Informing genetic management of small populations of threatened species

Emily L. Weiser

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Abstract

Worldwide biodiversity faces a variety of anthropogenic threats, including habitat loss and predation by introduced species. Reintroduction has been increasingly successful as a conservation tool to address these threats, but until recently, little attention was paid to securing the genetic health of reintroduced populations. The two major genetic threats to small or bottlenecked populations are inbreeding and loss of allelic diversity. Inbreeding can immediately reduce fitness and increase the risk of extinction, while loss of allelic diversity threatens long-term adaptability. Management for allele retention will also minimise inbreeding and is an effective strategy to maximise genetic viability. In some cases, though, a population may be particularly affected by inbreeding, which then becomes the immediate concern.

Assessing options for genetic management requires accurately predicting inbreeding effects and allele loss under various management scenarios. Such predictions require the use of probability-based individual simulation models, but available models have limitations in being applied to wild, managed populations. Likewise, inbreeding effects are difficult to quantify and include in predictions of viability. The computer tools used to inform genetic management could therefore be greatly improved.

With this thesis, I first describe a new model that I developed to facilitate evaluation of management options for maximising allele retention (Chapter 2). This model is highly flexible and freely available to inform reintroduction planning of a wide variety of taxa. I use the model to explore how demography affects allele retention, finding that while there are broad patterns across taxa, management strategies will need to be tailored to each population of interest (Chapter 3). I also demonstrate application of the model in a context of complex metapopulation management, showing that even small, fragmented populations can be successfully managed for long-term viability (Chapter 4). Appendices A and B exhibit further applications of this model to real-world examples.

I then consider a case in which a great deal of allelic diversity has already been lost and extreme inbreeding has occurred: the black robin. I first demonstrate that further inbreeding produces a mix of positive and negative fitness effects in this species, with important interactions among an individual's inbreeding coefficient and those of its parents (Chapter 5). Next, I apply these findings in a population viability analysis framework to evaluate the net effect of inbreeding in this species, which is positive and

produces a very high probability of persistence if conditions remain stable (Chapter 6). I use this example to demonstrate how other studies could incorporate complex inbreeding effects into population predictions, providing a detailed tutorial in Appendix C. Finally, I assess management options that could be used to improve retention of allelic diversity in the black robin and thus its long-term adaptability to any change (Chapter 7).

Throughout my research, I have collaborated closely with conservation managers to ensure that my analyses are relevant to their work and that my results are available for their use. In Chapter 8, I synthesise the work presented in Chapters 2-7 and explore the general implications of my findings for small or reintroduced populations.

Acknowledgments

Conducting this research has been a very rewarding experience, thanks in large part to the people with whom I have had the privilege of working. I have particularly enjoyed collaborating with conservation managers and scientists who are acting to save some of our special, threatened birds. My PhD research was jump-started by a seemingly simple question from Jess Scrimgeour (Department of Conservation) at just the right time: "How many kiwi are needed to start a new population?" This question led to the development of AlleleRetain, the model that underlies a large part of my thesis, and prompted me to begin exploring the concepts of genetic conservation that are integral to my research as a whole.

Development of AlleleRetain was greatly facilitated by Murray Efford (University of Otago), who kindly allowed me to work from the source code for his model called mohuasim; and Michelle Reynolds (U.S. Geological Survey) provided the impetus for further development of AlleleRetain so that it would be applicable to the Laysan duck in Hawaii. Discussions with both Michelle and Jess were particularly helpful in ensuring that AlleleRetain would be useful for real management scenarios. Oliver Overdyck, Tertia Thurley, John Innes, Ian Flux, Rhys Burns, and the rest of the Kokako Recovery Group played an integral role in applying AlleleRetain to prioritise management of kokako populations, which is certainly the most comprehensive and complex application of my work to date. Their support, dedication, and attention to detail were invaluable in making the modelling work both possible and useful. I must also extend my personal gratitude to Tertia for showing me my first wild kokako!

A key goal in developing AlleleRetain was to make it useful for modelling a wide variety of species in a range of management contexts. Although the model is sufficiently flexible, it is currently accessible only to users familiar with (or willing to learn) R. AlleleRetain will soon become vastly more useful thanks to Lynn Adams and Kate McInnes at the Department of Conservation's National Office, along with Kevin Parker (Massey University), who are overseeing development of a graphical user interface that will make this model available to a wide range of on-the-ground conservation managers. I am grateful to all of them for their enthusiasm and skill in helping to make the model much more practically applicable.

