
Long-term consequences of genetic rescue on island populations of South Island robins.

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Abstract

Population bottlenecks can lead to the loss of genetic diversity, including genetic variants that may be adaptive. In turn, small population size and isolation increases the chances that related individuals mate, and the risk of inbreeding depression and extinction. One method that can help sustain bottlenecked species is the translocation of individuals from genetically diverse populations into genetically depauperate populations to increase genetic diversity and fitness – a strategy known as genetic rescue. However, few studies have looked at the long-term effects of attempted genetic rescue and how often it leads to sustained increases in fitness. The objective of this thesis was to examine the long-term effects of attempted genetic rescue on two populations of South Island robin (*Petroica australis*), located on Motuara Island and Allports Island, at the northern end of New Zealand's South Island. Each population was established in 1973 with five birds, but by 1999, both populations were showing signs of inbreeding depression. In an attempt to alleviate the inbreeding, a genetic rescue attempt was made during 2008-2009. This rescue attempt was unusual in that it involved a reciprocal translocation of inbred donors. I assess both the dynamics of genetic change and fitness-related measures ranging from breeding success, survival, population size, immune system function and cognitive abilities, and when possible, compare these between at the start of the genetic rescue attempt to the present day. This broad approach is necessary as focusing on just a few life stages over a short time frame can underestimate inbreeding depression and the extent to which genetic rescue can lessen its effects.

Population-wide genetic diversity has changed over the 10 years since the translocations. Following the initial increase in genetic diversity in interbred birds in 2008-10, genetic diversity declined by 2016-18. However, this decline has been relatively slow; both expected heterozygosity (H_e) and allelic richness (A_r) are still higher than pre-translocation levels. Similarly, individual fitness as measured by a number of traits, remains higher in 2016-18 than before the rescue. In both populations, juvenile robins with higher genetic diversity were significantly more likely to survive to one year of age. Interbred F1-F3 individuals arising from the 2008-10 translocation were also significantly more likely to survive to 10 years of age than their pre-translocation counterparts. However, I found no clear evidence for correlations between genetic diversity and individual measures of breeding success within the current populations. The increased survival of both juveniles and F1-F3 interbred adults could increase lifetime reproductive success, and may explain the increase in population size on both islands since 2008-10.

I next assessed immune system function as possible drivers of increased survival. I artificially challenged robin with a phytohaemagglutinin (PHA) test, and measured ecto- and endo-parasite loads, and body morphology. I then assessed correlations between these traits with individual genetic diversity. Birds on both islands continued to show strong reactions to the PHA (as with the 2008-10 interbred individuals), but overall there was no correlation between genetic diversity and immune system response. Mite loads showed a significant inverse correlation with genetic diversity, but overall, there was only weak evidence that genetic diversity was correlated with parasite load and morphology.

Despite the potential impact of genetic diversity on fitness, few studies have investigated the relationship between genetic variation and intelligence. I assessed several cognitive measures in the robin populations, including male song structure and the performance of individuals in two experimental cognitive tasks. I found that genetic diversity affected some aspects of cognitive performance in the tasks, but not in song complexity.

My study confirms that the genetic rescue attempt has been successful in two South Island robin populations via reciprocal translocation of inbred donors. Increases in fitness were still evident 8-10 years later. To ensure the long-term viability of small populations, it is recommended that conservation management include genetic rescue, as well as traditional approaches of habitat restoration and predator control.



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General Introduction

Chapter 1

The Habsburgs, rulers of Spain from 1516-1700, sought to consolidate their power by the frequent use of consanguineous marriages. They were said to have a proverb that “The best spouse for a Habsburg is another Habsburg”. This resulted in a cumulatively deleterious effect on their gene pool. Marriages between first cousins, or between uncle and niece, were commonplace in the family (Fig. 1). As a result, the inbreeding coefficient scores for the Spanish Habsburg kings increased strongly along the generations, from $F = 0.025$ for King Philip I (founder of the dynasty), to $F = 0.254$ for Charles II, the last Spanish Habsburg (Fig. 1). Charles II had a genome comparable to that of a child born to a brother and sister mating, and several other members of the family also had inbreeding coefficients higher than 0.20 (Alvarez et al. 2009, Ceballos and Álvarez 2013). Charles II was infertile, physically and mentally disabled from birth, and described as "short, lame, epileptic, senile and completely bald before 35, always on the verge of death but repeatedly baffling Christendom by continuing to live” (Durant and Durant 2011), but eventually dying at 38 years of age. A study of 4,000 family members over 20 generations suggests that inbreeding directly led to the extinction of the Spanish Habsburgs by the end of the 17th century (Ceballos and Álvarez 2013).

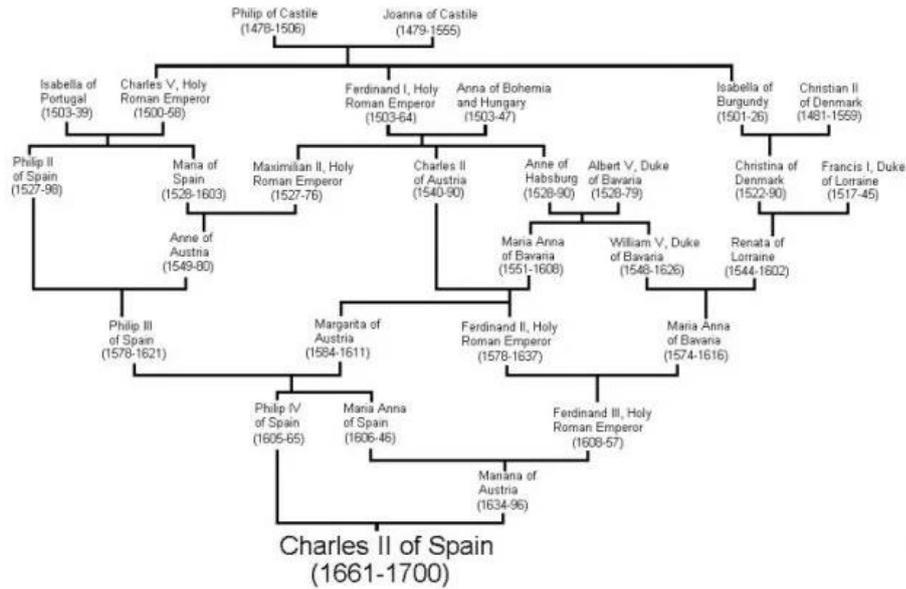


Figure 1.2. Spanish Habsburg family tree for Charles II of Spain showing close inbreeding between relations, eventually culminating in Charles II, the last of the Habsburgs. Figure from Lec CRP1 translated at English Wikipedia.

The extinction of the Habsburgs is not just a curious footnote of history but an analogy for a process now being played out across the natural world. Anthropogenic influences such as habitat destruction and fragmentation, over hunting, and predation from introduced pests and diseases, now affect an ever-increasing collection of species around the world. It is estimated that ~12% of the world’s bird species are now threatened with extinction (Birdlife International 2008). Consequently, many species are currently undergoing rapid population declines, and in some cases they are passing through severe population bottlenecks. Population bottlenecks can lead to the rapid loss of alleles, including those that may be adaptive (Frankham et al. 2002). This increases extinction risk by reducing evolutionary potential of the population (Barrett and Schluter 2008). In turn, small population size and isolation (due to habitat fragmentation or range restriction to island refuges) increase the chance that related individuals will mate, thereby increasing the risk of inbreeding (i.e., the “Habsburgs” problem).

Inbreeding can decrease fitness in two main ways, both of which are linked to reduced genetic diversity: (1) by decreasing allelic diversity across the genome, and (2) by increasing homozygosity across the genome (i.e., decreasing heterozygosity). Both can make individuals and populations less able to adapt to challenging circumstances (Caughley 1994). Increased homozygosity also increases the likelihood that recessive, deleterious alleles will be expressed. These deleterious alleles are often linked to negative phenotypic expressions (Frankham et al. 2002), such as reduced fertility and survival. Together, reduced allelic diversity and increased homozygosity can result in a reduction in fitness-related traits in the offspring of related individuals versus the offspring of unrelated individuals, a phenomenon termed “inbreeding depression” (Nei et al. 1975, Bashi 1977, Newman and Pilson 1997).

Ever since Darwin (1876), numerous studies have examined the potential negative consequences of inbreeding. Evidence suggests that population bottlenecks and subsequent inbreeding depression can affect a wide range of life history traits, from survival and immune system function to cognitive performance (Bashi 1977, Hale and Briskie 2007, Bellingham et al. 2010, Heber 2012, Frankham et al. 2014, Whiteley et al. 2015). Nevertheless, it is possible that a bottleneck event could have a positive effect on population wide fitness if it leads to genetic purging (Leberg and Firmin 2008). Genetic purging occurs when potentially lethal or semi-lethal alleles are eliminated from the population during a bottleneck (Frankham et al. 2002). In contrast, alleles of small effect will be much more difficult to purge because they are effectively invisible to selection (Charlesworth and Charlesworth 1987, Lande et al. 1999). Instead, these small-effect detrimental mutations are likely to become fixed in small populations and thus cause a further increase in the genetic load (Hedrick 1994). Even in a population of little spot kiwi (*Apteryx owenii*) which passed through a bottleneck of five individuals followed by another bottleneck of two, purging did not eliminate all the deleterious alleles that can lead to inbreeding depression (Taylor et al. 2017). As such, there

is large uncertainty regarding how purging in a bottleneck event will affect a population in relation to minimising inbreeding depression. Combined with the increased likelihood that purging depresses populations through increased fixation of low to mid-level deleterious alleles, it is generally considered likely to have an overall negative effect (Leberg and Firmin 2008, Taylor et al. 2017).

In theory, the negative effects resulting from population bottlenecks and inbreeding are not necessarily permanent and can be halted, or even reversed, and genetic variability restored through the introduction of new, relatively ‘outbred’ individuals into the genetically compromised population. When successful (i.e., resulting in an increase in population size), this is referred to as ‘genetic rescue’ (Westemeier et al. 1998, Vila et al. 2003, Madsen et al. 2004, Hedrick and Fredrickson 2010, Adams et al. 2011, Benson et al. 2011, Frankham et al. 2011, Weeks et al. 2011). If no population increase occurs, attempted genetic rescue could still be successful as a form of genetic restoration, especially if it results in positive outcomes for the rescued population, such as reduced genetic load and increased genetic diversity. Genetic rescue can thus be a cost-effective method at reducing genetic load, increasing genetic diversity and alleviating inbreeding depression (Tallmon et al. 2004, Frankham 2015, Whiteley et al. 2015).

Genetic rescue is not without its risks and could have deleterious consequences. For example, if genetic divergence between source and donor populations is great, translocating individuals from one population to another may lead to a reduction in fitness due to outbreeding depression, where co-adapted gene complexes became disrupted in subsequent generations (Edmands 1999, 2007, Frankham et al. 2012). Such negative effects may not be detected until several generations have passed. Furthermore, theoretical models suggest that if a rescued population remains small (i.e., population growth is restricted due to lack of suitable habitat), genetic variation can again be eroded and inbreeding depression increased

(Foose 1993). This situation could be exacerbated if additional deleterious alleles were introduced into the population by the donors (Bijlsma et al. 2010). Finally, even if genetic rescue proves beneficial in the long term, there is the risk that the higher fitness of interbred or hybrid individuals could result in swamping genetic variants in the recipient population (Hedrick 2005) and lead to greater between population genetic homogenisation, which will reduce the genetic variability within a species or among populations (Olden et al. 2004).

An extensive review of 156 studies of genetic rescue attempts found that 145 (93%) resulted in increased fitness benefits for the recipient population (Frankham 2015). For example, increased population growth and interbred/hybrid fitness was found in mountain pygmy possums (*Burramys parvus*) (Weeks et al. 2017), increased population growth and individual fitness in Florida panthers (*Puma concolor cougar*) (Johnson et al. 2010), increased litter sizes, survival and immune response in African lions (*Felis leo*) (Trinkel et al. 2010, Trinkel et al. 2011), increased reproductive success, survival, and five fitness-related traits in bighorn sheep (*Ovis canadensis*) (Hogg et al. 2006, Miller et al. 2012), and increased population growth in adders (*Vipera berus*) (Madsen et al. 2004). Other studies have observed genetic restoration following genetic rescue attempts, such as observed with greater prairie-chickens (*Tympanuchus cupido pinnatus*) (Bateson et al. 2014), and with the introduction of a single male wolf (*Canis lupus*) into a population on Isle Royale in Lake Superior, which resulted in an increase in population-wide levels of genetic diversity (Adams et al. 2011). In spite of these successes, genetic rescue for conservation purposes has remained underused and practical applications of attempted genetic rescues have often been short term, with the recipient population monitored for only a few years after the introduction of donors (Tallmon et al. 2004). Few studies have monitored the effects of genetic rescue over the long term or for more than a few generations (Whiteley et al. 2015, Ralls et al. 2018).

Monitoring the outcomes of attempted genetic rescue in wild populations over long periods of time raises several practical problems. Aside from a lack of funding for long-term studies, problems arise in the ability to accurately monitor trends in genetic diversity and individual/population wide fitness correlations over time. One option is to use genetic marker-based estimators of population-wide genetic diversity, which can be calculated from microsatellites and/or single nucleotide polymorphisms (SNPs). Individual and population-wide fitness correlations (e.g., heterozygosity fitness correlations (HFCs)), can also be used to see how rescue events affect the fitness of both individuals and populations.

Heterozygosity fitness correlations are a common method in conservation genetics (Hansson and Westerberg 2002, Coltman and Slate 2003, Chapman et al. 2009, Miller and Coltman 2014) to provide an estimate of genome-wide heterozygosity, which itself represents the individual level of inbreeding and will predict fitness if the fitness trait being measured is susceptible to inbreeding (Hansson and Westerberg 2002). Such correlations can be used as evidence of inbreeding depression and can be especially useful in situations where direct measures of inbreeding (such as from a pedigree) are not available, as is often the case for wild populations (Grueber et al. 2008, Ruiz-López et al. 2012).

The corresponding decrease in heterozygosity at neutral markers (e.g. microsatellites), due to consanguineous mating, has led to studies on the effects of inbreeding through HFCs (Grueber et al. 2008). This is the correlation between heterozygosity of markers and fitness components (e.g. survival or breeding success). However, the capacity of microsatellite loci to accurately estimate individual inbreeding has been questioned, as studies have indicated that molecular estimates of heterozygosity do not always correlate well with local versus general effect (Slate et al. 2004, Szulkin et al. 2010). The statistical power of a microsatellite data base will depend on the number of markers, the variability of the loci selected, and the variance in inbreeding in the individuals sampled (Grueber et al. 2008). Tests such as

heterozygosity-heterozygosity correlations (HHC) and the g^2 test can be used to assess the statistical power of microsatellites for determining HFCs attributable to inbreeding depression and their accuracy (David et al. 2007, Szulkin et al. 2010, Stoffel et al. 2016). Given a powerful enough microsatellite panel, HFCs can be a useful tool in examining whether there is a correlation between genome-wide heterozygosity and fitness traits (Bichet et al. 2018). SNPs would ultimately allow more power to detect HFCs, but this is not always a cost-effective tool for conservation projects where large panels or microsatellites have already been developed for the project or where the objective is to compare to previously collected data were run using microsatellites as part of a long term study. This is the case in this study.

In many populations, dispersal (especially by juveniles) lessens the probability that inbreeding will occur and thereby results in inbreeding avoidance without the need for mating bias against relatives during post-dispersal life stages (Part 1996, Szulkin and Sheldon 2008, Lebigre et al. 2010). This outcome arises irrespective of whether dispersal evolved due to selection for inbreeding avoidance or due to other forces such as local resource availability or kin competition (Pusey 1987, Lehmann and Perrin 2003). However, when dispersal is limited or constrained, mechanisms to avoid inbreeding could evolve through selective mate choice among philopatric relatives (Blouin and Blouin 1988, Reid et al. 2005, Jamieson et al. 2009). In theory, random mating would normally result in low rates of close inbreeding, so it is thought the pressure to evolve kin recognition and avoid inbreeding would be negligible (Jamieson et al. 2009). Consequently, many threatened species are unlikely to have a natural built-in mechanism for avoiding close inbreeding (Jamieson et al. 2009). As many endangered native species in New Zealand are now confined to small refuges (typically offshore islands), the opportunities for dispersal are more limited than would have been the case prior to human arrival. Coupled with the potential lack of a built-in mechanism for

avoiding mating with kin, inbreeding and inbreeding depression is often widespread in isolated populations of threatened species (Keller and Waller 2002, Frankham 2015).

Until recently, it was thought that threatened bird species in New Zealand might be less susceptible to inbreeding depression than species elsewhere (Craig 1991, 1994), in part because of repeated bottlenecks and genetic purging. Craig's (2000) review on conservation issues in New Zealand summarised inbreeding in New Zealand's small populations with only one sentence: "New Zealand's wildlife might be less susceptible to inbreeding depression than species elsewhere". This conclusion stems from earlier work that questioned whether genetic models and associated management guidelines derived for threatened species in continental regions of the Northern Hemisphere apply to species from small, insular populations in places like New Zealand that may have undergone prior genetic purging (due to the small number of founders and the naturally reoccurring small population size of many species). It is important to note that the assumption that bottlenecked populations have been purged of deleterious recessive alleles has not been verified empirically and Craig's (2000) conclusion are now at odds with recent views (Byers and Waller 1999, Keller and Waller 2002, Jamieson 2009, Innes et al. 2010). Furthermore, a review by Jamieson (2015) found no evidence for Craig's (1991, 1994) hypothesis. For example, despite a history of repeated bottlenecks, little spotted kiwi still retain a high genetic load, with between 10 and 15 diploid lethal equivalents acting on survival and recruitment into the adult population in at least one location (Taylor et al. 2017). This is consistent with an average of 12.3 lethal equivalents found in other wild populations suffering from inbreeding depression (O'Grady et al. 2006).

At present, there is no reason to believe that birds in New Zealand or other insular populations are less susceptible to inbreeding depression than those elsewhere. Many of New Zealand's surviving bird species have recently undergone population bottlenecks and persist only as small isolated populations, typically on offshore islands that are free of introduced

mammalian predators (Jamieson 2015). A number of studies have confirmed that population bottlenecks in New Zealand birds decrease genetic diversity (Robertson 2006, Sainsbury et al. 2006, Boessenkool et al. 2007, Jamieson 2015, Taylor et al. 2017), and that this in turn, leads to reduced fitness, such as reduced fertility and survival (Jamieson and Ryan 2000, Briskie and Mackintosh 2004, Mackintosh and Briskie 2005, Boessenkool et al. 2007, Taylor et al. 2017). Although case studies in New Zealand so far show that such inbred populations can recover (Briskie and Mackintosh 2004, Hooson and Jamieson 2004, Boessenkool et al. 2007, Jamieson 2007), the low hatching success or reduced juvenile survival associated with such populations (Jamieson 2007) means that their recovery in the short term is lower than that potentially possible if the population had not been bottlenecked. A recent summary (Environment Aotearoa 2019) paints a grave future for New Zealand's native biota, with the extinction risk increasing for 86 species in the past 15 years.

The need for understanding the benefits and pitfalls of genetic rescue is particularly acute in New Zealand, where a third of extant bird species are now endangered (Holdaway et al. 2001, Robertson et al. 2013). Many of these endangered birds survive only as small populations on predator-free islands or in small and intensively managed populations on the mainland (Robertson et al. 2013). Even species that have recovered through management may exhibit signs of inbreeding depression such as increased hatching failure, infertility, compromised immune function, and low juvenile survival (Briskie and Mackintosh 2004, Hale and Briskie 2007, Heber et al. 2013). Decreased genetic diversity also threatens the viability of populations in the long term if it reduces their ability to adapt to threats such as new diseases, climatic changes, invasive species, or increased competition. For example, studies have demonstrated that South Island robins (*Petrocia australis*) in severely bottlenecked populations show a reduced immune response in comparison to more genetically diverse conspecifics (Hale and Briskie 2007, Bellingham et al. 2010, Heber

2012). It has also been suggested that several disease outbreaks in birds in New Zealand were likely driven by inbreeding and reduced immune response (Bellingham et al. 2010), including the premature death of three female kākāpō (*Strigops habroptilus*) (Ballance 2007), high mortality of translocated hihi (*Notiomystis cincta*) (Armstrong et al. 2007), and the increased susceptibility of South Island saddlebacks (*Philesturnus carunculatus*) to disease (Hale and Briskie 2009). Given the practical difficulties and expense of controlling introduced predators on the main islands of New Zealand, it is likely that most native species will remain restricted to small, isolated island populations for the foreseeable future.

Unfortunately, traditional genetic rescue using outbred donors is not always possible for many threatened species, as there are often no longer any relatively outbred individuals available to use as donors. This is an especially common problem in New Zealand and combined with the restriction of many endangered species to small islands, which set a cap on population growth, genetic rescue attempts are likely to be difficult and might mean that genetic restoration is a more likely outcome. For example, the South Island saddleback and little spotted kiwi only survive as a few genetically depauperate populations on offshore islands as their mainland populations went extinct through predation from introduced mammals. This is also the case for kākāpō and takahē (*Porphyrio mantelli*) (Jamieson and Ryan 2000, Briskie and Mackintosh 2004). Similar problems are found elsewhere in the world, such as the confinement of animal populations within enclosed game reserves in South Africa that allow little or no dispersal and put limits on the size of the populations in each reserve. This creates a high risk of inbreeding in these fragmented, fenced and isolated populations, which may be compounded by a lack of management (Trinkel et al. 2010). Thus, understanding the practical and theoretical consequences of genetic rescue in New Zealand will be essential for assessing both its usefulness and its limitations as a tool for the management of endangered species and wherever small populations are at risk of extinction.

Inbreeding and genetic rescue of the South Island robin

This study focuses on two isolated island populations of South Island robins. South Island robins are small (35 g) passerines that are endemic to New Zealand. Their population is considered at risk and declining, but they can be found throughout much of the South Island in mature forest habitat (Heather and Robertson 2000). Robins exhibit a moderate degree of sexual dimorphism; adult males have a dark-grey head and body with a sharply defined white to yellowish-white breast and belly and are slightly larger (5 g on average) than females. Females are a lighter grey with a less distinct white breast (Heather and Robertson 2000). Robins spend much of their time foraging on the forest floor feeding on insects and other invertebrates. They form socially monogamous pair bonds, and males are territorial and often hold the same territory over multiple years (Heather and Robertson 2000).

I studied two populations of South Island robins, located on Motuara Island and Allports Island, at the northern end of New Zealand's South Island (Fig. 1.3). Each population was established in 1973 with five birds. The Motuara population was founded with five birds from Nukuwaiata Island in Pelorous Sound and the Allports Island population was founded with five birds from a mainland source population near Kaikoura (Fig 1.3) (Ardern et al. 1997). Despite the small number of founders and small size of the islands, both populations increased steadily after their introduction, with 56 adults reported on Allports Island and an estimated 250 adults reported on Motuara Island by 1989 (Maloney 1991). By 2008-2010, ~60 adults (3.75 birds/ha) were present on Allports Island and ~300 adults (5.01 birds/ha) on Motuara Island. Both populations appear to have maintained similar densities for 20 years, suggesting that both populations were reaching carrying capacity by 1989 (Heber 2012, Maloney 1991). Both populations also exceeded densities of mainland sites. For example in Kowhai Bush, the non-bottlenecked source population for Allports Island, had an estimated density of only 0.75-0.84 birds/ha in 1980 (Gill 1980, Maloney 1991), and this

population has since declined further due to predation (Heather and Robertson 2000). Both islands in this study are located a minimum of 1.4 km from the nearest shoreline in the Queen Charlotte Sound. Coupled with few to no robin populations in the surrounding mainland areas (Robertson 2007), and the low capacity of robins to sustain flight over long distances (Taylor et al. 2005, Boessenkool et al. 2007), the two island populations are considered relatively closed (i.e., genetically isolated) systems. After the initial introduction of robins onto both islands the populations were then left unmanaged for 35 years with no human-assisted migration or gene flow. This isolation was confirmed by subsequent genetic analyses showing low genetic variation in each population (Ardern et al 1997, Heber et al. 2013).

By 1999, both the Motuara and Allports islands populations of South Island robin were showing signs of inbreeding depression, including reduced hatching success, reduced sperm quality and impaired immune system function (Byrne 1999, Mackintosh and Briskie 2005, Hale and Briskie 2007, Heber et al. 2013). In an attempt to alleviate the inbreeding thought to be occurring in each population, a genetic rescue attempt was made during 2008-2009. This rescue attempt was unusual in that it involved a reciprocal translocation of birds between Motuara Island and Allports Island, each of which were thought to be relatively inbred due to the severe bottlenecks experienced when founded. The rationale for using donors from bottlenecked populations (rather than outbred donors) was that, although each population was known to be genetically depauperate, the genetic composition of each population would possibly differ as they were created from different founder stock. Thus, switching birds between populations could introduce new genetic diversity and possibly lead to genetic rescue. A total of 31 female robins were translocated between Allports and Motuara islands, with 15 females translocated from Allports Island to Motuara Island, and 10 females translocated from Motuara to Allports in 2008. Additional translocations of three females from each island was carried out in 2009. This ensured that more than five females

were recruited into each recipient population (Heber 2012). The translocation of only females was undertaken to force pairings between donor females and resident males (i.e., to induce ‘interbred’ pairings).

To obtain estimates of genetic variability in each island population prior to the reciprocal translocation (2008), and in the two years that followed (2009 and 2010), Heber et al (2013) collected blood samples from 658 individuals across the two islands (488 on Motuara and 170 on Allports). All birds were fitted with individually identifiable unique metal and colour bands. Comparisons of genetic diversity and fitness traits were then made between pure bred pre-translocation birds and interbred during translocation birds (i.e., F1-F3 interbred birds). Individuals were considered F1-F3 interbred birds if they were progeny of a translocation pair, defined as offspring from a translocated female with a male native to the island or if either one or both parents were interbred F1 or F2 birds. Individuals that were not from translocation couples were still considered pure breed birds. After the attempted rescue, F1-F3 interbred generations showed greater genetic diversity, survival, immunocompetence, and sperm quality than the inbred birds (Heber 2012, Heber et al. 2013). Thus, at least in the short-term, this genetic rescue experiment using inbred donors had led to a degree of genetic restoration and a decrease in some measures of inbreeding depression. It was not clear, however, whether the long-term implications of the reciprocal translocation would be sustained restoration, true genetic rescue, or a degree of outbreeding depression.

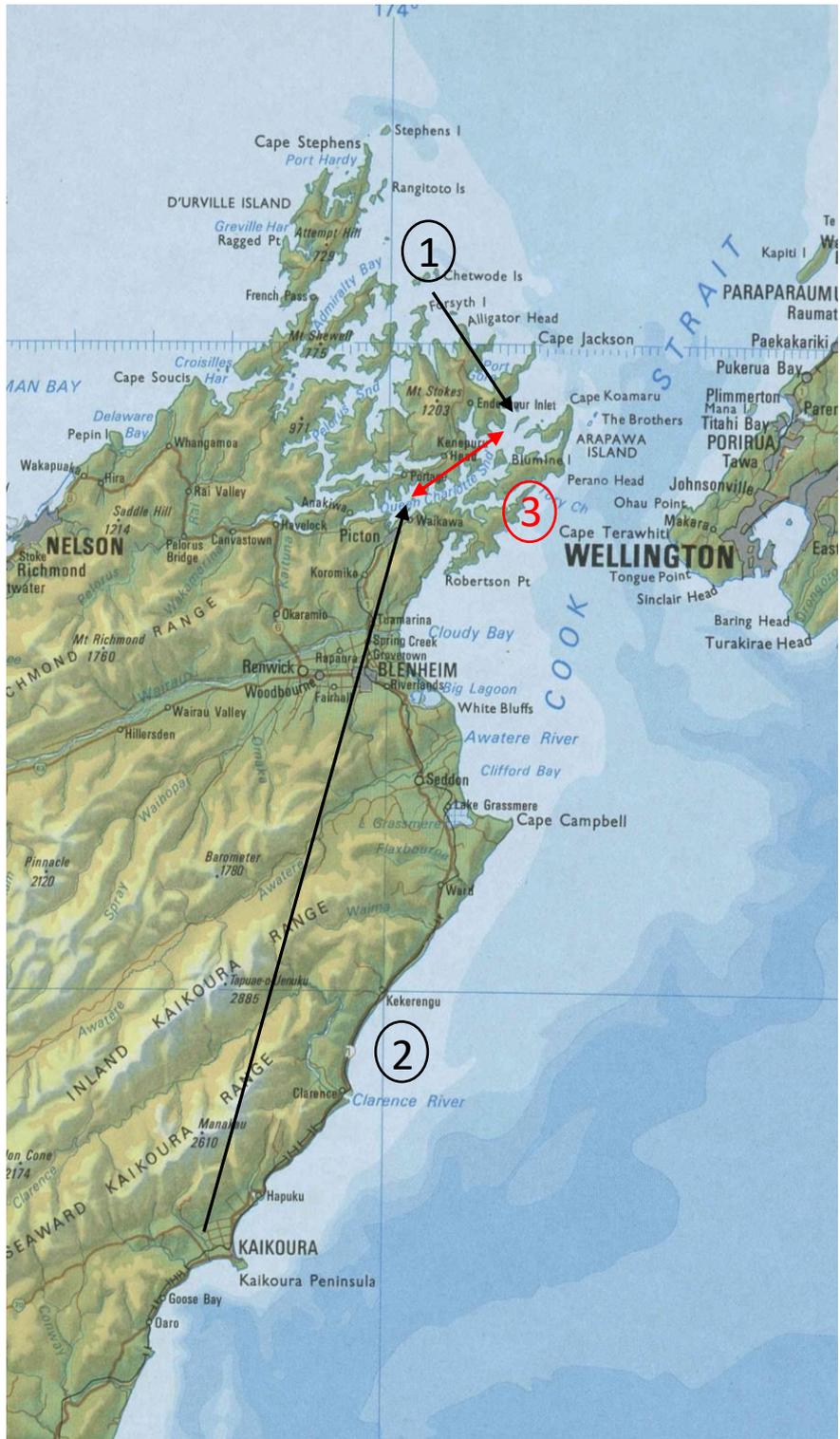


Figure 1.3. A map of the northern end of the South Island showing the locations of the study islands and the source populations of South Island robins. Number one shows the transfer of robins from Nukuwaiata Island to Motuara Island in 1973. Number two shows the transfer of robins from Kaikoura to Allports Island in 1973. Number three, marked in red, shows the reciprocal translocation between Motuara Island and Allports Island in 2008-2009. Map was downloaded from Land Information New Zealand - Toitū te whenua:

<https://www.linz.govt.nz/>.

Objectives of the thesis

At the start of this study, eight years had elapsed since the initial reciprocal translocation by Heber et al. (2013). During this time, only limited monitoring of either population had occurred. This presented an excellent opportunity to study the mid to long-term effects of attempted genetic rescue on two small, isolated populations. By using data collected for this project (2016 to 2019) and that collected during the initial attempted genetic rescue effort (2008 to 2010), I examined the effects of the reciprocal translocation event on South Island robins in their genetic diversity, population size, and a range of phenotypic traits.

In my thesis, I take two approaches to studying the effects of the genetic rescue attempt on the two robin populations. First, when possible, I compare changes that have occurred on a longitudinal time scale, comparing genetic and fitness measures I made with those collected previously in the 2008-2010 study. Second, I examine how current genetic variation in the populations might be affecting individual fitness of robins. Together, my overall objective is to determine the fitness consequences of this attempted genetic rescue as a guide to assess its effectiveness as a key tool for the management of threatened birds.

Chapter two of this thesis is a co-authored paper (*unsubmitted*) and was written by my assistant supervisor, Dr Helen Taylor. This chapter is designed to give context and background for the rest of the thesis by presenting as yet unpublished data that assesses how the genetic makeup of the two focal populations in this thesis has changed over the past decade. The chapter also compares genetic diversity in the two focal sites for this study with their respective source populations and two unrelated mainland populations of South Island robins.

In chapter three, I focus on fitness components related to reproductive success of the two focal populations, and how the attempted genetic rescue has affected survival both over the short term and long term, and the effect it has had on the population demographics.

In chapter four, I investigate whether the positive benefits of increased genetic diversity are correlated with immune system response, parasite burdens and morphology in the two robin populations.

In chapter five, I examine whether individual genetic diversity influences a range of cognitive functions such as song structure, motor control, inhibitory control, executive functions and learning.

Finally, in chapter six, I summarise the main findings of my study and evaluate the effect of the reciprocal translocation on the robin populations over time. I conclude by assessing the value and future of using genetic rescue as a management tool in the conservation of threatened species.

Note on contributions of work for each chapter

Chapter two was written by my assistant supervisor, Dr Helen Taylor, and is a large multi-lab collaborative paper; I was responsible for collecting the blood samples, formatting collected data and undertaking some of the statistical analyses, but the bulk of the analyses were conducted by Dr Taylor, the DNA extractions were conducted in the Gemmell Lab at the University of Otago, and the sequencing of the samples and scoring of the microsatellites were conducted at the Kempenaers lab at the Max Planck Institute for Ornithology in Seweisen, Germany. When this work is published, I will be one of the junior authors.

For chapters 1, 3, 4, 5 and 6, I was responsible for collecting all the field data, all analyse of data, and the writing of these chapters, with support from my supervisors. When the work from these chapters is published, I will be first author.

Chapter layout

All research chapters (2-5) were written to be standalone papers, as such, there is some repetition between the chapters in the methods.

Glossary

Below is a list of terms commonly used throughout this thesis;

Purebred pre-translocation individuals – these birds are descended from the original 1974 founders and have not received any new alleles since. In Heber (2013) and in parts of this thesis these individuals are referred to as inbred birds.

Pre-translocation pure bred breeding pairs – these breeding pairs comprise males and females that are purebred pre translocation individuals and are considered native to their island. Their progeny would be purebred birds.

Translocated breeding pairs – these breeding pairs included a female in the pair who is not native to its resident island or the breeding pair is formed with at least one F1-F2 interbred individual.

F1-F3 interbred individuals – these individuals are descendants between translocation breeding pairs and pairs that formed with at least one F1-F2 interbred individual in the pair. In Heber (2013) they are referred to as hybrid F1-F3 individuals and share a mixture of alleles from the two islands.

Post translocation individuals – these birds are of an unknown heritage, hatched post 2010, and may or may not comprise a mixture of alleles from both islands.

Post translocation breeding pairs – these pairs formed post 2010 and are from a mixed and unknown heritage that may or may not include mixed genetic material from both islands.

