Conservation Genetics of North Island Kōkako (*Callaeas wilsoni*)

Meghan Milner-Jones

A thesis submitted for the degree of Master of Science

Department of Zoology

University of Otago, Dunedin

New Zealand

June 2018

Abstract

Conservation genetics has recently been recognised as an important, although often overlooked, aspect of wildlife management. Applying molecular techniques and genetic concepts to management strategies has the potential to significantly improve current and future recovery efforts. Wildlife translocations are often used for threatened species management in New Zealand, in which the selection of appropriate source populations is an important decision. North Island kōkako (*Callaeas wilsoni*) are an endemic, threatened songbird distributed across 24 relict and translocated populations in the North Island of New Zealand. Translocations have significantly influenced kōkako recovery. As such, kōkako are one of the most commonly translocated species in New Zealand. Integrating genetic techniques and recommendations into source population selection will help ensure the long-term success of future translocation projects.

Te Hauturu-o-Toi is currently the largest population of kōkako but is thought to have been established by a founding population of as few as nine individuals. The aim of this research was to assess the suitability of the Hauturu kōkako population as a future source for translocations using molecular methods. In particular, the aim was to address two aspects of a source population that contribute to the overall success of a translocation; genetic diversity and disease status. To do so, the level of genetic variation across six populations and the presence of Psittacine Beak and Feather Disease on Hauturu was examined. Such research provides conservation managers with important genetic information that can be applied to best protect kōkako in future.

Conserving genetic diversity is important for long-term survival of endangered species. As translocations involve a small number of individuals and create a genetic bottleneck, sourcing founder birds from genetically diverse populations helps ensure long-term translocation success. I developed 21 kōkako-specific microsatellite primers using a whole kōkako genome, that were then multiplexed to infer genetic diversity and population structure in one translocated (Hauturu) and five relict (Mapara, Mangatutu, Te Urewera, Waipapa and Bay of Plenty) kōkako populations. A similar level of genetic diversity was apparent across all populations, providing evidence to support the use of Hauturu as a source for future translocations. Both multivariate and Bayesian methods recognised a clear population structure (K=3), identifying kōkako from Hauturu and Mapara as genetically

distinct from the other four sites. Understanding the differentiation between kōkako populations will allow managers to plan translocations that maximise genetic diversity and hence translocation success.

Disease risk and spread has the potential to induce a translocation failure. A kōkako on Hauturu developed aberrant coloured plumage and features (claws, legs, beak) between sampling seasons (2013–2015), reminiscent of Psittacine Beak and Feather Disease (PBFD). PBFD typically infects psittacine species, but due to high recombination rates can host-switch for infection in non-psittacine species. The Hauturu population was screened for PBFD using polymerase chain reaction, with all individuals (n=31) testing negative. There is no evidence for a host switching event from red-crowned parakeets (*Cyanoramphus novaezelandiae*) on Hauturu into kōkako, therefore, a translocation from Hauturu is unlikely to facilitate PBFD spread. Following this result, the melanocortin-1 receptor (MC1R) gene was amplified as an alternative explanation, looking for sequence variants between the before (normal), after (aberrant) and seven normal kōkako (also from Hauturu). The MC1R gene was selected as it has a pleiotropic role in regulating both melanin deposition and physiological stress responses. No mutational differences were found, and it is recommended that future research includes screening of alternative candidate genes or explores epigenetic mechanisms to provide an explanation for the observed phenotype.

Acknowledgements

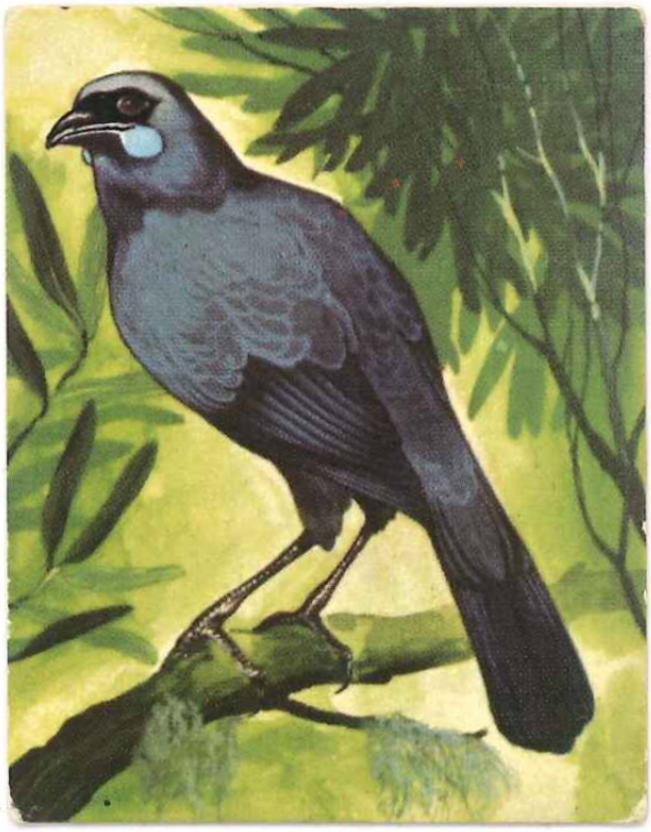
I am incredibly grateful for the support of my supervisor, Assoc. Prof. Bruce Robertson. Despite my very limited background in genetics, Bruce was confident in my ability right from the beginning of this project. Thank you for allowing me the freedom to explore my own ideas, knowing that I had the safety net of your thoughtful guidance. I would also like to thank Dr. Graeme Oatley, for your endless patience in the lab, for helping me navigate the overwhelming task of analysing genetic data and for all your advice and feedback. Without your support, this research would have not been possible. I am also thankful for the financial support from the University of Otago Master's scholarship, which allowed me to focus on my research. And to Imogen Foote, for your friendship and support both in and out of the lab.

I would like to extend a huge thank you to the Kōkako Specialist Group, from those who collected blood and feather samples over the past 20+ years, to those who were enthusiastic about my project at the KSG meeting last year (despite me being the only person in the room to have never seen nor heard a kōkako!). The Department of Conservation and Kōkako Specialist Group provided funding for this project, which supported my laboratory work. A special thank you to Laurence Barea, Tertia Thurley, John Innes and Ian Flux, whose expertise, advise and passion for kōkako inspired and improved my project.

Thank you to all of the staff and students in the Zoology department, for making it such an enjoyable and supportive place to be in. I would especially like to acknowledge the help provided by Luke Easton, Dr. Tania King, Dr. Nic Dussex and Dr. Andrew Veale. Thank you to everyone in the Wildlife Management room, for the great company, advice, support and endless cups of tea. A special thanks to Jamie McAulay, for introducing me to kōkako.

And to my friends and family who have all helped me through the trials and tribulations of my thesis. Thank you for the endless encouragement and support.

Finally, to Dad for being my inspiration. Without you I would have never started, let alone finished this thesis.



2

NORTH ISLAND KOKAKO (CROW)

Now found only in forests between Auckland and East Cape-Mt. Egmont. Cannot make sustained flight. Quite friendly to men, the crow feeds on insects, fruit and berries, eating leaves in winter when berries have gone. Nests in tree branches.

This is one in a series of 40 Cards in GREGG'S JELLY PACKETS.

Send 6d. in stamps to "Birds," P.O. Box 844, Dunedin, for a beautiful mounting album.

North Island kōkako for Gregg's Rare and Endangered Birds of New Zealand; a series of 40 collectable cards from Gregg's Jelly packets.

Artist: Eileen Mayo. Publisher: W. Gregg and Company Ltd. Dunedin. 1977.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter One:
General Introduction

1.1 Conservation genetics

Biodiversity is being lost at an unprecedented rate worldwide (Hoffmann et al. 2010; Pereira et al. 2010; Ceballos et al. 2015). At present, the planet is considered to be in the midst of the sixth mass extinction event, with modern extinction rates greatly exceeding natural background rates (Ceballos et al. 2015). The earth is now experiencing unparalleled biodiversity loss, largely due to the impacts of human activities such as habitat loss, exploitation and climate change (Tilman et al. 2017; Ceballos & Ehrlich 2018). To mitigate and minimise the impacts of human activity on threatened species, conservation biology has emerged as a discipline that aims to reduce the rate of extinction and to preserve biodiversity (Fiedler & Jain 1992). Conservation genetics has become recognised as a particularly important component of conservation biology, in terms of preserving evolutionary potential, minimising diversity loss and preventing extinction (Frankham 2003; Allendorf et al. 2012). Without sufficient genetic diversity, species lack the evolutionary potential to adapt to future change (Allendorf et al. 2012), therefore is an essential component of long-term survival of endangered species (Spielman et al. 2004).

The field of conservation genetics is an evolving discipline, that applies evolutionary and molecular genetics to the conservation of threatened species and biodiversity (Frankham 2010a; Allendorf et al. 2012). Conservation genetics has developed considerably over the past 40 years (Allendorf 2017; DeSalle & Amato 2004). This has primarily been the result of emerging technology that has facilitated the development of increasingly advanced molecular techniques and driven the complexity of questions that can be addressed (Allendorf 2017; DeSalle & Amato 2004; Galla et al. 2016; Ouborg et al. 2010). Early conservation geneticists used protein electrophoresis to describe and compare levels of genetic diversity across a wide range of different species in the 1960s (reviewed in Allendorf 2017; Charlesworth & Charlesworth 2017). Following this, the ability to isolate mitochondrial DNA and microsatellite loci allowed insights into phylogeny and made the isolation of a large number of neutral markers in a variety of different sample types possible (see Allendorf 2017). Nowadays, the field of conservation genetics is much broader (Allendorf 2017; DeSalle & Amato 2004). Significantly more complex issues can now be addressed, such as resolving taxonomic uncertainties (e.g. Hay et al. 2010), addressing wildlife forensic issues (Alacs et al. 2010) or exploring landscape genetics (Richardson et al. 2016). The ability to isolate single nucleotide polymorphisms (SNPs) and to sequence whole

genomes has significantly advanced the field of conservation genetics in recent decades and is expected to progress dramatically in the near future (Allendorf, Hohenlohe & Luikart 2010; Galla et al. 2016; Ouborg et al. 2010; Primmer 2009).

1.2 Genetics informing management

When managing wild populations, the application of molecular methods has the potential to greatly improve and enhance management efforts. Wildlife managers are often faced with uncertainties regarding the identification, movement or biology of individuals, all of which genetics can help address (Frankham, 2003). For example, molecular methods can establish mating systems of threatened species (e.g. O'Brien et al. 2018), dispersal patterns (e.g. van Dijk et al. 2015), detect hybridisation (e.g. van Riemsdijk et al. 2018) or resolve population structure (e.g. Collins et al. 2017) to name a few (see Frankham, 2010a for extensive review). One of the most significant threats to endangered species is the loss of genetic diversity associated with small and fragmented populations (Fahrig 2003; Jamieson et al. 2006; Allendorf et al. 2012; Frankham et al. 2017). The impact of diversity loss has been recently recognised in New Zealand, with the current New Zealand Threatened Species Strategy Plan (draft for consultation) acknowledging the importance of conserving genetic diversity in terms of both protecting and enhancing populations of endangered species (*New Zealand's Threatened Species Strategy: Draft for Consultation* 2017). Additionally, the New Zealand Biodiversity Action Plan (2016–2020) also recognises the protection of genetic diversity within one of the main strategic goals (*New Zealand Biodiversity Action Plan: 2016–2020* 2016).

Despite the emphasis on genetics and the advances in technology over recent years, genetic techniques are still poorly integrated into conservation and wildlife management worldwide. Many management and conservation strategies still fail to include genetic findings and recommendations. In 2003, Moyle et al. reviewed 181 recovery plans in the US, finding that only 22% identified genetic threats and that very little was integrated into management. More recent research suggests that the situation is improving, and that the US has the greatest inclusion of genetic factors in recovery plans (across seven countries) (Pierson et al. 2016). Although genetic threats are included more frequently in recovery plans, 37% of US plans have no consideration of genetic risk factors (Pierson et al. 2016). This figure is higher in countries such as Australia (45%) and the United Kingdom (67%)

(Pierson et al. 2016). In New Zealand there is also a perceived mismatch between geneticists and conservation practitioners, which has been termed a "conservation genetics gap" (Taylor, Dussex & van Heezik 2017). This has been reiterated in research worldwide (Howes et al. 2009; Milner-Gulland et al. 2010; Sutherland et al. 2010). It has been suggested that researchers need to be more involved in recovery planning (Howes et al. 2009) and that conservation genetics literature may need to be made more accessible for conservation managers (Milner-Gulland et al. 2010; Sutherland et al. 2010).

In this thesis, I aim to apply molecular methods in ways that will assist the future recovery of North Island kōkako (*Callaeas wilsoni*). Specifically, I will test for the presence of Psittacine Beak and Feather Disease and assess levels of genetic diversity in a proposed source population for future translocation projects. In doing so, this research will provide conservation managers with valuable information that can be applied in future to maximise the success of translocation projects (see below).

1.3 Wildlife translocations

Intentionally moving species as a means to enhance or maintain biodiversity has become a vital tool in conservation management and population recovery (Armstrong & Seddon 2008; Seddon 2010). Translocations are broadly defined by the International Union of Conservation of Nature (IUCN) as the movement of living organisms from one area, with free release into the other (IUCN 1987). This encompasses the intention to establish (introduce outside historical range), re-establish (movement within historical range) and augment (restock existing populations) populations (IUCN 1987). Historically, 90% of early translocations (between 1973 and 1986) involved the movement of game species (Griffith et al. 1989). Currently, translocations are utilized for a much greater diversity of taxa, particularly endangered species (Fischer & Lindenmayer 2000; Seddon et al. 2007; IUCN/SSC 2013). Research has identified, however, that there is a prevalent taxonomic bias when it comes to conservation translocations, particularly towards birds and mammals (Seddon et al. 2005; Champagnon et al. 2012). For amphibian, fish, reptile and invertebrate species, translocation efforts do not correlate to their prevalence in nature (Seddon et al. 2014). For example, mammals represent 30% of vertebrate species translocated, but equate to a proportion of approximately 8% of vertebrates in nature (Seddon et al. 2014). Regardless, translocations

play an important role in species recovery, with half of species recovery plans worldwide now recommending translocations as a management tool (Pierson et al. 2016).

For endangered species, translocations serve a number of purposes and in many ways, are invaluable for long-term species recovery. Returning to the IUCN (1987) definition, conservation translocations are focused around three broad aims; to reintroduce, augment or to introduce species into new habitats. Reintroductions are primarily used to re-establish self-sustaining populations of species within their historical range (Seddon et al. 2007; Seddon 2010). There are numerous examples of this in New Zealand, where mainland extinctions have occurred and translocations have been used to reintroduce species where they previously existed (e.g. saddleback (*Philensturnus carunculatus*) and New Zealand robins (*Petrocia sp.*), Taylor, Jamieson & Armstrong 2005). Augmentations on the other hand are used to supplement existing populations. This is often used as a method of enhancing genetic diversity at a particular population, reducing the likelihood of inbreeding, or promoting population growth (Seddon 2010). Finally, introductions are used primarily to fill a niche that has become empty from local extinctions, or as a method of future proofing populations for environmental changes (Seddon 2010; Weeks et al. 2011). The ultimate goal of translocations, regardless of whether it is to augment an existing population or to move species beyond their original range, is to improve and facilitate the long-term survival prospects of endangered species and to create self-sustaining populations (IUCN 1987; IUCN/SSC 2013).

New Zealand is a world leader when it comes to wildlife translocations (Armstong & McLean 1995) with a large number of threatened species actively managed through translocation programmes (Sherley 2010; Miskelly & Powlesland 2013; Parker 2013a). Human colonisation in New Zealand coincided with habitat loss and the introduction of mammalian predators, resulting in a suite of highly endangered endemic species (King, 1990). Conservation efforts were quickly directed towards recovering and maintaining the large cohort of threatened species (Parker 2013a). Some of the pioneering wildlife translocations were undertaken in New Zealand, with Richard Henry moving kākāpō (*Strigops habroptilus*), little spotted kiwi (*Apteryx owenii*) and South Island brown kiwi (*Apteryx australis*) off Resolution Island between 1885 and 1907 for conservation purposes (Hill & Hill 1987). The number and success rate of translocations has substantially increased

since these early attempts, as techniques have been improved and refined (Parker 2008, 2013a).

Across all taxa, birds have been translocated most often both historically (Clout & Saunders 1995; Sherley 2010) and at present (Cromarty & Alderson 2013; Miskelly & Powlesland 2013; Van Andel et al. 2016). Cromarty and Alderson (2013) reviewed translocation proposals in New Zealand (between 2002 and 2010) finding that five times more proposals are submitted for birds, and comprise 74% of approved proposals. The authors suggest that greater knowledge about bird translocations (than any other taxa) and popularity are likely to explain the high number of avian translocations (Cromarty & Alderson 2013). Indeed, 1100 separate releases of New Zealand birds were undertaken between 1863 and 2012 (Miskelly & Powlesland 2013). However, the number of reptile and invertebrate translocations has been increasing since the 1990s (Sherley 2010).

1.4 Managing translocations

The contemporary translocation literature is focused towards having guidelines to best manage and utilise translocation attempts as an effective conservation tool (Seddon et al. 2007; Armstrong & Seddon 2008; Cassey et al. 2008; Sutherland et al. 2010; Pérez et al. 2012; Batson et al. 2015). Translocation success in the past has been variable, with many translocations ending in failure (Griffith et al. 1989; Wolf et al. 1996; Fischer & Lindenmayer 2000; Soorae 2008). In New Zealand, translocation practice has developed considerably from initially utilizing offshore predator-free islands as safeguarded sanctuaries, to now translocating species between mainland sites (Parker 2013b, 2013a). Offshore island translocations are considerably more successful than those between mainland sites (Miskelly & Powlesland 2013). As the ultimate goal of translocations is to improve and facilitate the long-term survival and self-sustainability of endangered species, management is currently focused towards maximising translocation success (Armstrong & Seddon 2008; Sutherland et al. 2010; Batson et al. 2015). To achieve this, a number of different aspects must be considered, two of which are population genetics and disease.

1.4.1 Genetic management

It has been recommended that as reintroduction biology develops as a science, genetics should be better incorporated into the planning and evaluation of translocation programmes

(Seddon et al. 2007; Armstrong & Seddon 2008; IUCN/SSC 2013). As translocations primarily involve threatened species, managers are frequently dealing with populations that have experienced a considerable decrease in population size at some point in time (Frankham 2005; Griffith et al. 1989; Wolf et al. 1996). Moreover, translocation strategies typically involve the movement of a relatively small number of individuals, creating a genetic bottleneck where the diversity of the source population is not reflected in the new population (Nei 1973; Mock et al. 2004; Tracy et al. 2011). There are a number of genetic risks associated with translocation, which are primarily related to a loss of genetic diversity (Stockwell et al. 1996; Mock et al. 2004; Sigg 2006; Tracy et al. 2011), including reduced fitness, increased inbreeding, genetic divergence or limited adaptability (Frankham 1995, 1999; Lande 1988; Mock et al. 2004; Singer, Papouchis & Symonds 2000). Ultimately, long term, this can lead to increased extinction risk (Spielman et al. 2004).

As discussed above, the fundamental goal of translocations involves long-term persistence and population recovery, of which genetics plays an important role. Indeed, the latest IUCN guidelines now acknowledge the need to understand genetic diversity of the source populations and to include genetic monitoring for long-term persistence and translocation success (IUCN/SSC 2013). A recent review that aimed to provide a detailed summary of the use of translocation tactics (strategies that can be employed to increase the likelihood of translocation success post-release) found that genetic tactics are rarely mentioned in scientific articles or case studies published on mammal and bird translocations (less than 12% of studies) (Batson et al. 2015). This included genetic selection tactics, where individuals are selected based on genetic traits (e.g. heterozygosity or differentiation) and genetic composition, where the genetic make-up of translocated individuals is intentionally controlled (e.g. maximising diversity of translocated individuals). This review identifies that although genetics is now included as an IUCN translocation guideline (IUCN/SSC 2013), it is rarely incorporated into translocation literature (Batson et al. 2015).

Sourcing founders from a genetically diverse population is a valuable way to maximise the success and long-term persistence of a translocated population (Frankham et al. 2002; Armstrong & Seddon 2008; Allendorf et al. 2012). Research has clearly indicated that translocated populations often undergo a loss of genetic diversity relative to the source population (Stockwell et al. 1996; Houlden et al. 1996; Maudet et al. 2002; Mock et al. 2004;

Sigg 2006). To best ensure long-term translocation success, managers are therefore advised to maximise genetic diversity of the source population (IUCN/SSC 2013). Introducing animals from differentiated source populations also increases genetic diversity (Madsen et al. 1999; Maudet et al. 2002; Dresser et al. 2017; Proft et al. 2018). Populations founded from multiple sources have been found to have higher diversity than the original source population, and populations that were founded from only a single source (Bodkin et al. 2001). This has implications for evolutionary change, and has been suggested that mixing source populations will promote adaptive potential (Broadhurst et al. 2008; Sgrò et al. 2011; Proft et al. 2018).

Although theory and empirical research emphasize the value of capturing maximum genetic diversity from the source populations, some of the most well-known translocation success stories come from severely bottlenecked species such as the Guam rail (*Gallirallus owstoni:* Haig, Ballou & Derrickson 1990; Jenkins 1979) Chatham Island black robin (*Petroica traversi:* Ardern & Lambert 1997) and the Seychelles warbler (*Acrocephalus sechellensis:* Komdeur 1994; Komdeur, Kappe & van de Zande 1998; Richardson, Bristol & Shah 2006). Moreover, some species such as the saddleback successfully established and lost little diversity following sequential translocations (Lambert et al. 2005; Taylor & Jamieson 2008). This is understood to be a function of the source populations, in that there was low genetic diversity in saddlebacks prior to translocations, therefore little diversity to lose in subsequent translocations (Taylor & Jamieson 2008). Findings such as these highlight the need to assess the amount of genetic diversity present in contemporary populations to understand whether loss of genetic diversity might be an issue for translocation projects (IUCN/SSC 2013).

The number of individuals translocated is another important way to maximise the success of a translocation and prevent against a founding bottleneck (Tracy et al. 2011; Allendorf et al. 2012). By introducing a small number of individuals, the gene pool of the source population is unlikely to be represented in the new, translocated population (Stockwell et al. 1996; Mock et al. 2004, 2004; Tracy et al. 2011). Moreover, it is unlikely that all individuals will breed and contribute to future generations, meaning that an even smaller proportion of the gene pool will be passed on (Tracy et al. 2011). Small numbers of founders can lead to loss of genetic diversity and rapid genetic differentiation (Frankham 1996; Larson et al. 2002; Sigg 2006). To overcome this, it is recommended that a large number of founders

from genetically diverse populations are used to establish new populations (Griffith et al. 1989; Bodkin et al. 2001; Mock et al. 2004; Tracy et al. 2011). Indeed, modelling simulations have found that a large founding population helps to maintain and prevent diversity loss (Ottewell et al. 2014), as has been reflected in real world translocations (e.g. hihi, *Notiomystis cincta*; Brekke et al. 2011). In practice, the number of individuals needed to capture sufficient genetic diversity is unspecified. When establishing captive populations, previous guidelines have endorsed 20 founders as an adequate size (Lacy 1989; Willis & Wiese 1993). For wild populations however, there is no specific number as this is generally species-specific. Some research has attempted to calculate the number of founders required to maintain diversity in a population (e.g. Miller et al. 2009; Taylor & Jamieson 2008). Modelling frameworks have since been developed as a tool that allows genetic goals (e.g. retaining rare alleles) to be translated into a founding population number while including species-specific demographic parameters (Tracy et al. 2011; Weiser et al. 2012). Although the exact number of founders is unknown, it is suggested that a larger founding population (c.60 individuals) is required to retain genetic diversity over time (Tracy et al. 2011).

1.4.2 Disease management

The movement of animals from one location to another is important for the spread of disease (Cunningham 1996; Fèvre et al. 2006). When it comes to translocations, there is always a risk of an infectious disease outbreak (Woodford 1993; Dalziel et al. 2017) which can then become a serious threat to the viability and success of translocations (Aiello et al. 2014). This is particularly concerning, as translocations are used as a tool to manage and enhance populations that are often severely threatened (Frankham 2005; Griffith et al. 1989; Seddon 2010). Moreover, as translocations commonly involve small populations, the negative impacts of disease will be more pronounced than would be observed in a larger population (Cunningham 1996). Disease can not only result in death, but also increase individual susceptibility to other diseases and lower reproductive capacity (Scott 1988). Unfortunately, when evaluating translocation success, disease is a component that has historically been overlooked (e.g. Wolf et al. 1996).

Early translocation projects were undertaken without regard and consideration of the impacts of disease and disease spread (Griffith et al. 1993). Nowadays, there is a much greater appreciation for the impacts of disease (Aiello et al. 2014) and it is often cited as a significant

threat for conservation (Scott 1988; Daszak et al. 2000). In New Zealand, the Department of Conservation do recognize the impacts that a disease introduction during translocation would have (McInnes et al. 2004). However, researchers still emphasize that disease management needs to be better incorporated into reintroduction programmes (Ewen et al. 2012), that managers need to better appreciate that translocations can impact both disease prevalence and mechanisms of spread (Aiello et al. 2014), and the importance of disease screening (Parker et al. 2006).

There are two main disease risks that can ultimately lead to the failure of a translocation (Rideout et al. 2017). The first concerns the movement of individuals to a site that already has pathogens to which they are immunologically naïve (Cunningham 1996; Parker et al. 2006; Kock et al. 2010). Individuals from distant populations are likely to lack the immunity for infections that are present at other populations (Kock et al. 2010). For example, between 3% and 30% brush-tailed possums (*Trichosurus vulpecula)* in New Zealand are thought to be currently infected with bovine tuberculosis, a disease that does not occur naturally in possums from Tasmania (Hickling 1991). As possums were introduced from Tasmania to New Zealand in 1837, it is understood that the translocated possums were exposed to infected dairy cattle upon release and they were immunologically naïve to the disease (Hickling 1991). Secondly, translocations can introduce disease into an environment or population where it did not exist previously (Cunningham 1996; Parker et al. 2006; Kock et al. 2010; Sainsbury & Vaughan-Higgins 2012). For example, in 1985, North American racoons (*Procyon lotor*) were translocated from Texas to West Virginia (USA) to supplement local populations, but at the same time parvoviral enteritis was introduced to the local population (Allen 1986).

Various aspects of translocation procedures can increase the prevalence of disease in the wild. For one, translocations are often stressful for the animals involved (Dickens et al. 2010). This includes stress related to transportation, conditions in captivity, capture, release and increased competition at either the source or recipient site (Kock et al. 2010; Sainsbury & Vaughan-Higgins 2012). When animals are under stress, their susceptibility to disease and parasites can increase due to physiological changes (Dickens et al. 2010; Aiello et al. 2014). Moreover, stress can also change the relationship between host and disease, potentially altering the dynamic and moving from a latent infectious disease to a more harmful

relationship (Dickens et al. 2010; Kock et al. 2010). For example in the Eurasian crane (*Grus grus*), commensal microparasites (Coccidia) can cause disease when juvenile birds become stressed (Sainsbury & Vaughan-Higgins 2012). The number of founders can also influence disease prevalence. As previously discussed, current translocation guidelines encourage large founding population sizes to maximise genetic diversity (Bodkin et al. 2001; Griffith et al. 1989; Mock et al. 2004; Tracy et al. 2011). This, however, can facilitate the spread of disease, by increasing the likelihood of transmission, or increasing the number of susceptible hosts (Aiello et al. 2014). Other factors such as dispersal from release site or population connectivity can further influence disease prevalence and facilitate spread (Aiello et al. 2014).

To manage and minimise the disease risks associated with wildlife translocations, stress management, disease screening and risk assessments are recommended. Recognition of disease risk in translocations has improved in recent years. For example, guidelines have been established for disease risk analysis by the World Organisation for Animal Health (OIE) and the International Union for Conservation of Nature (IUCN) (OIE & IUCN 2014). Disease risk is also well recognised in New Zealand; the Department of Conservation (DOC) permit process requires mandatory disease screening (McInnes et al. 2004) and an online national database has been developed to manage and share disease testing results (Department of Conservation 2018). For many New Zealand species, best practice documents have been recently established for those that are commonly translocated such as North Island kōkako (Collen et al. 2016), robins (Collen et al. 2014a) and kākāriki (*Cyanoramphus sp*. Collen et al. 2014b). All best practice guidelines include clear instructions surrounding stress minimisation and disease screening protocols during the translocation process, all of which contribute to the long-term success of translocation endeavours.

1.5 North Island kōkako

The kōkako (*Callaeas wilsoni*) is a large (c. 38 cm), forest-dwelling songbird, belonging to the endemic wattlebird family *Callaeidae*. Wattlebirds are one of the oldest New Zealand avian lineages, and were present 82–85 mya when New Zealand split from Gondwana (Shepherd & Lambert 2007). Kōkako are the only members of this lineage that survive in remnant populations on the New Zealand mainland (King, Innes & Hay 2015). There are two subspecies of kōkako: South Island kōkako (*Callaeas cinerea*) and North Island kōkako

(*Callaeas wilsoni*). The South Island kōkako was endemic to the South Island of New Zealand, and distinguishable by their orange (as opposed to blue in *C. cinera*) coloured wattles. Unlike the North Island subspecies, South Island kōkako mainly inhabited beech forest, nested in the sub-canopy and foraged close to the ground (Clout & Hay 1981). It is suspected that these characteristics predisposed SI kōkako to strong predation pressures, especially during beech masting events (Clout & Hay,1981). Although reports surrounding the presence of SI kōkako still occur (Milne & Stocker 2014), the last verified sighting occurred in 1967 (McBride 1981). Consequently, South Island kōkako are currently classified as data deficient by the New Zealand Threat Classification system and are thought to be at least functionally, if not officially, extinct (Robertson et al. 2017).

North Island kōkako (hereafter "kōkako") are a territorial, elusive species, spending the majority of their time in the forest canopy where they both feed (Powlesland 1987) and nest (Flux, Bradfield & Innes 2006). Kōkako are sexually monogamous, and form pair bonds that co-defend territories over many years (Flux et al. 2006). Breeding generally occurs during the New Zealand summer, beginning in October and ending in February (Flux et al. 2006; Hay, Best & Powlesland 1985), but occasionally ends as late as May (Flux et al. 2006). During the breeding season, kōkako can fledge up to three clutches, therefore producing as many as six chicks in a successful season (Flux et al. 2006). Nesting years are thought to be dependent on food availability, with greater clutch sizes in years that have a greater abundance of fruit (Flux et al. 2006; Innes et al. 1996). Kōkako have a reasonably long lifespan for a passerine species, known in some cases to reach more that 20 years of age (Innes & Flux 1999).

Similar to most New Zealand avifauna, kōkako occupied a significantly larger range across North Island forests prior to human colonisation (Lavers 1978). Kōkako populations became limited to small remnant forest patches, decreasing to as few as 330 pairs in 13 relict mainland populations, and 70 pairs (non-relict populations) on offshore islands in 1990 (Innes, Molles & Speed 2013). Many threats contributed to kōkako decline. Polynesian arrival and establishment on the mainland of New Zealand resulted in forest clearance and the introduction of mammalian predators (i.e. Kiore; *Rattus exulans*) (Holdaway 1989). Highly dependent on mature, complex forest (Clout & Hay 1981), habitat clearance is thought to have initially reduced population sizes, which were then impacted by introduced

predatory mammals (Leathwick et al. 1983). Williams (1976) recognised that *Callaeidae* decline preceded the introduction of browsing mammals, indicating that initially, ship rats and kiore were primarily responsible for kōkako decline. Following this, diet overlap is understood to have further contributed to kōkako decline with browsing mammals depleting the supply of native plant species (Best & Bellingham 1990;. Hay et al. 1985; Leathwick et al. 1983; Powlesland 1987).

Presently, kōkako populations suffer predation from rats, possums, mustelids and harriers (Basse, Flux & Innes 2003; Innes et al. 1996; Innes et al. 2010). An extensive, eight-year experiment on kōkako population dynamics examined predation in three mainland populations (Innes et al. 1999). This study identified that predation was primarily responsible for kōkako decline, compared to other pressures such as habitat or food limitation. Kōkako populations are most vulnerable to predation by brushtail possums (*T. vulpecula*) and ship rats (*R. rattus*) (Innes et al. 1996; Innes et al. 1999; Leathwick et al., 1983). The primary limitation of population growth is predation of both eggs and nestlings during the breeding season (Innes et al., 1999), with nest failure commonly occurring within the first two weeks (Flux et al. 2006). The number of nesting attempts does not change when predators are controlled, but nesting success and the number of breeding pairs does increase (Innes et al. 1996). A comparison between populations with and without predator control clearly reveals the differences in breeding success. Innes et al. (1999) found a large difference between breeding success in populations with and without mammalian predators, with 80% successfully fledging on Little Barrier Island (no predators) and only 17% in Rotoehu (no predator control). Productivity is limited even further with female mortality, being preyed on whilst nesting, often leading to a male bias in populations (Innes et al. 2013).

Kōkako are considered to be one of the leading conservation success stories in New Zealand, with coordinated and intensive recovery efforts leading to a dramatic increase in population size and number. In 1999, there were an estimated 400 pairs across 13 mainland, and two offshore island populations (Innes et al. 2013). The latest census indicates that as of January 2017, there were c.1589 pairs across 24 populations (both offshore and mainland) (*North Island Kōkako (Callaeas wilsoni) Recovery Plan 2017–2025. Draft* 2018). Translocations have been a central objective of kōkako recovery. Early translocations were used to establish safe populations on predator free offshore islands (Kapiti and Hauturu) and

to protect kōkako from forest felling in the early 1980s (Innes et al. 2013). Translocations have since been used to re-establish populations within the former kōkako range, augment small populations and to introduce kōkako into suitable sites (Innes et al. 2013). Kōkako are among the most commonly translocated species in New Zealand (Miskelly & Powlesland 2013), with a total of 94 translocations undertaken as of 2011 (Innes, Molles & Speed 2013). Predator control has also been instrumental for population recovery, and is currently implemented across all mainland sites (Innes & Flux 1999). As a result of conservation efforts, the New Zealand Threat Classification has been downgraded from Nationally endangered to At Risk, Recovering (Robertson et al. 2017).

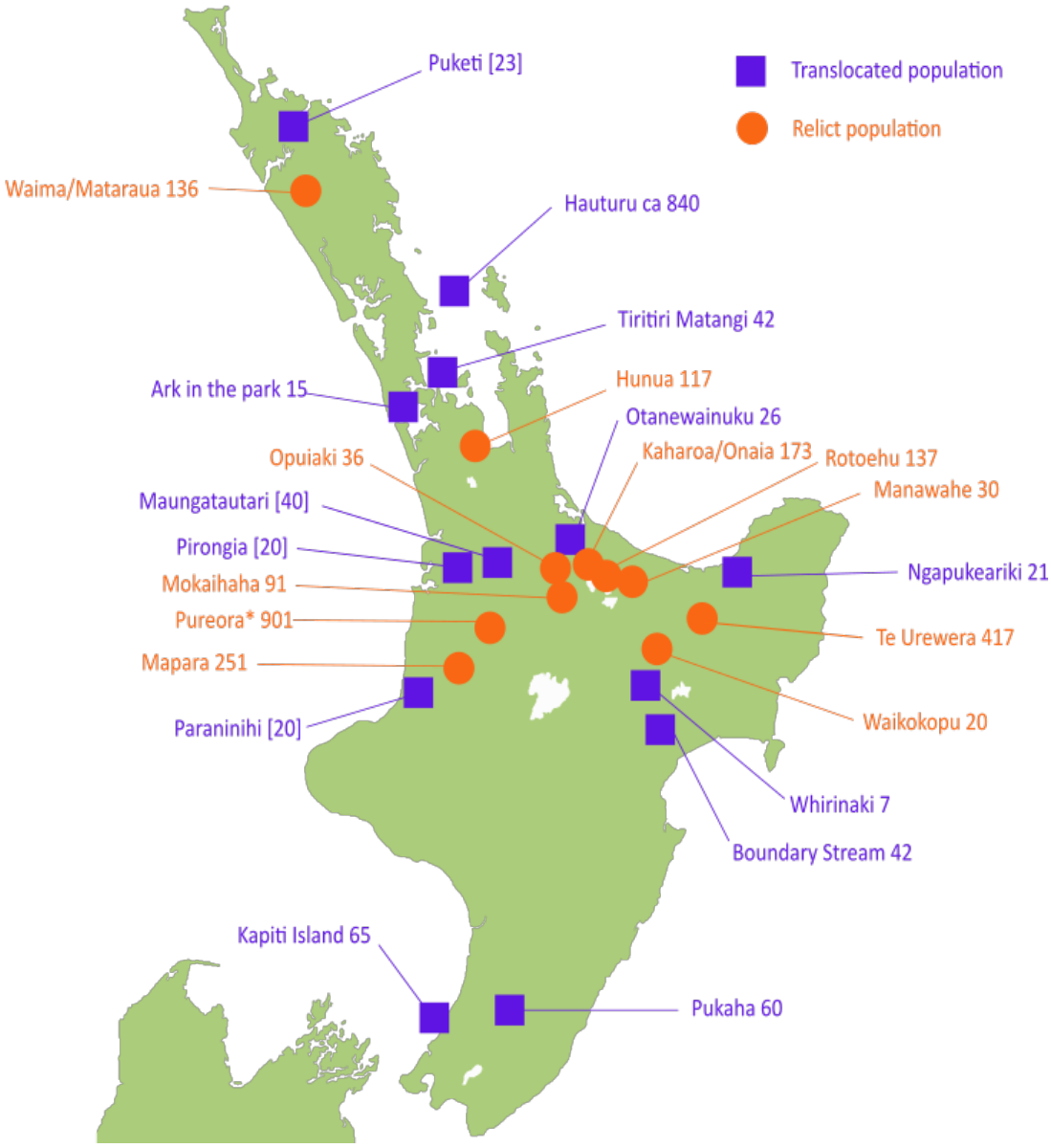


Figure 1.1. Map showing locations and population sizes (excluding juveniles and sub-adults; Square brackets indicate recently established sites) of translocated (n=13) and relict (n=11) populations of North Island kōkako as of January 2017 (Population estimates from *North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft 2018*). *Pureora forest includes: Mangatutu, Waipapa, Okahukura and Tunawaea populations.

1.6 Te Hauturu-o-Toi

Little Barrier Island (Te Hauturu-o-Toi) is a 28 km^2 volcanic island in the Hauraki Gulf. Hauturu was New Zealand's first wildlife sanctuary, officially declared as a reserve in 1895 (*Te Hauturu-o-Toi. Little Barrier Island Nature Reserve Management Plan* 2017). The island has been largely reforested, with successful eradications of cats (*Felis catus*) completed in 1980 (Veitch 2001) and kiore (*R. exulans*) in 2004 (Bellingham et al. 2010). In the absence of introduced predatory mammals and with a diverse and mature native forest, Hauturu is

one of the most intact ecosystems in New Zealand. For this reason, Hauturu is considered to be one of the most important wildlife reserves in New Zealand (Bellingham et al. 2010). Many endemic species thrive in the absence of predators on Hauturu, and in a number of cases endemic species would be otherwise extinct had it not been for strong-hold populations on the island (e.g. hihi; Thorogood et al. 2013; New Zealand storm petrol, *Fregetta maoriana*; Stephenson et al. 2008; Rayner et al. 2015).