Another large part of my thesis consists of my assessment of inbreeding and management options for black robins. This research was possible and relevant only because of the tireless efforts of countless field workers (most prominently Don Merton, but also many others) who saved the black robin from the brink of extinction. My work would have been equally impossible if Euan Kennedy (Department of Conservation) had not painstakingly collated, error-checked, and summarised decades of field data from the black robin project. I am also indebted to him for patiently imparting a small fraction of his great knowledge of the species to me. The month I spent on Rangatira Island (courtesy of both the Department of Conservation and research funding from the University of Otago's Department of Zoology) was an invaluable opportunity to learn about black robins from Euan and others, especially Tansy Bliss and Annette Harvey, and to get to know the birds and their environment. My work has also benefited from collaborating with Melanie Massaro (now at Charles Sturt University), whose experience and perspective on the species have been invaluable. Thanks also to Dave Houston (Department of Conservation) and the rest of the Black Robin Recovery Group for their input and assistance, especially in ensuring that the questions I've addressed are grounded in reality and will be directly useful for planning further management of this iconic species. Finally, implementation of the black robin population viability analysis was made possible by key pieces of advice from Bob Lacy and other users of the VORTEX listserv; I am indebted to them for their time and assistance.

A version of each chapter of my thesis has been prepared or submitted for publication, and many of the people mentioned above have contributed to these manuscripts as co-authors. I am also grateful to others who have commented on drafts of the published versions, including Michael Schwartz, Fred Allendorf, Gordon Luikart, Scott Mills, Suzanne Alonzo, Leigh Simmons, Shinichi Nakagawa, and several anonymous reviewers. Many of their recommendations are incorporated in the chapters presented here.

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The University of Otago's Department of Zoology has also been an integral part of my work, providing research funding and logistical support as well as a fantastic, friendly environment. Thanks to everyone there for making the department such a welcoming workplace that runs so smoothly! I would especially like to extend my gratitude to Marty Krkosek and Bruce Robertson for serving on my supervisory committee, and to Hamish Spencer for being ready to step forward as an additional co-supervisor if the need arose.

Special thanks to the Threatened Bird Research Group for being wonderfully supportive and reliably providing an opportunity to wind down after work. I am particularly grateful to Jolene Sutton and Sheena Townsend for hours of work-related discussion both in and outside the office, their advice around all aspects of being a PhD student, their friendship and unwavering support, and of course the entertaining games of Catan over delicious meals. Almost as importantly, they both showed me just how grateful I should be that I didn't have to do any lab work for my thesis! Kerry Weston was also a great source of support and encouragement, brought me to some of New Zealand's most beautiful places, and showed me that cycling up a hill was not so bad after all - just in time for cycling to become a much-needed part of my work-life balance. Thanks also to Robert Schadewinkel for letting me tag along on field work when I couldn't stare at a computer any longer!

Finally, and perhaps most importantly, I have been extremely fortunate to work with two fantastic supervisors: Catherine Grueber and Ian Jamieson. Catherine, thank you especially for your constant assistance with analytical methods and making yourself available on a daily basis for questions big and small. Your expertise in genetics was vital to ensure that my work and conclusions made sense, as genetics were not my strong point when I started this research! Ian, it was your foresight and collaborative networks that made my work useful and relevant, which has been the most rewarding part of this experience for me. Your unwavering confidence in me was also crucial as I learned to apply my research to real-world situations, and I've learned a great deal about advising conservation management from the way that you approach it so successfully yourself. Thank you both for your constant support and encouragement, patience, excellent feedback, and invaluable discussions, all of which have immeasurably improved my career as an aspiring scientist. It has been an absolute privilege to work with both of you.

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Chapter 1. General introduction



Mammalian predator-proof fence at Orokonui Ecosanctuary, a 307-ha cloud forest reserve near Dunedin, New Zealand.

Worldwide biodiversity faces a variety of anthropogenic threats, including habitat loss or modification, overexploitation, and competition from or predation by introduced species (Hoffmann et al. 2010). A large repertoire of tools and strategies has been developed to mitigate these threats. When the former distribution range of a species has been greatly reduced, one of the most useful conservation strategies is the reintroduction of that species to areas from which it had been extirpated, but that have since been restored to a state suitable for the species (Seddon et al. 2012).