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Long-term genetic consequences of a reciprocal translocation between two inbred populations of South Island robins/kakaruwai

Chapter 2

Helen R. Taylor, A.E.T.MacFarlane, B.Kempenaers, S. Heber, J.V.Briskie

Note from main author: This chapter is intended to provide background and context to the rest of the work conducted in this thesis. It also details how microsatellite genotype data for this thesis was generated. As such, the introduction and discussion are brief and cover only what is necessary to outline the study design for this chapter and interpret the results. The wider themes of population bottlenecks, reduced genetic diversity, and genetic rescue are touched on briefly here, but covered in much greater detail elsewhere in this thesis.

2.1 Introduction

The original genetic study of South Island robins/kakaruwai on Motuara and Allports Islands found that genetic diversity on both islands increased following a reciprocal translocation of birds between the two islands (Heber et al. 2013). This was despite the fact that birds on each island were suspected to be highly inbred due to each closed, island population having been founded with just five individuals in 1973 (Armstrong 2000). The improvements in genetic diversity and fitness seen in admixed birds resulting from the reciprocal translocation suggested that, while each population was thought to be inbred, they were genetically divergent, having been founded with individuals from two relatively geographically distant populations (Figure 2.1). This innovative study provided an exciting hint that inbred donors could potentially be used for genetic rescue when no outbred populations were available.

Though valuable, the original study did not have access to samples from the source populations for either the Motuara or Allports Island South Island robin/kakaruwai populations (Nukuwaiata Island and Kaikoura, respectively) or from any other relatively outbred South Island robin/kakaruwai population. Thus, it has not previously been possible to estimate how much genetic diversity might have been lost by founding the Motuara and Allports Island populations with just five birds, how genetically different these populations were and how much genetic diversity was restored relative to the source populations following the reciprocal translocation event.

Theory would predict that, if the source populations of Nukuwaiata Island and Kaikoura were relatively genetically diverse, then five birds would not have been enough to capture this diversity and at least some genetic diversity would have been lost when the Motuara and Allports populations were founded (Luikart and Cornuet 1998). Depending on how genetically divergent the source populations were originally, transferring birds between Motuara and Allports Islands might have increased genetic diversity, but may not have

restored the alleles that were lost when each island was founded with five birds. Finally, given that both Motuara and Allports Islands returned to being closed populations after the reciprocal translocations, with no known migration from any other population, we would expect that genetic diversity in each population might have eroded over time. The degree to which this has occurred will depend on the effective population size and number of generations that have passed in each case (Allendorf et al. 2013, pp 101).

Thanks to the sampling conducted subsequently in the source populations for Motuara and Allports Islands, in other mainland South Island robin/kakaruwai populations, and of the modern day Motuara and Allports Island populations themselves, it is now possible to ask the following questions:

1. How much genetic diversity was lost when the Motuara and Allports Islands South Island robin/kakaruwai populations were founded compared to their respective source populations?
2. How much (if any) of this genetic diversity was restored via the reciprocal translocation of birds between Motuara and Allports Islands?
3. Have the positive genetic consequences that directly followed the reciprocal translocations persisted over the past 10 years?

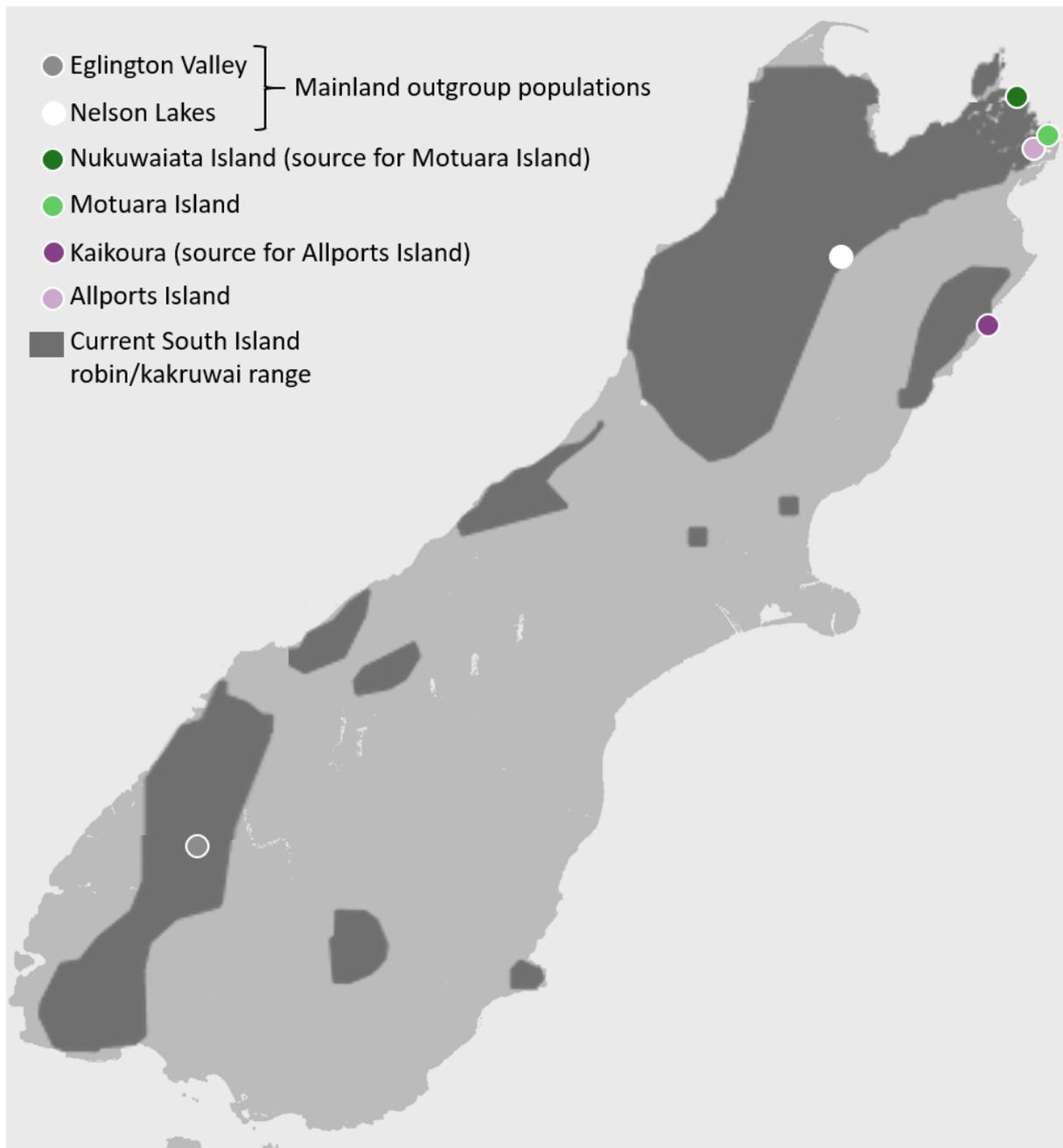


Figure 2.1: Map of the South Island of New Zealand showing sampling locations for this study. Current range of South Island robin/kakaruwai taken from Birdlife International (2016).

2.2 Methods

2.2.1 Sample sets and collection

Genetic samples for this study were collected from six locations (Figure 2.1; sample size in Table 2.1). These sites consist of two mainland populations included as reference

sample sets (Eglington Valley and Nelson Lakes) and two pairs of sites consisting of a source population and the population founded with individuals from that source (Nukuwaiata Island and Motuara Island, Kaikoura and Allports Island).

Two of these locations (Motuara Island and Allports Island) were sampled over two time ranges: 2008-10 and 2016-18. Samples from 2008-10 for each site are divided into purebred individuals, and interbred individuals that resulted from the reciprocal translocation event. Individuals were considered F1-F3 interbred birds if there were progeny of a translocation pair, defined as offspring from a translocated female with a male native to the island or if either one or both parents were F1 or F2 interbred bird resulting from the prior translocated pairings.

The other four sites were sampled at one time point/range; Eglington Valley 2010, Nelson Lakes 2010-12, and Nukuwaiata Island and Kaikoura in 2014. Dividing the data set by geographical location, time and, in the case of Motuara and Allports 2008-10, purebred and interbred individuals, gives 10 sample sets for comparison of genetic diversity and genotypic composition (Table 2.1).

All blood samples were collected via brachial venepuncture. Approximately 10 – 30 ml of blood was stored in 1 ml of Queen's Lysis Buffer (0.01 M Tris-HCl, 0.01 M NaCl, 0.01 M Na-EDTA (pH 7.5), 1% (v/v)n-lauroylsarcosine; pH 7.5) at room temperature or 4°C.

2.2.2 DNA extraction and genotyping

DNA was extracted from blood samples using either an E.Z.N.A. Blood DNA Kit D3392 (Omega Bio-Tek) (2008-10 samples only) or a NucleoSpin® Blood QuickPure DNA Kit (Machery-Nagel) (all other samples) according to the manufacturers' instructions. All PCR and microsatellite sequencing were conducted in the Kempnaers lab at the Max-Planck Institute for Ornithology according to the protocols detailed in Heber et al. (2013). To ensure

comparisons could be made between more recent samples, and those collected in 2008-10, we selected the same 28 microsatellite loci used in the final analysis in Heber et al. (2013). Microsatellite genotypes for all samples were scored in Kempenaers lab, again ensuring consistency of scoring across all samples sets taken at different time points.

2.2.3 Analyses

We used Genepop (Raymond and Rousset 1995) to assess deviation from Hardy-Weinberg proportions for each locus within each sample set and to test for gametic disequilibrium between pairs of loci in each sample set. In both cases, we adjusted for conducting multiple tests of significance using a sequential Bonferroni correction (Holm 1979). We calculated expected and observed heterozygosity (H_e and H_o), allelic diversity and richness (A and A_r), F_{IS} , F_{ST} , and the associated bootstrapped 95% confidence intervals in the R package HIERFSTAT (Goudet 2005). We visualised differences in genotypic composition among sample sets using a Principal Components Analysis (PCA) in R via the package adegenet (Jombart 2008). We also used BAYESIAN clustering in the programme STRUCTURE (Pritchard et al. 2000, Falush et al. 2003, 2007, Hubisz et al. 2009) to assess differences in the genotypic structure of sample sets and changes in this composition over time on Motuara and Allports Islands. We ran STRUCTURE for K values of 1-10 using the admixture model with correlated allele frequencies and performed 10 replicate runs of 3×10^6 Markov Chain Monte Carlo (MCMC), after an initial burn-in period of 10^6 repetitions. We summarised the STRUCTURE results using STRUCTURE HARVESTER (Earl and vonHoldt 2012). We used CLUMPAK (Kopelman et al. 2015) to average cluster probabilities for the 10 runs for each value of K and visualise the results.

2.3 Results

In total, we genotyped 1,236 South Island robins/kakaruwai at 28 microsatellite loci across 10 sample sets. No loci were found to be out of Hardy-Weinberg proportions in more than two of the 10 sample sets and no pairs of loci were found to be in gametic disequilibrium across all 10 sample sets. Considering this, and prior mapping of loci to the zebra finch genome to assess probability of physical linkage (Heber et al. 2013), all 28 loci were retained for subsequent analyses.

Four of the 10 sample sets had F_{IS} values that were significantly different from zero, suggesting these populations to be out of Hardy-Weinberg proportions (Table 2.1 and Figure 2.2A). Nelson Lakes and the Motuara purebred 2008-10 sample sets exhibited a deficit of heterozygotes whereas both the Motuara and Allports 2008-10 interbred sample sets exhibited an excess of heterozygotes.

Table 2.1. Change in genetic variation in South Island robin/kakaruwai populations following reciprocal translocations for the purposes of genetic rescue based on 28 microsatellite loci. N = number of birds genotyped. Mainland outgroups presented for comparison. Percent change in H_e is relative to the respective source population. Percentage change in () is relative to previous listed sample set. A_r = allelic richness.

Group	Sample set	N	Mean H_e	% Change H_e	Mean F_{IS}	Mean A_r
Mainland outgroups	Eglington Valley	23	0.63	NA	0.02	4.9
	Nelson Lakes	108	0.64	NA	0.03*	6.1
Motuara Island Group	Nukuwaiata Island (Source)	50	0.57	NA	0.00	3.8
	Motuara Purebred 2008-10	443	0.49	-13.8%	0.02*	2.9
	Motuara Interbreds 2008-10	30	0.55	(+10.5%)	-0.10*	3.6
	Motuara Post translocation 2016-18	274	0.52	-8.8% (-5.5%)	0.01	3.4
Allports Island Group	Kaikoura (Source)	21	0.57	NA	-0.02	4.6
	Allports Purebred 2008-10	112	0.45	-22.1%	-0.03	2.7
	Allports Interbreds 2008-10	43	0.54	(+21.5%)	-0.11*	3.6
	Allports Post translocation 2016-18	132	0.53	-7.0% (-2.7%)	0.00	3.5

*Significant departure from Hardy-Weinberg Proportions – F_{IS} significantly different from zero determined by bootstrapped 95% confidence intervals

2.3.1 Genetic diversity

Overall, genetic diversity (both H_e and A_r) was highest in the mainland outgroup populations (Table 2.1 and Figure 2.2B and C). The lowest genetic diversity was found in the purebred birds from the 2008-10 sample sets taken from Motuara and Allports Islands. Purebred robins from both Motuara and Allports Islands showed lower genetic diversity than their respective source populations, Nukuwaiata Island and Kaikoura (13.8% and 22.1% decreases in H_e , respectively). As presented in previous work on the Motuara and Allports populations (Heber et al. 2013), the interbred individuals resulting from the reciprocal translocation between the two islands show greater genetic diversity than their purebred counterparts from the same time period.

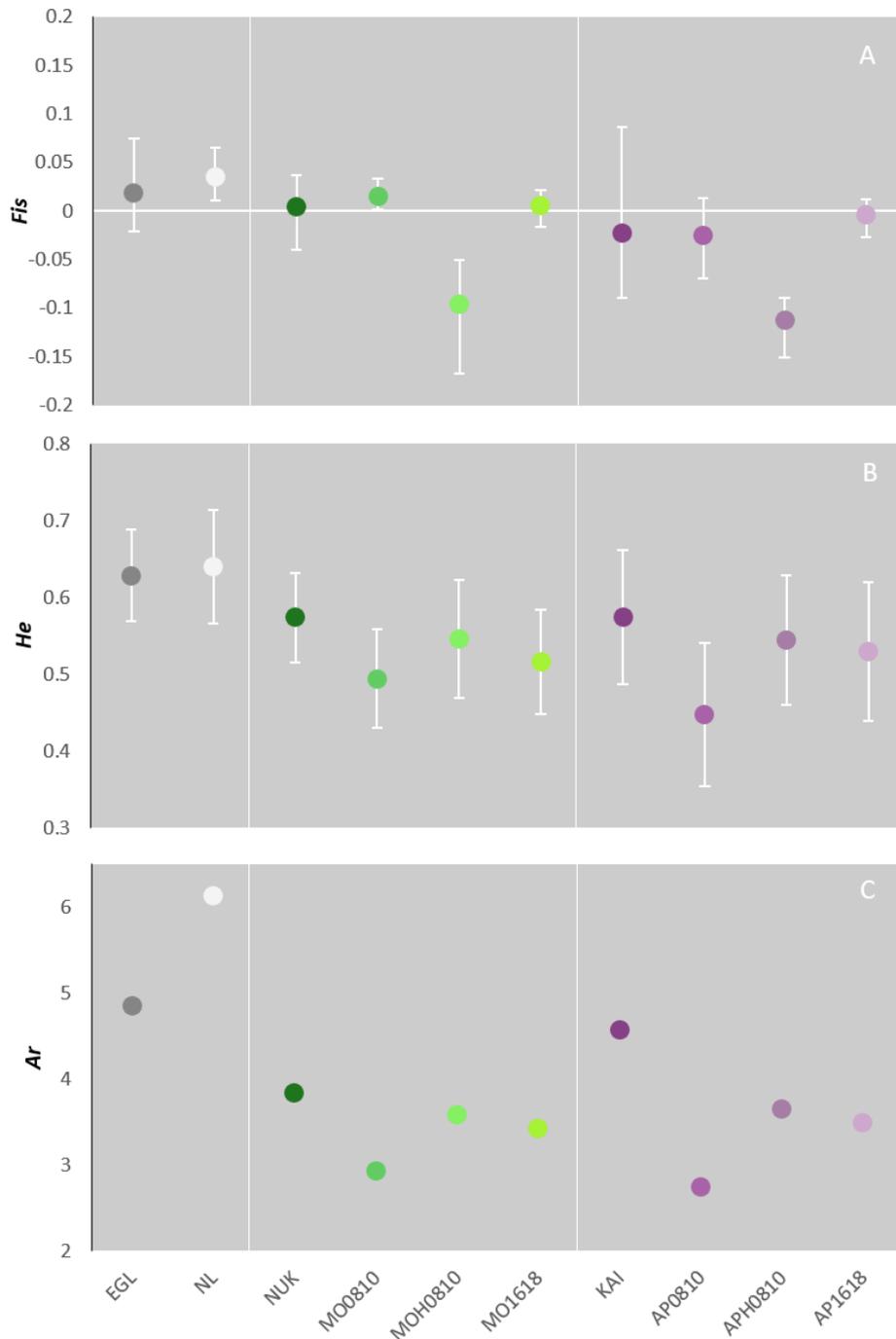


Figure 2.2: Change in genetic variation in South Island robin/kakaruwai populations following reciprocal translocations for the purposes of genetic rescue based on 28 microsatellite loci. A = F_{is} ; B = expected heterozygosity; C = allelic richness. In A and B, error bars represent 95% confidence intervals. In all cases, populations are coded as follows: Mainland outgroups: EGL = Eglington Valley; NL = Nelson Lakes; Motuara Island data set: NUK = Nukuwaiata Island (source); MO0810 = Motuara Island purebred birds sampled in 2008-10; MOH0810 = Hybrids of crosses between Motuara Island birds and Allports Island birds that were translocated to Motuara Island sampled in 2008-10; MO1618 = Motuara Island birds sampled in 2016-18; Allports Island data set: KAI = Kaikoura (source); AP0810 = Allports Island purebred birds sampled in 2008-10; APH0810 = Hybrids of crosses between Allports Island birds and Motuara Island birds that were translocated to Allports Island sampled in 2008-10; AP1618 = Allports Island birds sampled in 2016-18.

With this data set, we can also compare the genetic diversity of the interbred birds with that of the respective source populations for each island and show that, while interbreeds did show increased H_e and A_r compared to purebred Motuara and Allports birds, the reciprocal translocation did not fully restore genetic diversity to that seen in the source populations for these islands (Table 2.1 and Figures 2.2B and C). The samples taken on Motuara and Allports Islands in 2016-18 show declines in both H_e and A_r compared to interbred individuals, but genetic diversity in both sites is still higher than that seen in the 2008-10 purebred birds.

2.3.2 Genetic differentiation

Pairwise F_{ST} values, PCA, and STRUCTURE analyses taken together suggest that genetic differentiation between South Island robin/kakaruwai populations is generally low, but that the Motuara and Allports islands populations had drifted to quite different and genetically uniform states prior to reciprocal translocations. Following the reciprocal translocations in 2008-09, these two island populations became more genetically similar, and the resulting admixture has persisted more noticeably on Allports than Motuara Island.

The Pairwise F_{ST} values for the sample sets considered were all significant (Table 2.2 and Table S1) and clearly demonstrate that genetic similarity between Motuara Island and Allports Island increased following reciprocal translocations but that the two populations subsequently drifted apart again. The two mainland outgroup populations sampled for this study are approximately 508 km apart as the crow flies and have an F_{ST} of 0.06. The two source populations for Motuara and Allports Islands, Nukuwaiata Island and Kaikoura, are more genetically differentiated than the mainland outgroups ($F_{ST} = 0.12$). Prior to reciprocal translocation, Motuara and Allports Island had drifted to become differentiated from their respective source sites ($F_{ST} = 0.11$ and 0.12, respectively). However, the highest genetic

differentiation seen in this study ($F_{ST}=0.26$) is between purebred Motuara and purebred Allports birds.

Following the reciprocal translocations in 2008-09, genetic similarity between the Motuara and Allports Islands populations increased (interbred individuals 2008-10: $F_{ST}=0.03$). By the time of sampling in 2016-18, genetic similarity between these two populations had decreased again, but not to the same degree as in the purebred birds sampled in 2008-10 ($F_{ST}=0.10$). The reciprocal translocation has done little to restore similarity between Motuara Island and Allports Islands and their respective source populations (F_{ST} drops from 0.12 purebred to 0.10 in 2016-18, and 0.11 purebred to 0.08 in 2016-18, respectively).

In the PCA, PC1 and 2 explained the majority of the variance. PC1 shows the genetic similarity of the two mainland outgroup populations, the differences between the two source populations, and the increased genetic similarity between Motuara and Allports following the reciprocal translocation. Meanwhile, PC2 splits the mainland and source populations from the Motuara and Allports groups (Figure 2.3).

Our STRUCTURE analysis was not designed to define the best K for this dataset but explore how individuals grouped together genetically. However, based on the STRUCTURE HARVESTER results, our data most likely partitions into two or three genetic clusters (Figure S1). Although we illustrate K = 3 here as it gives the clearest indication of the trends, a similar pattern can be seen across Ks of 2-10 (Figure S2).

The STRUCTURE analysis neatly illustrates the major impacts of the reciprocal translocation on the Motuara and Allports populations. When K = 3, the data suggest the mainland outgroup populations as clear genetic outgroups to Motuara and Allports Islands,

Table 2.2. Genetic differentiation measured via F_{st} between 10 groups of South Island robins/kakaruwai following reciprocal translocations for the purposes of genetic rescue based on 28 microsatellite loci. Mainland outgroups presented for comparison. All values are significantly different from zero based on bootstrapped 95% confidence intervals.

	Eglinton Valley	Nelson Lakes	Nukuwaiata	Motuara Inbred 2008-10	Motuara Interbred 2008-10	Motuara 2016-18	Kaikoura	Allports Inbred 2008-10	Allports Interbred 2008-10	Allports 2016-18
Eglinton Valley	0.00									
Nelson Lakes	0.06	0.00								
Nukuwaiata	0.12	0.08	0.00							
Motuara Inbred 2008-10	0.18	0.14	0.12	0.00						
Motuara Interbred 2008-10	0.10	0.07	0.10	0.08	0.00					
Motuara 2016-18	0.16	0.11	0.10	0.01	0.05	0.00				
Kaikoura	0.09	0.07	0.12	0.19	0.08	0.16	0.00			
Allports Inbred 2008-10	0.18	0.16	0.22	0.26	0.11	0.22	0.11	0.00		
Allports Interbred 2008-10	0.12	0.09	0.12	0.11	0.03	0.08	0.08	0.08	0.00	
Allports 2016-18	0.12	0.10	0.14	0.14	0.03	0.10	0.08	0.05	0.00	0.00

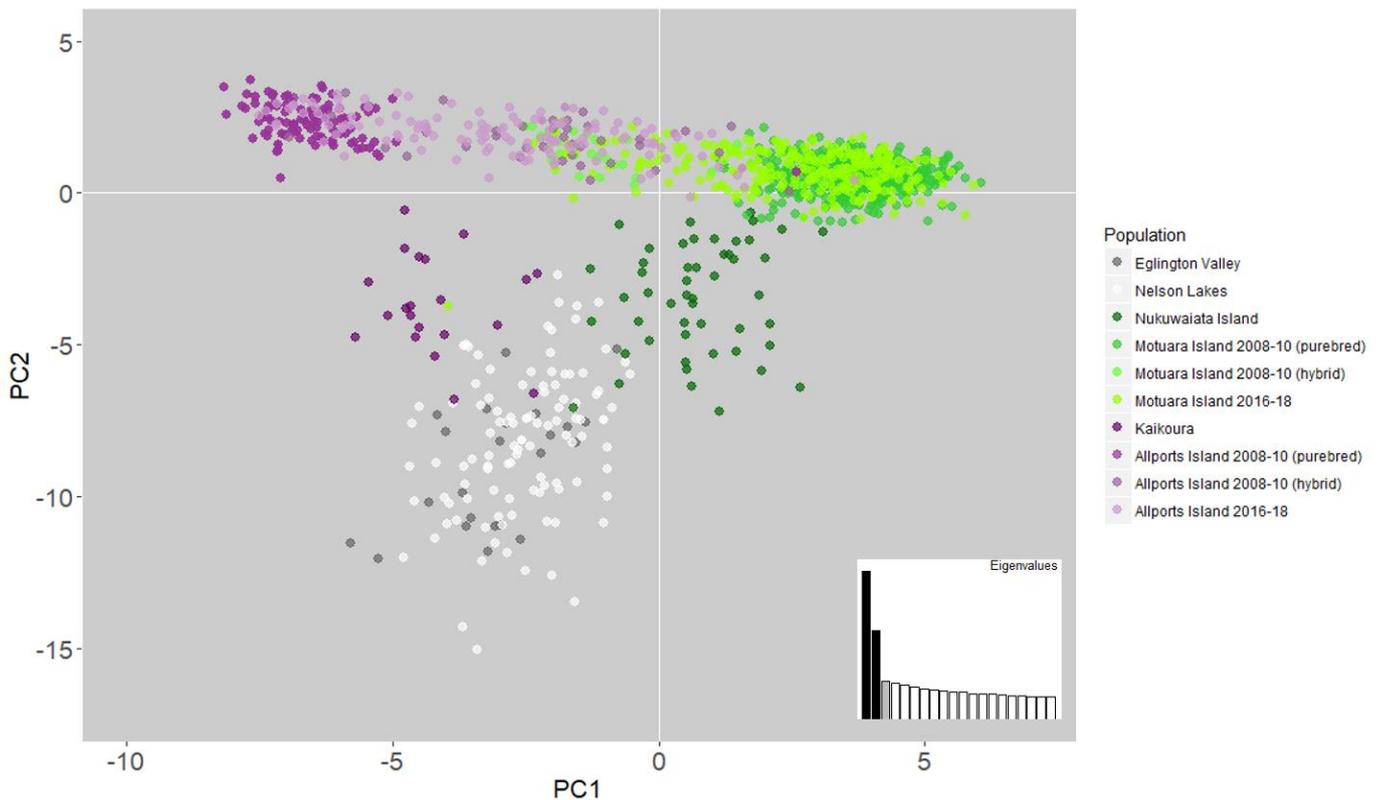


Figure 2.3. Results of PCA analysis showing convergence of genetic composition between two island populations of South Island robins/kakaruwai following a reciprocal translocation for attempted genetic rescue and comparing those island populations with their respective source populations and two mainland populations of the same species that act as outgroups.

but sharing large proportions of genetic material with the source populations on Nukuwaiata Island and in Kaikoura (Figure 2.4). The purebred Motuara and Allports birds sampled in 2008-10 are almost uniform in their genetic make-up within each island, but completely different to each other. Following the reciprocal translocation, the interbred birds sampled in 2008-10 are a clear mix of genetic material from Motuara and Allports island.

Data from the 2016-18 samples suggest that, while Motuara and Allports island are still more admixed and similar to one another than before the reciprocal translocation, the Motuara population is drifting back towards its pre-translocation state, whereas the Allports population still shows a degree of admixture similar to that seen in the Allports interbreds of 2008-10 (Figure 4). Again, while the reciprocal translocation has improved genetic diversity

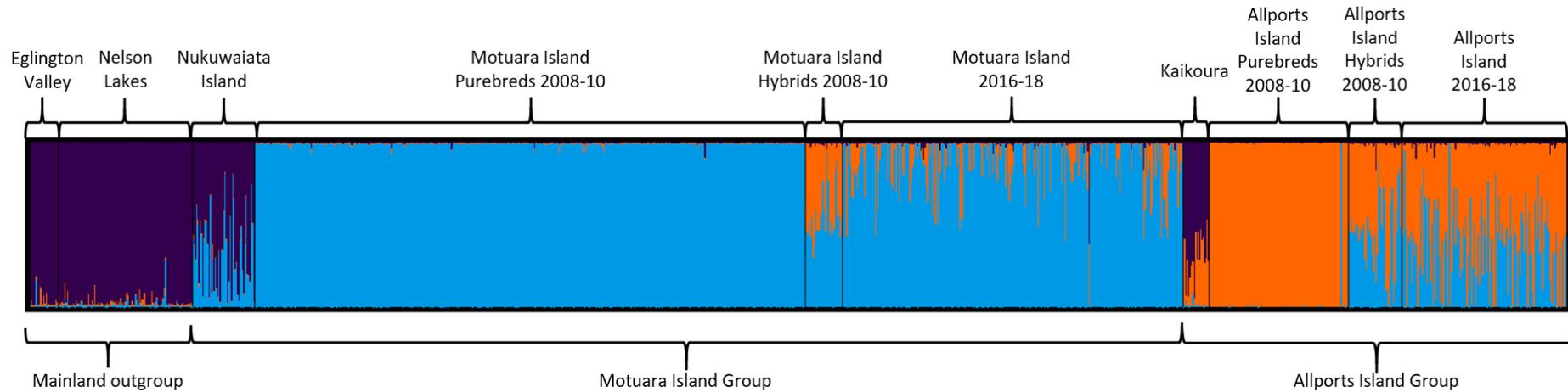


Figure 2.4. Results of STRUCTURE analysis for $K=3$ showing the initial increase in genetic diversity in two island populations of South Island robins following a reciprocal translocation for attempted genetic rescue and demonstrating that the increase in genetic diversity has been more long-lasting and pervasive in the smaller, Allports Island population than in the Motuara Island population. Source populations for each island and two mainland populations are included for comparison purposes.

in both the Motuara and Allports island populations, it has not restored all the diversity lost during the founding events for these populations; the source populations, Nukuwaiata Island and Kaikoura, each possess genetic material that is not present in their respective island-founded populations.

2.4 Discussion

Founder effects

Founding Motuara and Allports Islands with just five birds each caused clear losses in genetic diversity when compared to their respective source populations of Nukuwaiata Island and Kaikoura. Both heterozygosity and allelic diversity were impacted by the small founder numbers at each site. Allelic diversity tends to suffer greater losses than heterozygosity during bottleneck events (Allendorf 1986) and we can see that around half the allelic richness observed in each source population is missing from the Motuara and Allports Islands purebred birds compared to losses of 13.8-22.1% in heterozygosity.

The losses of genetic diversity observed here are likely underestimated as the data presented are for the source populations in 2014 rather than 1973. Nukuwaiata Island is an isolated, closed population, and Kaikoura is an isolated forest fragment experiencing its own population decline due to a lack of control of introduced predators (*J.V.Briskie, pers.comm.*). Thus, both source populations have likely seen losses of heterozygosity since 1973 due to genetic drift. Unfortunately, samples for these populations from 1973 do not, to our knowledge, exist and so it is not possible to judge how greatly we are underestimating the losses of genetic diversity that occurred when the Motuara and Allports Islands populations were founded. However, we can make comparisons with the genetic diversity seen in the mainland outgroup populations and observe that the purebred Motuara and Allports Islands birds are extremely genetically depauperate compared to Eglington Valley and Nelson Lakes

birds, with significantly lower expected heterozygosity and substantial losses of allelic richness.

Genetic alteration rather than restoration

Given the results above, it is fair to say that Heber *et al.* (2013) were correct to assume that both the Motuara and Allports Islands populations of South Island robins/kakaruwai were genetically impoverished and in need of assisted migration to increase genetic diversity. It is also clear from Heber *et al.* (2013) that the reciprocal translocations they conducted between Motuara and Allports Islands in 2008-9 resulted in an increase in genetic diversity in each population. However, what has been unclear until now is whether the reciprocal translocations restored genetic diversity to pre-bottleneck levels and whether the specific alleles lost during each bottleneck were restored via the translocation.

The first part is simple; genetic diversity has not been restored to pre-founding bottleneck levels via the reciprocal translocation. While interbred birds on both Motuara and Allports Islands show increased genetic diversity compared to their purebred counterparts, both heterozygosity and allelic richness in these birds are still lower than in the source populations on Nukuwaiata Island and in Kaikoura. As mentioned above, these comparisons are likely underestimates.

Perhaps more important is not just the amount of genetic diversity, but how the genotypic composition of each population changed following the reciprocal translocation. From the data presented here, we can also see that following the reciprocal translocation, the Motuara and Allports Islands populations became more alike, genetically. Though they have drifted apart again over time, these populations were still more genetically similar in 2016-18 than prior to the reciprocal translocation. However, as can be seen from the F_{ST} values, PCA and STRUCTURE analyses, the reciprocal translocations did not restore the diversity from the source populations that was lost during founding with five birds.

The Motuara and Allports Islands 2016-18 sample sets show slightly less divergence from their respective source populations than the purebred 2008-10 sample sets in each case. However, elements of the genotypic composition of each source population remain lost from the Motuara and Allports Islands populations, even after the reciprocal translocation. The source populations of Nukuwaiata Island and Kaikoura are, themselves, genetically divergent and differ in their genotypic composition. Thus, the Motuara and Allports populations could never contain all the genetic variants seen in the opposite source populations and a reciprocal translocation could not restore these variants. The post-translocation populations therefore contained more genetic diversity, but still differ from their source populations in terms of genotypic composition. This is important in terms of adapting to future changes and challenges (Allendorf 1986). An investigation is underway to see how these patterns are reflected in functional, immune loci (*H.R.Taylor, unpublished data*).

Continuing genetic erosion

The data presented here show that on Motuara and Allports Islands, allele frequencies have changed, and genetic diversity eroded over time following the reciprocal translocation. This is what we would expect in a closed population with no migration due to genetic drift (Allendorf et al. 2013 pp. 100). Although heterozygosity and allelic diversity have not dropped to pre-reciprocal translocation levels, the slight downward trend in each metric at each site suggests continued erosion of genetic diversity over time if both sites are left as closed populations.

The effect of the reciprocal translocation on genotypic composition has been more persistent on Allports than on Motuara Island. The STRUCURE analysis suggests that the Allports Island 2016-18 sample set contained a degree of genetic admixture similar to that seen in the original interbred birds on that island that resulted from the translocation. In contrast, the Motuara Island 2016-18 sample set shows a genotypic composition more similar

to the purebred birds at that site. This is also reflected in the F_{ST} values for each population. Given the relative sizes of these two robin populations (in 2008, Motuara Island ~300 birds, Allports Island ~60 birds) and the fact that similar numbers of females were moved between the islands (18 from Allports to Motuara and 13 from Motuara to Allports), it is perhaps not surprising that the translocation had a bigger genetic impact on the Allports Island population. All things being equal, there was a greater probability of Motuara Island genetic material persisting in the Allports Island population than the other way around.