Hauturu is also an important environment for kōkako. Between the years of 1981 and 1994, 32 birds were translocated to Hauturu in an effort to establish a safe offshore island population (Appendix 1; Innes et al. 2013). Many aspects of these translocations would fail to meet contemporary guidelines; for example, a high proportion of the translocated birds were male as a result of female mortality (Innes et al. 2013). Additionally, very few of the birds are understood to have bred, a number as low as nine founding birds (I. Flux, pers. comm.). This population is now the largest of all sites, in excess of 422 ± 115 pairs (Flux, Thurley, McKenzie & McAulay 2013). Population growth in Hauturu is much higher than mainland populations (Innes et al. 1996; Innes et al. 1999), and is understood to be a consequence of the lack of predators (Innes et al. 1996).

1.7 Thesis rationale and aims

The Hauturu kōkako population has been suggested to be nearing carrying capacity (J. Innes, pers. comm.), and the Kōkako Specialist Group (KSG) have expressed interest in utilising Hauturu as a source population for future translocations. Given the current size and growth rate on Hauturu, this population would present as a valuable source for translocations. The population size (c.422 pairs; Flux et al. 2013) of Hauturu is important from a genetic management perspective, as a large number of founders could be harvested from the island to capture sufficient genetic diversity (Tracy et al. 2011; Weiser et al. 2012). However, when considering the recent history of the Hauturu population, in terms of the low number of founders (I. Flux, pers. comm.), concerns have been raised as to the current levels of genetic diversity and inbreeding on the island, and hence the suitability as a translocation source. Moreover, a recent capture of a kōkako with aberrant plumage and features that resemble the symptoms of psittacine beak and feather disease (Doneley 2016; Pass & Perry 1984; Ritchie et al. 1989) has raised further concern about potential disease within Hauturu kōkako (T. Thurley, pers. comm.). As discussed above, when managing and planning translocations,

minimising risk and maximising long-term success is strongly emphasised in management guidelines and throughout the literature. Both genetic and disease risk management is therefore important if Hauturu is to be used in future proposed translocations. If the population is lacking in genetic diversity, sourcing founder birds from Hauturu may be ill-advised. Furthermore, if there is disease present then this will have implications for the spread to potentially immunologically naïve sites such as the possum example (e.g. Hickling 1991).

The use of Hauturu as a source population is a clear example of why both genetic and disease risk factors need to be considered when planning and managing translocations of endangered species. On face value, the Hauturu population appears as a large, productive site. However, without considering important risk factors such as genetic diversity and disease, a translocation from Hauturu may ultimately hinder kōkako recovery, rather than enhance it. In this thesis, I will use molecular tools to look into potential disease in the Hauturu kōkako population and measure genetic diversity across a number of kōkako sites (including Hauturu). In doing so, this research will assist conservation managers with decision making and future planning of kōkako recovery, as well as contribute to the wider literature in terms of population genetics of North Island kōkako.

The overall aim of this thesis is to use molecular methods to assess the utility of Hauturu as a source population for future kōkako translocations. To do so I will: 1) quantify genetic diversity of kōkako on Hauturu compared to relict, mainland populations, and 2) test for the presence of Psittacine Beak and Feather Disease in the Hauturu population. This research will therefore examine two important factors that contribute to the long-term success of a translocation and the overall suitability of a source population. As such this research is an example of how conservation genetics can be integrated into threatened species management in New Zealand.

The specific objectives of this research are to:

- Identify whether kōkako from the Hauturu population have an increased level of relatedness and decreased level of genetic diversity when compared to relict mainland populations, given the suspected founding population of nine birds
- Determine whether there is significant genetic differentiation between Hauturu and five relict mainland populations

- Assess whether the aberrant coloured kōkako has contracted a novel strain of Psittacine Beak and Feather Disease
- Consider the management implications of the findings, in terms of recommendations for future translocation projects

1.8 Thesis structure

The main body of this research is presented within three data chapters (Chapters 2, 3 and 4). The final chapter considers the management implications of the findings and discusses the value of integrating genetic research and conservation management for threatened species recovery.

Chapter Two describes the development of 21 novel kōkako-specific microsatellite loci from a whole genome that was sequenced by BGI genomics (China) as part of the 10,000 bird sequencing project (Zhang 2015). These loci are then used in chapter three to compare genetic diversity and population structure of kōkako from Hauturu and five mainland populations (see Chapter 3 for map).

Chapter Three compares the level of genetic diversity on Hauturu to five remnant mainland populations using standard measures of genetic diversity. In addition, population structure across all six populations is examined using multivariate and Bayesian methods to determine whether geographic isolation has led to significant differentiation between populations. This chapter uses blood samples collected by the Kōkako Specialist Group between 1997 and 2016, that were genotyped in the present study. Together these findings provide an updated and more reliable measure of the genetic variation within and between kōkako populations (Hudson et al. 2000; Murphy et al. 2006).

Chapter Four explores two molecular explanations for the observed phenotypic aberrations in a kōkako captured on Hauturu in 2015. The first uses polymerase chain reaction to screen kōkako from the Hauturu population for Psittacine Beak and Feather Disease (PBFD). As the population tested negative, sequences of the melanocortin-1 receptor (MC1R) gene were isolated and examined for mutational differences. This gene was selected as it has a pleiotropic function in regulating both melanin deposition and stress related responses. This chapter uses blood samples from Hauturu collected by the Kōkako Specialist Group between

two sampling seasons (2013 and 2015), from which genomic DNA was extracted and the regions of interest were amplified in the present study. Together, these findings contribute to a growing body of literature on PBFD and the role of the MC1R gene in melanic phenotypes.

Chapter Five considers the findings of Chapters three and four together in terms of the utility of Hauturu as a source population. I also discuss the wider implications of this research for translocation management of kōkako, the importance of integrating genetics and conservation management and directions for future research.

Chapter Two:

Development and characterization of 21 novel microsatellite loci for North Island kōkako

(*Callaeas wilsoni*)

North Island kōkako (*Callaeas wilsoni*). Source Matt Binns.

2.1 Introduction

The North Island kōkako (*Callaeas wilsoni*) is an endemic, forest dwelling passerine existing as 24 remnant and translocated populations in New Zealand. Kōkako are one of two surviving members of the ancient avian family Callaeidae (wattlebirds), alongside the saddleback (*Philesturnus carunculatus*) and extinct Huia (*Heteralocha acutirostris*) (Hay et al. 1985; King et al. 2015). As with many New Zealand species, North Island kōkako (hereafter "kōkako") experienced a significant decrease in population size and range following human colonization (Innes & Flux 1999). Presently, kōkako exist in a fragmented distribution across mainland sanctuaries and predator free offshore islands *(North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft* 2018). Kōkako are classified as At Risk, Recovering in the New Zealand Threat Classification System, based on the number of individuals (1000 to 5000 mature individuals), total population area (≤ 100 ha) and predicted increase greater than 10% over the next 10 years (Robertson et al. 2017).

Conservation management of kōkako has been particularly successful, with the re-establishment of 13 populations through translocation as of January 2017 (*North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft* 2018). Based on the frequency of translocations, population history and current fragmented distribution of kōkako, it is important to understand the level of genetic diversity and population structure (Frankham et al. 2017). At present, our understanding is limited with only two studies exploring conservation genetics of kōkako to date (Hudson et al. 2000; Murphy et al. 2006). Moreover, the only study to examine nuclear genetic diversity utilised four kōkako-specific loci across three kōkako populations (Hudson et al. 2000). Low numbers of microsatellite markers can limit the accuracy and statistical power of population based studies (Wan et al. 2004; Hubisz et al. 2009). It is therefore important to update the current understanding of genetic diversity and population structure among kōkako populations. By doing so, such research has the potential to facilitate and improve future conservation efforts and management decisions.

Here I develop 21 microsatellite marker primers specifically for North Island kōkako using whole genome sequence data. These markers will be used to examine genetic diversity and population structure of kōkako (Chapter 3). Such research will assist kōkako recovery efforts, particularly in terms of assessing the suitability of source populations for future translocation projects.

2.2 Methods

Genomic DNA was extracted from one North Island kōkako whole blood sample (stored in Queen Lysis buffer) was sent to BGI Genomics (China) for whole genome sequencing. This was part of the 10,000 bird sequencing project (Zhang 2015), which returned the genome in over four million 150bp reads. These were assessed for di-, tri- and tetra-nucleotide microsatellite repeats (≥ six repeat units in length) using MSatCommander 1.0.8 (Faircloth 2008). Only a subset of the genome (approximately one million reads) was screened with the programme, which identified 9378 microsatellite-containing reads.

Primer pairs were designed in flanking regions of suitable microsatellites using Primer3web V.4.1.0 (Koressaar & Remm 2007; Untergasser et al. 2012). Appropriate primers could not be designed within microsatellite repeat regions that were too close to the edge of the 150bp, in turn ruling out the read containing the microsatellite. Primer pairs were successfully designed in 32 microsatellite repeat regions (25 di-nucleotide reads, two tri-nucleotide reads and five tetra-nucleotide reads; Table 2.2). Forward primers were tagged at the 5' end with a M13 sequence (sequence: 5'-TGTAAAACGACGGCCAGT-3') to enable the use of fluorescently-tagged M13 primers (Schuelke 2000).

The 32 microsatellite primers were tested for functionality and polymorphism in seven kōkako individuals that represented the greatest geographical separation of populations (Table 2.1). This approach was used because the selected individuals were most likely to capture a significant proportion of the genetic diversity present across all 149 kōkako to be subsequently genotyped, (Schuelke 2000). Loci were assigned to one of eight multiplexes for amplification using polymerase chain reaction (PCR) (Table 2.2).

Table 2.1. Details of the North Island kōkako individuals genotyped (n=7) to test the functionality and polymorphism in the 32 microsatellite loci.

Location	Date collected	Date DNA extracted	Sample number
Hauturu (LBI)	11.08.15	05.06.17	E212429
Mapara	15.06.16	24.05.17	E232334
Mangatutu	11.09.16	24.05.17	E221375
Kaharoa	10.9.10	13.09.17	E212407
Te Urewera	11.2.98	04.09.17	E189469
Kapiti Island	4.05.00	08.06.17	E113824
Mataraua	15.12.97	08.06.17	E189953

The 2 μL PCR reaction included, 1 μL of DNA (15–68 ng, dried), 1 μL of 2 x Type-it Multiplex PCR Master Mix (Qiagen), 0.04 μM (per locus) of each reverse primer, 0.16 μM (per locus) of each M13-labelled forward primer, 0.16 μM of a universal M13 primer 5'labelled with a fluorescent dye; 6-FAM, VIC, NED or PET (DS-33 dye: Applied Biosystems) and PCR grade water (Sigma Aldrich). Reaction conditions were as follows; Initial denaturation at 94 °C for 15 minutes, eight cycles of 94 °C for 30 seconds, 60 °C (touchdown 1 °C per cycle) for 90 seconds and 72 °C for 60 seconds. Then a further 25 cycles of 94 °C for 30 seconds, 52 °C for 90 seconds and 72 °C for 60 seconds, and a final elongation step of 60 °C for 30 minutes. Genotyping of PCR products (2 μL of PCR product, and 7.8 μL Hi-Di Formamide) was performed using GeneScan 500 LIZ (Applied Biosystems Genescan™) as a size standard (0.2 μL) on an ABI 3730xl DNA Analyser (Applied Biosystems). Alleles were scored automatically using Geneious software v.9.1.8 (Kearse et al. 2012). Visual searching was undertaken to detect alleles that the software had missed, or that had been incorrectly labelled as peaks, and bins were predicted using the Geneious v.9.1.8 algorithm (Kearse et al. 2012).

Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium between pairs of loci were assessed using a Markov Chain Monte Carlo (MCMC) approximation of Fisher's exact test using Genepop v.4.0.10 (Raymond & Rousset 1995) to test the performance of each loci as reliable genetic markers. A test for null alleles, scoring errors due to stuttering and large allele dropout was performed using MICROCHECKER v.2.2.3 (Van Oosterhout et al. 2004). As part of the wider study examining genetic diversity and population structure (see Chapter 3), the number of alleles per locus, observed and expected heterozygosity and number of private alleles were explored on a larger sample (110 individuals; six populations) using GenAlEx v.6.5 (Peakall & Smouse 2012). Genotyping error was quantified by repeat-genotyping 20 individuals across all populations and calculating the number of mismatched alleles (Hoffman & Amos 2005; Pompanon et al. 2005).

2.3 Results and discussion

Of the 32 loci that were screened in this study, 25 amplified successfully across more than three of the seven individuals tested (see Table 2.2). Twenty-four (96%) of the loci were polymorphic, however, when amplified across a larger sample (110 individuals from six populations) only twenty-two amplified consistently. As only c. quarter of the kōkako genome reads were screened for microsatellite loci, many more reads are available if more microsatellite loci are needed for future analyses.

When amplified across all 110 individuals, seven loci were identified as containing potential null alleles, however, only four possessed a null allele frequency greater than 0.2. In each of the four loci, null alleles were no detected in all populations, with only two of the four displayed a high null allele frequency in more than one population. Exclusion of these four loci did not change the outcome of any results (for genetic diversity or population structure analyses), therefore all loci were retained in the study. Highly significant linkage disequilibrium was identified between one pair of alleles across all populations (Cwil04 and Cwil08; $P < 0.001$). One locus of the pair was excluded from any further analyses (Cwil04).

Three loci significantly departed from HWE proportions at $P < 0.05$ following Bonferroni adjustments for multiple comparisons (Cwil13 in the Hauturu population; Cwil22

in the Mapara population; Cwil19 in the BOP population). As there were no consistent departures across all populations, these loci were retained in the dataset.

A total of 240 alleles were scored across 110 individuals (see Chapter 3), with the number of alleles ranging from one to 8 (mean = 5.960 ± 0.196 (SD)). Overall, observed and expected heterozygosity was 0.678 ± 0.018 (SD) and 0.687 ± 0.014 (SD) respectively (Table 2.3). Of the microsatellite loci that amplified successfully, the level of polymorphism was particularly high (96%). This was higher than observed in other New Zealand species such as the takahe (*Porphyrio hochstetteri,* 17.3%; Grueber et al. 2008), blue duck (*Hymenolaimus malacorhynchos*, 54%; Abdelkrim et al. 2009) or rock wren (*Xenicus gilviventris*, 90%; Weston & Robertson 2014).

These 21 loci were subsequently used to examine contemporary genetic diversity and population structure across six North Island kōkako populations (Chapter 3). This research aimed to increase the current understanding of the genetic status of kōkako populations and to assist future recovery and conservation efforts. Previously, only four kōkako-specific microsatellite markers had been developed and were available for use in genetic research (Hudson et al. 2000). The development and use of this significantly larger microsatellite library will increase the validity and accuracy of future studies.

Table 2.2. Details of 24 kōkako-specific microsatellite primers that amplified successfully in primer trial (n=7). M13 label (5' – TGTAAAACGACGGCCAGT – 3') added to forward primer only.

Locus	Primer sequences (5' to 3')	Repeat motif	A	Size (bp)	H_o	H_e	Dye (Multiplex no.)
Cwil_01	F: GCCTGTGTCGCTGAGTGTAT R: TGCCTTCAAACAGAAAGCAA	$(GT)_{20}$	8	154–180	1.00	0.847	6-Fam(1)
Cwil_05	F: GGTATCTGTGTGTGGGCGTA R: TGGAGGACTGAGGTAGCACAA	$(CA)_{12}$	3	123–137	0.5	0.594	Vic (1)
Cwil_07	F: CACAAAATTGGGTGTGGATG R: TGATGCTGACAGATTTGAAGAC	$(TG)_{11}$	6	136–146	1.00	0.847	VIC (3)
Cwil_08	F: GTGGTGAACAGAAAAGCAGGT R: TTGAGCTAAGTAATGAAGATTGACC	$(GA)_{13}$	5	160–164	0.60	0.72	VIC (4)
Cwil_09	F: CAGACAGATGCCACCTGAAA R: TGCCATATGTGCAAAGGAGA	$(CA)_{17}$	2	146–148	0.286	0.408	NED (1)
Cwil_13	F: AAATAGCCCACAAAATCTTCCTT R: TGGAGGTGAGCCCTTCAG	$(CA)_{16}$	4	156–168	0.429	0.70	PET (1)
Cwil_14	F: TGCAGTCAGTGGCCTTTAGTT R: GACTGCAGACAGAGAGCACAA	$(TC)_{17}$	4	122–128	0.571	0.735	PET (2)
Cwil_15	F: GCTACCAACAGGGCAACTTT R: CAAAAGCCGGTTTTCTTCCT	$(GT)_{14}$	7	126–146	0.714	0.816	PET (3)
Cwil_16*	F: AAACCCGTAGGGGAGGTG R: GGACACAAATCCAGGAGGAA	$(GA)_{15}$	2	119–121	NA	NA	PET (4)
Cwil_17	F: GATGTGTGTGTGGTCTGCTC R: CCCAAGGACTCAGGAGCTAC	$(GT)_{19}$	7	114–136	0.50	0.806	6 FAM (5)
Cwil_18	F: TCAGTCAGGGCAAAGAGAGG R: AGACTAGATCCCTTCCAACTGA	$(ATCC)_{15}$	3	126–138	0.40	0.34	6-FAM (6)
Cwil_19	F: CTGAAATTCCTAGCCCCTCATAT R: CCCCAGCCACCCAAAATCTA	$(GT)_{18}$	7	118–131	0.714	0.816	6-FAM (7)
Cwil_20*	F: AGCAGAGCCTTGTGTGACAT R: TGGGATTGATGGAGACTGACC	$(ATCC)_{12}$	6	109–163	NA	NA	6-FAM (8)
Cwil_21	F: TGATTCCATCCCTCCTTCCC R: GGCCTGGTTCCTACTTAATTGG	$(AC)_{23}$	5	102–124	0.429	0.67	VIC (5)
Cwil_22	F: CCTTCCTCTCTGCTCCTCTG R: ACACTCCACCTTTGACTCTGT	$(AC)_{16}$	7	117–159	0.714	0.816	VIC (6)
Cwil_23	F: GCTTACCCACACTGTTCACC R: CCACAGACAAGAGGAGCAGA	$(ATCC)_{13}$	3	130–173	0.60	0.54	VIC (7)
Cwil_24	F: CATGACTTTTGAGGCGCAGA R: ACCAGTCCCCACACATTTGA	$(AC)_{19}$	6	106–125	0.286	0.408	VIC (8)
Cwil_25	F: TGCTACGGGAAAGGAGGAAG R: ACAAGTCAATGTACCATCCTCTC	$(AGAT)_{19}$	6	109–124	0.667	0.79	NED (5)
Cwil_27*	F: AGCTTCATCCCTCTGTCTCC R: CAAAGAGCAAAGCAGACCCC	$(ATCC)_{16}$	4	108–153	NA	NA	NED (7)
Cwil_28	F: CCGTTTGACTAGAGACCACCT R: GCCCTTAAGATCAGCCCTGC	$(GAT)_{9}$	4	135–144	0.571	0.66	NED (8)

Cwil_29	F: TGGAAGGAAAAGAGGTGAGATTT R: TTTTCCCCTACATTTGCGCG	$(GT)_{13}$	4	149–157	1.00	0.719	PET (5)
Cwil_30	F: TCATCCTGACTCTGCTGGTC R: TGAACATCCTCAGGGTGCAG	$(GT)_{18}$	5	120–135	1.00	0.68	PET (6)
Cwil_31	F: AGTCACTGGCTCTGAATAGGT R: ATGCTCCTCGAAGTAACAAGAT	$(GT)_{15}$	5	150–165	0.571	0.765	PET (7)
Cwil_32	F: TCTTGTTCTGACCATCCCTCC R: TAGAGGAAAGTGTGCTGGGG	$(ATC)_{10}$	5	116–144	0.571	0.67	PET (8)

** Indicates not used in study examining genetic diversity and population structure of kōkako (monomorphic, messy amplification)*

A = total number of alleles at each locus

Table 2.3. Genetic variation at 21 microsatellite loci within six North Island kōkako populations across the North Island of New Zealand (110 individuals). In Chapter Three.

Population	N	A	AR	H_o	H_e	PA	Population size*
Bay of Plenty	8	5.762 (0.382)	4.358 (1.123)	0.701 (0.046)	0.698 (0.036)	10	Rotoehu: 54 Kaharoa: 77
Hauturu	27	6.571 (0.481)	3.772 (0.925)	0.614 (0.037)	0.690 (0.030)	13	ca 420
Mapara	35	6.905 (0.643)	3.871 (1.031)	0.657 (0.045)	0.692 (0.041)	17	122
Mangatutu	23	6.810 (0.376)	4.103 (0.808)	0.706 (0.040)	0.722 (0.028)	12	429†
Te Urewera	6	4.190 (0.290)	3.691 (1.046)	0.681 (0.058)	0.636 (0.041)	5	188
Waipapa	11	5.524 (0.382)	3.905 (0.945)	0.707 (0.035)	0.682 (0.036)	6	429†
Overall	*110*	*5.960 (0.196)*	*3.950 (0.244)*	*0.678 (0.018)*	*0.687 (0.014)*	*NA*	Total: c.1589

**Population size (number of estimated breeding pairs) from draft kōkako recovery plan (North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft* 2018). *Total is across 20 populations.*

†Population estimate for Pureora forest, Mangatutu and Waipapa alone is not available

N, sample size; A, mean number of alleles; AR, mean allelic richness; PA, number of private alleles. Standard error provided in parentheses.

Chapter Three:
High genetic diversity in a genetically distinct, translocated population of North Island kōkako (*Callaeas wilsoni*):

Te Hauturu-o-Toi

Little Barrier Island (Te Hauturu-o-Toi). Source Jake Osborne.

3.1 Introduction

Selecting an appropriate source population is a fundamental aspect of any translocation project. The long and short-term success of a translocation is largely dependent on the founding individuals becoming established and reproducing in the new site (Armstrong & Seddon 2008; Sutherland et al. 2010; Batson et al. 2015). Understanding the genetic diversity of source populations is acknowledged as a translocation guideline (IUCN/SSC 2013) and has been emphasized repeatedly in the literature (Frankham et al. 2002; Armstrong & Seddon 2008; Allendorf et al. 2012). Translocations are common practice in New Zealand, often used as a management tool for threatened species recovery (Sherley 2010; Miskelly & Powlesland 2013; Parker 2013a). Due to the impact of human colonisation on New Zealand avifauna (Atkinson & Cameron 1993; Holdaway, Worthy & Tennyson 2001; King 1990), managers are frequently presented with populations that have been small, isolated and fragmented for significant periods of time (Jamieson et al. 2006; Jamieson 2009). As such, many avian populations in New Zealand are suffering from concerning genetic issues such as loss of genetic diversity and increased genetic differentiation (Jamieson et al. 2006; Jamieson 2009).

3.1.1 Loss of genetic diversity

Conserving genetic diversity is crucial for ensuring the long-term survival of endangered species (Hedrick & Kalinowski 2000; Spielman et al. 2004; Frankham 2005; Frankham et al. 2017). Whether human induced or due to natural processes, a decrease in population size can result in a genetic bottleneck, where the diversity of the original population's gene pool is not reflected in the resulting population (Nei et al. 1975; Allendorf 1986). Population bottlenecks have been found to reduce genetic diversity both experimentally (England et al. 2003) and in natural populations of threatened species (e.g. Eithiopian wolves (*Canis simensis*), Mauritius kestrels (*Falco punctatus*), Florida torreya trees (*Torreya taxifolia*); Spielman et al., 2004). A second concern is the loss of genetic diversity in small populations through genetic drift, where allele frequencies fluctuate between generations due to chance (Keller & Waller 2002; Frankham et al. 2017). This random fluctuation in allele frequency usually has a greater effect in small populations, as there are fewer genes to be sampled from at each new generation (Allendorf 1986). Over time, this can lead to the fixation or loss of alleles at a particular locus, decreasing genetic variation within the population (Stockwell et al. 1996; Tarr et al. 1998; Keller & Waller 2002). A further consequence of small population size is the increased likelihood of inbreeding, as with fewer mating opportunities individuals

are more likely to mate with relatives (Briskie & Mackintosh 2004). This impacts genetic diversity in terms of decreased heterozygosity (Frankel & Soulé 1981; Frankham et al. 2017) and increased expression of deleterious alleles (Frankham et al. 2017; Lynch & Walsh 1998).

3.1.2 Increased genetic differentiation

When populations lose connectivity, and become increasingly isolated from one another, the genetic differentiation between them can increase over time (Frankham et al. 2009; Hamrick et al. 1991, Ellstrand & Elam 1993). Similar to the evolutionary forces acting on populations that have decreased in size, over time, selection and genetic drift can lead to differences in allele frequencies and increase the differentiation among isolated populations (Frankham et al. 2017). Population size and connectivity both influence the rate of genetic drift, therefore small, isolated populations are likely to become differentiated from one another relatively quickly (Frankham 1998; Funk et al. 2016). This level of differentiation is dependent on the amount of gene flow between populations (Templeton et al. 1990; Segelbacher et al. 2003), which varies in relation to the distance between populations (Slatkin 1987) and the dispersal ability of individuals (Bohonak 1999; Frankham 2003). Research on the red-cockaded woodpecker (*Picoides borealis*) has clearly demonstrated the impacts of habitat fragmentation on genetic differentiation. Red-cockaded woodpeckers historically existed in a continuous distribution across eastern America. However, the contemporary distribution is highly fragmented, limited to isolated patches and the population size is approximately 1% of the original size (Kulhavy et al. 1995). Consequently, the isolated populations have been found to show increased genetic differentiation due to a lack of gene flow (Stangel et al. 1992).

3.1.3 Conservation and small isolated populations

From a conservation perspective, loss of genetic diversity and increased differentiation pose significant threats to the survival of endangered species. It is well established that genetic diversity and individual fitness are related and can decrease long-term population survival. For example, hatching failure is significantly greater among New Zealand avian species that have experienced a population bottleneck, when compared to those that did not (Briskie & Mackintosh 2004). Disease resistance can also be compromised in populations with reduced genetic diversity; for instance decreased immunocompetence has been observed in bottlenecked populations of New Zealand robin (*Petroica australis*; Hale & Briskie 2007),

and variability at the major histocompatibility complex (MHC) is often lower in many species (Seddon & Baverstock 2002; Sutton et al. 2011). Decreased sperm quality (Gage et al. 2006; White 2013; Losdat et al. 2014), reduced predator avoidance (Møller & Nielsen 2015) and increased proportion of abnormal offspring (Madsen et al. 1996, 1999) are among the many other documented impacts reduced diversity can have on individual fitness. On a broader scale, genetic variation is important for the maintenance of adaptive potential, therefore a loss of diversity can increase extinction risk (Reed & Frankham 2003; Willi et al. 2006; Allendorf et al. 2012; Allendorf 2017; Frankham et al. 2017). As a consequence, conserving genetic diversity is now recognised as a crucial aspect of conservation biology and species recovery (Frankham 2003; Allendorf et al. 2012; Allendorf 2017; Frankham et al. 2017).

For conservation management, it is important to have a clear understanding of the genetic differentiation and structuring between isolated populations (Crandall et al. 2000; Frankham 2015; Waples & Gaggiotti 2006). Identifying genetically differentiated populations can ensure that such populations are managed appropriately (Moritz 1994; Lesica & Allendorf 1995; Waples & Gaggiotti 2006). However, many authors suggest that the identification of differentiated populations should first encourage questions about the genetic issues (e.g. inbreeding, loss of diversity) within these populations before suggesting independent management (Frankham et al. 2017; Ralls et al. 2017). Alternatively, identifying similar populations can direct management towards retaining the evolutionary uniqueness and adaptive potential of a species (Crandall et al. 2000; Green 2005), using techniques to facilitate gene flow such as translocations, genetic restorations and genetic rescue (Frankham et al. 2017; Hedrick 1995; Weeks et al. 2011).

3.1.4 Translocations and small isolated populations

In the same way that loss of diversity and increased differentiation can affect natural populations, the aforementioned concerns are also relevant for translocation management (Armstrong & Seddon 2008; IUCN/SSC 2013; Seddon, Armstrong & Maloney 2007). In fact, translocations have the potential to further exacerbate the effects of fragmentation and isolation, as they typically involve a small number of individuals and effectively expose populations to an additional genetic bottleneck event (Nei 1973; Mock et al. 2004; Tracy et al. 2011). The overall success of a translocation is impacted by genetic factors at both an individual (reduced fitness) and population (decreased evolutionary potential) level

(Frankham et al. 2002; Armstrong & Seddon 2008; Allendorf et al. 2012). As such, it is recommended that individuals are selected in ways that maximise the genetic diversity of the founding population (IUCN/SSC 2013). This includes selecting individuals from populations with high genetic diversity, and from multiple sources to utilise genetic variation that is present across multiple sites (Madsen et al. 1999; Maudet et al. 2002; Armstrong & Seddon 2008; Allendorf et al. 2012). Consequently, if managers understand the diversity and population structure of potential source populations, they can select founders in a way that will maximise the success of a translocation.

3.1.5 Kōkako and translocation management

The North Island kōkako (*Callaeas wilsoni*) is an endemic, forest dwelling passerine. One of two surviving members of the ancient avian family Callaeidae (Hay, Best & Powlesland 1985; King, Innes & Hay 2015) wattlebirds are one of the oldest New Zealand avian lineages and were present 82–85 mya when New Zealand split from Gondwana (Shepherd & Lambert 2007). Kōkako are the only members of this lineage that survive in populations on the mainland (King et al. 2015). Similar to the history of most endemic New Zealand avifauna, kōkako occupied a significantly larger range and distribution prior to human colonization. Sub-fossil remains indicate that, historically, kōkako were distributed throughout the North Island (Lavers, 1978). However, following forest clearance and logging in the late 19th century, kōkako populations became limited to small remnant forest populations in the upper half of the island by the 1960s (Innes & Flux 1999). By 1999, total population size had declined to as few as 330 breeding pairs in 13 relict mainland populations, and 70 pairs on offshore islands (Innes et al. 2013). Habitat loss (Clout & Hay 1981), diet overlap with introduced browsing mammals (Leathwick et al. 1983; Hay et al. 1985; Powlesland 1987; Best & Bellingham 1990) and predation (Flux, Bradfield & Innes 2006; Innes et al. 1999; Innes et al. 1996; Leathwick et al. 1983) are all understood to have contributed to this historic decline in both population size and number of populations.

The persistence of kōkako today is largely due to intensive management efforts, with populations increasing substantially in the past 20–30 years (Innes et al. 1999b, 2013; Sinclair et al. 2006). Recovery has been relatively rapid, where the population has increased 297% from 400 pairs in 1999 to 1589 pairs as of January 2017 (*North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft* 2018). Past and present management has

maintained a strong focus on re-establishing populations through translocation, resulting in a near 50:50 split in the number of translocated to relict populations (11 relict and 13 translocated as of August 2017; J. Innes, pers. comm.). Kōkako are among the most commonly translocated species in New Zealand (Cromarty & Alderson 2013). As of 2011, 94 kōkako translocations had been undertaken, moving a total of 286 birds to 16 different locations (Innes et al. 2013). The effect of this sequence of translocations has created insurance populations, increased genetic diversity of inbred populations and reintroduced birds back to their former range (Bradley et al. 2012; Innes et al. 2013).

Conservation management of kōkako has been largely successful to date, as is evident with the decrease in threatened status (Robertson et al. 2017) and surpassing the population size target of 1000 pairs by 2017 (*North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft* 2018) as outlined in the 1999 recovery plan (Innes & Flux 1999). To develop robust, self-sustaining populations, future translocations of kōkako should include careful consideration of genetic diversity (Armstrong & Seddon 2008; Allendorf et al. 2012). Genetic research on kōkako is limited; low to moderate population differentiation has been identified between three relict populations using four polymorphic microsatellite loci (Hudson et al. 2000). It has since been suggested that low numbers of microsatellite loci can limit the validity, statistical power and accuracy of population based genetic studies (Wan et al. 2004; Hubisz et al. 2009). This raises significant questions as to the reliability of previous estimates of kōkako genetic diversity. Additionally, when examining evolutionary history, Murphy, Flux and Double (2006) found an unusual pattern of high haplotype but low nucleotide diversity which they suggest reflects recent genetic drift. Whilst this research provided valuable insights into the evolutionary history of kōkako, contemporary population structure is yet to be examined using microsatellite data. Such research would elucidate similarities or differences among existing populations and allow management to be developed appropriately (Frankham 2005; Frankham et al. 2017; Lesica & Allendorf 1995; Slatkin 1987).

There has been recent interest in utilising Hauturu as a source population for future translocation efforts due to the size and population growth rate (J. Innes, pers. comm.). Hauturu was established from 32 founders between 1981 and 1994 (Appendix 1), only nine of which are thought to have bred (I. Flux, pers. comm.). A founding population of nine birds

is likely to have been a significant bottleneck event and raises concern in regard to the current level of genetic variation present in kōkako on Hauturu. No previous research on kōkako genetic variation has included individuals from Hauturu, therefore the utility of using Hauturu as a source population is unclear at present.

3.1.6 Research aims

The aim of this chapter is to compare the level of diversity within a translocated island population (Te Hauturu-o-Toi) to five relict, mainland kōkako populations. Specifically, I aim to determine the levels of genetic diversity within six geographically isolated populations using standard measures of molecular diversity. This will build upon previous research by using both a larger set of microsatellite markers and number of populations, across a greater geographical range of kōkako populations (Hudson et al. 2000). I also aim to explore whether historic fragmentation and isolation has led to contemporary population wide genetic differentiation. To do so, I will use both Bayesian and multivariate methods to determine whether or not there is an observable level of genetic differentiation between any of the sampled populations. The findings of this research will help conservation managers determine the suitability of Hauturu as source population for future translocation projects.

3.2 Methods

3.2.1 Sample collection

Between 1998 and 2017, the Department of Conservation (DOC) and the Kōkako Specialist Group (KSG; Formerly the Kōkako Recovery Group) collected kōkako blood and feather samples during various translocation projects, for research projects (Hudson et al. 2000) and from Hauturu for the purposes of this study. A total of 147 unrelated individuals from thirteen different populations were available to include in the present study (Figure 3.1; for details on sample type and location see Appendix 3). The individuals sampled from Te Urewera, Rotoehu and Mapara populations were originally collected for the Hudson et al. (2000) study that analysed genetic diversity of kōkako using four microsatellite markers. Blood samples that were stored appropriately and of reasonable condition were DNA extracted from blood and included in the present study. It was informative to include these samples as a way to increase the sample size, the number of populations and to compare to the findings of the previous research using a larger set of kōkako specific microsatellite markers.

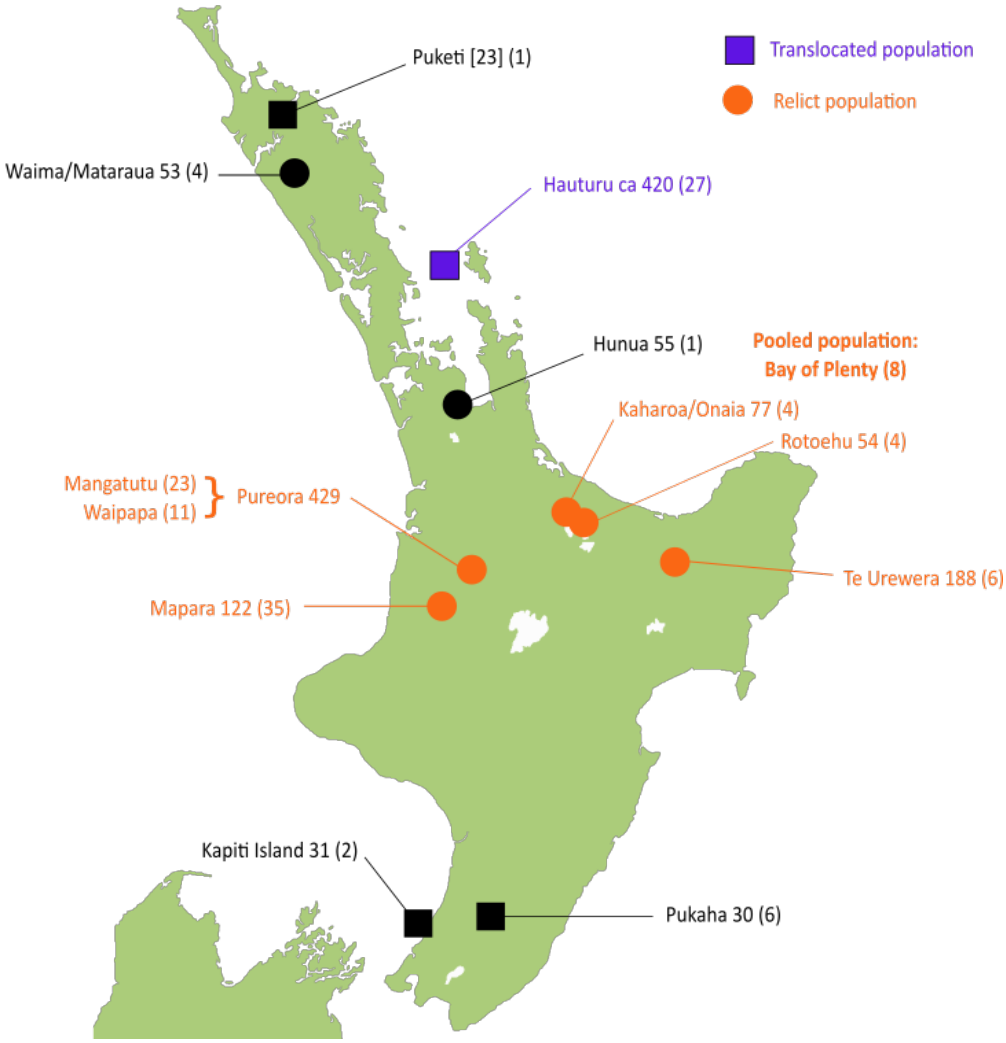


Figure 3.1. Map showing sampling locations of North Island kōkako (*Callaeas wilsoni*) with number of estimated breeding pairs (square brackets indicate recently established sites) as of January 2017 (*North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft* 2018). Sample sizes are shown in parentheses. Locations in black were excluded from analyses due to small sample sizes (Pukaha excluded as it was a captive population).

3.2.2 Te Hauturu-o-Toi

Hauturu is both the largest (>400 pairs) and most successful population of kōkako, in terms of breeding success and population growth (Flux et al. 2013; Innes et al. 1999, 1996). However, as few as nine individuals are understood to have bred successfully when the population was first established by translocation (33 birds) between 1981 and 1994 (Brown et al. 2004; Innes et al. 2013). Concerns have therefore been raised regarding the current level of genetic diversity on Hauturu (T. Thurley, pers. comm.). DOC caught (by mist-netting) and genetically sampled 31 individual kōkako between 2013 and 2015. The sampling location

(see Figure 3.2) is an area where sub-adult and non-territorial adults from across the island congregate to feed (I. Flux, pers. comm.). Individuals sampled from this location are therefore considered to represent diversity across the entire population.