Reintroduction has been used as a conservation tool for at least the past 100 years (Seddon et al. 2012). Success rates of reintroductions were previously low, with failures often being attributable to unsuitable habitat or geographic range, a small number of individuals being released, or specialised life-history traits (Griffith et al. 1989; Wolf et al. 1996). Methods for planning and implementing reintroductions have progressed in recent decades, improving success for many taxa (Parker et al. 2012; Soorae 2010). Demographic concerns associated with the population bottlenecks, initial low densities, and small population sizes often imposed by reintroductions have also received wide attention in the literature (Seddon et al. 2007). More recently, there has been a growing awareness that in addition to these methodological and demographic concerns, securing the genetic health of reintroduced populations is critical for long-term success (Armstrong & Seddon 2008; Groombridge et al. 2012; Jamieson & Lacy 2012; Weeks et al. 2011).

The genetic health of a population can be threatened by two major factors: inbreeding and loss of genetic diversity. Negative effects of inbreeding on fitness, termed inbreeding depression, have been well documented in wild populations (Keller & Waller 2002). Inbreeding commonly reduces fitness in wild populations and can increase the risk of extinction (e.g. Charlesworth & Willis 2009; Crnokrak & Roff 1999; Keller & Waller 2002; O'Grady et al. 2006; Saccheri et al. 1998), so minimising inbreeding is an important consideration for small or bottlenecked populations. Although inbreeding avoidance mechanisms have been documented in a variety of taxa (Pusey & Wolf 1996), these mechanisms may break down in small populations, where individuals have a limited choice of mates. Management action may then be necessary to prevent or mitigate inbreeding. Inbreeding depression in reintroduced populations has begun to receive more attention in recent years (e.g. Brekke et al. 2010; Ewing et al. 2008; Favé et al. 2007; Jamieson 2011; Jamieson & Ryan 2000; Keller et al. 2012; Marshall & Spalton 2000), but the extent to which inbreeding can affect population viability remains unclear (Keller et al. 2007).

Like other bottlenecked populations, reintroduced populations are also vulnerable to loss of genetic diversity, which is typically measured as either heterozygosity or allelic diversity (Allendorf & Luikart 2007). Generally, individuals that are more heterozygous tend to show higher fitness than those that are more homozygous (Chapman et al. 2009; Markert et al. 2004; Szulkin et al. 2010). Populations that have experienced a reduction of heterozygosity, e.g. through bottlenecks or inbreeding events, could therefore be less viable than outbred founder populations. In practice, however, even an extreme bottleneck causes little loss of heterozygosity if the bottleneck is temporary (Allendorf 1986; Stockwell et al. 1996). In contrast, a substantial portion of allelic diversity may be lost during even brief population bottlenecks (Allendorf 1986). Alleles are also lost over time in small populations as a result of genetic drift (Allendorf & Luikart 2007). Allelic diversity is important to retain because it defines the capacity for a population to adapt and survive in changing conditions (Allendorf & Luikart 2007; Markert et al. 2010). For example, high allelic diversity at loci associated with disease resistance enables a population to survive in the presence of a wide variety of pathogens (Edwards & Hedrick 1998). More alleles will give the population more options to respond to natural selection; if the allele that codes for a favoured characteristic is not present, the population will not be able to adapt and may not persist under changing conditions.

Maintaining allelic diversity through bottlenecks and over time is therefore an important consideration for managers of reintroduced populations. Rare and selectively neutral alleles are particularly difficult to retain in small populations (Allendorf 1986; Luikart et al. 1998; Sutton et al. 2011), and even alleles that are adaptive under current conditions may be lost (Radwan et al. 2010; Sutton et al. 2011). Rare alleles that are currently selectively neutral may be critical in preserving a population's ability to persist in the face of novel pathogens (Slade & McCallum 1992), so losing any such alleles may put a population at risk. Alleles that are lost can only be replaced by mutation over a very long time span (thousands of generations, thus not feasible for species under immediate threat); or by immigration, if there is some connectivity with another population of the same species. Allele loss is thus virtually irreversible for populations that cannot be supplemented with immigration, e.g. for species that persist in only one population or for taxa that are particularly difficult to move. Minimising loss of alleles is therefore critical to maintaining long-term viability of isolated populations, but predicting the effectiveness of management options to reduce allele loss is challenging in most populations (Allendorf 1986). Further work is therefore needed to assess options for retaining allelic diversity in

reintroduced populations (Armstrong & Seddon 2008; Groombridge et al. 2012; Jamieson & Lacy 2012).

Retaining allelic diversity requires a less restrictive bottleneck, larger carrying capacity, or higher degree of connectivity among populations than would be necessary to mitigate other small-population concerns, such as loss of heterozygosity, inbreeding, and demographic vulnerability to stochastic events (Allendorf 1986; Allendorf & Luikart 2007). Managing a population to retain rare alleles is therefore a conservative approach that will inherently protect a population from other genetic issues. However, in some cases, a species or population may have been subject to a particularly severe bottleneck. While retaining allelic diversity is still important in these cases, inbreeding depression may be a more immediate concern. Such situations benefit from assessment of the effects of inbreeding (e.g. Charlesworth & Willis 2009; Crnokrak & Roff 1999; Keller & Waller 2002) and the consequences of those effects for population viability (e.g. Keller et al. 2007; O'Grady et al. 2006; Thévenon & Couvet 2002).