Future management

The goal of the 2008-09 reciprocal translocations of South Island robins/kakaruwai between Motuara and Allports Islands was to increase genetic diversity and fitness in each population. In the short term, both aims were achieved, with interbred individuals on both islands showing greater genetic diversity and improved fitness compared to purebred birds. The rest of this thesis deals with the long-term fitness consequences of this reciprocal translocation and whether or not this experiment can be termed a successful genetic rescue (i.e., it led to an increase in population growth). Here, we examine purely the genetic consequences of the reciprocal translocation in the long-term.

As mentioned above, genetic erosion continues to decrease genetic diversity in both the Motuara and Allports islands South Island robin/kakaruwai populations and will continue to do so as long as these populations remain closed to migration. Simultaneously, the translocation has created two populations that are more similar to one another in genotypic composition than either is to its respective source population. If movement were facilitated between Motuara and Allports Islands in the future, the robin populations would become increasingly similar until the two islands eventually represented one, larger genetic population.

Whether or not this is considered a problem depends on the long-term goal for the Motuara and Allports Island populations. If the goal is to create a metapopulation consisting of Motuara and Allports Islands, then more reciprocal translocations between the sites would be advisable, involving larger numbers of birds (especially to Motuara Island) and at more regular intervals. Conversely, if there were a desire to restore genetic diversity from the source populations for each island, then future translocations could come from Nukuwaiata Island to Motuara and Kaikoura to Allports Island. Finally, if the goal is to create as genetically diverse a population as possible on each island, then translocations from mainland populations such as Eglington Valley and Nelson Lakes, which have much higher genetic diversity than any of the other populations considered here, would be advisable.

Before any management decisions regarding translocations are made, it is important to examine functional genetic diversity in Motuara and Allports Islands South Island robins/kakaruwai and their respective source populations to understand whether the trends seen here in neutral genetic diversity are reflected in coding regions of the genome. This work, along with simulations of various future translocation scenarios is currently underway, and will help inform both future management of isolated South Island robin/kakaruwai populations, and other species in fragmented, isolated populations where losses of genetic diversity could lead to reduced chance of persistence.

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Supplementary information

Table S1: F_{ST} values with bootstrapped lower and upper 95% confidence intervals for each pair of sample sets in this study. As no confidence intervals for any pair overlap zero, all F_{ST} are considered significantly different from zero.

Sample set pairs	F_{ST}	Lower 95% CI	Upper 95% CI
E_B109901, vs NL10_B109934	0.06	0.04	0.07
E_B109901, vs NUK_B109466,	0.12	0.10	0.14
E_B109901, vs MO0810_B104960,	0.18	0.14	0.24
E_B109901, vs MOH0810_B109208,	0.10	0.08	0.13
E_B109901, vs MO1618_B109151,	0.16	0.11	0.21
E_B109901, vs KAI_B104599,	0.09	0.07	0.12
E_B109901, vs AP0810_B109129,	0.18	0.15	0.24
E_B109901, vs APH0810_B109197,	0.12	0.10	0.14
E_B109901, vs AP1618_B109226,	0.12	0.10	0.16
NL10_B109934 vs NUK_B109466,	0.08	0.06	0.10
NL10_B109934 vs MO0810_B104960,	0.14	0.10	0.19
NL10_B109934 vs MOH0810_B109208,	0.07	0.05	0.09
NL10_B109934 vs MO1618_B109151,	0.11	0.07	0.16
NL10_B109934 vs KAI_B104599,	0.07	0.06	0.10
NL10_B109934 vs AP0810_B109129,	0.16	0.13	0.18
NL10_B109934 vs APH0810_B109197,	0.09	0.06	0.12
NL10_B109934 vs AP1618_B109226,	0.10	0.08	0.13
NUK_B109466, vs MO0810_B104960,	0.12	0.08	0.15
NUK_B109466, vs MOH0810_B109208,	0.10	0.07	0.13
NUK_B109466, vs MO1618_B109151,	0.10	0.07	0.13
NUK_B109466, vs KAI_B104599,	0.12	0.07	0.16
NUK_B109466, vs AP0810_B109129,	0.22	0.17	0.27
NUK_B109466, vs APH0810_B109197,	0.12	0.08	0.16
NUK_B109466, vs AP1618_B109226,	0.14	0.10	0.20
MO0810_B104960, vs MOH0810_B109208,	0.08	0.06	0.10
MO0810_B104960, vs MO1618_B109151,	0.01	0.00	0.01
MO0810_B104960, vs KAI_B104599,	0.19	0.15	0.24
MO0810_B104960, vs AP0810_B109129,	0.26	0.21	0.31
MO0810_B104960, vs APH0810_B109197,	0.11	0.08	0.15
MO0810_B104960, vs AP1618_B109226,	0.14	0.10	0.18
MOH0810_B109208, vs MO1618_B109151,	0.05	0.03	0.06
MOH0810_B109208, vs KAI_B104599,	0.08	0.05	0.10
MOH0810_B109208, vs AP0810_B109129,	0.11	0.08	0.14
MOH0810_B109208, vs APH0810_B109197,	0.03	0.02	0.04
MOH0810_B109208, vs AP1618_B109226,	0.03	0.02	0.05
MO1618_B109151, vs KAI_B104599,	0.16	0.12	0.20
MO1618_B109151, vs AP0810_B109129,	0.22	0.18	0.27
MO1618_B109151, vs APH0810_B109197,	0.08	0.06	0.10
MO1618_B109151, vs AP1618_B109226,	0.10	0.08	0.14
KAI_B104599, vs AP0810_B109129,	0.11	0.08	0.15
KAI_B104599, vs APH0810_B109197,	0.08	0.05	0.10

KAI_B104599, vs AP1618_B109226,	0.08	0.05	0.11
AP0810_B109129, vs APH0810_B109197,	0.08	0.06	0.10
AP0810_B109129, vs AP1618_B109226,	0.05	0.04	0.06
APH0810_B109197, vs AP1618_B109226,	0.00	0.00	0.01

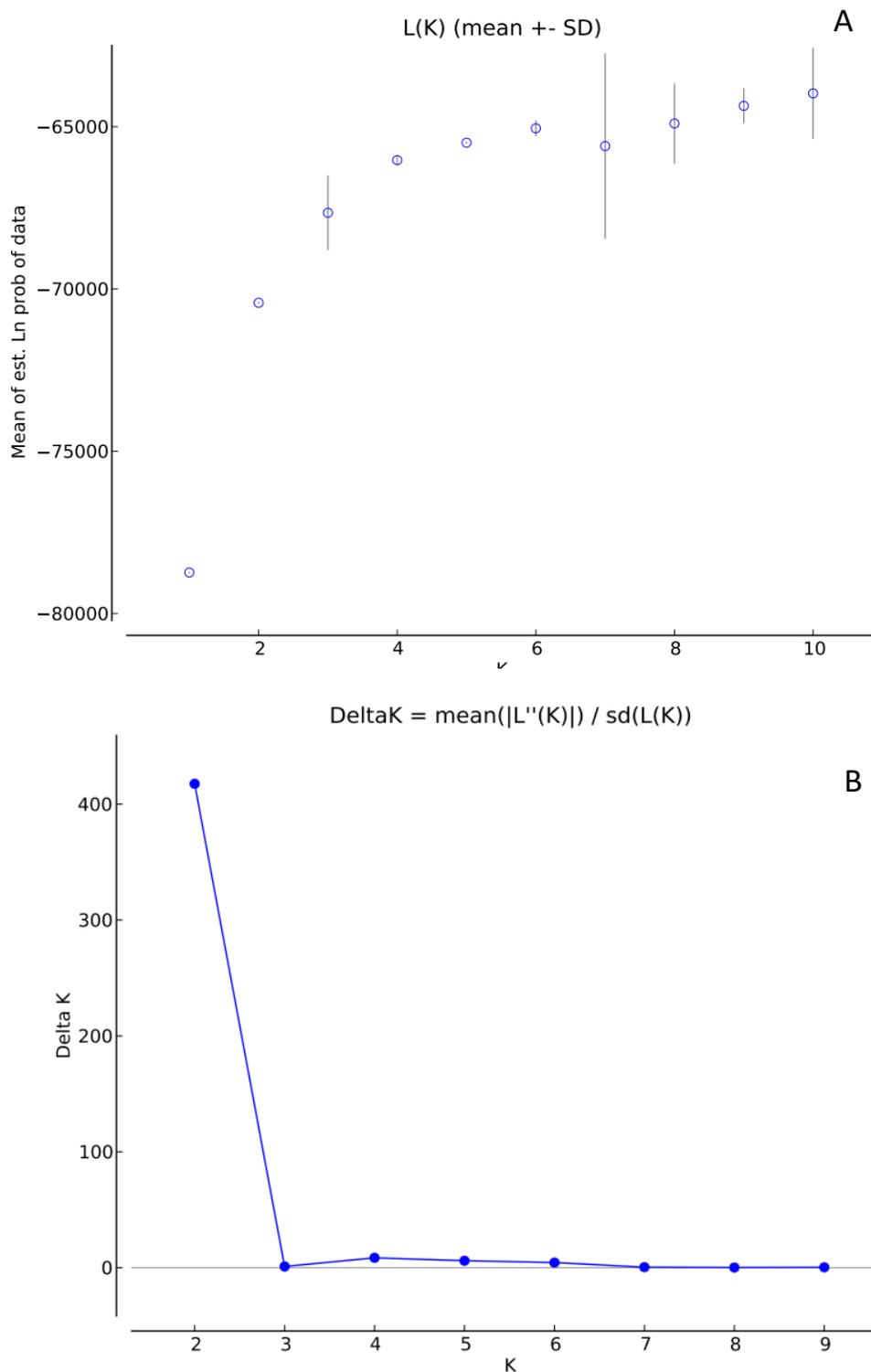


Figure S1: Results from STRUCTURE HARVESTER. A = LnP(D) plot for STRUCTURE analysis. The point at which the curve asymptotes and points start to overlap is considered the most likely value of K ; B = Delta K plot for STRUCTURE analysis for K 1-10. The value of K that corresponds to the highest Delta K value is associated with the greatest change in likelihood $L(K)$ and is considered the most likely value of K .

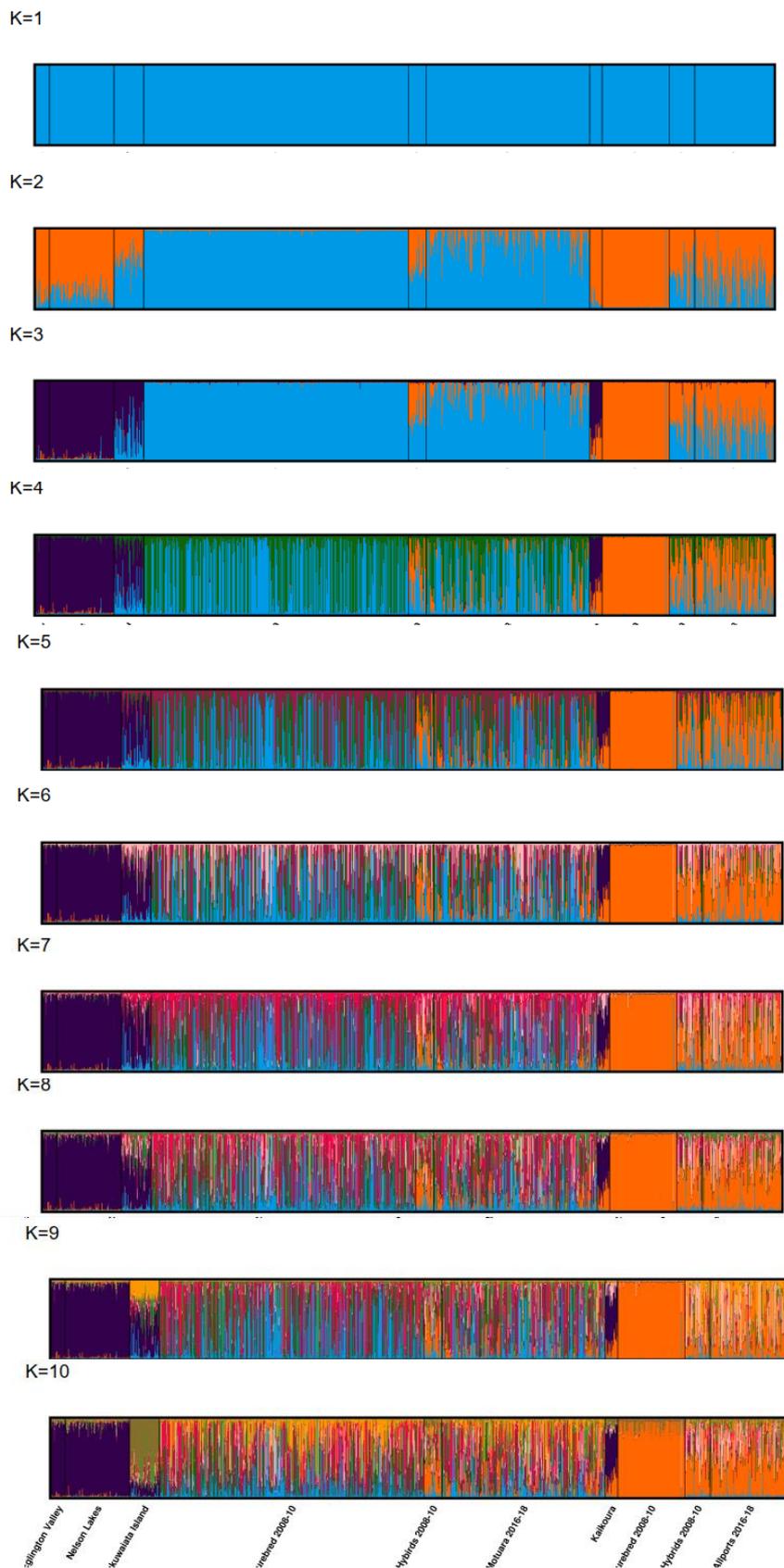


Figure S2: most probable STRUCTURE results across each value of K simulated.

Has attempted genetic rescue between two inbred South Island robin populations resulted in an increase in reproductive success, survival and population growth after 8-10 years?

Chapter 3



Figure 3.1 A male South Island robin (*Petroica australis*) on Motuara Island caring for a juvenile. Once chicks fledge male robins will often become the sole carer for the fledglings while the female re-nests.

Abstract

In 2008, a novel approach to genetic rescue was trialled using inbred donors in a reciprocal translocation between two island populations of South Island robin (*Petroica australis*). The reciprocal translocation was a success in the short-term, increasing genetic diversity and survival for F1-F3 interbred birds in both populations. However, the long-term effects of this reciprocal translocation on breeding success, survival and population demographics is unknown. Furthermore, to be considered a successful genetic rescue event there needs to be a sustained population increase. This presents a challenge for many of New Zealand's native species restricted to offshore islands that likely have limited carrying capacity. To establish the long-term outcomes of the attempted genetic rescue event in 2008-2009, the two populations were revisited from 2016-2019 to measure current breeding success, survival and population demographics, and to assess how the populations have changed since the translocation. A significant heterozygosity fitness correlation (HFC) was detected for juvenile survival to one year of age, within the 2016-2018 populations. Although both sexes showed positive HFCs for survival to one year of age, this trend was stronger for female survival to one year of age than for males. A similar pattern of survival was observed with adults transitioning to old age, as interbred F1-F3 individuals were significantly more likely to survive to 10 years of age than their pre-translocation counterparts. Although there was no variation in breeding success in relation to individual genetic diversity, increased survival for both juveniles and F1-F3 interbred adults likely led to increased opportunity to reproduce over their lifetimes. Overall, there was a considerable increase in the robin populations on both islands, likely driven by the increased survival of juveniles and adults. This indicates that a successful genetic rescue event has occurred. My results highlight the usefulness of genetic rescue for two reasons: (1) the donors that were used in these translocations were from inbred populations and, (2) the island environments they were transferred between had pre-existing populations that were expected to already be at carrying capacity. With many national and international endangered bird species confined to island refuges with set carrying capacities and with limited chances of migration it is important to know how attempted genetic rescue events could impact these populations.

3.1 Introduction

Many of New Zealand's threatened bird species have recently experienced population bottlenecks. Most of these species now persist only as small, isolated populations, typically on offshore islands that are free of introduced mammalian predators (Jamieson 2015). This has resulted in not only genetic bottlenecks, but limited population size and inadequate or limited gene flow due to the isolated nature of the islands. Limited options for mate selection within these small populations can result in increased inbreeding (mating between relatives), which will act to further erode genetic diversity over time (Robertson 2006, Sainsbury et al. 2006, Boessenkool et al. 2007). This in turn, leads to reduced fitness through decreased allelic diversity and increasing homozygosity, referred to as "inbreeding depression". Such effects have been found to reduce fertility and survival, as observed with increased rates of hatching failure (Jamieson and Ryan 2000, Briskie and Mackintosh 2004, Mackintosh and Briskie 2005, Boessenkool et al. 2007, Taylor et al. 2017), greater susceptibility of South Island saddlebacks (*Philesturnus c. carunculatus*) to disease (Hale and Briskie 2009), reduced immune system function in South Island robins (Grueber et al. 2012, Heber 2012), and high rates of infertility in kakapo (*Strigops habroptilus*) and takahe (*Porphyrio mantelli*) (Jamieson and Ryan 2000, Briskie and Mackintosh 2004).

The negative effects brought about from population bottlenecks and inbreeding can be halted, or even reversed, and genetic variability can be restored through the introduction of new alleles from relatively 'outbred' individuals into the genetically compromised population. This is referred to as 'genetic rescue' when it is successful (Frankham et al. 2002). Practical applications of attempted genetic rescue have often been short term, with the recipient population monitored for only a few years after the introduction of donors (Tallmon et al. 2004), with few studies monitoring the effects of attempted genetic rescue over the long term. Furthermore, 'attempted' genetic rescue is only considered 'successful' genetic rescue

once there is a sustained population increase brought about from an increase in the recipient population's fitness due to the rescue event (Frankham et al. 2002). With many of New Zealand's remaining endangered bird species confined to island refuges, with expected population caps due to limited island size, sustained population growth may not be possible. Thus, it is not clear if successful genetic rescue is possible, nor what the long-term consequences of attempted genetic rescue are in such situations.

An alternative approach to genetic rescue was trialled in 2008 (Heber et al. 2013), using inbred individuals as donors in two isolated island populations of South Island robins. Both recipient populations were founded from different source populations, with interbreeding unlikely due to the geographical distance between the islands. Due to the low number of founders, both island populations of robins had limited levels of genetic diversity, and showed signs of inbreeding depression (Briskie and Mackintosh 2004, Mackintosh and Briskie 2005, Hale and Briskie 2007). Immediately after the reciprocal translocation, interbred offspring in the subsequent F1-F3 generations showed greater genetic diversity at both neutral and functional loci, survival, immunocompetence, and sperm quality compared to inbred birds in either population (Grueber et al. 2012, Heber 2012, Heber et al. 2013). Although the value of genetic rescue with inbred donors was demonstrated within the first couple of generations of robins (Heber et al. 2013), it is unclear whether these benefits will persist.

Key to assessing the mechanisms behind a successful genetic rescue is an understanding of changes to the genetic make-up of the recipient population and whether/how these changes affect the fitness of individuals in the population. One tool for genetic monitoring is using an individual's heterozygosity, measured using a set of neutral markers, in association with a particular fitness trait, such as survival or reproductive success (Mitton 1993). Such heterozygosity fitness correlations (HFCs), using multi-locus heterozygosity

(MLH) as a measure of individual heterozygosity, can be used to see how the rescue event affects the fitness of individuals. Significant HFCs may indicate that inbreeding depression is occurring and can be especially useful in situations where a direct measure of inbreeding (such as from a pedigree) is not available (Grueber et al. 2008, Ruiz-López et al. 2012). It is also important to assess whether relationships between genetic diversity and fitness might be sex specific for given fitness traits, as sex-specific HFCs are often reported (Shaner et al. 2013, Arct et al. 2017, Bichet et al. 2018).

In this study, I investigated two populations of South Island robin that have undergone attempted genetic rescue 8-10 years previously, to determine the long-term effects on fitness measures and how this relates to current estimates of population and individual genetic variation on: (1) reproductive success in the recipient populations, (2) survival over both the short term and long term, and (3) the effects on population demographics.

3.2 Methods

3.2.1 Study sites

The two study populations were located on Motuara and Allports islands, at the northern end of the South Island. Each population was established in 1973 with five birds. The Motuara population was founded with five birds from Nukuwaiata Island and the Allports population was founded with five birds from a mainland source population near Kaikoura (Flack 1974, Ardern et al. 1997). Both islands differ in size (Allports Island: 16 ha, Motuara Island: 59 ha), and in the density of robins (Allports: ~104 adult individuals, 6.5 birds/ha; Motuara: 437 adult individuals, 7.4 birds/ha; A.MacFarlane, unpubl. data 2019). Both Allports and Motuara islands are located a minimum of 1.4 km from the nearest shoreline in the Queen Charlotte Sound. Coupled with few robin populations in the surrounding mainland areas (Robertson 2007) and the low capacity of robins to sustain flight

(Taylor et al. 2005, Boessenkool et al. 2007), the two island populations are considered relatively closed (i.e., genetically isolated) systems. In 2008 and 2009, Heber et al. (2013) attempted genetic rescue by transferring a total of 31 female robins between Allports and Motuara islands. At the time of the translocations, the populations had thus been isolated for nearly four decades. Despite being at expected carrying capacity, the populations were showing signs of inbreeding depression with reduced hatching success, reduced sperm quality, impaired immune system function and considerably lower genetic diversity in comparison to source populations (Byrne 1999, Mackintosh and Briskie 2005, Hale and Briskie 2007, Heber et al. 2013). However, after the rescue, offspring in subsequent generations from donors crossed with resident pairs showed greater genetic diversity, survival, immunocompetence, and sperm quality (Heber 2012, Heber et al. 2013).

3.2.2 Breeding success

During each breeding season from August – December 2016 – 2018, a population census for all surviving robins was conducted, covering all of Allports Island and an extensive search within a 22 ha grid on Motuara Island and opportunistic sightings over the rest of the island. All encountered birds with unique identifiable leg bands were logged onto a Garmin 64s GPS at the location they were seen. Territorial pairs were marked together and were defined as territory holders if they nested or showed a combination of breeding behaviours, including defending a territory with the male additionally courting and feeding the same female. The recorded GPS points for observed birds was used to: (1) determine the survival of juvenile and adult robins from one year to the next (if a juvenile was identified the following breeding season after it fledged it was designated as one year of age; if not detected by the end of breeding season it was classified as dead), (2) determine the overall size of the populations on the two islands, (3) to determine the location and status of territorial breeding individuals and allow relocation during the breeding season, and (4) allow the relocation of

active nests. All adult birds that were not already banded with uniquely identifiable colour bands were captured and fitted with leg bands. Blood samples (10–30 μ l) were collected from these individuals via brachial venepuncture, using a 29 Gx 1/2" insulin needle, and then stored in Queen's lysis buffer (Seutin et al. 1991) until DNA extraction at a later date; see chapter 2.2.2 for DNA extraction and genotyping methods. Birds that were incubating or feeding chicks in the nest were not captured or bled until after the chicks had fledged. This minimised observer interference which could have increased the possibility that individuals might abandon nests after handling. To calculate genetic variation, individuals were genotyped at 28 microsatellite loci (Heber et al. 2013). All PCR and microsatellite sequencing were conducted in the Kempnaers lab at the Max-Planck Institute for Ornithology according to the protocols detailed in Heber et al. (2013).

Extensive nest searches were carried out at similar times on both islands. When active nests were encountered, they were recorded as either incubation stage, chicks in the nest, or as fledglings currently being feed by adults and nest height was recorded. Nests were periodically checked every three days, however due to difficulties monitoring two populations on separate islands simultaneously it was not possible to monitor nests in this way indefinitely. Instead, nests were monitored over an 8-12-day rotation as I swapped between islands. The status of eggs and chicks (alive, dead or fledged) was recorded at each nest check. When nestlings reached pin-break stage, they were banded and bled in all nests that were accessible. Most chicks that did not fledge were found dead in the nest. Missing chicks that were not ready to fledge were assumed to have died and either been removed from the nest by the parents or attempted to fledge then died.

I analysed breeding success using four measures, as well as overall nesting success. First, I recorded the number of eggs laid by each female (i.e., clutch size). Most nests were found after they had hatched, however, unhatched eggs were not removed from the nest by

the adults. Therefore, it was possible to estimate clutch size after the chicks had hatched by including number of chicks and unhatched eggs in the nest. To be conservative I did not use nests with chicks that were found past pin break stage. This controlled for the possibility that as the chicks grew, they may have broken or damaged the unhatched eggs. I then recorded hatching success, a measure of how many eggs were laid in a clutch and the number of these that hatched. Nests that were predated or were abandoned during the incubation phase were removed from this measure. Eggs that fail to hatch therefore reflect either infertility or embryonic death, which is expected to increase with inbreeding (Heber and Briskie 2010). Fledging success was defined as the total number of chicks that hatched and survived to fledge. Nests that were i.e. predated, abandoned, died due to adverse weather conditions or starvation were still retained in the measure, as this was a measure of parental reproductive success during this stage. The survival of fledglings to one month of age was measured by the number of chicks that were still alive one month after fledging. Finally, I calculated overall success from the start of laying until survival at one month of age. Typically, robin fledglings are fed by their parents for around 25-50 days of age before dispersing (Heather and Robertson 2000).

For comparison, data on breeding were pooled from Heber (2012), who used the same populations and methods to observe breeding success in 2008 – 2010. This included pre-translocation pure bred breeding pairs (no mixed heritage), translocation breeding pairs (which included at least one individual in the pair who was from a different island), and F1-F3 interbred individuals (descendants between translocation breeding pairs).

3.2.3 Survival

In each breeding season, any juveniles that were banded in the previous breeding season were identified by their individual colour leg bands and their location recorded on a

Garmin 64 GPS. All juveniles that survived one winter were considered to have survived to one year of age. It was possible to calculate survival to two years of age for individuals that were banded in 2016 and observed again in the 2018 breeding season. Adult survival to over 8-10 years of age could be determined for some individuals that were banded as chicks by Heber (2012) between 2008-2010. Robins are relatively long lived and have been recorded reaching ages of 16 years (Heather and Robertson 2000). For these chicks the pedigrees were already known so it was possible to split them into two groups: individuals from a heritage of pre-translocation individuals, and interbred F1-F3 individuals. Subsequently, some of these individuals were observed in 2016-2019.

3.2.4 Population density

Although individuals were recorded each year on a GPS, population density was only calculated in 2018. This was considered the most accurate year for a total population census, because almost all birds had been banded and located over the previous two years, and only a few unbanded individuals remained in the two populations. The population census was also conducted at the start of the breeding season (August-September), before juveniles had fledged to avoid inflating population size. It was not possible to survey the entirety of Motuara Island due to its larger size. Instead, a thorough search of a 22 ha area was conducted. Although birds from outside may have flown into the census area, resulting in an over-estimate, I minimised this by distinguishing resident pairs from such 'floaters'. Residential pairs were less likely to move out of the area and were observed on multiple occasions at the same location while displaying territory ownership behaviours. Birds therefore passing through territories were considered unpaired floater birds. Nevertheless, it is possible I missed observing some individuals, thus counts from 2018 represent minimum population estimates. The total population for Motuara Island was then estimated by

extrapolating density from the 22 ha census area to the island area (59 ha). Allports Island was censused in its entirety and my estimate is a direct count for the entire population. To determine whether population size has changed since genetic rescue attempt in 2008, I compared my estimates with that made by Heber (2012) and earlier censuses from Maloney (1991). Similar methods were used to estimate population size for Heber (2012) and Maloney (1991), where both of these observers attempted to band all adult robins on both of the islands.

3.2.5 Genetic analyses - Heterozygosity-fitness correlations (HFCs)

A method of testing for inbreeding depression is to test for correlations between multi-locus heterozygosity and various fitness components. Such correlations are termed heterozygosity-fitness correlations (HFCs). These correlations are not always driven by inbreeding depression; there are three main hypotheses that serve to explain the occurrence of HFCs, these are: 1) The general effect hypothesis; where multilocus heterozygosity (MLH) reflects genome wide heterozygosity and thus accurately reflects the inbreeding coefficient of the individual, or how much of an individual's genome is identical by descent (IBD) (David 1997, 1998, Slate et al. 2004). Individuals with related parents will have a higher proportion of the genome IBD as they will have inherited much of their genome from ancestors common to both their mother and their father. The more inbreeding that has occurred, the greater the proportion of the genome that will be IBD, the higher the inbreeding co-efficient, and the lower genome-wide heterozygosity will be. So, the key requisite of the general effect hypothesis is that heterozygosity measured at a given panel of markers is correlated with the proportion of an individual's genome that is IBD. 2) The local effect hypothesis; that HFCs are caused by linkage disequilibrium between genotyped loci and nearby loci that are under selection, through the non-random association of these loci (Hill and Robertson 1968, Ohta

1971, David 1998, Chapman et al. 2009, Szulkin et al. 2010). 3) The direct effect hypothesis; that HFCs are caused by selection acting on the genotyped loci (Frydenberg 1963, Houle 1989, Lynch and Walsh 1998, Mitton 2000). As we use microsatellites for our analyses, which are assumed to be neutral markers, hypothesis three can be discounted. Testing for the remaining two hypotheses is crucial for understanding whether an observed HFC is being driven by inbreeding depression or not.

One key requirement of the general effect hypothesis is that inbreeding and thus genome-wide heterozygosity varies among individuals (Weir and Cockerham 1973, Slate et al. 2004, Szulkin et al. 2010, Kardos et al. 2014). Such variation in heterozygosity among individuals will result in correlations in heterozygosity across an individual's genome (Weir and Cockerham 1973, Slate et al. 2004, Szulkin et al. 2010). This correlated heterozygosity across an individual genome is termed identity disequilibrium (ID) (Szulkin et al. 2010). By testing for ID between pairs of loci throughout the genome, we can test whether an observed HFC could be caused by inbreeding depression (Slate et al. 2004, Szulkin et al. 2010, Kardos et al. 2014). We would predict that we would detect a significant ID within our populations.

I used the two most common methods available to test for the magnitude of identity disequilibrium (ID) across the loci in this study: heterozygosity–heterozygosity correlations (HHC) (Balloux et al. 2004) and the g^2 statistic (David et al. 2007). HHC repetitively estimates the correlation coefficient between heterozygosity at one half of a panel of loci with heterozygosity calculated from the other half of the panel of loci in the data set being used (Balloux et al. 2004). When the HHC correlation coefficient is significantly greater than zero, it suggests that ID is present. The g^2 statistic measures the excess of double heterozygotes at two loci relative to the expectation of random association (i.e. covariance in heterozygosity), standardized by the average heterozygosity (Szulkin et al. 2010). A g^2 significantly different from zero indicates that heterozygosity across loci correlates with individual inbreeding,

which increases the probability of detecting HFCs (Szulkin et al. 2010). My estimates of g^2 were obtained using the R package *inbreedR* (Stoffel et al. 2016a) by running 100 bootstraps. The 95% confidence intervals were defined using 100 permutations. Using the same package, I also estimated identity disequilibrium through HHC by dividing the 28 loci into two random subsets, testing the correlation in heterozygosity between the two subsets and repeating this by running this 1000 times, in order to obtain the HHC mean, SD and confidence interval at 95%.

Individual heterozygosity was calculated as standardised multi-locus heterozygosity (sMLH) for males and females with the same microsatellite data using the function “sMLH” from the R package “inbreedR” (Stoffel et al. 2016b). Multi-locus heterozygosity (*MLH*) is a simpler metric than sMLH because it measures the proportion of heterozygous loci within an individual divided by the number of loci typed in the focal individual (Bamford et al. 1995). However, MLH does not correct for differences in average observed heterozygosity in the sampled population over the subset of loci typed in the individual and sMLH does (Coltman et al. 1999, Stoffel et al. 2016a).

3.2.6 Statistical analysis

Male sMLH and female sMLH in each monitored pair was used as the predictors for breeding success at different nesting stages, including number of eggs laid, number of eggs hatched, number of chicks fledged, number of fledglings surviving to one month of age, and overall success from laying until the chick was one month of age. Breeding success was pooled from data collected in 2016-2018 and using the breeding data from Heber (2012) collected in 2008-2010. Generalised Linear Mixed Models fitted in a Bayesian framework using Markov chain Monte Carlo techniques (*MCMCglmm*), from functions implemented in the *MCMCglmm* version 2.29 package (Hadfield 2010), were used for the statistical analysis.

For all models 100,000 iterations were specified; the first 10,000 iterations were discarded, and the thinning interval was set to 1. For each breeding stage multiple models were tested, firstly with male sMLH and female sMLH as an interaction, as the breeding success of one sex might be dependent on the opposite sex, and secondly without the interaction. Males were removed from the model for analysis of clutch size (as males do not lay eggs). Models were compared with the model select function from the MuMIn package v-1.43.6 using the Bayesian information criterion (BIC) to select the best overall model. Identity of female and male parents was added as a random effect, because many pairs laid multiple clutches per year and bred in multiple years. To control for possible effects of island and climatic variation each year, location was added as a factor and year was added as a random factor.

To determine if there were differences in the reproductive success of robins from different observational periods, using the additional breeding data from Heber (2012), I divided the data into three “groups”: (1) pre-translocation, which consisted of ‘inbred’ adults that were alive prior to the genetic rescue in 2008, (2) translocation pairs, which included pairs that were formed from a combination of Allports and Motuara birds from 2008-2010 or pairs that included at least one known interbred individual descended from these translocation pairs (including individuals in generations F1-F3 after the genetic rescue), and (3) post-translocation, which were breeding individuals from my study from 2016-18. Nesting success was compared between the three observed groups (i.e., pre-translocation, translocation pairs, and post-translocation), for each stage in the nesting cycle and was analysed using a Kruskal-Wallis anova.

Survival for juveniles monitored in 2016-2018 to one and two years of age was calculated with a General Linear Model (GLM) using a binomial fit. Individuals that were located at one year of age were designated with 1 and individuals that could not be located a 0. Survival for both sexes combined was analysed first, followed by separate analyses for

males and females, to determine if there were sex specific HFCs related to survival. Individual genetic diversity, measured as individual sMLH, was used as the predictor and survival as the response. To control for possible effects of island and climatic variation each year, location and year were added as factors to the models. An additional term, banded, was added to the models. This was to control for the time of year juveniles were banded, as some were banded in the nest as chicks while others as juveniles post fledging. Similar to reproductive success of adults, juvenile survival was also divided into three “groups”: (1) pre-translocation juveniles, which consisted of ‘inbred’ juveniles that had two inbred parents, (2) interbred F1-F3 juveniles, which had parents that were formed from a crossing of Allports and Motuara birds from 2008-2010 or pairs that included at least one known interbred individual descended from these translocation pairs (including individuals in generations F1-F3 after the genetic rescue), and (3) post-rescue juveniles, which were juveniles descended from breeding individuals from 2016-18. A GLM with a binomial error structure was used to analyse differences in survival rates between the three groups. An ANOVA using a chi-squared output was run on the model output to see if there were significant overall differences between the three groups. A post hoc Tukey test was performed on the GLM output to compare differences between each group. All selected models were checked for goodness of fit (Agresti and Kateri 2011), with the gof function, R package aods3 v0.4-1.1. Long term survival was calculated with a Chi-square test comparing number of inbred juveniles and interbred F1-F3 juveniles alive in 2008-2010 to the number recorded alive in 2016-2018.