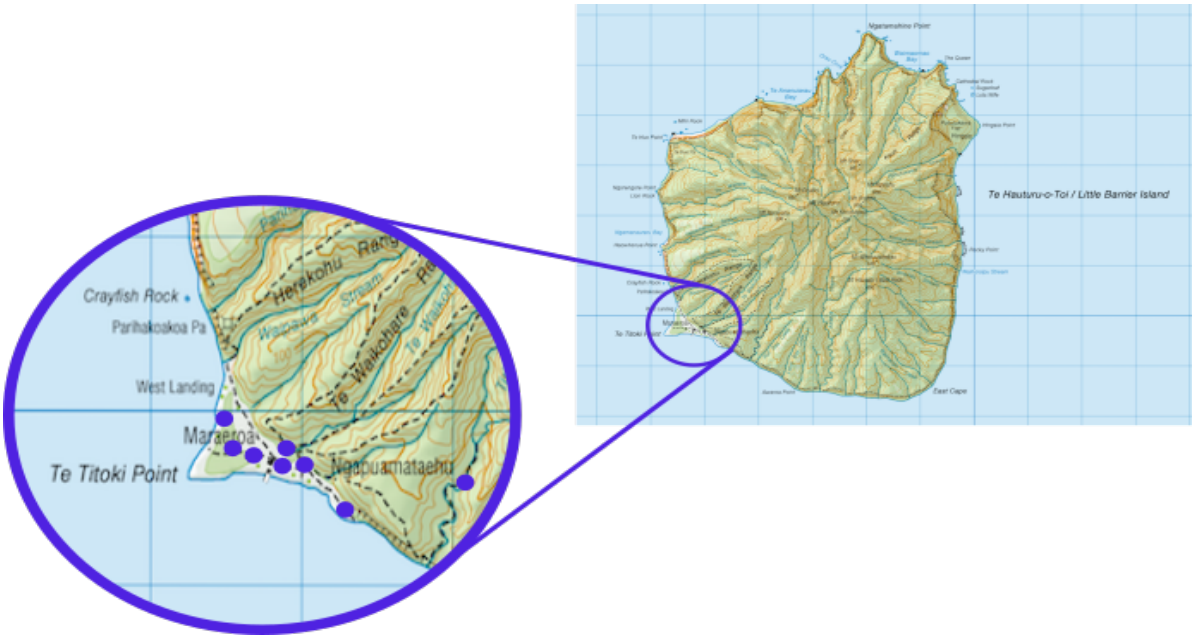


Figure 3.2. Map showing the sampling locations on South West Hauturu (8 locations, 31 birds). Image from Land Information New Zealand.

3.2.3 Multiplex microsatellite genotyping

Genomic DNA was extracted from whole blood (stored in Queens lysis buffer or 70% ethanol) and feather samples using a modified 5% Chelex protocol (Walsh et al. 1991). Twenty-four polymorphic, kōkako-specific microsatellite primers were designed for this study (Chapter 2) and multiplexed to achieve efficient application of the loci (Table 2.2, Chapter 2). The number of alleles, allele size ranges and primer sequences were entered for analysis. One million iterations were run, where the maximum number of loci per reaction was set to four, a minimum distance of 20 bp between loci of the same dye colour and a complementary threshold of 7. Four multiplexes were consequently designed, two with only one locus per dye due to large allele size ranges (Table 3.1).

Table 3.1. Details of the 21 polymorphic microsatellite loci used on North Island kōkako (n=110) from six populations.

Locus	Primer sequences (5' to 3')	Repeat motif	A	Size (bp)	Dye (MPX no.)
Cwil_07	F: CACAAAATTGGGTGTGGATG R: TGATGCTGACAGATTTGAAGAC	$(TG)_{11}$	9	131–149	6-FAM (1)
Cwil_04	F: GTGGTGAACAGAAAAGCAGGT R: TTGAGCTAAGTAATGAAGATTGACCA	$(GA)_{13}$	8	154–172	6-FAM (1)
Cwil_05	F: GGTATCTGTGTGTGGGCGTA R: TGGAGGACTGAGGTAGCACAA	$(CA)_{12}$	7	123–137	VIC (1)
Cwil_13	F: AAATAGCCCACAAAATCTTCCTT R: TGGAGGTGAGCCCTTCAG	$(CA)_{16}$	11	149–167	VIC (1)
Cwil_14	F: TGCAGTCAGTGGCCTTTAGTT R: GACTGCAGACAGAGAGCACAA	$(TC)_{17}$	12	107–143	NED (1)
Cwil_01	F: GCCTGTGTCGCTGAGTGTAT R: TGCCTTCAAACAGAAAGCAA	$(GT)_{20}$	18	146–190	NED (1)
Cwil_15	F: GCTACCAACAGGGCAACTTT R: CAAAAGCCGGTTTTCTTCCT	$(GT)_{14}$	17	127–169	PET (1)
Cwil_21	F: TGATTCCATCCCTCCTTCCC R: GGCCTGGTTCCTACTTAATTGG	$(AC)_{23}$	12	97–129	6-FAM (2)
Cwil_09	F: CAGACAGATGCCACCTGAAA R: TGCCATATGTGCAAAGGAGA	$(CA)_{17}$	4	143–149	6-FAM (2)
Cwil_24	F: CATGACTTTTGAGGCGCAGA R: ACCAGTCCCCACACATTTGA	$(AC)_{19}$	12	89–137	VIC (2)
Cwil_29	F: TGGAAGGAAAAGAGGTGAGATTT R: TTTTCCCCTACATTTGCGCG	$(GT)_{13}$	9	141–157	VIC (2)
Cwil_19	F: CTGAAATTCCTAGCCCCTCATAT R: CCCCAGCCACCCAAAATCTA	$(GT)_{18}$	17	102–140	NED (2)
Cwil_08	F: GTGGTGAACAGAAAAGCAGGT R: TTGAGCTAAGTAATGAAGATTGACC	$(GA)_{13}$	9	154–172	NED (2)
Cwil_25	F: TGCTACGGGAAAGGAGGAAG R: ACAAGTCAATGTACCATCCTCTC	$(AGAT)_{19}$	10	82–126	PET (2)
Cwil_31	F: AGTCACTGGCTCTGAATAGGT R: ATGCTCCTCGAAGTAACAAGAT	$(GT)_{15}$	12	150–178	PET (2)

Cwil_32	F: TCTTGTTCTGACCATCCCTCC R: TAGAGGAAAGTGTGCTGGGG	$(ATC)_{10}$	12	112–144	6-FAM (3)
Cwil_22	F: CCTTCCTCTCTGCTCCTCTG R: ACACTCCACCTTTGACTCTGT	$(AC)_{16}$	11	118–170	VIC (3)
Cwil_17	F: GATGTGTGTGTGGTCTGCTC R: CCCAAGGACTCAGGAGCTAC	$(GT)_{19}$	20	112–158	NED (3)
Cwil_28	F: CCGTTTGACTAGAGACCACCT R: GCCCTTAAGATCAGCCCTGC	$(GAT)_{9}$	5	138–150	PET (3)
Cwil_18	F: TCAGTCAGGGCAAAGAGAGG R: AGACTAGATCCCTTCCAACTGA	$(ATCC)_{15}$	7	121–145	6-FAM (4)
Cwil_30	F: TCATCCTGACTCTGCTGGTC R: TGAACATCCTCAGGGTGCAG	$(GT)_{18}$	14	112–152	VIC (4)
Cwil_23	F: GCTTACCCACACTGTTCACC R: CCACAGACAAGAGGAGCAGA	$(ATCC)_{13}$	12	117–169	NED (4)

A = total number of alleles at each locus

3.2.4 Microsatellite amplification conditions

For each multiplex plan, 1 µL of DNA sample was dried in 96-well PCR plates (using a 95 °C hold or air-dried at room temperature), with the inclusion of a negative control to detect contamination. The 2 µL PCR reaction included, 1 µL of the dried DNA, 1 µL of 2 x Type-it Multiplex PCR Master Mix (Qiagen), 0.16 µM (per locus) of each reverse primer, 0.04µM (per locus) of each M13-labelled forward primer, 0.16 µM of universal M13 primer labelled with fluorescent dye; 6-FAM, VIC, NED or PET (DS-33 dye: Applied Biosystems) and PCR grade water (Sigma Aldrich). Reaction conditions were as follows; Initial denaturation at 94 °C for 15 minutes, eight cycles of 94 °C for 30 seconds, 60 °C (touchdown 1 °C per cycle) for 90 seconds and 72 °C for 60 seconds. Then a further 25 cycles of 94°C for 30 seconds, 52 °C for 90 seconds and 72 °C for 60 seconds, and a final elongation step of 60 °C for 30 minutes. Following amplification, PCR products were diluted with 25 µL of PCR grade water (Sigma Aldrich), and dyes were pooled for each multiplex plan prior to genotyping. Genotyping of PCR products (2 µL of PCR product, and 7.8 µL Hi-Di Formamide) was performed using GeneScan 500 LIZ (Applied Biosystems Genescan™) as a size standard (0.2 µL) on an ABI 3730xl DNA Analyser (Applied Biosystems).

Alleles were scored automatically using Geneious software v.9.1.8 (Kearse et al. 2012). Visual searching was undertaken to detect alleles that the software had missed, or that had been incorrectly labelled as peaks, and bins were predicted using the Geneious v.9.1.8 algorithm (Kearse et al. 2012).

3.2.5 Analyses

Populations with a sample size too small to analyse (Wan et al. 2004) were excluded from the study (Kapiti Island, n=2; Puketi Forest, n=1; Mataraua Forest, n=4; Hunua Ranges, n=1); Mt Bruce (n=6) was also excluded because it is a captive population. This decreased the number of kōkako samples included in the study from 147 to 133. Of the 133 kōkako sampled, those that failed to amplify at more than seven of the loci were removed from the dataset prior to analysis (n=23). After correcting for missing data, the Rotoehu and Kaharoa populations were both too small to be included in subsequent analyses (n=4 for both). The two populations were pooled together to represent the Bay of Plenty region. This gave a final sample size of 110 kōkako across six populations (Bay of Plenty n=8; Little Barrier Island n=27; Mapara n=35; Mangatutu n=23; Waipapa n=11; Te Urewera n=6; see Appendix 3).

Genotyping errors can occur for a variety of reasons, such as interactions between DNA molecules, human error, biochemical artefacts or equipment errors (for a review see: Pompanon, Bonin, Bellemain & Taberlet 2005). To quantify genotyping error and assess the reliability of the dataset, error rate can be calculated by measuring the mismatches between original and replicated scores of genotypes (Pompanon et al. 2005). To estimate genotyping error rate, 20 randomly selected individuals, across all locations (14% of samples), were repeat-genotyped across all loci (n=24) as a measure of accuracy (Hoffman & Amos 2005). The error rate per allele was calculated by dividing the number of mismatched alleles by the total number of repeat genotyped alleles (Hoffman & Amos 2005; Pompanon et al. 2005).

3.2.6 Preliminary data checking

Allele sizes (bp) across all populations (6) and loci (22) were exported from Geneious v.9.1.8 (Kearse et al. 2012) into Microsoft Excel v.15.40. GenAlEx v.6.5 (Peakall & Smouse 2012) was used to label missing alleles (0) and export formatted data files. A test for null alleles, scoring errors due to stuttering and large allele dropout was performed using MICROCHECKER v.2.2.3 (Van Oosterhout et al. 2004). The presence of null alleles is

known to lead to an overestimation of genetic distance and F_{ST}, especially in populations with low levels of gene flow (high level of genetic differentiation) and can skew population genetics results (Chapuis & Estoup 2007).

To test the performance of each loci as reliable population genetic markers, deviations from Hardy-Weinberg equilibrium (HWE) were assessed for each population using a Markov Chain Monte Carlo (MCMC) approximation of Fisher's exact test in Genepop v.4.0.10 (Raymond & Rousset 1995). The dememorization number, number of batches and number of iterations per batch were all set to 1,000. Linkage disequilibrium between pairs of loci was also assessed using Genepop v.4.1.10 (Raymond & Rousset 1995) using the log likelihood ratio statistic. The significance levels for both tests were adjusted using a standard Bonferroni correction for multiple comparisons (P=0.05/number of tests), to reduce Type 1 errors across multiple tests among loci (Rice 1989).

3.2.7 Genetic diversity

Standard measures of genetic diversity (number of alleles per locus, observed and expected heterozygosity and number of private alleles) were initially explored using GenAlEx v.6.5 (Peakall & Smouse 2012). FSTAT v.2.9.3.2 (Goudet 2001) was used to calculate allelic richness, a measure of allelic diversity measure corrected for sample size. A one-way analysis of variance (ANOVA) and tukey test were performed in R 3.4.3 (R Core Team 2014) to test for significant differences in allelic richness among populations.

Pairwise genetic differentiation among the six populations was estimated using F_{ST} and Jost's D estimator of differentiation (Jost 2008) in the diveRsity package (v 1.9.90) (Keenan et al. 2013) in R 3.4.3 (R Core Team 2014). Significance was determined using bias corrected 95% confidence intervals, using 999 bootstrap replicates. Comparisons were considered significant if confidence intervals did not overlap zero. The utility of F_{ST} as a measure of genetic differentiation has been debated recently as many assumptions are violated in natural populations (Jost 2008; Ryman & Leimar 2009; Whitlock 2011). For example, F_{ST} can be downward biased when comparing differentiation in fragmented populations using microsatellites, as within-population variance is often high in relation to between population variation (Hedrick 2005; Gerlach et al. 2010; Meirmans & Hedrick 2011; Allendorf et al. 2012). As a result, many different estimators have been developed, some of

which have been standardised to vary between 0 and 1. To determine the most appropriate estimator, the corPlot function in diveRsity was used, and plots for each of the estimators (F_{ST}, G_{ST}, G'_{ST} and D_{JOST}) were compared.

It is well established that small populations are susceptible to inbreeding, which can reduce survival, reproduction and hence increase extinction risk (Frankel & Soulé 1981; Lynch & Walsh 1998; Frankham 2003; Briskie & Mackintosh 2004; Jamieson et al. 2006; Frankham et al. 2017). I used three different methods to estimate inbreeding and relatedness within each population. Firstly, Wright's inbreeding coefficient (F_{IS}) was calculated using diveRsity (v 1.9.90) (Keenan et al. 2013), which provides a measure of the extent of inbreeding within sub-populations (Wright 1922). Bias corrected confidence intervals were obtained using 999 bootstrap replicates. Secondly, I used two alternative inbreeding measures; internal relatedness (Amos et al. 2001) and heterozygosity by loci (Aparicio et al. 2006). Internal relatedness (IR) counts the frequencies of shared alleles between two individuals to give a measure of homozygosity, where rare shared alleles are weighted higher than common shared alleles (Amos et al. 2001). HL weighs loci according to their heterozygosity, under the assumption that more variable loci are more informative (Aparicio et al. 2006). It has been suggested that heterozygosity by loci (HL) is sometimes more appropriate when the population has immigration, rare alleles and a low number of microsatellite markers (Aparicio et al. 2006). Both estimators were included in the analysis and estimated using IRMacroN4 in excel (Amos et al. 2001). Values range from –1 to 1, with more positive IR or HL values reflecting homozygous individuals (Amos et al. 2001; Aparicio et al. 2006). Finally, genetic relatedness of individuals was estimated using GenAlEx v.6.5 (Peakall & Smouse 2012), using the Queller and Goodnight (1989) index of relatedness (r). Permutation and bootstrap numbers were both set to 999, and 95% confidence intervals were estimated using 10,000 bootstraps. Relatedness values range from –1 to 1, with 0.5 indicating full sibling or parent-offspring relationships and zero indicating unrelated (Queller & Goodnight 1989).

The number of effective breeders (N_b) can be estimated in species with overlapping generations, by only taking a single generation and cohort of the species into account (Amos et al. 2001). To do so, the unbiased linkage disequilibrium method (LDNe) was implemented (Waples 2006; 2008) in the programme NeEstimator 2.0 (Do et al. 2014). The mating system

was specified as monogamous (Flux et al. 2006), and all alleles with a frequency < 0.05 were excluded. Confidence intervals for N_b were calculated using a jackknife and parametric method.

3.2.8 Population structure

To determine the presence of population structure, an individual-based Bayesian cluster methodology was implemented in STRUCTURE v.2.3 (Pritchard et al. 2000; Pritchard 2010). STRUCTURE uses a Bayesian algorithm to identify the number of genetically distinct clusters (K), using the assumption that each cluster is characterised by specific allele frequencies (Pritchard et al. 2000). This method assigns individuals to clusters based on their unique genotype at multiple loci and can be applied using a number of different markers.

STRUCTURE was run using the admixture model with correlated allele frequencies, in which individuals are assigned to clusters (K) without prior knowledge of population membership (Pritchard 2000). Fifteen iterations (Markov Chain Monte Carlo length 300,000 steps, burn-in period 100,000) were performed for each K (1 to 6) populations. The most probable number of distinct clusters was inferred using the Evanno method (ΔK) implemented in STRUCTURE HARVESTER (Earl & Vonholdt 2012). This method calculates the mean-likelihood value of K, based on the ad-hoc statistic ΔK (Evanno et al. 2005). CLUMPP software (Jakobsson & Rosenberg 2007) was then used to average the individual assignment coefficients (q) from the 15 iterations to each of the genetic clusters. Individuals with a threshold of $q > 0.8$ were assigned to cluster, and those with $q < 0.8$ were considered to have mixed membership (Bergl & Vigilant 2007; Nsubuga et al. 2010). To visualise the output produced by CLUMPP, a bar plot showing individual admixture and cluster assignment was created using DISTRUCT v.1.1 (Rosenberg 2004). Based on the identified genetic clusters (K), the differentiation between the genetic groupings was assessed using an analysis of molecular variation (AMOVA) in Arlequin v.3.5 (Excoffier & Lischer 2010).

A limitation of STRUCTURE analysis is the reliance it has on assumptions of HWE and linkage disequilibrium within populations. In non-equilibrium populations, a bias towards a higher value of K can occur, limiting the accuracy of cluster estimation (Kaeuffer et al. 2007). A discriminant analysis of principal components (DAPC) is an alternative,

multivariate method, that is capable of identifying population clusters and has proven to perform as well as STRUCTURE in simple population models (Jombart, Devilliard & Balloux 2010). Additionally, DAPC is considered advantageous over STRUCTURE when population models are more complicated (Jombart 2008; Jombart et al. 2010), and has the advantage of not relying on the HWE and LD assumptions. This alternative, non-model based method uses discriminant functions find linear allele combinations that maximise between group variation, but minimise variation within the clusters (Jombart et al. 2010). A Principal Component Analysis (PCA) is initially run in the model (which looks at genetic variation), and then discriminant functions are applied. Probabilities are determined for each individual in terms of membership to each of the different clusters (Jombart et al. 2010).

The DAPC was conducted using the adegenet 1.4.2 (Jombart 2008; Jombart et al. 2010) package in R 3.4.3 (R Core Team 2014). DAPC uses a K-means clustering approach to determine the number of clusters. This is a sequential clustering algorithm that compares a specified number of potential clusters (for this study K was set from 1 to 6) using the Bayesian Information Criterion (BIC). The data is first transformed using a PCA, and then K-means clustering is applied to determine the optimal number of clusters to best describe the data, using the find.clusters function in adegenet (Jombart 2008). The number of principal components (PCs) retained at this point was set to 200, to ensure that they are all retained, as the model is not sensitive to PC retention at this stage. Each K then has an associated statistical model and likelihood, which is assessed using the BIC (Jombart et al. 2010). The model (K) that minimized the BIC was selected.

As a PCA is used to transform the dataset, the number of principal components to retain must be selected. Retaining too few PCs will lead to a low power of discrimination, whereas retaining too many can overfit the discriminant functions (Jombart et al. 2010). A cross-validation approach can be used to find the balance between retaining too many or too few PCs and find the most appropriate number of PCs objectively. This method uses the xval function in adegenet, and runs a training set with a variable number of PCs, to determine how accurately they predict group membership using a validation set of excluded individuals (Jombart 2008; Jombart et al. 2010). This was run 30 times, with a 90% membership used in the training set and 10% in the validation set. Due to the small number of clusters (K), all discriminant functions were retained (Jombart et al. 2010). Results were visualised in a

DAPC scatterplot, with colours corresponding to distinct clusters. For comparison with the STRUCTURE results, a DAPC was also run for K=2 and K=4.

3.3 Results

Twenty-two of the 24 loci were successfully amplified for analysis of genetic diversity and population structure of North Island kōkako. Cwil20 and Cwil27 were excluded from the analyses due to not amplifying cleanly and consistently across individuals. Genotyping error rate (error rate per allele) was found to be low (0.0258 errors per allele, n=20 samples). Twenty-three samples failed to amplify at more than seven loci and were subsequently excluded from data analyses. Many of these samples were 20 years old (n=11) or had a low concentration of DNA from feather extraction (n=4). A total of 110 kōkako, from six populations were successfully genotyped at more than 15 of the 22 loci.

No evidence for large allele dropout or stuttering was detected in any of the six populations or 22 loci using MICROCHECKER v.2.2.3 (Van Oosterhout et al. 2004). Seven loci (Cwil19, 15, 31, 18, 22, 01, and 17) showed signs of null alleles at one or more population (Table 3.2). However, only four (Cwil19, 15, 31 and 17) loci had a null allele frequency greater than 0.2 (Van Oosterhout value). Two (Cwil15 and 17) of these four loci were identified as null alleles in more than one population (Table 3.2). There were no consistent null alleles across all populations, and removal of the four loci with an allele frequency greater and 0.2 did not change the outcome of the genetic diversity or population structure results. All loci were therefore retained in all analyses.

Table 3.2. Loci identified as potential null alleles using the Van Oosterhout method in MICROCHECKER.

Population	N	Loci	Total expected homozygotes	Total observed homozygotes	Combined prob. of all homozygote classes	Allele frequency (Van Oosterhout)
Bay of Plenty	8	Cwil19	1.375	5	< 0.01	0.264
Hauturu	27	Cwil15	6.919	16	< 0.001	0.244
	27	Cwil19	5.625	10	> 0.05	0.123
	27	Cwil31	3.717	8	> 0.05	0.111
	27	Cwil18	10.374	15	*	0.167
Mapara	35	Cwil31	8.954	14	> 0.05	0.118
	35	Cwil22	7.249	16	< 0.001	0.180
Mangatutu	23	Cwil01	3.325	9	< 0.025	0.170
	23	Cwil22	9.249	14	*	0.188
Te Urewera	6	Cwil31	1.416	4	< 0.025	0.275
Waipapa	11	Cwil17	1.863	6	< 0.01	0.218

** More than 50% of alleles at this locus are of one size class, therefore binomial analysis could not be performed*
N = sample size

One pair of alleles (Cwil08 and Cwil04) displayed highly significant linkage disequilibrium across all populations ($P < 0.001$). This was significant in all but one population (Bay of Plenty) when tested individually ($P < 0.001$). One loci from the pair (Cwil04) was consequently removed from further analyses.

Of the 110 tests for HWE at 21 loci, 17 significantly departed from HWE proportions at $P< 0.05$ significance level. Following a standard Bonferroni correction, only three loci departures remained significant (Cwil13 in the Hauturu population; Cwil22 in the Mapara population; Cwil19 in the BOP population). There were therefore no consistent departures from HWE across all populations for any loci, and all were retained. This meant that a total of 21 loci were used to analyse genetic diversity and population structure.

3.3.1 Genetic diversity

Across the 110 individuals from six kōkako populations, a total of 240 alleles were scored. The average number of alleles per locus was 5.960 ± 0.196 (SD), ranging from one to 16 alleles. The mean number of alleles varied across kōkako populations, with the highest observed in Mapara (6.905 ± 0.643, Population size as of Jan 2017 = 122 breeding pairs) and the lowest at Te Urewera (4.190 ± 0.290, Population size as of Jan 2017 = 188 breeding pairs; Table 3.3). The mean allelic richness was also lowest in the Te Urewera population (3.691 ± 1.046) and highest in Bay of Plenty (4.358 ± 1.123; Table 3.3). Across all populations however, mean allelic richness scores were similar and did not differ significantly (Tukey HSD: $P > 0.05$). Observed (H_o) and expected (H_e) heterozygosities were similar among populations (Table 3.3). The number of private alleles ranged from five (Te Urewera) to 17 (Mapara; Table 3.3), and were all from different loci (only two had no private alleles).

Table 3.3. Genetic variation at 21 microsatellite loci within six North Island kōkako populations across the North Island of New Zealand (110 individuals).

Population	N	A	AR	H_o	H_e	PA	Population size*
Bay of Plenty	8	5.762 (0.382)	4.358 (1.123)	0.701 (0.046)	0.698 (0.036)	10	Rotoehu: 54 Kaharoa: 77
Hauturu	27	6.571 (0.481)	3.772 (0.925)	0.614 (0.037)	0.690 (0.030)	13	ca 420
Mapara	35	6.905 (0.643)	3.871 (1.031)	0.657 (0.045)	0.692 (0.041)	17	122
Mangatutu	23	6.810 (0.376)	4.103 (0.808)	0.706 (0.040)	0.722 (0.028)	12	429†
Te Urewera	6	4.190 (0.290)	3.691 (1.046)	0.681 (0.058)	0.636 (0.041)	5	188
Waipapa	11	5.524 (0.382)	3.905 (0.945)	0.707 (0.035)	0.682 (0.036)	6	429†
Overall	110	5.960 (0.196)	3.950 (0.244)	0.678 (0.018)	0.687 (0.014)	NA	Total: c.1589

**Population size (number of breeding pairs) from draft kōkako recovery plan (North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft* 2018). *Total is across 20 populations.*

†Population estimate for Pureora forest, Mangatutu and Waipapa alone is not available

N, sample size; A, mean number of alleles; AR, mean allelic richness; PA, number of private alleles. Standard deviation provided in parentheses.

The global inbreeding coefficient was 0.0692, and was significant based on the 95% bias corrected confidence interval (Table 3.4). Across all populations, inbreeding coefficients were lower than 0.07, with the highest value observed in the Hauturu population (F_{IS} = 0.0648; Table 3.4). Three populations had negative F_{IS} values (Table 3.4) however is likely to be due to small sample sizes (n=6 to 11). The only value that differed significantly from zero was in the Waipapa population, and was negative (Table 3.4).

Table 3.4. Inbreeding coefficients (F_{IS}) within six North Island kōkako populations. The 95% bias corrected confidence interval (F_{IS} lower and upper) calculated using 999 bootstrap replicates. Significant values indicated in bold when interval does not overlap zero.

Population	N	F_{IS}	F_{IS} lower	F_{IS} upper
Hauturu	27	0.0648	–0.0100	0.1253
Mapara	35	0.0242	–0.0469	0.0952
Mangatutu	23	0.019	–0.0427	0.0764
Te Urewera	6	–0.077	–0.289	0.0458
Waipapa	11	**–0.0881**	–0.182	–0.0123
Bay of Plenty	8	–0.054	–0.2005	0.0491
Overall	110	**0.0692**	0.0347	0.1013

N = sample size

Both internal relatedness (IR) and heterozygosity by loci (HL) produced the same pattern of results, therefore only IR is presented (HL presented in Appendix 4). All individuals within all populations produced positive values, indicative of higher homozygosity (Figure 3.3). There was little difference between the median IR (or HL) across any population, ranging from 0.2 to 0.4 (Figure 3.3). Hauturu had the highest median IR value (0.4; Figure 3.3), however was only marginally larger than the other populations (Figure 3.3).

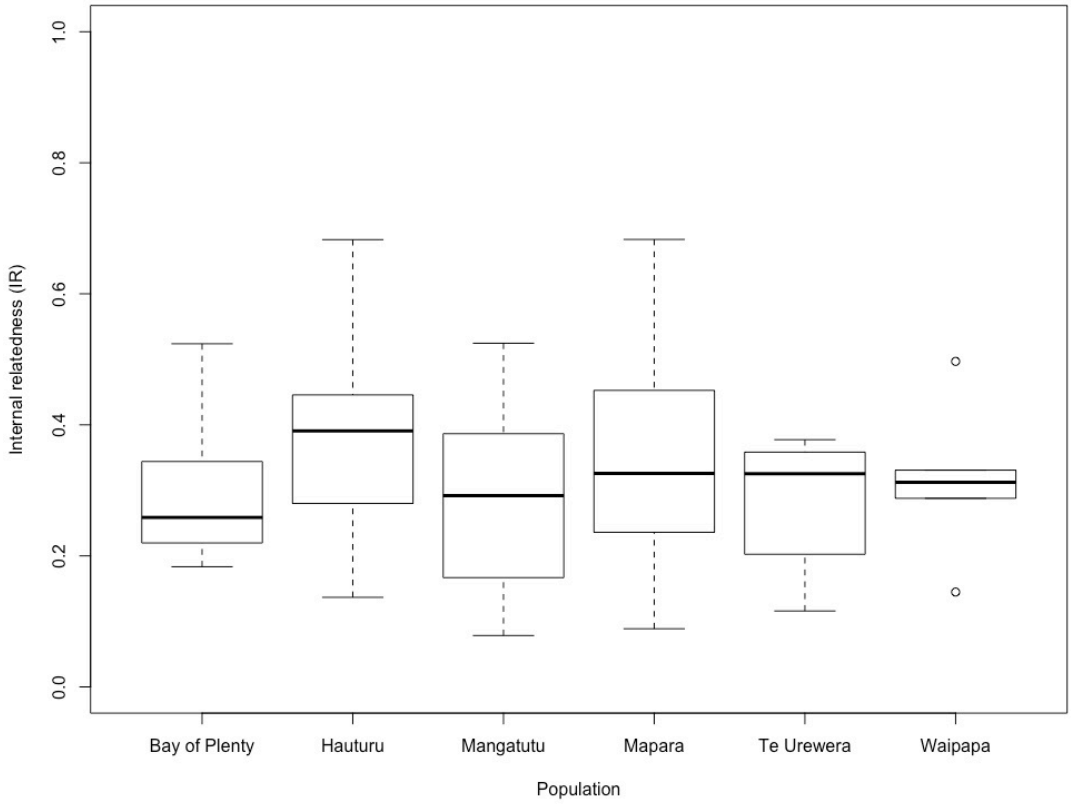


Figure 3.3. Boxplots showing the levels of internal relatedness (IR) with median, upper and lower quartile values, in six kōkako populations (Bay of Plenty: n=8, Hauturu: n=27, Mapara n=35, Mangatutu: n=23, Te Urewera n=6, Waipapa n=11).

In terms of relatedness, the mean pairwise values ranged from 0.05 to 0.2 (Figure 3.4). Kōkako in the Te Urewera population were the most related (Figure 3.4), whereas those in the Bay of Plenty population were the most unrelated (Figure 3.4). As the parameters of this relatedness estimator are between –1 and 1, all populations show an elevated level of relatedness (Queller & Goodnight 1989). Most populations had a relationship coefficient close to 0.15, which is lower than a half sibling level of relatedness (0.25; Queller & Goodnight 1989). Kōkako in the Te Urewera approached this value, but there is a lot of uncertainty in the estimate (Figure 3.4).

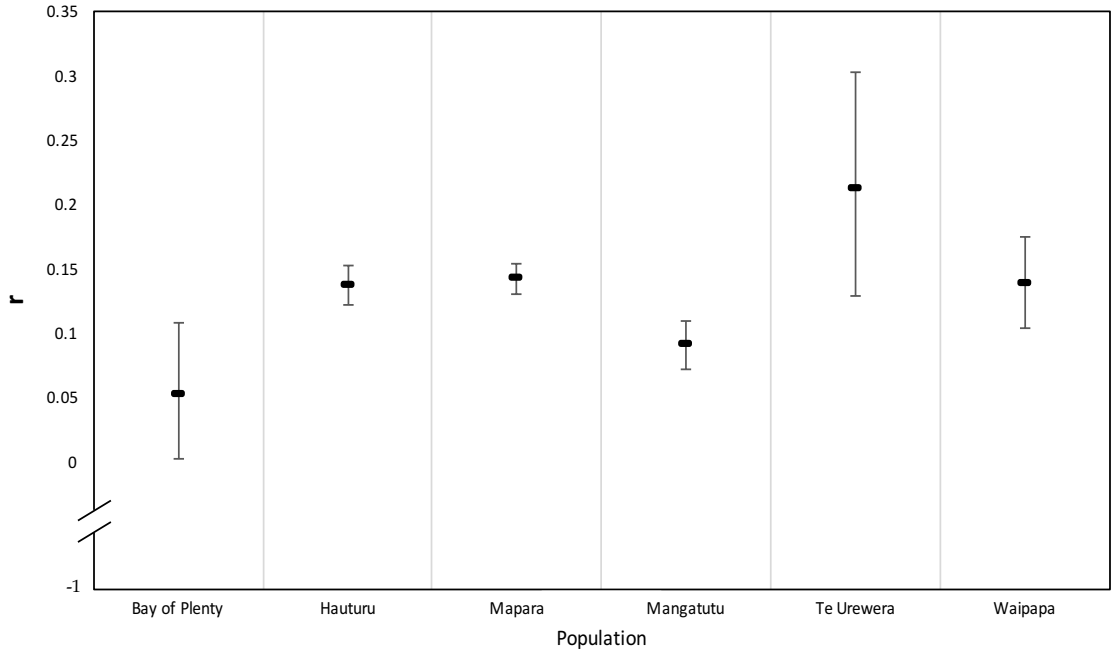


Figure 3.4. Figure showing the mean pairwise genetic relatedness (error bars are 95% confidence intervals) in six kōkako populations (Bay of Plenty: n=8, Hauturu: n=27, Mapara n=35, Mangatutu: n=23, Te Urewera n=6, Waipapa n=11).

3.3.2 Number of effective breeders

Due to small sample sizes, only three populations were included in this analysis. Based on the LDNe estimation method, the effective number of breeders (N_b) of Hauturu was 72.9 individuals. This value lies between 54.4 and 105.9 based on the parametric confidence intervals (Table 3.5), however, Jackknifing gives a much larger confidence interval (Table 3.5). Despite this, Jackknifing estimates the smallest possible number of breeders as 32.1 individuals, as seen by the lower CI value (Table 3.5). The Mapara population N_b is estimated at 37.8 individuals, and there is much more confidence in this value (Table 3.5). Mangatutu has the largest N_b, but could not define an upper CI with Jackknifing (Table 3.5).

Table 3.5. Effective number of breeders (N_b) estimates for three North Island kōkako populations and the associated parametric and Jackknife 95% confidence intervals.

Population	N	N_b	Parametric CI low	Parametric CI high	Jacknife CI low	Jacknife CI high
Hauturu	840	72.9	54.4	105.9	32.1	1268.3
Mapara	251	37.8	32.6	44.3	23.6	69.2
Mangatutu	901*	132.5	82.5	300.5	53.3	infinite

*N = Population size (excluding juveniles and sub-adults) from draft kōkako recovery plan (North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft* 2018).

**Population estimate for Pureora forest, Mangatutu alone is not available*

3.3.3 Genetic differentiation among kōkako populations

Using the corPlot function in DiveRsity (Keenan et al. 2013), the appropriateness of four F_{ST} type statistics (F_{ST}, G_{ST}, G'_{ST} and D_{JOST}) was compared (Figure 3.5). A negative relationship between F_{ST} and the mean number of alleles, but a strong positive relationship between D_{JOST} and mean number of alleles was observed (Figure 3.5). This indicates that there may be a strong bias in the data (Keenan et al. 2013). Both estimators are reported, but the F_{ST} results are therefore interpreted with caution.

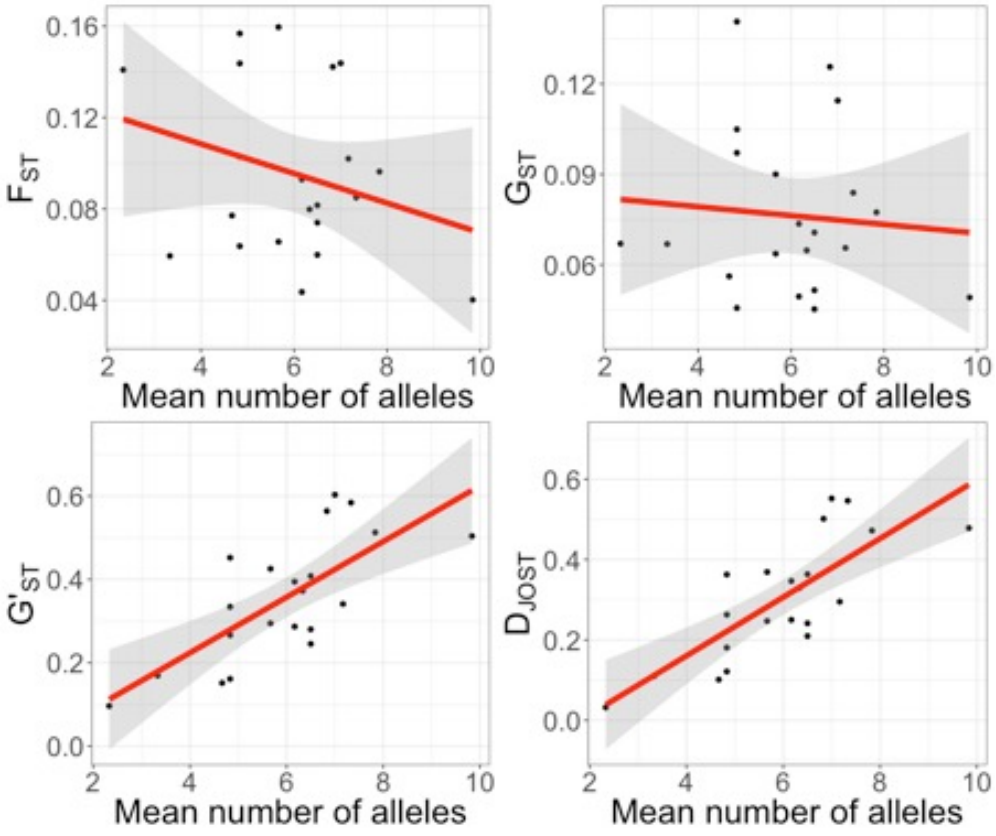


Figure 3.5. Four estimators of genetic differentiation (F_{ST}, G_{ST}, G'_{ST} and D_{JOST}) and the relationship with the mean number of alleles across all 21 loci.

The two measures of genetic differentiation (F_{ST} & D_{JOST}) provided inconsistent results (Table 3.6). When using F_{ST}, differentiation between all six populations was significant (Table 3.6) and the overall F_{ST} value was moderate (F_{ST} = 0.0949). This included the two populations within Pureora (Mangatutu and Waipapa) which were the closest geographically (Table 3.6). Alternatively, only Mapara, Hauturu and Te Urewera populations differed significantly from all other populations when using the D_{JOST} estimator (Table 3.6). No significant differentiation was observed between the two populations within Pureora forest (Mangatutu and Waipapa; Table 3.6). The Bay of Plenty population did not differ significantly from these two populations (Table 3.6). The overall D_{JOST} value was 0.2434 (CI; 0.1975, 0.2995 = significant).

Table 3.6. Pairwise D_{JOST} and F_{ST} values among North Island kōkako populations. F_{ST} above diagonal, D_{JOST} below. Values in bold indicate significant comparisons, as the bias corrected 95% confidence interval does not overlap zero.

