (P < 0.001) (Table 3a). All four age-sex classes of bellbirds have similar patterns of high LIN1 prevalence (36-50%) when body condition is low (-1.3 to -1.7) during winter, except that HY males have low LIN1 prevalence (0%, N = 0/3) during winter (Figure 6.3a; for sample sizes see Appendix A.1). At Tawharanui two interaction terms, both of which involved significant seasonal variation, and a single-term ‘sex’ were significant (Table 3a). We found significant autumn and spring peaks in LIN1 prevalence at Tawharanui for AHY male birds (autumn: 62%, N = 8/13; spring: 30%, N = 6/20) but not for females (autumn: 13%, N = 1/8); spring: 17%, N = 1/6) (Figure 6.3b and 6.4b). Overall, LIN1 infections have significantly lower prevalence in females (7%, N = 3/41) than males (24%, N = 18/75) at Tawharanui (Figure 6.3a and 4d). HY birds, however, have vastly different LIN1 and body condition patterns from AHY

Table 6.3 Significant influences on seasonal variation in the prevalence on LIN1 *Plasmodium* infections in bellbirds using the (a) full dataset and (b) AHY (adult bird) dataset only. Final step model effects and parameter estimates from backward logistic regressions are shown. The independent variable (1 = infected, 0 = not infected) was modelled against factors (season, host age, host sex), a linear covariant (residual body condition index, RBCI) and their interactions. Model effects were retained if their removal caused significant change ($P < 0.05$) in model deviance.

Model effects	Parameter estimate	X^2	df	*P*
(a) LIN1 *Plasmodium* sp. full dataset				
Hauturu				
Model Summary		45.5	17	< 0.001
Age x Sex x RBCI x Season	4.7 ± 1.4	11.1	1	< 0.001
Age x RBCI x Season	2.4 ± 1.2	3.8	1	0.05
Sex x Season	3.6 ± 1.0	12.4	1	< 0.001
constant	-4.0 ± 0.8	25.9	1	< 0.001
Tawharanui				
Model Summary		27.7	7	< 0.001
Sex x RBCI x Season	-2.0 ± 1.0	4.0	1	0.045
Age x Season	3.3 ± 0.9	12.9	1	< 0.001
Sex	-2.5 ± 1.0	1.0	1	0.02
constant	-2.8 ± 0.8	14.0	1	< 0.001
Tiritiri Matangi				
Model Summary		37.7	32	0.2
constant	-1.5 ± 0.2	64.8	1	< 0.001
(b) LIN1 *Plasmodium* sp. AHY dataset only				
Hauturu				
Model Summary		14.9	1	< 0.001
RBCI x Season	-0.5 ± 0.2	10.3	1	0.001
constant	-2.9 ± 0.7	19.3	1	< 0.001
Tawharanui				
Model Summary		11.6	2	0.003
Sex	-2.0 ± 0.8	6.2	1	0.01
Season	-0.4 ± 0.2	3.9	1	0.049
constant	0.6 ± 0.8	0.6	1	0.4
Tiritiri Matangi				
Model Summary		12.3	9	0.2
constant	-1.5 ± 0.2	50.9	1	< 0.001

Figure 6.3 (Next page) Seasonal variation in host factors of sex, age (adult = AHY, juvenile = HY) and body condition index (studentized residual from regression: PC1 of skeletal measurements against cube-root of body mass) that influence LIN1 prevalence at three Hauraki Gulf locations. The ‘1 Apr-30 May’ and ‘1 Sep-15 Nov’ seasons were not sampled at Hauturu. Significant *post hoc* parameter estimates for these results are located in Appendix C: Table C.2.

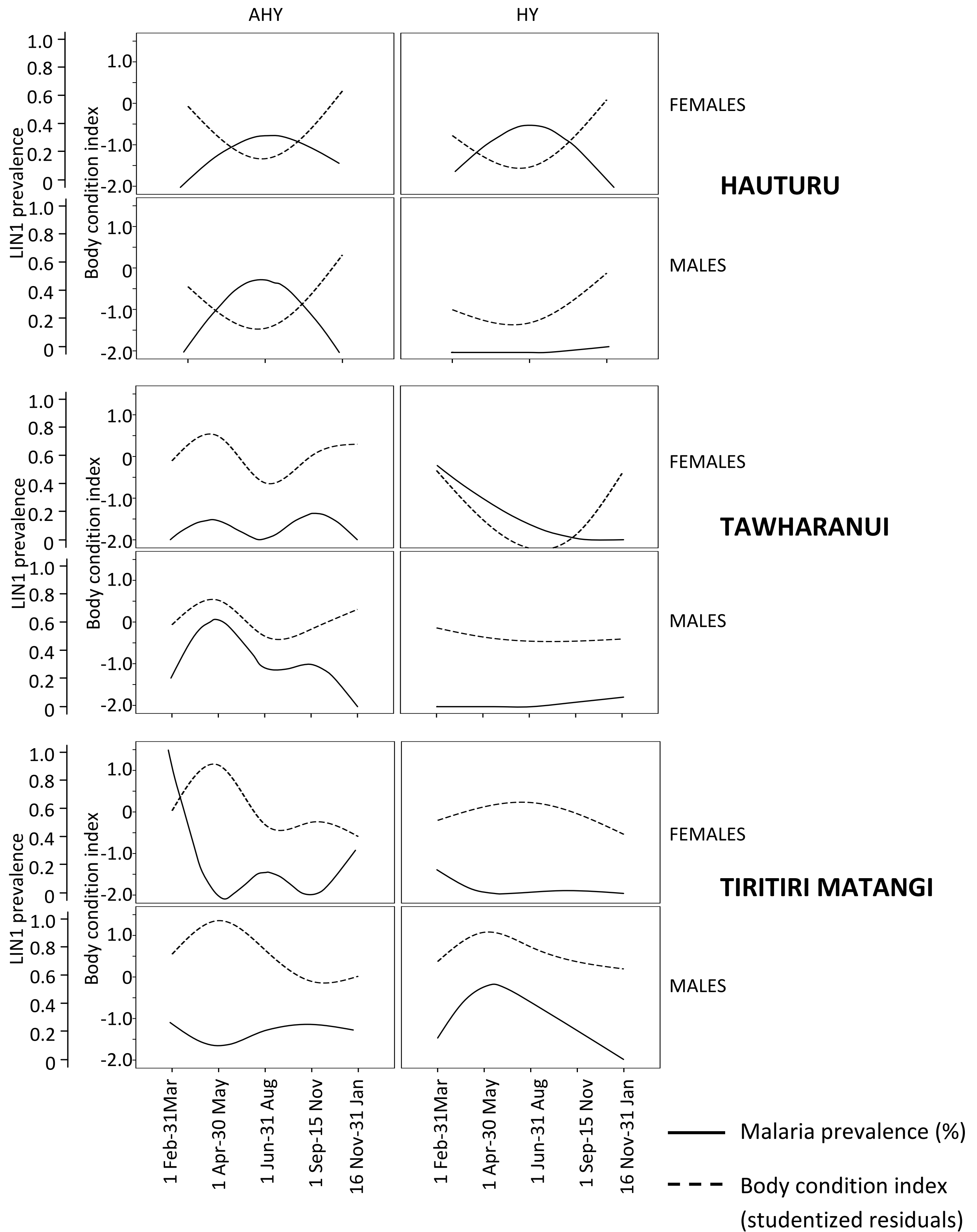


Figure 6.3.

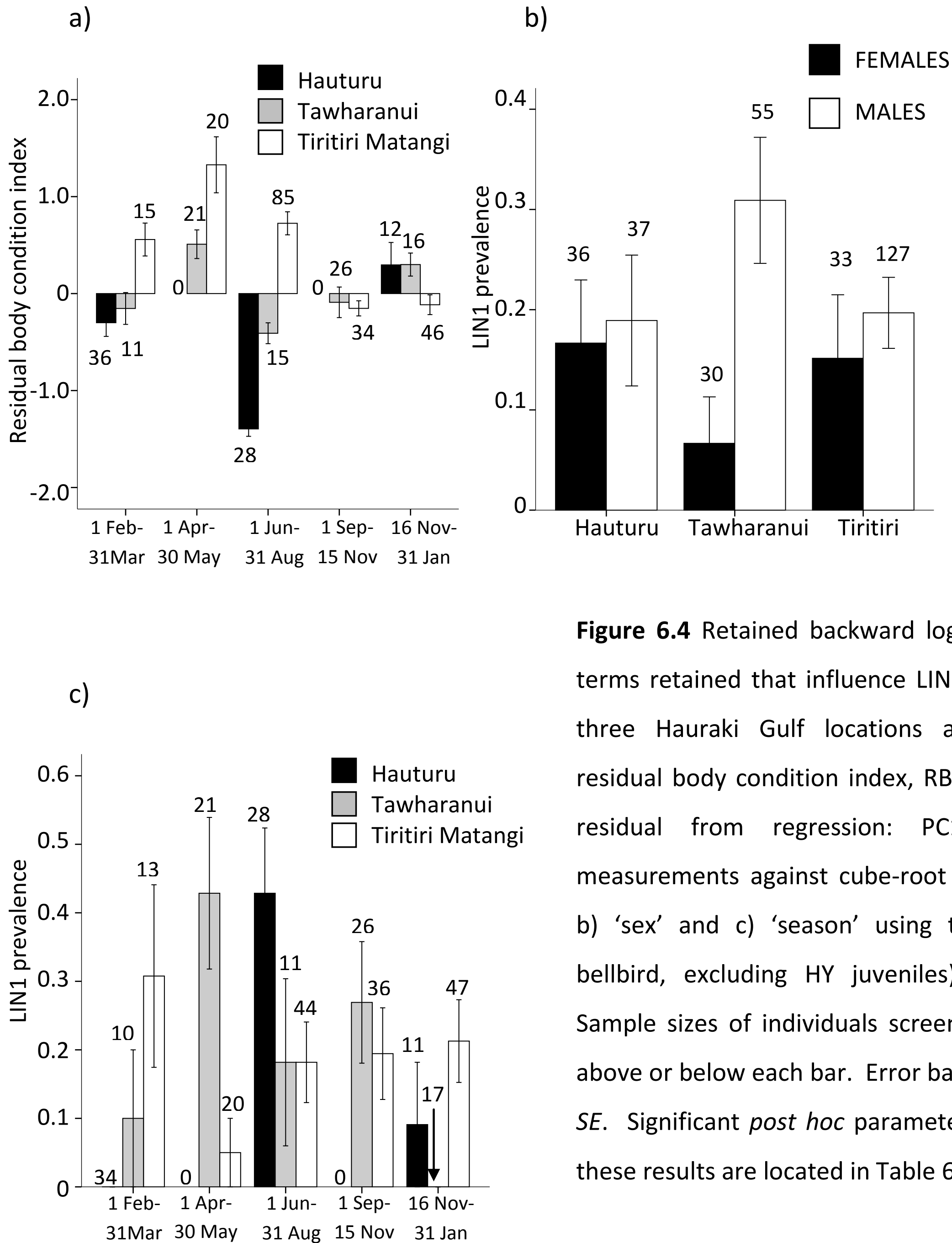


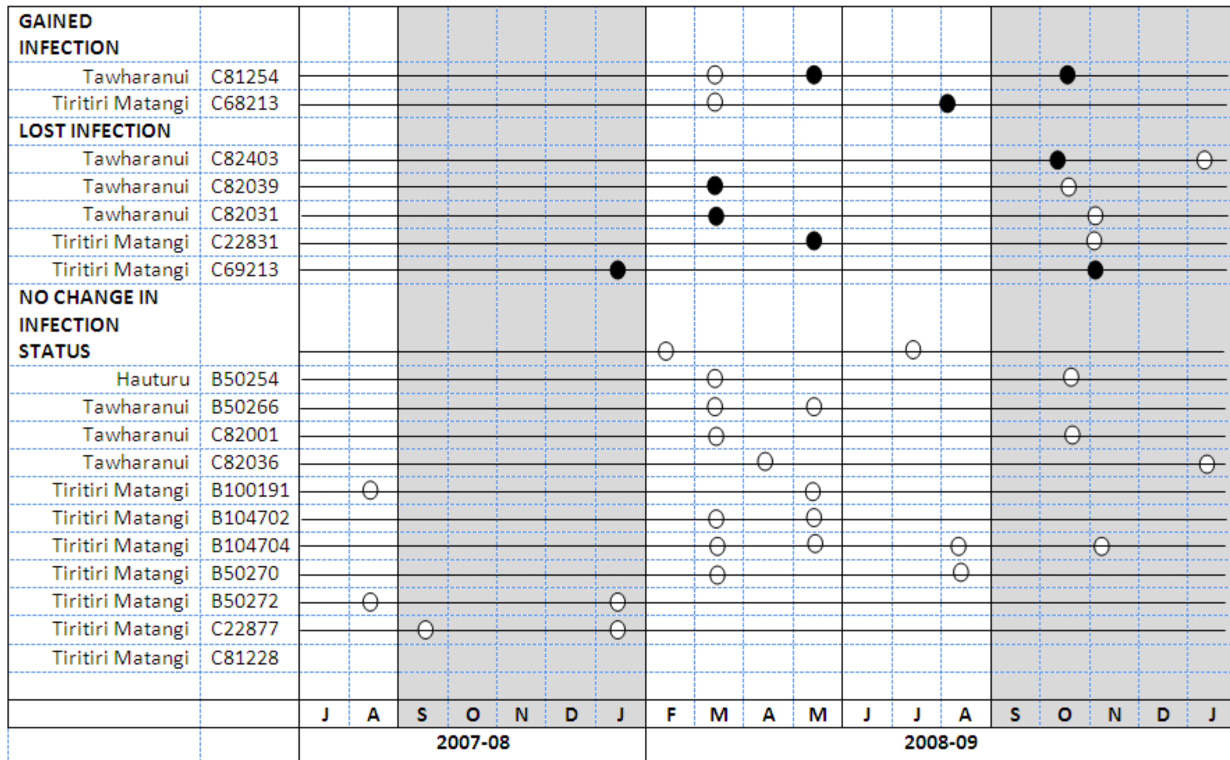
Figure 6.4 Retained backward logistic regression terms retained that influence LIN1 prevalence at three Hauraki Gulf locations a) 'season and residual body condition index, RBCI' (studentized residual from regression: PC1 of skeletal measurements against cube-root of body mass), b) 'sex' and c) 'season' using the AHY (adult bellbird, excluding HY juveniles) only dataset. Sample sizes of individuals screened are located above or below each bar. Error bars represent ± 1 *SE*. Significant *post hoc* parameter estimates for these results are located in Table 6.3b.

birds (see Figure 6.3b). These age class differences in LIN1 infections at Tawharanui appear to be largely due to low HY sample sizes (ranging from 0-1 individuals screened between April and November). Unlike the other two locations, at Tiritiri Matangi the overall regression model was not statistically significant (P = 02), thus LIN1 prevalence does not vary significantly according to sex, age, body condition and season at that host subpopulation (Figure 6.3c; Table 3a).

Because of the possible influence of data gaps and low sample sizes in the HY dataset, especially at Tawharanui, we performed the backward regression analyses a second time with only the AHY dataset (Table 6.3b). The significant terms retained explained that 1) at Hauturu LIN1 infections have a direct negative relationship with body condition that varies by season (see Figure 6.4a), 2) at Tawharanui sex differences and seasonal variation mainly influence LIN1 infections (Figure 6.4b; Table 6.3b) and 3) at Tiritiri Matangi there was no statistical variation in LIN1 infections by sex, body condition and season (see Figure 6.4a-c; Table 6.3b). Using AIC model selection in an information theoretic approach, we found that the single-term model 'season' best explained LIN1 variation at both Hauturu and Tawharanui (Appendix C: Table C.2). At Tiritiri Matangi on the other hand, the 'sex' model had the lowest AIC score (Appendix C: Table C.2).

6.4.3 *Gain and loss of infection*

Eighteen bellbirds were sampled more than once within a 3-10 month period (Figure 6.5). Thirty-nine percent (N = 7/18) of these birds tested positive at least once for LIN1 infection in the peripheral blood stream: 2 gained, 4 lost, and 1 retained pre-existing infections (Figure 6.5). The two birds that gained infection both tested negative in late autumn, and are malaria positive when tested again during winter. One of these birds, when tested a third time was found to have retained infection from autumn through summer. Of the four birds that lost infections, three were positive in late autumn/winter, but had lost infection by summer. The fourth bird to lose an infection, originally tested positive in early breeding season and then tested negative by late summer. Infection status of one bird was positive when tested during two consecutive breeding seasons 2007-08 and 2008-09 (Figure 6.5).



N = 18; $N_{\text{NOT INFECTED}}$ = 11 (61 %); N_{INFECTED} = 7 (39 %): $N_{\text{GAINED INFECTION}}$ = 2; $N_{\text{LOST INFECTION}}$ = 4; $N_{\text{NO CHANGE IN INFECTION STATUS}}$ = 1

Figure 6.5 Results from PCR on cytochrome *b* mitochondrial DNA screening for *Plasmodium* infection in individual bellbirds (*Anthornis melanura*) that were sampled more than once during the period of July 2007 and January 2009. The black dots represent birds that tested positive for LIN1 infections, clear dots indicate negative infections. No *Plasmodium* lineages other than LIN1 were detected more than once in any given individual host. The grey shading areas demark the breeding season, and vertical lines divide among seasons designated in this study.