3.3 Results

3.3.1 Breeding success

From 2008 to 2018, using samples from Allports Island, Motuara Island, Kaikoura and Nukuwaiata Island, a total of 1105 robins were banded and genotyped at 28

microsatellite loci. Between 2008-2010 and 2016-2018, a total of 322 nests with eggs were monitored. Additional nests were found during the pullus stage, increasing the total nests monitored to 366. However, it was only possible to monitor 344 of the 366 nests to fledgling stage. A total of 312 clutches that fledged were then monitored to one month of age. For combined nests in 2016-2018 robins on average nested 3.7m off the ground (range = 0-13.2m, SE = 0.2m, N = 180 nests).

Clutch size was on average 2.0 (SE = 0.01) eggs per clutch and ranged from 1-3 eggs. Hatching success varied from 0% (all eggs failed to hatch) to 100%, with an average of 77% of the monitored eggs hatching (average number of eggs hatching per nest, 1.61, SE = 0.04, range 0-3, N eggs monitored = 582, N eggs hatched = 445). A total of 66% of the hatched chicks subsequently fledged, with an average of 1.06 chicks fledging/nest (range = 0-3, SE = 0.04, N chicks monitored to fledging = 340). For nests that fledged, a total of 70% of individuals survived to one month of age (average number of fledglings surviving one month/nest = 0.75, SE = 0.04, range = 0-3, N fledglings monitored surviving one month = 247). When comparisons were made across all life stages from laying to one month after fledging, the overall likelihood of an individual chick hatching and surviving to one month of age was 35% (N chicks surviving from lay until plus one month after fledging = 198).

When possible the cause of nest failure was recorded for nests from 2016-2018. In total, 169 nests were monitored during incubation phase and 155 during pullus phase. Failure due to predation was rare and was determined when the nest was dismantled or with the partially depredated parts of the eggs/chick visible in the nest. The rate of nests predated during incubation was 0.6% (N =1) and 4.5% during the pullus stage (N = 7). It was not possible to determine the identity of the predators, but it was most likely due to resident New Zealand falcons (*Falco novaeseelandiae*) or Australasian harrier (*circus approximans*) on both islands. Although morepork owls (*Ninox novaeseelandiae*) do predate robin nests they

were never directly observed or heard during the three years of this study on Motuara or Allports island. Other causes of failure included nests falling out of the tree (1.7%, N = 3), but this was only recorded during pullus stage. Although difficult to determine if nests failed due to abandonment or infertility during the incubation phase, this could be determined for 1.1% (N = 2) of nests where the female abandoned before incubation was complete. The cause of the remaining nests that failed during incubation phase were unknown (N = 25). For pullus stage, 11% (N=17) of the nests failed with the chicks recorded dead in the nest due to abandonment or adverse weather, while the remaining causes of death are unknown (N = 15).

Breeding success models indicated that an interaction between males and females did not provide the best overall fit for the models and as such all models are presented without an interaction but retaining both male sMLH and female sMLH in the same model. No significant correlation between male or female genetic diversity and fitness measures were observed at any stage of the nesting cycle or overall, nor did female genetic diversity correlate with clutch size (Table 3.1). Thus, there was no indication that variation in reproductive success, as measured in this study, was related to individual level of genetic variation. There were significant differences between Motuara Island and Allports Island for number of chicks fledged: Allports Island parents fledged more chicks per nest (Mean \pm SE: 1.09 ± 0.067 chicks/nest) than on Motuara Island (1.02 ± 0.055 chicks/nest) (Table 3.1). Similarly, parents on Allports Island raised more chicks to one month of age (Mean \pm SE, 0.751 ± 0.063) than on Motuara Island (0.703 ± 0.05). This resulted in the number of chicks surviving overall per nest being higher on Allports Island (Mean \pm SE; 0.71 ± 0.07 chicks/nest) than on Motuara Island (0.68 ± 0.06 chicks/nest) (Table 3.1).

When comparisons of nesting success were made between the three groups (pre-translocation pairs, translocation pairs, and post translocation pairs), no significant differences were observed at any stage: clutch size, hatching success, number of chicks

fledged, number of chicks raised to one month of age, and overall nesting success (Table 3.2, Fig. 3.2a-e). However, there was a trend for post-translocation females to have smaller clutches in 2016-2018 compared to pre-translocation females (Table 3.2, Fig. 3.2a).

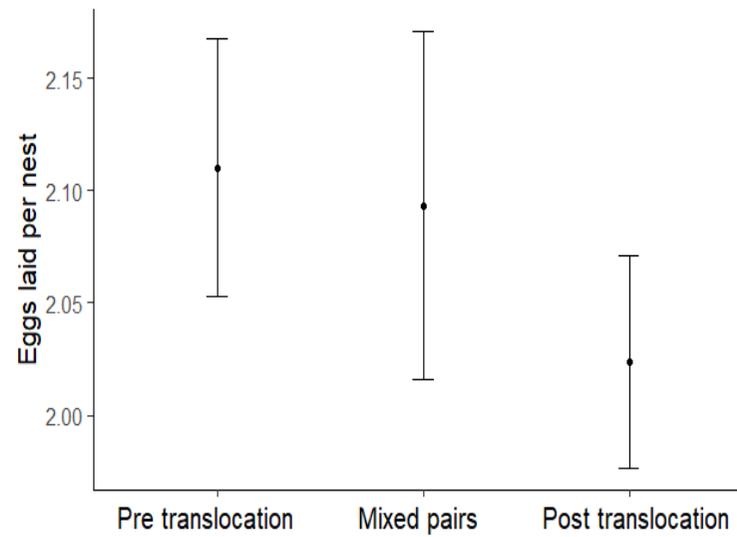
Table 3.1. MCMCglmm outputs for number of eggs laid, number of eggs hatched, number of chicks fledged, number of fledglings surviving to one month of age and overall success from laying until the chick was one month of age. Presented are the post means, lower and upper 95% confidence intervals, effective sample size from the iterations, and pMCMC. Significant results are highlighted in bold.

Trait	Factors	Post mean	Lower 95% CI	Upper 95% CI	eff samp	pMCMC
Eggs laid	Intercept	2.149	1.956	2.344	54730	<0.001
	Female sMLH	-0.072	-0.268	0.119	88426	0.47
	Location	-0.092	-0.169	-0.014	32368	0.02
Chicks hatched	Intercept	1.886	1.252	2.531	17067	<0.001
	Female sMLH	-0.105	-0.572	0.347	18000	0.652
	Male sMLH	-0.082	-0.504	0.365	17125	0.707
	Location	-0.140	-0.328	0.055	18000	0.156
Chicks fledged	Intercept	1.419	0.738	2.095	19124	<0.001
	Female sMLH	-0.168	-0.650	0.317	18000	0.499
	Male sMLH	0.020	-0.451	0.465	18000	0.926
	Location	-0.337	-0.537	-0.134	17597	0.001
Fledglings survival to one month	Intercept	0.970	0.289	1.622	5835	0.004
	Female sMLH	-0.033	-0.515	0.448	17137	0.894
	Male sMLH	-0.006	-0.459	0.463	13177	0.984
	Location	-0.270	-0.471	-0.069	15360	0.008
Survival overall	Intercept	0.857	0.136	1.565	90000	0.019
	Female sMLH	-0.086	-0.597	0.432	88579	0.739
	Male sMLH	0.125	-0.391	0.640	87591	0.633
	Location	-0.258	-0.475	-0.038	90000	0.021

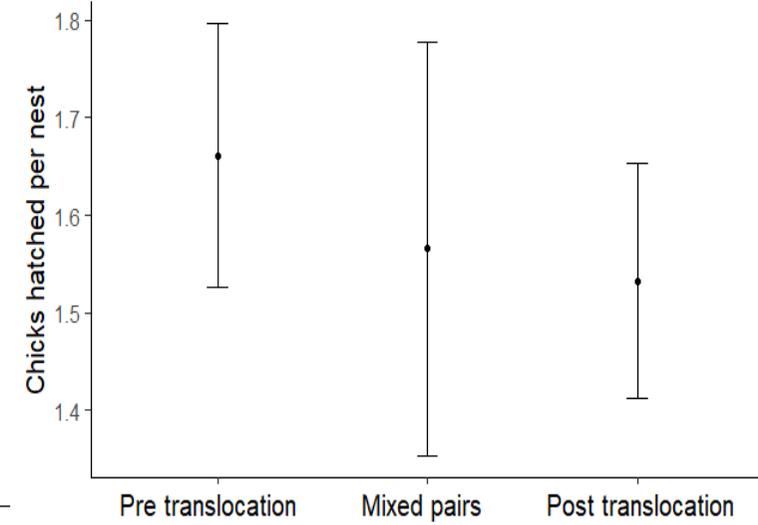
Table 3.2. Kruskal-Wallis anova test between the three different groups (pre-translocation, translocation pairs and post-translocation pairs) for clutch size, number of eggs hatched, number of chicks fledged, number of fledglings surviving to one month of age and overall success from laying until the chick was one month of age.

Trait	χ^2	DF	P
Eggs laid	5.320	2	0.069
Eggs hatched	1.764	2	0.414
Chicks fledged	0.613	2	0.736
Chicks raised to one month of age	3.562	2	0.168
Overall nesting success	1.018	2	0.601

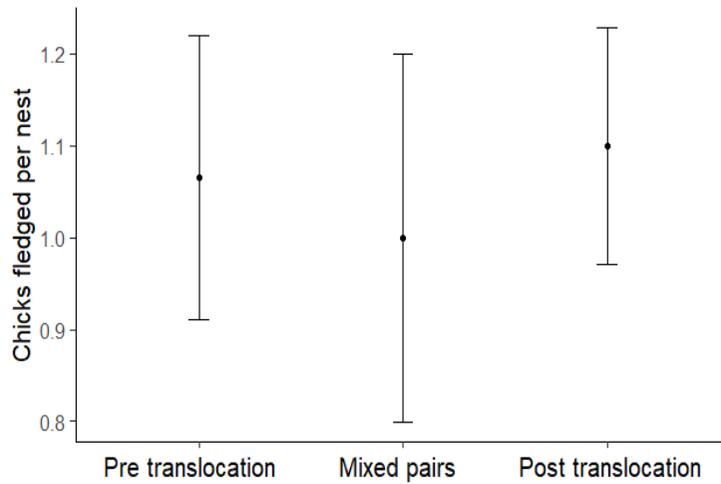
Figure 3.2. Breeding success over the four stages of nesting cycle and overall breeding success of South Island robins. Models are (a) number of eggs laid per nest, (b) number of eggs hatched, (c) number of chicks fledged, (d) number of fledglings surviving to one month of age, and (e) overall success from laying until chick was one month of age. All figures are presented with mean and 95% confidence intervals.



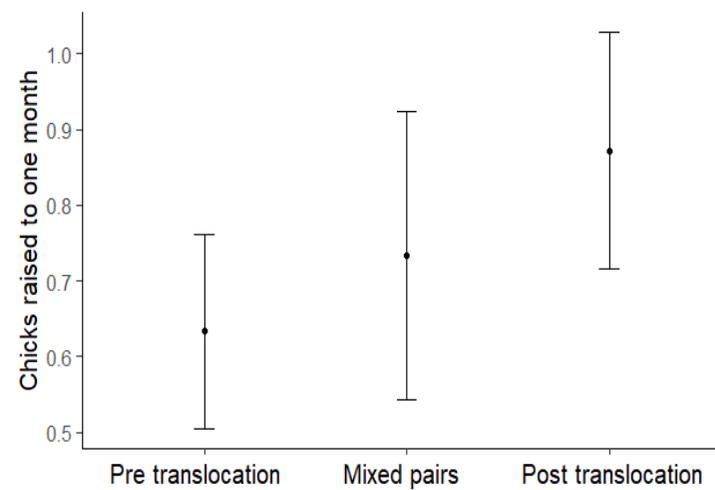
a



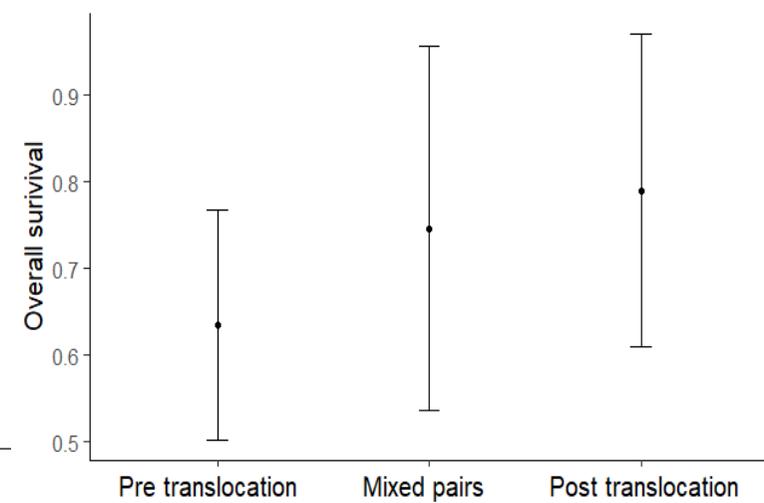
b



c



d



e

3.3.2 Survival

In total, 232 juveniles were monitored to one year of age between 2016 and 2018. The survival rate for these individuals was 52%. A significant HFC was detected for survival to one year of age (Table 3.3), as juveniles with higher levels of individual genetic diversity were more likely to survive to one year of age (Fig. 3.3). This pattern was primarily driven by female survival to one year of age, where a significant sex-specific HFC was detected. The HFC was not quite significant for males (Table 3.4). Year also had a significant effect on female survival, with juvenile females from 2016-2017 (Mean \pm SE: 0.63 ± 0.05) having higher survival than juvenile females from 2017-2018 (Mean \pm SE: 0.42 ± 0.07) (Table 3.5). There was no difference between years in male survival (Table 3.4).

There were significant differences in survival to one year of age between the three groups (pre-translocation juvenile, interbred individuals, and post-translocation juveniles) (Table 3.5). Interbred F1-F3 (71% survival to one year of age), and post-translocation individuals (51.7% survival to one year of age) were significantly more likely to survive than pre-translocation juveniles (31% survival to one year of age) (Table 3.6, Fig.3.4). Of the juveniles that survived to one year of age in 2016, it was possible to monitor the survival of 78 juveniles to two years of age in 2018, but no HFC was detected (Table 3.7, Fig. 3.5). Overall, 83% of individuals that survived to one year of age were detected at two years of age. A relatively small percentage (8%, $N = 23$), of individuals from the juvenile cohorts from 2008-2010 survived until 2017-2018. In this group, the F1-F3 interbred individuals were significantly more likely to survive to old age in comparison to pre-translocation individuals ($\chi^2 = 152.36$, $df = 2$, $p < 0.001$). interbred F1-F3 individuals made up 28% of the recorded juveniles in 2008 but formed 43% of the resighted adults in 2016-2018.

Table 3.3. Results of a General Linear Model with binomial structure for survival to one year of age. Juveniles with higher genetic diversity were more likely to survive to one year of age. Estimated regression parameters, estimate, standard errors, z-values, and P-values are presented. Significance is indicated in bold.

Factors	Estimate	Std. Error	z	Pr(> z)
Intercept	-1.375	0.840	-1.637	0.103
sMLH	2.059	0.753	2.735	0.007
Year	-0.639	0.324	-1.969	0.051
Location	-0.561	0.299	-1.879	0.062
Sex	-0.319	0.283	-1.126	0.261
Banded	0.234	0.384	0.610	0.543

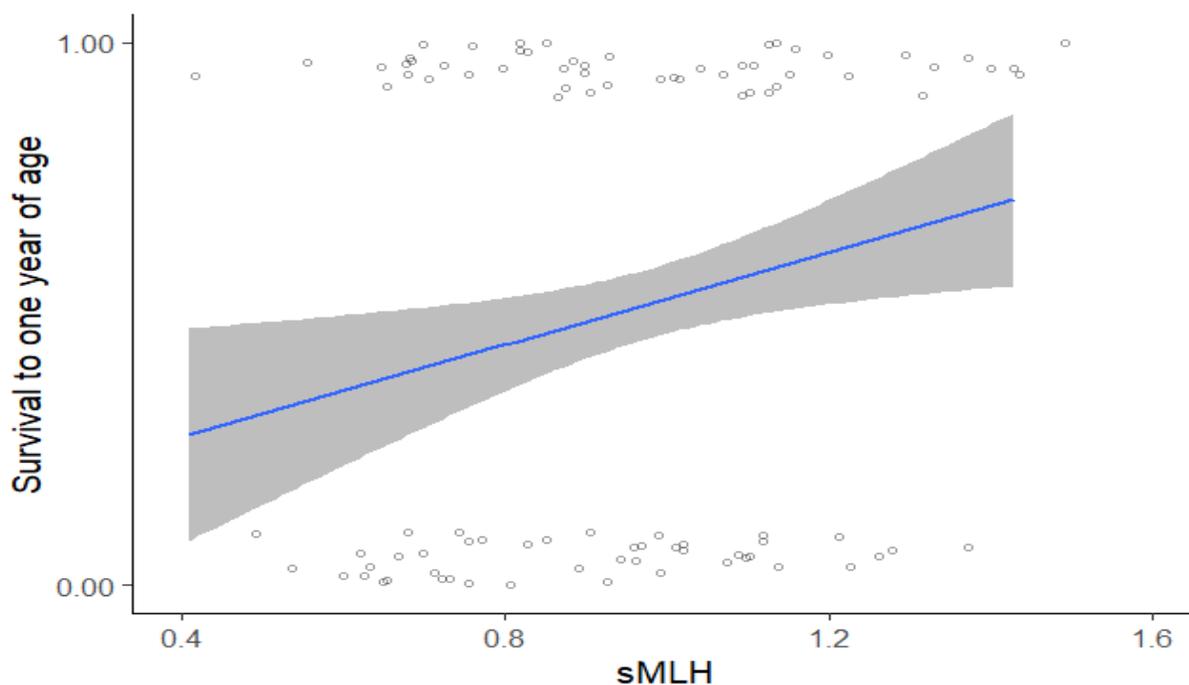


Figure 3.3. Correlation between sMLH and survival to one year of age with 95% confidence intervals. A value of 1 on the y-axis denotes that the bird survived while a value of 0 indicates the bird was unaccounted for and assumed to be dead. The x axis represents individual genetic diversity as measured by sMLH.

Table 3.4. Results of a General Linear Model with binomial structure for sexual differences in survival to one year of age. Presented first is the output for females followed by the output for males. Estimated regression parameters, estimate, standard errors, z-values, P-values are presented for females and males. Significance is indicated in bold.

Female				
Factors	Estimate	Std. Error	z	Pr(> z)
Intercept	-1.614	1.080	-1.495	0.135
sMLH	2.658	1.118	2.378	0.017
Year	-1.186	0.422	-2.809	0.005
Location	-0.476	0.391	-1.216	0.224

Males				
Factors	Estimate	Std. Error	z	Pr(> z)
Intercept	-1.367	1.065	-1.284	0.199
sMLH	1.851	1.003	1.845	0.065
Year	-0.274	0.418	-0.654	0.513
Location	-0.651	0.446	-1.459	0.145

Table 3.5. Output from General Linear Model binomial analysis for differences between pre-translocation birds, interbred individuals, and post-translocation juveniles. Presented is the degrees of freedom, deviance, residual degrees of freedom, residual deviance and P-values. Significance is indicated in bold.

Factors	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
Null			380	526.79	
Group	2	22.1462	378	504.64	<0.001
Location	1	5.9993	377	498.64	0.014

Table 3.6. Tukey post hoc tests for General Linear Model binomial analysis between pre-translocation birds, interbred individuals, and post-translocation juveniles. Presented is the estimate, standard error, z value and p value. Significance is indicated in bold.

	Estimate	Standard error	z value	P
Pre translocation vs F1 - F3 interbred	-1.5946	0.4653	-3.427	<0.001
Pre translocation vs Post translocation	0.8966	0.2389	3.753	<0.001
F1 - F3 interbred vs Post translocation	0.6279	0.4449	-1.569	0.116

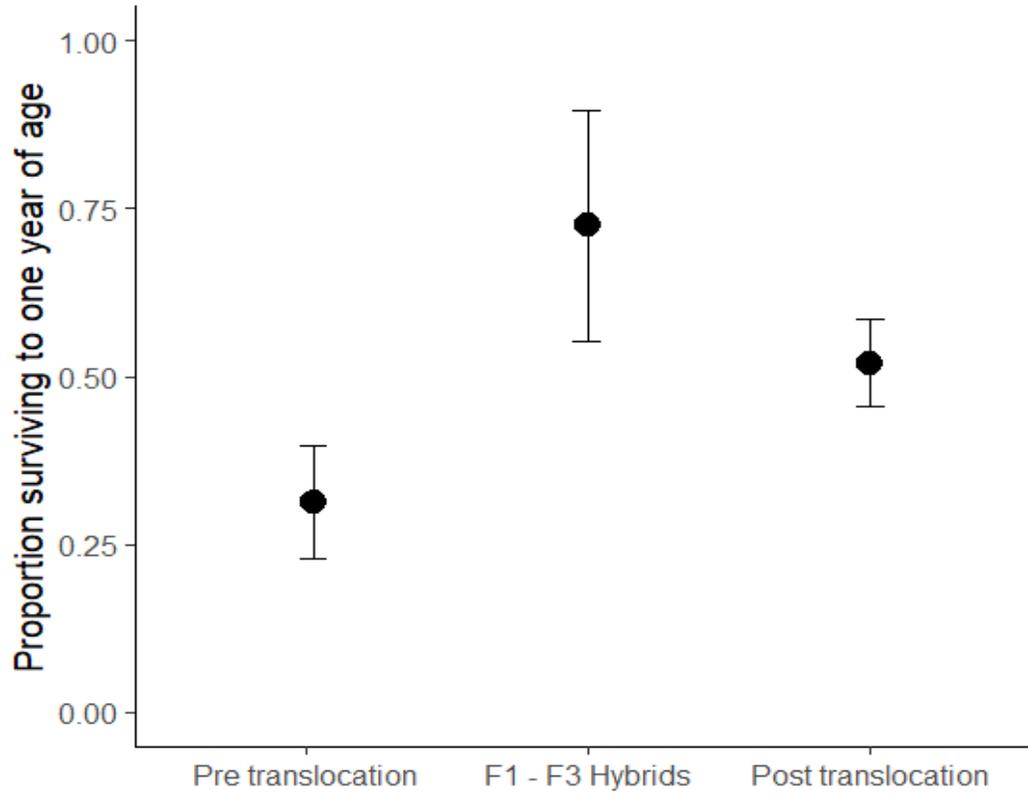


Figure 3.4. Comparisons between pre-translocation juveniles, interbred juveniles and post-translocation juveniles. Presented is the mean proportion of juveniles surviving to one year of age with 95% confidence intervals.

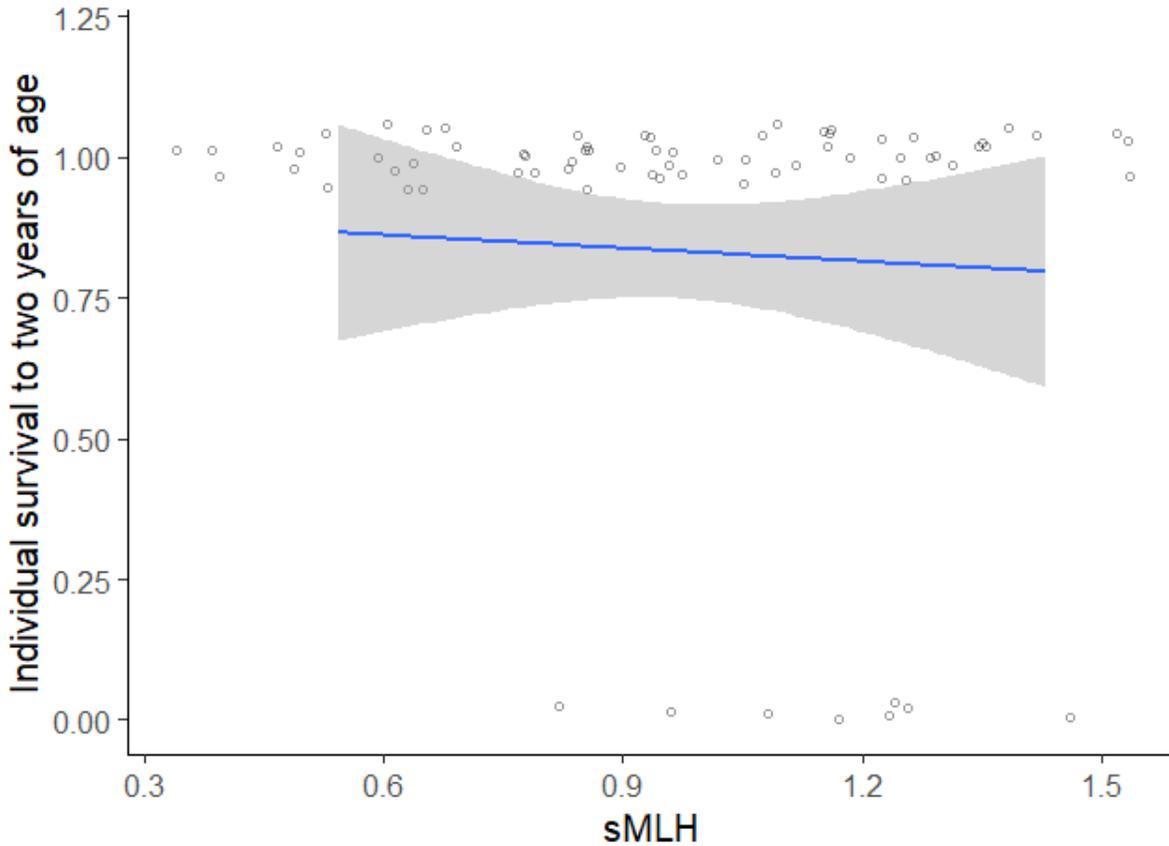


Figure 3.5. Model output for survival to two years of age with 95% confidence intervals. A value of 1 on the y-axis denotes that the bird survived while a value of 0 indicates the bird was unaccounted for and assumed to be dead. The x axis represents individual genetic diversity as measured by sMLH.

Table 3.7. Results of a General Linear Model with binomial structure for the analysis of survival to two years of age. Juveniles with higher genetic diversity significantly more likely to survive to one year of age but there was no pattern at two years of age. Estimated regression parameters, estimate, standard errors, z-values, P-values are presented.

Factors	Estimate	Std. Error	z	Pr(> z)
Intercept	2.203	1.572	1.401	0.161
sMLH	-1.256	1.603	-0.784	0.433
Location	0.544	0.628	0.866	0.387
Sex	1.031	0.737	1.399	0.162

3.3.3 Population demographics

In comparison to Heber (2012) and Maloney (1991), I recorded a considerable population increase in 2018 (Fig. 3.6 and 3.7). In 2008-2010, approximately 60 adult robins were observed on Allports Island (density of ~ 3.75 birds/ha) and ~ 300 adult robins were censused on Motuara Island (density of ~ 5.1 birds/ha, (Heber 2012)). By my study in 2018, the Allports Island population had increased to a minimum total of 104 resident adult birds, and at least 3 additional unbanded birds. Density also increased to 6.5 birds/ha and in total there were 39 territory holding pairs (this excluded 3 possible pairs where only one individual from the pair could be identified feeding chicks after fledging). On Motuara Island, the population within the 22 ha grid was censused at 186 adult birds and a density of 8.45 birds/ha. Extrapolating from the 22 ha counts to the 59 ha area of Motuara Island represents a total population of 498 adult robins. However, caution should be taken with this estimate as it is possible that floater birds not living in the 22 ha grid could have led to an over-estimate. If I used just the number of territorial pairs (74), as these birds were observed multiple times and known to have territories in the 22 ha area, a more conservative estimate would be 437 adult residents over the entire island (density of 7.4 birds/ha). Thus, my censuses show an increase on Allports from 3.75 birds/ha to 6.5 birds/ha (173% increase in population size) and a similar increase on Motuara from 5.1 birds/ha to 7.4 birds (145% increase in population size).

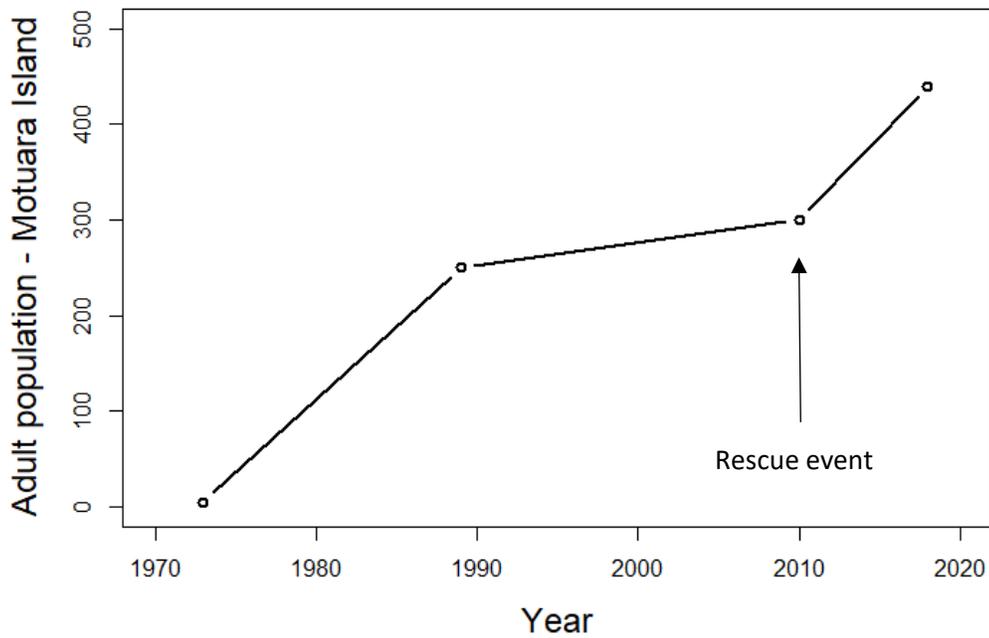


Figure 3.6. Change in adult robin populations from 1974 to 2018 on Motuara Island. Population measures were collected in 1989 (Maloney 1991), 2008-2010 (Heber 2012) and in my study in 2018.

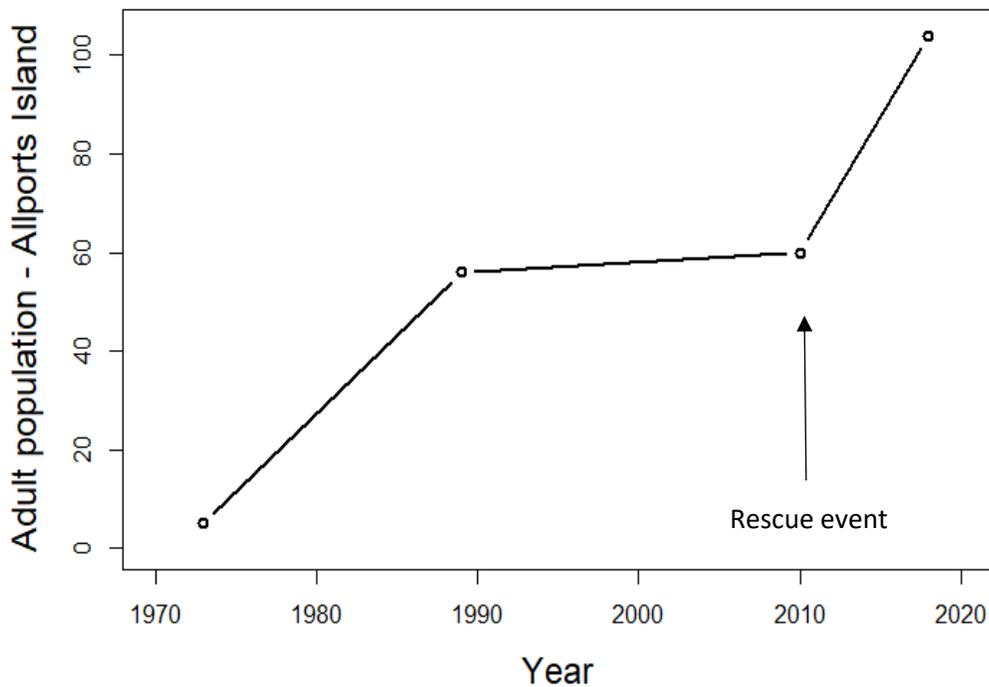


Figure 3.7. Change in adult robin populations from 1974 to 2018 on Allports Island. Population measures were collected in 1989 (Maloney 1991), 2008-2010 (Heber 2012) and again in 2018.

3.3.4 Identity disequilibrium

The g_2 estimator of identity disequilibrium calculated for all markers was positive.

Heterozygosity differed significantly from zero across loci ($n = 1,380$, $g_2 = 0.019$, $se = 0.0017$, 95% CI = 0.0161, 0.0226, $p = 0.01$; and HHC = 0.25 ± 0.049 , CI = 0.143, 0.341, Fig. 3.8). These results suggest that marker heterozygosity is representative of genome wide heterozygosity (Szulkin et al. 2010), and means that any HFCs detected would be indicative of inbreeding depression.