Assessing options for genetic management requires accurately predicting inbreeding effects and allele loss under various management scenarios. However, calculating loss of allelic diversity beyond the first generation is problematic (Allendorf 1986), especially for species with overlapping generations (Wang et al. 2010). The chance of retaining any given allele over multiple generations in a population depends on a series of probabilities, including survival rates, reproductive success and variance, and the chance of each offspring inheriting a given allele from each parent. As each probability is compounded over successive years and generations, deterministic calculations cannot predict allele loss.

Instead, individual-based computer models that simulate these factors can be used to predict retention of allelic diversity and evaluate management options to minimise allele loss (e.g. Tracy et al. 2011). Although there are several such tools available, each has limitations in being applied to wild populations. In particular, available models (described in Chapter 2) cannot necessarily simulate all the management options that may be of interest, especially for reintroduced populations. A model that remedies these limitations and can accurately simulate a wide range of life history traits would be very useful to guide planning and management of specific reintroduced populations.

Likewise, computer models can be used to assess inbreeding depression and possible mitigation strategies. Effects of inbreeding have been shown to vary widely among taxa, among populations of the same species, and even among years within the

same population (Crnokrak & Roff 1999; Keller 1998; Keller & Waller 2002). Statistical techniques such as generalised linear mixed effects models and model averaging can be useful tools for assessing effects in a population of interest (Grueber et al. 2011). However, these assessments alone are not sufficient to evaluate the effects of inbreeding on a population's chance of persisting long-term. Population viability analysis (PVA), a tool to evaluate population growth and persistence (Beissinger & McCullough 2002), is a useful method for assessing the population-level consequences of inbreeding effects. Inbreeding depression has only rarely been fully addressed in PVAs, owing to factors such as the difficulty of quantifying inbreeding depression and the technical challenges of implementing those effects in a PVA using the software currently available (Allendorf & Ryman 2002).

Thus, the extent to which computer tools are used to inform management of small or reintroduced populations could be greatly improved. With this thesis, I develop and demonstrate computer tools that can be used to test hypotheses about loss of genetic diversity in small, reintroduced populations. I demonstrate how these models can be used to guide management options toward maximising long-term viability of such populations. To explore implications for wild populations, I draw on examples of well-studied species with various life histories and management considerations. Most of these examples are New Zealand birds, which are under threat from introduced mammals such as rats (ship rat *Rattus rattus*, Norway rat *Rattus norvegicus*, and Pacific rat or kiore *Rattus exulans*), cats (*Felis catus*), mustelids (weasels *Mustela nivalis*, stoats *Mustela ermina*, and ferrets *Mustela furo*), and Australian brushtail possums (*Trichosurus vulpecular*) (Clout 2001; Innes et al. 2010). New Zealand conservation managers have pioneered methods for controlling mammalian predators (Clout & Russell 2006; Towns & Broome 2003), and eradications on offshore islands or mainland reserves are often followed by reintroductions of native species to areas of their former range (Innes et al. 2010; Towns & Broome 2003). Many of these secured areas are small, raising concern about long-term genetic viability of reintroduced populations that are often critical to conservation of a species. New Zealand therefore provides a wealth of examples of reintroduction for conservation and diverse opportunities to assess management options for small populations.

With this thesis, I first describe a new model that I developed to facilitate evaluation of management options that will maximise allele retention in reintroduced populations (Chapter 2). I then use the model to explore the effects of demography and life history traits on allele retention, and show that generalised guidelines for management

cannot be applied across populations or species (Chapter 3). Next, I demonstrate application of the model to real populations with various management considerations, using my work with North Island kokako (*Callaeas wilsoni*) to show how the model can be used to guide management of metapopulations in a wide variety of realistic scenarios (Chapter 4). Appendices A and B provide further examples of application of this model to real-world examples (see details below).