Location	Hauturu	Mapara	Mangatutu	Te Urewera	Waipapa	Bay of Plenty
Hauturu	-	**0.1167**	**0.1146**	**0.1492**	**0.1335**	**0.0539**
Mapara	**0.2529**	-	**0.0855**	**0.1236**	**0.0925**	**0.0852**
Mangatutu	**0.2359**	**0.1869**	-	**0.0566**	**0.0509**	**0.0511**
Te Urewera	**0.2845**	**0.2715**	**0.1187**	-	**0.0893**	**0.0865**
Waipapa	**0.2742**	**0.2016**	0.0607	**0.1322**	-	**0.0633**
Bay of Plenty	**0.0901**	**0.2132**	0.0703	**0.1452**	0.0806	-

3.3.4 Population structure

Results of the population subdivision analysis using STRUCTURE are presented for K=2 to K=4, where distinct clusters are indicated by different colours (Figure 3.6). Using Evanno's ΔK metric, it was identified that a K=3 was the most likely number of K genetic clusters (ΔK=236; Figure 3.7). The first cluster included 24 assigned (and three unassigned) individuals from Hauturu (Table 3.7; Figure 3.6). The second cluster was the largest, and included 41 individuals from the remaining four populations (Mangatutu, Te Urewera, Bay of Plenty and Waipapa; Figure 3.6). This cluster had the greatest number of unassigned individuals (seven from Bay of Plenty; Table 6). The final cluster included 31 individuals from Mapara (and four unassigned; Table 3.7; Figures 3.6 & 3.8).

A total of 96 individuals were successfully allocated to one of the three inferred clusters (based on $q > 0.8$), meaning that only 12.7% could not be assigned to a cluster. In the Bay of Plenty population, eight individuals were sampled, only one of which could be assigned to a cluster based on $q > 0.8$ (Cluster 2; Table 3.7). All other populations had high assignment values to one of the three clusters (0.93 to 0.98; Table 3.7).

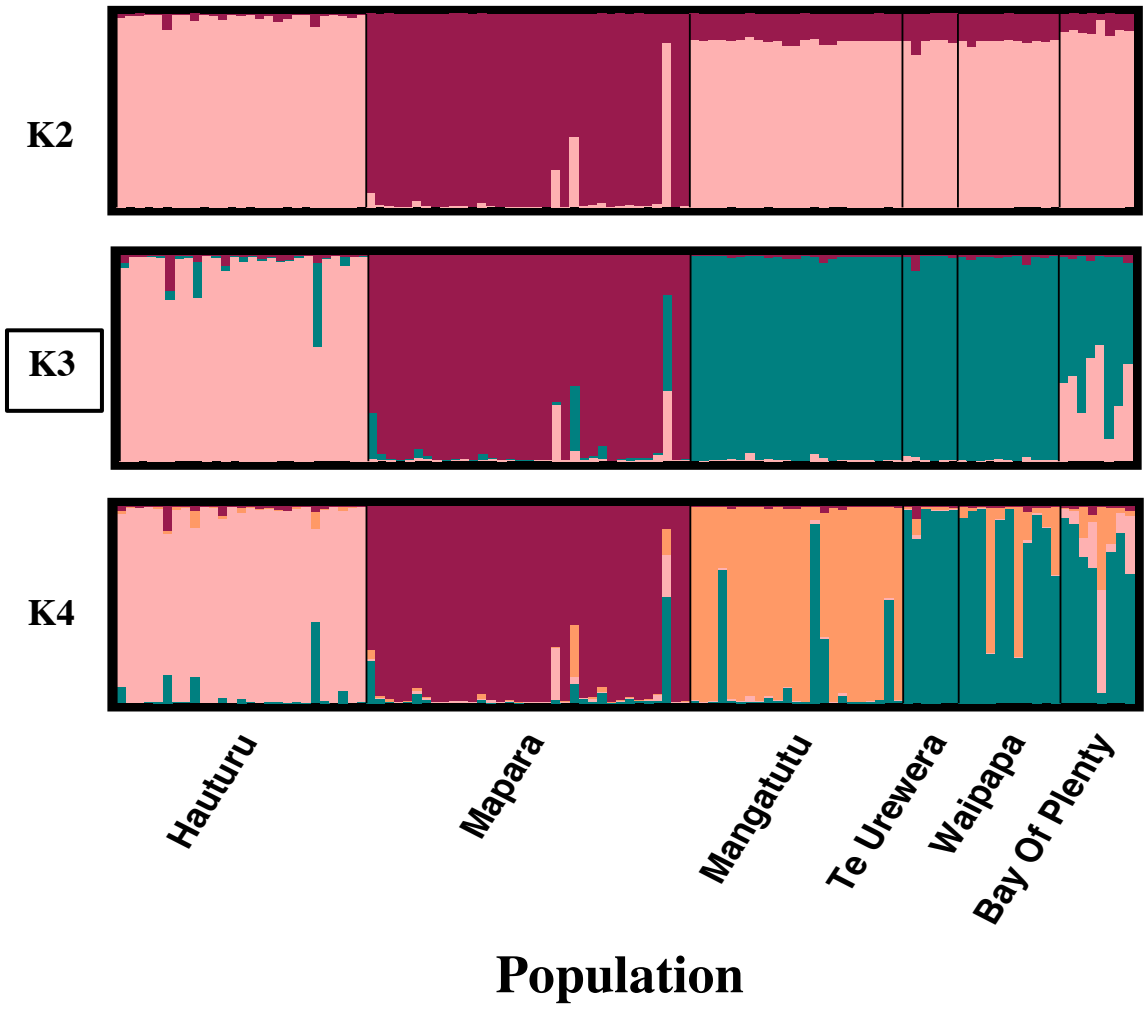


Figure 3.6. Individual clustering assignment implemented in STRUCTURE for three clustering scenarios (K2–4; most likely value of K=3) for six North Island kōkako populations. Vertical bars represent individuals, colours correspond to cluster membership, and black bars separate populations.

Table 3.7. Number of kōkako with q ≥ 0.8 membership in each of the three clusters inferred by STRUCTURE, from each population. Unassigned individuals are defined as those with q < 0.8. Bold values indicate the most likely cluster.

		Inferred cluster				
Population	N	1	2	3	Assigned	Unassigned
Bay of Plenty	8	0.37	**0.62**	0.01	1	7
Hauturu	27	**0.94**	0.03	0.01	24	3
Mapara	35	0.03	0.03	**0.93**	31	4
Mangatutu	23	0.01	**0.98**	0.01	23	0
Te Urewera	6	0.01	**0.97**	0.02	6	0
Waipapa	11	0.01	**0.98**	0.01	11	0

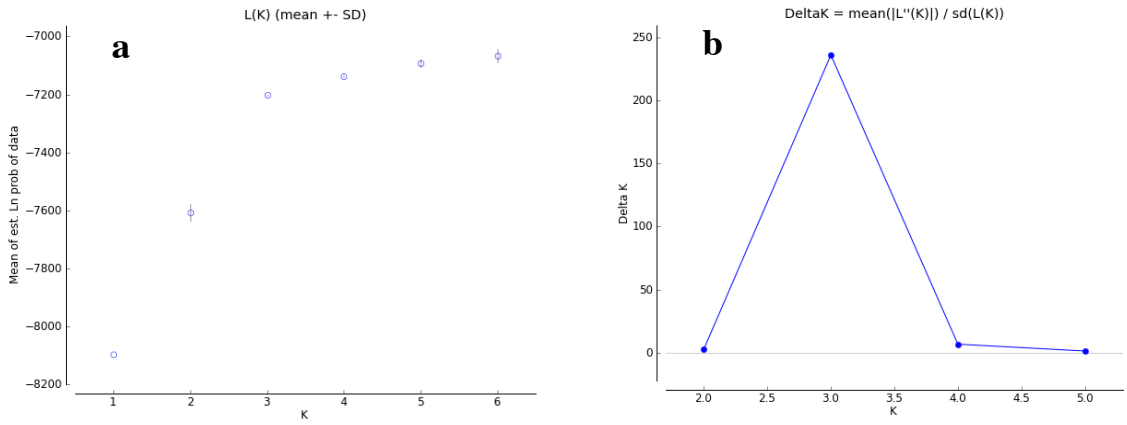


Figure 3.7. Results of STRUCTURE analysis for North Island kōkako *Callaeas wilsoni* (n=110) with (**a**) LnP(K) values and (**b**) the ad-hoc statistic DK values when testing for 1–6 clusters.

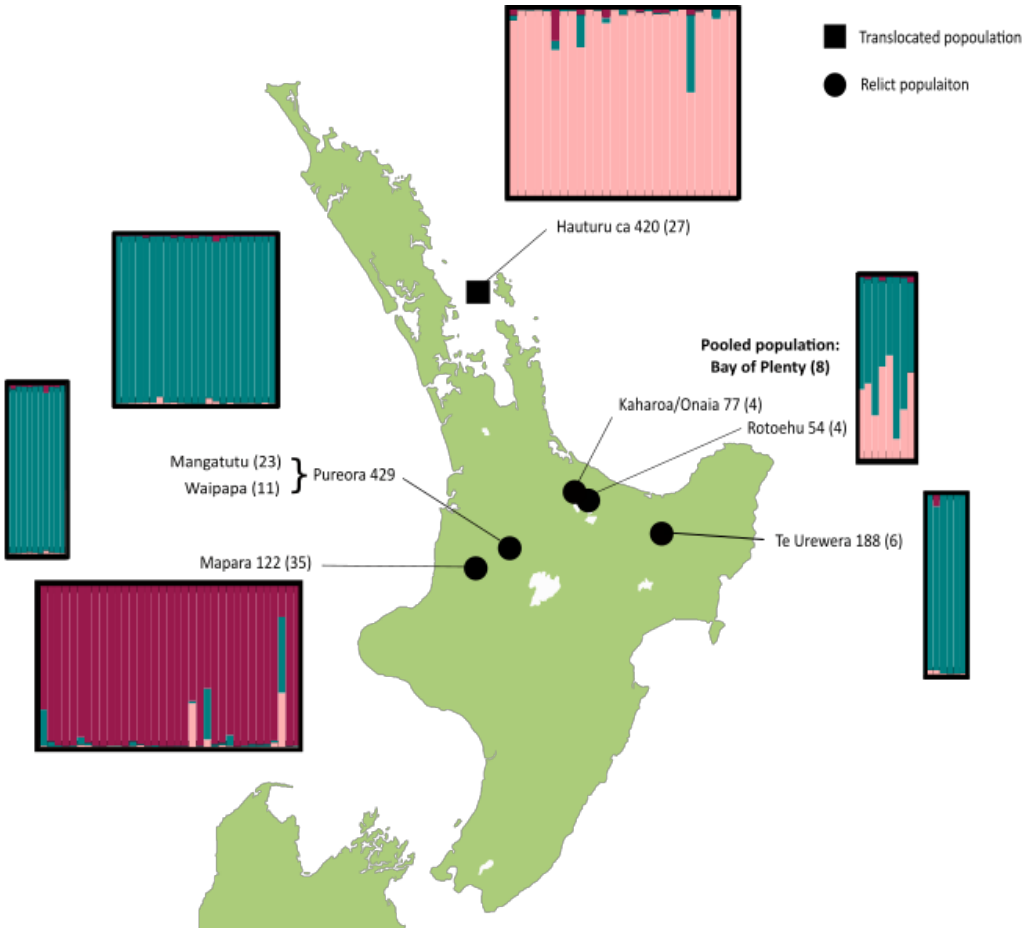


Figure 3.8. Map of sampled populations with STRUCTURE plots (K=3). Vertical bars represent individuals, colours correspond to cluster membership. Number of breeding pairs as of January 2017 (*North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017–2025. Draft* 2018) is given for each population, with sample sizes in parentheses.

3.3.5 Discriminant Analysis of Principal Components

For the K-means clustering approach in the DAPC, 200 principal components were retained and the BIC values declined until K=3 (BIC=235.7). Following this, the BIC values increased, indicating that the most likely number of clusters was three (Figure 3.9). This was concordant with the STRUCTURE results, both indicating the presence of three distinct genetic clusters.

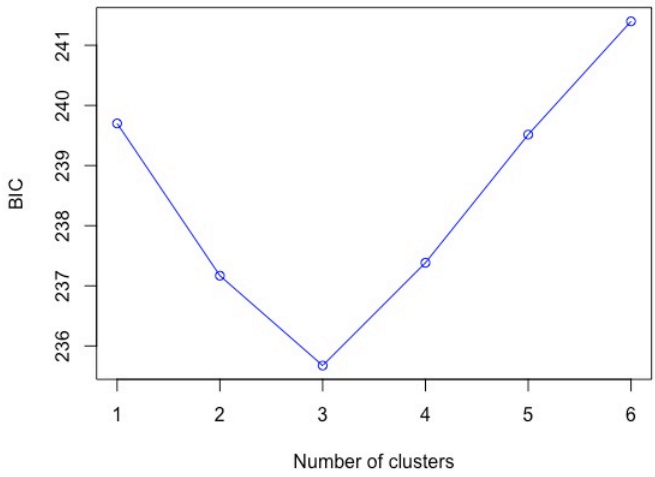


Figure 3.9. Plot showing the BIC values in the DAPC analysis for each K assessed from the K-means clustering approach. The optimum number of clusters is indicated by lowest BIC value.

Based on the cross-validation approach, where a training and validation set is used to determine the optimal number of PCs, ten principal components were retained. These PCs accounted for 38.7% of the variation within the microsatellite dataset to produce the DAPC scatterplot (Figure 3.10).

Results of the population subdivision analysis using DAPC are presented for K=3 and K=4, where distinct clusters are indicated by different colours (Figure 3.10). Scatterplots could not be produced for K=2. Analogous with the STRUCTURE findings, cluster one was dominated by individuals from Mapara, and cluster three by Hauturu individuals (Figure 3.10a; Table 3.8). Cluster two had mixed membership, but included all kōkako from the Mangatutu and Te Urewera populations, and the majority of those from Waipapa (n=10) and Bay of Plenty (n=4) (Table 3.8). The first of the two DAPC principal components separate all clusters from one another (Figure 3.10a). There is a strong separation of cluster 1 (Mapara) and cluster 3 (Hauturu) across the first DA component (eigenvalue 326.4). Across the second DA component, there is weaker separation between Cluster 2 (mixed) and the other two clusters (Eigenvalue 221.5; Figure 3.10a).

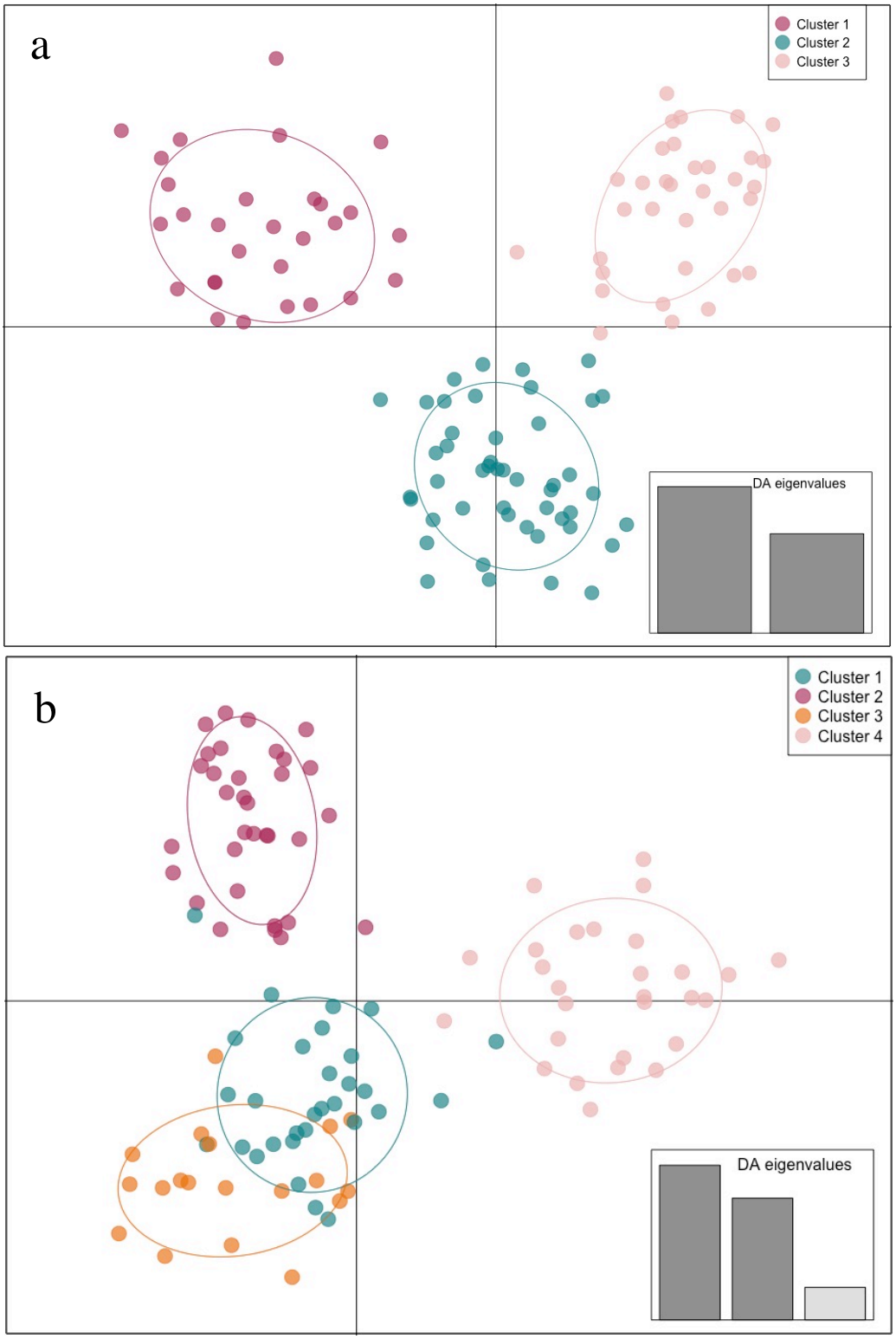


Figure 3.10. Figure showing clusters in discriminant space using 10 principal components for (**a**) K=3, and (**b**) K=4. The x-axis represents the first discriminant factor, and the y-axis represents the second. Eigenvalues representing the variance explained by discriminant factors are shown in the bar plots. Individuals are represented using dots, clusters are colour-coded and depicted by 95% inertia ellipses.

Table 3.8. Number of kōkako attributed to each cluster in the DAPC analysis (K=3) from each of the six populations.

Population	Cluster one	Cluster two	Cluster three
Hauturu	0	2	25
Mapara	32	2	1
Mangatutu	0	23	0
Te Urewera	0	6	0
Waipapa	1	10	0
Bay of Plenty	1	4	3
Total	*33*	*47*	*29*

3.4 Discussion

Twenty-two polymorphic microsatellite loci were used to assess the level of genetic diversity and population genetic structure among six isolated populations of North Island kōkako. This is the most extensive examination of genetic diversity in kōkako to date, and is the first to compare the population genetic structure using Bayesian and multivariate based approaches. The level of genetic variation in kōkako from Hauturu was similar to that of the five relict populations across all diversity measures. I found that kōkako have among the highest levels of genetic diversity observed in an endemic avian species (in NZ), that may be related to historical or evolutionary processes. Evidence of population structuring was observed, identifying Hauturu and Mapara as distinct clusters, and this finding was backed up by fixation indices. From a genetics point of view, the observed level of genetic variation presents no genetic barrier to using any of the studied populations as a source. The differentiation between sites would also be valuable to utilise in future translocations, as a way of maximising the genetic diversity in the founding population. These results should advance kōkako recovery in terms of having a more comprehensive and reliable understanding of genetic variation and population structuring, that can inform future translocations.

3.4.1 Utility of Hauturu as a source population

3.4.1.1 Genetic diversity

Across all genetic diversity measures, the Hauturu kōkako population was comparable to the other five relict populations in the study. The mean number of alleles was moderate; however, when corrected for sample size (allelic richness) was lower than most populations (Table 3.3). This difference was not statistically significant, meaning there was no clear distinction between the level of diversity in kōkako on Hauturu and the five mainland populations. Hauturu is the only translocated population that was examined in this study, suggesting that there was no significant loss of diversity due to a founding bottleneck in 1982 (Innes et al. 2013).

The finding that the level of diversity in kōkako on Hauturu was comparable to the other relict populations was unexpected, given the translocation history (Innes et al. 2013). Hauturu was established from 32 birds between 1981 and 1994, with the majority translocated in 1982 (13 birds, Innes et al. 2013; see Appendix 1). It is understood, however, that as few as 9 of the founding birds bred (I. Flux, pers. comm.). Both theoretical and empirical literature classify a breeding population of nine birds to be an extreme founding bottleneck (Nei 1973; Maruyama & Fuerst 1985; England et al. 2003; Briskie & Mackintosh 2004; Jamieson 2011; Tracy et al. 2011). Although the number of founders required to maintain genetic diversity and reduce inbreeding in wild populations is undefined and species specific (Miller et al. 2009; Taylor & Jamieson 2008; Tracy et al. 2011; Weiser, Grueber & Jamieson 2012), there is a general consensus that a large founding (>10 individuals) population is required to maintain and prevent significant diversity loss (Bodkin et al. 2001; Mock et al. 2004; Tracy et al. 2011; Ottewell et al. 2014). As such, in the interest of maintaining genetic diversity and reducing inbreeding, current translocation guidelines recommend a founding population much larger than nine breeding individuals (IUCN/SSC 2013).

Given that Hauturu is an isolated island, 80 km from the mainland of New Zealand, kōkako cannot disperse to or from the island. Ongoing gene flow therefore cannot explain the observed level of genetic diversity on Hauturu (Hedrick 1995; Kelly & Phillips 2015; Frankham 2015, 2016; Hedrick & Garcia-Dorado 2016). This raises questions as to whether there were only 9 founders, and if the breeding population is larger than previously thought.

If the founding population was only nine birds, it is possible that the breeding system of kōkako could be contributing to the observed diversity levels. Flux, Bradfield and Innes (2006) studied the breeding biology of kōkako at Mapara and found that although kōkako were generally monogamous, 7% of pairs separated per year. In a 5-year period, the greatest number of observed pairings by a female was four (different males, Flux et al. 2006). Although many birds appear to be sexually monogamous, true genetic monogamy is particularly rare in birds with 86% of species displaying additional paternity (Griffith et al. 2008). Extra pair paternity has been previously related to high levels of genetic diversity. For example, Brekke et al. (2011) found that there was no loss of genetic diversity in hihi (*Notiomystis cincta*) populations founded through reintroduction, when compared to the source. The high diversity in hihi populations has since been attributed to the breeding system of hihi, in which there is a high level of extra-pair copulation due to the presence of floater males (Brekke et al. 2012). I cannot explore this potential hypothesis in kōkako in the present study, but it does appear unlikely that this level of diversity could be explained by a founding population of only nine birds.

It is important to note that the level of diversity found in this study is higher than that previously reported for kōkako (Hudson et al. 2000). Using four microsatellite markers, Hudson et al. (2000) found a moderate level of diversity across three relict populations (A = 3.8, H_e = 0.56). Twice as many populations and an additional 17 markers were examined in this study (A = 5.5, H_e = 0.679). Empirical (Koskinen et al. 2004) and computer simulation studies (Takezaki & Nei 1996) have found that an increased number of microsatellite loci significantly increases the stability of genetic distances and the ability to accurately determine evolutionary relationships. Moreover, Hale, Burg and Steeves (2012) quantified the number of samples required to provide accurate population allele frequencies using microsatellites, and suggested that between 25 and 30 individuals would suffice. Together, this suggests that the present findings are a more reliable measure of contemporary kōkako diversity (than previous research) given that a larger sample size and greater number of microsatellite loci were used in this study.

3.4.1.2 Inbreeding and relatedness

The low F_{IS} values observed (Global = 0.0692, range –0.0881 to 0.065) provide limited evidence for inbreeding across any of the six populations. Frankham, Briscoe and Ballou

(2002) suggest that an inbreeding coefficient of 0.25 is great enough to reduce fitness, even when the effects of inbreeding are not visible. The value across kōkako populations is consequently much lower than a level that would warrant concern (Frankham et al. 2002), and indicates that mating is random across the studied kōkako populations. The highest value was observed in Hauturu ($F_{IS} = 0.065$), a population that was thought to have been established from a very low number of founders (Brown et al. 2004; Innes et al. 2013). Hauturu is the most successful and largest population of kōkako (Flux et al. 2013; Innes et al. 1999, 1996). Population size is often used as a measure of population recovery (Campbell et al. 2002) and could be taken as an indication that a population is not inbred. Cryptic inbreeding depression can occur in large populations, however, suggesting that population size is not necessarily a proxy for population health (Burt et al. 2016; Taylor et al. 2017). It would be valuable to examine the Hauturu population for signs of inbreeding depression in future research.

There was an elevated level of relatedness across all populations, and all relatedness measures (IR, HL and r). There were some discrepancies across each of the measures in terms of the population with the most related individuals. For example, the greatest median HL and IR was observed at Hauturu, whereas Te Urewera had the highest mean relatedness coefficient. In the case of Te Urewera, however, this might be a sampling artefact (see limitations below). Although there was an elevated level of relatedness at all sites, this was much lower than a full sibling or parent-offspring level of relationship (Queller & Goodnight 1989). These results highlight that even kōkako in the large, relict populations examined (e.g. Te Urewera or Mangatutu) were distantly related.

3.4.1.3 Population structure

Clear population structure was observed among the six kōkako populations, with both the Bayesian clustering and DAPC methods identifying the presence of three distinct genetic clusters. Hauturu and Mapara were identified as significantly different (genetically) in both cases, as was supported by the high, significant fixation indices between all population comparisons (F_{ST} and D_{JOST}). The largest cluster contained the four remaining populations, indicating that these populations did not differ significantly from one another (Pritchard et al. 2000; Bergl & Vigilant 2007; Nsubuga et al. 2010). Studies have recently questioned the validity of STRUCTURE analysis, and its ability to find real clusters (Kalinowski 2011; Puechmaille 2016). A recent meta-analysis found it was largely biased towards defining K

as two (two clusters), which provides additional confidence in the identification of three clusters in this study (Janes et al. 2017). Moreover, the DAPC and D_{JOST} fixation index supported the clusters identified by STRUCTURE providing more evidence for the identified groupings.

The kōkako populations on Hauturu and Mapara are genetically distinct from the other four relict populations examined in this study. Genetic bottlenecks, where populations are reduced to a fraction of the original size, rarely reflect the gene pool of the original population (Nei et al. 1975; Allendorf 1986). Both Hauturu and Mapara are known to have experienced population bottlenecks, with Hauturu being established via various translocations between 1981 and 1994 (Innes et al. 2013) and the Mapara population experiencing a crash in 1989 (Double & Murphy 2000). It is likely that these two events contribute to the observed genetic differentiation (Frankham 1998; Frankham et al. 2017; Funk et al. 2016). The N_b estimates support this hypothesis, where the contemporary number of effective breeders at Mangatutu is larger than that of Mapara and Hauturu. There was some uncertainty in the estimate (as was indicated by large confidence intervals); however, the analysis suggested that the number of effective breeders on Hauturu is 72 and 37 at Mapara, so 36 and 18 pairs respectively. Once again, this finding is not in agreement with previous research, where no significant genetic difference between Mapara and two other mainland populations was identified (Te Urewera and Rotoehu; Hudson et al. 2000).

The mixed membership in the Bay of Plenty population, between the Hauturu and mixed population clusters (Figures 3.6 & 3.8), may be related to the translocation history of Hauturu. The fixation index (D_{JOST}) suggested that Hauturu and BOP were significantly different, however, STRUCTURE could only assign one individual to a cluster based on $q > 0.8$ (Table 3.7). Between 1981 and 1983, six individuals from Kaharoa and five from Rotoehu were translocated to Hauturu (Innes et al. 2013). The number of females, and number of individuals that successfully bred is unknown. However, these 11 birds represent 37.5% of the total number of founders that established Hauturu (Innes et al. 2013; Appendix 1). No birds from any of the other four examined populations were used to establish Hauturu. Mixed membership within a population is often attributed to gene flow between populations (Pritchard et al. 2000). Dispersal between these populations is not possible based on distance and location, therefore would not explain the observed result. It must be noted that the sample

size for the pooled BOP population was small (Kaharoa = 4; Rotoehu = 4), so the results must be interpreted with caution (see limitations).

3.4.1.4 Hauturu as a source population: Genetics informing management

Together, my findings indicate that Hauturu presents as a valuable source given that: the level of diversity is similar to that of the other studied populations (Table 3.3); that Hauturu is the largest population of kōkako (c.420 breeding pairs; *North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017-2025. Draft* 2018); and that kōkako on Hauturu possess genetic diversity at microsatellite loci not present in other populations (Figure 3.8). Although the highest median IR was reported on Hauturu, there was little difference between the measures of inbreeding and relatedness across all populations (Table 3.4; Figures 3.3 & 3.4). This means that there should be no greater concern in using Hauturu as a source, in terms of inbreeding, than any of the other relict populations. Together this provides genetic evidence to support the use of Hauturu as a source population in future translocation projects.

Sourcing individuals from multiple, genetically differentiated sources has been repeatedly recommended as a method of increasing the diversity of founding populations (Madsen et al. 1999; Bodkin et al. 2001; Maudet et al. 2002; Broadhurst et al. 2008; Sgrò et al. 2011). This is important for the long term success of translocations (IUCN/SSC 2013), as it is suggested this will promote adaptive potential of the new population (Broadhurst et al. 2008; Sgrò et al. 2011). Based on this recommendation, it would be valuable to draw from each of the three genetic clusters found in this study when establishing new sites (Figure 3.8). This would mean selecting individuals from populations such as Hauturu, Mapara and Mangatutu as a method of capturing the maximum genetic variation present across all kōkako populations.

3.4.2 High genetic diversity across six kōkako populations

A high level of genetic diversity was revealed across all North Island kōkako populations examined in the present study (A = 5.9 H_e = 0.679). This was consistent across all diversity measures (A, AR, H_o and H_e) and all six populations. Genetic diversity of kōkako presented here is greater than the reported diversity of most endemic avian species with similar histories of decline (Boessenkool, et al. 2006; Brekke et al. 2011; Jamieson 2009; Taylor & Jamieson 2008), and threatened avian species on a global scale (Evans & Sheldon 2008). When

surveying the published literature, I failed to find a greater level of expected heterozygosity in an endemic New Zealand avian species. A close second is the hihi, of which also have a lower reported number of alleles per locus (A = 4.86, H_e = 0.645, n = 269; Brekke et al. 2011). Interestingly, the observed level of diversity in kōkako is comparable to that of a common, widespread species, the bellbird (*Anthornis melanura*) (A = 5.43, H_e = 0.621, n = 315; Baillie et al. 2014). Although bellbird populations have experienced significant range reductions and local extirpations in the past (Craig & Douglas 1984), genetic diversity is high and similar across the present geographical range (Baillie et al. 2014). Moreover, they are considered a species of least concern by the IUCN (Birdlife international 2016). Another endemic species with a comparable level of diversity is the North Island brown kiwi (*Apteryx mantelli*) (A = 7.0, H_e = 0.605, n = 35; Shepherd & Lambert, 2006), the most common of the four kiwi species with an estimated 26,600 individuals across four populations in 2015 (Birdlife International 2017). North Island kōkako therefore appear to have a level of diversity comparable to largely abundant and widespread endemic species in New Zealand.

3.4.2.1 High genetic diversity in a historical context

Historical levels of genetic diversity, gene-flow or ancient processes (such as existing in glacial refugia) are all likely to contribute to the level of diversity and patterns in the genetic variation observed today (Paxinos et al. 2002; Pertoldi et al. 2001; Taylor, Jamieson & Wallis 2007). Kōkako were widespread and abundant prior to European colonisation in the 1800s (Lavers 1978). Preceding deforestation across the North Island, continuous stretches of native forest would have connected remnant populations that exist today as isolated fragments (King, 1990). This may have facilitated sufficient gene flow between connected populations, and could serve as a hypothesis as to why the observed level of diversity is high. Murphy, Flux and Double (2006) also hypothesized that dispersal is likely to have occurred pre-deforestation, and may explain the widespread distribution of mitochondrial haplotypes that they observed. It is also possible that historically kōkako had a significantly higher level of diversity.

Taylor, Jamieson and Wallis (2007) compared contemporary and historic genetic diversity in South Island Saddleback (*Philesturnus carunculatus*), a sub-species of the closest extant kōkako relative. South Island saddlebacks were extirpated from the mainland in 1905 due to introduced predators, and became restricted to offshore islands (Hooson & Jamieson

2003). The authors found that a significant amount of diversity had been lost with this mainland extinction (H_e = 0.5 to 0.1; Taylor et al. 2007). Interestingly, the historic level of diversity in saddlebacks on the mainland was very similar to that observed in kōkako today (saddleback: A = 5.8, AR = 5.71, H_e = 0.53), whereas contemporary levels of diversity in saddleback are much lower (e.g. Kaimohu Island: A = 1.41, AR = 1.40, H_e = 0.10; Taylor et al. 2007). This could suggest that kōkako would have had a high level of genetic diversity historically, that has been partially maintained in contemporary populations. To investigate the possibility of high gene flow or the maintenance of genetic diversity from historic populations, future research would need to include museum samples across the historical range of kōkako (Paxinos et al. 2002; Pertoldi et al. 2001; Taylor et al. 2007).

Across a greater timescale, the evolutionary history of kōkako may also explain diversity levels observed today. The existence of populations following glaciation events can explain contemporary levels of genetic diversity. For example, two contrasting phylogenetic patterns have been proposed to explain differences in contemporary genetic diversity in the North Island brown (*Apteryx mantelli*) and little spotted kiwi (LSK; *A. owenii*) (Shepherd et al. 2012). It is suggested that the low level of diversity in LSK can be attributed to the existence in a single refuge, whereas a much greater level of diversity in the NI brown kiwi may be attributed to historical existence in multiple refuges (Shepherd et al. 2012) The same two hypotheses have been proposed for the evolutionary history of NI kōkako (Murphy et al. 2006). That they either existed in one Pleistocene refuge or existed in multiple and then genetically diverged and dispersed widely. My results would provide further support for the multiple refugia hypothesis, as if they were limited to a single refuge it would be expected that genetic diversity be reduced through a bottleneck as observed in LSK (Shepherd et al. 2012). As previously described, the contemporary level of diversity is comparable to that of the NI brown kiwi, so it may be feasible that they had similar evolutionary histories (Shepherd & Lambert 2006). Ancient DNA would also be required to test this hypothesis.

3.4.2.2 High genetic diversity in a contemporary context

Gene flow is understood to counteract the effects of genetic drift in small populations (Slatkin 1987; Bohonak 1999; Kelly & Phillips 2015; Frankham 2015). In the aforementioned Taylor et al. (2007) study, a comparatively more vagile species the New Zealand Robin (*Petroica australis*) suffered a much smaller diversity loss when compared to historical samples (H_e =

0.4 to 0.3), and was attributed to the dispersal capabilities of robins. It is unclear whether contemporary gene flow in kōkako populations is a valid explanation for the observed level of diversity. Hudson et al. (2000) ruled out dispersal as a method of contemporary gene flow across kōkako populations, suggesting they are poor fliers and reluctant to fly across open habitat. Recently a kōkako released in Ark in the Park in 2010, a conservation area in the Waitakere ranges, was found three years later in Glendowie, a suburb in Auckland NZ 30 km East of the sanctuary (Knight & Rivera 2015). This may be a rare occurrence, but does suggest that kōkako are capable of dispersing across urban environments. Survival between population fragments does need to be considered, as without predator control kōkako survival is known to be poor (Innes et al. 1999b; Basse et al. 2003). Theoretical research has suggested that one migrant per generation is the minimum required to reduce the effects of genetic drift (Slatkin 1987; Mills & Allendorf 2002). Moreover, as kōkako are territorial, sub-adult males often disperse for territories (Innes & Flux 1999). Further research that includes monitoring of kōkako (dispersal) would be required to determine the validity of this hypothesis.

3.4.2.3 Management implications

Whether due to historical or more recent processes, it is clear that the level of diversity across kōkako populations (in this study) is relatively high. As discussed, it is important to consider genetic diversity and population differentiation when undertaking a translocation (Armstrong & Seddon 2008; Allendorf et al. 2012; IUCN/SSC 2013; Frankham et al. 2017). Reduced genetic diversity has the potential to reduce both individual and population fitness, and therefore impact the long-term success of a translocation. The observed level of genetic diversity is promising for the future of kōkako recovery. From a conservation genetics perspective, all populations included in this study present as valuable source populations. Kōkako from all examined relict populations have been used as a source in previous translocations (Innes et al. 2013). This research therefore provides some retrospective confidence in these projects, given that birds from these populations have been used to establish and augment existing populations in the past. Going forward, these findings should assist managers to make informed decisions that will advance the future of kōkako recovery.

3.4.3 Limitations

Ascertainment bias may have occurred in this study, in which a tendency to select the most polymorphic loci might have biased the results (Eriksson & Manica 2011). Studies that use

species-specific microsatellite markers tend to display higher genetic diversity than those who use cross- species loci (Evans & Sheldon 2008), which may explain why the level of diversity reported in this study is higher. However, this is a similar level of diversity as has been reported in hihi, in which 15 species-specific microsatellite loci were used (Brekke et al. 2011). In addition, many of the samples from the Te Urewera, Mapara and Rotoehu (BOP) populations were from the same study (Hudson et al. 2000), therefore using a greater number of microsatellite markers on many of the same individuals produced higher diversity estimates. Ascertainment bias is considered to be a considerably greater concern in datasets that use single nucleotide polymorphisms (Eriksson & Manica 2011), therefore should not have significantly biased the findings of this research.

The number and quality of the samples provided for this study meant that I was limited in terms of how many populations I could examine, and lead to the pooling of some populations (Rotoehu and Kaharoa to represent Bay of Plenty region). There was some uncertainty in certain measures (HL and IR) for populations with a small sample size, which could not be avoided in this study. However, there was a large sample size for Hauturu which was the main focus of this research, meaning that we can be confident in the Hauturu results (Hale et al. 2012). As discussed, this research utilised a much larger set of microsatellite loci than previous research, which provides additional confidence in the findings (Takezaki & Nei 1996; Koskinen et al. 2004; Wan et al. 2004; Hubisz et al. 2009).

3.4.4 Conclusions

The aim of this research was to assess the suitability of using Hauturu as a source population for future translocation projects. I found that the level of diversity on Hauturu was comparable to the five relict populations, across all diversity measures. Moreover, significant differentiation between Hauturu and the other populations was present at the examined microsatellite loci. From a conservation genetics point of view, it appears that Hauturu presents as a valuable source population for future translocations, as do the five relict sites. In addition, when compared to other endemic avian species kōkako have a reasonably high level of genetic diversity. Future research should be able to discern the reasoning behind this. This study contributes to the wider literature, in terms of increasing our understanding of the genetic diversity of an endemic threatened bird species. In addition, this study will provide

conservation managers with important and valuable information that will improve future translocation efforts for this taonga species.

Chapter Four:
An assessment of two potential molecular explanations for the aberrant colouration of a North Island kōkako (*Callaeas wilsoni*)

An adult kōkako with aberrant plumage and features from Te Hauturu o-Toi. Source Ian Flux.