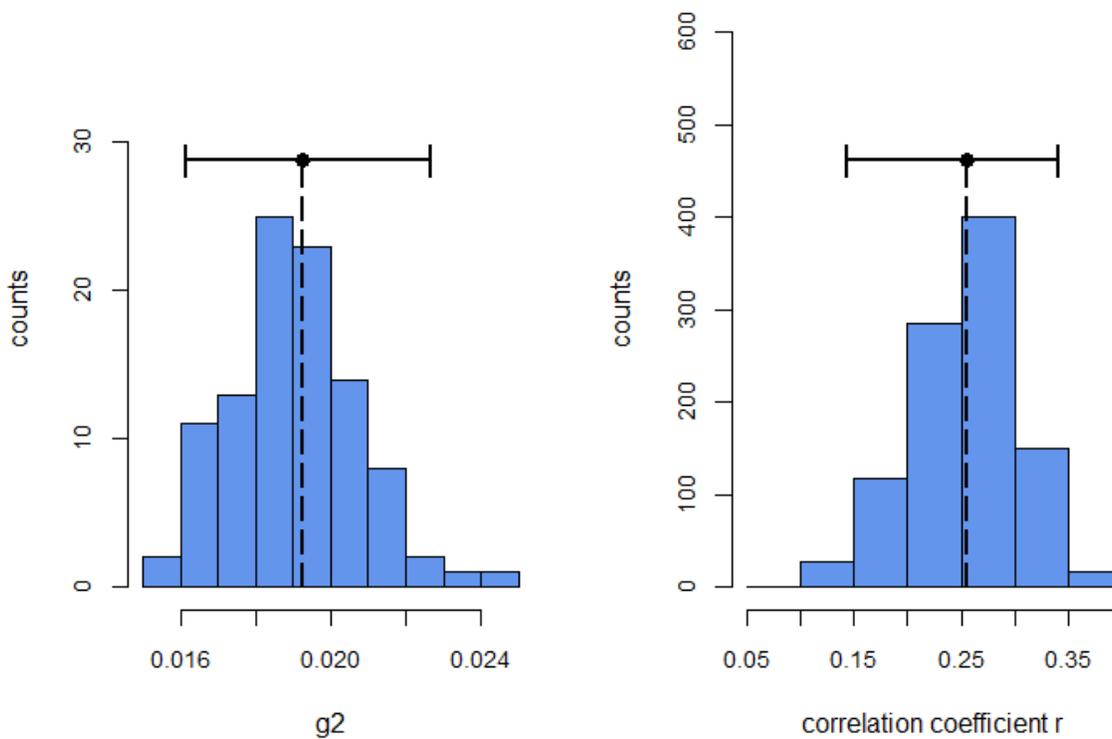


Figure 3.8. Outputs for g_2 and HHC (correlation coefficient r) tests. In both cases, the 95% confidence intervals do not overlap with zero, indicating the results were significant.

3.4 Discussion

A genetic rescue is considered successful if there is a sustained increase in the population. With many of New Zealand's remaining endangered bird species confined to island refuges with expected population caps, due to the limited of size islands, this begs the question whether genetic rescue attempts can ever result in successful genetic rescue (i.e., with concomitant population increase) or would it more likely result in genetic restoration (i.e., only an increase in genetic diversity but little change in population size)? In my study, both robin populations increased considerably on the two island refuges, indicating that a successful genetic rescue event has occurred. This is a promising outcome for two reasons: (1) the donors that were used in these translocations were from inbred populations that had lower genetic diversity than relatively outbred mainland populations (*unpub.data chapter 2*) and, (2) the island environments they were transferred between had pre-existing populations that were expected to already be at carrying capacity. This suggests that these robin populations were not previously at carrying capacity and this may have been the result of inbreeding and low levels of genetic diversity.

The increase in population size I observed was likely driven by increased survival from juveniles and adults which had higher levels of individual genetic diversity. A significant HFC was detected for juvenile survival to one year of age, within the 2016-2018 populations. Interestingly, although both sexes showed positive correlations with increasing genetic diversity and for survival to one year of age, this trend appeared to be driven by female survival to one year. When comparisons were made between the juveniles across time (from pre- to post-translocation), the interbred F1-F3 and post-translocation juveniles were significantly more likely to survive to one year of age than pre-translocation individuals. This pattern was further reflected with some adults surviving to old age, as interbred F1-F3 individuals from 2008-2010 were significantly more likely to survive to 10 years of age than their pre-translocation counterparts. Increased survival for both juveniles and F1-F3 interbred adults can, in turn, provide these

individuals with the increased opportunities to reproduce. Considered together, it supports the idea that increased population size is a result of increased survival, leading to increased number of offspring per lifetime, and both are the consequence of increased genetic diversity on the islands as a result of the genetic rescue. Interestingly, genetic diversity between individuals did not appear to significantly influence any of individual nesting success measures, suggesting the population increase must have been largely due to increased individual survival.

Observed changes in breeding success

Overall, I found no significant HFCs for any of the breeding phases or overall for male and female genetic diversity. This result was similarly reflected with comparisons made between pre-translocation pairs, translocation pairs and post-translocation pairs, where no significant differences were detected. These observations are at odds with some previous studies that found increases in reproductive success with increased genetic diversity. For example, Hawaiian crow (*Corvus hawaiiensis*) embryos from individuals with low genetic diversity have a five times lower probability of survival, suggesting that inbreeding depression is particularly severe in early life traits (Hoeck et al. 2015). Similarly, in a captive breeding program of Attwater's prairie chicken (*Tympanuchus cupido attwateri*), a reduction in mean parental relatedness in pairs resulted in a significant increase in the proportion of the clutch surviving to five weeks post-hatch (Hammerly et al. 2016). Other researchers have similarly found inbreeding effects on sperm quality (Fitzpatrick and Evans 2009, Zajitschek et al. 2009) and abnormalities in embryogenesis (Keller and Waller 2002). Yet my findings were not unexpected as Heber et al. (2013) likewise found no clear evidence for a difference in reproductive success between inbred and interbred birds. There is no expectation that all reproductive and life history traits are equally affected by population bottlenecks and inbreeding, and it may be that the reproductive

traits I studied in robins were robust to the degree of genetic variation that was lost when the populations were founded.

Genetic rescue, survival and population increase

Survival to one year showed a significant HFC with juveniles in 2016-2018 with higher genetic diversity being significantly more likely to survive to one year of age. In contrast, there was no significant HFC detected for survival from one to two years of age, as most birds that survived to one year of age managed to survive to two years of age. This would suggest that the first year after fledging is a critical stage in a juvenile robin's development, and subsequently if they could pass this milestone their chances of surviving in the following years was greatly enhanced. The first year of independence, between the end of parental care and adulthood, for juveniles in many bird species has been found to be a period of high mortality with strong selection pressure for higher fitness traits, such as increased mass, tarsus length or immunocompetence (Maness and Anderson 2013, Naef-Daenzer and Gruebler 2016). For example, in alpine marmots (*Marmota marmota*) there was a positive correlation between heterozygosity and survival in juveniles, but not in yearlings, 2-year-olds or adults, which may be explained by selection against low heterozygous individuals (Cohas et al. 2009). Similar findings have been made with deer mouse (*Peromyscus maniculatus*) (Schwartz and Mills 2005), and rainbow trout (*Oncorhynchus mykiss*) (Tymchuk et al. 2007). The detection of a significant HFC for survival to one year of age is therefore not unexpected.

Although robins have been known to live up to 16 years (Heather and Robertson 2000), I was somewhat surprised that a number of individuals from the 2008-2010 study were still alive during my census. Long-term survival of F1-F3 interbred individuals was significantly higher than inbred individuals of the same cohort. The survival probability for individuals can have a major effect on population dynamics (Maness and Anderson 2013), as increased survival can provide individuals with

the increased opportunities to reproduce. This combination of increased survival and reproduction would likely lead to a substantial population increase. Similar results have been observed with Florida panthers (*Felis concolor coryi*) where interbred individuals had greater survival which resulted in the population tripling in size (Benson et al. 2011). Detailed and rigorous demographic analyses suggest that an annual population growth rate of 4% replaced a 5% population decline following translocation in Florida panthers. Extrapolating the change in population size I observed in robins would have required a yearly population growth rates of 7.1% on Allports Island and 4.8% on Motuara Island from 2010-2018, a similar growth rate to the Florida panthers.

An unpublished report for the Department of Conservation on the robin populations in 1989 on both Motuara Island and Allports Island suggested similar population sizes that Heber (2012) recorded at the time of the translocation. The population on Allports Island in 1989 was estimated at 56 adults and on Motuara Island the population was estimated at 250 adult individuals (Maloney 1991). Although there appears to have been a slight increase in the robin population between 1989 and 2010 for Allports Island and Motuara Island this change was not as remarkable as that seen between 2010 and 2018, further suggesting that genetic rescue was the cause of the increase that occurred after the translocation.

The differences in sex specific HFCs detected in this study could be due to heterogametic sex selection (Lindström 1999, Pike and Petrie 2003). Deleterious recessive alleles that are linked to the sex chromosomes cannot be masked as easily in female birds as they are the heterogametic sex, and it could be expected that females with lower genetic diversity should exhibit lower fitness than males (Charlesworth and Charlesworth 1999). This was observed in this study for juvenile survival to one year of age; although both sexes showed trends towards increased survival with increased genetic diversity this trend was much stronger in females. Unfortunately, due to the low variation in survivorship, with

most individuals surviving to two years of age, it was not possible to analyse the sexes separately to see if this trend was repeated the following season.

Genetic rescue and outbreeding depression

In spite of the recognised benefits of translocations for conservation purposes, concerns have been raised about genetic rescue leading to outbreeding depression, due to the disruption of locally adapted gene complexes (Tallmon et al. 2004, Edmands 2007, Frankham et al. 2011). Yet few studies have been able to monitor populations over the long term to assess if outbreeding depression is occurring. My study is one of the few that has been able to monitor two populations of the same species concurrently over a 10-year period to see if outbreeding is occurring. The effects of outbreeding depression are not typically expressed until after the F2 generation, and effects of outbreeding depression can be delayed to the F3 generation and beyond (Marshall and Spalton 2000, Tallmon et al. 2004), as this is when the recombination of original parental gene fragments occur, potentially affecting local adaptations. Although I was not able to measure all traits likely to be affected by outbreeding depression, the high survival of robins with increased individual genetic variation (including F1-F3 robins) and similar levels in a number of metrics of reproductive success (e.g., hatching success), suggests any negative consequences have been outweighed by the benefits of greater genetic diversity. Overall, I found no evidence for outbreeding depression, and robins 10 years after genetic rescue now have similar breeding success, a higher population size and higher survival than they did pre-rescue.

Is a change in habitat availability the cause of the population increase?

Although I have attributed the population increase as a consequence of genetic rescue, it is also possible the change could be the result in increased habitat availability or habitat quality. Both islands were cleared for farmland but were then abandoned in the 1930s and left to regenerate naturally. As the

forest structure transitions through ecological succession, this could increase habitat availability and thereby the population size for robins. After almost 90 years both islands are now dominated by mature kānuka (*Kunzea ericoides*) stands with successional broadleaf woody species growing through the understory. Yet regeneration is a relatively slow process for similar island environments once they reach secondary succession. Based on regeneration sequences on northern offshore islands, kānuka stands usually require over 100 years before they are replaced by broadleaf woody species (Atkinson 2004). Similar results were also observed on Tiritiri Matangi Island where ecological succession was relatively slow (Mitchell 2013). As a result, it seems likely that time scale of native forest succession was too long to result in the substantial population increases I observed on Motuara and Allports islands between 2008 and 2018. By 2008, succession was well advanced and both islands had entirely closed canopies with relatively deep leaf litter (pers. obs.). Furthermore, although robins prefer mature forest they readily live in areas of scrub, and exotic plantations so long as there are areas of open understorey under a closed canopy with fertile soils. Both in 2008-2010 and in my study, there were no areas of either island that were unoccupied, and thus the increase in population was not due to an increase in habitat area but an increase in density of robins across the entire island. If an increase in habitat quality was the cause of increasing robin populations, we would expect to have seen similar population increases from 1989 to 2010, as the forest matured during this time. Indeed, there was a slight increase in the robin population during this time, it was not as large as the change observed from 2010 to 2018. Although I cannot rule out that changes in habitat due to forest succession may have helped augment the two populations growth, it is unlikely to be the main cause of the large population growth on the two islands.

Conclusion

With many of New Zealand's endangered bird species confined to island refuges with set carrying capacities and with limited chances of dispersal, it is important to know how attempted genetic

rescue could impact these populations. Observations from this study show that the introduction of new alleles into the population resulted in increased survival of both adults and juveniles and that the likely long-term consequence of the increased survival was an increase in population size on the two islands. This suggests that genetic rescue may be a useful tool to increase the population size of endangered birds suffering from inbreeding, even for species in New Zealand that are restricted to small islands and in which the scope for population growth seems limited by island size.

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**Does genetic diversity in a small passerine
correlate with immune system response,
parasite load and morphology?**

Chapter 4



Figure 4.1 the collection of a blood sample and morphological measurements from a South Island robin chick on Motuara Island.

Abstract

In 2008, a novel approach using inbred donors in a reciprocal translocation between two highly inbred island populations of South Island robin (*Petroica australis*) was undertaken. The reciprocal translocation was a success in the short-term, with increased genetic diversity resulting in increased survival and immune response in both populations. In 2016-2018, I revisited these two populations and repeated similar measures. Initial findings showed that there was still a significant correlation between genetic diversity and survival for juveniles to one year of age (Chapter 3). Whether this correlation was mirrored with immune system response and parasite loads was unknown. Therefore, immune systems function was reassessed by challenging robins with the phytohaemagglutinin (PHA) test, measuring ecto- and endo-parasite loads and two morphological measures, individual mass and tarsus length. Birds on both islands continued to show a strong reaction to the PHA challenge, but overall there was only a weak positive correlation between genetic diversity and immune system response. For ecto- and endo-parasite loads only one HFC was detected: as genetic diversity increased mite loads decreased. No significant correlations were observed for morphological measures. Overall, there was only weak evidence that genetic diversity was correlated with a range of measures related to immune system response, parasite load and morphology. This was possibly due to a range of factors, including microsatellites being a poor indicator for immune response, a lack of genetic variation between the observed individuals, and the age of the robins as only young birds were tested.

4.1 Introduction

Traditionally, success in conservation, both in New Zealand and around the world, has been largely assessed through the positive demographic growth of the species or populations that are declining (Pacioni et al. 2013). However, demographic success can often belie a series of fitness-related problems directly attributable to the loss of genetic variation and inbreeding depression (Frankham et al. 2002), such as reduced immune system function (e.g. through loss of functional gene diversity at major histocompatibility complexes (MHC) and toll like receptors (TLR) (Grueber et al. 2017)). Reduced genetic diversity in populations limits their ability to evolve in response to new pests, climatic changes, and introduced or evolving parasites (Sorci et al. 1997, Frankham et al. 2002, Spielman et al. 2004). With increased rates of human-assisted spread of pathogens and changes in pathogen distribution from climate change it is becoming increasingly important to understand how loss of genetic variation could potentially impact recovering populations. For example, in 2015 the saiga antelope (*Saiga tatarica*) suffered a mass die off, with 120,000 confirmed deaths from a population of 250,000 animals. Although the cause was attributed to bacterial infections carried by the saigas (Zimmer 2015), the high level of mortality was likely a consequence of reduced genetic diversity due to recent populations bottlenecks (Campos et al. 2010).

Many of New Zealand's endangered birds have been similarly subjected to severe population bottlenecks and although conservation measures have successfully increased the population size in some species, there is mounting evidence that the loss of genetic variation may have increased their susceptibility to pathogens (Hale and Briskie 2009), and reduced immune system function (e.g., South Island robins (Grueber et al. 2012, Heber 2012)). In some species, it has been suggested that disease outbreaks were likely driven by inbreeding depression and reduced immune response (Bellingham et al. 2010), including the premature death of three female kākāpō (*Strigops habroptilus*) (Ballance 2007), high levels of mortality of translocated hihi (*Notiomystis cincta*) (Armstrong et al. 2007) and the

increased susceptibility of saddlebacks (*Philesturnus carunculatus*) to disease (Hale and Briskie 2009). Diseases and parasites are therefore of particular concern in the management of endangered species that have undergone a population bottleneck (Frankham et al. 1999).

In theory, the negative effects on immune system function brought about from population bottlenecks and inbreeding depression can be reversed through a degree of hybridization, genetic rescue or genetic restoration, which may improve the viability of endangered species by mitigating problems related to inbreeding and loss of genetic variation (Amos and Balmford 2001). Typically, attempts to reduce inbreeding depression involve the translocation of donor individuals into the bottlenecked population, with the aim to increase population size, levels of genetic variation and immune system function (Whiteley et al. 2015). Assessing the effectiveness of genetic rescue on immune system function, both in the immediate and long-term, thus requires reliable estimates of genetic variation of the focal population as well as direct and indirect measures of immune system function. Calculating heterozygosity at neutral markers and correlating it with individual fitness (e.g., heterozygosity fitness correlations (HFCs)), can be a powerful tool to show how the loss of genetic variation influences phenotypic qualities (Szulkin et al. 2010, Bichet et al. 2019), such as immunocompetence and parasite loads.

To assess how genetic diversity impacts individual and population-wide immunocompetence in wild populations, it is important to assess a range of phenotypic health measures, such as immune system response, parasite loads including parasite diversity and morphological measures related to immunocompetence (Biard et al. 2015). Immune system response in birds can be assessed relatively easily by artificially challenging their immune system with a mitogen, such as phytohaemagglutinin (PHA) (Smits et al. 1999). The PHA challenge is an inexpensive, easily applied skin test which provides a measure of the proliferative response potential of circulating T lymphocytes to the injection site (Smits

et al. 1999). PHA is not harmful but elicits an immune response. When PHA is injected under the skin it causes an increased intrusion of leucocytes in the postcapillary venules, and is seen externally as an inflammation at the injection site (Stadecker et al. 1977). The size of the inflammation can be used as a general index of cell-mediated immunity (Scheuerlein and Ricklefs 2002), with an increase in leucocytes at the injection site causing a larger swelling and indicating a stronger immune system response. Similarly, ecto- and endoparasite levels can give an indication of individual and population-wide health, as individuals with higher parasite burdens are generally less healthy and have increased mortality rates (Brown et al. 1995).

Apart from direct measures of immune system response and estimates of parasite loads, changes in body mass and morphology can be used to assess the condition of an individual over the longer term. For example, a review of 109 European bird species found that body mass was strongly correlated with survival (Sæther 1989), where heavier individuals have higher survival rates. Similarly, tarsus length in house martin (*Delichon urbica*) and great tit (*Parus major*) is strongly associated with conditions during early development in the nestling period in birds (Christe et al. 1998, Heeb et al. 2000). Both tarsus length and body size during the nestling period can be reduced when there are increased stressors from parasites or from inadequate parental care (Fitze et al. 2004). This can result in higher mortality later in life (Fitze et al. 2004).

The effectiveness of attempted genetic rescue to increasing survival and immune response was demonstrated with a study of South Island robins (*Petroica australis*) from 2008-2010. Even though the donors came from bottlenecked populations, the interbred offspring in the F1-F3 generations showed greater levels of genetic diversity, higher survival and increased immune response to PHA (Heber et al. 2013). Since this study, a period of 8-9 years has passed (with potentially up to 8 new cohorts of robins added to the two populations) and it is possible that immune system function has changed as a result

(e.g., due to loss of genetic variation through drift). In chapter 2, I showed that genetic diversity within the two robins populations, although declining since the translocation in 2008, was still higher than before the rescue attempt, and fledglings with higher levels of genetic diversity had significantly higher survival rates to one year of age (chapter 3). In this chapter, I examine whether the positive benefits from increased genetic diversity are correlated with immune system response, parasite burdens and morphology in the two robin populations a decade after they were subject to a genetic rescue attempt.

4.2 Methods

4.2.1 Study sites

I studied immune system response and parasite loads in two South Island robin populations on Motuara and Allports islands, at the northern end of the South Island. See chapter 3.2.1 p74-75 for more information on study populations.

4.2.2 PHA immune challenge

PHA tests were carried out in the austral autumn during March 2017 and March 2018. In accordance with Heber et al. (2013), and in order not to interfere with potential nesting success by removing adults from their nests for extended periods, only individuals that fledged in the prior breeding season were used in the experiments. These birds had fledged between 3 to 7 months previously and were independent of parental care, and in this study are subsequently referred to as juveniles. The use of only juveniles in the PHA test was also a means to standardising age group.

To measure the strength of an individual's immune response, I used the phytohaemagglutinin (PHA) test following the protocols used by Heber et al. (2013), which were adapted from Smits et al. (1999). A total of 1 mg of PHA-P (Sigma, L-8754) was placed into a sterile Eppendorf tube (1 mg is the amount needed for 4 birds, each bird received 250 µg), and 200 µl of pyrogen-free saline solution added

by pipette. A new Eppendorf was used for each solution to avoid contamination. Before capture, birds were feed a minimum of 10 mealworms (*Tenebrio molitor*), or until they started caching the mealworms. This ensured that they were satiated at capture. On capture morphological measures of mass (to nearest 0.5 g), tarsus length (to 1 mm) and wing length (to 1 mm) were measured (see morphology section below). Patagium thickness was then measured with a micrometer (Mitutoyo, 395-371, Tokyo, Japan). It was not possible to use the manufacturer-adjusted pressure without distorting the patagium's thickness through compression. Instead, using methods from Smits et al. (1999), thickness was measured once the micrometre made contact with the patagium, defined as the point at which the skin began to distort or twist with the micrometre head. Contact was then gently released until the skin orientation returned back to normal. A reading was taken at this point. The patagium was measured three times with the micrometer set back to 0 between each measurement. An average of the three measures was used to calculate patagium thickness. The patagium was sterilized with a cotton swab dipped in ethanol before injection. Fifty μ l (0.05 ml) of the PHA suspension was drawn into a sterile syringe (Terumo U-100 insulin syringe, 29G x1/2), making sure that there were no air bubbles present. The injection site was always in the centre of the left patagium of the selected bird. Birds were then kept overnight for a period of 14 hours in individual holding cages fitted with a perch, and with *ad libitum* access to mealworms and water, in accordance with Heber et al. (2013). Cages were kept in camp so that individual birds could be monitored for stress but covered with a thick cloth to minimise disturbance. Holding birds overnight and only for 14 hours minimised the risk of interfering with territory loss caused by the prolonged absence of the territory holder (reduced daylength in the autumn also minimised absence from territory during daylight hours). The following morning, patagium thickness was measured at the injection site using the same protocols as above. Cell-mediated immune response was calculated as the difference between pre- and post-injection measurement averages of patagium

thickness. The genetic profile of the birds was unknown at the time of the injection. The sex of the bird was determined at a later date by using each individual genetic profile. Female sex, the heterogametic sex, was determined if both the CHD-W and CHD-Z bands were present, and male sex, homogametic sex, was assigned if a single CHD-Z band was present (Fridolfsson and Ellegren 1999).

Twelve birds were excluded from the final analysis due to inconsistencies while injecting the PHA solution. This was due to the loss of some of the solution during the injection process.

4.2.3 Estimates of parasite burdens

To screen individuals for burdens of coccidial oocysts, faecal samples were collected from robins in the PHA test. A layer of baking paper was placed at the bottom of each cage prior to use and all faeces that were deposited overnight were collected the following morning and individually stored in 50 ml containers. Containers were couriered to Massey University, School of Veterinary Science, within 7 days of collection and analysed by Barb Adlington, for the presence or absence of oocysts. Two metrics were collected; (1) presence of oocysts in faecal samples and, (2) estimated number of oocysts per gram of faecal matter in samples that were of an adequate quality. The total number of oocysts present per gram of faecal matter was given a categorical score from 0 to 7: 0=no oocysts, 1 = 0–9, 2 = 10–99, 3 = 100–999, 4 = 1000–999, 5 = 10 000–99 999, 6 = 100 000–999 999, and 7 = 1 000 000+ oocysts.

When robins were captured, the absolute number of feather mites was obtained for each individual by examining the primary feathers of the left wing and counting each individual (2017 only). Each bird was then placed in a paper bag lining a cotton bag and held until it was ready to be processed (2017 and 2018). To estimate loads of feather mites, chewing lice, and other feather parasites, a dust-ruffling method with flea powder (19.5 g/kg permethrin) was used following Walther and Clayton (1997). The number of hippoboscids flying off each bird during the handling and ruffling process was counted. Due to the size difference, it was possible to classify hippoboscids into one of two

genera as they left the bird, *Ornithomya* spp. or *Ornithoica* spp. On removal from the bag, the bird was held over a large funnel with a 35 ml vial attached to its mouthpiece. Flea powder was thoroughly distributed throughout the bird's feathers by hand and the plumage 'ruffled' for a period of 2 minutes over the funnel to dislodge parasites. The bird was then returned to the paper bag for a period of 3 minutes. After 3 minutes the ruffling process was repeated for a further 2 minutes, without the addition of more flea powder, before the bird was released. The contents of the paper bag were then emptied into the funnel, before the funnel was flushed with 70% ethanol into the holding vial. Vials were sealed with parafilm wax to reduce desiccation and labelled. To avoid contamination, a new paper bag was used for each bird, the funnel was swabbed with alcohol and a new set of latex gloves was used. Parasite species were identified to the genus level in the laboratory at a later date. The number of individuals in each parasite species group was counted using a stereomicroscope.

4.2.4 Morphological measures

To determine if body mass or size was correlated with levels of genetic variation, I measured robins when they were captured, either for the PHA test (see above) or for routine banding in the subsequent spring (i.e., 5 months after PHA tests and when juveniles were now approaching 1 year of age). Body mass was measured using a Pesola 100g Micro-Line 20100, to the nearest 0.5 g, while tarsus length was measured using Mitutoyo callipers to nearest 0.1 mm. As different robins were measured at two time periods (autumn and spring), I tested whether this seasonal difference could affect my subsequent analyses. A Student's t-test was conducted on the weight differences between the two groups (spring vs. autumn), and although not significant (Mean bird mass autumn = 37.5 g, mean spring = 36.5 g, $t = 1.84$, $df = 152$, $p = 0.068$), an additional factor was added into the morphological models (season) to control for this difference.

4.2.5 Heterozygosity-fitness correlations (HFCs)

All adult birds were fitted with leg bands and uniquely identifiable colour bands. Blood samples (10–30 µl) were collected from these individuals via brachial venepuncture, using a 29 Gx ½” insulin needle, and then stored in Queen’s lysis buffer (Seutin et al. 1991) until DNA extraction at a later date; see chapter 2.2.2 for DNA extraction and genotyping methods. To calculate genetic variation, individuals were genotyped at 28 microsatellite loci (Heber et al. 2013). All PCR and microsatellite sequencing were conducted in the Kempenaers lab at the Max-Planck institute for Ornithology according to the protocols detailed in (Heber et al. 2013). Multilocus heterozygosity for juveniles was calculated with microsatellite data and the function “sMLH”, which measures the proportion of analysed loci that are heterozygous for that individual, divided by the average heterozygosity of the loci, using the R package “inbreedR” (Stoffel et al. 2016). The magnitude of identity disequilibrium (ID), heterozygosity correlations across loci, were evaluated; See 3.2.5 genetic analyses - heterozygosity-fitness correlations (HFCs) p79-81 for more information on how individual heterozygosity was calculated, as standardised multi-locus heterozygosity (sMLH) for males and females with the same microsatellite data.

4.2.6 Statistical analysis

Linear regression models were used to test individual genetic diversity as a continuous predictor, measured as sMLH, against individual mass (g) and tarsus length (mm) as responses. Site (Allports or Motuara Island), year (2017 and 2018), sex (male and female) and season (spring and autumn) were added as factors to models to explain total variance and act as control for them. For linear models, instead of using a step-wise model simplification method, all models were first fitted with the maximum possible number of interactions with genetic diversity, before these interactions were gradually removed, but predictors were retained regardless of their significance level. Each individual model was checked for the best overall fit (Bolker et al. 2009), using the model.sel function, R package MuMIn. The model

with the best overall fit was used as the final linear model. These methods were repeated in all the following analyses. Diagnostic plots for the selected linear models were visually inspected for normality and homoscedasticity of residuals as well as to detect any potential outliers. Selected models were similarly checked for goodness of fit (Agresti and Kateri 2011), with the `gof` function, R package `aods3` v0.4-1.1. When the diagnostic plots of the models were inspected, one bird was observed to have significant leverage for the `mas` observations and was acting as an outlier; this bird was 14 g lighter than its counter parts (Mean male mass = 36.6 g), and thus was likely recorded incorrectly in the field. When this bird was removed from the data set, improved normality and goodness of fit was achieved.

PHA analyses were conducted with the same linear models to determine if genetic diversity predicted immune response, but I removed season as a factor from this model as all observations were carried out in the same month. The factors of site (Motuara, Allports), year and sex were retained, and the model was checked with the same diagnostic methods as the morphology analyses.

Parasite loads were analysed using GLM with a Poisson distribution. However, due to the nature of the count data there were considerable variation between individuals in relation to parasite burdens, with many birds carrying large parasite burdens or no parasites at all. This resulted in overdispersion for many of the models. When overdispersion occurred, a negative binomial model was used. A likelihood ratio test was also used to compare GLM Poisson and negative binomial models to check that the appropriate statistical analysis was used with the data. All models retained factors site, year and sex, and the model was checked with the same model select methods as the morphology analyses. For mites (*Pedanodectes* spp.) and lice (Phthiraptera spp.), relatively low counts were observed for several of the species. To increase sample sizes, all species of mite and lice were grouped together into their respective families for analyses. Analyses were run on: (1) parasite diversity (number of parasite species observed on each bird), (2) total observed parasites (number of individual parasites on each bird, all species

combined), (3) combined number of *Pedanodectes* spp., (4) number of feather mites, (5) combined number of Phthiraptera spp, (6) number of *Ornithomya* spp., (7) number of *Ornithoica* spp., and (8) number of combined hippoboscid spp. Estimates for total parasite load, combined Phthiraptera spp., feather mites, *Pedanodectes* spp., *Ornithomya* spp., and combined hippoboscid species were over-dispersed for GLM Poisson models and lacked goodness of fit. Instead a negative binomial model was used for the data. However, there was an excessive of zeros for Phthiraptera spp (77% of birds did not have lice present). As a result, a zero-inflated negative binomial model, Package *pscl* version 1.5.2 (Zeileis et al. 2008), was used for analysis of Phthiraptera spp. For coccidia burdens, a GLM model using a binomial error structure was used, with the response measured as presence (1) or absence (0) of oocysts in the faecal sample and individual genetic diversity as the predictor. This was followed by a GLM with a Poisson error structure for the categorical analysis. All figures were created with ggplot and are presented with 95% confidence intervals.

4.3 Results

4.3.1 PHA response

A total of 85 juvenile South Island robins' immune systems were challenged using the PHA test (49 in 2017 and 36 in 2018; Mean change (mm) for both years combined \pm SE, 1.26 ± 0.03 , Range 0.39-2.12 mm). Sample year and genetic diversity as an interaction provided the best overall fit in the models. Genetic diversity and sample year as an interaction did not correlate with immune response (Table 4.1, Fig. 4.2). There was a significant difference between the locations; birds on Allports Island (Mean \pm SE, 1.34 ± 0.05) had larger reactions than birds on Motuara Island (Mean \pm SE, 1.18 ± 0.05).

Table 4.1. Correlation between genetic diversity and PHA immune response, for analysis a linear regression model was used, no transformations or outliers were removed. The model with the best overall fit included a main interaction between sMLH, and year. All additional factors are presented. Estimates, standard errors, t-values and P-values for all factors are presented. Significance is indicated in bold.

Factors	Estimate	Std. Error	<i>t</i>	P
(Intercept)	1.188	0.204	5.809	< 0.001
sMLH	0.017	0.195	0.089	0.930
Sex	-0.017	0.070	-0.248	0.805
Location	-0.201	0.063	-3.180	0.002
Year	-0.184	0.342	-0.536	0.593
sMLH:Year	0.540	0.325	1.663	0.100

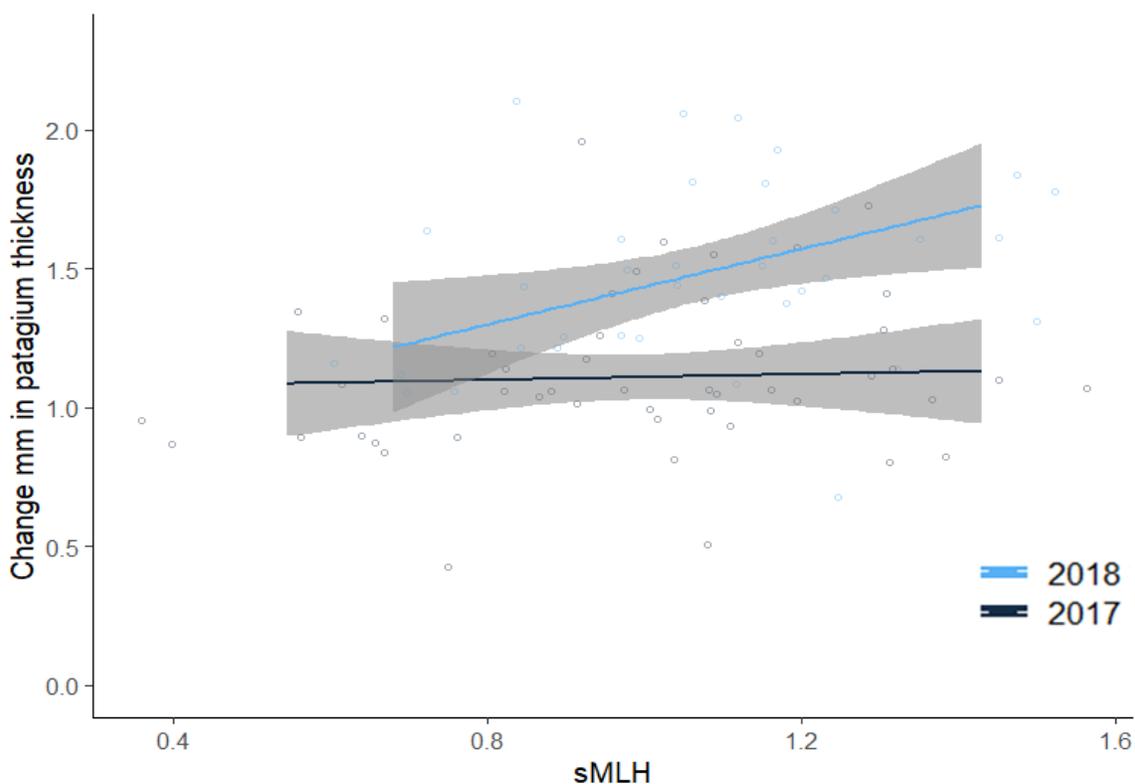


Figure 4.2. Correlation interaction between genetic diversity, sMLH, and year (2017 and 2018) for immune response as measured by change of in patagium thickness (mm) for PHA tests with South Island robins.

4.3.2 Faecal parasite loads

Faecal samples were collected from 84 robins; coccidia oocysts were present in 71% of faecal samples. Genetic diversity did not predict the presence of oocysts (Estimate -0.6333, Std. Error 0.1421, $z = -0.446$, $P = 0.656$) (Table 4.2, Fig. 4.3). Oocyst burden scores were also not significantly correlated with genetic diversity (Estimate -0.508, Std. Error 0.555, $z = -0.917$, $P = 0.359$). There was a significant difference between the two islands for presence of oocysts in faecal samples; only 50% of the birds on Allports Island had oocysts in faecal samples while 87% of birds on Motuara Island had oocysts in their samples.