6.5 Discussion

6.5.1 *Spatial and temporal malaria patterns within a host metapopulation*

We found that four distinct avian malaria lineages exist among three very different host subpopulations. Despite considerable habitat and demographic (stable island population or re-

colonizing mainland population) heterogeneity, the bellbird parasite assemblages we observed are homogeneous among subpopulations. In a previous study on the population and re-colonization genetics of the bellbird host within the Hauraki Gulf, we illustrate high levels of bellbird dispersal among Hauturu, Tawharanui and Tiritiri Matangi (Chapter Three; see also Brunton et al. 2008). Our results imply that active dispersal among subpopulation components of a metapopulation can have a homogenizing effect on the parasite population. It is obvious that the invertebrate vector(s) essential for completion of the parasite life cycle for LIN1, LIN2 and LIN3 is present at all three locations. However, in another study on the biogeography of bellbird malaria parasites, we found that the global distribution of LIN1 parasites may be restricted to the Hauraki Gulf region, or at least north of the central North Island of New Zealand (Baillie and Brunton 2011; Chapter Five). Essentially, there are obvious limits to which host dispersal can maintain parasite population connectivity.

Though parasite assemblages and overall prevalence do not vary within the Hauraki Gulf bellbird metapopulation, bellbird parasites exhibit substantial plasticity in seasonality dependent on location. LIN1 prevalence at Tawharanui and Tiritiri Matangi peaks in autumn and spring, though not statistically significantly at the latter location. Findings at these two locations are consistent with the Beaudoin theoretical model of seasonal autumn and spring peaks in avian malaria parasite prevalence (Beaudoin et al. 1971). On the contrary, we detected a marked peak in prevalence at Hauturu during winter that was not consistent with the Beaudoin model. This anomaly of seasonal variation in infection at Hauturu is unlikely the result of fluctuations in vector transmission or parasite-mediated population cycles (Hudson et al. 2002), because vector activity is likely low during winter. The Hauturu subpopulation is genetically most diverse the three subpopulations studied seasonally as suggested by mitochondrial and microsatellite DNA data (Chapter 4), suggesting that reduced patterns of parasite resistance is not due to lack of variability (see Little and Ebert 2001; Westerdahl et al. 2004). There is a clear need for more studies on other diseases present at these locations and the relationships between landscape heterogeneity, vector abundance and host effects on host infection (Wood et al. 2007).

6.5.2 *Sex-biased infection varies among different host subpopulations*

A key finding of this study is that the influence of both sex and body condition on parasite prevalence not only exhibited temporal variation, it varied differentially among three locations. Our results show that malaria infections in adult males (24%) at Tawharanui are more than three times higher than in adult females (7%), especially at the end of the breeding season (LIN1 prevalence in males > 60%). These marked sex differences in infection at Tawharanui, however, are not exhibited at Hauturu or Tiritiri Matangi. In most vertebrate systems, males generally have higher parasite levels than females and this is attributed to an energetic trade-off in which males balance the costs of immune function against the benefits of territory and mate acquisition and/or maintenance (Poulin 1996; Schalk and Forbes 1997; McCurdy et al. 1998; Nunn et al. 2009). Bellbird aggression levels are visibly high, and such behaviour may exist at an energetic cost. The sex-age dominance hierarchy assorts adult males at the top and juvenile females at the bottom, thus adult male bellbirds could indeed experience greater energetic costs in establishment and maintenance of territory (Craig et al. 1982; Craig and Douglas 1986). Females also defend territory, but this behaviour appears to be more temporally restricted, e.g., during incubation (SMB, personal observation). According to the Bateman's Principle, the dispersing sex is thought to trade-off immunity for resource gains (Rolff 2002; see Perez-Tris and Bensch 2005). In bellbirds, however, females are the dispersing sex (see Chapter Three). Contrary to that rationale based on mammalian systems, we propose that in bird populations that socially aggregate in high numbers dispersal may be an effectual strategy by which the genetic benefits far outweigh the costs and allow for greater diversity in genetic resistance to disease.

The disparity in sex-biased malaria infection rates among different subpopulations, however, presents an interesting conundrum. Though males have higher infection rates than females at Hauturu and Tiritiri Matangi, these differences are not statistically significant. Thus we consider that sex differences may be situation dependent. The most parsimonious explanation for differences among locations in sex-biased malaria prevalence has its

foundations in population growth stage and acquisition of new territory and nesting sites. At the re-colonizing Tawharanui subpopulation, new unclaimed territories were still being acquired at the time of our study, whereas territories and nesting sites are long established at Hauturu and Tiritiri Matangi with high site fidelity of the mating pair (Dianne Brunton, unpublished data). Simply, at re-colonizing subpopulations malaria prevalence in males will be higher than females, due to comparatively greater energetic cost of territory instability than at demographically stable subpopulations at carrying capacity. It could be that sex-bias in infection will abate in future as territory establishment stabilizes at Tawharanui. Ultimately, the spatiotemporal variation we observed among the Hauraki Gulf bellbird metapopulation, indicates a plasticity in sex-biased immune response possibly due to heterogeneity in demographic history. It is interesting to note that testosterone might indirectly influence immunity by altering male behaviour and hence resource acquisition (e.g., Buchanan et al. 2001). This would not conflict with the idea of sexually dimorphic immunity but would question the mechanism that guarantees honest signalling as proposed by Folstad and Karter (1992) in the immunocompetence handicap hypothesis.

6.5.3 *Paradoxical relationships between parasite prevalence and body condition in bellbirds*

Body condition in bellbirds paradoxically varied both positively or negatively with malaria prevalence among seasons depending on location. This apparent discrepancy can be philosophically resolved in a trade-off between reproductive effort and immunity (Hamilton and Zuk 1982; Sheldon and Verhulst 1996; Moller 1997). Avian malaria parasitism has been shown experimentally to exact a cost to its host in terms of reproductive success and post-breeding body condition where infected birds experience a decrease in body condition (Moller et al. 2000; Marzal et al. 2008). Oppositely, infected birds have also been found to increase body condition in order to maximize current reproduction, but this can only occur when energetic resources are not limiting (Marzal et al. 2008). A similar paradoxical relationship in resistance to blood parasites to that revealed in our study, has been detected in other studies where high infection rates are associated with both lower (Merino et al. 2000; Marzal et al. 2005) and higher (Ots and Horak 1998; Wiehn et al. 1999; Marzal et al. 2008) reproductive

output and body mass. Based on the findings in this study, we suggest that density-dependent immunosuppression (Stjernman et al. 2004; Valera et al. 2006; Dolnik and Hoi 2010) associated with resource limiting factors on islands might explain the observed differences in the 'sign' (positive or negative) of the relationship between body condition and parasite prevalence among different subpopulations. At Hauturu competition for food is at a premium during winter compared to Tiritiri Matangi where food supply is in surplus (because the diet is supplemented with unlimited sugar water in artificial feeders). Thus the negative sign of the relationship at Hauturu may reflect food supply as a limiting factor. In less densely populated locations, this relationship can be reversed and infected birds gain body fat upon infection to offset the cost of infection when resources are plentiful (Marzal et al. 2008). Interestingly, we find no sex differences (in adult birds) in the sign of the relationship between malaria infections and body condition implying that the interaction between limited resources and parasite costs are exacted upon both sexes similarly in this bellbird system. In the direct sense, dense host populations facilitate disease by facilitating contact of hosts. Indirectly, however, density is related to resource limiting factors that can tip the scales of energetic costs such that they compromise the host's ability to resist disease. Separating susceptibility from exposure given patterns of infection in natural populations is one of the greatest challenges in the study of disease dynamics in wild populations today (Paterson and Piertney 2011).

6.5.4 *Conclusions*

In conclusion, we outline four main findings from this study on spatial and seasonal variation in avian malaria parasites infecting an endemic host. First, overall levels of avian malaria parasite prevalence did not vary among three different subpopulations suggesting that dispersal within metapopulations homogenizes parasite assemblages. Second, seasonality of parasite infections did not follow a strict bimodal pattern and there is substantial plasticity in seasonality dependent on location. Third, male sex-bias in malaria infection rates was most pronounced at a newly re-colonizing host subpopulation. Thus, it is apparent that sex-biased immune response is plastic due to heterogeneity in demographic history (stable versus re-colonizing populations). Fourth, body condition in bellbirds can vary positively or negatively with malaria

prevalence. The sign of this relationship is likely influenced by food supply as a limiting factor that constrains allocation of a host's energy to immune function. A lack of long-term disease records collected over a network of spatial locations is a serious limiting factor to this and most studies involving quantitative analyses and predictive models of ecological and disease patterns (Pascual and Dobson 2005). We encourage ongoing research in this population, which is an excellent model for study of host-parasite-vector ecology. An engaging frontier approach to understanding host-pathogen ecology in the natural environment is the application of 'wild immunology' where qPCR can be used to profile different immune components, including parasitemia levels, in wild host populations (Knowles et al. 2011; Paterson and Piertney 2011; Pedersen and Babayan 2011). Identifying the immunological, ecological and environmental factors underlying variation in prevalence and parasitemia in avian malaria is a critical step toward predicting and understanding how long-term environmental changes will impact the evolution and health of host-parasite-vector systems.

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CHAPTER SEVEN

Synthesis and future research directions

7.1 Introduction

The main motivation for my thesis, and a central goal in ecology, is to elucidate the processes that structure biological community. Studies, such as in this thesis, using common and mobile model species can contribute to the resolution of how climate, geography and ecological interactions determine community composition and evolution (Hickerson et al. 2010). Genetic issues need to be considered when designing means to alleviate the effects of habitat loss and global climate change on wild populations (Frankham 2010). A mosaic of relatively homogeneous patches within a continuous metapopulation is the evolutionary ideal distribution of genotypes for many species (Parisod and Bonvin 2008). By altering our global ecology, we humans have generated a large departure from this evolutionary ideal in the forms of accelerated global climate change, habitat loss and fragmentation and the re-distribution of predators and exotic diseases to novel prey and hosts (Reusch and Wood 2007). Small landmasses and endemic hotspots, such as Pacific islands, are especially vulnerable to anthropogenic perturbation and tend to undergo frequent local extirpations and severe bottlenecks (MacArthur and Wilson 1963, 1967; Laurance 2010). With this thesis, I explore how vagile avian dispersers might maintain gene flow if habitat protection is tactically emplaced (Fuller et al. 2010).

Ultimately with this concluding chapter, I aim to demonstrate the contributions my thesis makes to science in three interconnected areas 1) re-colonization and genetic connectivity of metapopulations across fragmented landscapes, 2) biogeographical origins of a host species and its host-parasite relationships and 3) evolution of immune phenotype with

regard to immune defense trade-offs and sex dimorphism in parasite resistance. Additionally, I outline several points of improvement to my study and where future work could be targeted to expand on the findings of this research.

7.2 Main findings

7.2.1 *Metapopulation dynamics and genetic connectivity*

My work, for the first time outlines the importance of dispersal ability of the bellbird in shaping its broad genetic diversity and distribution today. Contrary to many other endemic species among the island archipelago of New Zealand for which genetic studies have been documented (e.g., short-tailed bat, Lloyd 2003; Campbell's *Maoricicada*, Hill et al. 2009; the *Kikihia subalpina* complex, Marshal et al. 2009; kokako, Murphy et al. 2006; robin, Miller and Lambert 2006; brown kiwi, Baker et al. 1995, Burbidge et al. 2003), bellbirds have high levels of dispersal. Most bellbird populations were in mutation-drift equilibrium at the time of this study. Specifically, a continuous distribution migration model with strong female sex-biased and often bi-directional dispersal best characterizes the mode of genetic differentiation in bellbirds. These findings indicate that bellbirds would have dispersed continuously through the New Zealand landscape, at least in recent history.

However, there are departures from migration-drift equilibrium at the urban site and the previously deforested remnant islands adjacent to mainland areas from which the bellbird has been extirpated. A genetic bottleneck was detected in only one of these cases, Tiritiri Matangi which is a replanted reserve that had been intensively farmed in the recent past. Taken together, even vagile species with high re-colonization probability are subject to the limiting effects of poor habitat quality. Generally though, bellbirds do exhibit island to mainland genetic connectivity and the re-colonization potential of island sites appears to be high due to dispersal activity, with the exceptions of the Poor Knights Islands and the Sub-Antarctic Auckland Islands. Finally, natural re-colonizations in bellbirds can be characterized by high founder numbers, a lack of genetic bottleneck and rapid population growth.

The findings in this study indicate that additional future habitat loss will lead to further reductions in gene flow even for the more mobile species (Laurance 2010). A re-thinking of the New Zealand reserve system toward a more spatially conscious conservation strategy that encourages secondary contact and genetic admixture among population fragments would be prudent (Palsboll et al. 20007; Fuller et al. 2010). Such a strategy will allow bellbirds to re-colonize sites based on their own habitat preferences rather than relying on intensive artificial feeding and translocation. The findings of this thesis provide an exemplar model for a functional spatial genetic metapopulation that can be used as a guideline for natural genetic processes that can be imitated through translocation of more endangered species.

7.2.2 *Biogeography of birds and their parasites*

My findings have filled a wide gap in our knowledge about the processes underlying the genetic variation observed in a more mobile and understudied species in New Zealand and mechanisms that prevent genetic divergence and allow birds to adapt to changing forest, physical barrier and climatic environment conditions. Support for a phylogeographic hypothesis of repeated periods of allopatry followed by secondary contact among two, a northern and a southern, distinct lineages of bellbirds is given within this thesis. These two lineages are co-distributed and genetically admixed throughout the country today. The ancient 'incomplete' barrier to gene flow was associated with the present day Cook Strait, which has periodically drained and flooded due to sea level oscillations of the Pleistocene. This research provides evidence of an interesting idea that ancestors of bellbirds at the modern day Poor Knights Islands would have arrived on the islands no earlier than the mid- to late Pleistocene, *c*. 200 kyr.

From a more contemporary perspective on biogeography, the distribution of bellbirds and their *Plasmodium* infections is highly heterogeneous, i.e., not all parasites completely overlap geographically with the bellbird. Three of the four parasite lineages detected here have wide host and geographical distributions hence can be considered to be generalist parasites. The only malaria lineage present throughout the country was a cosmopolitan species, LIN2 or *P. elongatum* GRW06 (an exact match with GenBank lineage Acc. No. DQ368381). My

phylogenetic analysis provides strong evidence that this lineage and the two other putatively exotic lineages [the unclassified predominantly African lineages LIN3 *P.* (*Novyella*) sp. and LIN4 *P*. (*Haemaboeba*) *relictum*] have been recently introduced to New Zealand. An unclassified LIN1 *P.* (*Novyella*) sp. comprises 80% of positive infections in bellbirds. This lineage is restricted to the Hauraki Gulf bellbird metapopulation. These parasites are likely endemic to New Zealand because they are phylogenetically distinct from other malaria parasites and their closest matches are two parasite lineages that infect taxa closely related to the bellbird. The LIN3 and LIN4 parasites are similarly restricted to the northern sampling locations as LIN1, however, the vectors for these exotic lineages may not have established yet (Derraik et al. 2004). It is unknown as to whether these are 'sleeper' emergent diseases in which virulence may increase as exotic mosquitoes establish or relationships with endemic mosquitoes develop (Tompkins and Gleeson 2006).

7.2.3 *Evolution of immune phenotype*

Immune phenotype is likely influenced simultaneously by compounding factors, e.g., host density, insularity, climate and other diseases (Lee 2006). In this study, I found that parasite seasonality was bimodal with spring and autumn prevalence peaks at two of three locations. These bimodal patterns reflect general expectations for malaria seasonality in temperate climates (Beaudoin 1971; Cosgrove et al. 2008; Knowles et al. 2011). The unimodal pattern in parasite prevalence at Hauturu is distinctive among bird malaria studies and informative on a different level. Here, the peak in prevalence during winter may reflect energy resource limitations, especially considering low body condition during winter. Additionally, I detected a unique paradoxical relationship between parasite prevalence and body condition. The 'sign' of this relationship is 'negative' on islands where the host population is at carrying capacity, and 'positive' at the growing/re-colonizing mainland population. Though the effects of insularity and population growth stage cannot be controlled for, I suggest that a density-dependent effect is the most parsimonious explanation for 'sign' differences and this could be indirectly related to conspecific competition for food resources. Another major finding in this thesis is

that parasite prevalence is sex-biased and higher in males, but significantly only at the growing colony where territory establishment would be most unstable. I posit that sex differences in *Plasmodium* infections will dissipate with time as the population becomes established and more stable. Finally, the sign of the relationship between parasite prevalence and body condition did not vary among the sexes. This is another important and novel finding because under ICHH and according to our male bias in parasite prevalence, we would expect the sign to be negative for males and positive for females if males invest more in reproduction or costly territory defense than females. It could be that energetic investment by bellbirds is buffered by equal partitioning of energy devoted to territory defense and parental care, whereas in a less egalitarian and more polygamous mating system such as that of the hihi, the 'sign' might vary among the sexes. A longitudinal study on sex differences in nutrition food, life-history (e.g., longevity, territory defense, parental investment) and fitness consequences of malarial infection will be illuminating.