4.1 Introduction

A North Island kōkako (*Callaeas wilsoni*) with aberrant white plumage and features was captured on Little Barrier Island (Te Hauturu-o-Toi) in August 2015 (Figure 4.1). This individual was initially captured in June 2013 and was considered healthy, with no observable phenotypic abnormalities (Appendix 2). Upon recapture in 2015, a lack of pigmentation was apparent on all areas of the body and substantial changes to the plumage, bill, legs and claws had occurred (Figure 4.1; see method) raising concern that the abnormalities were a result of a viral disease (T. Thurley, pers. comm.). Hauturu is a particularly valuable population of kōkako, in terms of population stability, with the largest population size (c.420 pairs; *North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017-2025. Draft* 2018), high population growth (Innes et al. 1996; Innes and Flux 1999) and a lack of predators (Bellingham et al. 2010; Veitch 2001). Considering this, Hauturu has been identified as a potential source population for future translocations. Minimising and preventing disease spread is an important component of translocation management (see Chapter 1). The introduction and spread of disease has the ability to undermine the positive implications of a translocation (through mortality and reduced fitness), as they are designed to enhance populations that are already compromised (Cunningham 1996; Kock, Woodford & Rossiter 2010; Sainsbury & Vaughan-Higgins 2012). Translocating from a site that has tested positive for viral disease carries the risk of introducing disease to an immunologically naïve population (Cunningham 1996; Kock, Woodford & Rossiter 2010; Parker, Brunton & Jakob-Hoff 2006; Sainsbury & Vaughan-Higgins 2012). To assess the suitability of Hauturu as a source population, it is essential to clarify why this bird has developed abnormal colouration and whether or not it is a consequence of viral disease. In this chapter I will explore two potential explanations for the observed phenotype by testing for the presence of Psittacine Beak and Feather Disease Virus and by comparing sequences of the melanocortin-1 receptor gene (MC1R).

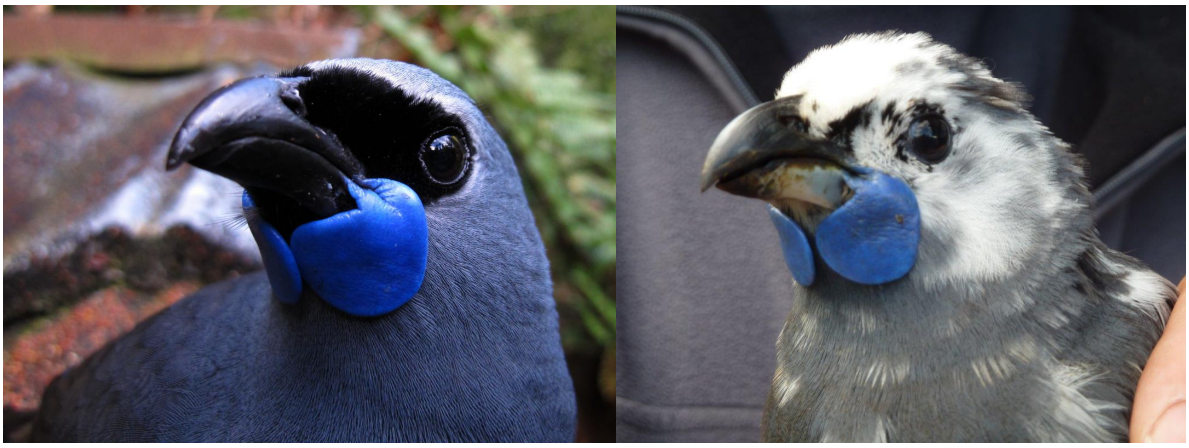


Figure 4.1. Plumage comparison of a normal and the aberrant kōkako captured on Hauturu in August 2015 (no before photographs of kōkako E212419 were taken). Showing the changes to the bill, black mask and plumage over head and body. Sources Matt Binns, Ian Flux.

4.1.1 Psittacine beak and feather disease (PBFD)

Psittacine beak and feather disease (PBFD) is recognized as one of the most common, chronic viral avian diseases worldwide (Raidal & Peters 2018). PBFD has a worldwide distribution in captive populations (Khalesi et al. 2005), however, the extent and prevalence of this virus is poorly understood in wild populations (Fogell, Martin & Groombridge 2016). Research on wild species has largely emerged in the last five years, the majority conducted on Australian and New Zealand species (Fogell, Martin & Groombridge 2016). PBFD threatens the persistence of many species at risk of extinction, such as the Mauritius parakeet (*Psittacula echo*; Kundu et al. 2012), critically endangered orange-bellied parrot (*Neophema chrysogaster*; Peters et al. 2014; Raidal, Sarker & Peters 2015) and swift parrot (*Lathamus discolour*; Sarker, Ghorashi, Forwood & Raidal 2013). Sixty percent of reported PBFD cases are within declining populations (Fogell, Martin & Groombridge 2016) and has directly lead to the loss of a founder population of Mauritius parakeet (Tollington et al. 2015). Consequently, this disease is recognized as a significant conservation threat to avian populations (Raidal & Peters 2018).

PBFD is a viral disease, that is caused by the beak and feather disease virus (BFDV). BFDV is a member of the viral family *Circoviridae* due to the circular ssDNA, ambisense, non-enveloped genome (Pass & Perry 1984; Todd et al. 1991; Bassami et al. 1998). Two main open reading frames that encode the capsid (*cap*) and replication-associated (rolling circle replication initiator protein gene; *rep*) proteins are present within the BFDV genome (see Figure 4.2; Raue et al. 2004). These regions are conserved between viral variants and

are commonly used to detect the presence of the virus in infected individuals by polymerase chain reaction (PCR) (Ypelaar et al. 1999; Fogell et al. 2016). The BFDV viral ssDNA is between 14 and 16 nm in length (particle diameter) and between 1.7 to 2.0 kb in size (Bassami et al. 1998; Ritchie et al. 1989; Todd et al. 1991), making it the smallest known autonomously replicating viral genome (Todd 2000). BFDV is prone to mutation, has experienced a large number of recombination events and regular cross-species transmission (Julian et al. 2013; Raidal & Peters 2018; Sarker et al. 2014). As such, BFDV is a pathogenic host-generalist capable of host switching between both psittacine and non-psittacine species (Raidal & Peters 2018).

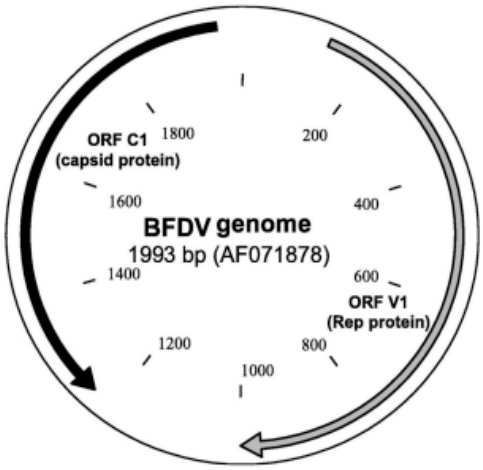


Figure 4.2. Diagram of the BFDV genome, showing the two major open reading frames encoding the Rep (ORF V1) and capsid proteins (ORF C1). Diagram from Raue et al. (2004).

PBFD typically targets follicle cells in the beak and feathers, leading to abnormalities in each (Pass & Perry 1984; Ritchie et al. 1989). Symptoms include loss of contour, down and tail feathers (Pass & Perry 1984; Ritchie et al. 1991) with replacement by irregular dystrophic feathers, many of which stop growing after emerging from the follicle (Ritchie et al. 1989). Changes to feather pigmentation have also been reported, with blue feathers replaced by white and green with yellow (Doneley 2016). Beak abnormalities typically result in the development of lesions, palatine necrosis and transverse or longitudinal cracking (Doneley 2016; Pass & Perry 1984; Ritchie et al. 1989). PBFD also targets the lymphoid cells, leading to immunosuppression that can result in lethal secondary infections (Ritchie et al. 1989; Todd 2000).

4.1.1.1 Prevalence in New Zealand

Although published studies relating to the presence and prevalence of PBFD in New Zealand are limited, PBFD has been detected in populations of both wild (4) and captive (8) psittacine species in New Zealand (Appendix 5). The virus has been detected in both wild and captive populations of red-crowned parakeet (*Cyanoramphus novaezelandiae***;** Ha et al. 2009; Jackson et al. 2015; Massaro et al. 2012; Ortiz-Catedral et al. 2009) and sulphur-crested cockatoo (*Cacatua galerita*; Ha et al. 2007; Ritchie, Anderson & Lambert 2003). Three endemic species have tested positive for PBFD; Antipodes Island parakeet (captive) (*Cyanoramphus unicolor*; Ha et al. 2009), red-crowned parakeet and yellow-crowned parakeet (*Cyanoramphus auriceps*; Appendix 5). The prevalence of PBFD in wild, native populations of New Zealand psittaciforms is thought to be relatively low, estimated at less than 7% (Ha et al. 2009). It has been suggested, however, that exotic species such as eastern rosellas (*Platycercus eximius*) and sulphur cockatoos are a concerning reservoir of PBFD in the wild and that there is a surprisingly high prevalence of PBFD in these species (Ha et al. 2007). Managing PBFD in New Zealand is therefore considered a conservation priority (Department of Conservation 2011) as it has the potential to compromise the persistence of endangered species (Ha et al. 2007; Ortiz-Catedral et al. 2009).

4.1.1.2 PBFD in non-psittacine species

PBFD is a host-generalist virus capable of switching between psittacid species (Raidal & Peters 2018), with recent research suggesting all Psittacine species may be susceptible to all genotypes of PBFD (Khalesi et al. 2005; Raidal et al. 2015). The rate at which the virus is known to host-switch across Psittaciformes is high (Sarker et al. 2015). Occasionally, this switch extends to non-psittacine species. Indeed, there have been cases of PBFD in species such as rainbow bee eaters (*Merops ornatus*; Sarker et al. 2015), powerful owls (*Ninox strenua*; Sarker et al. 2016) laughing kookaburra (*Dacelo novaguineae*; Amery-Gale et al. 2017) and Gouldian finches (*Chloebia gouldiae*; Circella et al. 2014). This, however, is an area of research that is limited due to a lack of research. Indeed, transmission of PBFD to non-psittacine species has been identified as a knowledge gap (Department of Sustainability, Environment, Water, Population and Communities 2012). Emerging evidence suggests that the prevalence of PBFD in non-psittacine species is much higher than was once thought, with 20% of non-psittacine birds testing positive for the disease in Australia, often without

showing symptoms (Amery-Gale et al. 2017). There are no published cases of PBFD in a non-psittacine species in New Zealand (Appendix 6).

4.1.1.3 Threat to kōkako recovery

Beak and feather disease virus has been detected on Hauturu in the population of red-crowned parakeets (Jackson et al. 2015; Ortiz-Catedral et al. 2009). It was originally suggested that this may be a native strain of PBFD (Ortiz-Catedral et al. 2009), however more recent research suggests that the genome isolates share a high similarity with (NZ) eastern rosellas and yellow-crowned parakeets with PBFD (Massaro et al. 2012). Kōkako are a recovering species, of which a significant amount of conservation effort is focused towards translocation efforts (Innes & Flux 1999; Innes et al. 2013). The aberrant kōkako has many symptoms that resemble PBFD infection, including irregular feather replacement and beak abnormalities (Doneley 2016; Pass & Perry 1984; Ritchie et al. 1989). If a case of PBFD were present, this would be a significant threat for the future of kōkako recovery. Considering that translocations need to be managed in ways that minimise the spread of disease (Chapter 1), a case of PBFD may compromise the utility of Hauturu as a future source population. The Department of Conservation have introduced a national ban on translocating parrots from areas with known PBFD status to areas in which the status is unknown or negative (Department of Conservation 2011). Moreover, there are currently no management strategies on Hauturu to minimise the spread of PBFD, and it has been suggested that the disease could spread rapidly across the island (Knafler et al. 2016).

4.1.2 Melanins and plumage colouration

There are alternative reasons for plumage variation, aside from the abnormalities caused by disease. If the aberrant white phenotype seen in the Hauturu kōkako is not a viral symptom, the next logical explanation is to examine whether or not a mutation in the pathway of melanin synthesis is involved. As kōkako are dark grey in colour, the genetic basis of a change in phenotype would be related to melanin synthesis pathway, as eumelanin is primarily responsible for dark phenotypic features (McGraw et al. 2005; Roulin & Ducrest 2013; Galván & Solano 2016).

Birds are the most colourful group of vertebrates, showing incredible diversity in both colour and pattern. Plumage colouration is primarily a reflection of pigment deposition in the feathers (McGraw et al. 2005; Mundy 2005; Roulin & Ducrest 2013). Carotenoid pigments

are responsible for the yellow, orange and red colour variants, whereas melanins regulate black, brown and grey phenotypes (McGraw et al. 2005). Two melanic pigments, eumelanin (black, brown and grey) and phaeomelanin (red-brown), have been described in birds (McGraw et al. 2005; Galván & Solano 2016). The relative ratio of eumelanin and phaeomelanin determines the overall phenotype, with greater deposition of eumelanin responsible for dark coloured birds (McGraw et al. 2005; Roulin & Ducrest 2013). Melanocytes produce eumelanin and phaeomelanin, and are specialised cells present in the epidermis, eye and feather follicles (Galván & Solano 2016). When there is a disruption in the process of pigment deposition, or the structural properties of feathers during development, this results in abnormal plumage colouration (van Grouw 2013).

4.1.2.1 Melanocortin-1 receptor (MC1R)

The melanocortin-1 receptor (MC1R) is an important component in the synthesis pathway of melanin (Hoekstra et al. 2006; Hofreiter & Schöneberg 2010; Mundy 2005; Mundy et al. 2004; Roulin & Ducrest 2013). The MC1R gene is highly conserved among vertebrates (Selz et al. 2007; Hubbard et al. 2010) and has been used to describe phenotypic variation in a large number of species. MC1R encodes a seven-transmembrane G protein-coupled receptor (Fredriksson & Schiöth 2005; Guernsey et al. 2013; Jackson 1994) expressed in the melanocytes of developing feather or hair follicles (Robbins et al. 1993; Baião et al. 2007; Roulin & Ducrest 2013). Eumelanin and phaeomelanin are heteropolymers, produced by melanosomes within the melanocytes of feather and hair follicles (Roulin & Ducrest 2013). The production of eumelanin and phaeomelanin is primarily regulated by MC1R, the relative ratio of each determining the phenotype or overall colour of an organism (Hubbard et al. 2010). Generally speaking, increased MC1R activity stimulates the synthesis of eumelanin and therefore results in a darker phenotype (Ollmann et al. 1998; García-Borrón et al. 2005; Guernsey et al. 2013). Alternatively, if MC1R activity is decreased eumelanin synthesis is also reduced and phaeomelanin production increases (Rees 2003; Fredriksson & Schiöth 2005).

Phenotypic variation in colour has recently been linked to mutations in the MC1R gene in a number of different species, many of which can be traced to non-synonymous amino acid substitutions (Andersson 2003; Mundy 2005; Takeuchi et al. 1996). Such mutations have been identified across the length of the gene (Manceau et al. 2010) but are often within

the coding region (Zhang et al. 2013). Loss of function mutations decrease eumelanin production and therefore result in a lighter phenotype. An example of this is seen in the coat colour of Labrador retrievers (Everts et al. 2000). A substitution at Arginine (position 306) was found to produce a premature stop codon in all individuals with the light coat colour (Everts et al. 2000). Similarly, a non-synonymous point substitution (Arg65Cys) in the coding region of MC1R in the beach mouse (*Peromyscus polionotus*), gives a light coat colour (Hoekstra et al. 2006; Mullen et al. 2009). For darker phenotypes, gain-of-function mutations increase MC1R activity and hence eumelanin production (Mundy 2005; Roulin & Ducrest 2013).

4.1.2.2 MC1R and plumage colour

Melanins have been well studied in avian species, as variation in eumelanic and pheomelanic pigments often reflect differences in plumage colouration. As such, both within and between species variation has been linked to point mutations and hence amino acid changes in the MC1R gene (Mundy 2005; Bourgeois et al. 2012; Roulin & Ducrest 2013). Sequence variations have been studied in a number of different bird species such as the Arctic Skua (*Stercoarius parasiticus;* Mundy et al. 2004), Japanese Quail (*Coturniz japonica;* Zhang et al. 2013), Bananaquit (*Coereba flaveola;* Theron et al. 2001) and Eleonora's falcon (*Falco eleonorae;* Gangoso et al. 2011). Some species possess a white colour morph, where a lack of pigmentation results in a fully or partially white phenotype. Significant links between MC1R mutation and white phenotypes have been identified in a number of studies, across many unrelated species (Baião et al. 2007; Mundy et al. 2004; Roulin & Ducrest 2013; Vidal et al. 2010; Zhan et al. 2012). For example Johnson, Ambers and Burnham (2012) found strong support linking MC1R variation with plumage colour in Gyrfalcons (*Falco rusticolus*), where a non-synonymous point substitution (Val128Ile) was perfectly associated with white and melanic birds. Similarly, a missense SNP mutation (Ile58Val) in Japanese quails was found to be associated with black (as opposed to white or maroon) plumage (Zhang et al. 2013).

4.1.2.3 Pleiotropy and MC1R

The genes that are responsible for regulating melanin synthesis pleiotropically influence a number of life history traits via the melanocortin system (Ducrest, Keller & Roulin 2008; Roulin 2016). Studies have identified correlations between melanin based colouration and

the resistance to oxidative stress (Galván et al. 2010; Roulin et al. 2011), regulation of stress responses (Almasi et al. 2010) and ability to cope with parasitism and variable food supply (Piault et al. 2009; Lei et al. 2013). On Hauturu, the kōkako population is approaching (or has already reached) carrying capacity (J. Innes, pers. comm.). As such, density dependent factors may be acting upon the population, and it is suggested that the observed phenotypic changes might be due to environmental stress. Recently, pharmacological research has confirmed the pleiotropic function of MC1R suggesting that this gene also regulates immunological responses (Loser et al. 2010). MC1R is expressed in many cells of the immune system: monocytes, macrophages, neutrophils, B lymphocytes, natural killer cells, cytotoxic and $CD8^+$ T cells (reviewed in Gangoso et al. 2011). In addition, research has shown that the binding of melanocortins to melanocortin receptors (including MC1R) can regulate physiological functions such as inflammatory or cytotoxic immune processes, stress responses and exocrine gland activity (Luger et al. 2003; Catania et al. 2004; Ducrest et al. 2008; Loser et al. 2010; Lei et al. 2013). For example, inflammatory responses in Eleonora's falcon has been found to be morph specific (Gangoso et al. 2011; Gangoso et al. 2015), a species in which colour morphs are found to be perfectly associated with a four amino acid deletion in the MC1R gene (Gangoso et al. 2011). Although correlative, this research provides convincing evidence for the pleiotropic role that MC1R has, influencing both melanic colouration and physiological functions.

Physiological stress often lowers the fitness of organisms and has a number of environmental causes such as increased competition, depletions in food supply, disease, immunological and predation pressures. In vertebrates, physiological stress often results in growth abnormalities in keratin structures such as feathers, claws, hair, horns or nails (Fuchs 1995; Johnstone et al. 2012). In birds, environmental stress often phenotypically presents as fault bars in the feather structure due to abnormalities in keratin deposition (Murphy et al. 1989; Prum & Williamson 2001; Jovani & Blas 2004; Jovani & Rohwer 2017). Feather replacement during a moult is an energetically expensive process, that is compromised when an individual is stressed (Murphy & King 1992; Lindström et al. 1993; Strochlic & Romero 2008; DesRochers et al. 2009). Due to the aforementioned associations between melanocortin receptors and physiological responses such as stress and immunity, it is possible that stress has an effect on MC1R and melanin deposition in keratin structures at moult. In the aberrant kōkako, it appears that melanic keratin structures are being replaced by irregular, non-

melanic structures at moult (Figures 4.3, 4.4 & 4.5). Thus, it is important to examine the MC1R gene in the aberrant and phenotypically normal kōkako to compare whether mutational differences provide an explanation for the observed phenotypic abnormalities.

4.1.3 Research aims

It is clear that the kōkako captured on Hauturu in 2015 has undergone substantial phenotypic changes in a relatively short period of time. PBFD is significant threat to endangered species (Raidal & Peters 2018) and can induce similar abnormalities in the skin, feathers and beak to that seen in the kōkako. Alternatively, as pigmentation has a genetic basis, it is possible that the observed phenotypic abnormalities are the result of a genetic mutation. The primary aim of this chapter is to use molecular techniques to explore two potential explanations for the aberrant plumage seen in the Hauturu kōkako. Firstly, I will use polymerase chain reaction to test for PBFD using PCR primers that have successfully amplified the virus in a number of non-psittacine species (Amery-Gale et al. 2017). Secondly, I will examine MC1R gene sequences for differences between the white kōkako (2015 capture), the before sample (2013 capture) and a range of kōkako from other populations. To do so, the MC1R gene will be amplified using the primers MSHR9 and MSHR72 (Mundy et al. 2004). Sequences will be compared to look for mutations that may be associated with a change in the function of MC1R, and subsequent amino acid changes.

4.2 Methods

4.2.1 Sample collection

A healthy adult kōkako (band number E212419) was captured on Hauturu in June 2013 during sampling efforts for genetic analysis (Chapter 3; Appendix 2). Blood samples were taken and stored in Queens lysis buffer. This individual exhibited no external symptoms of poor condition (see Appendix 2 for measurements) and was a member of a territorial pair (I. Flux, pers. comm.). This individual was recaptured in August 2015, and had undergone several changes to the plumage, bill and claws as seen in Figures 4.3, 4.4 and 4.5. Areas of the bill and legs were pale in colour, and many of the claws were slender and also pale in colour (Figure 4.5). Standard body measurements were taken on recapture, with no significant changes in body condition. Blood samples were also taken upon recapture and stored in Queens lysis buffer.

Figure 4.3. Image showing loss of pigmentation on all areas of adult kōkako; head, body, tail and wing feathers. Source Ian Flux.

Figure 4.4. Image showing loss of pigment to scales on leg, and loss of pigment and slender claws. Source Ian Flux.

Figure 4.5. Image showing beak abnormalities and loss of black mask. Source Ian Flux.

4.2.2 Beak and feather disease detection

Blood samples collected from Hauturu (n=29) to investigate the level of genetic diversity (see Chapter 3) were included in this study to screen the whole population for PBFD. Genomic DNA was extracted from whole blood (stored in Queens lysis buffer) using a standard 5% Chelex protocol (Walsh et al. 1991). A feather sample from a Major Mitchell Cockatoo (*Lophochroa leadbeateri*) was also extracted using a modified 5% Chelex protocol to be used as a positive control (J. Kenny, pers. comm.).

4.2.3 PCR screening

Molecular screening for PBFD followed the methodology as described in Amery-Gale et al. (2017). Polymerase chain reaction (PCR) is the most commonly used screening method for detecting PBFD, with 49% of studies between 1984 and 2015 utilising this molecular technique (Fogell et al. 2016). Due to the structure of the PBFD virus (Figure 4.2) and conserved regions between species, PCR has been successfully utilised to identify cases of PBFD in a wide range of both psittacine and non-psittacine species of bird (Khalesi et al. 2005; Amery-Gale et al. 2017). PCR screening is both a fast and reliable method that successfully detects the virus across a number of samples, when compared to a technique such as Haemagglutination Assays (Khalesi et al. 2005). An oligonucleotide primer pair that

target the capsid protein encoding region (ORF C1) of the circovirus genome were used (Table 4.1), a region approximately 495 bp in length (Hulbert et al. 2015; Amery-Gale et al. 2017). These primers were adapted from Ogawa, Yamaguchi and Fukushi (2005) and have previously been used to successfully amplify PBFD in a number of non-psittacine species (Amery-Gale et al. 2017).

Table 4.1. Primer sequences targeting the Capsid protein-encoding region (ORF C1) of the PBFD genome.

Direction	Target region	Primer sequence	Reference
Forward: PPBFD-F	Capsid protein (*Cap*)	5'-GGGTCCTCCTTGTAGTGGGATC-3'	(Amery-Gale et al. 2017; Hulbert et al. 2015)
Reverse: PPBFD-R	Capsid protein (*Cap*)	5'-CAGACGCCGTTTCACAACCAATAG-3'	(Amery-Gale et al. 2017; Hulbert et al. 2015)

The 25 µl reaction included 1 µl (5–50 ng) of extracted DNA, 0.2 µl (0.5 U) BioTaq polymerase (Bioline Ltd), 0.63 µl (8 mM) each dNTP, 1.25 µl (50 mM) $MgCl_2$, 0.25 µl of 1x Taq buffer, 5 µl (500 nM) each primer (Sigma-Aldrich) and PCR grade water (Sigma Aldrich). Each PCR setup included both a negative (PCR grade water) and positive control (Major Mitchell Cockatoo). The PCR thermal profile was as follows; Initial denaturation at 94 °C for 3 minutes, 40 cycles of 94 °C for 20 seconds, 63 °C for 20 seconds, 72 °C for 30 seconds, and a final elongation step at 72°C for three minutes (Amery-Gale et al. 2017). PCR products were visualised by transillumination following gel electrophoresis (1% agarose 1 x TAE gel containing SYBR safe DNA gel stain; Invitrogen). A 1000 bp ladder DNA size marker was used to estimate amplicon size and positive results were determined visually with a band at 495 bp. All samples were repeat amplified once.

4.2.4 MC1R gene amplification

The MC1R gene was amplified within eight different kōkako (Table 4.2). The two samples collected from the aberrant kōkako (E212419) were included to provide a before and after comparison of the aberrant phenotype. Samples were randomly selected from populations representing the greatest geographical separation, to capture diversity at this gene. Samples

were therefore collected from six different locations between 1997 and 2016 (Table 4.2). Genomic DNA was extracted from whole blood (stored in Queens lysis buffer) using a standard 5% Chelex protocol (Walsh et al. 1991). Sequencing difficulties reduced the sample size from 33 to 8 individuals (Table 4.2).

Table 4.2. Details of the nine kōkako samples the MC1R gene was amplified within.

Location	Sample (band) number	Sample type	Date collected
Hauturu	E212419	Blood	25.06.13
Hauturu	E212419 (recapture)*	Blood	10.08.15
Kapiti Island	E113824	Blood	04.05.00
Mangatutu	E212800	Blood	19.09.15
Mangatutu	E232323	Blood	16.04.16
Mapara	E177065	Blood	02.11.97
Mapara	NA	Blood	12.04.98
Mataraua	E189953	Blood	15.12.97
Waipapa	E196124	Blood	01.09.98

* *Recapture is individual with aberrant plumage.*

An 817 bp segment of the 945 bp avian MC1R sequence was amplified using primers MSHR72 and MSHR9 (Table 4.3). This region contains all the known functional sites of the MC1R and amino acid positions that been previously correlated to white plumage. These primers exclude the 3' and 5' coding regions of MC1R (Mundy et al. 2004).

Table 4.3. Primer details for amplification of 817 bp targeted region of MC1R.

Primer	Primer sequence	Reference
Forward: MSHR72	5'-ATGCCAGTGAGGGCAACCA-3'	(Mundy et al. 2004)
Reverse: MSHR9	5'-CTGGCTCCGGAAGGCATAGAT-3'	(Mundy et al. 2004)

Polymerase chain reaction (PCR) was used to amplify the region of interest in an 11 µl volume including 1 µl (5–50 ng) of extracted DNA template, 1 µl of 1x Taq buffer, 0.3 µl (50 mM) $MgCl_2$, 0.5 µl (500 nM) of each primer (MSHR9 & MSHR72), 0.25 µl (8 mM) each DNTP, 0.1 µl of (0.5 U) BioTaq Polymerase (Applied Biosystems) and PCR grade water (Sigma Aldrich). Reaction conditions followed a standard touchdown PCR as follows; Initial denaturation at 94 °C for 5 minutes, ten cycles of 94 °C (touchdown 1 °C per cycle) for 30 seconds (denaturation), 62 °C for 30 seconds (annealing) and 72 °C for one minute (extension). Then, a further 30 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds and 72 °C for one minute. PCR products were visualised by transillumination following gel electrophoresis (1% agarose 1 x TAE gel containing SYBR safe DNA gel stain; Invitrogen). A 1000 bp ladder DNA size marker was used to estimate amplicon size.

After a number of failed reactions, an alternative PCR protocol was used. This included a 10 µl volume including 1 µl (15 ng) of extracted DNA template, 5 µl of 2 x MyFi™ DNA Polymerase (BioLine), 0.2 µl (500 nM) of each primer (MSHR9 & MSHR72) and PCR grade water (Sigma Aldrich). Reaction conditions were the same as above.

PCR products were purified on a clean-up plate (PALL AcroPrep) and sequenced in both directions on an ABI 3730xl Genetic Analyser (Genetic Analysis Services at Otago).

4.2.5 Sequence alignment

Forward and reverse sequences from each individual were trimmed and aligned using de-novo assembly with the highest sensitivity in Geneious R9 v.9.1.8 (Kearse et al. 2012). Consensus sequences for all individuals were extracted. Dobson et al. (2012) collated avian MC1R sequences with melanic colour morphs and compared SNPs associated with plumage morphs to their species of interest (all eight subspecies of Australian magpie; *Cracticus tibicen*). Following this methodology, I used the subset of avian MC1R sequences that included a white colour morph (with available accession numbers) and updated this with more recent findings (Table 4.4). All nine kōkako sequences were aligned to a chicken sequence (*Gallus gallus;* Kerje et al. 2003) using MUSCLE alignment with 8 iterations in Geneious R9 v.9.1.8 (Kearse et al. 2012). This allowed me to confirm the amino acid translation and visually search for SNPs that have been previously correlated to colour differences (Table 4.4).

Table 4.4. Comparison of polymorphic MC1R amino acid sites (numbered) across bird species with a white colour morph. Gene numbered after *Gallus gallus*, with asterisks indicating consensus with the top row. Modified table from Dobson et al. (2012).

Common name	Species	Plumage form	Accession no.	85	92	119	207	230
Chicken	*Gallus gallus*	Red Junglefowl	AY220303	Val	Glu	Asp	His	Arg
		White Leghorn	AY220304	**	Lys	**	**	**
Lesser snow geese	*Anser c. caerulescens*	Blue phenotype	AY521183	Met	**	**	**	**
		White phenotype	AY521184	**	**	**	**	**
Arctic skua	*Stercoraius parasiticus*	Dark phenotype	AY521215	**	**	**	**	His
		Light phenotype	AY521217	**	**	**	**	**

Amino acid changes at position 119 and 207 are correlated to colour changes in bird species without white morphs (Dobson et al. 2012).

4.3 Results

4.3.1 Psittacine beak and feather disease virus

No evidence for psittacine beak and feather disease virus was detected using the PCR screening method (Amery-Gale et al. 2017). Initial screening that included four normal samples from Hauturu, a before and after sample of the aberrant kōkako, and a positive control (Major Mitchell Cockatoo) only amplified the targeted region (ORF C1) in the Major Mitchell Cockatoo (Figure 4.6). There was no amplification of the capsid protein-encoding region in the original nor the recapture sample of the aberrant kōkako from Hauturu (Ko1, Ko2; Figure 4.6). An additional repeat amplification returned identical results.

Further screening resulted in no amplification of the ORF C1 across all Hauturu individuals (n=29; Figure 4.7). Repeat amplification also returned identical results. There is therefore no molecular evidence for PBFD in kōkako, that were screened in this study, on Hauturu.

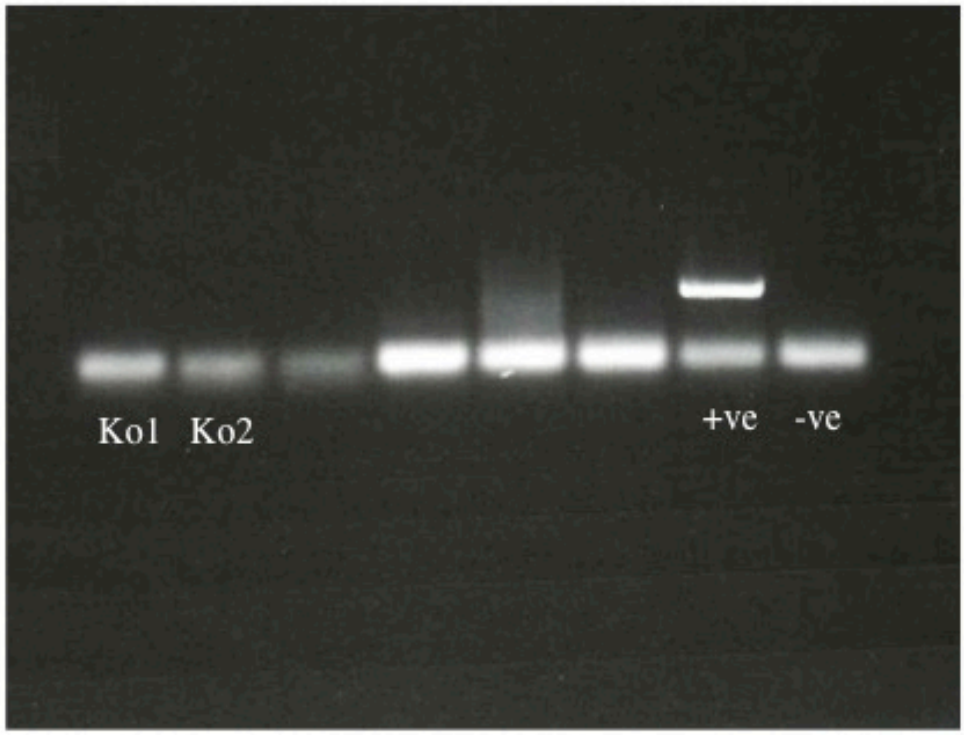


Figure 4.6. PCR screening in six kōkako from Hauturu, for 495 bp of the Capsid protein-encoding region (ORF C1) of the PBFD genome. Ko1 is the pre-leucistic kōkako, Ko2 is the leucistic kōkako, and the positive control is the Major Mitchell Cockatoo.

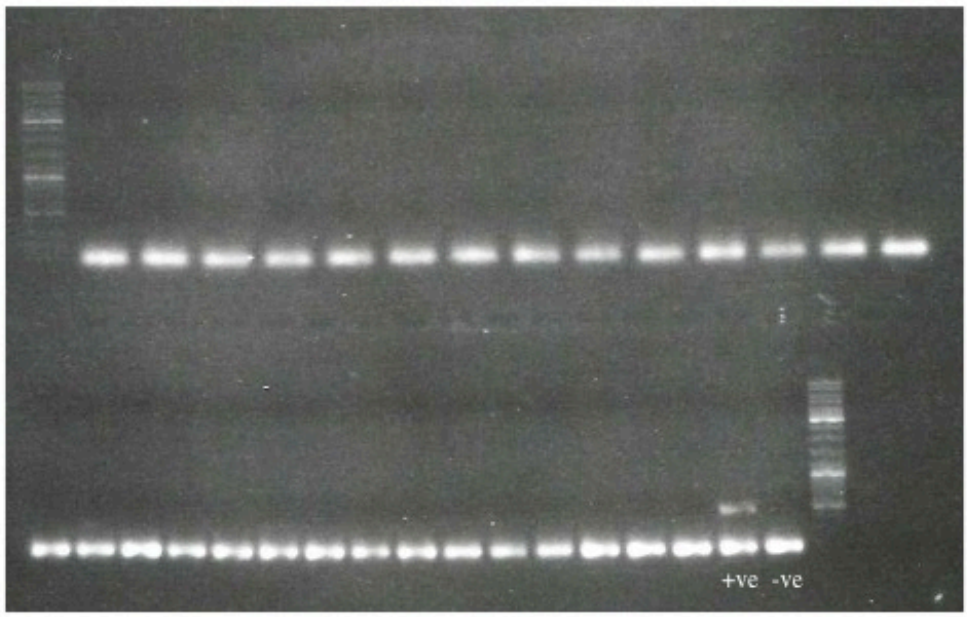


Figure 4.7. PCR screening in all 29 Hauturu individuals for 495 bp of the Capsid protein-encoding region (ORF C1) of the PBFD genome. Positive control is the Major Mitchell Cockatoo.

4.3.2 MC1R gene

The coding region of the kōkako MC1R consisted of 837 nucleotides that encoded a receptor of 283 amino acids. The amplified region (837 nucleotides) represented the majority of the MC1R gene, which is c.954 bp in vertebrates (Wlasiuk & Nachman 2007). The kōkako sequences shared between 89–90% identity to the *Gallus gallus* reference sequence, and represented 279 out of the 315 amino acids (positions 18 to 297). Sequence differences meant that 26 different amino acids were coded for in kōkako MC1R when compared to *Gallus gallus*.

There were no sequence variations between the before and after samples of the aberrant kōkako. Across all nine kōkako sequences three polymorphic sites were identified in three individuals with normal plumage (Table 4.5). These substitutions were synonymous, therefore did not result in an amino acid change. There were no further differences between any of the nine kōkako sequences.

Table 4.5. Nucleotide positions of the synonymous point substitutions identified in three individual kōkako. Positions are numbered in reference to *Gallus gallus* (Kerje et al. 2003).

Individual	Nucleotide site	Change	Amino Acid
Ko29	165	C → G	Leucine
Ko73	525	C → T	Valine
Ko93	576	C → T	Glycine

Amino acid sequences that are known to be associated with plumage differences in other avian species (Table 4.4) were searched across the kōkako individuals. Across all five of these amino acid positions, and all nine individuals, kōkako were identical (Table 4.6). The only amino acid difference between the *Gallus gallus* and kōkako (at the known variable regions) was at site 92, where all kōkako sequences coded for Arginine as opposed to Glutamic acid (Table 4.6).

Table 4.6. Comparison of polymorphic MC1R amino acid sites (numbered) between kōkako and the reference sequence Gallus gallus, with consensus indicated by asterisks. Amino acids numbered after Gallus gallus.

Common name	Species	Plumage form	Accession no.	85	92	119	207	230
Chicken	*Gallus gallus*	Red Junglefowl	AY220303	Val	Glu	Asp	His	Arg
Kōkako	*Callaeas wilsoni*	Dark kōkako	NA	**	Arg	**	**	**
		Aberrant kōkako	NA	**	Arg	**	**	**

4.4 Discussion

In 2013, a healthy kōkako was captured for genetic sampling to assess the level of diversity of kōkako on Hauturu (Chapter 3). Upon recapture, the kōkako presented with white pigmentation across all areas of the body including the plumage, bill, claws and facial mask. The aim of this study was to use genetic techniques to explore explanations for the dramatic phenotypic changes. I found no evidence for psittacine beak and feather disease virus (even when screened across samples drawn from the whole Hauturu population), despite the virus being present on the island (Ortiz-Catedral et al. 2009). Given this, the aberrant plumage is not an instance of the PBFD virus undergoing a host switching event, for infection within kōkako. I also failed to find any evidence to suggest that mutations in the melanocortin-1 receptor gene have led to the observed phenotype.

4.4.1 No evidence for psittacine beak and feather disease virus

PBFD is a prevalent, widespread and debilitating disease that is recognised as a significant conservation threat for avian populations worldwide (Raidal & Peters 2018). PBFD is genetically diverse, prone to mutation (Julian et al. 2013; Sarker et al. 2014) and is capable of host-switching and creating infection in non-psittacine species (Amery-Gale et al. 2017; Circella et al. 2014; Sarker et al. 2015, 2016). As such, non-psittacine birds in Australia (Amery-Gale et al. 2017; Sarker et al. 2015, 2016) and Italy (Circella et al. 2014) have recently tested positive for PBFD, often without showing clinical symptoms (Amery-Gale et al. 2017). There is yet to be a published incidence of a spill-over infection into any non-psittacine species in New Zealand (see Appendices 5 & 6). My research also supports this trend, failing to find any molecular evidence for PBFD in kōkako on Hauturu. The observed

white phenotype is therefore not a PBFD viral symptom, nor evidence for a host-switching event. This is a finding that we can be confident in as this PCR protocol has successfully amplified the 495 bp ORF C1 region of the virus in both psittacine and non-psittacine species previously (Hulbert et al. 2015; Amery-Gale et al. 2017). The capsid protein (ORF C1) is conserved between viral variants (Ypelaar et al. 1999; Fogell et al. 2016), therefore would amplify even if the virus had mutated for infection in kōkako. Moreover, PBFD DNA was successfully amplified in the positive control (Figures 4.6 & 4.7).