Table 4.2. Oocyst prevalence in the faecal samples correlated with individual robin genetic diversity. A General Linear Model with binomial structure was used for the analysis. All additional factors are presented. Estimated regression parameters, estimate, standard errors, z-values, P-values are presented. Significance is indicated in bold.

Factors	Estimate	Std. Error	z	P
(Intercept)	-1.382	1115.000	-0.001	0.999
sMLH	-0.633	1.421	-0.446	0.656
Sex	-0.709	0.572	-1.240	0.215
Location	2.052	0.588	3.490	<0.001
Year	0.001	0.553	0.002	0.998

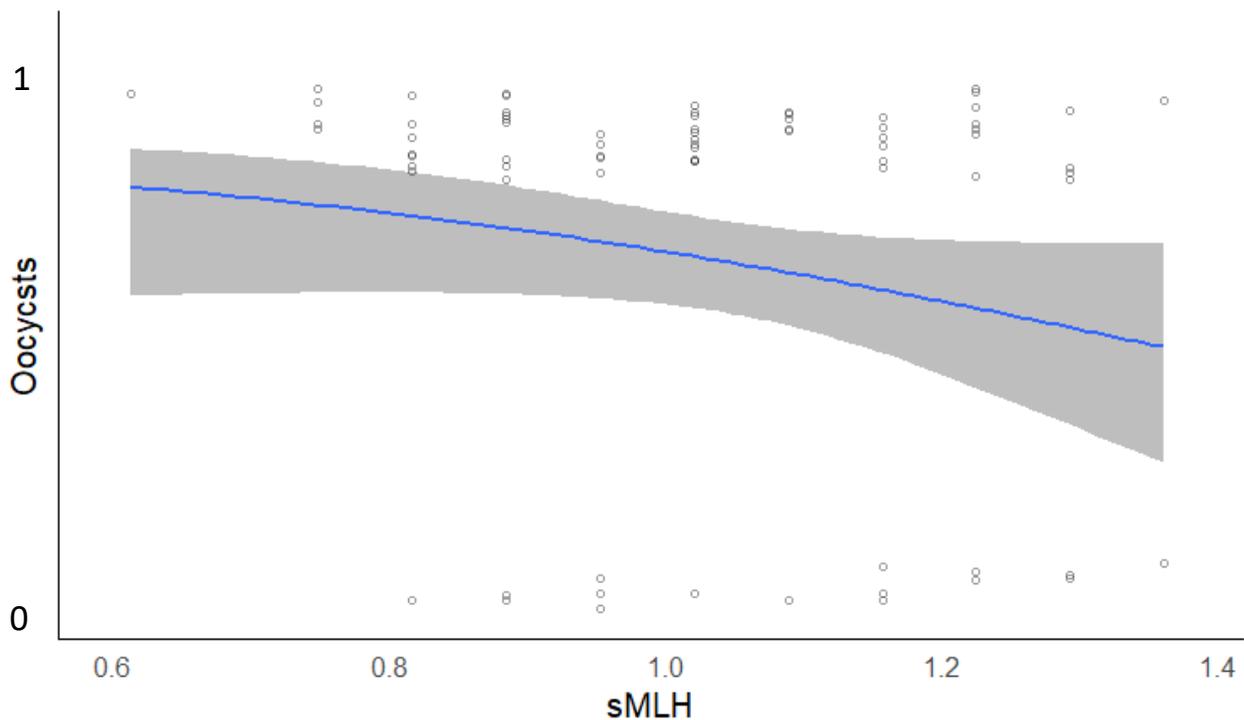


Figure 4.3. Binomial General Linear Model output for presence of oocysts in faecal samples South Island robins, a score of a 1 indicates presence, and absence 0, compared with genetic diversity

4.3.3 Ectoparasite loads

A total of 84 South Island robins were dust bathed, 48 in 2017 and 36 in 2018 (Table 4.3).

Seven ectoparasite species groups were identified from South Island robins. These included two species of hippoboscid flies (*Ornithomya* sp. and *Ornithoica* sp., Family Hippoboscidae), one species of chewing louse (*Menacanthus eurysternus*, Family Menoponidae), two species of feather mite (*Pedanodectes* sp., Family Proctophyllodidae, and a quill mite *Paralges* sp., Family Dermoglyphidae), one species of free-living, stored-products mite (*Tyroborus lini*, Family Acaridae), and one species of blood-sucking nest mite (*Ornithonyssus bursa*, Family Macronyssidae). When comparisons were made between genetic diversity and parasite diversity on each bird as well as overall total parasite load per bird there were no significant correlations (Table 4.4, Fig. 4.4 and 4.5). There was a significant correlation between genetic diversity and mite loads collected from birds during the dust ruffling

process. Birds with increasing genetic diversity had significantly fewer mites (Table 4.5, Fig. 4.6). Lice were present on 23% of the birds. When comparisons were made for presence of lice, the zero-inflated model output indicated genetic diversity did not predict presence of lice on individual birds. However, in the zero-inflated count model, if lice were present on a bird, individuals with higher genetic diversity were more likely to have more lice (Table 4.5). For the remaining parasite species, genetic diversity did not correlate with any of the parasite species separately (Table 4.5, Fig. 4.7, 4.8 and 4.9).

Feather mites were counted on 46 birds in 2017, when tested genetic diversity did not correlate with the number of feather mites present (Table 4.5, Fig. 4.10). For several of the parasite load estimates, there were significant differences between the two islands. For total parasite loads, birds on Motuara Island had more parasites on average (Mean \pm SE, 16.97 \pm 4.54) in comparison to Allports Island (Mean \pm SE, 7.35 \pm 1.46). Birds on Motuara Island also had more parasitic *Ornithomya* flies (Mean \pm SE, 3.34 \pm 0.41) than birds on Allports Island (Mean \pm SE, 0.74 \pm 0.15). Not unexpectedly, this meant that birds on Motuara had in total more Hippoboscid flies present (Mean \pm SE, 4.63 \pm 0.44) than Allports Island (Mean \pm SE, 1.79 \pm 0.24). Similar results were also observed for lice: Motuara Island birds had significantly more lice (Mean \pm SE 18.4 \pm 7.6) than birds on Allports Island (Mean \pm SE, 6.21 \pm 1.49).

Table 4.3. Estimates of parasite loads collected from South Island robins by dust bathing. Presented is the total number of birds sampled, combining both 2017 and 2018. Feather mites samples were only collected in 2017. Mean parasite loads is presented with standard error, and range (lowest to highest) for the eight parasites.

Parasite loads	Sample size	Mean	SE	Range
Parasite diversity	84	2.1	0.1	0-4
Total parasites	84	12.04	2.4	0-160
Feather mites	46	18.46	3.78	0-116
<i>Ornithomya</i> sp	84	2.01	0.25	0-13
<i>Ornithoica</i> sp	84	1.2	0.1	0-3
Hippoboscid spp	84	3.18	0.29	0-13
Pedanodectes spp	84	6.74	2.13	0-153
<i>Phthiraptera</i> spp	19	9.4	0.69	1-41

Table 4.4. Model outputs for parasite diversity of South Island robins and total parasite loads. Estimated regression parameters, estimate, standard errors, z-values, P-values are presented for all factors for both models. Significance is indicated in bold.

Observation	Factors	Estimate	Std. Error	z	P
Total parasite diversity - GLM Poisson	(Intercept)	75.142	312.886	0.240	0.810
	sMLH	0.054	0.398	0.136	0.892
	Sex	0.048	0.151	0.315	0.753
	Location	0.197	0.154	1.283	0.199
	Year	-0.037	0.155	-0.238	0.812
Total parasite loads - GLM negative binomial	(Intercept)	-266.588	447.120	-0.596	0.551
	sMLH	-0.316	0.570	-0.554	0.580
	Sex	0.230	0.216	1.068	0.286
	Location	0.540	0.219	2.463	0.014
	Year	0.133	0.222	0.601	0.548

Table 4.5. Estimates of parasite loads collected from South Island robins by dust bathing. Presented are the individual parasite groups (*Phthiraptera* spp and *Pedanodectes* spp, parasitic flies *Ornithoica* sp and *Ornithomya* sp) as well as combined parasitic fly count of Hippoboscid spp and wing bar mites observation. For each analysis the model type is presented with; estimate, standard errors, z-values, P-values for all factors. Significance is indicated in bold.

Trait	Factors	Estimate	Std. Error	z	P
Wing bar mites – GLM Negative binomial	(Intercept)	2.651	1.147	2.312	0.021
	sMLH	0.020	1.081	0.018	0.985
	Sex	0.126	0.427	0.295	0.768
	Location	0.358	0.420	0.851	0.395
<i>Pedanodectes</i> spp – GLM Negative binomial	(Intercept)	-118.957	760.470	-0.156	0.876
	sMLH	-2.598	0.966	-2.689	0.007
	Sex	0.3145	0.3645	0.863	0.38826
	Location	0.5602	0.369	1.519	0.129
	Year	0.0608	0.3771	0.161	0.87189
<i>Ornithomya</i> sp – GLM Negative binomial	(Intercept)	-327.671	419.782	-0.781	0.435
	sMLH	-0.396	0.550	-0.720	0.471
	Sex	0.052	0.205	0.255	0.799
	Location	1.452	0.232	6.269	<0.001
	Year	0.162	0.208	0.781	0.435
<i>Ornithoica</i> sp - GLM Poisson	(Intercept)	812.417	429.282	1.893	0.058
	sMLH	0.592	0.526	1.125	0.260
	Sex	-0.142	0.201	-0.709	0.478
	Location	0.219	0.203	1.077	0.281
	Year	-0.403	0.213	-1.893	0.058
Total Hippoboscid spp - GLM Negative binomial	(Intercept)	129.911	321.395	0.404	0.686
	sMLH	0.041	0.414	0.098	0.922
	Sex	-0.002	0.155	-0.014	0.989
	Location	0.963	0.165	5.837	<0.001
	Year	-0.064	0.159	-0.402	0.687
Count model Phthiraptera spp - zero-inflated negative binomial	(Intercept)	-2.064	0.781	-2.642	0.008
	sMLH	3.403	0.632	5.387	<0.001
	Sex	0.391	0.231	1.691	0.091
	Location	-0.613	0.312	-1.966	0.049
Zero-inflated model - Phthiraptera spp	(Intercept)	1.004	1.767	0.568	0.570
	sMLH	-0.133	1.576	-0.084	0.933
	Sex	1.664	0.706	2.358	0.018
	Location	-0.406	0.590	-0.688	0.492

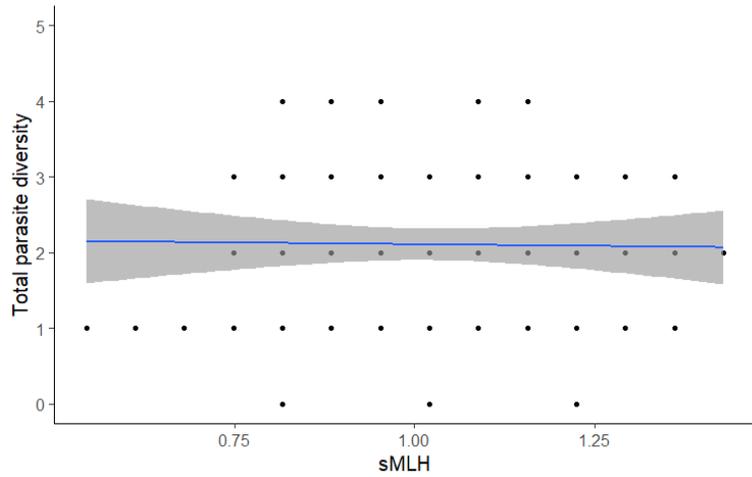


Figure 4.4 Correlation between parasite diversity and genetic diversity for South Island robins.

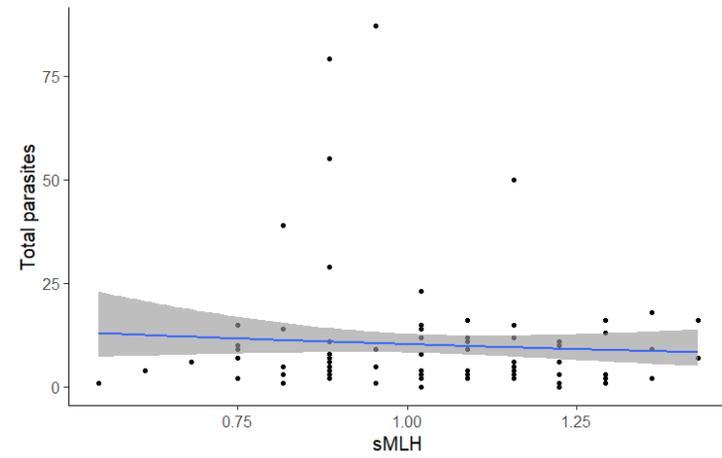


Figure 4.5. Correlation between total number of parasites and genetic diversity South Island robins.

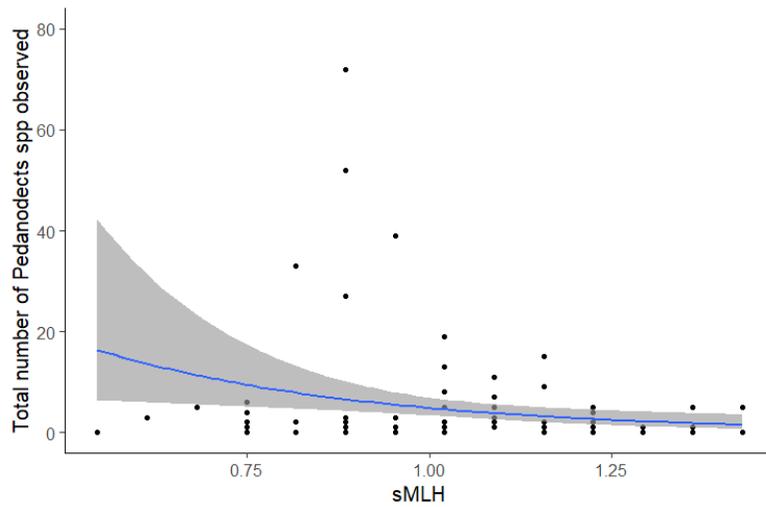


Figure 4.6. Correlation between Pedanodectes spp observed on individual birds and genetic diversity for South Island robins.

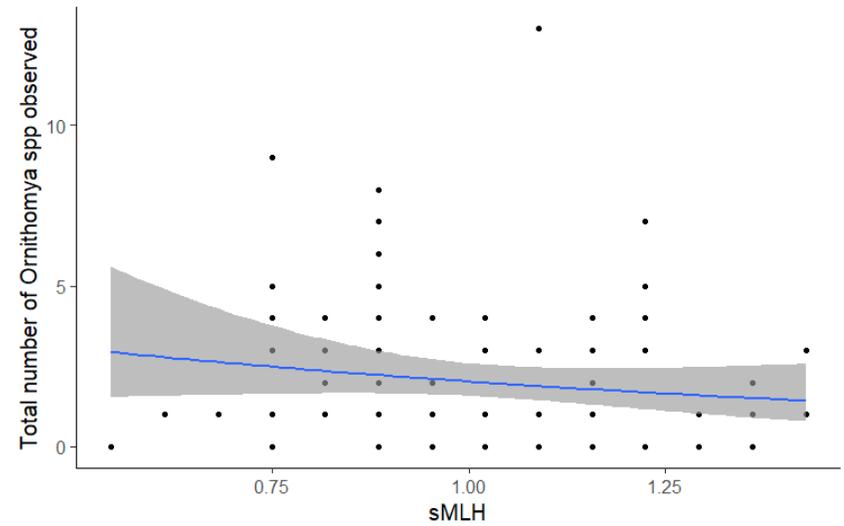


Figure 4.7 Correlation between total number of *Ornithomya* spp flies and genetic diversity for South Island robins.

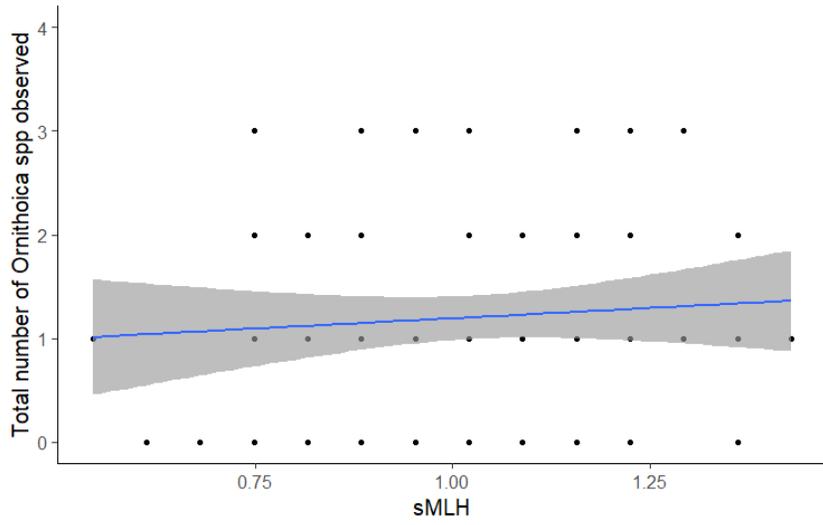


Figure 4.8. Correlation between total number of *Ornithoica* spp files and genetic diversity for South Island robins.

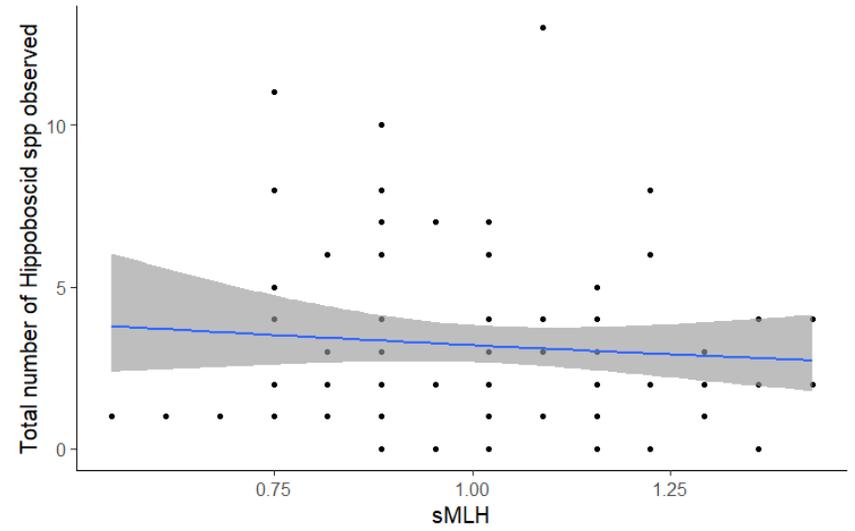


Figure 4.9. Correlation between total number of Hippoboscid flies observed on each bird and genetic diversity for South Island robins.

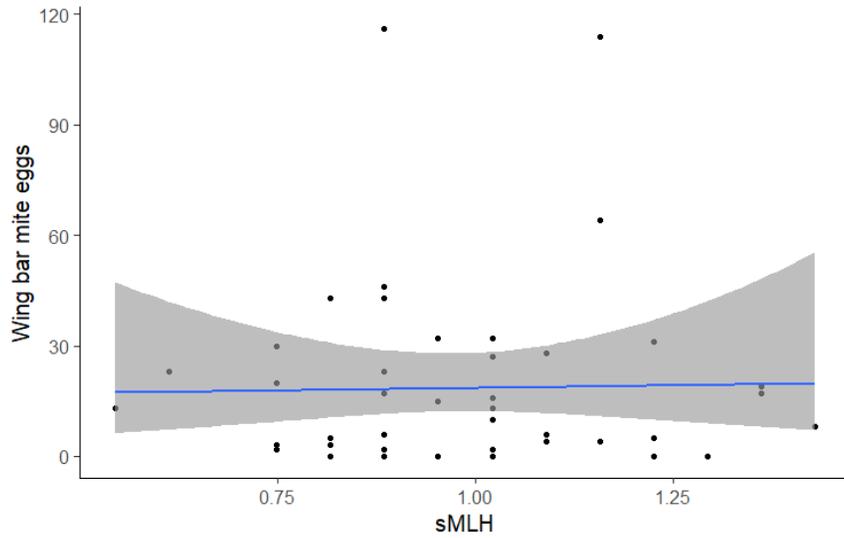


Figure 4.10. Correlation between total feather mites and genetic diversity for South Island robins.

4.3.4 Morphological measures

Estimates of mass were collected from 132 juveniles (Mean (g) \pm SE, 36.6 ± 0.25 , Range 29.5-45). Body mass was not significantly correlated with genetic diversity (Table 4.6, Fig. 4.11). There was a significant difference between males and females for body mass: males (Mean (g) \pm SE, 47.5 ± 1.46) were heavier than females (Mean (g) \pm SE, 35.0 ± 0.26). For tarsus length, 67 juveniles were measured (Mean (mm) \pm SE, 37.4 ± 0.18 , Range 33.4-40.9). As with body mass, there was no significant correlation between tarsus length and individual level of genetic diversity (Estimate -0.561, Std. Error 1.593, $z = -0.352$, $P = 0.728$) (Table 4.6, Fig. 4.12). There was a significant difference between males and females for tarsus length; males tarsus (Mean (g) \pm SE, 39 ± 0.29) were longer than females (Mean (g) \pm SE, 37 ± 0.2).

Table 4.6. Results of linear regression models between individual level of genetic diversity (measured as sMLH) on body mass and tarsus length of South Island robins. No transformations were used, and one outlier of body mass was removed (see text). All factors used in the model are presented, significance is indicated in bold. Estimated regression parameters, estimate, standard error, t-values, and P-values in the linear regression models are presented.

Observation	Factors	Estimate	Std. Error	<i>t</i>	P
Bird mass (g) linear regression output	(Intercept)	34.017	1.048	32.458	< 0.001
	sMLH	1.478	0.979	1.510	0.133
	Sex	3.242	0.389	8.341	< 0.001
	Location	-0.023	0.413	-0.057	0.955
	Year	0.600	0.469	1.278	0.203
	Season	-0.374	0.440	-0.850	0.397
Observation	Factors	Estimate	Std. Error	<i>t</i>	P
Tarsus length (mm) linear regression output	(Intercept)	36.915	0.988	37.373	< 0.001
	sMLH	-0.070	0.959	-0.073	0.942
	Sex	1.660	0.325	5.103	< 0.001
	Location	-0.472	0.353	-1.338	0.186
	Year	-0.072	0.422	-0.172	0.864
	Season	0.211	0.380	0.555	0.581

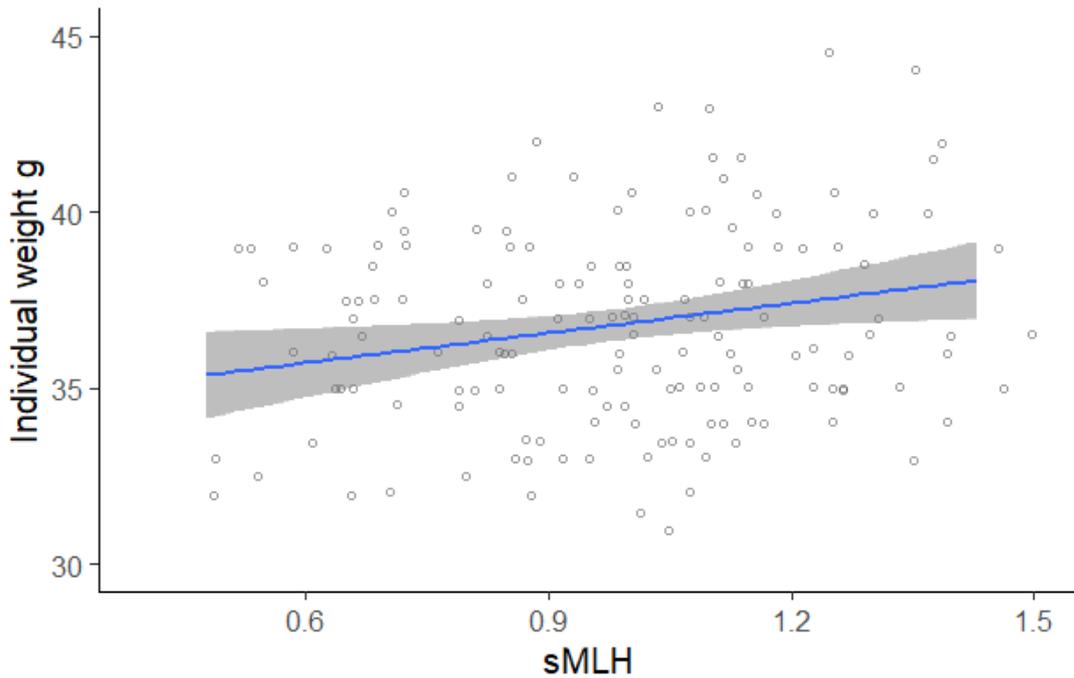


Figure 4.11. Correlation between individual body mass (g) of South Island robins and their level of genetic diversity as measured by sMLH.

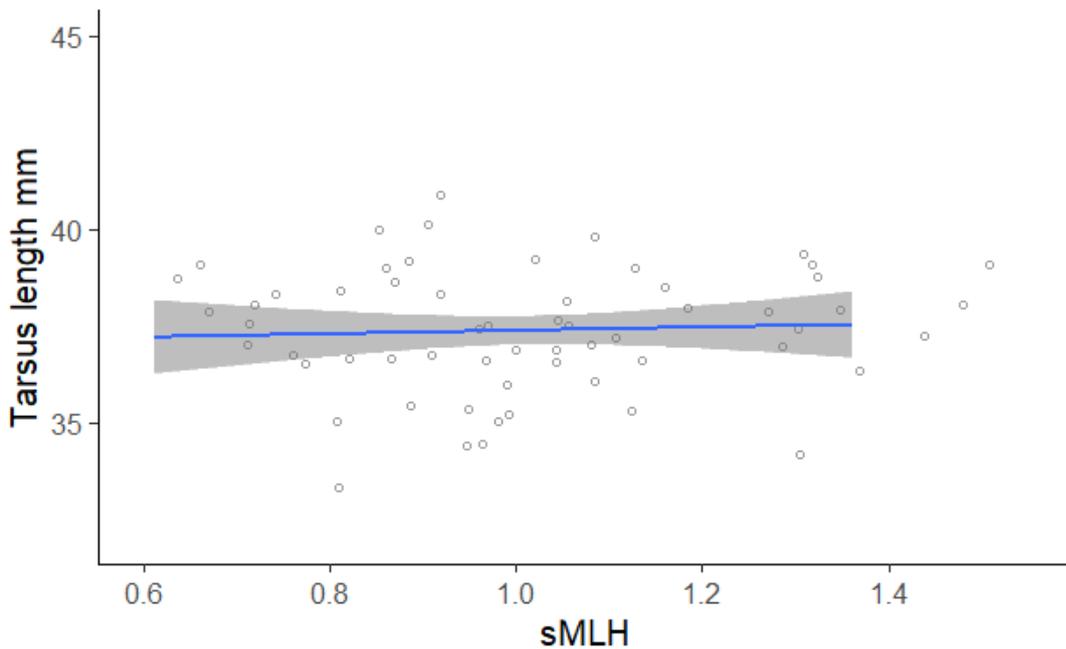


Figure 4.12. Correlation between individual tarsus length (mm) of South Island robins and their level of genetic diversity as measured by sMLH.

4.3.5 Identity disequilibrium

See chapter 3.3.4 p97 for identity disequilibrium out puts.

4.4 Discussion

It is well documented that severe genetic bottlenecks can lead to reduced genetic diversity (Frankham et al. 2002), but how and if the loss of genetic diversity manifests in decreased fitness related to immune response is often less clear. One concern for the management of endangered species is that increased inbreeding can lead to increased susceptibility to pathogens and a reduction in the immune system function for individuals with lower genetic diversity (Coltman et al. 1999, Keller and Waller 2002, Acevedo-Whitehouse et al. 2003, Spielman et al. 2004). In this study, I assessed if genetic diversity in two South Island robin populations that have undergone attempted genetic rescue correlated with individual levels of immune system response, parasite loads and two morphological measures. Overall, there was weak evidence that genetic diversity correlated with immune system responses and parasite loads in these two populations. Genetic diversity correlated with one parasite load measure; *Pedanodectes* spp, and that birds with higher genetic diversity were less likely to have mites. However, remaining parasite load estimates were similar to Heber (2012), who found that genetic diversity did not correlate with parasite diversity. Although not significant there was a trend towards increased immune response and genetic diversity.

Genetic diversity and immunocompetence measures

PHA response has been used as a reliable predictor for individual survival (Møller and Cassey 2004, Møller and Saino 2004) and individuals with higher genetic diversity have been shown to have stronger reactions to the mitogen (Heber et al. 2013). A weak non-significant but positive trend was observed, within the two robin populations, when

correlations were made between cell-mediated immune response and individual genetic diversity. This result is not unexpected and there are several reasons why this may have occurred. Comparing interbred F1 - F3 individuals with their inbred counterparts, in the original Heber et al. (2013) analysis, is the equivalent of comparing two population extremes, i.e., the very best vs the very worst. Heterosis occurs within the first F1 – F2 generations and it is expected that these generations will be the healthiest, with fitness related benefits declining over succeeding generations (Edmands 1999). In contrast this study was conducted 8 – 9 years after the initial introduction of new alleles into the population. Although juvenile genetic diversity in 2017-2018 was higher than that of pre-translocation robins in 2008, it was lower than that of interbred F1-F3 generations in 2009-2010 (chapter 2). It is possible the population is more homogenized genetically and there is no longer a large disparity between individual levels of genetic diversity in 2017-2018 which would make a significant HFCs for immune system response more difficult to detect.

Prior to genetic rescue immune system response in the two populations had been exceptionally low or non-existent (Hale and Briskie 2007, Heber 2012). In Heber et al. (2013) only interbred F1-F3 robins showed a marked increase in immune response, while inbred birds had almost no reaction. PHA tests on Motuara Island, by Hale and Briskie (2007), found a negative response in autumn. The use of the PHA tests can be subjective and comparisons between different observers is not recommended (Smits et al. 1999). Instead, it is only possible to infer pattern within a study. Nevertheless, the fact that I obtained measurable swelling response in all the robins I tested (Fig. 4.2) contrasts with the lack of any response in many of the robins tested by Hale and Briskie (2007). This suggests that the immune response of robins continues to remain higher than that before the genetic rescue.

Only one of the seven parasite load measures, *Pedanodectes* spp loads, indicated a significant HFC. There is evidence that some mites live commensally with their hosts

(Proctor and Owens 2000, Blanco et al. 2001), but once they reach very high density, they can reduce body condition or plumage condition (Thompson et al. 1997, Harper 1999). The mite numbers dislodged by dusting in this study were generally low, but there was a significant trend towards birds with higher genetic diversity to have lower mite loads. A significant relationship was also made for lice load counts, where birds with higher genetic diversity higher lice loads, however, genetic diversity in this case did not predict if lice would be present or absent on birds.

Study limitations

The use of estimates of parasite diversity and abundance to infer the strength of individual immune system should be viewed with caution as immune response is subject to both seasonal change and error that can mask underlying differences in immunocompetence. For example, if a severe infection occurs in an individual, it might quickly lead to mortality, leading to an under representation of infection rates. Similarly, some pathogens only affect an individual's health when they become stressed. As I only sampled juvenile birds outside of the breeding season, it is expected that most birds would be under low stress. For both the PHA test and parasite load estimates, all tested birds were under one year of age and although the use of juveniles allowed me to standardise age, it may not have been a good representation of population wide immune function. Acquired immunity can be modulated by environmental factors, such as stress, resource availability and age (Gustafsson et al. 1994). Considering robins can live for 12+ years, juvenile birds would be unlikely to have experienced strenuous environmental impacts, and the tempering effects on their immune systems (Gustafsson et al. 1994). This situation is similar to comparing the immune system and fitness response of a group of 18-year-old humans to their 70-80-year-old counterparts. This former would generally be considered relatively fit and healthy with little variation. In

contrast, the latter group is likely to have very diverse life histories where you would expect a larger disparity in health conditions. In a way, youth standardises health and it would be worthwhile to screen older robins for parasites in the future.

Some pathogens such as Ornithonyssine mites or fleas might spread and carry more virulent pathogens (Skoracki et al. 2006, Bitam et al. 2010). It was not possible to screen for such pathogens in my study, but mite and flea loads might not be indicative of the robin's health and the virulent pathogens they transferred could be. For practical reasons I also could not assess all potential parasites on individual robins (e.g. tape worms, liver flukes), as this would have involved the euthanizing and dissection of the robin. Thus, it is likely that other types of parasites might be present, and which have negative effects what we did not record.

The use of microsatellites and immunocompetence measures

The use of microsatellites may not have correlated well with loci related to immune system function. Although microsatellites can be used to predict the direction of diversity change at functional regions, these neutral loci are often poor predictors of the magnitude of change (Sutton et al. 2011). Thus, evaluating changes in immune response should also include an examination of immunogenetic loci, such as toll like receptors (TLR) and major histocompatibility complex (MHC) (Grueber et al. 2017). TLRs function in recognizing structural molecules derived from invading microbes and in activating immune cell responses (Delneste et al. 2007). The relationship between the different degrees of individual inbreeding and aspects of immune response are thought to be mediated through reduced genetic diversity particularly at MHC and TLR loci (Potts and Wakeland 1990, Carrington et al. 1999). These functional genes may be under more intense selection than neutral loci and not subject to the same processes of genetic drift as neutral loci (Jamieson 2015). A meta-analysis of 109 populations by Sutton et al. (2011) showed a strong correlated loss between neutral and

functional diversity during bottleneck events, but that loss of MHC variation was 15% greater than neutral diversity. Yet, only a limited number of studies have quantified how the immune system and immune system loci change during genetic rescue events (Madsen et al. 1999). For example, Grueber et al. (2015) observed individual-level heterozygosity was poorly correlated with TLRs and microsatellites in 10 species of birds and demonstrated that sequence variations of TLRs can provide additional data about individual diversity over neutral loci alone. Finding evidence that TLRs experience pathogen-mediated selection in birds, using robins from Motuara Island and Allports Island as a case study, demonstrated that a small number of donors increased TLR diversity in the recipient populations, but with little change in the MHC variation (Grueber et al. 2017). The authors concluded that there was relatively modest potential for the translocations to increase the diversity of either populations' MHC, and suggested that innate immune gene families (i.e., TLRs) be used over adaptive immune gene families (MHC) when comparing individual genetic diversity and pathogen loads. It therefore plausible that the PHA scores and parasite loads I measured are influenced by TLR variation in our populations. Currently TLR analysis for samples collected in my study in 2017 are underway and, it is hoped that with the addition of these samples will allow me to determine the role functional immunological loci play with PHA response and parasite loads in the future.