7.2.4 *Filling in the gaps*

The following is a list of knowledge gaps and improvements to study design which are adapted into my outline for future research below:

1. A foremost improvement to my study design with regard to metapopulation dynamics and host-parasite interactions is to extend the study time frame over at least several years, if not decades to provide a comprehensive longitudinal investigation that more accurately depicts the ecological complexity of this system.
2. Equally important is a spatial expansion of the sampling regime. Inclusion of the Coromandel Peninsula and the Hens and Chickens Islands, especially, will provide an almost complete representation of the Hauraki Gulf metapopulation. More intensie sampling of both the North and South Island mainland is required. Moreover, my conclusions on the genetic population structure and barriers to gene flow will be better supported by utilizing sampling locations intermediate to those in this study. A more comprehensive spatial

sampling regime will permit biogeographic exploration within a landscape genetics approach that incorporates biotic and abiotic habitat variation with genetic variation.

3. Information on measures of bellbird fitness, e.g., reproductive rates, nestling survival, longevity, are required to assess the fitness costs of malaria infections.
4. The measures of intensity of malarial infection, or parasitemia (the density of parasites), are scarce in the literature today. However, parasitemia is more informative than prevalence with respect to host immunocompetence after initial infection. In March 2011, a first field test of new molecular technique was published that allows quantification of malaria parasitemia using qPCR (see Knowles et al. 2011). Any future research on bellbird malaria should incorporate this revolutionizing technique.
5. Knowledge of other diseases affecting the bellbird host would help assess parasite-parasite interactions that greatly influence hosts, e.g., perhaps the Hauturu bellbirds are immune compromised by a form of disease not measured in this study.
6. Future research regarding how to measure differential habitat effects on parasite prevalence and host body condition might involve measuring 'absolute' food intake using the carbon and nitrogen measures as well as the relative influence of carbohydrate intake to protein.
7. The bellbird phylogenetic trees should be rooted before publication in peer reviewed journals with the tui sister taxon control region mitochondrial DNA sequence data. Though my overall results are unlikely to change, a more closely related taxon will provide a better sequence alignment.
8. Phylogenetic concordance of several more genetic markers should be used in future to assess to allopatry, isolation with migration and introgressive hybridization. Autosomal and sex-linked markers are beginning to be used in biogeographic studies, and can give information on selective pressures.

7.3 Conclusion

A better understanding of the genetic influences on immune phenotype is key to our assessment of future effects of environmental and climate change on wild animal metapopulations (Reusch and Wood 2007). My research in this thesis provides invaluable insight into the fundamental biology of bellbird metapopulation dynamics by highlighting the relationship between habitat and genetic diversity, as well as the ecological complexity of avian malaria infections in natural populations. My observations on sex dimorphism in malaria prevalence lead me to suggest that non-exclusive proximate factors involving host population demographics and the relationship between parasite prevalence and body condition are ultimately related to habitat quality.

There are only a handful of studies, as presented in this thesis, on wild model systems that detail the spatiotemporal nature of host-parasite interactions, e.g., great reed warbler in Sweden (Westerdahl et al. 2004; Perez-Tris and Bensch 2005; Bensch et al. 2007); Hawaiian honey creepers (Foster et al. 2007; Freed et al. 2007; Atkinson and Samuel 2010); blue tit in England (Wood et al. 2007; Cosgrove et al. 2008; Knowles et al. 2011). My study on the New Zealand bellbird is unique to the Southern Hemisphere and complements the research in Sweden, England and Hawaii by offering a model system with a different suite of ecological, biological and behavioral interactions. The bellbird is insular as opposed to continental. They are non-migratory, thus parasites and dispersal can be studied year-round. Bellbirds have an evolutionary history with malaria and mosquitoes, whereas honey creepers in Hawaii do not (Atkinson et al. 1995). Thus, the effects of endemic versus exotic malaria can be compared using only one host species, the bellbird. Furthermore, unlike the other studies, the bellbird exhibits morphological sex dimorphism and females sing most of the year in territory defense (Brunton et al. 2008). This thesis and study system stand out in a global forum for comparison with ongoing and future studies on host-specificity, parasite biogeography and the future ability of wild mobile bird species to adapt to human-induced habitat barriers to gene flow, climate change and habitat loss. Moreover, the consequences of genetic diversity for immune response and fitness in natural populations are increasingly viewed as informative to human

epidemiology and immunology (Pedersen and Babayan 2011). This thesis serves the directive to understand how habitat loss relates to genetic diversity and in turn affects resistance to disease and the evolutionary potential of natural populations.

7.4 Future research directions

If we can decipher the additive influences of genetic diversity and habitat quality on host-parasite interactions it will help us better predict how animals will cope with environmental change through time. Recent studies have shown that immune responses to disease are the result of genetic variability, e.g., major histocompatibility complex (MHC) alleles; infection history, e.g., current prevalence, parasitemia; physiological condition, e.g., sex, age, life history strategy, reproductive state; resource availability, e.g., diet; abiotic conditions, e.g., seasonality and temperature; co-evolutionary history between host and parasite (Pedersen and Babayan 2011). Despite extensive research since the development of molecular PCR techniques on the distribution and complex interactions of birds, their environment and malaria parasites, very little is understood about the biological processes underlying variation in malaria prevalence (Knowles et al. 2011). As I outline in the previous section, integration of longitudinal field observation, landscape genetics and epidemiological approaches using exciting new and developing molecular techniques, e.g., qPCR, RNA transcriptome-wide analyses and next generation sequencing, will help future researchers characterize the genes both directly and indirectly related to immune phenotype (Paterson and Piertney 2011).

Building upon ongoing research of DHB and the findings within this thesis, a research program focusing on the Hauraki Gulf host-parasite-vector system could be established. In this manner, individual studies on the host, parasite and vector components could be integrated into a larger cohesive ecological study. In future, it is imperative that malaria parasitemia levels and prevalence be quantified using qPCR and feathers continue to be collected for nutritional intake assays of bellbirds. Also, different immune system components can be profiled using real-time PCR (Jackson et al. 2011). Approaching host-parasite interactions in this

comprehensive manner is becoming easier and less expensive as new molecular techniques are developed (Paterson and Piertney 2011). One aspect of host-parasite-vector research that I am particularly interested in is the relationship between habitat quality and sex dimorphism in immune phenotype and genotype. If habitat resources are driving female sex-biased dispersal in birds (Lee 2006), then I expect that food availability (as measured through stable isotope ratios; C:N:S) varies significantly with malaria prevalence and parasitemia. Also, dispersal and dispersal distances increase when times are tough (Coulon et al. 2008, 2010) and we need to recognize the consequences of animals dispersing further afield to mate amongst more genetically differentiated population patches. There is immense potential for novel scientific and 'deep immunological' discovery on evolutionary strategies that cope with the negative effects of reduced genetic diversity due to poor quality habitat.

7.5 Literature cited

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Appendix A: *A. melanura* microsatellite DNA data

Table A.1 Characterization of eight microsatellite loci in the New Zealand bellbird pooled for Poor Knights Islands, Hauturu, Tawharanui, Tiritiri Matangi, Hunua, Tongariro, Kapiti, Kaikoura, Dunedin and Auckland Islands a priori populations. Parameters shown: number of individuals genotyped, *N*; number of gene copies, *k*; observed heterozygosity, H_O; expected heterozygosity, H_E.

Locus	Acc. No.	*N*	Allelic size range	*k*	H_O	H_E
Ame6	GU294309	742	206	16	0.39353	0.51315
Ame7	GU294310	742	210	15	0.52291	0.84105
Ame10	GU294312	742	193	8	0.65768	0.76231
Ame11	GU294313	742	225	9	0.60377	0.7127
Ame14	GU294314	742	225	18	0.55795	0.90868
Ame18	GU294316	742	219	6	0.28571	0.3841
Ame20	GU294317	742	245	6	0.54178	0.67819
Ame25	GU294319	742	240	11	0.65768	0.79181

Table A.2 Probability of deviation from Hardy–Weinberg equilibrium, P_{HWE}, as calculated in ARLEQUIN, for each of 8 microsatellite loci in the New Zealand bellbird *Anthornis melanura* at eight sampling locations. Poor Knights, Poor; Tiritiri Matangi, Tiri; Tawharanui, Tawh; Hauturu, Haut; Tongariro, Tong; Kapiti, Kapi; Kaikoura, Kaik; Dunedin, Dune; Auckland Islands, Auck. A sequential Bonferroni correction using $p_i = \alpha/(1 + k - i)$ at $\alpha = 0.05$ was used to correct for over significance of P_{HWE} .

Locus	Poor	Tiri	Tawh	Haut	Tong	Kapi	Kaik	Dune	Auck
Ame6	0.304	0.535	0.233	0.341	0.354	0.560	0.626	0.017	0.150
Ame7	1.000	0.016†	0.789*	0.000*†	0.052	<0.001*	0.962	0.175	<0.001*
Ame10	0.629	0.000*	0.746	0.749	0.153	0.821	0.435	0.663	0.663
Ame11	0.179	0.090	0.596	0.401	0.812	0.357	0.136	0.423	0.940
Ame14	0.034	0.000*†	0.904*	<0.001*	0.009	0.041	0.122	<0.001*†	<0.001*†
Ame18	0.717	0.367	0.417*	0.414	0.528	0.058	0.089	0.146	1.000
Ame20	1.000	0.287	0.588	0.762	0.949	0.107	0.192	<0.001*	0.434
Ame25	0.049	0.390	0.801	0.146	0.604	0.648	0.831	0.294	0.773

* Significant deviation from HWE following sequential Bonferroni correction

† Indicates where null alleles had been detected in MICROCHECKER

Table A.3 Percent missing data for eight microsatellite loci from 371 individual New Zealand bellbirds.

Locus	No. alleles	No. missing genes	Total no. genes	% missing data
Ame6	15	3	736	0.4
Ame7	14	6	730	0.8
Ame10	7	8	726	1.1
Ame11	8	7	728	1.0
Ame14	17	18	706	2.5
Ame18	3	6	730	0.8
Ame20	3	7	728	1.0
Ame25	10	9	724	1.2

Table A.4. Results of tests on linkage disequilibrium for each sampling location before Bonferroni correction as implemented in GENEPOP. Bonferroni's adjustment based on 28 paired-comparisons per sampling location: lower the *P*-value from 0.05 to 0.0017857.

Number of populations detected : 11
Number of loci detected : 8
Markov chain parameters
Dememorisation : 1000
Batches : 100
Iterations per batch : 1000

Pop	Locus#1	Locus#2	P-value	SE
Poor	Ame11	Ame20	0.08483	0.005618
Poor	Ame18	Ame20	0.11886	0.004794
Poor	Ame10	Ame18	0.1346	0.007023
Poor	Ame20	Ame25	0.14932	0.005131
Poor	Ame10	Ame14	0.15988	0.009707
Poor	Ame18	Ame25	0.19103	0.006964
Poor	Ame7	Ame10	0.21537	0.008612
Poor	Ame7	Ame25	0.23724	0.011528
Poor	Ame11	Ame18	0.24439	0.012734
Poor	Ame11	Ame14	0.24852	0.016211
Poor	Ame7	Ame14	0.26463	0.012063
Poor	Ame6	Ame10	0.33189	0.012733
Poor	Ame6	Ame25	0.3605	0.011237
Poor	Ame6	Ame18	0.45835	0.011252
Poor	Ame6	Ame20	0.56628	0.006752
Poor	Ame14	Ame20	0.5772	0.007323
Poor	Ame6	Ame14	0.58323	0.012998
Poor	Ame7	Ame20	0.61052	0.005814
Poor	Ame10	Ame11	0.65412	0.013243
Poor	Ame6	Ame11	0.66314	0.014239
Poor	Ame11	Ame25	0.76253	0.012441
Poor	Ame14	Ame18	0.82234	0.007421
Poor	Ame10	Ame20	0.82495	0.003935
Poor	Ame7	Ame11	0.89517	0.008103
Poor	Ame10	Ame25	0.90793	0.004867
Poor	Ame7	Ame18	0.9249	0.004201
Poor	Ame14	Ame25	0.9742	0.002549
Poor	Ame6	Ame7	1	0
Tiri	Ame6	Ame10	0.02269	0.003858
Tiri	Ame6	Ame18	0.02625	0.003892
Tiri	Ame10	Ame20	0.03068	0.003381
Tiri	Ame6	Ame14	0.03889	0.010836
Tiri	Ame10	Ame25	0.12395	0.006108
Tiri	Ame10	Ame18	0.15951	0.005404
Tiri	Ame6	Ame20	0.1701	0.020209
Tiri	Ame14	Ame18	0.17508	0.009414
Tiri	Ame10	Ame11	0.20248	0.009175
Tiri	Ame10	Ame14	0.20326	0.01199
Tiri	Ame6	Ame7	0.21449	0.026598
Tiri	Ame14	Ame25	0.33634	0.025585
Tiri	Ame7	Ame10	0.37168	0.013068
Tiri	Ame20	Ame25	0.46364	0.020824
Tiri	Ame11	Ame20	0.47944	0.017746
Tiri	Ame7	Ame25	0.5727	0.026349
Tiri	Ame11	Ame25	0.61985	0.01743
Tiri	Ame14	Ame20	0.62155	0.024628
Tiri	Ame18	Ame20	0.63785	0.01038
Tiri	Ame7	Ame11	0.64683	0.022472
Tiri	Ame7	Ame20	0.71535	0.02402
Tiri	Ame6	Ame25	0.79975	0.019159

Pop	Locus#1	Locus#2	P-value	SE
Tiri	Ame18	Ame25	0.81148	0.007231
Tiri	Ame6	Ame11	0.8431	0.014943
Tiri	Ame7	Ame18	0.95981	0.003461
Tiri	Ame7	Ame14	0.96959	0.008784
Tiri	Ame11	Ame18	0.97913	0.001517
Tiri	Ame11	Ame14	0.98824	0.003226
Tawh	Ame7	Ame10	0.00081	0.00081
Tawh	Ame14	Ame18	0.04939	0.01666
Tawh	Ame18	Ame25	0.06864	0.018098
Tawh	Ame10	Ame25	0.11053	0.024975
Tawh	Ame6	Ame11	0.12532	0.013383
Tawh	Ame11	Ame25	0.13347	0.020969
Tawh	Ame14	Ame20	0.13516	0.028881
Tawh	Ame7	Ame25	0.14289	0.028561
Tawh	Ame7	Ame14	0.16254	0.032891
Tawh	Ame10	Ame18	0.20545	0.023751
Tawh	Ame14	Ame25	0.21682	0.037226
Tawh	Ame18	Ame20	0.23117	0.019575
Tawh	Ame6	Ame7	0.24496	0.028032
Tawh	Ame20	Ame25	0.25192	0.030444
Tawh	Ame7	Ame18	0.2924	0.032662
Tawh	Ame6	Ame14	0.31136	0.035138
Tawh	Ame7	Ame11	0.3761	0.030354
Tawh	Ame11	Ame14	0.47365	0.03762
Tawh	Ame10	Ame14	0.53362	0.044432
Tawh	Ame10	Ame20	0.57988	0.029538
Tawh	Ame11	Ame20	0.67536	0.020488
Tawh	Ame6	Ame10	0.73148	0.026895
Tawh	Ame7	Ame20	0.73571	0.027434
Tawh	Ame11	Ame18	0.85003	0.014788
Tawh	Ame6	Ame20	0.87583	0.014676
Tawh	Ame6	Ame18	0.89345	0.014882
Tawh	Ame10	Ame11	0.97547	0.00693
Tawh	Ame6	Ame25	0.99507	0.002312
Haut	Ame7	Ame10	0.01278	0.006423
Haut	Ame11	Ame18	0.03763	0.006575
Haut	Ame7	Ame20	0.16165	0.026695
Haut	Ame11	Ame20	0.18707	0.01989
Haut	Ame14	Ame20	0.21421	0.029059
Haut	Ame18	Ame20	0.23695	0.012724
Haut	Ame14	Ame18	0.24869	0.027598
Haut	Ame7	Ame18	0.35168	0.030326
Haut	Ame14	Ame25	0.35784	0.043426
Haut	Ame18	Ame25	0.43907	0.019415
Haut	Ame6	Ame11	0.45594	0.04165
Haut	Ame10	Ame25	0.47527	0.034396
Haut	Ame6	Ame20	0.49022	0.033524
Haut	Ame6	Ame18	0.49331	0.027522
Haut	Ame10	Ame18	0.49665	0.020932
Haut	Ame10	Ame14	0.50744	0.044637