It is important to note that this finding does not suggest that kōkako are incapable of developing PBFD, just on the strength of this evidence, it is apparent that PBFD was not present in kōkako as of 2015. PBFD is present on Hauturu, first detected in red-crowned parakeets in 2008 (Ortiz-Catedral et al. 2009), with prevalence at c.10% of the red-crowned parakeet population in 2012 (Massaro et al. 2012). PBFD is excreted via faeces and feather dust and has a high environmental persistence (Todd 2000). Phylogenetic analysis of PBFD genomes suggest there is a level of ongoing viral flow between island populations (including Hauturu) of red-crowned parakeets but that the viral prevalence is low (Jackson et al. 2015). There is a possibility that kōkako may become infected in the future, especially if the population of red-crowned parakeets and kōkako continue to grow, as with increased population density there is also an increased likelihood of disease transmission (Hess 1996). However, PBFD is yet to be detected in any species other than red-crowned parakeet on Hauturu (Massaro et al. 2012), and it could be argued that the likelihood it will spread to other psittacid species (e.g. Kākāpō, *Strigops habroptilus*; kea, *Nestor notabilis;* or other species of parakeet) is greater than to a non-psittacine species such as kōkako. This finding confirms that a translocation of kōkako from Hauturu is unlikely to facilitate the spread of (non-psittacine viral variant) PBFD to naïve populations. Ongoing monitoring and disease screening for PBFD outbreak should be undertaken in the future, in both psittacine and non-psittacine species (Knafler et al. 2016).

4.4.2 MC1R and the aberrant kōkako

MC1R presented as an ideal candidate gene to study due to the pleiotropic association between physiological functions and melanin expression. Variation in the MC1R gene has been linked to plumage differences in a number of bird species (Bourgeois et al. 2012; Gangoso et al. 2011; Mundy, 2005; Roulin & Ducrest, 2013; Theron et al. 2001; Zhang et al.

2013), many of which include white colour morphs (Baião et al. 2007; Johnson et al. 2012; Zhan et al. 2012; Guernsey et al. 2013). Despite this clear association, the observed white aberrant plumage and body changes in the Hauturu kōkako do not appear to be related to variation in this gene. No differences were found between the before and after phenotypic samples, nor were there any differences between the aberrant kōkako and any of the other sampled birds from across the North Island. The only nucleotide differences were in three of the normal coloured individuals, but did not result in any amino acid changes.

As I did not obtain sequence for the amino acid codons at either end of the reference sequence, there is a possibility that functional mutations may have occurred in these un-sequenced regions. This appears to be unlikely, however, as the 837 bp kōkako region included all amino acid sites that have been previously associated plumage variation in birds (Table 4.4). Moreover, the amplified kōkako region contained all of the transmembrane regions of the gene (Mundy, 2005), which is where variation is usually found (Roulin & Ducrest 2013). As such, all known variable sites were carefully examined in kōkako, only one of which was found to differ in regard to the reference sequence (Arginine, amino acid position 92). Colour-related mutations such as the Val85Met in lesser snow geese (Mundy et al. 2004) and red footed boobies (Baião et al. 2007) or Arg230His reported in the Arctic skua (Mundy et al. 2004) were not observed in this study.

It is interesting that all kōkako sequences at amino acid position 92 coded for Arginine as opposed to Glutamine (Table 4.6). This is a variable site in other species, often correlated to plumage differences. This site is within the second transmembrane region of the gene (Mundy, 2005), which has been previously identified as an important region for MC1R function (Roulin & Ducrest 2013). Glu92Lys is the most common variant in birds (Theron et al. 2001; Kerje et al. 2003; Nadeau et al. 2006) and has also been identified in red lemurs (*Varecia rubra:* Mundy & Kelly 2003). In the avian examples, the amino acid substitution is associated with increased MC1R activation and therefore produces a darker phenotype. Benned-Jensen, Mokrosinski and Rosenkilde (2011) characterised cellular expression and activity of this substitution in mice, and found that cAMP accumulation was increased with a Glu→Lys substitution. Intracellular cAMP production induces the transcription factor Mitf which then increases the activity of eumelanin enzymes and proteins such as Tyr and Tyrp1 (Walker & Gunn 2010). Increased cAMP activity can therefore lead to increased eumelanin

production and greater eumelanin deposition (Mundy 2005; Roulin & Ducrest 2013). Although speculative, there may be a relationship between the Arginine 92 position and dark colouration of kōkako, an observation that could be explored with further research.

This is not the first study to fail to provide evidence for an association between phenotype and MC1R. Researchers could not associate colour differences with MC1R variation in a number of bird species such as Australian magpies (*Cracticus tibicen;* Dobson, Schmidt & Hughes 2012) carrion and hooded crows (*Corvus corone;* Haas et al. 2009), old world leaf warblers (*Phylloscopus collybita;* MacDougall-Shackleton et al. 2003), blue-crowned manakins (*Lepidothrix caronata;* Cheviron, Hackett & Brumfield 2006) or Reunion grey white eyes (*Zosterops borbonicus:* Bourgeois et al. 2012). The mounting number of studies that have failed to find an association suggest that the MC1R gene is not always involved in melanin-based changes. Indeed, pigment deposition is a complex process that involves a large number of genes and a number of different developmental mechanisms (Roulin & Ducrest 2013). More recently, a number of different candidate genes have been studied in relation to colour variation that could instead be related to the observed aberrant phenotype (see below).

4.4.3 Alternative explanations and future research

In terms of future research, it would be valuable to find the same aberrant kōkako and assess how white it is now, three years later. There are a number of different possible phenotypic outcomes, which would help direct future research efforts. This would allow us to determine whether or not the colouring is a gradual process, in which the feathers are being replaced by white feathers after moulting. If so, the bird may now be completely white. Alternatively, the bird may have the same mottled pattern as seen in 2015 (Figure 4.3). This would be an important distinction to make, in terms of the genes that should be examined in future research.

4.4.3.1 Candidate genes

Plumage variation has been linked to a number of alternative genes in the melanocortin pathway. Some authors argue that MC1R variation is related more to whole body phenotypic changes, whereas alternative genes can explain more discrete plumage patterning (MacDougall-Shackleton et al. 2003; Haas et al. 2009). As discussed, no relationship between MC1R and plumage morphs were found in carrion and hooded crows (Haas et al.

2009), which the authors attributed to more fine-scale plumage differences. More recently, carrion and hooded crow plumage differences now been attributed to alternative genes such as MITF, NDP and RAGSGRF1 (Poelstra et al. 2015). Similarly, phenotypic differences in the Tawny owl are unrelated to the MC1R gene, but instead attributed to pro-opiomelanocortin (POMC) and Agouti (ASIP) genes (Emaresi et al. 2013). Both POMC and ASIP are genes that interact with melanocortin receptors, and regulate the function of MC1R (Horrell, Boulanger & D'Orazio 2016). These genes have been linked to plumage colour in a number of different species (Hiragaki et al. 2008; Roulin et al. 2011b; Zhang et al. 2013) and would be valuable to explore in kōkako. Another gene that would be valuable to examine is the endothelin receptor B-2 (EDNRB2) gene. Abnormalities in this gene have been linked to white spotted phenotypes, in which pigment synthesis is abnormal and results in white feathers and pink skin (Kinoshita et al. 2014; Li et al. 2015; Miwa et al. 2007; Wu et al. 2017).

A similar candidate gene approach (as in this study) could be taken, sequencing some of these alternative melanin related genes in the aberrant and a number of normal coloured birds. To do so, future studies could follow the methodology of Bourgeois et al. (2016), who looked at seven candidate genes (ASIP, Corin, DCT, POMC, SLC24A5, TYR and TYRP1) to explain phenotypic variation in Reunion grey white-eyes, as previous research found no association with MC1R (Bourgeois et al. 2012). Alternatively, future research could use a full genome approach. Genomics is changing the way that researchers are able to compare and analyse gene sequences. Whole genome sequencing has allowed researchers to explore phenotypic differences across a large number of melanogenesis related genes, relatively easily (Poelstra et al. 2015; Yang et al. 2017; San-Jose & Roulin 2017). The aforementioned carrion crow study used whole genome sequencing and an extensive candidate gene search (428 melanin-related genes) to find the genes attributed to colour differences (Poelstra et al. 2015). An alternative approach would be to use genome-wide association analysis to identify genes associated with eumelanin variation, as has been used in chickens (Yang et al. 2017). This would be an alternative, less labour intensive (than sequencing seven or more genes) method of screening a large number of genes and identifying variations.

4.4.3.2 Epigenetic mechanisms

Epigenetics is the study of changes in gene function and expression that are not explained by mutations in a DNA sequence (Bird 2007; Kilvitis et al. 2014). As such, epigenetic mechanisms can activate, suppress or reduce the function of a particular set of genes (Bossdorf et al. 2007; Bird 2007; Kilvitis et al. 2014). As predicted, the phenotypic changes might still be related to the MC1R gene, but be a result of epigenetic processes instead of a mutational difference as examined in this study. DNA methylation of cytosines is the most well-studied epigenetic mechanism (Schrey et al. 2013), that has been found to directly influence phenotype (Bird 2007; Bock 2012). Moreover, DNA methylation can be influenced directly by environmental factors such as stress (Angers et al. 2010). This might explain the lack of pigmentation, as hypo-methylation of the MC1R gene could suppress the melanocortin receptors and reduce melanin deposition (Bossdorf et al. 2007; Bird 2007; Kilvitis et al. 2014). Examining epigenetic mechanisms such as DNA methylation would be a valuable avenue for future research.

4.4.4 Conclusions and management implications

In this study, I utilised genetic techniques in an attempt to explain why a kōkako on Hauturu has developed aberrant phenotypic features. As such, neither of the two explanations I explored could elucidate why the individual has changed in this way. Although I have presented two negative findings, they are still valuable contributions to the wider literature. Firstly, I can confirm that the phenotypic changes are not a symptom of psittacine beak and feather disease virus. This means that, despite a number of viral host switching events into non-psittacine species occurring recently in Australia (Amery-Gale et al. 2017), and Italy (Circella et al. 2014; Appendix 6), this is yet to be reported in New Zealand. In terms of kōkako conservation management this has a number of positive implications. Proposed kōkako translocations from Hauturu are unlikely to facilitate PBFD spread to immunologically naïve areas. Furthermore, as Hauturu is the largest and most productive kōkako population, the findings of this study confirm that (as of 2015) PBFD is not a threat to the population persistence of such a valuable site.

This study is not the first to find a lack of association between the MC1R gene and the aberrant phenotypic kōkako features. An increasing number of studies have failed to find an association between MC1R and plumage colour, many of which have instead found that

answers lie within alternative candidate genes. As such, this gives rise to a wealth of future research, which may benefit from developing genomic based technologies. It would be valuable to determine what the aberrant kōkako looks like now, as this could help direct research efforts and narrow down potential candidate genes. Regardless, this study adds to the body of research that suggests melanin deposition and plumage colouration is a complex process that is not always related to sequence differences in the melanocortin-1 receptor gene.

Chapter Five:
General Discussion

5.1 Summary

Assessing the suitability of Hauturu as a future source population for kōkako translocations was the focus of this thesis and is a primary concern for the Kōkako Specialist Group (KSG). As an introduced population established from as few as nine breeding individuals, there were reservations about the level of genetic diversity, the relatedness of individuals and therefore the usefulness of Hauturu as a source of kōkako for future translocations. Also, the discovery of a kōkako on Hauturu with aberrant plumage and features similar to Psittacine Beak and Feather Disease (PBFD) raised concerns that the Hauturu population might be infected with the disease. Furthermore, managers were concerned that the combination of PBFD and potential low genetic variation might make the kōkako on Hauturu vulnerable to a viral disease outbreak and population crash. The scope of this thesis therefore covered two important genetic considerations (genetic diversity and disease) that contribute to the overall success of a translocation (see Chapter 1) and should be considered when selecting a source site (Armstrong & Seddon 2008; IUCN/SSC 2013; Carter et al. 2017; Dalziel et al. 2017). The findings of this research have addressed the aforementioned concerns, revealing that the genetic diversity of kōkako on Hauturu is comparable to relict populations (Chapter 3) and that there is no genetic evidence of PBFD in kōkako (Chapter 4).

Translocations involve the movement of a small number of individuals, where populations often experience a loss of genetic diversity, relative to the source population (Stockwell et al. 1996; Houlden et al. 1996; Maudet et al. 2002; Mock et al. 2004; Sigg 2006). Consequently, to maximise the long-term success of a translocation project, it is recommended that individuals are sourced from genetically diverse populations (Armstrong & Seddon 2008; Allendorf et al. 2012; IUCN/SSC 2013). Levels of genetic diversity, inbreeding and relatedness across six kōkako populations were examined to compare diversity estimates between kōkako on Hauturu (a potential source population) to relict, mainland sites (Chapter 3). I found that the level of diversity on Hauturu was comparable to mainland populations and that the level of genetic diversity across all kōkako populations was higher than typically observed for other threatened New Zealand avifauna (Chapter 3). As translocating individuals from multiple, genetically differentiated source populations can increase genetic diversity (Madsen et al. 1999; Maudet et al. 2002; Dresser et al. 2017; Proft et al. 2018) and promote adaptive potential of the new population (Broadhurst et al. 2008; Sgrò et al. 2011; Proft et al. 2018), the genetic population structure of kōkako was also

examined. I found that there was a significant population structure (three distinct clusters), with Hauturu and Mapara identified as genetically distinct populations. Together, these findings provide genetic evidence to support the use of Hauturu as a source for future translocations (Chapter 3). I also discussed that the high level of genetic diversity across all kōkako populations may be explained by the possibility of historically high levels of genetic diversity, the existence of multiple refugia, and dispersal between contemporary populations.

Disease is a significant conservation concern (Scott 1988; Daszak et al. 2000) that can lead to the failure of a translocation (Aiello et al. 2014; Rideout et al. 2017). When planning translocations, it is important to understand the disease status of a source to prevent the spread into immunologically naïve populations (Cunningham 1996; Parker et al. 2006; Kock et al. 2010; Sainsbury & Vaughan-Higgins 2012). Two molecular explanations for the aberrant phenotype of a kōkako captured on Hauturu were explored in Chapter four, as the phenotypic abnormalities were reminiscent of PBFD (Doneley 2016; Pass & Perry 1984; Ritchie et al. 1989). I found that the white plumage and abnormal features were not a symptom of PBFD or evidence for a viral host-switching event. Feather abnormalities following a moult often arise when an individual is under physiological stress (Murphy & King 1992; Lindström et al. 1993; Strochlic & Romero 2008; DesRochers et al. 2009). As the melanocortin-1 receptor gene (MC1R) has a role in regulating melanin deposition (Hoekstra et al. 2006; Hofreiter & Schöneberg 2010; Mundy 2005; Mundy et al. 2004; Roulin & Ducrest 2013) and stress responses (Ducrest et al. 2008; Almasi et al. 2010; Roulin 2016), I examined MC1R sequences for mutational differences as an alternative molecular explanation. There were no mutational differences within the MC1R gene of the aberrant kōkako (before and after the phenotype change), or between the aberrant and a number of normal-phenotype kōkako. The findings of this research suggest that (as of 2015) PBFD is not a threat to the largest population of kōkako, however, further monitoring in the future is recommended, especially before any translocation.

5.2 Translocation management

5.2.1 Hauturu as a source population

Populations established by a small number of founding individuals typically have reduced genetic diversity (Nei et al. 1975; Allendorf 1986; Stockwell et al. 1996; Tarr et al. 1998; Keller & Waller 2002) and an increased likelihood of inbreeding (Frankel & Soulé 1981;

Lynch & Walsh 1998; Briskie & Mackintosh 2004; Frankham et al. 2017). Loss of diversity and increased inbreeding can reduce individual fitness and hence long-term population survival (Reed & Frankham 2003; Willi et al. 2006; Allendorf et al. 2012; Allendorf 2017; Frankham et al. 2017). To ensure short and long-term success of a translocation, the genetic diversity of the source population must therefore be considered (IUCN 1987; Armstrong & Seddon 2008; Allendorf et al. 2012). As Hauturu was founded from as few as nine birds (I. Flux, pers. comm.) I examined standard measures of genetic diversity, relatedness and inbreeding of kōkako to assess the utility as a source. Across all diversity measures (number of alleles per locus, observed and expected heterozygosity, number of private alleles and allelic richness), Hauturu was comparable to all five mainland sites (Chapter 3). Similarly, there was little difference between the level of inbreeding and relatedness across any of the six populations. Consequently, using Hauturu as a source population in future translocations presents no greater genetic risk to the long-term persistence of a translocated population than using any of the other relict populations. In addition, analysis of genetic differentiation showed that Hauturu is a genetically diverse and distinct population, which could be used in to maximise the success of future translocations (Broadhurst et al. 2008; Sgrò et al. 2011; Proft et al. 2018).

With PBFD present on Hauturu (Jackson et al. 2015; Ortiz-Catedral et al. 2009) and an aberrant kōkako displaying phenotypic characteristics similar to the symptoms of PBFD (Doneley 2016; Pass & Perry 1984; Ritchie et al. 1989), it was important to test for PBFD disease presence on Hauturu. PBFD primarily infects psttacine species (Khalesi et al. 2005; Raidal et al. 2015), but non-psittacine species can also harbour the disease (Bert et al. 2005; Sarker et al. 2015b; Amery-Gale et al. 2017). Approximately 25% of the total kōkako population are found on Hauturu (*North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017-2025. Draft* 2018). PBFD risk was not only a concern for proposed translocations, but also threatened the persistence of the largest population of kōkako (*North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017-2025. Draft* 2018). The molecular investigation of the aberrant kōkako detected no evidence of PBFD on Hauturu (Chapter 4). This study was the first to examine PBFD in a non-psittacine species in New Zealand and confirms that kōkako on Hauturu were not infected with the virus as of August 2015 (Chapter 3). As such, a translocation from Hauturu is unlikely to facilitate the spread of PBFD to immunologically

naïve kōkako populations or contribute to translocation failure in future (Cunningham 1996; Parker et al. 2006; Kock et al. 2010; Sainsbury & Vaughan-Higgins 2012).

A number of characteristics of Hauturu make it a good source population in future recovery efforts for kōkako. As noted above, Hauturu represents c.25% of the total protected population of kōkako (*North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017-2025. Draft* 2018). It is predator free (Bellingham et al. 2010), closed to the public, and has strict biosecurity measures for those that do visit the island (*Te Hauturu-o-Toi. Little Barrier Island Nature Reserve Management Plan* 2017). Birds on Hauturu are not only more abundant (*North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017-2025. Draft* 2018), but also easier to catch (J. McAulay, pers. comm.). Translocations are an expensive procedure, especially for kōkako, due to the difficulty, time taken to capture birds and required skill level of contractors to capture the birds (Collen et al. 2016). In conservation, scarce money and resources must be prioritised (Joseph et al. 2009), with research advocating for the use of cost utility and cost effectiveness analyses to make economically sound decisions (Fairburn et al. 2004). As birds on Hauturu are abundant and easy to catch, using Hauturu as a source has the potential to significantly reduce the cost of translocations for the KSG. Translocations are an integral part of kōkako recovery, therefore having more money and flexibility allows managers to direct resources towards populations that require attention. Hauturu presents as a new, unique, safe and productive population of birds and offers an opportunity to advance kōkako recovery.

5.2.2 Utilising genetic differentiation

The present genetic analysis has wider implications for translocation management of kōkako because three distinct genetic clusters were identified through the STRUCTURE and DAPC analyses (Chapter 3). There are three general aims of translocations; to augment, to establish or to reintroduce populations (IUCN 1987; Seddon 2010). Depending on the overall aim of a translocation project, the observed genetic differentiation can be utilised in different ways. For example, as previously discussed, using multiple sources to establish new populations has increased genetic diversity in simulations and in practice (Madsen et al. 1999; Maudet et al. 2002; Armstrong & Seddon 2008; Sgrò et al. 2011; Allendorf et al. 2012; Dresser et al. 2017). When the aim of a translocation is to establish a new population, sourcing individuals from the most differentiated populations will ensure that the diversity of the new population

is maximised. Alternatively, the observed differentiation can also be utilised when the goal of a translocation is to augment an existing populations (Hedrick & Fredrickson 2010; Frankham et al. 2017). Genetic rescue is an example of an augmentation, where individuals are translocated into an existing population with low genetic diversity to reduce inbreeding and increase genetic variation (Allendorf et al. 2012; Frankham 2015, 2016). In this instance, it would be advisable to select individuals that are the most differentiated to the inbred population, as a way of increasing the overall genetic diversity (Frankham et al. 2017).

5.2.3 Relict populations

Outside of Hauturu, the five relict populations examined in this study also present as good source populations (Chapter 3). None of the relict sites have significant heterozygosity deficits, or particularly elevated levels of relatedness. In line with the recommendations of Hudson et al. (2000), the findings of the present study suggest that there are no genetic barriers to translocating between any of the studied populations. However, the way in which we came to this conclusion is different. Hudson et al. (2000) suggested that there was no barrier to translocating between populations due to the absence of any population structure. This previous study, however, might have lacked the power to detect fine scale genetic structure due to the low number of microsatellite loci employed (Hubisz et al. 2009). I did find that there were three clear genetic clusters, with Mapara and Hauturu being genetically distinct from the other four populations (Chapter 3).

In the past, the discovery of significant population structure has lead researchers to recommend that isolated populations should be managed as independent units (see Frankham et al. 2017). For example, separate management was recommended for the endangered Eastern Bristlebird (*Dasyornis brachypterus*) in Australia when four geographically isolated populations were found to be genetically differentiated (Roberts et al. 2011). The level of differentiation however was small (Roberts et al. 2011) and the authors cautioned against mixing due to the concerns regarding outbreeding depression. Outbreeding depression is the reduction in reproductive fitness of offspring after the crossing of parents from two genetically divergent populations (Lynch 1991). Early conservationists stressed the risk of outbreeding depression, however, it is now considered to be a predictable and uncommon phenomenon (Weeks et al. 2011; Frankham et al. 2017). In geographically isolated but similar environments, it is suggested that it takes several thousands of generations before

outbreeding depression becomes likely (reviewed in Frankham et al. 2017). In the case of kōkako, there are unlikely to be sufficient environmental differences between populations (based on geographic location) to warrant concerns of outbreeding depression. Instead, the observed genetic differentiation should be utilised when establishing new sites to maximise the genetic diversity of the founding population (Broadhurst et al. 2008; Sgrò et al. 2011; Proft et al. 2018).

5.2.4 Translocations and stress

Despite the MC1R gene pleiotropically influencing both stress responses and melanic deposition (Luger et al. 2003; Catania et al. 2004; Ducrest et al. 2008; Loser et al. 2010; Lei et al. 2013), I found no sequence differences between normal and the aberrant kōkako (Chapter 4). This gene was selected as it was suspected that the phenotypic change might be related to stress. Although no difference was found, this does not rule out the possibility that stress was responsible for the aberrant plumage and features. As Hauturu is assumed to be at carrying capacity there might be density dependent factors acting on the population (J. Innes. pers. comm.). As feather replacement is an energetically expensive process (Murphy & King 1992; Lindström et al. 1993; Strochlic & Romero 2008; DesRochers et al. 2009), physiological stress in birds often presents as feather abnormalities (Murphy et al. 1989; Prum & Williamson 2001; Jovani & Blas 2004; Jovani & Rohwer 2017). If this is an indication that birds on Hauturu are stressed, this could mean that the population has reached capacity.

Translocations are a stressful procedure (Dickens et al. 2010; Kock et al. 2010; Sainsbury & Vaughan-Higgins 2012). Moreover, physiological stress can increase an individual's susceptibility to disease (Dickens et al. 2010; Aiello et al. 2014) or alter the relationship between host and disease (Dickens et al. 2010; Kock et al. 2010). As such, best practice emphasizes the importance of minimising the stress on translocated individuals, including guidelines for kōkako translocations (Collen et al. 2016). If the phenotypic changes are an indicator of physiological stress, managers might want to avoid translocating birds with this phenotype, as they may already be stressed and a translocation could further impact their fitness (Dickens et al. 2010). It is therefore important that future research is directed towards determining the underlying cause for the aberrant phenotype.

5.2.5 Additional genetic considerations

Here I used molecular techniques to assess the suitability of Hauturu as a source population. When managing translocations, genetic factors have a wider application than just evaluating the appropriateness of source populations. Once the source population(s) has been identified, managers must decide how many individuals will be taken from a source. A sufficient number of individuals must be used to establish a population, in order to capture a reasonable level of genetic diversity and prevent diversity loss (Tracy et al. 2011; Allendorf et al. 2012; Ottewell et al. 2014). Following this, it is also important that the birds become established in the new site (Armstrong & Seddon 2008; Richardson et al. 2010). If individuals do not breed or disperse outside of the new population they will not genetically contribute to the subsequent generation and their genetic variation will be lost. As kōkako are territorial, the size and capacity of the new site becomes particularly important for this, as it limits the number of birds that can be translocated. From a genetic perspective, I have emphasized the importance of mixing source populations, whereas in reality this can be complicated. Previous translocations have found that kōkako tend to form pair bonds with birds that have a similar local dialect (Brown et al. 2004; Rowe 2007; Bradley et al. 2013; Collen et al. 2016). Consequently, for kōkako, local dialect should be considered when mixing distinct source populations (Valderrama et al. 2013). As kōkako are monogamous (Flux et al. 2006), this means that the new genetic information will not become integrated until the following generation breed, when local dialect is no longer a barrier to forming pair bonds (Collen et al. 2016). Finally, supplementary top-up translocations can be used to introduce additional genetic variation after the initial population founding (Armstrong & Seddon 2008; Frankham et al. 2017). If, as discussed above, many of the founding birds do not breed, the founding population might be exposed to genetic risks associated with small populations such as genetic drift and inbreeding (Keller & Waller 2002; Frankham et al. 2017). To minimise these risks, several follow up translocations can reintroduce additional genetic diversity back into the founding population (Bouzat et al. 2009; Frankham 2016; Frankham et al. 2017).

There are also a number of non-genetic factors that must be considered when assessing source population suitability. Genetic diversity, population structure and disease cover only a fraction of the factors that must be considered to ensure translocation success (IUCN/SSC 2013). For example, local adaptation of individuals in the source location may prevent them from becoming successfully established in the new site (Seddon et al. 2007;

Weeks et al. 2011). As discussed above there is geographical variation in the dialect of kōkako, that has been found to influence mate selection in previous kōkako translocations (Collen et al. 2016). Translocation management must also consider the impact of harvesting on the source population (Seddon et al. 2007; Armstrong & Seddon 2008). This includes considering the impact on genetic diversity of the source, and therefore ensuring that populations are not harvested excessively (e.g. reducing source to low level). As such, although this research provides important information about the genetic suitability of source populations, other factors must be considered before translocating birds from a source (Armstrong & Seddon 2008; IUCN/SSC 2013). Genetics and disease are just some of many important components that come into consideration when undertaking the complex procedure that is a wildlife translocation.

5.3 Integrating genetic research and conservation management

Together, the findings of this research are a clear example of how genetics can be used to facilitate and improve conservation management. With an increased understanding of the genetic and disease status of populations, managers can use such information to improve the success of future translocations. Through my research, I found that kōkako populations had a high level of genetic diversity (Chapter 3). This is uncharacteristic of New Zealand avifauna (Ardern & Lambert 1997; Jamieson et al. 2006; Jamieson 2009). Undertaking similar genetic research on threatened species that have not been studied previously may therefore result in alternative management recommendations. Following the same methodology, researchers can identify populations that are inbred or lacking genetic diversity and would benefit from the addition of new genetic material (genetic rescue; Frankham 2015; Hedrick and Fredrickson 2010; Frankham 2016). Moreover, populations that lack genetic diversity are likely to be more susceptible to a disease outbreak (Seddon & Baverstock 2002; Hale & Briskie 2007; Sutton et al. 2011). Introducing small numbers of individuals has been found to restore the diversity in the Toll-like receptor regions (immunity genes) of inbred populations (Grueber et al. 2017). This again reinforces that an increased understanding of genetic diversity and disease risk has important practical applications that should be integrated into conservation management.

Although the importance of genetic research is well understood, it is often poorly integrated into conservation management (Moyle et al. 2003; Howes et al. 2009; Pierson et

al. 2016; Taylor et al. 2017b). Because of this, it has been suggested that geneticists should be more involved in recovery planning (Howes et al. 2009) and that genetics literature should be more accessible to conservation managers (Milner-Gulland et al. 2010; Sutherland et al. 2010). This thesis reflects this new approach to conservation management, as it arose from the KSG approaching the university with specific concerns and questions relevant to kōkako recovery. Moreover, the latest government policies for threatened species recovery in New Zealand now emphasize the need to include genetic consideration in recovery projects (*New Zealand Biodiversity Action Plan: 2016-2020* 2016; *New Zealand's Threatened Species Strategy: Draft for Consultation* 2017), which is clearly being integrated into kōkako recovery. If anything, this research highlights the importance of having well-funded and co-ordinated recovery groups such as the Kōkako Specialist Group. Unfortunately, many threatened species lack this form of organised recovery which might form a barrier to integrating similar genetic research into future recovery efforts.

5.4 Future of conservation genetics: Genomics

With advances in technology, whole genome sequencing and bioinformatic analysis is becoming increasingly accessible to researchers (Allendorf, Hohenlohe & Luikart 2010; Galla et al. 2016; Ouborg et al. 2010; Primmer 2009). As such, this is driving the field of conservation genetics, increasing the complexity of questions that can be answered and the speed of analyses and data collection (Primmer 2009; Ouborg et al. 2010; Allendorf et al. 2010; Galla et al. 2016). A whole kōkako genome sequence was used in this research to facilitate the development of a large number of kōkako-specific microsatellite primers (Chapter 2). As such, I was able to develop a sufficient number of markers to reliably estimate population structure and diversity (Chapter 3), a factor that has limited population genetic studies in the past (Wan et al. 2004; Hubisz et al. 2009). In terms of future research, genomic technology could also further the findings of Chapter four. As no link between the MC1R gene and phenotypic aberrations were found (Chapter 4), whole genome approaches could allow a much larger number of candidate genes to be screened (Poelstra et al. 2015; Bourgeois et al. 2016; Yang et al. 2017; San-Jose & Roulin 2017).

Recent research has identified that genomics has the potential to have a significant impact on the way that conservation management is undertaken in the future (He et al. 2016; Flanagan et al. 2017; Wellband & Heath 2017; Corlett 2017; Malone et al. 2018). Genomics

is a field that is still developing and has had limited practical application thus far. Despite this, it has been identified that genomic information will assist managers in areas such as minimizing adaptation to captivity, prioritising sites to protect under projected climate change or identifying hybridization (see Flanagan et al. 2017). Of particular relevance to this thesis is the role that genomics might play in selecting individuals and source populations for translocation (He et al. 2016; Flanagan et al. 2017; Malone et al. 2018). I have emphasized that source populations can be selected in ways that maximise the success of a translocation (Chapters 3 & 4). He, Johansson and Heath (2016) have a similar view, arguing that genomics and transcriptomics can be utilised to select individuals that are most likely to successfully adapt to a new environment. They suggest that individuals at a potential source population should be profiled to select those with the most functional genetic variation (and therefore adaptive potential) and tolerance to potential environmental stress (He et al. 2016). The authors' research follows a similar approach that I have taken throughout this thesis, but suggests that new techniques can further this research by applying an individual-based approach to source population selection. Once again, this is returning to the idea (as discussed above) that translocations are stressful, that new environments can be stressful, and that it is difficult to predict how individuals will respond. Together, this suggests that genomics will have an important role in future translocation projects and that it will complement research similar to mine by taking an individual based approach (He, Johannson & Heath 2016).

It seems likely that genomics will assist managers in the future to maximise the success of translocations (He et al. 2016; Flanagan et al. 2017; Wellband & Heath 2017; Corlett 2017; Malone et al. 2018). Although genomic technology is rapidly advancing, it is important for geneticists to maintain communication with conservation managers. Amid the advances in genomic technology, researchers have identified concerns that genomic research has the potential to widen the 'gap' between managers and researchers (Flanagan et al. 2017; Taylor, Dussex & van Heezik 2017b). Taylor, Dussex and van Heezik (2017) consulted with conservation practitioners, and found that 92% of those surveyed did not understand the difference between genetic research and genomics. As genomics becomes more widely used, and provides researchers with the ability to address more complex questions, there is potential for research to become more academic focused as opposed to driven by the needs of conservation managers. There is clearly a balance that needs to be established, in that

genetic research in the future meets the needs of both geneticists and that of managers (Flanagan et al. 2017; Taylor et al. 2017b).

5.5 Future directions

There are a number of important and interesting directions that future research could take. Firstly, it would be important to gain a better understanding as to why the aberrant kōkako has developed white features (Chapter 4). To do so, a genomics or transcriptomes based approach could be used to screen a greater number of candidate genes (Poelstra et al. 2015; San-Jose & Roulin 2017; Yang et al. 2017). Another direction that future research should take is to use ancient DNA or museum samples to compare historical or contemporary levels of genetic diversity (see Chapter 3). In doing so, this might explain why the current levels of genetic diversity across all populations are high, and allow researchers to test hypotheses regarding the historical level of diversity or existence in multiple refugia. Availability and quality of samples limited the number of populations that I was able to examine in this study. There are 24 kōkako populations at present, so it would be valuable to compare diversity and structure across the whole range in future. Finally, genomics presents as an exciting and important direction for the future of conservation research that kōkako recovery could benefit from.

References

Abdelkrim J, Robertson B, Stanton J-A, Gemmell N. 2009. Fast, cost-effective development of species-specific microsatellite markers by genomic sequencing. BioTechniques **46**:185–192.

Aiello CM, Nussear KE, Walde AD, Esque TC, Emblidge PG, Sah P, Bansal S, Hudson PJ. 2014. Disease dynamics during wildlife translocations: disruptions to the host population and potential consequences for transmission in desert tortoise contact networks. Animal Conservation **17**:27–39.

Alacs EA, Georges A, FitzSimmons NN, Robertson J. 2010. DNA detective: a review of molecular approaches to wildlife forensics. Forensic Science, Medicine, and Pathology **6**:180–194.

Allen TJ. 1986. Evaluation of movements, harvest rate, vulnerability and survival of translocated racoons in southern West Virginia. Transactions of the Northeast Section of the Wildlife Society **43**:64.

Allendorf FW. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology **5**:181–190.

Allendorf FW. 2017. Genetics and the conservation of natural populations: allozymes to genomes. Molecular Ecology **26**:420–430.

Allendorf FW, Aitken SN, Luikart GH. 2012. Conservation and the Genetics of Populations 2nd Edition. John Wiley & Sons Ltd, Hoboken, United States.

Allendorf FW, Hohenlohe PA, Luikart G. 2010. Genomics and the future of conservation genetics. Nature Reviews Genetics **11**:697–709.

Almasi B, Jenni L, Jenni-Eiermann S, Roulin A. 2010. Regulation of stress response is heritable and functionally linked to melanin-based coloration. Journal of Evolutionary Biology **23**:987–996.

Amery-Gale J, Marenda MS, Owens J, Eden PA, Browning GF, Devlin JM. 2017. A high prevalence of beak and feather disease virus in non-psittacine Australian birds. Journal of Medical Microbiology **66**:1005–1013.

Amos W, Wilmer JW, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T. 2001. The influence of parental relatedness on reproductive success. Proceedings of the Royal Society of London: Biological Sciences **268**:2021–2027.

Andersson L. 2003. Melanocortin receptor variants with phenotypic effects in horse, pig, and chicken. Annals of the New York Academy of Sciences **994**:313–318.

Angers B, Castonguay E, Massicotte R. 2010. Environmentally induced phenotypes and DNA methylation: how to deal with unpredictable conditions until the next generation and after. Molecular Ecology **19**:1283–1295.

Aparicio JM, Ortego J, Cordero PJ. 2006. What should we weigh to estimate heterozygosity, alleles or loci? Molecular Ecology **15**:4659–4665.

Ardern SL, Lambert DM. 1997. Is the black robin in genetic peril? Molecular Ecology **6**:21–28.

Armstong D, McLean I. 1995. New Zealand translocations: theory and practice. Pacific Conservation Biology **2**:39–54.

Armstrong DP, Seddon PJ. 2008. Directions in reintroduction biology. Trends in Ecology & Evolution **23**:20–25.

Atkinson IAE, Cameron EK. 1993. Human influence on the terrestrial biota and biotic communities of New Zealand. Trends in Ecology & Evolution **8**:447–451.

Baião PC, Schreiber E, Parker PG. 2007. The genetic basis of the plumage polymorphism in red-footed boobies (*Sula sula*): a melanocortin-1 receptor (MC1R) analysis. The Journal of Heredity **98**:287–292.

Baillie SM, Ritchie PA, Brunton DH, Voelker G. 2014. Population genetic connectivity of an endemic New Zealand passerine after large-scale local extirpations: a model of re-colonization potential. Ibis **156**:826–839.

Bassami MR, Berryman D, Wilcox GE, Raidal SR. 1998. Psittacine beak and feather disease virus nucleotide sequence analysis and its relationship to porcine circovirus, plant circoviruses, and chicken anaemia virus. Virology **249**:453–459.

Basse B, Flux I, Innes J. 2003. Recovery and maintenance of North Island kōkako (*Callaeas cinerea wilsoni*) populations through pulsed pest control. Biological Conservation **109**:259–270.

Batson WG, Gordon IJ, Fletcher DB, Manning AD. 2015. Review: Translocation tactics: a framework to support the IUCN guidelines for wildlife translocations and improve the quality of applied methods. Journal of Applied Ecology **52**:1598–1607.

Bellingham P, Towns D, Cameron E, J. Davis J, Wardle D, Wilmshurst J, Mulder C. 2010. New Zealand island restoration: seabirds, predators, and the importance of history. New Zealand Journal of Ecology **34**.

Benned-Jensen T, Mokrosinski J, Rosenkilde MM. 2011. The E92K melanocortin 1 receptor mutant induces cAMP production and arrestin recruitment but not ERK activity indicating biased constitutive signaling. PloS One **6**:e24644.

Bergl RA, Vigilant L. 2007. Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). Molecular Ecology **16**:501–516.

Bert E, Tomassone L, Peccati C, Navarrete MG, Sola SC. 2005. Detection of beak and feather disease virus (BFDV) and avian polyomavirus (APV) DNA in psittacine birds in Italy. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health **52**:64–68.

Best HA, Bellingham PJ. 1990. A detailed habitat study of North Island kōkako in Puketi Forest, Northland. Page Science and Research Internal Report No. 103. Department of Conservation, Wellington, New Zealand.

Bird A. 2007. Perceptions of epigenetics. Nature **447**:396–398.

Birdlife International. 2017. *Apteryx mantelli*. The IUCN Red List of Threatened Species 2017. Available from http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T45353580A119177586.en (accessed April 28, 2018).

Bock C. 2012. Analysing and interpreting DNA methylation data. Nature Reviews Genetics **13**:705–719.

Bodkin JL, Ballachey BE, Cronin MA, Scribner KT. 2001. Population demographics and genetic diversity in remnant and translocated populations of sea otters. Conservation Biology **13**:1378–1385.

Boessenkool S, Taylor SS, Tepolt CK, Komdeur J, Jamieson IG. 2006. Large mainland populations of South Island robins retain greater genetic diversity than offshore island refuges. Conservation Genetics **8**:705–714.