Conclusion

The use of genetic rescue to increase population wide health is well documented. In this study there was only weak evidence that genetic diversity was correlated with a range of measures related to immune system response, parasite load and morphology. Only one HFCs was detected that showed as genetic diversity increases *Pedanodectes* spp loads decrease. This was not unexpected due to a range of factors, including the possibility that microsatellites could be a poor predictor for immune response. It is also possible that a

narrow range of age groups and narrow range of genotypes in the population may have limited my ability to detect HFCs. Future work using TLR loci should clarify the effect of genetic rescue event on immune system response.

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**Can the introduction of new alleles into
a genetically depauperate South Island
robin population increase cognitive
functions?**

Chapter 5



Figure 5.1 An inquisitive male robin participating in stage three of the detour task.

Abstract

Despite the potential importance of genetic diversity on fitness, few studies have examined the relationships between genetic variation and intelligence in birds. Instead, most studies focus on the effects of population bottlenecks on inbreeding and primary fitness traits, such as fertility and survival. How inbreeding affects avian intelligence in wild populations is not clear. To examine the links between individual levels of genetic diversity and cognitive performance in birds, I used two isolated island populations of South Island robin (*Petroica australis*) that were subject to an attempted genetic rescue experiment in 2008. These populations now support growing populations of robins with varying levels of individual genetic variation. The aim of this study was to test the effects of inbreeding on two parameters that constitute individual phenotype: song performance and cognitive performance. I found a significant increase in the ability of robins with higher genetic diversity to complete one of the most difficult parts of the detour task. Additional non-significant trends were observed towards individuals with higher genetic diversity completing tests faster and successfully completing more tasks in the final phase. No correlation was found between individual genetic variation and the complexity of male South Island robin song. My results support the hypothesis that higher genetic diversity is linked to some aspects of cognitive performance in robins, and that genetic rescue may provide a method to reverse cognitive loss in other species suffering from inbreeding.

5.1 Introduction

Within the Order Passeriformes it is thought that song structure and cognitive ability are closely linked, and that both are under selection as secondary sexual characteristics (Bolhuis and Gahr 2006, Boogert et al. 2011b, Cardoso and Hu 2011). In some birds, such as oscine passerines, song structure is a learned display and subject to cognitive constraints by the costs associated with learning (Gil and Gahr 2002, Nowicki and Searcy 2004), including limitations imposed by a male's genetic makeup and/or environmental aspects that affect a male's quality (Nowicki and Searcy 2004, Woodgate et al. 2014). As a result, female choice based on features of male song (e.g., repertoire size, syllable diversity), may be a means by which sexual selection can act on cognitive traits in songbirds (Boogert et al. 2011a, Peters et al. 2014, Anderson et al. 2017). Song structure could thus act as an honest indicator of fitness in adults (Searcy 1984, Lampe and Saetre 1995). Although it is not easy to define or measure, "general intelligence" and broad mental capacity that influence cognitive ability have been linked to higher survival and mating success (Peters et al. 2014).

Despite the potential importance of genetic diversity on fitness (Frankham et al. 2002, Reed and Frankham 2003), few studies have examined the relationships between levels of genetic variation and avian song complexity or general intelligence in birds (Aspi 2000, Joron and Brakefield 2003, Van Oosterhout et al. 2003, Drayton et al. 2007, de Boer et al. 2016). Instead, most studies focus on the effects of population bottlenecks and inbreeding on primary fitness traits, such as fertility and survival (Jamieson and Ryan 2000, Briskie and Mackintosh 2004, Mackintosh and Briskie 2005, Boessenkool et al. 2007, Taylor et al. 2017).

As with other fitness traits, a low level of genetic diversity is likely to negatively affect cognitive function as the development of those regions of the brain required for learning are hypothesized to be costly and readily disrupted by stressors (Nowicki et al. 2002). Those individuals that can develop better song control nuclei and associated neural structures should be able to learn more complex songs and are likely to have higher

intelligence overall (Nowicki and Searcy 2004). If an individual has low genetic diversity (e.g., due to inbreeding or a severe population bottleneck), the development of complex song control nuclei and neural structures could be disrupted and thus reduce cognitive performance. Similarly, early developmental stressors such as nutritional deficiencies can affect both song development and learning, leading to adults with reduced repertoire sizes and cognitive ability (Nowicki et al. 2002, Peters et al. 2014, Farrell et al. 2016). In some cases, developmental stressors could be exacerbated if one or both parents are inbred, if this prevents them from providing adequate nutritional care for their chicks. Several studies have indicated that sexually selected traits, including intelligence and song structure, would suffer with increasing levels of inbreeding, and perhaps even more than morphological traits (Hamilton and Zuk 1982, Zuk et al. 1990, Cotton et al. 2004).

How inbreeding affects both avian intelligence and song complexity in wild populations is not clear (Seddon et al. 2004). To date only a few experiments have studied the effects of inbreeding on song structure. For example, in song sparrows (*Melospiza melodia*), repertoire size was observed to increase with increasing levels of genetic diversity (Pfaff et al. 2007). Similarly, full-sibling matings in zebra finches (*Taeniopygia guttata*) was found to have a strong negative effect on song rate (Bolund et al. 2010). Increased inbreeding in canaries (*Serinus canaria*) also affected song structure by reducing syllable phonetics (de Boer et al. 2016).

South Island robins (*Petrocia australis*) have complex song structures, with a high diversity of syllables (Hay 1975), and both sexes create numerous food caches throughout the day which they then retrieve before dusk (Powlesland 1980). Thus, they need to have good memory to recall both the locations of their caches and the songs in their repertoire. The closely related North Island robin (*P. longipes*) has similar caching behaviour to South Island robins and through a variety of cognitive tests has been shown to have sophisticated learning

capabilities (Shaw et al. 2015, Shaw 2017, Shaw and Schmelz 2017, MacKinlay and Shaw 2018). As both song structure and learning behaviour could be negatively affected by inbreeding, the South Island robin provides an ideal species to investigate the relationship between individual levels of genetic diversity, cognitive abilities and song.

To date, all experiments testing the effect of inbreeding on singing behaviour and cognitive ability have been lab based. Confirming that similar effects occur in wild populations is important because lab based studies may temper the expression of deleterious alleles, given that exposure to harsh environmental stressors are reduced in captive animals (e.g. with *ad libitum* food and shelter; (Boogert et al. 2011a)). To examine the links between individual levels of genetic diversity and cognitive performance in birds, I used two isolated island populations of South Island robin that were subject to an attempted genetic rescue experiment in 2008. Both populations had been through severe population bottlenecks ($n = 5$ birds each) in 1973 and were showing signs of inbreeding depression by the 1990s and 2000s (Briskie and Mackintosh 2004, Mackintosh and Briskie 2005, Hale and Briskie 2007, Heber 2012). Whether cognitive impairment is a consequence of low levels of genetic diversity and can be reversed through genetic rescue has not been studied in the wild to date. By 2019, the attempted genetic rescue experiment had increased levels of genetic variation in both populations (chapter 2), but with each consisting of a mixture of birds with varying levels of individual genetic diversity. The aim of this study was to test how cognitive ability, as measured by song complexity and achievement in a series of cognitive tests, differs among a genetically diverse range of South Island robins. My objectives were to test if: (1) individual genetic diversity influences song complexity (syllable diversity) and song structure (total phrases, phrase length, and frequency), and (2) if individual level of genetic diversity influences cognitive functions such as motor control, inhibitory control, executive functions and learning.

5.2 Methods

5.2.1 Study species

South Island robins are small (35 g) passerines. Adult males are darker and slightly larger (on average 5 g) than females (Heather and Robertson 2000). Robins are territorial and remain as a monogamous pair, holding the same territory, often over multiple years (Heather and Robertson 2000). They spend much of their time foraging on the forest floor feeding on invertebrates; insects are dismembered and portions are cached in the territory, often in multiple caching locations for up to 4-5 days (Powlesland 1980). Robins have been observed regularly moving cached invertebrates between different locations, possibly to reduce the likelihood that caches will be pilfered by other robins (Powlesland 1980). Thus, they need to have good spatial memory. As South Island robins evolved in the absence of mammalian predators, they are generally fearless towards people and readily investigate novel objects. Coupled with their complex caching behaviour, this makes them an excellent species to study complex cognitive tasks in the wild (Burns and Van Horik 2007, Shaw et al. 2015, Shaw et al. 2017, Shaw and Schmelz 2017, MacKinlay and Shaw 2018).

5.2.2 Study populations

I studied song structure and cognitive function in two South Island robin populations on Motuara and Allports islands, at the northern end the South Island. See chapter 3.2.1 p74-75 for more information on study populations.

5.2.3 Male song and repertoire measurements

Male South Island robin song is complex, with wide variation in call types (Hay 1975). Hay (1975) was able to distinguish between full song, sub-song, downscale song and 17 other recognisable call types. Full song is sung predominantly within 2 hours of dawn but may be sung at other times throughout the day as well. Sub-song is a quieter non-stereotyped version of the full song and is sung throughout the day. Lastly, the downscale song is a series of short phrases taken from the dawn chorus and/or sub-song with clicks used by the males

(Powlesland 1983). Males also use the sub-song and downscale song to call mates and fledglings when feeding. Both full song and sub-songs consist of a variety of phrases, each containing several identical syllables (Fig.2). Each syllable is repeated in sequence to form a single phrase; multiple phrases are then sung in combination for an extended period of time (Fig. 2) following a Markov chain (Hay 1975). Females call but do not produce a full song type, instead giving short phrases when establishing territories and nesting sites, or feeding offspring (Powlesland 1983). Males sing year-round but singing peaks between August and December, coinciding with the breeding season (Hay 1975).

Male song of robins on my study sites was recorded in the breeding season between 20 August and 20 November 2016-2018. To control for male song type (full-song, sub-song and downscale song), recordings were only made prior to or within 15 minutes of dawn, and therefore only included full-song. To individually identify males while collecting recordings in the dark, the location of the focal male's singing site was established beforehand and then on the day of the recording, I arrived in this location before the male started singing. The identity of the bird was confirmed with a head torch or once it became light enough to see its colour bands. Only recordings in which the male could be identified were used. This method meant that only one male could be recorded per-day. Recordings were not collected in high wind or during rain. A ZOOM SGH-6 shotgun microphone attached to a ZOOM H5 handy recorder was used to collect all recordings. All songs were recorded for a minimum of five minutes. This is the recommended time to capture the repertoire size for the closely related North Island robin (Jones 1999, MacKinlay and Shaw 2018). In the South Island robin most of the variation in repertoire size is captured within the first one to three minutes of a recording of the dawn chorus (Hay 1975, Byrne 1999).

Several metrics were extracted from the recordings to quantify song complexity: (1) number of unique syllables sung by each individual, defined as vocalizations that produced a

continuous black trace on a sonogram (Fig. 5.2) during the 5-minute bout; (2) total number of phrases sung during the 5-minute bout; (3) average phrase length; (4) average high frequency, and (5) average low frequency for each song (Fig. 5.2). Sonograms were visually inspected using RavenPro 1.5, an acoustic analysis software. It was expected that higher values of each of these metrics (e.g., more unique syllables/5 min) were indicative of greater song complexity.

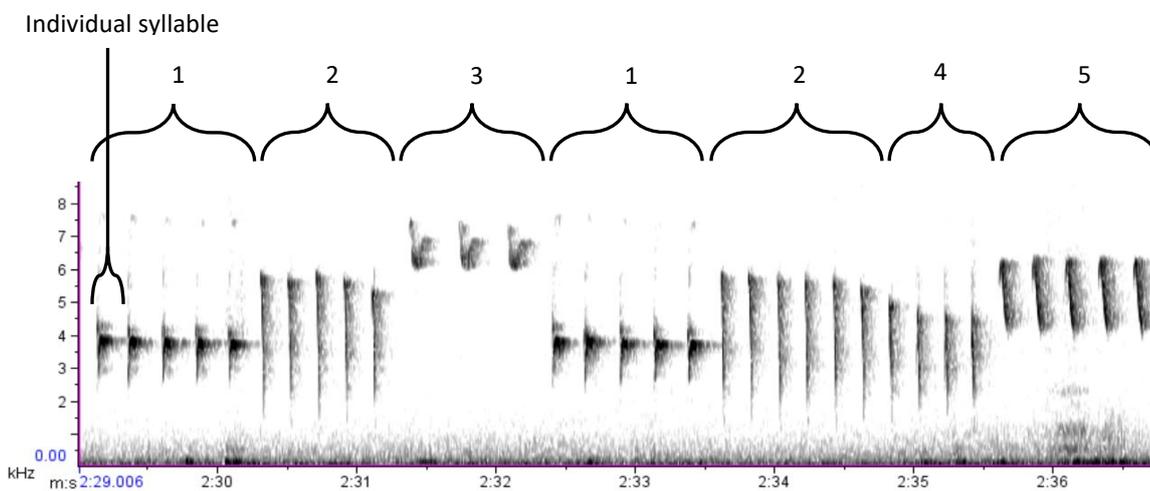


Figure 5.2. An example of a seven second recording from a male robin's song (band B109331); the first bracket delineates a single syllable. Each corresponding set of brackets indicates a phrase, numbered corresponding to the syllables that make up each phrase. Phrases sung together form the song. Each new syllable that appears in a song is added towards the individual's repertoire score.

5.2.4 Cognitive tests

In addition to measuring song complexity, two cognitive tests were conducted on robins between 25 February and 30 August, 2017-2018. Cognitive tests were not performed during the breeding season to ensure that birds were motivated for food only for their own needs rather than to feed offspring. All tests were conducted in the same location in each individual's territory. Tests were not conducted when other robins were present to reduce the

likelihood that other birds would learn from watching the focal bird. This was not always possible as a focal bird's partner was often present during a trial, in which case one bird from the pair was chosen, and this was usually the more dominant male. However, females did participate in the experiments and all analyses includes both sexes. Shaw et al. (2015) recommended feeding individuals prior to starting cognition experiments to standardise motivation. Therefore, each robin was fed between 10-15 mealworms (*Tenebrio molitor*) before each experiment or until they started caching. In all tests, the observer was positioned a minimum distance of 5 m from the test apparatus.

5.2.5 Detour reaching task

Detour reaching tasks have been used successfully with North Island robins (Shaw et al. 2015, MacKinlay and Shaw 2018). The detour task is a measure of inhibitory control (an individual's ability to inhibit a prepotent response, such as reaching through a clear barrier), executive functions (cognitive processes such as attention, inhibition, memory and cognitive flexibility) and learning (Shaw et al. 2015, Shaw 2017). The protocols were adapted from Shaw et al. (2015) and Shaw and Schmelz (2017) (Fig. 5.3a-d). The goal was to see if the bird could successfully learn to retrieve food from a hidden location and how many trials an individual required to inhibit a prepotent response to reach for food through a transparent barrier (Fig. 5.3d). There were four phases to the experiment. Birds were allowed a maximum of 20 trials per phase and they had up to two minutes to complete each trial. This differs from Shaw (2015 and 2018) where there was no cap on the number of trials conducted per phase and instead birds were tested until they had past each level. I set a cap in this experiment as I viewed the inability of a robin to pass a test in 20 trials as a measure of its limited cognitive ability. This meant there was a cut-off for each level and many birds failed to complete all four levels of the experiment. The first phase was a test of neophobia and habituation. Once a

focal bird was identified, it was shown a freshly killed mealworm. The worm was placed on a wooden platform measuring 20 x 30 cm (Fig. 5.3a). To graduate to the next phase the robin had to retrieve the mealworm from the centre of the platform within two minutes in three consecutive trials. This was applicable for phases one to three. In the second phase, a white barrier with a gap in front was placed on the platform so that it half covered an opaque cylinder (Fig. 5.3b). In the third phase, the white cover was positioned so that it completely covered the opaque tube (Fig. 5.3c). This ensured that a robin could not directly approach an open end of the cylinder and instead had to approach the cylinder wall. Finally, in phase four, the opaque tube was swapped for a clear tube (Fig. 5.3d). To pass the robin had to retrieve the mealworm without first pecking the clear tube. If a robin pecked the tube first it was not allowed to retrieve the worm; this acted as a penalty. If the robin did not peck the tube six times in a row it was considered to have passed. For each phase several metrics were recorded: (1) whether the bird passed or failed, (2) time taken to retrieve the mealworm, (3) total number of trials it took to pass onto the next phase and, (4) total number of trials passed in the phase four test.



Figure 5.3a. A plain board of wood 20 x 30 cm, one mealworm was placed in the centre and robins had 60 seconds to retrieve the worm.

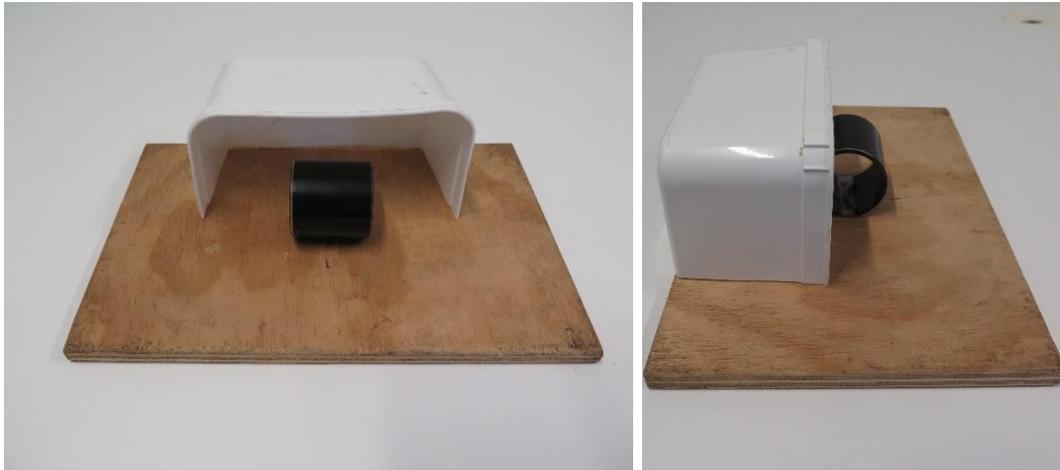


Figure 5.3b. An opaque tube is fitted onto the board and a cover is placed over half the tube. Birds can approach from any angle from the front and had 120 seconds to retrieve the worm from the tube.

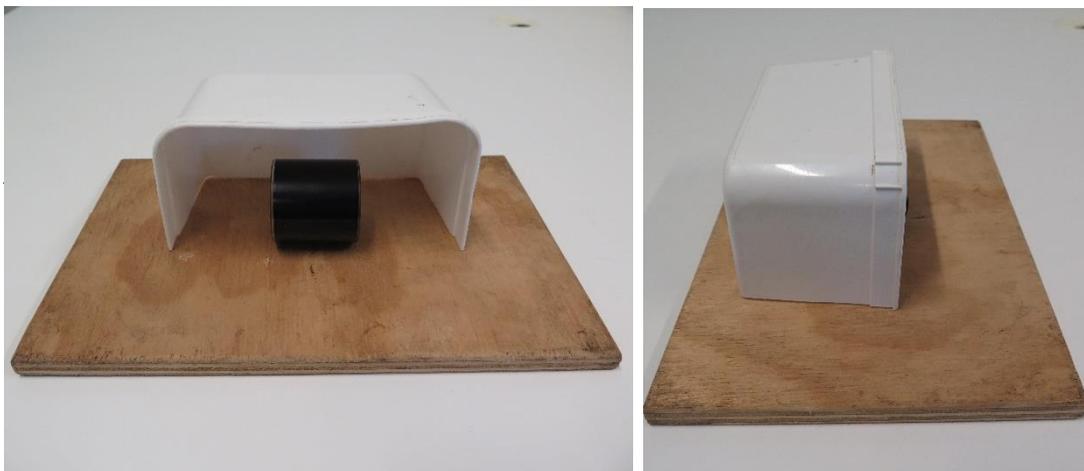


Figure 5.3c. The same set-up and method as in 2b but the lid fully covers the open sides of the tube and the bird is forced to approach directly from the front and birds had to actively look in through the sides to find the mealworm. Robins had 120 seconds to retrieve the worm from the tube.

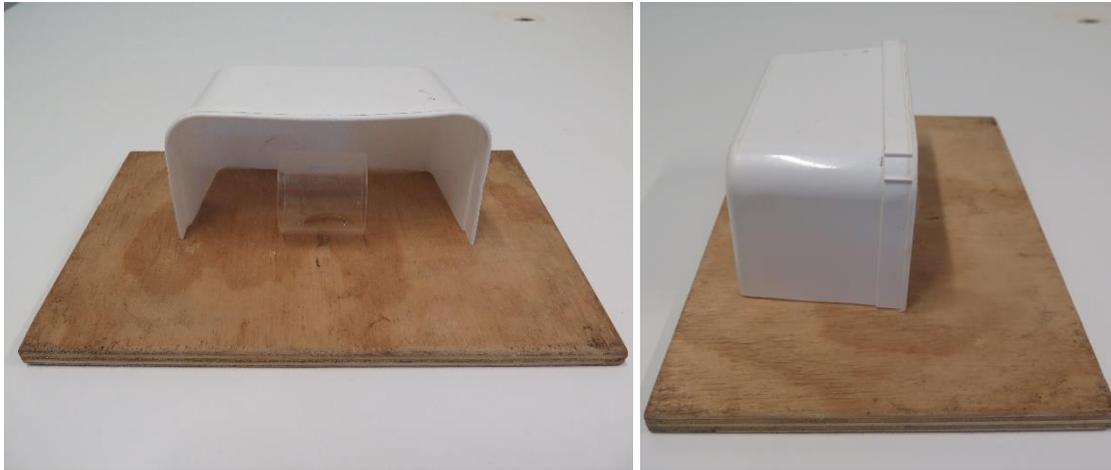


Figure 5.3d. Test phase, using the same methods as 2C except the opaque tube is swapped with a clear tube and birds had 120 seconds to retrieve the worm from the clear tube.

5.2.6 Learning task

The learning task was similar to the detour reaching task, but more emphasis was placed on learning and motor control (coordinated movements and skilled actions). This experiment was conducted on a combination of birds that completed the detour reaching task and birds that did not. If birds participated in the detour reaching task first, they had to wait a week before participating in the learning task. The task I used was modified by combining elements of the detour reaching task and a memory task from Shaw et al. (2015, 2017). A clear cylinder with a leather lid was fixed to a 20 x 30 cm wooden board (Fig. 5.4a). In this experiment the birds had to learn to remove the lid without pecking the clear cylinder that contained a large quantity of rewards (in the form of mealworms). As with the detour task, the learning task was carried out in four phases. In phase one, a habituation phase, 6-10 live mealworms were placed in the clear cylinder; the leather lid was left off and the bird was given 60 seconds to approach and remove a single mealworm (Fig. 5.4.a). Each bird was given a limit of 20 trials and needed to pass five trials in a row to progress to the next phase.

Fresh mealworms were added for each one that was removed: therefore, there was always 6-10 mealworms in the cylinder. In the second phase, the leather lid was repositioned so that it covered 7/8 of the cylinder (Fig. 5.4b). A robin had the option of removing the lid or pecking through the small gap to retrieve the mealworms. By the third phase, the leather lid was placed so that it covered 100% of the cylinder's top (Fig. 5.4C). To pass, the robin had to remove the lid within the 60 second time frame. Phase four was similar to the detour task: the robin had to remember to remove the lid without pecking the side of the tube first. Robins that pecked the clear cylinder first were penalised by being moved away from the experiment and denied a mealworm. If the robin did not peck the cylinder five times in a row it was considered to have passed. The same metrics as the detour reaching task were recorded in the learning task.



Figure 5.4a. A board of wood 20 x 30 cm was fitted with a clear pot and leather lid. The lid and pot were attached to the board via a screw to stop robins from flying away with these components. Robins had 60 seconds to retrieve a mealworm from the clear pot.



Figure 5.4b. The leather lid was positioned to cover 7/8 of the pot. Robins had 60 seconds to retrieve a mealworm from the clear pot by either removing the lid or pecking through the small gap.



Figure 5.4c. The leather lid covers 100% of the pot. This was the same for levels 3 and 4. Robins had 60 seconds to retrieve a mealworm from the clear pot by removing the lid.

5.2.7 Heterozygosity-fitness correlations (HFCs)

See 3.2.5 genetic analyses - heterozygosity-fitness correlations (HFCs) p79-81 for more information on how individual heterozygosity was calculated, as standardised multi-locus heterozygosity (sMLH) for males and females with the same microsatellite data.

5.2.8 Statistical analysis

5.2.8.1 Song structure

All analyses were conducted in R (v. 3.5.0). Individual genetic diversity (measured as sMLH) was used as the predictor in all analyses of song complexity, and the observed phenotypic traits from the song structure as the response. Generalised Linear Models (GLM) with Poisson error structures were first used for analyses. GLM models were inspected for overdispersion and checked with dispersiontest function (AER package R) and for goodness of fit gof() function (aods3 v0.4-1.1 package R). When overdispersion was detected, a negative binomial model was used instead of the GLM Poisson model. A likelihood ratio test was also used to compare GLM Poisson and negative binomial models to check that the appropriate statistical model was used. Average phase length was analysed using a GLM Gaussian error structures. Selected models were similarly checked for goodness of fit (Agresti and Kateri 2011), with the gof() function, R package aods3 v0.4-1.1. Location (Allports Island and Motuara Island) was added as an additional factor to the models to control for any site effects. The number of phrases sung in a 5-minute bout did not significantly predict the number of syllables sung by each individual (Estimate = -0.0002, Std error = 0.0007, $z = -0.355$, $P = 0.722$). Nonetheless, an offset function for total phrases sung in a 5-minute bout was added to the GLM model analysing total syllables. The total number of phrases and average high/low frequency for each song was tested using the same methods as above.

5.2.8.2 Cognition experiments

For both cognition tests (detour reaching and learning tasks), individuals that passed were designated with a 1 and individuals that failed 0. GLM models with a binomial error structure were used to test individual ability to pass each of the four phases, and to pass from phase one to phase four using individual sMLH as the predictor. For the detour task, all birds passed phases two and three, and these phases were excluded from the binomial analysis. For the learning task, all birds passed phases two and four; these phases were likewise excluded from the binomial analysis.

The number of trials each bird required to pass each of the four phases and average time (seconds) individuals took to retrieve the reward was analysed first with a GLM model using a Poisson error structure. Models were inspected for overdispersion and goodness of fit. When overdispersion occurred, a negative binomial model was used instead, and a likelihood ratio test was also used to compare the GLM Poisson and negative binomial models to check that the appropriate statistic model was used. Location and sex (male and female) were added as additional factors to control for their effect. For the learning task, detour was added as a factor to the models. This was used as a control for birds that had first participated in the detour task before the learning task and as such might be more familiar with the experimental setup. Factor location was removed from phase one analysis in the detour task as all birds on Motuara Island passed, which created low variation for this factor. Model select function indicated that the removal of location from this model increased the overall fit, and thus location was excluded for this reason. Sex was also removed as a factor from the learning task for phase one for individual ability to pass to the next phase as all females passed the initial phase which created low variation for this factor. For the detour experiment, three birds were excluded from the final stage as they stopped participating. All figures were created with ggplot and are presented with 95% confidence intervals.

5.3 Results

5.3.1 Male repertoire

Dawn chorus audio recordings were collected from a total of 26 males. There was considerable variation in syllable diversity between males (Mean \pm SE: 45 ± 1.17 , range 33 – 59 syllables). If male syllable diversity was predicted by genetic diversity, a positive relationship would be expected between sMLH and repertoire size, but no significant relationship was observed (Table 5.1), nor for any of the other measures related to male song structure: phrase length (seconds) (Mean \pm SE: 1.29 ± 0.039 , range 0.88– 1.6), number of phrases (Mean \pm SE: 228 ± 7.77 , range 180– 332), and average high (Mean kHz \pm SE: 6335 ± 58.4 , range 5697– 6818), and low frequency (Mean kHz \pm SE: 2580 ± 53.4 , range 1970– 3318) (Table 5.1). There were significant differences between the islands for total syllables sung and total number of phases used in each five-minute bout. Birds on Motuara Island had higher syllable diversity (Mean \pm SE: 49 ± 1.4) than birds on Allports Island (Mean \pm SE: 41 ± 1.3). However, birds on Motuara sung significantly fewer phrases in each five-minute bout (Mean \pm SE: 211 ± 6.5) than birds on Allports Island (Mean \pm SE: 248 ± 13).

Table 5.1. Male robin song structure in relation to individual level of genetic diversity (sMLH) and location. Presented is total syllables sung in a 5-minute bout (analysed with a Poisson distribution), total phrases sung, average phrase length (both analysed with a Gaussian distribution) and high/low frequencies (analysed with a negative binomial distribution). Estimated parameters, estimate, standard errors, z-values, P-values are presented for all factors. Significant effects are indicated in bold.

Traits	Factors	Estimate	Std. error	z	Pr(> z)
Total syllables - GLM Poisson output	Intercept	-1.724	0.138	-12.495	<0.001**
	sMLH	-0.075	0.14	-0.54	0.589
	Location	0.334	0.062	5.434	<0.001**
Total phrases - GLM negative binomial output	Intercept	5.641	0.132	42.817	<0.001**
	sMLH	-0.134	0.132	-1.015	0.31
	Location	-0.149	0.058	-2.572	0.01**
Phrase length - GLM gaussian output	Intercept	1.075	0.179	6.013	<0.001**
	sMLH	0.146	0.179	0.815	0.424
	Location	0.120	0.078	1.539	0.138
Low frequency - GLM negative binomial output	Intercept	7.855	0.084	93.141	<0.001**
	sMLH	-0.023	0.084	-0.274	0.784
	Location	0.024	0.037	0.638	0.524
High frequency - GLM negative binomial output	Intercept	8.781	0.044	201.024	<0.001**
	sMLH	-0.029	0.044	-0.662	0.508
	Location	0.001	0.019	0.027	0.978

5.3.2 Detour task

The detour reaching performance of 35 robins was tested. Most robins (31/35) completed the habituation phase and all birds that completed the habituation phase completed the next two training stages (when the cylinder was opaque; Fig. 5.3b & c). Once individuals reached the final stage there was variation in their ability to successfully complete the challenge, with only 11/28 birds successfully completing phase four. There was also variation in the minimum number of trials required to pass habituation (Mean \pm SE = 3.54 ± 0.85 , range 3-11 trials), and training phases (phase two, Mean \pm SE = 3.73 ± 0.45 , range 3-10 trials; phase three, Mean \pm SE = 3.54 ± 0.44 , range 3-9 trials). In comparison, there was even greater variation between the number of trials required to pass the final test phase, with individuals taking considerably more trials to pass (Mean \pm SE = 14.75 ± 1.8 , range 5-24 trials). Similar results were observed for the time taken to retrieve the reward: habituation (mean \pm SE = 23 ± 7.59 , range 2-105 seconds), training phases (phase two, mean \pm SE = 35 ± 8.12 , range 3-88 seconds; phase three, Mean \pm SE = 27 ± 7.74 , range 3-102 seconds) and final phase four (Mean \pm SE = 32 ± 8.29 , range 3-96).

If genetic diversity was influencing cognitive scores, I would expect that individuals with higher genetic diversity should take less time to retrieve the mealworm, need fewer attempts to complete the trial and overall pass onto higher phases. This was observed for average time individuals took to complete the final phase: birds with higher genetic diversity completed the trials significantly faster during the test phase (Table 5.2, Fig. 5.5). Weaker trends (not significant, but within 90% confidence intervals) were also observed in which individuals with higher genetic diversity completed the trials faster during phase three (Table 5.2) and completed more challenges for the final test phase (Table 5.3). For an individual's ability to pass/fail each phase and overall, no significant results or trends were observed (Table 5.4)

There were significant differences between the sexes for trials required to pass phase one: females (Mean \pm SE = 5 \pm 1.7, range 3-17) took significantly more tries to pass than males (Mean \pm SE = 3.5 \pm 0.34, range 3-11) (Table 5.3). The same was true for time taken to retrieve mealworms in phases one: females (Mean \pm SE = 37 \pm 8.6 seconds, range 4-105) took significantly longer to retrieve mealworms than males (Mean \pm SE = 18 \pm 3.9 seconds, range 2-95) (Table 5.2). There was also a significant difference between the islands for time required to retrieve rewards in phase four, with birds on Allports Island (Mean seconds \pm SE = 20 \pm 5, range 3-85) retrieving mealworms significantly faster than Motaura Island birds (Mean seconds \pm SE = 43 \pm 7, range 8-96) (Table 5.2).

Table 5.2. Detour task. A GLM with negative binomial error structure was used all four phases analyses. Estimated parameters, estimate, standard errors, z-values, P-values are presented for all factors. Significance with 95% confidence intervals is indicated in bold with **, while significance within 90% confidence intervals is indicated with *.

Trial	Factors	Estimate	Std. error	z	Pr(> z)
Phase one GLM negative binomial output	(Intercept)	4.543	0.662	6.868	<0.001**
	sMLH	-0.827	0.585	-1.415	0.157
	Location	-0.102	0.314	-0.326	0.745
	Sex	-0.736	0.351	-2.099	0.036**
Phase two GLM negative binomial output	(Intercept)	3.079	0.663	4.641	<0.001**
	sMLH	0.284	0.582	0.488	0.625
	Location	0.207	0.312	0.663	0.508
	Sex	0.035	0.353	0.098	0.922
Phase three GLM negative binomial output	(Intercept)	4.299	0.698	6.16	<0.001**
	sMLH	-1.149	0.615	-1.868	0.061*
	Location	0.35	0.331	1.058	0.29
	Sex	-0.062	0.373	-0.165	0.868
Phase four GLM negative binomial output	(Intercept)	4.437	0.554	8.016	<0.001**
	sMLH	-1.401	0.492	-2.847	0.004**
	Location	0.843	0.264	3.196	0.001**
	Sex	-0.181	0.292	-0.62	0.535

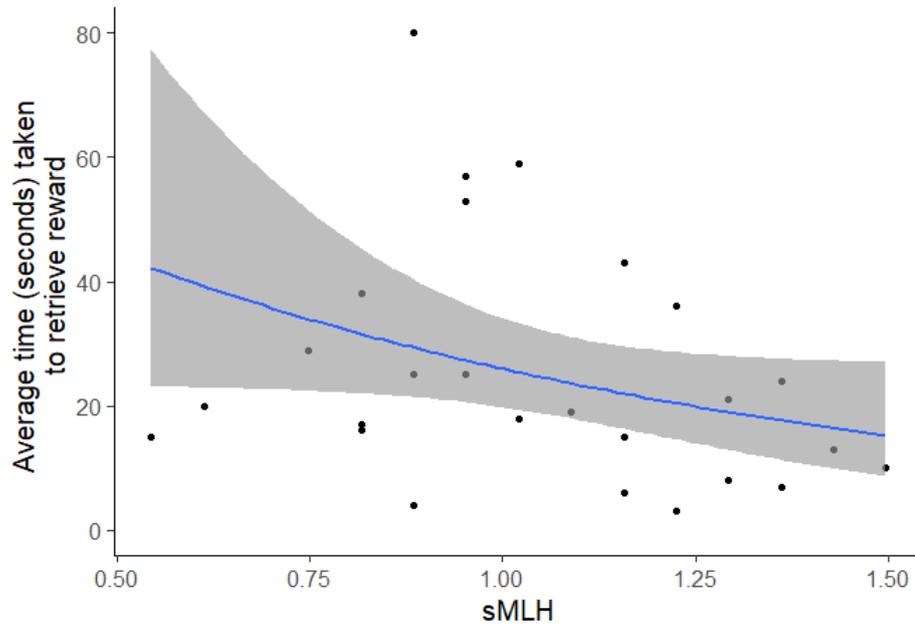


Figure 5.5. Correlation between average time (seconds) taken for South Island robin to retrieve the mealworms and their level of genetic diversity as measured by sMLH, presented with 95% confidence intervals using a negative binomial distribution.