Pop	Locus#1	Locus#2	P-value	SE
Haut	Ame7	Ame11	0.56292	0.041005
Haut	Ame6	Ame25	0.57022	0.035914
Haut	Ame10	Ame20	0.58771	0.024237
Haut	Ame6	Ame10	0.60405	0.036582
Haut	Ame11	Ame25	0.61176	0.03012
Haut	Ame20	Ame25	0.64246	0.026227
Haut	Ame7	Ame14	0.74429	0.041989
Haut	Ame10	Ame11	0.76251	0.023973
Haut	Ame6	Ame14	0.855	0.031902
Haut	Ame7	Ame25	0.85684	0.029473
Haut	Ame6	Ame7	0.87609	0.0276
Haut	Ame11	Ame14	0.90942	0.022197
Hunu	Ame7	Ame18	0.07067	0.004913
Hunu	Ame11	Ame25	0.17857	0.010595
Hunu	Ame7	Ame11	0.30509	0.011237
Hunu	Ame10	Ame18	0.33758	0.005946
Hunu	Ame11	Ame20	0.37333	0.00562
Hunu	Ame14	Ame18	0.43802	0.011811
Hunu	Ame18	Ame25	0.47259	0.011381
Hunu	Ame10	Ame11	0.50247	0.003667
Hunu	Ame7	Ame10	0.57203	0.007077
Hunu	Ame6	Ame20	0.62911	0.004794
Hunu	Ame11	Ame18	0.63974	0.006261
Hunu	Ame7	Ame20	0.78148	0.006342
Hunu	Ame6	Ame7	1	0
Hunu	Ame6	Ame10	1	0
Hunu	Ame6	Ame11	1	0
Hunu	Ame6	Ame14	1	0
Hunu	Ame7	Ame14	1	0
Hunu	Ame11	Ame14	1	0
Hunu	Ame6	Ame18	1	0
Hunu	Ame10	Ame20	1	0
Hunu	Ame14	Ame20	1	0
Hunu	Ame18	Ame20	1	0
Hunu	Ame6	Ame25	1	0
Hunu	Ame7	Ame25	1	0
Hunu	Ame14	Ame25	1	0
Hunu	Ame20	Ame25	1	0
Hunu	Ame10	Ame14	No information	
Hunu	Ame10	Ame25	No information	
Tong	Ame14	Ame20	0.06458	0.013551
Tong	Ame6	Ame11	0.08725	0.016942
Tong	Ame6	Ame20	0.10959	0.009893
Tong	Ame6	Ame25	0.11712	0.017994
Tong	Ame6	Ame7	0.13764	0.018103
Tong	Ame10	Ame25	0.14576	0.02308
Tong	Ame10	Ame11	0.18918	0.021102
Tong	Ame6	Ame14	0.20222	0.029278
Tong	Ame7	Ame14	0.38206	0.046461
Tong	Ame18	Ame25	0.38365	0.016561
Tong	Ame11	Ame20	0.46738	0.017516
Tong	Ame7	Ame18	0.49502	0.018763
Tong	Ame6	Ame10	0.53735	0.027907
Tong	Ame11	Ame18	0.61676	0.015899
Tong	Ame7	Ame20	0.62676	0.020382
Tong	Ame10	Ame18	0.6941	0.012958
Tong	Ame7	Ame10	0.70935	0.033681
Tong	Ame20	Ame25	0.77336	0.016003
Tong	Ame18	Ame20	0.87995	0.004057
Tong	Ame11	Ame25	0.88833	0.019583
Tong	Ame14	Ame18	0.90285	0.012998
Tong	Ame6	Ame18	0.91492	0.005882
Tong	Ame10	Ame20	0.94811	0.006349
Tong	Ame7	Ame11	1	0
Tong	Ame10	Ame14	1	0

Pop	Locus#1	Locus#2	P-value	SE
Tong	Ame11	Ame14	1	0
Tong	Ame7	Ame25	1	0
Tong	Ame14	Ame25	1	0
Kapi	Ame7	Ame25	0.01254	0.007717
Kapi	Ame6	Ame10	0.07081	0.013077
Kapi	Ame6	Ame20	0.08267	0.008988
Kapi	Ame6	Ame25	0.14059	0.022996
Kapi	Ame10	Ame25	0.15127	0.024813
Kapi	Ame14	Ame20	0.31473	0.03143
Kapi	Ame6	Ame14	0.32593	0.036104
Kapi	Ame10	Ame18	0.38818	0.021816
Kapi	Ame11	Ame20	0.42115	0.012699
Kapi	Ame6	Ame7	0.42904	0.039339
Kapi	Ame7	Ame18	0.49065	0.031329
Kapi	Ame10	Ame20	0.54092	0.020281
Kapi	Ame11	Ame25	0.55573	0.022649
Kapi	Ame11	Ame18	0.57652	0.010937
Kapi	Ame11	Ame14	0.69767	0.023372
Kapi	Ame10	Ame11	0.70677	0.016826
Kapi	Ame7	Ame10	0.73233	0.034548
Kapi	Ame7	Ame20	0.74177	0.022095
Kapi	Ame20	Ame25	0.7573	0.019735
Kapi	Ame10	Ame14	0.8167	0.028093
Kapi	Ame6	Ame11	0.82852	0.010383
Kapi	Ame7	Ame11	0.84259	0.014355
Kapi	Ame6	Ame18	0.86374	0.015665
Kapi	Ame18	Ame25	0.88167	0.015947
Kapi	Ame7	Ame14	0.90079	0.027249
Kapi	Ame14	Ame18	0.9181	0.01425
Kapi	Ame18	Ame20	0.98853	0.0019
Kapi	Ame14	Ame25	1	0
Kaik	Ame18	Ame20	0.02058	0.002191
Kaik	Ame7	Ame20	0.05074	0.010449
Kaik	Ame7	Ame11	0.09763	0.020239
Kaik	Ame7	Ame25	0.19944	0.033676
Kaik	Ame10	Ame18	0.22151	0.013117
Kaik	Ame6	Ame14	0.25571	0.027425
Kaik	Ame20	Ame25	0.28114	0.026067
Kaik	Ame6	Ame10	0.32362	0.023347
Kaik	Ame18	Ame25	0.54309	0.016732
Kaik	Ame14	Ame25	0.55423	0.046116
Kaik	Ame6	Ame7	0.55862	0.022639
Kaik	Ame6	Ame20	0.5897	0.015804
Kaik	Ame10	Ame20	0.62767	0.022936
Kaik	Ame6	Ame18	0.62874	0.008999
Kaik	Ame10	Ame11	0.63657	0.028297
Kaik	Ame11	Ame18	0.64559	0.010831
Kaik	Ame14	Ame20	0.66108	0.029217
Kaik	Ame11	Ame14	0.66871	0.037468
Kaik	Ame10	Ame14	0.67244	0.038984
Kaik	Ame14	Ame18	0.68541	0.017439
Kaik	Ame11	Ame20	0.69044	0.016849
Kaik	Ame6	Ame25	0.83367	0.018646
Kaik	Ame10	Ame25	0.84792	0.025428
Kaik	Ame7	Ame18	0.88908	0.008213
Kaik	Ame6	Ame11	0.89607	0.010233
Kaik	Ame7	Ame10	0.89762	0.018604
Kaik	Ame11	Ame25	0.98606	0.005127
Kaik	Ame7	Ame14	1	0
DBot	Ame6	Ame11	0.03824	0.010898
DBot	Ame14	Ame18	0.04138	0.005731
DBot	Ame20	Ame25	0.05226	0.007865
DBot	Ame6	Ame14	0.08423	0.016905
DBot	Ame7	Ame11	0.1675	0.019497
DBot	Ame10	Ame20	0.30065	0.015643

Pop	Locus#1	Locus#2	P-value	SE
DBot	Ame7	Ame25	0.39006	0.03481
DBot	Ame11	Ame14	0.42079	0.033892
DBot	Ame11	Ame25	0.44768	0.027475
DBot	Ame7	Ame20	0.45052	0.021531
DBot	Ame7	Ame14	0.53357	0.044129
DBot	Ame7	Ame18	0.53362	0.016845
DBot	Ame6	Ame20	0.57397	0.022276
DBot	Ame14	Ame20	0.62337	0.025425
DBot	Ame6	Ame25	0.62735	0.028683
DBot	Ame11	Ame20	0.64338	0.018482
DBot	Ame7	Ame10	0.6679	0.026249
DBot	Ame18	Ame25	0.69039	0.011883
DBot	Ame10	Ame25	0.69864	0.021029
DBot	Ame14	Ame25	0.70018	0.034167
DBot	Ame10	Ame18	0.70641	0.008618
DBot	Ame10	Ame11	0.70859	0.019635
DBot	Ame11	Ame18	0.78	0.009893
DBot	Ame10	Ame14	0.80023	0.025827
DBot	Ame6	Ame7	0.88096	0.019812
DBot	Ame6	Ame10	0.9405	0.009938
DBot	Ame18	Ame20	0.96772	0.002322
DBot	Ame6	Ame18	1	0
DSil	Ame11	Ame18	0.07069	0.010177
DSil	Ame6	Ame25	0.10535	0.01399
DSil	Ame18	Ame20	0.11515	0.013713
DSil	Ame10	Ame20	0.13479	0.015852
DSil	Ame7	Ame20	0.13658	0.017247
DSil	Ame10	Ame18	0.20226	0.014655
DSil	Ame6	Ame7	0.20571	0.020484
DSil	Ame11	Ame20	0.23769	0.0205
DSil	Ame7	Ame18	0.24411	0.021569
DSil	Ame14	Ame20	0.27384	0.02159
DSil	Ame10	Ame25	0.28305	0.023139
DSil	Ame10	Ame11	0.29507	0.017941
DSil	Ame6	Ame18	0.34784	0.018968
DSil	Ame14	Ame18	0.40413	0.023231
DSil	Ame10	Ame14	0.46695	0.027441
DSil	Ame6	Ame14	0.61665	0.029986
DSil	Ame7	Ame14	0.64024	0.035152
DSil	Ame6	Ame11	0.69397	0.018476
DSil	Ame14	Ame25	0.76232	0.025771
DSil	Ame6	Ame10	0.86369	0.013309
DSil	Ame11	Ame14	0.88726	0.016024
DSil	Ame7	Ame10	0.89399	0.015892
DSil	Ame7	Ame11	0.91719	0.014364
DSil	Ame18	Ame25	0.92323	0.009702
DSil	Ame6	Ame20	0.93338	0.008754
DSil	Ame11	Ame25	0.96033	0.006761
DSil	Ame20	Ame25	0.96734	0.006931
DSil	Ame7	Ame25	1	0
Auck	Ame6	Ame10	0.07096	0.003293
Auck	Ame7	Ame10	0.34792	0.010106
Auck	Ame6	Ame11	0.19061	0.012247
Auck	Ame7	Ame11	0.67665	0.022983
Auck	Ame10	Ame11	0.21233	0.008708
Auck	Ame6	Ame14	0.15817	0.015757
Auck	Ame7	Ame14	0.23306	0.028467
Auck	Ame10	Ame14	0.03714	0.003462
Auck	Ame11	Ame14	1	0
Auck	Ame6	Ame18	0.18017	0.005311

Pop	Locus#1	Locus#2	P-value	SE
Auck	Ame7	Ame18	0.96452	0.002589
Auck	Ame10	Ame18	0.04429	0.002469
Auck	Ame11	Ame18	0.15055	0.00848
Auck	Ame14	Ame18	0.81069	0.00905
Auck	Ame6	Ame20	0.37753	0.010291
Auck	Ame7	Ame20	0.70487	0.013817
Auck	Ame10	Ame20	0.98364	0.001255
Auck	Ame11	Ame20	0.97096	0.004346
Auck	Ame14	Ame20	0.61068	0.023036
Auck	Ame18	Ame20	0.85216	0.004703
Auck	Ame6	Ame25	0.92544	0.003329
Auck	Ame7	Ame25	0.78076	0.00989
Auck	Ame10	Ame25	0.32266	0.006522
Auck	Ame11	Ame25	0.73198	0.010468
Auck	Ame14	Ame25	0.77699	0.015913
Auck	Ame18	Ame25	0.19274	0.005627
Auck	Ame20	Ame25	0.47583	0.009525
Auck	Ame6	Ame7	0.48397	0.012443

***P*-value for each locus pair across all populations (*N* = 371 individual bellbirds)**

(Fisher's method)

Locus#1	Locus#2	Chi2	df	P-Value
Ame6	Ame7	17.847614	22	0.714984
Ame6	Ame10	25.912273	22	0.255468
Ame7	Ame10	33.792865	22	0.051558
Ame6	Ame11	22.934544	22	0.405434
Ame7	Ame11	16.095478	22	0.811119
Ame10	Ame11	17.16835	22	0.754002
Ame6	Ame14	27.989154	22	0.176041
Ame7	Ame14	14.139315	22	0.896461
Ame10	Ame14	19.21965	20	0.507592
Ame11	Ame14	7.987898	22	0.997192
Ame6	Ame18	17.417601	22	0.739906
Ame7	Ame18	17.301143	22	0.746527
Ame10	Ame18	30.181883	22	0.11411
Ame11	Ame18	23.164391	22	0.392497
Ame14	Ame18	24.058783	22	0.344156
Ame6	Ame20	20.958488	22	0.523305
Ame7	Ame20	20.178244	22	0.571891
Ame10	Ame20	18.217766	22	0.693002
Ame11	Ame20	20.318799	22	0.563106
Ame14	Ame20	22.275959	22	0.443524
Ame18	Ame20	23.722289	22	0.361953
Ame6	Ame25	17.785959	22	0.718601
Ame7	Ame25	22.552553	22	0.427352
Ame10	Ame25	23.72433	20	0.254642
Ame11	Ame25	13.707079	22	0.911549
Ame14	Ame25	10.284877	22	0.983533
Ame18	Ame25	19.813909	22	0.594679
Ame20	Ame25	20.045476	22	0.580195

Table A.5. Tables of allelic frequencies for each of eight microsatellite loci at each of eleven sampling locations. Allele sizes are labelled across the top of each table. In the first column of each table are the sampling location abbreviations: Poor Knights, Po; Tiritiri Matangi, Ti; Tawharanui, Tw; Hauturu, Ha; Hunua, Hu; Tongariro, To; Kapiti, Kp; Kaikoura, Kk; Dunedin Botanical Gardens, Db; Silver Stream, Dunedin, Ds; Auckland Islands, Auck. The final column shows the number of gene copies for each locus at each location.

Locus: Ame6

	130	135	140	145	150	155	160	165	175	180	185	190	195	200	206	
Po	0.891	0	0	0.016	0.094	0	0	0	0	0	0	0	0	0	0	64
Ti	0.231	0.417	0	0.046	0	0.009	0	0	0	0	0	0.056	0.241	0	0	108
Tw	0.875	0.036	0.027	0.036	0	0.009	0	0	0	0	0	0	0.018	0	0	112
Ha	0.673	0.1	0.027	0.045	0	0	0.009	0	0	0	0.018	0.018	0.109	0	0	110
Hu	0.667	0.167	0.056	0	0	0	0	0	0	0	0.111	0	0	0	0	18
To	0.833	0.033	0.033	0.017	0.017	0	0	0	0	0.033	0	0	0	0.033	0	60
Kp	0.758	0.032	0.097	0	0	0.081	0	0	0	0.016	0.016	0	0	0	0	62
Kk	0.742	0	0.097	0.081	0	0	0	0	0	0	0	0.032	0	0	0.048	62
Db	0.771	0.021	0.062	0	0	0	0	0.021	0.021	0.042	0.062	0	0	0	0	48
Ds	0.714	0.048	0.095	0	0	0	0	0	0.095	0	0	0.048	0	0	0	42
Au	0.66	0.02	0.28	0.04	0	0	0	0	0	0	0	0	0	0	0	50

Locus: Ame7

	131	158	162	166	170	174	178	182	186	190	194	198	206	210	
Po	0	0.906	0.047	0	0	0.031	0	0.016	0	0	0	0	0	0	64
Ti	0.019	0	0.009	0	0	0	0.259	0.398	0.241	0.074	0	0	0	0	108
Tw	0.01	0	0.394	0.067	0	0.048	0.26	0.106	0.058	0.048	0	0	0.01	0	104
Ha	0	0.055	0.118	0.036	0.073	0.018	0.309	0.182	0.091	0.109	0	0	0	0.009	110
Hu	0	0.056	0.5	0	0	0	0.167	0.056	0.056	0.056	0.111	0	0	0	18
To	0	0.121	0.172	0.121	0.017	0.017	0.103	0.241	0.103	0.103	0	0	0	0	58
Kp	0	0.065	0.065	0.032	0.161	0.113	0.226	0.323	0.016	0	0	0	0	0	62
Kk	0	0.25	0.047	0.031	0.016	0.078	0.234	0.312	0.031	0	0	0	0	0	64
Db	0	0.08	0.18	0.06	0.02	0.2	0.16	0.28	0.02	0	0	0	0	0	50
Ds	0	0.167	0.095	0	0	0.095	0.119	0.452	0.048	0	0	0.024	0	0	42
Au	0	0.04	0	0	0	0	0.22	0.62	0.12	0	0	0	0	0	50

Locus: Ame10

	165	173	177	181	185	189	193	
Po	0	0.172	0.516	0	0.281	0.031	0	64
Ti	0	0	0	0.12	0.435	0.444	0	108

Tw	0	0.056	0.222	0.176	0.435	0.065	0.046	108
Ha	0	0.047	0.264	0.245	0.311	0.123	0.009	106
Hu	0	0	0.143	0.357	0.5	0	0	14
To	0	0.083	0.283	0.217	0.367	0.017	0.033	60
Kp	0	0.065	0.355	0.113	0.387	0.048	0.032	62
Kk	0.032	0.032	0.339	0.258	0.226	0.113	0	62
Db	0	0	0.38	0.28	0.3	0.04	0	50
Ds	0	0	0.452	0.214	0.214	0.071	0.048	42
Au	0	0	0.66	0.34	0	0	0	50