Bohonak AJ. 1999. Dispersal, gene flow, and population structure. The Quarterly Review of Biology **74**:21–45.

Bossdorf O, Richards CL, Pigliucci M. 2007. Epigenetics for ecologists. Ecology Letters **11**:106–115.

Bourgeois YXC, Bertrand JAM, Delahaie B, Cornuault J, Duval T, Milá B, Thébaud C. 2016. Candidate gene analysis suggests untapped genetic complexity in melanin-based pigmentation in birds. Journal of Heredity **107**:327–335.

Bourgeois YXC, Bertrand JAM, Thébaud C, Milá B. 2012. Investigating the role of the melanocortin-1 receptor gene in an extreme case of microgeographical variation in the pattern of melanin-based plumage pigmentation. PLOS ONE **7**:e50906.

Bouzat JL, Johnson JA, Toepfer JE, Simpson SA, Esker TL, Westemeier RL. 2009. Beyond the beneficial effects of translocations as an effective tool for the genetic restoration of isolated populations. Conservation Genetics **10**:191–201.

Bradley DW, Molles LE, Valderrama SV, King S, Waas JR. 2012. Factors affecting post-release dispersal, mortality, and territory settlement of endangered kōkako translocated from two distinct song neighborhoods. Biological Conservation **147**:79–86.

Bradley DW, Molles LE, Waas JR. 2013. Local–foreign dialect discrimination and responses to mixed-dialect duets in the North Island kōkako. Behavioral Ecology **24**:570–578.

Brekke P, Bennett PM, Santure AW, Ewen JG. 2011. High genetic diversity in the remnant island population of hihi and the genetic consequences of re-introduction. Molecular Ecology **20**:29–45.

Brekke P, Wang J, Bennett PM, Cassey P, Dawson DA, Horsburgh GJ, Ewen JG. 2012. Postcopulatory mechanisms of inbreeding avoidance in the island endemic hihi (*Notiomystis cincta*). Behavioral Ecology **23**:278–284.

Briskie JV, Mackintosh M. 2004. Hatching failure increases with severity of population bottlenecks in birds. Proceedings of the National Academy of Sciences of the United States of America **101**:558–561.

Broadhurst LM, Lowe A, Coates DJ, Cunningham SA, McDonald M, Vesk PA, Yates C. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. Evolutionary Applications **1**:587–597.

Brown KP, Empson R, Gorman N, Moorcroft G. 2004. North Island kōkako (*Callaeas cinerea wilsoni*) translocations and establishment on Kapiti Island, New Zealand. Department of Conservation, Wellington, New Zealand.

Burt AJ, Gane J, Olivier I, Calabrese L, Groene AD, Liebrick T, Marx D, Shah N. 2016. The history, status and trends of the endangered Seychelles Magpie (*Copsychus sechellarum*). Bird Conservation International **26**:505–523.

Campbell SP, Clark JA, Crampton LH, Guerry AD, Hatch LT, Hosseini PR, Lawler JJ, O'Connor RJ. 2002. An assessment of monitoring efforts in endangered species recovery plans. Ecological Applications **12**:674–681.

Carter I, Sainsbury AW, Walsh K, Matthew H, Curson J, Ewen JG. 2017. Health and disease in translocated wild animals. EcoHealth **14**:5–6.

Cassey P, Blackburn TM, Duncan RP, Lockwood JL. 2008. Lessons from introductions of exotic species as a possible information source for managing translocations of birds. Wildlife Research **35**:193–201.

Catania A, Gatti S, Colombo G, Lipton JM. 2004. Targeting melanocortin receptors as a novel strategy to control inflammation. Pharmacological Reviews **56**:1–29.

Ceballos G, Ehrlich PR. 2018. The misunderstood sixth mass extinction. Science **360**:1080–1081.

Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM, Palmer TM. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science Advances **1**:e1400253.

Champagnon J, Elmberg J, Guillemain M, Gauthier-Clerc M, Lebreton J-D. 2012. Conspecifics can be aliens too: A review of effects of restocking practices in vertebrates. Journal for Nature Conservation **20**:231–241.

Chapuis M, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution **24**:621–631.

Charlesworth B, Charlesworth D. 2017. Population genetics from 1966 to 2016. Heredity **118**:2–9.

Cheviron ZA, Hackett SJ, Brumfield RT. 2006. Sequence variation in the coding region of the melanocortin-1 receptor gene (MC1R) is not associated with plumage variation in the blue-crowned manakin (*Lepidothrix coronata*). Proceedings. Biological Sciences **273**:1613–1618.

Circella E, Legretto M, Pugliese N, Caroli A, Bozzo G, Accogli G, Lavazza A, Camarda A. 2014. Psittacine beak and feather disease–like illness in gouldian finches (*Chloebia gouldiae*). Avian Diseases **58**:482–487.

Clout MN, Hay JR. 1981. South Island kōkako (*Callaeas cinerea cinerea*) in nothofagus forest. Notornis **28**:256–259.

Clout MN, Saunders AJ. 1995. Conservation and ecological restoration in New Zealand. Pacific Conservation Biology **2**:91–98.

Collen R, Armstrong D, Cromarty P, Empson R, Jamieson I, McArthur N, Parker K, Parlato E, Powlesland R, Ward-Smith T. 2014a. Best practice techniques for the translocation of North Island robins (*Petroica longipes*), South Island robins (*P. australis australis*) and Stewart Island robins (*P. a. rakiura*). Department of Conservation, Wellington.

Collen R, Cash B, Adams L, Ortiz-Catedral L, Booth M, Gray R, Fastier D, Nakagawa K, Ward-Smith T. 2014b. Best practice techniques for the translocation of red-crowned parakeet (kākāriki, *Cyanoramphus novazelandiae novazelandiae*) and yellow-crowned parakeet (kākāriki, *Cyanoramphus auriceps*). Department of Conservation, Wellington.

Collen R, Flux I, Innes J, Thurley T, Wills S. 2016. Best practice techniques for the translocation of North Island kōkako (*Callaeas wilsoni*). Department of Conservation, Wellington, N.Z.

Collins CJ, Chilvers BL, Osborne A, Taylor M, Robertson BC. 2017. Unique and isolated: population structure has implications for management of the endangered New Zealand sea lion. Conservation Genetics:1–13.

Corlett RT. 2017. A Bigger Toolbox: Biotechnology in Biodiversity Conservation. Trends in biotechnology **35**:55–65.

Craig JL, Douglas ME. 1984. Bellbirds in Auckland and Northland. Notornis **31**:82–86.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution **15**:290–295.

Cromarty PL, Alderson SL. 2013. Translocation statistics (2002-2010), and the revised Department of Conservation translocation process. Notornis **60**:55–62.

Cunningham AA. 1996. Disease risks of wildlife translocations. Conservation Biology **10**:349–353.

Dalziel AE, Sainsbury AW, McInnes K, Jakob-Hoff R, Ewen JG. 2017. A comparison of disease risk analysis tools for conservation translocations. EcoHealth **14**:30–41.

Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science **287**:443–9.

Department of Conservation. 2011. Psittacine beak and feather disease. A threat to our native parrots. Publishing Team, Department of Conservation, Wellington, New Zealand.

Department of Conservation. 2018. National wildlife health database project. Available from https://www.doc.govt.nz/our-work/wildlife-health/national-wildlife-health-database-project/ (accessed April 26, 2018).

Department of Sustainability, Environment, Water, Population and Communities. 2012. Review of the threat abatement plan for psittacine beak and feather disease affecting endangered psittacine species (2005). Australian Government, Canberra.

DeSalle R, Amato G. 2004. The expansion of conservation genetics. Nature Reviews Genetics **5**:702–712.

DesRochers DW, Reed JM, Awerman J, Kluge JA, Wilkinson J, van Griethuijsen LI, Aman J, Romero LM. 2009. Exogenous and endogenous corticosterone alter feather quality. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **152**:46–52.

Dickens MJ, Delehanty DJ, Michael Romero L. 2010. Stress: An inevitable component of animal translocation. Biological Conservation **143**:1329–1341.

Do C, Waples RS, Peel D, Macbeth GM, Tillet BJ, Ovenden JR. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources **14**:209–214.

Dobson AE, Schmidt DJ, Hughes JM. 2012. Sequence variation in the melanocortin-1 receptor (MC1R) does not explain continent-wide plumage color differences in the Australian Magpie (*Cracticus tibicen*). The Journal of Heredity **103**:769–780.

Doneley B. 2016. Avian Medicine and Surgery in Practice: Companion and Aviary Birds, Second Edition. CRC Press, Oakville, Canada.

Double M, Murphy S. 2000. Genetic variation within and among populations of North Island kōkako. Page Science and Research Internal Report No. 176. Department of Conservation, Wellington, New Zealand.

Dresser CM, Ogle RM, Fitzpatrick BM. 2017. Genome scale assessment of a species translocation program. Conservation Genetics:1–9.

Ducrest A, Keller L, Roulin A. 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends in Ecology & Evolution **23**:502–510.

Earl DA, Vonholdt BM. 2012. STRUTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources **4**:359–361.

Emaresi G, Ducrest A, Bize P, Richter H, Simon C, Roulin A. 2013. Pleiotropy in the melanocortin system: expression levels of this system are associated with melanogenesis and pigmentation in the tawny owl (*Strix aluco*). Molecular Ecology **22**:4915–4930.

England PR, Osler GHR, Woodworth LM, Montgomery ME, Briscoe DA, Frankham R. 2003. Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. Conservation Genetics **4**:595–604.

Eriksson A, Manica A. 2011. Detecting and removing ascertainment bias in microsatellites from the HGDP-CEPH panel. G3: Genes, Genomes, Genetics **1**:479–488.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology **14**:2611–2620.

Evans SR, Sheldon BC. 2008. Interspecific patterns of genetic diversity in birds: correlations with extinction risk. Conservation Biology **22**:1016–1025.

Everts RE, Rothuizen J, van Oost BA. 2000. Identification of a premature stop codon in the melanocyte-stimulating hormone receptor gene (MC1R) in Labrador and Golden retrievers with yellow coat colour. Animal Genetics **31**:194–199.

Ewen JG, Armstrong DP, Empson R, Jack S, Makan T, McInnes K, Parker KA, Richardson K, Alley M. 2012. Parasite management in translocations: lessons from a threatened New Zealand bird. Oryx **46**:446–456.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources **10**:564–567.

Fahrig L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics **34**:487–515.

Fairburn GA, Hughey KFD, Cullen R. 2004. Cost effectiveness of endangered species management: the kōkako (*Callaeas cinerea*) in New Zealand. New Zealand Journal of Ecology **28**:83–91.

Faircloth BC. 2008. msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. Molecular Ecology Resources **8**:92–94.

Fèvre EM, Bronsvoort BM de C, Hamilton KA, Cleaveland S. 2006. Animal movements and the spread of infectious diseases. Trends in Microbiology **14**:125–131.

Fiedler PL, Jain SK, editors. 1992. Conservation biology: the theory and practice of nature conservation preservation and management. Chapman and Hall, New York, USA.

Fischer J, Lindenmayer DB. 2000. An assessment of the published results of animal relocations. Biological Conservation **96**:1–11.

Flanagan SP, Forester BR, Latch EK, Aitken SN, Hoban S. 2017. Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. Evolutionary Applications **0**:1–18.

Flux I, Bradfield P, Innes J. 2006. Breeding biology of North Island kōkako (*Callaeas cinerea wilsoni*) at Mapara Wildlife Management Reserve, King Country, New Zealand. Notornis **53**:199–207.

Flux I, Thurley T, McKenzie K, McAulay J. 2013. A kōkako (*Callaeas wilsoni*) population estimate for Little Barrier Island (Hauturu): a trial of a novel sub-sampling design. Unpublished Report.

Fogell DJ, Martin RO, Groombridge JJ. 2016. Beak and feather disease virus in wild and captive parrots: an analysis of geographic and taxonomic distribution and methodological trends. Archives of Virology **161**:2059–2074.

Frankel O, Soulé ME. 1981. Conservation and Evolution. Cambridge University Press, New York, USA.

Frankham R. 1995. Conservation Genetics. Annual Review of Genetics **29**:305–327.

Frankham R. 1996. Relationship of genetic variation to population size in wildlife. conservation biology **10**:1500–1508.

Frankham R. 1998. Inbreeding and extinction: island populations. Conservation Biology **12**:665–675.

Frankham R. 1999. Quantitative genetics in conservation biology. Genetical Research **74**:237–244.

Frankham R. 2003. Genetics and conservation biology. Comptes Rendus Biologies **326**, **Supplement 1**:22–29.

Frankham R. 2005. Genetics and extinction. Biological Conservation **126**:131–140.

Frankham R. 2010a. Where are we in conservation genetics and where do we need to go? Conservation Genetics **11**:661–663.

Frankham R. 2010b. Challenges and opportunities of genetic approaches to biological conservation. Biological Conservation **143**:1919–1927.

Frankham R. 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology **24**:2610–2618.

Frankham R. 2016. Genetic rescue benefits persist to at least the F3 generation, based on a meta-analysis. Biological Conservation **195**:33–36.

Frankham R, Ballou JD, Ralls K, Eldridge M, Dudash MR, Fenster CB, Lacy RC, Sunnucks P. 2017. Genetic management of fragmented animal and plant populations. Oxford University Press, Oxford, New York.

Frankham R, Briscoe DA, Ballou JD. 2002. Introduction to conservation genetics. Cambridge University Press, New York, USA.

Fredriksson R, Schiöth HB. 2005. The repertoire of G-protein-coupled receptors in fully sequenced genomes. Molecular Pharmacology **67**:1414–1425.

Fuchs E. 1995. Keratins and the skin. Annual Review of Cell and Developmental Biology **11**:123–154.

Funk WC et al. 2016. Adaptive divergence despite strong genetic drift: genomic analysis of the evolutionary mechanisms causing genetic differentiation in the island fox (*Urocyon littoralis*). Molecular Ecology **25**:2176–2194.

Gage MJG, Surridge AK, Tomkins JL, Green E, Wiskin L, Bell DJ, Hewitt GM. 2006. Reduced heterozygosity depresses sperm quality in wild rabbits, *Oryctolagus cuniculus*. Current Biology **16**:612–617.

Galla SJ, Buckley TR, Elshire R, Hale ML, Knapp M, McCallum J, Moraga R, Santure AW, Wilcox P, Steeves TE. 2016. Building strong relationships between conservation genetics and primary industry leads to mutually beneficial genomic advances. Molecular Ecology **25**:5267–5281.

Galván I, Gangoso L, Grande JM, Negro JJ, Rodríguez A, Figuerola J, Alonso-Alvarez C. 2010. Antioxidant machinery differs between melanic and light nestlings of two polymorphic raptors. PLOS ONE **5**:e13369.

Galván I, Solano F. 2016. Bird integumentary melanins: biosynthesis, forms, function and evolution. International Journal of Molecular Sciences **17**:520.

Gangoso L, Grande JM, Ducrest A-L, Figuerola J, Bortolotti GR, Andrés JA, Roulin A. 2011. MC1R-dependent, melanin-based colour polymorphism is associated with cell-mediated response in the Eleonora's falcon. Journal of Evolutionary Biology **24**:2055–2063.

Gangoso L, Roulin A, Ducrest A, Grande JM, Figuerola J. 2015. Morph-specific genetic and environmental variation in innate and acquired immune response in a color polymorphic raptor. Oecologia **178**:1113–1123.

García-Borrón JC, Sánchez-Laorden BL, Jiménez-Cervantes C. 2005. Melanocortin-1 receptor structure and functional regulation. Pigment Cell Research **18**:393–410.

Gerlach G, Jueterbock A, Kraemer P, Deppermann J, Harmand P. 2010. Calculations of population differentiation based on GST and D: forget GST but not all of statistics! Molecular Ecology **19**:3845–3852.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indicies. Available from http://www. unil.ch/izea/softwares/fstat.html.

Green DM. 2005. Designatable units for status assessment of endangered species. Conservation Biology **19**:1813–1820.

Griffith B, Scott JM, Carpenter JW, Reed C. 1989. Translocation as a species conservation tool: status and strategy. Science **245**:477–480.

Griffith B, Scott MJ, Carpenter JW, Reed C. 1993. Animal translocations and potential disease transmission. Journal of Zoo and Wildlife Medicine **24**:231–236.

Griffith SC, Owens IPF, Thuman KA. 2008. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology **11**:2195–2212.

Grueber CE, King TM, Waters JM, Jamieson IG. 2008. Isolation and characterization of microsatellite loci from the endangered New Zealand takahe (Gruiformes; Rallidae; *Porphyrio hochstetteri*). Molecular Ecology Resources **8**:884–886.

Grueber CE, Sutton JT, Heber S, Briskie JV, Jamieson IG, Robertson BC. 2017. Reciprocal translocation of small numbers of inbred individuals rescues immunogenetic diversity. Molecular Ecology **26**:2660–2673.

Guernsey MW, Ritscher L, Miller MA, Smith DA, Schöneberg T, Shapiro MD. 2013. A Val85Met mutation in melanocortin-1 receptor is associated with reductions in eumelanic pigmentation and cell surface expression in domestic rock pigeons (*Columba livia*). PLOS ONE **8**:e74475.

Ha HJ, Alley MR, Cahill JI, Howe L, Gartrell BD. 2009. The prevalence of psittacine beak and feather disease virus infection in native parrots in New Zealand. New Zealand Veterinary Journal **57**:50–52.

Ha HJ, Anderson IL, Alley MR, Springett BP, Gartrell BD. 2007. The prevalence of beak and feather disease virus infection in wild populations of parrots and cockatoos in New Zealand. New Zealand Veterinary Journal **55**:235–238.

Haas F, Pointer M., Saino N, Brodin A, Mundy NI, Hansson B. 2009. An analysis of population genetic differentiation and genotype-phenotype association across the hybrid zone of carrion and hooded crows using microsatellites and MC1R. Molecular Ecology **18**:294–305.

Haig SM, Ballou JD, Derrickson SR. 1990. Management options for preserving genetic diversity: reintroduction of Guam rails to the wild. Conservation Biology **4**:290–300.

Hale KA, Briskie JV. 2007. Decreased immunocompetence in a severely bottlenecked population of an endemic New Zealand bird. Animal Conservation **10**:2–10.

Hale ML, Burg TM, Steeves TE. 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLoS ONE **7**:e45170.

Hay JM, Sarre SD, Lambert DM, Allendorf FW, Daugherty CH. 2010. Genetic diversity and taxonomy: a reassessment of species designation in tuatara (*Sphenodon Reptilia*). Conservation Genetics **11**:1063–1081.

Hay JR, Best HA, Powlesland RG. 1985. Kōkako. John McIndoe Limited, Dunedin.

He X, Johansson ML, Heath DD. 2016. Role of genomics and transcriptomics in selection of reintroduction source populations. Conservation Biology **30**:1010–1018.

Hedrick PW. 1995. Gene flow and genetic restoration: the Florida panther as a case study. Conservation Biology **9**:996–1007.

Hedrick PW. 2005. A standardized genetic differentiation measure. Evolution; International Journal of Organic Evolution **59**:1633–1638.

Hedrick PW, Fredrickson R. 2010. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. Conservation Genetics **11**:615–626.

Hedrick PW, Garcia-Dorado A. 2016. Understanding inbreeding depression, purging, and genetic rescue. Trends in Ecology & Evolution **31**:940–952.

Hedrick PW, Kalinowski ST. 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics **31**:139–162.

Hess G. 1996. Disease in metapopulation models: implications for conservation. Ecology **77**:1617–1632.

Hickling GJ. 1991. The ecology of brushtail possum populations infected with bovine tuberculosis. Pages 67–71 Symposium on Tuberculosis (Publication no. 132). Veterinary Continuing Education, Palmeston North, New Zealand.

Hill S, Hill J. 1987. Richard Henry of Resolution Island. John McIndoe Limited, Dunedin, New Zealand.

Hiragaki T, Inoue-Murayama M, Miwa M, Fujiwara A, Mizutani M, Minvielle F, Ito S. 2008. Recessive black is allelic to the yellow plumage locus in japanese quail and associated with a frameshift deletion in the ASIP gene. Genetics **178**:771–775.

Hoekstra HE, Hirschmann RJ, Bundey RA, Insel PA, Crossland JP. 2006. A single amino acid mutation contributes to adaptive beach mouse color pattern. Science **313**:101–104.

Hoffman JI, Amos W. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Molecular Ecology **14**:599–612.

Hoffmann M et al. 2010. The impact of conservation on the status of the world's vertebrates. Science **330**:1503–1509.

Hofreiter M, Schöneberg T. 2010. The genetic and evolutionary basis of colour variation in vertebrates. Cellular and Molecular Life Sciences **67**:2591–2603.

Holdaway RN. 1989. New Zealand's pre-human avifauna and its vulnerability. New Zealand Journal of Ecology **12**:11–25.

Holdaway RN, Worthy TH, Tennyson AJD. 2001. A working list of breeding bird species of the New Zealand region at first human contact. New Zealand Journal of Zoology **28**:119–187.

Hooson S, Jamieson IG. 2003. The distribution and current status of New Zealand Saddleback *Philesturnus carunculatus*. Bird Conservation International **13**:79–95.

Houlden BA, England PR, Taylor AC, Greville WD, Sherwin WB. 1996. Low genetic variability of the koala *Phascolarctos cinereus* in south-eastern Australia following a severe population bottleneck. Molecular Ecology **5**:269–281.

Howes BJ, Pither R, Prior KA. 2009. Conservation implications should guide the application of conservation genetics research. Endangered Species Research **8**:193–199.

Hubbard JK, Uy JAC, Hauber ME, Hoekstra HE, Safran RJ. 2010. Vertebrate pigmentation: from underlying genes to adaptive function. Trends in Genetics **26**:231–239.

Hubisz MJ, Falush D, Stephens M, Pritchard JK. 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources **9**:1322–1332.

Hudson QJ, Wilkins RJ, Waas JR, Hogg ID. 2000. Low genetic variability in small populations of New Zealand kōkako *Callaeas cinerea wilsoni*. Biological Conservation **96**:105–112.

Hulbert C, Chamings A, Hewson K, Steer P, Gosbell M, Noormohammadi A. 2015. Survey of captive parrot populations around Port Phillip Bay, Victoria, Australia, for psittacine beak and feather disease virus, avian polyomavirus and psittacine adenovirus. Australian Veterinary Journal **93**:287–292.

Innes J, Brown K, Jansen P, Shorten R, Williams D. 1996. kōkako population studies at Rotoehu Forest and on Little Barrier Island. Department of Conservation, Wellington, New Zealand.

Innes J, Flux I. 1999. North Island kōkako recovery plan 1999-2009. Department of Conservation, Wellington, New Zealand.

Innes J, Hay R, Flux I, Bradfield P, Speed H, Jansen P. 1999a. Successful recovery of North Island kōkako *Callaeas cinerea wilsoni* populations, by adaptive management. Biological Conservation **87**:201–214.

Innes J, Hay R, Flux I, Bradfield P, Speed H, Jansen P. 1999b. Successful recovery of North Island kōkako *Callaeas cinerea wilsoni* populations, by adaptive management. Biological Conservation **87**:201–214.

Innes J, Kelly D, Overton JM, Gillies C. 2010. Predation and other factors currently limiting New Zealand forest birds. New Zealand Journal of Ecology **34**:86–114.

Innes J, Molles LE, Speed H. 2013. Translocations of North Island kōkako, 1981-2011. Notornis **60**:107–114.

IUCN. 1987. The IUCN position statement on translocation of living organisms: introductions, re-introductions and re-stocking. IUCN, Gland, Switzerland.

IUCN/SSC. 2013. Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Page viiiii + 57. IUCN Species Survival Commission, Gland, Switzerland.

Jackson B, Varsani A, Holyoake C, Jakob-Hoff R, Robertson I, McInnes K, Empson R, Gray R, Nakagawa K, Warren K. 2015. Emerging infectious disease or evidence of endemicity? A multi-season study of beak and feather disease virus in wild red-crowned parakeets (*Cyanoramphus novaezelandiae*). Archives of Virology **160**:2283–2292.

Jackson IJ. 1994. Molecular and developmental genetics of mouse coat color. Annual Review of Genetics **28**:189–217.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: A cluster matching and pemutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics **23**:1801–1806.

Jamieson IG. 2009. Loss of genetic diversity and inbreeding in New Zealand's threatened bird species. Department of Conservation, Wellington, New Zealand.

Jamieson IG. 2011. Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. Conservation Biology **25**:115–123.

Jamieson IG, Wallis GP, Briskie JV. 2006. Inbreeding and endangered species management: is New Zealand out of step with the rest of the world? Conservation Biology **20**:38–47.

Janes JK, Miller JM, Dupuis JR, Malenfant RM, Gorrell JC, Cullingham CI, Andrew RL. 2017. The K = 2 conundrum. Molecular Ecology **26**:3594–3602.

Jenkins JM. 1979. Natural History of the Guam Rail. The Condor **81**:404–408.

Johnson JA, Ambers AD, Burnham KK. 2012. Genetics of plumage color in the gyrfalcon (*Falco rusticolus*): analysis of the melanocortin-1 receptor gene. Journal of Heredity **103**:315–321.

Johnstone CP, Reina RD, Lill A. 2012. Interpreting indices of physiological stress in free-living vertebrates. Journal of Comparative Physiology **182**:861–879.

Jombart T. 2008. Adegenet: an R package for the multivariate analysis of genetic markers. Bioinformatics **24**:1403–1405.

Jombart T, Devilliard S, Balloux F. 2010. Discriminany analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics **11**:94.

Joseph L, Maloney R, Possingham H. 2009. Optimal allocation of resources among threatened species: a project prioritization protocol. Conservation biology: the journal of the Society for Conservation Biology **23**:328–38.

Jost L. 2008. GST and its relatives do not measure differentiation. Molecular Ecology **17**:4015–4026.

Jovani R, Blas J. 2004. Adaptive allocation of stress-induced deformities on bird feathers. Journal of Evolutionary Biology **17**:294–301.

Jovani R, Rohwer S. 2017. Fault bars in bird feathers: mechanisms, and ecological and evolutionary causes and consequences. Biological Reviews of the Cambridge Philosophical Society **92**:1113–1127.

Julian L, Piasecki T, Chrzastek K, Walters M, Muhire B, Harkins GW, Martin DP, Varsani A. 2013. Extensive recombination detected amongst beak and feather disease virus isolates from breeding facilities in Poland. Journal of General Virology **94**:1086–1095.

Kaeuffer R, Réale D, Coltman DW, Pontier D. 2007. Detecting population structure using STRUCTURE software: effect of background linkage disequilibrium. Heredity **99**:374–380.

Kalinowski ST. 2011. The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. Heredity **106**:625–632.

Kearse M et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28**:1647–1649.

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA. 2013. diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. Methods in Ecology and Evolution **4**:782–788.

Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. Trends in Ecology & Evolution **17**:230–241.

Kelly E, Phillips BL. 2015. Targeted gene flow for conservation. Conservation Biology **30**:259–267.

Kerje S, Lind J, Schütz K, Jensen P, Andersson L. 2003. Melanocortin 1-receptor (MC1R) mutations are associated with plumage colour in chicken. Animal Genetics **34**:241–248.

Khalesi B, Bonne N, Stewart M, Sharp M, Raidal S. 2005. A comparison of haemagglutination, haemagglutination inhibition and PCR for the detection of psittacine beak and feather disease virus infection and a comparison of isolates obtained from loriids. The Journal of General Virology **86**:3039–3046.

Kilvitis HJ, Alvarez M, Foust CM, Schrey AW, Robertson M, Richards CL. 2014. Ecological epigenetics. Pages 191–210 Ecological Genomics. Springer, New York, USA.

King C. 1990. The Handbook of New Zealand mammals.

King CM, Innes JG, Hay JR. 2015. Protecting the wildlife from introduced predators. The drama of conservation: the history of Pureora Forest, New Zealand. Heidelberg: Springer; Department of Conservation, Wellington, New Zealand.

Kinoshita K, Akiyama T, Mizutani M, Shinomiya A, Ishikawa A, Younis HH, Tsudzuki M, Namikawa T, Matsuda Y. 2014. Endothelin receptor B2 (EDNRB2) is responsible for the tyrosinase-independent recessive white (mow) and mottled (mo) plumage phenotypes in the chicken. PLOS ONE **9**:e86361.

Knafler GJ, Ortiz-catedral L, Jackson B, Varsani A, Grueber CE, Robertson BC, Jamieson IG. 2016. Comparison of beak and feather disease virus prevalence and immunity-associated genetic diversity over time in an island population of red-crowned parakeets. Archives of Virology **161**:811–820.

Knight T, Rivera D. 2015. Ark in the Park kōkako breeding season report: 2014-2015. Page 19. Auckland Zoo, Auckland, New Zealand.

Kock RA, Woodford MH, Rossiter PB. 2010. Disease risks associated with the translocation of wildlife. Revue Scientifique Et Technique (International Office of Epizootics) **29**:329–350.

Komdeur J. 1994. Conserving the seychelles warbler *Acrocephalus sechellensis* by translocation from Cousin Island to the islands of Aride and Cousine. Biological Conservation **67**:143–152.

Komdeur J, Kappe A, van de Zande L. 1998. Influence of population isolation on genetic variation and demography in Seychelles warblers: a field experiment. Animal Conservation **1**:203–212.

Koressaar T, Remm M. 2007. Enhancements and modifications of primer design program Primer3. Bioinformatics (Oxford, England) **23**:1289–1291.

Koskinen MT, Hirvonen H, Landry P-A, Primmer CR. 2004. The benefits of increasing the number of microsatellites utilized in genetic population studies: an empirical perspective. Hereditas **141**:61–67.

Kundu S, Faulkes CG, Greenwood AG, Jones CG, Kaiser P, Lyne OD, Black SA, Chowrimootoo A, Groombridge JJ. 2012. Tracking viral evolution during a disease outbreak: the rapid and complete selective sweep of a circovirus in the endangered Echo parakeet. Journal of Virology **86**:5221–5229.

Lacy RC. 1989. Analysis of founder representation in pedigrees: Founder equivalents and founder genome equivalents. Zoo Biology **8**:111–123.

Lambert DM, King T, Shepherd LD, Livingston A, Anderson S, Craig JL. 2005. Serial population bottlenecks and genetic variation: Translocated populations of the New Zealand Saddleback (*Philesturnus carunculatus rufusater*). Conservation Genetics **6**:1–14.

Lande R. 1988. Genetics and demography in biological conservation. Science **241**:1455–1460.

Larson S, Jameson R, Bodkin J, Staedler M, Bentzen P. 2002. Microsatellite DNA and mitochondrial DNA variation in remnant and translocated sea otter (*Enhydra lutris*) populations. Journal of Mammalogy **83**:893–906.

Lavers R. 1978. Distribution of the North Island kōkako (*Callaeas cinerea wilsoni*): a review. Notornis **25**:165–185.

Leathwick JR, Hay JR, Fitzgerald AE. 1983. The influence of browsing by introduced mammals on the decline of North Island kōkako. New Zealand Journal of Ecology **6**:55–70.

Lei B, Amar A, Koeslag A, Gous TA, Tate GJ. 2013. Differential haemoparasite intensity between black sparrowhawk (*Accipiter melanoleucus*) morphs suggests an adaptive function for polymorphism. PLOS ONE **8**:e81607.

Lesica P, Allendorf FW. 1995. When are peripheral populations valuable for conservation? Conservation Biology **9**:753–760.

Li L, Li D, Liu L, Li S, Feng Y, Peng X, Gong Y. 2015. Endothelin receptor B2 (EDNRB2) gene is associated with spot plumage pattern in domestic ducks (*Anas platyrhynchos*). PLOS ONE **10**:e0125883.

Lindström Å, Visser GH, Daan S. 1993. The energetic cost of feather synthesis is proportional to basal metabolic rate. Physiological Zoology **66**:490–510.

Losdat S, Chang SM, Reid JM. 2014. Inbreeding depression in male gametic performance. Journal of Evolutionary Biology **27**:992–1011.

Loser K et al. 2010. The neuropeptide alpha-melanocyte-stimulating hormone is critically involved in the development of cytotoxic CD8+ T cells in mice and humans. PLOS ONE **5**:e8958.

Luger TA, Scholzen TE, Brzoska T, Böhm M. 2003. New insights into the functions of alpha-MSH and related peptides in the immune system. Annals of the New York Academy of Sciences **994**:133–140.

Lynch M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. Evolution **45**:622–629.

Lynch M, Walsh B. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Inc, Sunderland, Mass.

MacDougall-Shackleton EA, Blanchard L, Igdoura SA, Gibbs HL. 2003. Unmelanized plumage patterns in Old World leaf warblers do not correspond to sequence variation at the melanocortin-1 receptor locus (MC1R). Molecular Biology and Evolution **20**:1675–1681.

Madsen T, Shine R, Olsson M, Wittzell H. 1999. Conservation biology: Restoration of an inbred adder population. Nature **402**:34–35.

Madsen T, Stille B, Shine R. 1996. Inbreeding depression in an isolated population of adders *Vipera berus*. Biological Conservation **75**:113–118.

Malone EW, Perkin JS, Leckie BM, Kulp MA, Hurt CR, Walker DM. 2018. Which species, how many, and from where: Integrating habitat suitability, population genomics, and abundance estimates into species reintroduction planning. Global Change Biology **0**:1–20.

Manceau M, Domingues VS, Linnen CR, Rosenblum EB, Hoekstra HE. 2010. Convergence in pigmentation at multiple levels: mutations, genes and function. Philosophical Transactions of the Royal Society of London: Biological Sciences **365**:2439–2450.

Maruyama T, Fuerst PA. 1985. Population bottlenecks and nonequilibrium models in population genetics. Number of alleles in a small population that was formed by a recent bottleneck. Genetics **111**:675–689.

Massaro M et al. 2012. Molecular characterisation of beak and feather disease virus (BFDV) in New Zealand and its implications for managing an infectious disease. Archives of Virology **157**:1651–1663.

Maudet C, Miller C, Bassano B, Breitenmoser-Würsten C, Gauthier D, Obexer-Ruff G, Michallet J, Taberlet P, Luikart G. 2002. Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex (*Capra ibex*). Molecular Ecology **11**:421–436.

McBride. 1981. Sighting of South Island kōkako (*Callaeas cinerea cinerea*) in Mount Aspiring National Park. Notornis **28**:255–256.

McGraw KJ, Safran RJ, Wakamatsu K. 2005. How feather colour reflects its melanin content. Functional Ecology **19**:816–821.

McInnes K, Cromarty P, Ombler J. 2004. Standard operating procedure for the health management of terrestrial vertebrate species protected under the Wildlife Act. Document Number WGNCR-49724. Department of Conservation, Wellington.

Meirmans PG, Hedrick PW. 2011. Assessing population structure: FST and related measures. Molecular Ecology Resources **11**:5–18.

Miller KA, Nelson NJ, Smith HG, Moore JA. 2009. How do reproductive skew and founder group size affect genetic diversity in reintroduced populations? Molecular Ecology **18**:3792–3802.

Mills LS, Allendorf FW. 2002. The one-migrant-per-generation rule in conservation and management. Conservation Biology **10**:1509–1518.

Milne A, Stocker. 2014. Evidence for the continued existence of the South Island kōkako (*Callaeas cinerea*) drawn from reports collected between January 1990 and June 2012. Notornis **61**:137–143.

Milner-Gulland EJ, Fisher M, Browne S, Redford KH, Spencer M, Sutherland WJ. 2010. Do we need to develop a more relevant conservation literature? Oryx **44**:1–2.

Miskelly CM, Powlesland RG. 2013. Conservation translocations of New Zealand birds, 1863-2012. Notornis **60**:3–28.

Miwa M, Inoue-Murayama M, Aoki H, Kunisada T, Hiragaki T, Mizutani M, Ito S. 2007. Endothelin receptor B2 (EDNRB2) is associated with the panda plumage colour mutation in Japanese quail. Animal Genetics **38**:103–108.

Mock KE, Latch EK, Rhodes OE. 2004. Assessing losses of genetic diversity due to translocation: long-term case histories in Merriam's turkey (*Meleagris gallopavo merriami*). Conservation Genetics **5**:631–645.

Møller AP, Nielsen JT. 2015. Genetic variation in birds in relation to predation risk by hawks: A comparative analysis. Current Zoology **61**:1–9.

Moritz C. 1994. Defining "evolutionary significant units" for conservation. Trends in Ecology & Evolution **9**:373–374.

Moyle LC, Stinchcombe JR, Hudgens BR, Morris WF. 2003. Conservation genetics in the recovery of endangered animal species: a review of US endangered species recovery plan (1977-1998). Animal Biodiversity and Conservation **26**:85–95.

Mullen LM, Vignieri SN, Gore JA, Hoekstra HE. 2009. Adaptive basis of geographic variation: genetic, phenotypic and environmental differences among beach mouse populations. Proceedings. Biological Sciences **276**:3809–3818.

Mundy NI. 2005. A window on the genetics of evolution: MC1R and plumage colouration in birds. Proceedings of the Royal Society: Biological Sciences **272**:1633–1640.

Mundy NI, Badcock N., Hart T, Scribner K, Janssen K, Nadeau NJ. 2004. Conserved genetic basis of a quantitative plumage trait involved in mate choice. Science **303**:1870–1873.

Mundy NI, Kelly J. 2003. Evolution of a pigmentation gene, the melanocortin-1 receptor, in primates. American Journal of Physical Anthropology **121**:67–80.

Murphy ME, King JR. 1992. Energy and nutrient use during moult by white-crowned sparrows *Zonotrichia leucophrus gambelii*. Ornis Scandinavica **23**:304–313.

Murphy ME, Miller BT, King JR. 1989. A structural comparison of fault bars with feather defects known to be nutritionally induced. Canadian Journal of Zoology **67**:1311–1317.

Murphy SA, Flux IA, Double MC. 2006. Recent evolutionary history of New Zealand's North and South Island kōkako (*Callaeas cinerea*) inferred from mitochondrial DNA sequences. Emu - Austral Ornithology **106**:41–48.

Nadeau NJ, Minvielle F, Mundy NI. 2006. Association of a Glu92Lys substitution in MC1R with extended brown in Japanese quail (*Coturnix japonica*). Animal Genetics **37**:287–289.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences **70**:3321–3323.

Nei M, Maruyama T, Chakraborty R. 1975. The bottleneck effect and genetic variability in populations. Evolution **29**:1–10.

New Zealand Biodiversity Action Plan: 2016-2020. 2016. Page 60. Department of Conservation, Wellington.

New Zealand's Threatened Species Strategy: Draft for Consultation. 2017. Page 50. Department of Conservation, Wellington.

North Island Kōkako (*Callaeas wilsoni*) Recovery Plan 2017-2025. Draft. 2018. Department of Conservation, Wellington.

Nsubuga AM, Holzman J, Chemnick LG, Ryder OA. 2010. The cryptic genetic structure of the North American captive gorilla population. Conservation Genetics **11**:161–172.

O'Brien DM, Keogh JS, Silla AJ, Byrne PG. 2018. The unexpected genetic mating system of the red-backed toadlet (*Pseudophryne coriacea*); a species with prolonged terrestrial breeding and cryptic reproductive behaviour. Molecular Ecology **Accepted**

Author Manuscript. Available from http://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14737 (accessed June 19, 2018).

Ogawa H, Yamaguchi T, Fukushi H. 2005. Duplex shuttle PCR for differential diagnosis of budgerigar fledgling disease and psittacine beak and feather disease. Microbiology and Immunology **49**:227–237.