Table 5.3. Detour task. For phases one to three a GLM with a Poisson error structure was used and in phases four and overall number of trials required to pass a GLM with negative binomial error structure was used for the analysis. Estimated parameters, estimate, standard errors, z-values, P-values are presented for all factors. Significance with 95% confidence intervals is indicated in bold with **, while significance within 90% confidence intervals is indicated with *.

Trial	Factor	Estimate	Std. error	z	Pr(> z)
Phase one GLM Poisson output	(Intercept)	1.838	0.419	4.39	<0.001**
	sMLH	-0.292	0.383	-0.762	0.446
	Location	0.247	0.206	1.203	0.229
	Sex	-0.492	0.214	-2.299	0.022**
Phase two GLM Poisson output	(Intercept)	1.238	0.435	2.848	0.004**
	sMLH	0.043	0.38	0.112	0.911
	Location	0.151	0.203	0.743	0.457
	Sex	0.053	0.227	-0.234	0.815
Phase three GLM Poisson output	(Intercept)	1.492	0.426	3.505	<0.001**
	sMLH	0.21	0.376	-0.558	0.577
	Location	0.159	0.203	0.784	0.433
	Sex	0.042	0.228	-0.182	0.855
Phase four GLM negative binomial output	(Intercept)	2.356	0.723	3.26	0.001**
	sMLH	0.243	0.603	0.403	0.687
	Location	-0.215	0.306	-0.704	0.482
	Sex	0.195	0.337	0.579	0.562
Trials passed out of 20 for phase four GLM negative binomial output	(Intercept)	1.014	0.756	1.341	0.18
	sMLH	1.104	0.665	1.662	0.097*
	Location	0.46	0.355	-1.297	0.195
	Sex	0.021	0.391	0.052	0.958

Table 5.4. Detour task. Presented is the GLM analysis for pass/fail rates for birds correlated with genetic diversity, a GLM with binomial error structure for the analysis. Estimated parameters, estimate, standard errors, z-values, P-values are presented for all factors. Significance with 95% confidence intervals is indicated in bold with **, while significance within 90% confidence intervals is indicated with *. In phases two and three all of the birds successfully passed; therefore, these models were not presented.

Trials	Factors	Estimate	Std. error	z	Pr(> z)
Phase one GLM Binomial output	(Intercept)	3.86385	2.7979	1.381	0.167
	sMLH	-1.658	2.33692	-0.709	0.478
	Sex	-0.0845	1.23446	-0.068	0.945
Phase four GLM Binomial output	(Intercept)	-0.7986	1.7899	-0.446	0.655
	sMLH	0.5616	1.6051	0.35	0.726
	Location	-1.2843	0.9003	-1.427	0.154
	Sex	0.6788	0.9839	0.69	0.49
Overall GLM Binomial output	(Intercept)	-1.2136	1.7649	-0.688	0.492
	sMLH	0.6282	1.5254	0.412	0.68
	Location	-0.6621	0.7897	-0.838	0.402
	Sex	0.1509	0.8711	0.173	0.862

5.3.3 Learning task

The learning performance of 44 individuals was tested during the habituation phase. Most robins (40/44) completed the habituation phase. Similar to the detour task most birds that completed habituation phase completed phase two (37/40), while phase three was more challenging with only 26/37 robins passing. All birds that managed to complete phase three successfully completed phase four (26/26). This suggests that phase three was the more difficult of the tasks and may have weeded out poorly-performing individuals before the phase four test. There was variation in the minimum number of trials required to pass habituation (Mean \pm SE = 6.61 \pm 0.66, range 5-22 trials), and training phases (phase two, Mean \pm SE = 6.86 \pm 0.51, range 5-16 trials; phase three, Mean \pm SE = 7.73 \pm 0.91, range 5-24 trials). There was also substantial variation between the number of trials required to pass

the final test phase, with individuals taking more trials to pass than in earlier phases (Mean \pm SE = 8.19 ± 0.83 , range 5-19 trials). Similar results were observed for the time taken to complete each phase: habituation (Mean \pm SE = 21 ± 2.45 , range 3-60 seconds), training phases (phase two, Mean \pm SE = 23 ± 2.33 , range 2-56 seconds; phase three, Mean \pm SE = 27 ± 3.34 , range 4-60 seconds) and test phase (Mean \pm SE = 13 ± 1.68 , range 4-35.8 seconds).

As with the detour task, if individual levels of genetic diversity correlated with the cognitive measures, I would expect that individuals with higher genetic diversity should take less time to retrieve the mealworm, need fewer attempts to complete the trial and pass onto higher phases/overall. Overall, there was only a weak trend (within 90% confidence limits), for individuals with higher genetic diversity taking fewer trials to complete phase three (Table 5.5). No significant or weak trends were observed for correlations between levels of genetic diversity and time taken to retrieve mealworms (Table 5.6). The same was true for pass/fail rates for each phase and overall (Table 5.7).

There was a significant difference in the number of trials, in phase two, required for birds that had previously participated in the detour task and had subsequently gone onto the learning task to complete (Mean \pm SE = 5.7 ± 0.5 trials, range 5-12) in comparison to birds that had not participated in the detour task prior to participating in the learning task (Mean \pm SE = 7.9 ± 0.8 trials, range 5-16) (Table 5.5).

There were significant differences between the sexes for time taken to retrieve mealworms in phases one: females (Mean \pm SE = 9 ± 2 seconds, range 3-19 seconds) took significantly less time than males (Mean \pm SE = 25 ± 3 seconds, range 5-52 seconds) to retrieve the mealworms (Table 5.6). There was also a significant difference between the islands for time required to retrieve mealworms in phase one and four (Table 5.6). In phase one, birds on Allports Island (Mean \pm SE = 13.8 ± 2.2 seconds) retrieved mealworms significantly faster than Motaura Island birds (Mean \pm SE = 26.3 ± 3.7 seconds) (Table 5.6).

The reverse was true for phase four, with birds on Allports Island (Mean \pm SE = 18.2 \pm 0.4 seconds) retrieving mealworms significantly slower than Motaura Island birds (Mean \pm SE = 16.8 \pm 0.8 seconds) (Table 5.6). For an individual's ability to pass/fail each phase and overall for the learning tasks no significant results or trends were observed (Table 5.7)

Table 5.5. Learning task. Phases two, four and trials passed out of 20, were analysed with GLM Poisson error structure, while phase one and three were analysed with GLM with negative binomial error structure. Estimated parameters, estimate, standard errors, z-values, P-values are presented for all factors. Significance with 95% confidence intervals is indicated in bold with **, while significance within 90% confidence intervals is indicated with *.

Trials	Factors	Estimate	Std. error	z	Pr(>Z)
Phase one GLM negative binomial output	(Intercept)	1.388	0.506	2.744	0.006**
	sMLH	0.011	0.418	0.026	0.979
	Location	0.309	0.218	1.415	0.157
	Sex	0.249	0.263	0.945	0.344
	Detour	0.192	0.211	0.908	0.364
Phase two GLM Poisson output	(Intercept)	1.69	0.323	5.241	<0.001**
	sMLH	0.291	0.267	1.093	0.274
	Location	0.037	0.142	0.257	0.797
	Sex	0.055	0.17	0.322	0.748
	Detour	-0.306	0.143	-2.145	0.032**
Phase three GLM negative binomial output	(Intercept)	2.821	0.467	6.035	<0.001**
	sMLH	-0.702	0.398	-1.763	0.078*
	Location	-0.268	0.223	-1.204	0.229
	Sex	0.347	0.253	1.369	0.171
	Detour	-0.274	0.218	-1.254	0.21
Phase four GLM Poisson output	(Intercept)	1.55	0.474	3.272	<0.001**
	sMLH	0.455	0.391	1.165	0.244
	Location	0.277	0.213	1.302	0.193
	Sex	-0.139	0.226	-0.615	0.538
	Detour	-0.058	0.207	-0.281	0.779

Table 5.6. Learning task. A GLM with negative binomial error structure used for all four phases analysis. Estimated parameters, estimate, standard errors, z-values, P-values are presented for all factors. Significance with 95% confidence intervals is indicated in bold with **, while significance within 90% confidence intervals is indicated with *.

Trials	Factors	Estimate	Std. error	z	Pr(> Z)
Phase one GLM negative binomial output	(Intercept)	1.622	0.496	3.269	0.001**
	sMLH	0.354	0.406	0.871	0.384
	Location	0.473	0.222	2.129	0.033**
	Sex	0.881	0.265	3.325	0.001**
	Detour	0.007	0.21	0.035	0.972
Phase two GLM negative binomial output	(Intercept)	2.091	0.504	4.148	<0.001**
	sMLH	0.636	0.42	1.513	0.13
	Location	0.148	0.225	0.657	0.511
	Sex	0.49	0.262	1.873	0.061*
	Detour	-0.274	0.218	-1.254	0.21
Phase three GLM negative binomial output	(Intercept)	2.534	0.565	4.483	<0.001**
	sMLH	0.583	0.459	1.271	0.204
	Location	0.026	0.278	0.094	0.925
	Sex	0.4	0.304	1.318	0.187
	Detour	-0.308	0.262	-1.174	0.241
Phase four GLM negative binomial output	(Intercept)	1.516	0.45	3.367	<0.001**
	sMLH	0.529	0.369	1.433	0.152
	Location	0.838	0.207	4.042	<0.001**
	Sex	-0.348	0.208	-1.671	0.095*
	Detour	0.118	0.193	0.611	0.541

Table 5.7. Learning task. A GLM with binomial error structure was used for all four phases analysis. Estimated parameters, estimate, standard errors, z-values, P-values are presented for all factors. Significance with 95% confidence intervals is indicated in bold with **, while significance within 90% confidence intervals is indicated with *. In phases two and three all of the birds successfully passed; therefore, these models were not presented.

Trials	Factors	Estimate	Std. error	z	Pr(> Z)
Phase one	(Intercept)	1.249	2.681	0.466	0.641
GLM	sMLH	0.205	2.209	0.093	0.926
Binomial	Location	0.733	1.132	0.647	0.518
output	Detour	1.205	1.25	0.964	0.335
Phase three	(Intercept)	2.609	2.106	1.239	0.215
GLM	sMLH	-2.277	1.807	-1.26	0.208
Binomial	Location	1.394	0.907	1.536	0.125
output	Sex	-0.555	1.086	-0.511	0.609
	Detour	0.947	0.924	1.025	0.305
Overall passed	(Intercept)	1.923	1.805	1.065	0.287
- GLM	sMLH	-1.484	1.49	-0.996	0.319
Binomial	Location	1.002	0.791	1.267	0.205
output	Sex	-1.221	0.996	-1.226	0.22
	Detour	0.896	0.755	1.187	0.235

5.3.4 Identity disequilibrium

See chapter 3.3.4 p97 for identity disequilibrium out puts.

5.4 Discussion

After the translocation of robins between my two study populations there was an overall increase in population-wide genetic diversity (chapter 2), but how this may have affected robin cognitive function was not studied previously. I found that robins with higher genetic diversity performed better at some cognitive tasks, and the cognitive test that showed a significant HFC was considered the most difficult task to accomplish. Additional trends towards individuals with higher genetic diversity completing these tasks faster and successfully completing more tasks in the final phase were also observed. The detour task was likely the more difficult of the two experiments with only 11 of the original 35 individuals successfully finishing the task. In comparison, 26 of the original 44 birds that participated in the learning task completed it. Therefore, we might expect to detect HFCs in the detour task over the learning task, which was the case. No evidence was found for HFCs in relation to male South Island robin song complexity measures. Some researchers have predicted that there may be an association between song structure and cognitive function (Bolhuis and Gahr 2006, Boogert et al. 2011b, Cardoso and Hu 2011). But the results on the association between song complexity and cognitive performance are very mixed (Anderson et al. 2017, DuBois et al. 2018). Overall, my results support the idea that higher genetic diversity is linked to some aspects of cognitive performance in robin populations.

Effects of inbreeding on cognitive function

In avian cognition experiments, motor control, inhibitory control, executive functions and learning tests are widely implemented, including several studies on North Island robins (Shaw et al. 2015, Shaw 2017, Shaw and Schmelz 2017, MacKinlay and Shaw 2018). Yet to date, no studies have attempted to quantify how genetic diversity influences cognitive function in wild bird populations. The significant correlation between genetic diversity and

performance in the detour task indicates that inbreeding could indeed influence inhibitory control, executive functions or learning in robins. Other trials in both the detour task and learning task, although not significant, showed the same pattern, with individuals with higher genetic diversity completing more trials correctly, taking less time to retrieve the reward in phase three of the detour task, and completing phase three in the learning task with fewer attempts. Together, this supports the idea that higher genetic diversity is linked to some aspects of cognitive function.

Increased cognitive function could be important for survival in South Island robins. Although cognitive function is linked to breeding success (Peters et al. 2014), it is also closely linked to foraging and goal orientation related to foraging (Hills 2006). As robins display a complex caching behaviour, which is likely associated with specialised cognitive function in spatial memory, it would be expected that inbreeding could negatively affect brain development. Robins with increased intelligence may be able to feed more efficiently and to remember the location of caching sites, as well as the length of time food has been stored within caches. This would reduce the time and energy that robins would require to feed and allow for other activities such as preening, predator avoidance and territorial defence that could increase survival.

Song structure and inbreeding

In order to function as an honest indicator of fitness, ornaments and displays should be more costly to express for males with low fitness than for males with high fitness (Grafen 1990). As many female songbirds select mates based on song structure, song has been viewed as an honest indicator of male quality (Searcy 1984, Lampe and Saetre 1995). However, my study in robins and other studies looking at avian song complexity and inbreeding did not show any significant correlations between syllable diversity and individual genetic diversity. In zebra finches, only a weak effect of inbreeding on several structural song

traits was observed, with only syllable rate being significantly affected (Bolund et al. 2010). Similarly, inbreeding in song sparrows only affected syllable phonetics and not syllable diversity (de Boer et al. 2016). The most likely reason is that song structure and rates are difficult to disentangle in relation to mate choice studies and environmental effects (Bolund et al. 2010). What we might assume is an honest indicator of fitness may not be. As I did not know how mate choice was related to the variation in the song structure traits I measured, I was not able to pinpoint the mechanisms behind the lack of a pattern in robins.

It has been suggested that song structure in zebra finches may function as a way of recognising individuals (de Boer et al. 2016). Thus, the song structure of an individual might be used to signal an individual's identity rather than his quality. In robins, males sing both a full dawn chorus and sub-song. With the dawn chorus being more stereotyped than their sub-song (Hay 1975), there is possibly little difference between the dawn chorus and non-stereotyped sub-song other than volume and output. Therefore, song structure related to syllable diversity could be a form of individual identification and volume and overall output demonstrate fitness. Timing and duration of dawn chorus might also be an indicator of male quality. For example, one male on Allports Island, an F1 interbred with one of the highest SMLH scores (i.e., high individual genetic diversity), was usually the first bird to begin singing each morning and was observed on occasions singing up to half an hour before dawn. It is possible that the start of dawn chorus represents a form of male quality, with only the most fit (i.e., males with high genetic diversity) able to start singing earlier. Unfortunately, due to the difficulties of identifying birds and arriving on time to record the start of the dawn chorus, it was not possible to ascertain when each bird started singing in relation to one another.

Observer error could also cause mask a relationship between song structure and genetic diversity. All sonograms were inspected visually, as I was unable to use software to

analyse and dissect the syllables from the song as the robin song appears to be too complex. An automated analysis of the song structure would likely be more accurate if it were possible and less subjective than a human observer. Furthermore, the observer might be scoring the wrong features of the song, as females may select for particular syllables or phrases and not the overall repertoire size. This has been observed in canaries (*Serinus canaria*) where females select males that sing 'sexy' notes (Vallet et al. 1999). It is possible that particular notes are harder to sing, however it is not currently known whether this occurs in robins.

Relationship between cognitive ability and song structure

The relationship between avian song learning and cognitive abilities is thought to possibly be linked or mirrored (Bolhuis and Gahr 2006, Boogert et al. 2011b, Cardoso and Hu 2011). However, recent findings would suggest otherwise (Anderson et al. 2017, DuBois et al. 2018). In this study no link between song structure and cognitive ability was observed. Similar results have been observed with song sparrows (Anderson et al. 2017). No correlation was observed between the song measures and cognitive task performances in song sparrows, leading the authors to conclude that learned song may not be a reliable indicator of individual cognitive ability. Similar mixed or inconclusive results have been found with zebra finch (Boogert et al. 2011a, Templeton et al. 2014), swamp sparrow (*Melospiza georgiana*; Sewall et al. 2013, DuBois et al. 2018), and European starling (*Sturnus vulgaris*; Farrell et al. 2011). These findings lead Anderson et al. (2017) to summarise that measures of song complexity in previous studies show no correlation with cognitive function measures and are just as likely to show positive and negative results. They recommended that the best way to test learning related to song would be to compare songs learned by young males to those produced by older males, whereby accurate copying of the song would equate to superior song learning. However, this may not be practical in some wild populations, as it would require multiple

years in which the complex interactions and life stages with different individuals would have to be monitored. As such, it was not feasible to do this with this study.

MacKinlay and Shaw (2018) investigated a hypothesis proposed by Sewall et al. (2013) that cognitive demands associated with caching behaviour may result in differing trade-offs or associations with vocal learning. Comparing North Island robins to non-caching song sparrows, they concluded that caching behaviour is associated with cognitive specialisation, particularly spatial memory. Given that South Island robins use similar caching systems, they should have similar spatial memory specialisation to North Island robins. Therefore, although robins may perform well in cognitive tasks, this specialisation is not associated with similar neural pathways as song learning, as it may have greater adaptive value for caching species compared to non-caching species like song sparrows.

Conclusion

Few studies to date have attempted to quantify how inbreeding and genetic rescue influence cognitive function in wild avian populations. I found some evidence that the attempted genetic rescue has helped increase cognitive performance of wild populations of South Island robins. Yet, there were no similar HFCs for song complexity and genetic diversity. This is not at odds with prior experiments and could be due to a range of yet undetermined factors. Results from this study highlight the potential benefits from attempted genetic rescue by enhancing the allelic diversity of the recipient population and in doing so the cognitive function for the rescued species. Overall there is a need to further our understanding on how inbreeding affects the cognitive performance of wild populations of endangered species and whether genetic rescue has the potential to reverse any cognitive problems.

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General Discussion

Chapter 6

Doom and gloom

The Holocene extinction, the current sixth mass extinction event, is recognised as a direct consequence of human activity (Dirzo et al. 2014, Ripple et al. 2017, Ceballos and Ehrlich 2018). During this time almost all animal and plant families have experienced extinctions, with extinction rates estimated to be 100 – 1,000 times higher than natural rates (Pimm et al. 1995, Dirzo et al. 2014, Pimm et al. 2014). This process is ongoing, with many wild animal and plant species still undergoing population declines and range fragmentation. New Zealand is no exception as extensive extinctions have been occurring since the 14th century (Perry et al. 2014). Things have not improved despite increased awareness of the situation. The Environment Aotearoa (2019) summary still paints a grim future for our native biota: the extinction risk has worsened for 86 species in the past 15 years with the conservation status improving for only 26 species. If we are to stop this trend it is more imperative than ever to halt population declines and the problems associated with small populations.

Population bottlenecks brought about from population declines can lead to the rapid loss of alleles, thereby increasing the genetic load (Templeton and Read 1984, Hedrick 1994). These genetic bottlenecks often indiscriminately remove alleles including those which may be adaptive. This increases the extinction risk by reducing the evolutionary potential of the species (Barrett and Schluter 2008). In turn, small population size and isolation increases the

chances of related individuals mating, thereby increasing the risk of inbreeding. Inbreeding can further decrease fitness, by decreasing allelic diversity across the genome. This decrease in genetic diversity makes individuals and populations less able to adapt to challenging circumstances (Caughley 1994) and thus at greater risk of extinction. Close kin mating increases homozygosity across the genome increasing the likelihood that recessive, deleterious alleles will be expressed. These deleterious alleles are often linked to negative phenotypic expressions (e.g., reduced fertility and survival). Combined together, reduced allelic diversity and increased homozygosity, can result in a reduction in fitness-related traits, a process called “inbreeding depression” (Nei et al. 1975, Bashi 1977, Newaman and Pilson 1997).

An optimistic future?

Prospects for animals and plants suffering from small populations may appear grim at the moment, but the future may not be as dire as it seems. Sanderson et al. (2018) predicts three future global trends relevant to conservation: (1) the end of human population growth, (2) the end of poverty, and (3) increased urbanization. Although further human population growth is expected in the decades to come, within the next 100 years growth rates should stabilize, and the world’s population begin to decline. In the 1980s, more than half of the population within developing countries lived in poverty. By 2010, that proportion had fallen to 21%, despite increases in population size for many developing countries (Olinto et al. 2013). Further predictions suggest that by 2030 the percentage of extremely poor could be between 3%–7% globally (Cruz et al. 2015). Concurrent with the decline in poverty, all regions of the world have become increasingly urbanized over the last 100 years (Sanderson et al. 2018). Urbanization and reduced poverty have several effects on cultural norms related to points one and two (Sanderson et al. 2018). Urban families experience higher costs for

rent, food, and other necessities, without the offsetting benefits that greater labour from a larger family provide in agricultural economies. Thus, incentives for large families diminish as people move into towns, thereby further reducing population increases. Regional per capita incomes also increase with urbanization, thereby resulting in dramatic reductions in poverty. This allows increased education and technological breakthroughs for conservation from education. Currently, we are seeing changes in the perception of conservation practices and its benefits. For example, the Global Forest Resources Assessment (Sloan and Sayer 2015), showed that deforestation has slowed and re-forestation has increased globally from 1990 to 2015. At present, forest gains are primarily occurring within higher latitudes and in richer countries whilst forest loss continues in poor developing countries in the tropics (Sloan and Sayer 2015). This trend may change as poorer countries develop.

The global trends in human demographics and economies described above have important implications for conservation. In the future it is likely that the negative pressure affecting biota by human population growth will lessen. The challenge at hand is therefore keeping many declining species from disappearing until a time when they can be re-introduced into their original home ranges. For many species this may mean simply keeping the species alive and as a viable population for the next 100-150 years until enough human pressure has been removed from its original environment and they can once again be re-introduced as land previously devoted to human use reverts back to more natural habitats. Such may be the case for many native species in New Zealand if “predator free 2050” can be also achieved.

One method that can help sustain species through the coming human demographic peak and increase population health of threatened species is the use of genetic rescue, the translocation of donors between populations to increase genetic diversity within the rescued population. Genetic rescue has been shown to be a cost-effective means of increasing

population health and helping species survive (Whiteley et al. 2015). However, there are challenges for the use of genetic rescue, including a determination of the optimal number of donors, the effect of attempted genetic rescue events over the long term and how often rescue events may need to be repeated.

Genetic rescue within two South Island robin populations

South Island robins in this study were an ideal species for examining the effects of genetic rescue. The robins in my study sites were a typical example of an extreme population bottleneck: just 5 birds were released on Motuara Island in 1973, and the population soon expanded to 250 birds. Five birds were also released onto Allports Islands before the population expanded to 56 birds by 1989 (Maloney 1991). Both populations appeared to remain near this density for the next 20 years. My approach to assessing the outcome of attempted genetic rescue was ambitious as it encompassed monitoring both the dynamics of genetic changes and fitness-related measures that range from individual to population-wide genetic diversity, breeding success, individual survival, population change, immune system function and even the cognitive abilities of the birds, over a 10-year period. However, this broad approach was necessary as focusing on just a single or few life stages over a short time frame can lead to erroneous conclusions (Bilski et al. 2013). This study represents one of the few studies that have been able to monitor the effects of a genetic rescue event over 10 years.

When comparisons of genetic diversity were made across the 10 years, we found differences in mean levels of heterozygosity and allelic richness, which increased in both populations immediately after the translocation in 2008-2009, and within the first F1-F3 interbred generations. After the initial increase both observed heterozygosity and allelic richness gradually declined over time, as indicated by the lower levels observed in 2017-2019. However, this decline has been relatively slow, and it may take several decades before genetic diversity declines to the levels present before 2008.

Increases in genetic diversity in both robin populations was accompanied by an increase in fitness. Juvenile robins with higher genetic diversity were significantly more likely to survive to one year of age. This pattern was mirrored in adults transitioning to old age: interbred F1-F3 individuals were significantly more likely to survive to 10 years of age than their pre-translocation counterparts. Even though I found no clear evidence for correlations between genetic diversity and breeding success, increased survival for both juveniles and F1-F3 interbred adults can provide individuals with the increased opportunities to reproduce. In this study, both robin populations increased considerably, likely driven by the increased survival and flow on effect this would have had on lifetime reproductive output. This indicates that a successful genetic rescue event has occurred. It is a promising result for two reasons: (1) the donors that were used in these translocations were from inbred populations and, (2) the island environments they were transferred between had pre-existing populations that were expected to be already at carrying capacity. Furthermore, the population growth could explain why the decline in genetic diversity within these populations has been relatively slow. Large growing populations are less likely to lose genetic diversity than small populations, as the effects of genetic drift and inbreeding are less pronounced (Gilpin 1986). As the robin populations appear to have been growing over the past 10 years it would be expected that the loss of genetic diversity through genetic drift is low.

The benefits of genetic rescue should be more prominent in populations that live in environmental conditions that permit increased population expansion. In theory this is a problem for many of New Zealand's bird species which are confined to island refuges that have set population caps. With no possibility of expanding beyond the island refuge they will inevitably lose genetic variation and accumulate inbreeding (Jamieson 2011). The results from this study run contrary to this idea and support the possibility that pre-existing island

populations that are suffering from inbreeding depression could still have increased population growth if subject to genetic rescue.

Why might genetic rescue increase survival?

I investigated the possibility that immune system response and parasite burdens could be predicted by genetic diversity. There is mounting evidence that the loss of genetic variation increases individual and population susceptibility to pathogens (Hale and Briskie 2009) and reduces immune system function (Grueber et al. 2012, Heber et al. 2013). In some species, it has been suggested that disease outbreaks were likely driven by inbreeding depression and reduced immune response (Bellingham et al. 2010). Heber et al. (2013) found that interbred F1-F3 individuals had higher immune system response and increased survival. I also found a weak trend for some parasites (e.g., mite loads) which decreased with increasing genetic diversity. Overall, this suggests that individuals with higher genetic diversity may have less parasites and increased immune response which could help with long term survival, as seen in the longevity of the F1-F3 interbreeds. This lends weight to the idea that with increased genetic diversity there is increased immune response which can result in healthier individuals that may live longer. It is also likely that the use of microsatellites may not be the best tool to assess loci related to immune system function. Evaluating changes in immune response should therefore include examination of immunogenetic loci, such as toll like receptors (TLR) and major histocompatibility complex genes (MHC) (Grueber et al. 2017).

Differences in cognitive ability may have influenced survival as well. I found evidence for a link between genetic diversity and some cognitive tasks. Although cognitive function is often linked to breeding success it is also linked to foraging and goal orientation related to foraging (Hills 2006). Individuals with increased intelligence and cognitive function may be able to feed more efficiently, and in the case of robins, allow them to remember the location of caching sites, and the length of time food has been stored within

these caches. This would reduce the time and energy that robins would require to feed and allow for other activities such as preening and territorial defence.

Considering both immune system response and increased intelligence together could help explain why individuals with higher genetic diversity are surviving longer. In the long run, the genetic rescue event can lead to increased reproductive success and eventually increased population growth.

Implications from this study

With >400 species of endangered birds around the world confined to island refuges (BirdLife International 2000), there is an urgent need to develop effective methods for the long-term management of these species. It is crucial to ensure that they retain genetic diversity to cope with current selective pressures as well as the potential to adapt to whatever future environmental changes they may face (e.g., climate change). Considering my findings, the periodical introduction of new alleles into small populations through translocation events should be part of a wider management scheme for all island populations, including other measures such as habitat restoration and predator control. However, when such periodical introductions need to occur is still an unresolved issue. Initial results from this study would suggest that the introduction of new alleles into the two robin populations, at this stage, would not be necessary: allelic diversity and heterozygosity has remained relatively high for 10 years and there has been a substantial population increase. However, it would be instructive to continue to follow each population over the next 10 to 20 years as the rate of decrease may change, and further genetic rescue attempts may be needed. Although, there was a higher increase in genetic diversity on Allports island than Motuara island, it is possible that the smaller Allports Island population may in the long term require more

frequent intervention. Given that rates of genetic drift and thus loss of genetic variation is expected to be higher in smaller populations.

Concerns about genetic rescue

Despite the recognised benefits of translocations, concerns have been raised about the potential harmful effects of genetic rescue. Genetic rescue has been linked with outbreeding depression resulting from the disruption of locally adapted gene complexes (Edmands 1999, Tallmon et al. 2004, Edmands 2007, Frankham et al. 2011, Frankham et al. 2012). Few studies have tried to or been able to monitor populations long term to assess the risk of outbreeding depression. This study is one of the few that has been able to monitor two populations of the same species concurrently over a 10-year period to see if outbreeding or inbreeding depression is occurring. Long-term monitoring is needed as the effects of outbreeding depression can be delayed to the F3 generations and beyond (Marshall and Spalton 2000, Tallmon et al. 2004) when the recombination of original parental gene fragments occur, potentially affecting local adaptations. Robins breed at one year of age and then annually, so it is likely that there will be up to F8-F10 interbred generation progeny within the populations. It is expected that enough time would have passed to allow the development of considerable genetic variation between individuals, which would enable the detection of population-wide effects from outbreeding depression. Overall, I found no evidence that outbreeding depression was occurring. In fact, robins today had marginally higher survival rates and no difference in reproductive success compared to their pre-rescue 8-10 years ago. This result lends further weight to the idea that reciprocal translocation of individuals, even inbred individuals, between populations has positive benefits.

Limitations with attempted genetic rescue

Despite the positive effects detailed above, and in my study, there are limitations to attempted genetic rescue. Firstly, for many threatened species, there are no donor populations available to use for translocations of new individuals into a recipient population. Instead, hybridisation with a closely related species or subspecies could be a measure of last resort. This has been suggested for helmeted honeyeater (*Lichenostomus melanops cassidix*), which has been reduced to a population of 100 birds. Historically there was some gene flow between helmeted honeyeater and closely related subspecies *L. m. gippslandicus*. It has been suggested that *L. m. gippslandicus* could be used for genetic rescue events and that natural levels of gene flow between the two subspecies could be restored (Harrisson et al. 2016). In the Galapagos a recently introduced parasitic fly (*Philornis downsi*) has been causing significant mortality for many endemic finch species (Peters et al. 2019). Yet interbred Darwin's small tree finch (*Camarhynchus parvulus*) and Darwin's medium tree finch (*C. pauper*) had lower *Philornis downsi* loads than non- interbred pairs (Peters et al. 2019), suggesting that interbred, in this case, can increase overall population fitness as a mechanism to combat an invasive parasite (Peters et al. 2019).

Yet this method runs risks. For example, black stilts (*Himantopus novaezelandiae*) survived as a single population that passed through a bottleneck of just 23 birds. There are now no other surviving populations that can be used as donors (Reed et al. 1993). Although it would be possible to use pied stilts (*Himantopus leucocephalus*) as donors, it is not recommended as this could inevitably lead to the genetic swamping of the black stilt's gene pool from the much larger population of pied stilts and eventually result in the functional extinction of black stilts as a separate species. The use of different species for hybridisations also run a higher risk of increased incompatible gene complexes which leads to outbreeding depression in the long run. However, in extreme cases this might be the only option, as seen with the Norfolk Island boobook owl (*Ninox novaeseelandiae undulata*) which was reduced

to a single female in 1986. The deliberate introduction of a closely related male *N. n. novaeseelandiae* allowed the taxon to persist, albeit in a hybrid form (Garnett et al. 2011).

Further examples include black robins (*Petroica traversi*), which are all descended from a single breeding pair (Ardern and Lambert 1997). Through translocations there are now two separate populations on Mangere Island and Rangatira Island. Both populations have differentiated from one and other over time, likely due to genetic drift. As the two populations now have slightly different genetic compositions it has been suggested that translocations of individuals between the two populations may be used to stimulate gene flow, even between such isolated and closely related populations, and act as a form of genetic restoration (Forsdick et al. 2017).

For some species, such as the kakapo (*Strigops habroptilus*), it is unlikely any other species or populations could be used as a form of genetic rescue given its small single population, uniqueness and phylogenetic distance from other related species. For such species, the best strategy is to try to prevent further bottlenecks and inbreeding in the first place. Yet soon, it might be possible to select individuals that carry functional loci that may code for particular disease resistance and breed these individuals into the populations as priority birds. In the distant future it might even be possible to extract functional DNA from museum skins or from evolutionary related kaka or kea and splice this genetic material into the kakapo genome to increase genetic diversity. For the moment, such options are still ‘science fiction’, but if the world’s endangered species are to make it to the next century, conservation biologists must be prepared to test and adopt all the available options.

Conclusion

In this thesis, I was able to demonstrate that successful genetic rescue through inbred donors has occurred within two island habitats which were already thought to have reached a

set population cap. Both genetic diversity and population growth resulted from the rescue attempts. The resulting population growth should also have a positive effect on maintaining the genetic diversity of the two populations, by reducing the negative effects of genetic drift. My work confirms that genetic rescue can be an effective management tool, even for small island populations, but it needs to be complemented with other management strategies such as predator removal, habitat restoration and long-term monitoring to avoid loss of further genetic variation in the future.

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