Locus: Ame11

	205	209	213	215	217	221	223	225	
Po	0.25	0.641	0.031	0	0.078	0	0	0	64
Ti	0.019	0.444	0.139	0	0.398	0	0	0	108
Tw	0.055	0.591	0.255	0	0.082	0.018	0	0	110
Ha	0.056	0.519	0.25	0	0.148	0.028	0	0	108
Hu	0.188	0.562	0.125	0	0.125	0	0	0	16
To	0.117	0.333	0.3	0	0.183	0.033	0	0.033	60
Kp	0.016	0.306	0.581	0	0.097	0	0	0	62
Kk	0.034	0.414	0.379	0.017	0.052	0.086	0.017	0	58
Db	0.04	0.16	0.66	0	0.06	0.08	0	0	50
Ds	0.095	0.286	0.476	0	0.095	0.048	0	0	42
Au	0	0.28	0.12	0	0.02	0.52	0	0.06	50

Locus: Ame14

	140	145	150	155	160	165	170	175	180	185	190	195	200	205	210	215	225	
Po	0	0	0	0.20	0	0	0	0.469	0.016	0	0.30	0.02	0	0	0	0	0	64
Ti	0	0	0	0.01	0.38	0.49	0.02	0.02	0.049	0.01	0	0.02	0	0	0	0	0	102
Tw	0	0	0	0.22	0.12	0.08	0.09	0.05	0.06	0.13	0.08	0.04	0.03	0.08	0	0	0.02	100
Ha	0	0	0.01	0.13	0.05	0.05	0.17	0.06	0.12	0.06	0.09	0.08	0.04	0.08	0.01	0.05	0	100
Hu	0.11	0	0	0	0.06	0	0.22	0	0.056	0.11	0.11	0.22	0.056	0	0.056	0	0	18
To	0	0.03	0	0.03	0.05	0.08	0.15	0.117	0.15	0.07	0.12	0.07	0.1	0	0.033	0	0	60
Kp	0	0	0	0.02	0.05	0.23	0.05	0.113	0.177	0.11	0.13	0.07	0.016	0.081	0.016	0	0	62
Kk	0	0	0	0.11	0.06	0.19	0.22	0.109	0.094	0.02	0	0.13	0.016	0.062	0	0	0	64
Db	0	0	0	0	0.09	0.11	0.07	0.087	0.37	0.07	0.02	0	0.109	0.087	0	0	0	46
Ds	0	0	0	0.03	0.05	0.03	0.1	0.2	0.325	0.2	0	0	0.025	0.05	0	0	0	40
Au	0	0	0	0.14	0.08	0.04	0.2	0.16	0.1	0.08	0.12	0.04	0.04	0	0	0	0	50

Locus: Ame18

	203	207	211	215	219	
Po	0	0.226	0.758	0.016	0	62

Ti	0	0	0.808	0.192	0	104
Tw	0	0.145	0.764	0.045	0.045	110
Ha	0	0.12	0.806	0.074	0	108
Hu	0	0.222	0.778	0	0	18
To	0	0.117	0.75	0.133	0	60
Kp	0	0.097	0.839	0.065	0	62
Kk	0	0.141	0.859	0	0	64
Db	0	0.18	0.82	0	0	50
Ds	0.02	0.31	0.619	0	0.048	42
Au	0	0.28	0.72	0	0	50

Locus: Ame20

	229	233	237	241	245	
Po	0	0.609	0.391	0	0	64
Ti	0	0.123	0.453	0.34	0.085	106
Tw	0.142	0.642	0.17	0.047	0	106
Ha	0.139	0.481	0.343	0.037	0	108
Hu	0.222	0.667	0.111	0	0	18
To	0.15	0.533	0.317	0	0	60
Kp	0.242	0.29	0.468	0	0	62
Kk	0.156	0.438	0.391	0.016	0	64
Db	0.16	0.38	0.34	0.12	0	50
Ds	0.175	0.4	0.375	0.05	0	40
Au	0.3	0.64	0.06	0	0	50

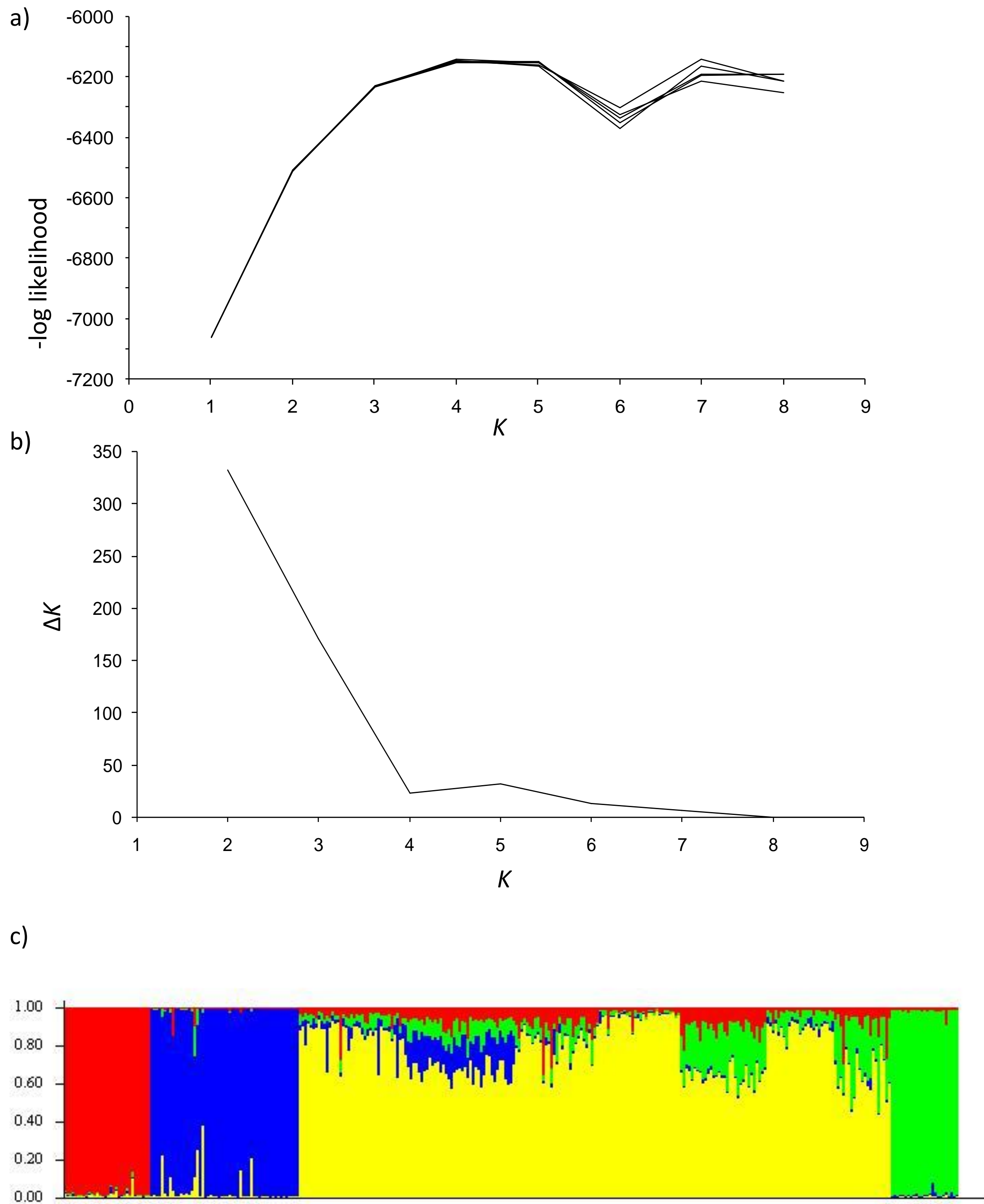
Locus: Ame25

	204	208	212	216	220	224	228	232	236	240	
Po	0	0	0	0.875	0.016	0.109	0	0	0	0	64
Ti	0.184	0	0	0.153	0.306	0.357	0	0	0	0	98
Tw	0.009	0.046	0.019	0.315	0.12	0.296	0.111	0.056	0.028	0	108
Ha	0.009	0.093	0	0.315	0.185	0.241	0.139	0.019	0	0	108
Hu	0.056	0.111	0	0.222	0.278	0.222	0.111	0	0	0	18
To	0	0.083	0	0.317	0.233	0.167	0.083	0.1	0.017	0	60
Kp	0	0.097	0	0.355	0.097	0.145	0.097	0.145	0.065	0	62
Kk	0	0	0	0.203	0.188	0.297	0.031	0.141	0.125	0.016	64
Db	0.02	0	0.02	0.42	0.1	0.18	0.2	0.06	0	0	50
Ds	0	0.048	0	0.238	0.119	0.381	0.143	0.071	0	0	42
Au	0	0	0	0.02	0.34	0.64	0	0	0	0	50

Table A.6 Confidence intervals (CI) for Jost's *D* estimates generated in program SMOGD for 8 microsatellite loci from bellbird locations of mainland New Zealand (including Hauturu and Kapiti islands). CI's show no overlap with zero and therefore panmixia cannot be rejected. The Jost *D* test shows supportive evidence for biologically significant genetic divergence among bellbird populations not only among Poor Knights, Tiritiri Matangi and the Auckland Islands populations, but across mainland New Zealand as well.

Locus	Bootstrapped Est.	Variance	Std. Error	95% CI min	95% CI max
Ame6	0.0280	0.0001	0.0003	0.0122	0.0488
Ame7	0.3142	0.0026	0.0016	0.2257	0.4046
Ame10	0.0867	0.0016	0.0013	0.0224	0.1569
Ame11	0.1652	0.0014	0.0012	0.1033	0.2351
Ame14	0.3775	0.0028	0.0017	0.2889	0.4731
Ame18	0.0236	0.0002	0.0005	0.0029	0.0552
Ame20	0.0849	0.0008	0.0009	0.0377	0.1389
Ame25	0.1154	0.0017	0.0013	0.0517	0.1985

Figure A.1 Evanno et al. (2005) method for determining number of population clusters, *K*. Shown are a) Log likelihood and b) Δ*K* plots by *K* for bellbird eight sampling locations, and c) the STRUCTURE bar plot generated using the admixture and *no priori* location settings.



Appendix B: *A. melanura* mitochondrial DNA data

Figure B.1 Partial mitochondrial cytochrome *b* sequence alignment for 6 New Zealand bellbird (*Anthornis melanura*) haplotypes.

```
SB389Ha   G C T A C T T C A C C A T C A T C C T A A T C C T A T T C C C C C T A G C A A G C A T T T T A G [ 48]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [ 48]
SB545Tw   - - - - - - T - - - - - - - - T - - - - - - - - - - - - - - - - - - - - C A - - - - T - - - - - [ 48]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - [ 48]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [ 48]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [ 48]

SB389Ha   A A A A C A A A C T C C T A A A C C T C T A A T C A A C T C T A A T A G T T T A T A A A A A C A [ 96]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [ 96]
SB545Tw   - - - - - - - A - - C C T - - - - - - - - - - - - - - - - - - - - T - - - - - - T A - - - A - - [ 96]
SB620To   - - - - - - - - - - - - - - - - - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - [ 96]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [ 96]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [ 96]

SB389Ha   T T G G T C T T G T A A A C C A A A G A T T G A A G A T T A T A C C C C T T C T T A G A G T T T [144]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [144]
SB545Tw   - - - - - - - T - T A - - - - - - - - - - - - A - - - - - A - - - C C - - - - - T - - - - - - - [144]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [144]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [144]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [144]

SB389Ha   T A C T G T C A G A A A G A A A G G A G T C G A A C C T T C A T C A C C A G C T C C C A A A G C [192]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [192]
SB545Tw   - - - - - - - A - A - A - - - A - - - - - - - - - - - T - - A - - - - - A - - - - - - - - - - - [192]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [192]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [192]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [192]

SB389Ha   T G G A A T T T T C A A C T A A A C T A C T T T C T G A C C C C C C A A T T A A A C C G C T C G [240]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - T - - - - - [240]
SB545Tw   - - - - - - - - - - - - - - - - - - - - - T - - - T - - - - - - C - A - T - - - - - T - C - - - [240]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [240]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - T - - - - - [240]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [240]

SB389Ha   A A T A G C C C C C C G A G A T A A C C C C C G C A C A A G T T C T A A A A C C A C A A A T A A [288]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [288]
SB545Tw   - - - - - - - - C - C - A - - - - A - - - - C - C - - - - - - - - - - - - - - - - - - - - - A - [288]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [288]
SB655Po   - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [288]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - A - - - - - - - [288]

SB389Ha   A G T T A A C A A C A A A C C T C A C C C A G C A A T C A A G A A A A A C C C C G C C C C C C A [336]
SB421Tw   - - - C - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - A - - - - - - - - - - - [336]
SB545Tw   A - - C - - - - A C - - - C - - - - - - - - - C A - - - - - G - - - - - A - - - - C - - - - - - [336]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [336]
SB655Po   - - - C - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - A - - - - - - - - - - - [336]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [336]

SB389Ha   A G A A T A A A A C A T A G C A A C A C C A T T A A A A T C C A A C C G A A C C A A A G A C A T [384]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [384]
SB545Tw   - - - - - - - - - - A - - - C - A - - - C - T - - A - - - - - - - - - - - - - - - - - - C - - [384]
SB620To   - - - - - - - - - - - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [384]
SB655Po   - - - - - - - - - - - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - [384]
SB700Kp   - - - - - - - - - - - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - [384]

SB389Ha   A C C C A C A T T A T C A A C C A A C C C C C C A T C C A A C A C A T A C C C A G A C A A A T C [432]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - - - - - - - - - - - - - - [432]
SB545Tw   - - - - A - A - - - - - - - - - A - - - - - - - - - - - - G - - - - - - - - - - - - - - - - - - [432]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [432]
SB655Po   - - - - A - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - - - - - - - - - - - - - - [432]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [432]

SB389Ha   C T C T C C C C C A A C A A A A A T C C C C A C C C C T A A A A C A A A G C C C A G T C C C A T [480]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - [480]
SB545Tw   - - - - - - C C C A A C C C - - - - C C - - - - - - - - - - - - - A - - - - - - - - C - - - - - [480]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - [480]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - [480]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - [480]
```

```
SB389Ha   A C C A T A C C C G A C A A C C C G T C A A T C C C C T C A A G A C T C A G G A T A A G C A T C [528]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [528]
SB545Tw   - - - - T - - C C - - - - - - - C - - - - - - - - - - - - A A - - - - - - - - - - - - - - - - - [528]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [528]
SB655Po   - - - - - - - - C - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [528]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [528]

SB389Ha   C G C C G C C A A C G A A A C C G A G T A C A C G A A A A C C A C C A A C A T T C C C C C T A A [576]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [576]
SB545Tw   - - - - - C - - - - - - - - - - - - - - - - - - - - - - - - - - - - - A - - - - - - - - - - - A [576]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [576]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [576]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [576]

SB389Ha   A T A C A C C A T C A C T A A C A C C A A A G A A A C G A A A G A A A C C C C C A A A C T T A C [624]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [624]
SB545Tw   - T A - - - C - - - - - - - - - - - - - - - - - - - - - - - A - - - - - - - - C A - - C - - - - [624]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [624]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [624]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [624]

SB389Ha   C A A T C A T C C A C A C C C C G C A A T A G A C G C T A C C A C T A A C C C C A C C A C C C C [672]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [672]
SB545Tw   C - A - - - - - C - - - - - - - - - - - - - - - - - - - - - - A - - - - - - - C - - - - - - - - [672]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [672]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [672]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - [672]

SB389Ha   G T A A T A A G G C G A A G G A T T G G A C G C A A C C [700]
SB421Tw   - - - - - - - - - - - - - - - - - - - - - - - - - - - - [700]
SB545Tw   - - - - - - - - - - - - - - - - - - - - - - - - - A - - [700]
SB620To   - - - - - - - - - - - - - - - - - - - - - - - - - - - - [700]
SB655Po   - - - - - - - - - - - - - - - - - - - - - - - - - - - - [700]
SB700Kp   - - - - - - - - - - - - - - - - - - - - - - - - - - - - [700]
```