OIE, IUCN. 2014. Guidelines for wildlife disease risk analysis. OIE in association with the IUCN and the Species Survival Commission, Paris.

Ollmann MM, Lamoreux ML, Wilson BD, Barsh GS. 1998. Interaction of Agouti protein with the melanocortin 1 receptor in vitro and in vivo. Genes & Development **12**:316–330.

Ortiz-Catedral L, McInnes K, Hauber ME, Brunton DH. 2009. First report of beak and feather disease virus (BFDV) in wild Red-fronted Parakeets (*Cyanoramphus novaezelandiae*) in New Zealand. Emu - Austral Ornithology **109**:244–247.

Ottewell K, Dunlop J, Thomas N, Morris K, Coates D, Byrne M. 2014. Evaluating success of translocations in maintaining genetic diversity in a threatened mammal. Biological Conservation **171**:209–219.

Ouborg NJ, Pertoldi C, Loeschcke V, Bijlsma R, Hedrick PW. 2010. Conservation genetics in transition to conservation genomics. Trends in Genetics **26**:177–187.

Parker KA. 2008. Translocations: providing outcomes for wildlife, resource managers, scientists, and the human community. Restoration Ecology **16**:204–209.

Parker KA. 2013a. Restoring Richard Henry's dream: the evolution of translocation in New Zealand conservation. Notornis **60**:1–2.

Parker KA. 2013b. Avian translocations to and from Tiritiri Matangi 1974-2011. New Zealand Journal of Ecology **37**:282–287.

Parker KA, Brunton DH, Jakob-Hoff R. 2006. Avian translocations and disease; implications for New Zealand conservation. Pacific Conservation Biology **12**:155–162.

Pass D A, Perry R A. 1984. The pathology of psittacine beak and feather disease. Australian Veterinary Journal **61**:69–74.

Paxinos EE, James HF, Olson SL, Ballou JD, Leonard JA, Fleischer RC. 2002. Prehistoric decline of genetic diversity in the nene. Science **296**:1827–1827.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics **28**:2537–2539.

Pereira HM et al. 2010. Scenarios for global biodiversity in the 21st century. Science **330**:1496–1501.

Pérez I, Anadón JD, Díaz M, Nicola GG, Tella JL, Giménez A. 2012. What is wrong with current translocations? A review and a decision-making proposal. Frontiers in Ecology and the Environment **10**:494–501.

Pertoldi C, Hansen MM, Loeschcke V, Madsen AB, Jacobsen L, Baagoe H. 2001. Genetic consequences of population decline in the European otter *(Lutra lutra*): an assessment of microsatellite DNA variation in Danish otters from 1883 to 1993. Proceedings. Biological Sciences **268**:1775–1781.

Peters A, Patterson EI, Baker BGB, Holdsworth M, Sarker S, Ghorashi SA, Raidal SR. 2014. Evidence of psittacine beak and feather disease virus spillover into wild critically endangered orange-bellied parrots (*neophema chrysogaster*). Journal of Wildlife Diseases **50**:288–296.

Piault R, Gasparini J, Bize P, Jenni-Eiermann S, Roulin A. 2009. Pheomelanin-based coloration and the ability to cope with variation in food supply and parasitism. The American Naturalist **174**:548–556.

Pierson JC, Coates DJ, Oostermeijer JGB, Beissinger SR, Bragg JG, Sunnucks P, Schumaker NH, Young AG. 2016. Genetic factors in threatened species recovery plans on three continents. Frontiers in Ecology and the Environment **14**:433–440.

Poelstra JW, Vijay N, Hoeppner MP, Wolf JBW. 2015. Transcriptomics of colour patterning and coloration shifts in crows. Molecular Ecology **24**:4617–4628.

Pompanon F, Bonin A, Bellemain E, Taberlet P. 2005. Genotyping errors: causes, consequences and solutions. Nature Reviews Genetics **6**:847–846.

Powlesland RG. 1987. The foods, foraging behaviour and habitat use of North Island kōkako in Puketi State Forest, Northland. New Zealand Journal of Ecology **10**:117–128.

Primmer CR. 2009. From conservation genetics to conservation genomics. Annals of the New York Academy of Sciences **1162**:357–368.

Pritchard JK. 2010. Documentation for STRUCTURE software: Version 2.3.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945–959.

Proft KM, Jones ME, Johnson CN, Burridge CP. 2018. Making the connection: expanding the role of restoration genetics in restoring and evaluating connectivity. Restoration Ecology **26**:411–418.

Prum RO, Williamson S. 2001. Theory of the growth and evolution of feather shape. Journal of Experimental Biology **291**:30–57.

Puechmaille SJ. 2016. The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Molecular Ecology Resources **16**:608–627.

Queller DC, Goodnight KF. 1989. Estimating relatedness using genetic markers. Evolution **43**:258–275.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available from Available from: http://R-project.org.

Raidal S, Sarker S, Peters A. 2015. Review of psittacine beak and feather disease and its effect on Australian endangered species. Australian Veterinary Journal **93**:466–470.

Raidal SR, Peters A. 2018. Psittacine beak and feather disease: ecology and implications for conservation. Emu - Austral Ornithology **118**:80–93.

Ralls K, Ballou JD, Dudash MR, Eldridge MDB, Fenster CB, Lacy R., Sunnucks P, Frankham R. 2017. Call for a paradigm shift in the genetic management of fragmented populations. Conservation Letters **0**. Available from http://onlinelibrary.wiley.com/doi/full/10.1111/conl.12412 (accessed April 15, 2018).

Raue R, Johne R, Crosta L, Bürkle M, Gerlach H, Müller H. 2004. Nucleotide sequence analysis of a C1 gene fragment of psittacine beak and feather disease virus amplified by real-time polymerase chain reaction indicates a possible existence of genotypes. Avian Pathology: Journal of the W.V.P.A **33**:41–50.

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86**:248–249.

Rayner MJ et al. 2015. Using miniaturized radiotelemetry to discover the breeding grounds of the endangered New Zealand storm petrel *Fregetta maoriana*. Ibis **157**:754–766.

Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. Conservation Biology **17**:230–237.

Rees JL. 2003. Genetics of hair and skin color. Annual Review of Genetics **37**:67–90.

Rice WR. 1989. Analyzing tables of statistical tests. Evolution **43**:223–225.

Richardson DS, Bristol R, Shah NJ. 2006. Translocation of the Seychelles warbler *Acrocephalus sechellensis* to establish a new population on Denis Island, Seychelles. Conservation Evidence **3**:54–57.

Richardson JL, Brady SP, Wang IJ, Spear SF. 2016. Navigating the pitfalls and promise of landscape genetics. Molecular Ecology **25**:849–863.

Richardson K, Ewen JG, Armstrong DP, Hauber ME. 2010. Sex-specific shifts in natal dispersal dynamics in a reintroduced hihi population. Behaviour **147**:1517–1532.

Rideout BA, Sainsbury AW, Hudson PJ. 2017. Which parasites should we be most concerned about in wildlife translocations? EcoHealth **14**:42–46.

Ritchie BW, Niagro FD, Latimer KS, Steffens WL, Pesti D, Lukert PD. 1991. Hemagglutination by psittacine beak and feather disease virus and use of hemagglutination inhibition for detection of antibodies against the virus. American Journal of Veterinary Research **52**:1810–1815.

Ritchie BW, Niagro FD, Lukert PD, Latimer KS, Steffens WL, Pritchard N. 1989. A review of psittacine beak and feather disease: Characteristics of the PBFD virus. Journal of the Association of Avian Veterinarians **3**:143–149.

Ritchie PA, Anderson IL, Lambert DM. 2003. Evidence for specificity of psittacine beak and feather disease viruses among avian hosts. Virology **306**:109–115.

Robbins LS, Nadeau JH, Johnson KR, Kelly MA, Roselli-Rehfuss L, Baack E, Mountjoy KG, Cone RD. 1993. Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell **72**:827–834.

Roberts DG, Baker J, Perrin C. 2011. Population genetic structure of the endangered Eastern Bristlebird, *Dasyornis brachypterus*; implications for conservation. Conservation Genetics **12**:1075–1085.

Robertson HA et al. 2017. Conservation status of New Zealand birds, 2016. Page 23 New Zealand Threat Classification Series 19. Department of Conservation, Wellington.

Roulin A. 2016. Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration. Biological Reviews **91**:328–348.

Roulin A, Almasi B, Meichtry-Stier KS, Jenni L. 2011a. Eumelanin- and pheomelanin-based colour advertise resistance to oxidative stress in opposite ways. Journal of Evolutionary Biology **24**:2241–2247.

Roulin A, Ducrest A. 2013. Genetics of colouration in birds. Seminars in Cell & Developmental Biology **24**:594–608.

Roulin A, Emaresi G, Bize P, Gasparini J, Piault R, Ducrest A. 2011b. Pale and dark reddish melanic tawny owls differentially regulate the level of blood circulating POMC prohormone in relation to environmental conditions. Oecologia **166**:913–921.

Rowe S. 2007. The influence of geographic variation in song dialect on post-translocation pair formation in North Island kōkako (*Callaeas cinerea wilsoni*). Notornis **54**:28–37.

Ryman N, Leimar O. 2009. G(ST) is still a useful measure of genetic differentiation - a comment on Jost's D. Molecular Ecology **18**:2084–2087.

Sainsbury AW, Vaughan-Higgins RJ. 2012. Analyzing disease risks associated with translocations. Conservation Biology **26**:442–452.

San-Jose L, Roulin A. 2017. Genomics of coloration in natural animal populations. Phil. Trans. R. Soc. B **372**:20160337.

Sarker S, Forwood JK, Ghorashi SA, Peters A, Raidal SR. 2015a. Beak and feather disease virus genotypes in Australian parrots reveal flexible host-switching. Australian Veterinary Journal **93**:471–475.

Sarker S, Ghorashi SA, Forwood JK, Raidal SR. 2013. Whole-genome sequences of two beak and feather disease viruses in the endangered swift parrot (*Lathamus discolor*). Genome Announcements **1**. Available from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3860835/.

Sarker S, Lloyd C, Forwood J, Raidal SR. 2016. Forensic genetic evidence of beak and feather disease virus infection in a Powerful Owl, *Ninox strenua*. Emu - Austral Ornithology **116**:71–74.

Sarker S, Moylan KG, Ghorashi SA, Forwood JK, Peters A, Raidal SR. 2015b. Evidence of a deep viral host switch event with beak and feather disease virus infection in rainbow bee-eaters (*Merops ornatus*). Scientific Reports **5**:14511.

Sarker S, Patterson EI, Peters A, Baker GB, Forwood JK, Ghorashi SA, Holdsworth M, Baker R, Murray N, Raidal SR. 2014. Mutability dynamics of an emergent single stranded DNA virus in a naïve host. PLOS ONE **9**:e85370.

Schrey AW, Alvarez M, Foust CM, Kilvitis HJ, Lee JD, Liebl AL, Martin LB, Richards CL, Robertson M. 2013. Ecological epigenetics: Beyond MS-AFLP. Integrative and Comparative Biology **53**:340–350.

Schuelke M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology **18**:233–234.

Scott ME. 1988. The impact of infection and disease on animal populations: Implications for conservation biology. Conservation Biology **2**:40–56.

Seddon JM, Baverstock PR. 2002. Variation on islands: major histocompatibility complex (MHC) polymorphism in populations of the Australian bush rat. Molecular Ecology **8**:2071–2079.

Seddon PJ. 2010. From reintroduction to assisted colonization: Moving along the conservation translocation spectrum. Restoration Ecology **18**:796–802.

Seddon PJ, Armstrong DP, Maloney RF. 2007. Developing the science of reintroduction biology. Conservation Biology **21**:303–312.

Seddon PJ, Griffiths CJ, Soorae PS, Armstrong DP. 2014. Reversing defaunation: Restoring species in a changing world. Science **345**:406–412.

Seddon PJ, Soorae PS, Launay F. 2005. Taxonomic bias in reintroduction projects. Animal Conservation **8**:51–58.

Segelbacher G, Höglund J, Storch I. 2003. From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. Molecular Ecology **12**:1773–1780.

Selz Y, Braasch I, Hoffmann C, Schmidt C, Schultheis C, Schartl M, Volff J. 2007. Evolution of melanocortin receptors in teleost fish: the melanocortin type 1 receptor. Gene **401**:114–122.

Sgrò CM, Lowe AJ, Hoffmann AA. 2011. Building evolutionary resilience for conserving biodiversity under climate change. Evolutionary Applications **4**:326–337.

Shepherd LD, Lambert DM. 2006. Nuclear microsatellite DNA markers for New Zealand kiwi (Apteryx spp.). Molecular Ecology Notes **6**:227–229.

Shepherd LD, Lambert DM. 2007. The relationships and origins of the New Zealand wattlebirds (Passeriformes, *Callaeatidae*) from DNA sequence analyses. Molecular Phylogenetics and Evolution **43**:480–492.

Shepherd LD, Worthy TH, Tennyson AJD, Scofield RP, Ramstad KM, Lambert DM. 2012. Ancient DNA analyses reveal contrasting phylogeographic patterns amongst kiwi (*Apteryx spp*.) and a recently extinct lineage of spotted kiwi. PLOS ONE **7**:e42384.

Sherley GH. 2010. Summary of native bat, reptile, amphibian and terrestrial invertebrate translocations in New Zealand. Page 39. 303, Science for Conservation. Department of Conservation, Wellington.

Sigg DP. 2006. Reduced genetic diversity and significant genetic differentiation after translocation: Comparison of the remnant and translocated populations of bridled nailtail wallabies (*Onychogalea fraenata*). Conservation Genetics **7**:577–589.

Sinclair ARE, Innes J, Bradfield P. 2006. Making endangered species safe: the case of the kōkako of North Island, New Zealand. New Zealand Journal of Ecology **30**:121–130.

Singer FJ, Papouchis CM, Symonds KK. 2000. Translocations as a tool for restoring populations of bighorn sheep. Restoration Ecology **8**:6–13.

Slatkin M. 1987. Gene flow and the geographic structure of natural populations. Science **236**:787–792.

Soorae PS. 2008. Global re-introduction perspectives: re-introduction case-studies from around the globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE.

Spielman D, Brook BW, Frankham R. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences of the United States of America **101**:15261–15264.

Stephenson B, Flood R, Thomas B, Saville S. 2008. Rediscovery of the New Zealand storm petrel (*Pealeornis maoriana*): two sightings that revised our knowledge of storm petrels. Notornis **55**:77–83.

Stockwell CA, Mulvey M, Vinyard GL. 1996. Translocations and the preservation of allelic diversity. Conservation Biology **10**:1133–1141.

Strochlic DE, Romero LM. 2008. The effects of chronic psychological and physical stress on feather replacement in European starlings (*Sturnus vulgaris*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **149**:68–79.

Sutherland WJ et al. 2010. Standards for documenting and monitoring bird reintroduction projects. Conservation Letters **3**:229–235.

Sutton JT, Nakagawa S, Robertson BC, Jamieson IG. 2011. Disentangling the roles of natural selection and genetic drift in shaping variation at MHC immunity genes. Molecular Ecology **20**:4408–4420.

Takeuchi S, Suzuki H, Yabuuchi M, Takahashi S. 1996. A possible involvement of melanocortin 1-receptor in regulating feather color pigmentation in the chicken. Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression **1308**:164–168.

Takezaki N, Nei M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics **144**:389–399.

Tarr CL, Conant S, Fleischer RC. 1998. Founder events and variation at microsatellite loci in an insular passerine bird, the Laysan finch (*Telespiza cantans*). Molecular Ecology **7**:719–731.

Taylor HR, Colbourne RM, Robertson HA, Nelson NJ, Allendorf FW, Ramstad KM. 2017a. Cryptic inbreeding depression in a growing population of a long-lived species. Molecular Ecology **26**:799–813.

Taylor HR, Dussex N, van Heezik Y. 2017b. Bridging the conservation genetics gap by identifying barriers to implementation for conservation practitioners. Global Ecology and Conservation **10**:231–242.

Taylor SS, Jamieson IG. 2008. No evidence for loss of genetic variation following sequential translocations in extant populations of a genetically depauperate species. Molecular Ecology **17**:545–556.

Taylor SS, Jamieson IG, Armstrong DP. 2005. Successful island reintroductions of New Zealand robins and saddlebacks with small numbers of founders. Animal Conservation **8**:415–420.

Taylor SS, Jamieson IG, Wallis. 2007. Historic and contemporary levels of genetic variation in two New Zealand passerines with different histories of decline. Journal of Evolutionary Biology **20**:2035–2047.

Te Hauturu-o-Toi. Little Barrier Island Nature Reserve Management Plan. 2017. Page 64. Department of Conservation, Wellington.

Templeton AR, Shaw K, Routman E, Davis SK. 1990. The genetic consequences of habitat fragmentation. Annals of the Missouri Botanical Garden **77**:13–27.

Theron E, Hawkins K, Bermingham E, Ricklefs RE, Mundy NI. 2001. The molecular basis of an avian plumage polymorphism in the wild: a melanocortin-1-receptor point mutation is perfectly associated with the melanic plumage morph of the bananaquit, *Coereba flaveola*. Current biology **11**:550–557.

Thorogood R, Armstrong DP, Low M, Brekke P, Ewen JG. 2013. The value of long-term ecological research: integrating knowledge for conservation of hihi on Tiritiri Matangi Island. New Zealand Journal of Ecology **37**:298–306.

Tilman D, Clark M, Williams DR, Kimmel K, Polasky S, Packer C. 2017. Future threats to biodiversity and pathways to their prevention. Nature **546**:73–81.

Todd D. 2000. Circoviruses: immunosuppressive threats to avian species: a review. Avian Pathology: Journal of the W.V.P.A **29**:373–394.

Todd D, Niagro FD, Ritchie BW, Curran W, Allan GM, Lukert PD, Latimer KS, Steffens WL, McNulty MS. 1991. Comparison of three animal viruses with circular single-stranded DNA genomes. Archives of Virology **117**:129–135.

Tollington S, Greenwood A, Jones CG, Hoeck P, Chowrimootoo A, Smith D, Richards H, Tatayah V, Groombridge JJ. 2015. Detailed monitoring of a small but recovering population reveals sublethal effects of disease and unexpected interactions with supplemental feeding. The Journal of Animal Ecology **84**:969–977.

Tracy LN, Wallis GP, Efford MG, Jamieson IG. 2011. Preserving genetic diversity in threatened species reintroductions: how many individuals should be released? Animal Conservation **14**:439–446.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. 2012. Primer3—new capabilities and interfaces. Nucleic Acids Research **40**:e115.

Valderrama SV, Molles LE, Waas JR, Slabbekoorn H. 2013. Conservation implications of song divergence between source and translocated populations of the North Island Kōkako. Journal of Applied Ecology **50**:950–960.

Van Andel M, McInnes K, Tana T, French NP. 2016. Network analysis of wildlife translocations in New Zealand. New Zealand Veterinary Journal **64**:169–173.

van Dijk RE, Covas R, Doutrelant C, Spottiswoode CN, Hatchwell BJ. 2015. Fine-scale genetic structure reflects sex-specific dispersal strategies in a population of sociable weavers (*Philetairus socius*). Molecular Ecology **24**:4296–4311.

van Grouw H. 2013. What Colour is that bird? The causes and recognition of common colour aberrations in birds. British Birds **106**:17–29.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes **4**:535–538.

van Riemsdijk I, van Nieuwenhuize L, Martínez-Solano I, Arntzen JW, Wielstra B. 2018. Molecular data reveal the hybrid nature of an introduced population of banded newts (*Ommatotriton*) in Spain. Conservation Genetics **19**:249–254.

Veitch CR. 2001. The eradication of feral cats (*Felis catus*) from Little Barrier Island, New Zealand. New Zealand Journal of Zoology **28**:1–12.

Vidal O, Araguas RM, Fernández E, Heras S, Sanz N, Pla C. 2010. Melanism in guinea fowl (*Numida meleagris*) is associated with a deletion of Phenylalanine-256 in the MC1R gene. Animal Genetics **41**:656–658.

Walker WP, Gunn TM. 2010. Shades of meaning: the pigment-type switching system as a tool for discovery. Pigment Cell & Melanoma Research **23**:485–495.

Walsh P, Metzger DA, Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques **10**:506–513.

Wan Q-H, Wu H, Fujihara T, Fang S-G. 2004. Which genetic marker for which conservation genetics issue? Electrophoresis **25**:2165–2176.

Waples RS, Gaggiotti O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology **15**:1419–1439.

Weeks AR et al. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evolutionary Applications **4**:709–725.

Weiser EL, Grueber CE, Jamieson IG. 2012. AlleleRetain: a program to assess management options for conserving allelic diversity in small, isolated populations. Molecular Ecology Resources **12**:1161–1167.

Wellband KW, Heath DD. 2017. Plasticity in gene transcription explains the differential performance of two invasive fish species. Evolutionary Applications **10**:563–576.

Weston KA, Robertson BC. 2014. Isolation and characterisation of 14 microsatellites for the New Zealand rock wren *Xenicus gilviventris*. Conservation Genetics Resources **6**:115–117.

White KL. 2013. The role of inbreeding in the reproductive fitness of kakapo (*Strigops habroptilus*). Master of Science. University of Otago, Dunedin, New Zealand. Available from http://hdl.handle.net/10523/4178.

Whitlock MC. 2011. G'ST and D do not replace FST. Molecular Ecology **20**:1083–1091.

Willi Y, Buskirk JV, Hoffmann AA. 2006. Limits to the adaptive potential of small populations. Annual Review of Ecology, Evolution, and Systematics **37**:433–458.

Willis K, Wiese RJ. 1993. Effect of new founders on retention of gene diversity in captive populations: A formalization of the nucleus population concept. Zoo Biology **12**:535–548.

Wlasiuk G, Nachman MW. 2007. The genetics of adaptive coat color in gophers: coding variation at MC1R is not responsible for dorsal color differences. The Journal of Heredity **98**:567–574.

Wolf CM, Griffith B, Reed C, Temple SA. 1996. Avian and mammalian translocations: update and reanalysis of 1987 survey data. Conservation Biology **10**:1142–1154.

Wolf Horrell EM, Boulanger MC, D'Orazio JA. 2016. Melanocortin 1 receptor: structure, function, and regulation. Frontiers in Genetics **7**. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4885833/ (accessed October 19, 2017).

Woodford MH. 1993. International disease implications for wildlife translocation. Journal of Zoo and Wildlife Medicine **24**:265–270.

Wright S. 1922. Coefficients of inbreeding and relationship. The American Naturalist **56**:330–338.

Wu N, Qin H, Wang M, Bian Y, Dong B, Sun G, Zhao W, Chang G, Xu Q, Chen G. 2017. Variations in endothelin receptor B subtype 2 (EDNRB2) coding sequences and

mRNA expression levels in 4 Muscovy duck plumage colour phenotypes. British Poultry Science **58**:116–121.

Yang L, Du X, Wei S, Gu L, Li N, Gong Y, Li S. 2017. Genome-wide association analysis identifies potential regulatory genes for eumelanin pigmentation in chicken plumage. Animal Genetics **48**:611–614.

Ypelaar I, Bassami MR, Wilcox GE, Raidal SR. 1999. A universal polymerase chain reaction for the detection of psittacine beak and feather disease virus. Veterinary Microbiology **68**:141–148.

Zhan XJ, Dixon A, Fox NC, Bruford MW. 2012. Missense SNP of the MC1R gene is associated with plumage variation in the Gyrfalcon (*Falco rusticolus*). Animal Genetics **43**:460–462.

Zhang G. 2015. Genomics: Bird sequencing project takes off. Nature **522**:34–34.

Zhang XH, Pang YZ, Zhao SJ, Xu HW, Li YL, Xu Y, Guo Z, Wang DD. 2013. The relationship of plumage colours with MC1R (Melanocortin 1 Receptor) and ASIP (Agouti Signaling Protein) in Japanese quail (*Coturnix coturnix japonica*). British Poultry Science **54**:306–311.

Appendix 1: Source locations of the 32 kōkako translocated to establish the Hauturu population between 1981 and 1994. Source: Innes et al. 2013.

Location	Date	Number transferred
Matawharau Block, Rotoehu	October 1981	1
Kaharoa	March 1982	6
Puwhenua, Mamaku Plateau	March 1982	2
Mangapapa Rr, Mamaku Plateau	June 1982	1
Oropi	June 1982	1
Oropi	November 1982	2
Te Rerenga Stream, Mamaku Plateau	December 1982	2
Puwhenua, Mamaku Plateau	December 1982	1
Matawharau Block, Rotoehu	March 1983	5
Matahina	July-October 1986	6
Matahina	February 1988	3
Great Barrier Island	1994	2

Appendix 2: Measurements of kōkako E212419 before (2013) and after developing aberrant phenotypic features (2015).

Band No.	Date	Age Class	Weight (g)	Tarsometatarsus (mm)	Wing Chord (mm)	Head and Bill (mm)	Blood taken
E212419	25.06.13	Adult	232	66.1	161	59.9	Y
E212419	10.08.15	Adult (aberrant)	243	65.3	161	60.6	Y

Appendix 3: Sample details of the 110 kōkako sampled from each of the six kōkako populations.

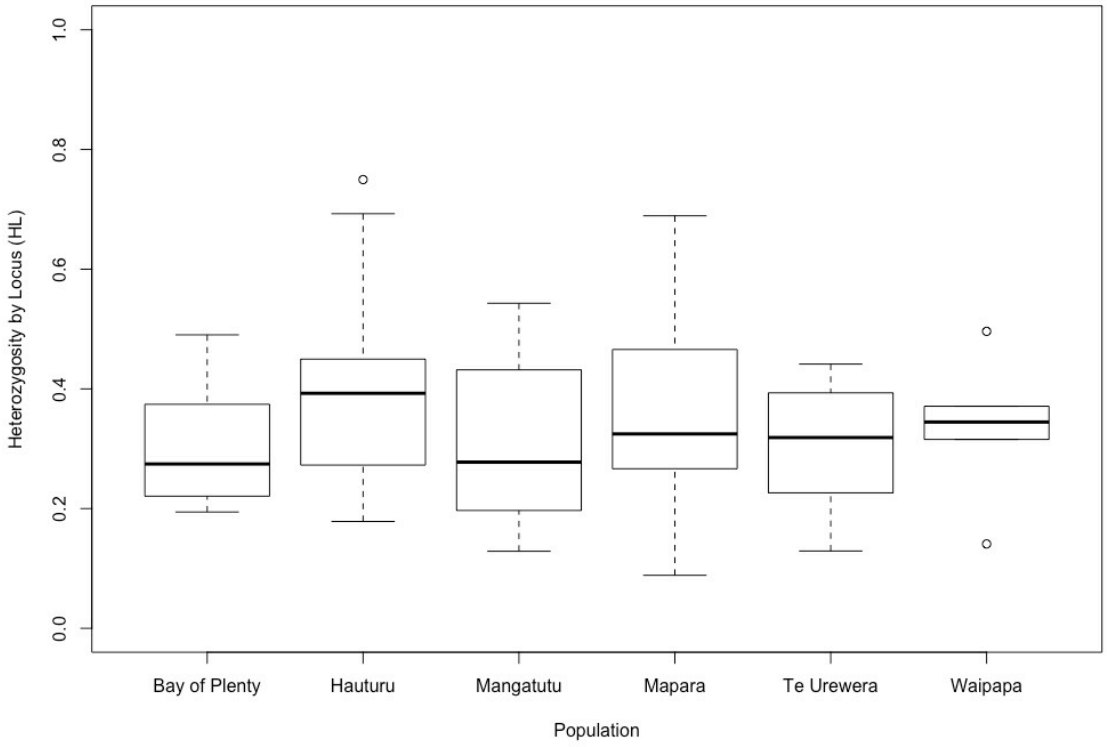
Location	Date collected	Date extracted	Sample (band) number	Sample type
Hauturu	01.06.13	27.04.17	E212754	Blood
Hauturu	05.08.15	08.06.17	E212423	Blood
Hauturu	12.08.15	08.06.17	E212783	Feathers
Hauturu	08.08.15	08.06.17	E212426	Feathers
Hauturu	02.06.13	27.04.17	E212758	Blood
Hauturu	03.06.13	27.04.17	E212760	Blood
Hauturu	02.06.13	27.04.17	E212759	Blood
Hauturu	09.08.15	05.06.17	E212773	Blood
Hauturu	10.08.15	05.06.17	E212776	Blood
Hauturu (Aberrant)	10.08.15	05.06.17	E212419	Blood
Hauturu	05.08.15	05.06.17	E212424	Blood
Hauturu	09.08.15	05.06.17	E212774	Blood
Hauturu	05.08.15	05.06.17	E212425	Blood
Hauturu	11.08.15	05.06.17	E212429	Blood
Hauturu	09.08.15	05.06.17	E212428	Blood
Hauturu	11.08.15	05.06.17	E212430	Blood
Hauturu	09.08.15	05.06.17	E212775	Blood
Hauturu	10.08.15	05.06.17	E212777	Blood
Hauturu	11.08.15	05.06.17	E212780	Blood
Hauturu	11.08.15	05.06.17	E212779	Blood
Hauturu	25.06.13	05.06.17	E212418	Blood
Hauturu	01.06.13	27.04.17	E212753	Blood
Hauturu	01.06.13	27.04.17	E212757	Blood
Hauturu	12.08.15	08.06.17	E212782	Blood
Hauturu	09.08.15	08.06.17	E212772	Blood
Hauturu	04.08.15	08.06.17	E212422	Blood
Hauturu	11.08.15	08.06.17	E212781	Blood
Kaharoa (Bay of Plenty)	11.09.10	13.09.17	E127126	Feathers
Kaharoa (Bay of Plenty)	10.09.10	13.09.17	E212407	Feathers
Kaharoa (Bay of Plenty)	14.09.10	13.09.17	E212408	Feathers
Kaharoa (Bay of Plenty)	16.08.16	24.05.17	E221368	Blood

Mangatutu	01.06.16	24.05.17	E232340	Blood
Mangatutu	11.06.16	24.05.17	E221357	Blood
Mangatutu	30.08.16	24.05.17	E221369	Blood
Mangatutu	01.09.16	24.05.17	E221372	Blood
Mangatutu	01.09.16	24.05.17	E221373	Blood
Mangatutu	19.10.16	24.05.17	E221383	Blood
Mangatutu	15.10.16	24.05.17	E221378	Blood
Mangatutu	19.09.16	24.05.17	E221376	Blood
Mangatutu	11.09.16	24.05.17	E221375	Blood
Mangatutu	28.10.15	31.05.17	E232308	Blood
Mangatutu	30.09.15	31.05.17	E232356	Blood
Mangatutu	25.10.15	31.05.17	E232306	Blood
Mangatutu	19.09.15	31.05.17	E212800	Blood
Mangatutu	28.09.15	31.05.17	E232354	Blood
Mangatutu	11.10.15	31.05.17	E232364	Blood
Mangatutu	23.08.15	31.05.17	E212787	Blood
Mangatutu	22.08.15	31.05.17	E212785	Blood
Mangatutu	30.09.15	31.05.17	E232357	Blood
Mangatutu	09.10.15	31.05.17	E232359	Blood
Mangatutu	09.10.15	31.05.17	E232360	Blood
Mangatutu	09.10.15	31.05.17	E232361	Blood
Mangatutu	11.10.15	31.05.17	E232369	Blood
Mangatutu	16.04.16	24.05.17	E232323	Blood
Mapara	12.05.16	24.05.17	E232327	Blood
Mapara	Oct-08	08.06.17	E67476	Feathers
Mapara	25.10.08	08.06.17	E67451	Feathers
Mapara	12.10.08	08.06.17	E190078	Feathers
Mapara	Oct-08	08.06.17	E67484	Feathers
Mapara	15.06.16	24.05.17	E232333	Blood
Mapara	15.06.16	24.05.17	E232334	Blood
Mapara	02.11.97	02.09.17	E187244	Blood
Mapara	01.11.97	02.09.17	E177065	Blood
Mapara	05.09.97	02.09.17	n/a (‘Shalimar’)	Blood
Mapara	01.11.97	02.09.17	E177098	Blood

Mapara	11.04.98	04.09.17	E177139	Blood
Mapara	15.09.15	31.05.17	E212798	Blood
Mapara	14.09.15	31.05.17	E212796	Blood
Mapara	08.09.15	31.05.17	E212790	Blood
Mapara	14.10.08	05.06.17	E131292	Blood
Mapara	14.10.08	05.06.17	E67455	Blood
Mapara	14.10.08	05.06.17	E67466	Blood
Mapara	05.11.97	05.06.17	E187222	Blood
Mapara	07.04.98	05.06.17	E131252	Blood
Mapara	06.01.98	05.06.17	E190085	Blood
Mapara	10.04.98	05.06.17	E196122	Blood
Mapara	18.12.97	05.06.17	E177103	Blood
Mapara	13.10.97	05.06.17	E190090	Blood
Mapara	08.04.98	08.06.17	E187213	Blood
Mapara	08.04.98	08.06.17	E196114	Blood
Mapara	12.04.98	08.06.17	n/a (‘Xerxes’)	Blood
Mapara	19.09.98	08.06.17	E190095	Blood
Mapara	03.03.99	08.06.17	E196133	Blood
Mapara	27.12.98	08.06.17	E196120	Blood
Mapara	10.09.98	08.06.17	E196126	Blood
Mapara	12.04.98	08.06.17	n/a (‘Fleaphry’)	Blood
Mapara	18.12.97	08.06.17	E187228	Blood
Mapara	09.09.15	31.05.17	E212793	Blood
Mapara	05.07.97	08.06.17	n/a (‘Shal__ar’)	Blood
Rotoehu (Bay of Plenty)	30.07.97	02.09.17	unbanded	Blood
Rotoehu (Bay of Plenty)	20.10.94	02.09.17	E127145	Blood
Rotoehu (Bay of Plenty)	29.08.98	04.09.17	E127115	Blood
Rotoehu (Bay of Plenty)	16.08.16	24.05.17	E221368	Blood
Te Urewera	28.12.98	02.09.17	n/a	Blood
Te Urewera	28.09.98	04.09.17	(T47)(10A)	Blood
Te Urewera	23.02.98	04.09.17	E189473	Blood
Te Urewera	11.02.98	04.09.17	E189469	Blood
Te Urewera	11.02.98	04.09.17	E189470	Blood
Te Urewera	18.02.98	04.09.17	E189471	Blood

Waipapa	20.06.17	28.08.17	E234101	Blood
Waipapa	21.06.17	28.08.17	E234103	Blood
Waipapa	25.06.17	28.08.17	E234107	Blood
Waipapa	25.06.17	28.08.17	E234109	Blood
Waipapa	27.06.17	28.08.17	E234111	Blood
Waipapa	03.07.17	28.08.17	E234114	Blood
Waipapa	02.08.17	28.08.17	E234117	Blood
Waipapa	04.08.17	28.08.17	E234118	Blood
Waipapa	04.08.17	28.08.17	E234119	Blood
Waipapa	05.08.17	28.08.17	E234120	Blood
Waipapa	01.09.98	08.06.17	E196124	Blood

Appendix 4: Boxplots showing the estimates of Heterozygosity by Locus (HL) with median, upper and lower quartile values, in six kōkako populations (Bay of Plenty: n=8, Hauturu: n=27, Mapara: n=44, Mangatutu: n=23, Te Urewera n=6, Waipapa n=11).



Appendix 5: Table depicting New Zealand species that have tested positive for PBFD (published). Includes source of infected individuals and Genbank accession numbers (where available).

Species	Source	Location	Accession number	Reference
Eastern rosella (*Platycercus eximius*)	Wild	Te Puke, Hutt Valley (Wellington), Auckland	**Hutt Valley & Te Puke:** n/a **Auckland:** JQ782196 to JQ782200 KF467251 to KF467254	**Hutt Valley & Te Puke:** Ha et al. 2007 **Auckland:** Massaro et al. 2012; Jackson et al. 2014
Yellow-crowned parakeet (*Cyanoramphus auriceps*)	Wild	Eglinton Valley (Fiordland)	JQ782201 to JQ782208	Massaro et al. 2012
Red-crowned parakeet (*Cyanoramphus novaezelandiae*)	Wild & Captive	**Wild:** Hauturu, Tiritiri Matangi Island	**Wild:** GQ396652 to GQ396656, GU936287 to GU936297, KM452734 to KM452744 **Captive:** n/a	**Wild:** Jackson et al. 2015, Massaro et al. 2012, Ortiz-Catedral et al. 2009 **Captive:** Ha et al. 2009
Sulphur-crested cockatoo (*Cacatua galerita*)	Wild & Captive	**Wild:** Turakina Valley	**Wild:** n/a **Captive:** AY148285 to AY148288, AY148290	**Wild:** Ha et al. 2007 **Captive:** Ritchie et al. 2003
Antipodes Island parakeet (*Cyanoramphus unicolor*)	Captive	-	-	Ha et al. 2009
Blue-streaked lorikeet (*Eos reticulate*)	Captive	-	AY148296, AY148297	Ritchie et al. 2003
Budgerigar (*Melopsittacus undulates*)	Captive	-	AY148301	Ritchie et al. 2003
Goldie's lorikeet (*Psitteuteles goldiei*)	Captive	-	AY148298	Ritchie et al. 2003
Long-billed corella (*Cacatua tenuirostris*)	Captive	-	AY148289	Ritchie et al. 2003
Rainbow lorikeet (*Trichoglossus haematodus*)	Captive	-	AY148294, AY148295, AY148300	Ritchie et al. 2003

Red collared lorikeet (*Trichoglossus haematodus rubritorquis*)	Captive	-	AY148291	Ritchie et al. 2003
Yellow-bibbed lorikeet (*Lorius chlorocercus)*	Captive	-	AY148292	Ritchie et al. 2003

Appendix 6: Table depicting non-psittacine species (and country) that have tested positive for PBFD (published). Includes source of infected individuals and Genbank accession numbers (where available).

Species	Source	Location	Accession number	Reference
Powerful Owl (*Ninox strenua*)	Wild	Australia (NSW)	KT008265 KY410369	Amery-Gale et al. 2017; Sarker et al. 2016
Rainbow bee eater (*Merops ornatus*)	Wild	Australia	KM823541 to KM823548	Sarker et al. 2015
Gouldian finches	-	Italy	JX131620	Circella et al. 2014
Tawny frogmouth (*Podargus strigoides*)	Wild	Australia	KY410377	Amery-Gale et al. 2017
Southern boobook owl (*Ninox boobook*)	Wild	Australia	KY410375 KY410378	Amery-Gale et al. 2017
Barn owl (*Tyto alba*)	Wild	Australia	-	Amery-Gale et al. 2017
Brown goshawk (*Accipiter fasciatus*)	Wild	Australia	KY410356	Amery-Gale et al. 2017
Australian magpie (*Cracticus tibicen)*	Wild	Australia	KY410350 KY410351 KY410352 KY410353	Amery-Gale et al. 2017
Australian raven *(Corvus coronoides)*	Wild	Australia	KY410354 DQ146997	Amery-Gale et al. 2017; Stewart, Perry & Raidal 2006
Australian white ibis (*Threskiornis moluccus*)	Wild	Australia	KY410355	Amery-Gale et al. 2017
Hardhead duck (*Aythya australis*)	Captive	Australia	-	Amery-Gale et al. 2017
Laughing kookaburras (*Dacelo novaguineae*)	Wild	Australia	KY410364 KY410365	Amery-Gale et al. 2017
Sacred kingfisher (*Todiramphus sanctus*)	Wild	Australia	-	Amery-Gale et al. 2017