Figure B.2 Mitochondrial control region Domain I sequence alignment for 26 New Zealand bellbird (*Anthornis melanura*) haplotypes.

```
Poo651   C C T G A G C C A G G A T T A A G G A T A T T A C T A A C A C C G G G T T G C T G A T T T C T C  [ 48]
Poo661   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Poo682   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Tir241   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Tir242   - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Hau283   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Hau386   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Hau442   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Ton648   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Ton629   - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Ton642   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Kap700   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Kap703   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Kap709   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Kap731   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Kap738   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Kai103   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Kai105   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Kai116   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Kai129   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Dun578   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Dun597   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Dun602   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Auc226   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Auc232   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
Hun755   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 48]
```

```
Poo651   G T G A G G G G A A C G A T T A A T A A A T A A C C A G G C T C T C T G G C T T G G G G G T A C  [ 96]
Poo661   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Poo682   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Tir241   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Tir242   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Hau283   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Hau386   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Hau442   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Ton648   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Ton629   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Ton642   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Kap700   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Kap703   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Kap709   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Kap731   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Kap738   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Kai103   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Kai105   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Kai116   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Kai129   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Dun578   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Dun597   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Dun602   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Auc226   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Auc232   - - - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
Hun755   - - - - - - - - G - - C - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [ 96]
```

```
Poo651   T T G A G G A C A T C G G T T A A T G T G A G T G T A T G G G C C C T G T G C A A T C A T A G T  [144]
Poo661   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Poo682   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Tir241   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Tir242   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Hau283   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Hau386   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Hau442   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Ton648   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Ton629   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Ton642   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Kap700   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Kap703   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Kap709   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Kap731   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Kap738   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Kai103   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Kai105   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Kai116   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Kai129   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Dun578   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Dun597   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Dun602   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Auc226   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]
Auc232   - - - - - - - - - - - - - - - - - - - - - - - - A - - - - - - - - - - - - - - - - - - - - - - -  [144]
Hun755   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [144]

Poo651   T A G T G T G G G T G G T T C C T C G T G T G G T G G A G T G T T T G T A T G T A C T A T T A A  [192]
Poo661   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Poo682   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Tir241   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Tir242   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Hau283   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Hau386   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Hau442   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Ton648   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Ton629   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Ton642   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Kap700   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Kap703   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Kap709   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Kap731   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Kap738   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Kai103   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Kai105   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Kai116   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Kai129   - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Dun578   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Dun597   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Dun602   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Auc226   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Auc232   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
Hun755   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [192]
```

```
Poo651   G C A T G T A C C G A T C T T G G G C G A C G C C T G T G T G T T A C T G G G C T G G A C G A C  [240]
Poo661   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [240]
Poo682   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Tir241   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Tir242   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Hau283   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Hau386   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Hau442   - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [240]
Ton648   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - A - - - T - - - - - - - - - - - - -  [240]
Ton629   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Ton642   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - A - - - T - - - - - - - - - - - - -  [240]
Kap700   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - C - - - - - - -  [240]
Kap703   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [240]
Kap709   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Kap731   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Kap738   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Kai103   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Kai105   - - - - - - - - - - - - - - C - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Kai116   - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [240]
Kai129   - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [240]
Dun578   - - - - - - G - - - - - - - - - - - T - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [240]
Dun597   - - - - - - G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - -  [240]
Dun602   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Auc226   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Auc232   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]
Hun755   - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - - - - T - - - - - - - - - - - - -  [240]

Poo651   C T G T G A G G G A T G T A C T G C G G T G G T A A T G G G G G C T A G A A T G A G T T C G T A  [288]
Poo661   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Poo682   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Tir241   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Tir242   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Hau283   - - - - - - - - - - - - - - G - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Hau386   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Hau442   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Ton648   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Ton629   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Ton642   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Kap700   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Kap703   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Kap709   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Kap731   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Kap738   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Kai103   - - - - - - - - - - - - - G A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Kai105   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Kai116   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Kai129   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Dun578   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Dun597   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Dun602   - - - - - - - - - - - - - G A C - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Auc226   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Auc232   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
Hun755   - - - - - - - - - - - - - - A - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - -  [288]
```

```
Poo651   T G A A T C G T C A G T C A T G G G A A C T A A T A T T T G A G T T G G G T T G G A A C T G G C   [336]
Poo661   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Poo682   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]
Tir241   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Tir242   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]
Hau283   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]
Hau386   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C A - - - - - - - - - - - - -   [336]
Hau442   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Ton648   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Ton629   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C A - - - - - - - - - - - - -   [336]
Ton642   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]
Kap700   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]
Kap703   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Kap709   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - -   [336]
Kap731   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]
Kap738   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - T - - - - - - - - - - - - -   [336]
Kai103   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Kai105   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]
Kai116   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - A - - - - - - - - - - - - -   [336]
Kai129   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Dun578   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Dun597   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Dun602   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [336]
Auc226   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]
Auc232   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]
Hun755   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - C - - - - - - - - - - - - - -   [336]

Poo651   A T G A C A G T C T A T G T G A A G T A T A C T A C A T T A A T A T A A T G A G T A A T G T G G   [384]
Poo661   - - - - - - - - - - - - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [384]
Poo682   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Tir241   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Tir242   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Hau283   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Hau386   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Hau442   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [384]
Ton648   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Ton629   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Ton642   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Kap700   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Kap703   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [384]
Kap709   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Kap731   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Kap738   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Kai103   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Kai105   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Kai116   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [384]
Kai129   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [384]
Dun578   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [384]
Dun597   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -   [384]
Dun602   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Auc226   - - - - - - - - - - - - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Auc232   - - - - - - - - - - - - - - - - - A - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
Hun755   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G - - - - - -   [384]
```

Poo651 A A A A T A A T G A T A T G C G A A G T A A T A C A T A C C C T A T A A A A A A G T T A T G G G [432]
Poo661 - [432]
Poo682 - [432]
Tir241 - [432]
Tir242 - [432]
Hau283 - [432]
Hau386 - [432]
Hau442 - [432]
Ton648 - [432]
Ton629 - [432]
Ton642 - [432]
Kap700 - [432]
Kap703 - [432]
Kap709 - [432]
Kap731 - [432]
Kap738 - [432]
Kai103 - [432]
Kai105 - [432]
Kai116 - [432]
Kai129 - [432]
Dun578 - [432]
Dun597 - [432]
Dun602 - [432]
Auc226 - [432]
Auc232 - [432]
Hun755 - [432]

Poo651 G G G G [436]
Poo661 - - - - [436]
Poo682 - - - - [436]
Tir241 - - - - [436]
Tir242 - - - - [436]
Hau283 - - - - [436]
Hau386 - - - - [436]
Hau442 - - - - [436]
Ton648 - - - - [436]
Ton629 - - - - [436]
Ton642 - - - - [436]
Kap700 - - - - [436]
Kap703 - - - - [436]
Kap709 - - - - [436]
Kap731 - - - - [436]
Kap738 - - - - [436]
Kai103 - - - - [436]
Kai105 - - - - [436]
Kai116 - - - - [436]
Kai129 - - - - [436]
Dun578 - - - - [436]
Dun597 - - - - [436]
Dun602 - - - - [436]
Auc226 - - - - [436]
Auc232 - - - - [436]
Hun755 - - - - [436]

Appendix C: *Plasmodium* spp. mitochondrial DNA data

Figure C.1 Mitochondrial cytochrome *b* sequences for four *Plasmodium* haplotypes sampled in bellbirds during this study (LIN1, LIN2, LIN3, LIN4).

```
LIN1   ATTTTAACTTATTTACATATTTTAAGAGGATTAAATTATTCATATTCA [ 48]
LIN2   --TTT-A----C-----T---------G-------------AT----- [ 48]
LIN3   ---C-T-----------------------------------T------ [ 48]
LIN4   ------------------------------------------------ [ 48]

LIN1   TATTTACCTTTATCTTGGATATCTGGATTAATTATATTTTTAATATCT [ 96]
LIN2   --------A-T---ATGGATT--AG---T---A-----CTT------- [ 96]
LIN3   --------A-----A--------A--------A--------------A [ 96]
LIN4   --------------A-----------------A-----C--------- [ 96]

LIN1   ATAGTTACAGCATTTATGGGATATGTATTACCTTGGGGTCAAATGAGT [144]
LIN2   --AGTA--T--T----TG--T--T-T-TT---------TC----G-GT [144]
LIN3   -----------T------------------------------------ [144]
LIN4   -----A-----T--------T--C------------------------ [144]

LIN1   TTTTGGGGAGCAACAGTAATTACAAATCTATTATATTTTATTCCTGGA [192]
LIN2   --CT----T--T--A-TC-T-ACT--TT--TTATAT-TT-T---AG-- [192]
LIN3   --C-----T-----T--T-----T---T-------------A-----T [192]
LIN4   --C-----T--T--C-----A--T---T-------C-----A------ [192]

LIN1   CTTGTTTCATGGATTTGTGGAGGATATCTTGTTAGTGATCCAACATTA [240]
LIN2   CT---AT-A-G-A-TTGT--T---------------GAC--------- [240]
LIN3   --------------------T--------------------------- [240]
LIN4   --------------A-----T-----------A-----C-----T--- [240]

LIN1   AAAAGATTTTTTGTATTACATTTTATATTTCCTTTTATAGCTTTATGT [288]
LIN2   --------C-----------C----C---C--A------------T-- [288]
LIN3   --------------T-----C-----------A--------------- [288]
LIN4   --------C----------------C---------------------- [288]

LIN1   ATTGTATTTATACATATATTTTTTCTACATTTACAAGGTAGCACTAAT [336]
LIN2   --------------------C---T-A-A------AG-TAG-A--A-T [336]
LIN3   --------C-----------C---------C-------------A--- [336]
LIN4   --------------------C---T-------------------A--- [336]

LIN1   CCTTTAGGGTATGATACTGCTTTAAAAATACCCTTTTATCCAAATCTT [384]
LIN2   --T---G-GTA------AG--------A-----T-C-----------A [384]
LIN3   -----------------A-----------------C------------ [384]
LIN4   -----------------A-----------------C------------ [384]

LIN1   TTAAGCTTAGATGTTAAAGGATTTAATAATATATTAGTATTATTTTTA [432]
LIN2   ------C-T---A----------T-----TGT----G-T-T-TT-TT- [432]
LIN3   -----TC-T---A-------------------TC----T-----C--- [432]
LIN4   -----TC-T---A-----------------G-------T--------- [432]

LIN1   GCTCAAAGTTTATTCGGAATACTACCATTATCACATCCAGATAATGC [479]
LIN2   TCTC-----TTATTTG----TT----A-----AC-T--AGA-A-TGC [479]
LIN3   --A------C----T------T------------------------- [479]
LIN4   --A-----------T------T------------------------- [479]
```

Table C.1 Sample sizes for number of bellbirds screened ($N_{SCREENED}$) for and infected ($N_{INFECTED}$) by *Plasmodium* sp. LIN1 parasites and prevalence estimates (%) for each sex-age class during five biologically meaningful seasons at three bellbird host subpopulations, Hauraki Gulf, New Zealand.

		Females			Males		
Season	Age	$N_{SCREENED}$	$N_{INFECTED}$	%	$N_{SCREENED}$	$N_{INFECTED}$	%
Hauturu							
Early autumn	AHY	16	0	1.00	18	0	0.00
	HY	9	1	0.11	11	0	0.00
Winter	AHY	14	5	0.36	14	7	0.50
	HY	7	3	0.43	3	0	0.00
Summer	AHY	6	1	0.17	5	0	0.00
	HY	20	0	0.00	22	1	0.05
Tawharanui							
Early autumn	AHY	5	0	0.00	5	1	0.20
	HY	2	1	0.50	5	0	0.00
Late autumn	AHY	8	1	0.13	13	8	0.62
	HY	0	-	-	1	0	0.00
Winter	AHY	4	0	0.00	7	2	0.29
	HY	0	-	-	0	-	-
Spring	AHY	6	1	0.17	20	6	0.30
	HY	1	0	0.00	0	-	-
Summer	AHY	7	0	0.00	10	0	0.00
	HY	8	0	0.00	14	1	0.07
Tiritiri Matangi							
Early autumn	AHY	1	1	1.00	12	3	0.25
	HY	6	1	0.17	7	1	0.14
Late autumn	AHY	9	0	0.00	11	1	0.09
	HY	1	0	0.00	2	1	0.50
Winter	AHY	7	1	0.14	37	7	0.19
	HY	7	0	0.00	5	2	0.40
Spring	AHY	6	0	0.00	30	7	0.23
	HY	0	-	-	0	-	-
Summer	AHY	10	3	0.30	37	7	0.19
	HY	4	0	0.00	4	0	0.00

Table C.2 *AIC* model selection results from tests of factors affecting bellbird malaria prevalence at three Hauraki Gulf locations. Terms with the lowest *AIC* are bolded and marked with an asterisk, and relevant statistics are bolded.

	Hauturu			**Tawharanui**			**Tiritiri Matangi**		
Term	-2LL	AIC	df	-2LL	AIC	df	-2LL	AIC	df
Season x Sex x Age x RBCI	-33.87	93.74	129	-43.17	120.34	95	-78.04	192.07	172
Sex x Age x RBCI	-41.77	93.54	137	-50.08	110.16	107	-88.15	186.30	185
Season x Age x RBCI	-36.47	86.94	135	-47.13	114.25	102	-85.25	190.50	180
Season x Sex x RBCI	-37.8	89.57	135	-47.24	116.48	101	-85.69	193.37	179
Season x Sex x Age	-30.38	84.76	130	-36.56	105.13	96	-79.05	192.05	173
Age x RBCI	-42.94	91.89	139	-51.45	108.91	109	-89.14	184.29	187
Sex x RBCI	-44.68	95.35	139	-50.89	107.77	109	-88.58	183.17	187
Sex x Age	-48.67	105.34	138	-49.02	110.04	106	-87.92	183.83	186
Season x RBCI	-37.95	83.90	138	-46.71	101.43	108	-87.36	186.73	184
Season x Age	-33.31	78.61	136	-43.26	104.53	103	-84.97	187.94	181
Season x Sex	-34.00	80.60	136	-40.41	100.81	102	-84.61	189.21	180
RBCI	-44.92	93.84	140	-52.02	108.03	110	-89.26	182.52	188
Age	-49.33	102.67	140	-50.50	105.01	110	-89.06	182.12	188
Sex*	-51.97	107.95	140	-49.81	103.63	110	**-88.50**	**180.99**	**188**
Season*	**-34.01**	**74.01**	**139**	**-44.69**	**99.38**	**107**	-88.31	186.62	185

Figure C.2 LogDet (Tamura-Kumar) pair-wise sequence divergence estimates as implemented in MEGA. Numbers correspond to sequence names denoted below. Lineages of malaria parasite which are closely related (within a genetic distance of < 5%) to LIN1 (SB00050), LIN2 (SB0455), LIN3 (SB0295) and LIN4 (SB0437) are marked in bold font.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1																						
2	0.210																					
3	0.195	0.051																				
4	0.195	0.051	0.000																			
5	0.191	0.052	0.011	0.011																		
6	0.213	0.062	0.019	0.019	0.023																	
7	0.209	0.059	0.016	0.016	0.019	0.003																
8	0.194	0.056	0.014	0.014	0.004	0.026	0.023															
9	0.194	0.054	0.009	0.009	0.002	0.021	0.017	0.005														
10	0.215	0.082	0.066	0.066	0.076	0.070	0.074	0.080	0.074													
11	0.208	0.091	0.062	0.062	0.072	0.082	0.078	0.076	0.070	0.066												
12	0.208	0.064	0.066	0.066	0.076	0.077	0.073	0.079	0.073	0.058	0.070											
13	0.200	0.097	0.074	0.074	0.078	0.080	0.076	0.082	0.076	0.092	0.080	0.079										
14	0.198	0.095	0.077	0.077	0.080	0.082	0.078	0.084	0.078	0.095	0.082	0.077	0.002									
15	0.198	0.095	0.077	0.077	0.080	0.082	0.078	0.084	0.078	0.095	0.082	0.077	0.002	0.000								
16	0.208	0.002	0.051	0.051	0.053	0.063	0.059	0.056	0.055	0.082	0.093	0.067	0.095	0.093	0.093							
17	0.208	0.002	0.051	0.051	0.053	0.063	0.059	0.056	0.055	0.082	0.093	0.067	0.095	0.093	0.093	0.000						
18	0.195	0.051	0.000	0.000	0.011	0.019	0.016	0.014	0.009	0.066	0.062	0.066	0.074	0.077	0.077	0.051	0.051					
19	0.198	0.077	0.078	0.078	0.088	0.081	0.086	0.092	0.086	0.058	0.066	0.019	0.075	0.073	0.073	0.079	0.079	0.078				
20	0.181	0.079	0.064	0.064	0.069	0.067	0.071	0.073	0.067	0.078	0.071	0.074	**0.049**	0.051	0.051	0.077	0.077	0.064	0.063			
21	0.208	**0.002**	0.051	0.051	0.053	0.063	0.059	0.056	0.055	0.082	0.093	0.067	0.095	0.093	0.093	**0.000**	**0.000**	0.051	0.079	0.070		
22	0.204	0.068	0.069	0.069	0.079	0.073	0.077	0.083	0.077	0.050	0.066	**0.012**	0.075	0.073	0.073	0.070	0.070	0.069	**0.007**	0.062	0.077	
23	0.204	0.051	**0.007**	**0.007**	**0.017**	**0.019**	**0.016**	**0.021**	**0.016**	0.074	0.070	0.073	0.082	0.085	0.085	0.051	0.051	**0.007**	0.086	0.077	0.071	0.05

1 *P. reichenowi* (mammalian) AF069610
2 *P.* sp. AY33088
3 *P. relictum* CINCQ01 DQ659560
4 *P. relictum* FOUSEY01 DQ659561
5 *P. relictum* GRW04 AY099041
6 *P. relictum* SGS01 AF495571
7 *P. relictum* GRW11 AY831748
8 *P. relictum* GRW12 DQ368378
9 *P. relictum* DQ659556
10 *P.* sp. GEOTRI01
11 *P. rouxi* AY178904
12 *P.* sp. AFTRU04
13 *P.* sp. MELANA01 AY714198
14 *P.* sp. MELNOT01 AY714197
15 *P.* sp. TUMIG02 DQ847261
16 *P. elongatum* GRW06 DQ368381
17 *P. elongatum* DQ659588
18 *P.* sp. DQ839046
19 *P.* sp. AFTRU08 EU810633
20 SB0050
21 SB0455
22 SB0295
23 SB0437

Appendix D: Bellbird morphological analyses

Figure D.1 Residual body condition index (RBCI: studentized residual of linear regression of cubed root of mass and PC1 for skeletal measurements) is significantly linearly related to body fat content (scale of 0 to 4) for bellbirds ($P < 0.001$) in a generalized linear regression (GLZ) analysis. The slope of the relationship is similar among males (M) and females (F) ($P = 0.5$).

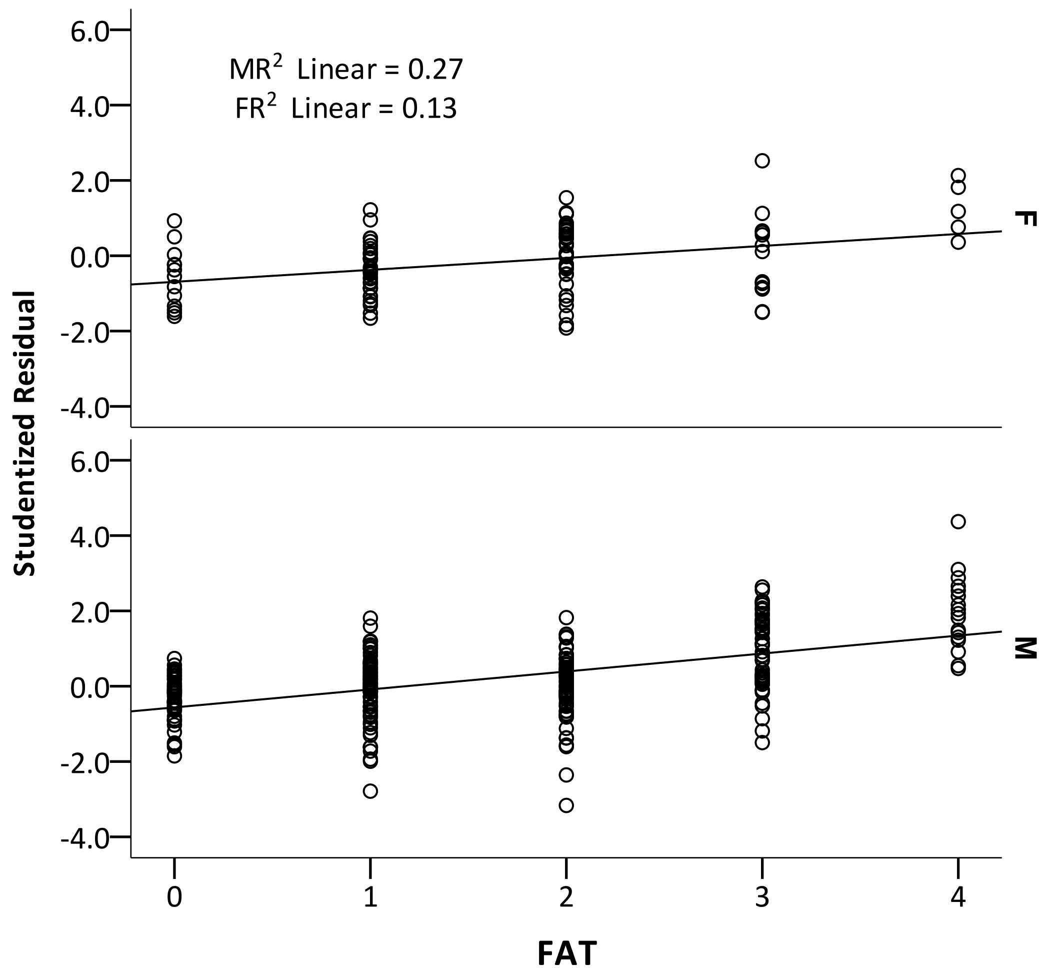


Figure D.2 Histograms and significance of *t*-test results comparing morphological measurements between bellbird males (M) and females (F). Histogram frequency is on the y-axis and the x-axis is A) mass (g), B) tarsus length (mm), C) head-bill length (mm), D) unflattened wing length (mm) and E) tail retrices length (mm) and. For analyses on feather lengths, only birds that were not molting were used.

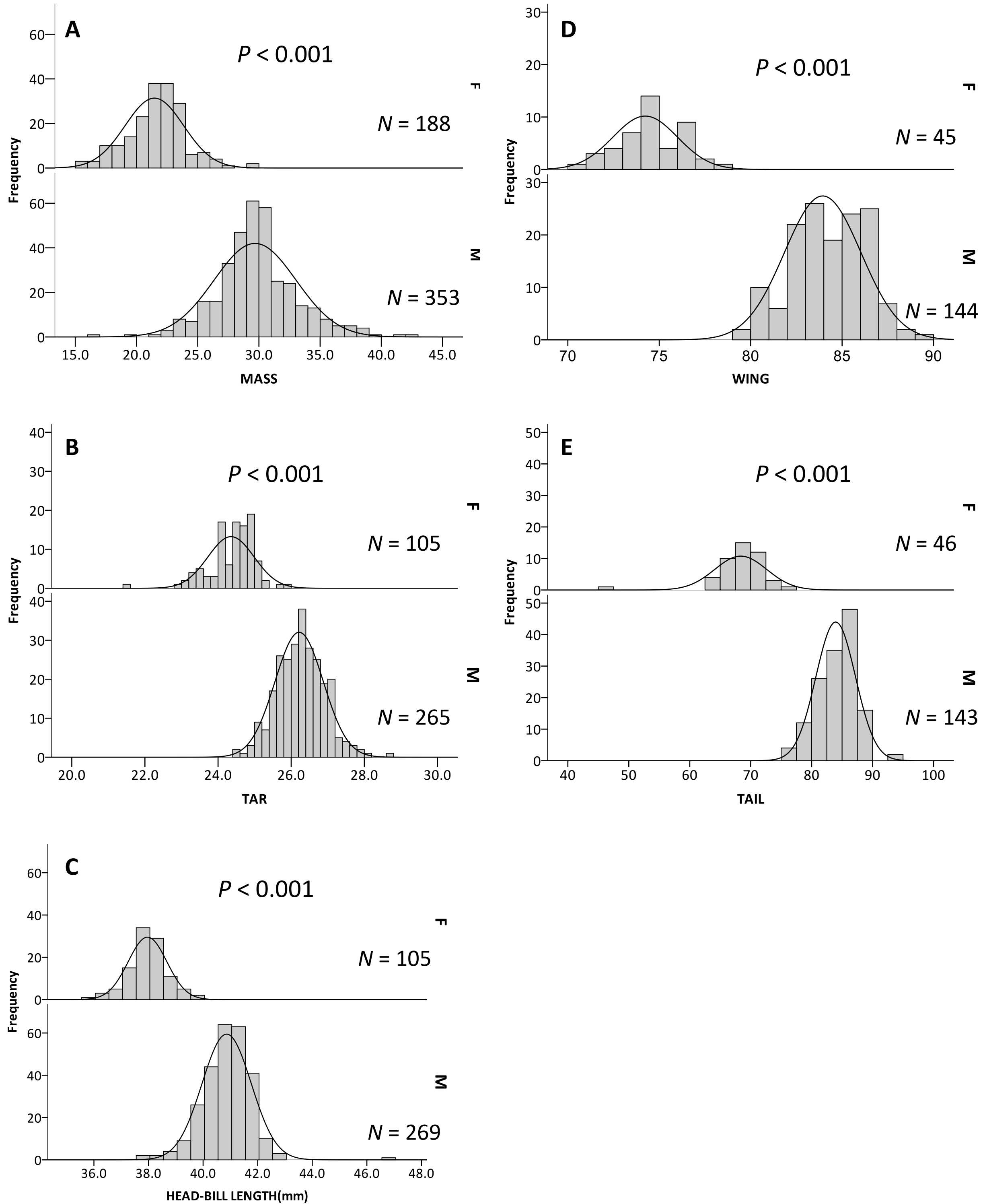


Table D.1 Standardized discriminant function analysis (DFA) coefficients for phenotypic traits, proportion of variance explained and Wilk's Lambda test results for each function. The first three discriminant functions explained 98.5 and 99.2% of variation in body size for females and males, respectively.

	Females			Males		
Trait	Function 1	Function 2	Function 3	Function 1	Function 2	Function 3
Tarsus	0.227	0.789	0.722	0.430	0.827	-0.219
Head-bill	0.514	-0.124	-0.545	0.174	0.172	0.460
Wing length	0.453	0.102	-0.624	0.332	-0.552	1.064
Tail length	0.314	-0.713	0.817	0.509	-0.156	-1.200
% variance explained	77.0	14.2	7.3	78.8	16.9	3.5
Canonical correlation	0.62	0.33	0.24	0.78	0.50	0.25
χ^2_{df}	130.4_{28}	35.1_{18}	13.7_{10}	513.0_{28}	144.7_{18}	32.0_{10}
P-value	<0.001	0.009	0.189	<0.001	<0.001	<0.001

Figure D.3 Discriminant function analysis (DFA) results for A) females and B) males.

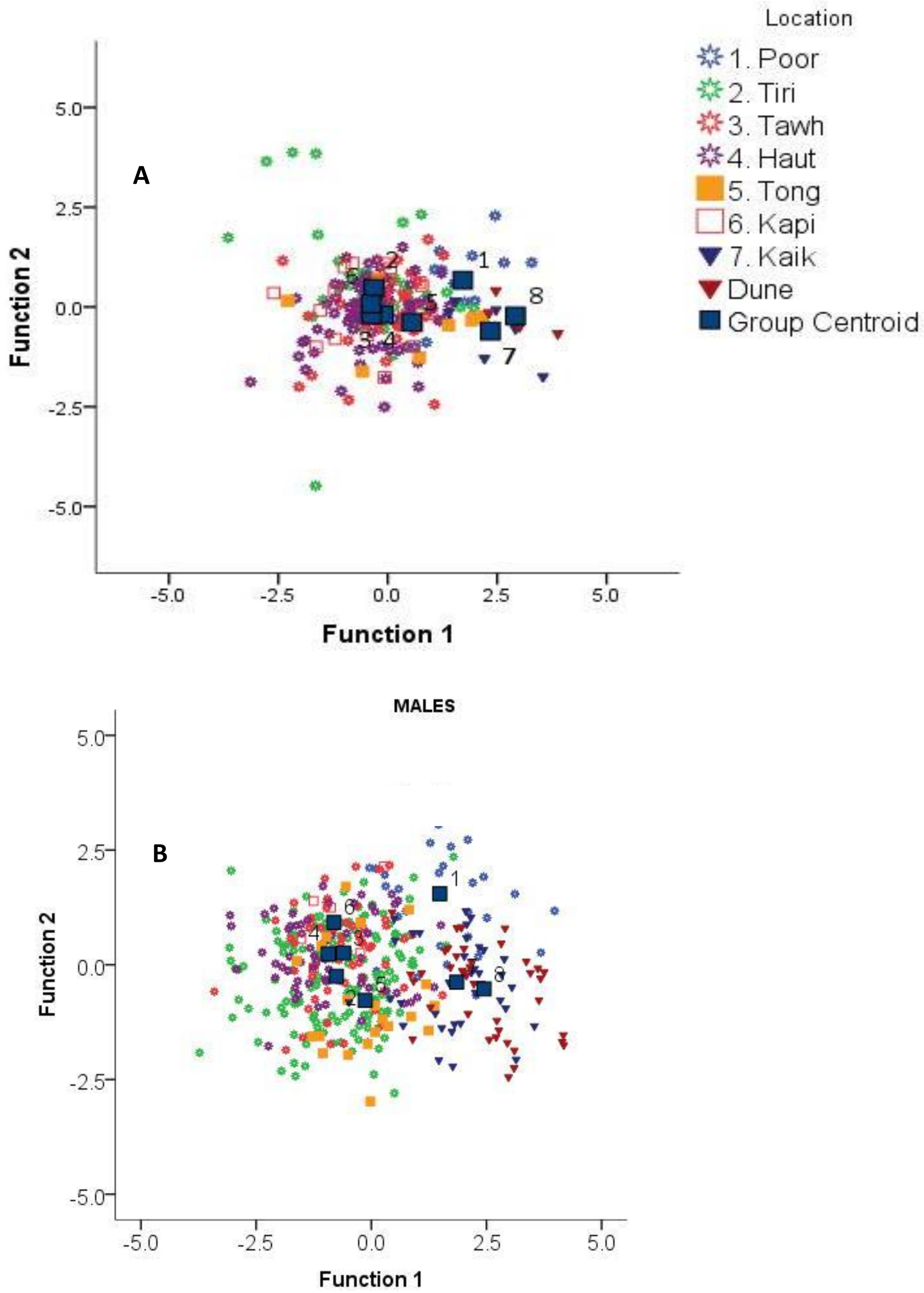


Table D.2 Assignment test results showing cross-validated percent and count (in parentheses) of individuals assigned to each sampling location for DNA data (a) microsatellite loci, and based on phenotype (tarsus, head-bill, wing and tail) b) males and (c) females. Bolded numbers indicate assignment to correct sampling location; 61%, 38% and 31% of individuals were assigned correctly based on microsatellite data, and phenotype of males and females, respectively. Bellbird sampling location abbreviations: Poor Knights, Poor; Tiritiri Matangi, Tiri; Tawharanui, Tawh; Hauturu, Haut; Tongariro, Tong; Kapiti, Kapi; Kaikoura, Kaik; Dunedin, Dune.

(a)

	Poor	Tiri	Tawh	Haut	Tong	Kapi	Kaik	Dune	N
Poor	**96.9 (31)**	0	0	0	0	0	3.1 (1)	0	32
Tiri	0	**89.1 (49)**	1.8 (1)	7.3 (4)	0	0	0	1.8 (1)	55
Tawh	1.8 (1)	0	**53.6 (30)**	16.1 (9)	10.7 (6)	3.6 (2)	5.6 (3)	8.9 (5)	56
Haut	0	0	18.2 (10)	**45.5 (25)**	18.2 (10)	3.6 (2)	10.9 (6)	3.6 (2)	55
Tong	0	0	6.7 (2)	20.0 (6)	**50.0 (15)**	6.7 (2)	10.0 (3)	6.7 (2)	30
Kapi	0	0	3.2 (1)	9.7 (3)	6.5 (2)	**48.4 (15)**	6.5 (2)	25.8 (8)	31
Kaik	0	0	9.4 (3)	9.4 (3)	3.1 (1)	15.6 (5)	**50.0 (16)**	12.5 (4)	32
Dune	2.2 (1)	0	2.1 (1)	13.0 (6)	2.2 (1)	13.0 (6)	10.9 (5)	**56.5 (26)**	46

(b)

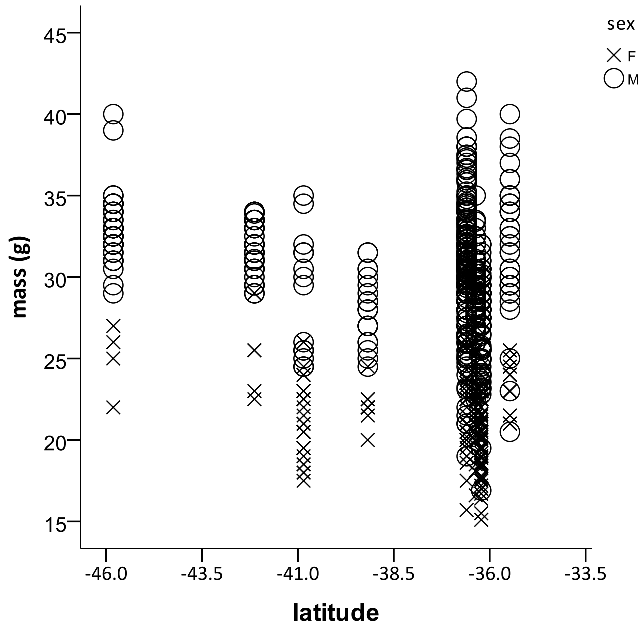
	Poor	Tiri	Tawh	Haut	Tong	Kapi	Kaik	Dune	N_{males}
Poor	**66.7 (22)**	0	0	3.0 (1)	3.0 (1)	3.0 (1)	9.1 (3)	15.2 (5)	36
Tiri	5.5 (8)	**30.3 (44)**	11.7 (17)	16.6 (24)	22.8 (33)	9.7 (14)	3.4 (5)	0	145
Tawh	5.2 (3)	13.8 (8)	**22.4 (13)**	17.2 (10)	19.0 (11)	20.7 (12)	1.7 (1)	0	58
Haut	3.0 (2)	16.7 (11)	19.7 (13)	**33.3 (22)**	9.1 (6)	13.6 (9)	4.5 (3)	0	66
Tong	0	0	0	14.3 (3)	**57.1 (12)**	14.3 (3)	14.3 (3)	0	21
Kapi	0	0	0	16.7 (1)	0	**83.3 (5)**	0	0	6
Kaik	17.1 (6)	0	2.9 (1)	0	11.4 (4)	2.9 (1)	**34.3 (12)**	31.4 (11)	35
Dune	7.3 (3)	0	0	0	4.9 (2)	0	31.7 (13)	**56.1 (23)**	41

(c)

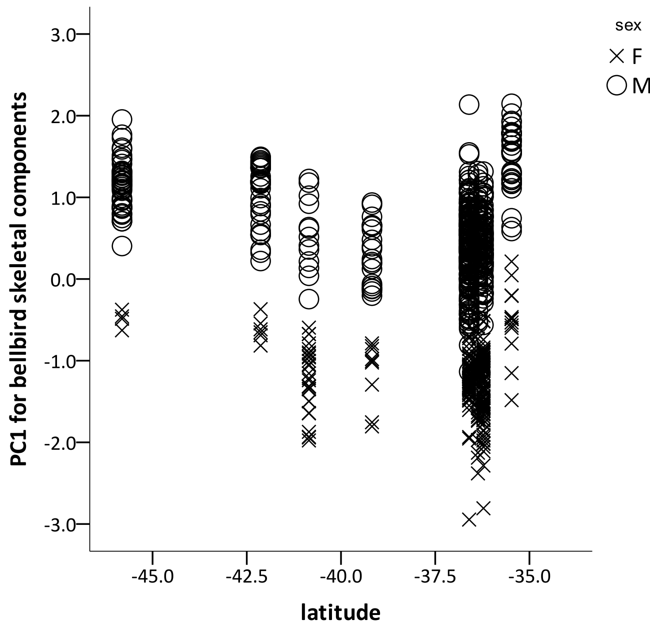
	Poor	Tiri	Tawh	Haut	Tong	Kapi	Kaik	Dune	$N_{females}$
Poor	**75.0 (9)**	0	0	0	16.7 (2)	0	8.3 (1)	0	12
Tiri	4.8 (2)	**31.0 (13)**	4.8 (2)	9.5 (4)	11.9 (5)	31.0 (13)	4.8 (2)	2.4 (1)	42
Tawh	5.7 (3)	14.3 (5)	**14.3 (5)**	17.1 (6)	25.7 (18)	22.9 (8)	0	0	35
Haut	1.4 (1)	20.5 (15)	1.4 (1)	**24.7 (18)**	24.7 (9)	27.4 (20)	0	0	73
Tong	0	0	11.1 (1)	11.1 (1)	**22.2 (2)**	22.2 (2)	0	33.3 (3)	9
Kapi	10.5 (2)	5.3 (1)	5.3 (1)	5.3 (1)	15.8 (3)	**57.9 (11)**	0	0	19
Kaik	16.7 (1)	0	0	0	16.7 (1)	0	**33.3 (2)**	33.3 (2)	6
Dune	25.0 (1)	0	0	0	0	0	50.0 (2)	**25.0 (1)**	4

Figure D.4 Bellbird body size increases significantly ($P < 0.001$) from north to south throughout New Zealand for A) mass (g) and b, B) factor 1 (PC1) of PCA on tarsus length (mm) and head-bill length (mm). However, the Hauraki Gulf and Poor Knights Islands bellbirds are larger than expected ($P < 0.001$) assuming this clinal trend. All birds were measured by a single researcher, Shauna M Baillie, in order to standardize comparisons among locations.

A)



B)



Appendix E: Bellbird nest success and chick growth

Figure E.1 Field notes on bellbird nest locations that were regularly visited during September 2007 to January 2008 at the north end of Tiritiri Matangi, Hauraki Gulf.

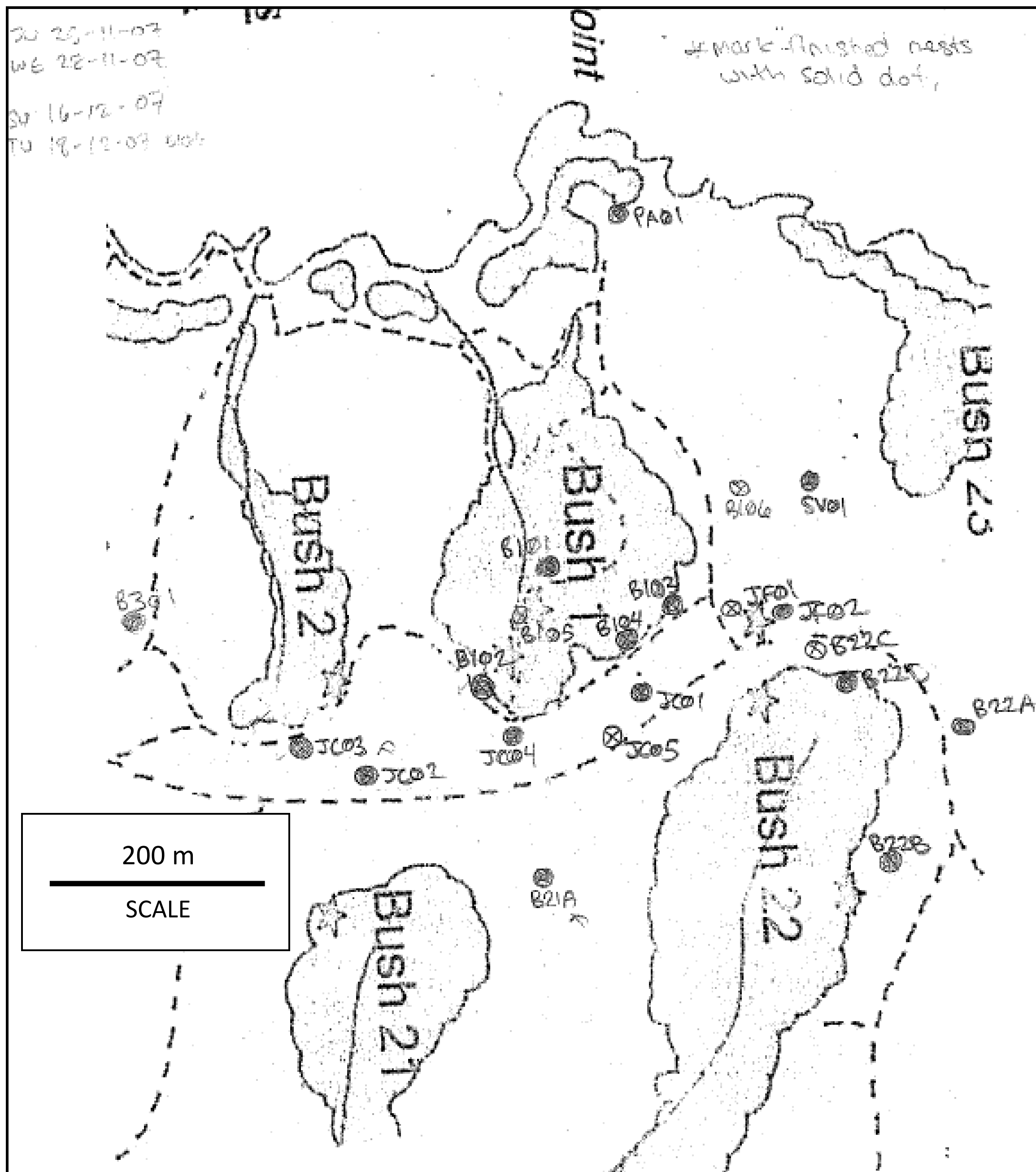


Table E.1 Description of 21 New Zealand bellbird nests at Tiritiri Matangi, Hauraki Gulf during 2007. Nest locations are shown in Figure E.1.

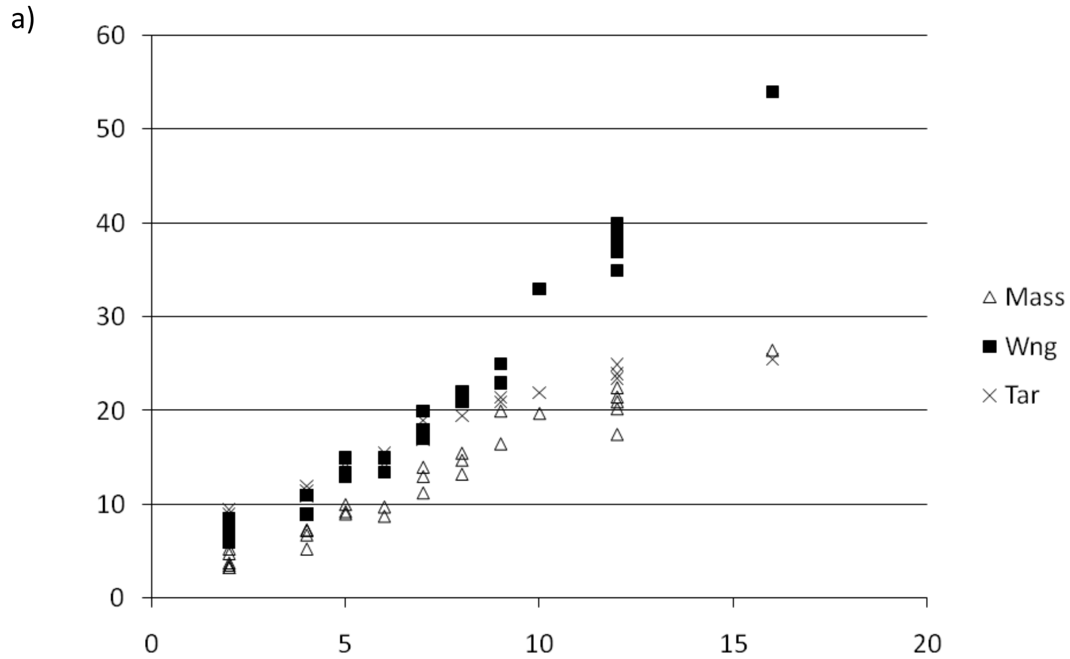
Nest No.	Nest ID	Date found	Nest contents	Tree	Epiphyte	Tree height (m)	Nest aspect	Habitat	Female bellbird ID	Male bellbird ID	Comments
1	B101	18/09/2007	Build	*Melicytus ramiflorus*	*Muhlembeccia*	8	NE	mature forest	WR-WM	YBk-BkM	Not actually found until had chicks on 29 Nov 07
2	JC01	19/09/2007	Build	*Kunsia ericoides*	.	6	SE	mature forest	WB-RM	W-MR	First nest; abandoned
3	nil	17/10/2007	not found	N/A	N/A	N/A	N/A	mature forest	WR-OM	unknown	Seen carrying nest material
4	JC02	29/10/2007	3 eggs	*Cordyline australis*	*Muhlembeccia*	1.7	W	shrub	UBF	UBM	
5	B102	31/10/2007	3 eggs	*Cordyline australis*	.	2	S	edge mature forest	UBF	UBM	
6	B103	1/11/2007	Build	*Myrsine australis*	.	4	SW	edge mature forest	GG-GM	B-GM	
7	JC03	2/11/2007	3 eggs	*Cordyline australis*	.	2.5	W	immature forest	UBF	B-RM	
8	JF01	13/11/2007	Build	*Cordyline australis*	.	7	NE	immature forest	Y-BM	W-M	
9	SV01	15/11/2007	Build	unknown	*Muhlembeccia*	2	NE	savannah	BBk-M	GR-M	
10	B104	17/11/2007	2 chks	unknown	unknown	2.5	E	edge mature forest	WB-RM	W-MR	Second nest
11	B22A	19/11/2007	2 chks/1 egg	unknown	*Muhlembeccia*	2.5	NW	immature forest	W-dkGM	YR-M	
12	B22B	19/11/2007	3 chks	*Cordyline australis*	.	1.7	N	shrub	UBF	UBM	
13	B22C	19/11/2007	Build	unknown	unknown	2	N	shrub	UBF	UBM	
14	B22D	19/11/2007	2 chks/1 egg	*Cordyline australis*		2	N	immature forest	UBF	UBM	
15	B105	20/11/2007	Build	*Cordyline australis*		3	W	edge mature forest	RB-RM	RB-M	
16	JC04	20/11/2007	2+ chks	*Knightia excelsa*	.	4.5	unknown	immature forest	GBk-GM	unknown	
17	JF02	20/11/2007	Build	*Cordyline australis*	.	1.5	S	immature forest	GM-Y	RW-M	
18	B21A	21/11/2007	3 cks	*Phormium tenax*	.	1.5	W	shrub	UBF	BR-YM	
19	JC05	21/11/2007	3 eggs	Cordyline australis	.	2	unknown	immature forest	UBF	UBM	
20	B301	21/11/2007	unknown	unknown	unknown	2.5	NE	immature forest	check	check	
21	B106	18/12/2007	eggs	*Cordyline australis*	.	4	SE	immature forest	BBk-M	GR-M	They abandoned SV01 built this one right away

Table E.2 Nest success for 21 New Zealand bellbird nests at Tiritiri Matangi, Hauraki Gulf during 2007. This table shows data summary up to 20 December 2007. Nest locations are shown in Figure E.1.

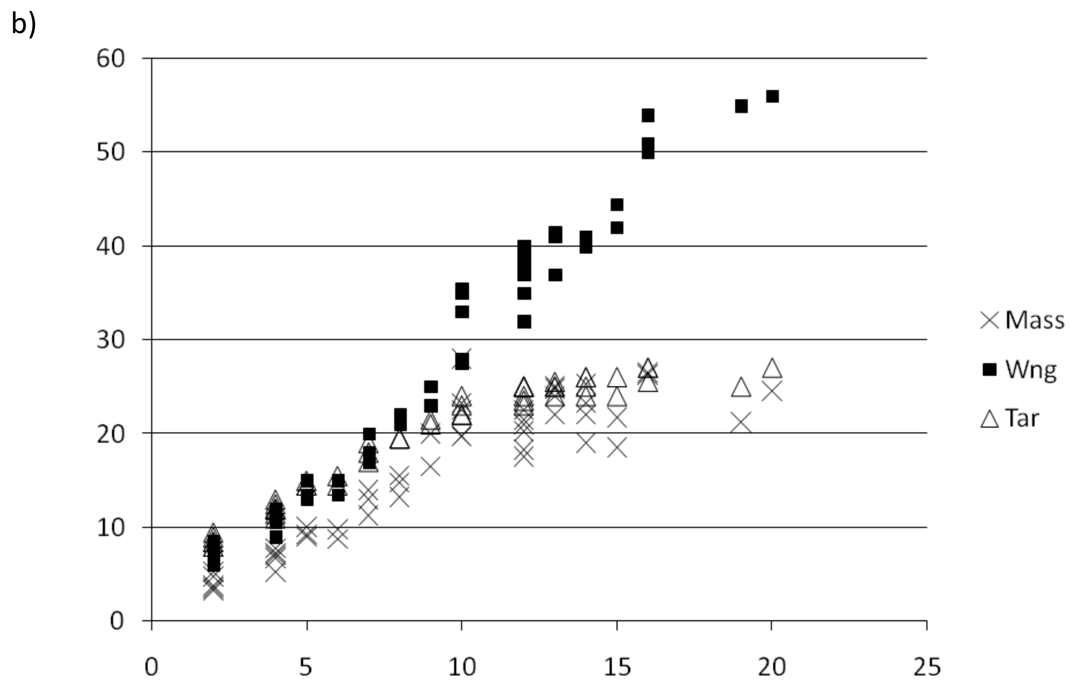
Record No.	Nest ID	N_{EGGS}	N_{CHICKS}	$N_{CHICKS\ FLEDGED}$	Hatch success	Fledge success	Hatch*fledge success
1	B101	unk	2+	unk	.	.	
2	JC01	unk	0	0	0.00	.	0.00
3	JC02	3	2	2	0.67	1.00	0.67
4	B102	3	3	3	1.00	1.00	1.00
5	B103	unk	0		0.00	.	0.00
6	JC03	3	1	0	0.33	0.00	0.00
7	JF01	unk	3	unk	.		
8	SV01	0	0	0	.	.	.
9	B104	unk	2	2	.	1.00	
10	B22A	3	2	2	0.67	1.00	0.67
11	B22B	3	3	3	1.00	1.00	1.00
12	B22C	3	2	2	0.67	1.00	0.67
13	B22D	3	2	1	0.67	0.50	0.33
14	B105	unk	0	0	0.00	.	0.00
15	JC04	3	3	unk	1.00	.	.
16	JF02	2	0	0	0.00		0.00
17	B21A	3	3	3	1.00	1.00	1.00
18	JC05	3	3	3	1.00	1.00	1.00
19	B301	.	.	.	.	.	.
20	B106	unk	unk	unk	.	.	.
Summary		2.7 eggs/nest	1.7 eggs hatched /nest	1.5 chicks fledged /nest	62%	85%	53%

Figure E.2 New Zealand bellbird chick growth curves for mass (g), maximum wing length, Wng (mm), and tarsus length, Tar (mm), based on a) eight chicks of known age and b) all 20 chicks that were visited and measured every three days Tiritiri Matangi, Hauraki Gulf during 2007. Body size regressions for known-age chicks were used to estimate age for unknown-age chicks.

a)



b)



Appendix F: Statement of author contribution forms

I. **Baillie SM, Ritchie PA, Brunton DH (*in revision*) Population genetic connectivity and diversity of a highly mobile forest passerine endemic to New Zealand.** *Conservation Genetics* COGE-S-11-00044

II. **Baillie SM, Rioux Paquette S, Ritchie PA, Parker K, Brunton DH (*in progress*) Genetic analysis of natural re-colonization by an endemic New Zealand passerine .** *Molecular Ecology*

III. **Baillie SM, Ritchie PA, Brunton DH (*in progress*) Ice-age phylogeography of the New Zealand bellbird *Anthornis melanura*.** *Journal of Biogeography*

IV. **Baillie SM, Brunton DH (2011) Diversity, distribution and biogeographical origins of Plasmodium parasites from the New Zealand bellbird (*Anthornis melanura*).** *Parasitology* doi:10.1017/S0031182011001491

V. **Baillie SM, Brunton DH (*in progress*) Avian malaria seasonality, sex-biased infections and a paradoxical relationship between body condition and parasite prevalence in New Zealand bellbirds *Anthornis melanura.*** *International Journal of Parasitology*