I then consider a case in which a great deal of allelic diversity has already been lost, extreme and prolonged inbreeding has occurred, and inbreeding depression could impact long-term viability: the Chatham Island black robin (*Petroica traversi*). I first describe the fitness consequences of inbreeding in this species (Chapter 5), and then apply these findings to evaluate the black robin's long-term probability of persistence (Chapter 6). I use the PVA described in Chapter 6 to demonstrate how other studies could incorporate complex inbreeding effects into population predictions, and provide a detailed tutorial in Appendix C. My analyses indicate that under current conditions, inbreeding will not be a threat to the black robin, so I finally assess management options that could be used to improve retention of allelic diversity in this species and maximise its capacity to adapt to changing conditions (Chapter 7). Finally, I synthesise the work presented in Chapters 2-7 and explore the implications of these analyses to other small or reintroduced populations in New Zealand and elsewhere (Chapter 8).

Although the case studies in this thesis are representative examples that I have used to demonstrate broadly relevant small-population concerns, the work presented here is also useful for guiding management of these species and particular populations. Throughout my research, I have collaborated closely with conservation managers to ensure that my analyses have been relevant to their work, and that my results are available for their use. I have participated in species recovery group meetings for kiwi, kokako, and black robins, providing advice which has helped to inform their national strategies for conservation. I have also provided written reports to the kiwi recovery group for populations of two species of kiwi. Those reports are included in this thesis as Appendices A and B. Similarly, I have used my model to advise management of a population of Laysan ducks. Those results were included in a manuscript published with scientists at the U.S. Geological Survey in Hawaii (Reynolds et al. 2013). The work presented in Chapter 4 was specifically requested by the Kokako Recovery Group, and has been provided to the group in an unpublished report. Finally, my work on black robins (Chapters 5-7) has been conducted in collaboration with the Black Robin Recovery Group, particularly Euan

Kennedy (ESK), who collated the historic black robin data for his own thesis (Kennedy 2009) and made possible the analyses presented here.

In accordance with the broader relevance of this work, most of the chapters of this thesis are revised versions of manuscripts intended for publication in international journals. Chapters 2 and 3 have been revised from two manuscripts published with my supervisors, Ian Jamieson (IGJ) and Catherine Grueber (CEG), as co-authors. Chapter 5 is drawn from a manuscript (in review) co-authored with ESK as well as IGJ and CEG. Chapters 6 and 7 and are revised from manuscripts (in preparation for submission) co-authored with ESK, IGJ, CEG, and Melanie Massaro, who has also been involved with black robin research. Co-authorship on these manuscripts reflects the collaborative nature of this research, especially with respect to making my work applicable in an immediate and practical sense. My work has benefited greatly from this collaboration, and the co-authors have provided valuable perspectives on the concepts addressed here as well as advice on the analyses and suggestions on written drafts. However, each manuscript describes work that is chiefly my own; I am the lead author on each, and I have performed the analysis, drafted the manuscript, and revised it for inclusion in this thesis.

As a whole, the work presented here advances computer tools that can assist planning of reintroductions and management of small populations, especially with respect to genetic viability. The results and conclusions from this thesis are not only immediately applicable to particular populations of threatened species, but also demonstrate concepts and patterns relevant to a variety of managed taxa around the world.

Chapter 2. A new tool for assessing management options to conserve genetic diversity in small populations



Adult North Island robin on Kapiti Island, New Zealand.

A version of this chapter has been published as: Weiser, E. L., C. E. Grueber, and I. G. Jamieson. 2012. AlleleRetain: A program to assess management options for conserving allelic diversity in small, isolated populations. Molecular Ecology Resources 12:1161-1167.

Abstract

Preserving genetic health is an important aspect of species conservation. Allelic diversity is particularly important to conserve, as it provides capacity for adaptation and thus enables long-term population viability. Allele loss is difficult to predict beyond one generation for real populations with complex demography and life history traits, so I developed a computer model to simulate allele retention in small populations. This model, called AlleleRetain, is an individual-based simulation model implemented in R that can be applied to assess management options for conserving allelic diversity in small populations of animals with overlapping generations. AlleleRetain remedies the limitations of similar existing software, and its source code is freely available for further modification for specific case studies. The model and its supporting materials are available on CRAN (cran.r-project.org), the major online repository for R packages.

Introduction

Allelic diversity is important to retain in managed populations because it defines the capacity for a population to adapt and survive in changing conditions (Allendorf & Luikart 2007; Markert et al. 2010). If allele loss can be predicted, management can be planned accordingly to minimise loss (e.g. Tracy et al. 2011). However, expected loss of allelic diversity is difficult to calculate beyond the first generation, especially for species with overlapping generations (Allendorf 1986). This is partly because the probability of retaining a selectively neutral allele over multiple generations in a population depends on several factors, such as allele frequency in the source population (for introduced populations), bottleneck size (if relevant), reproductive success and variance, and frequency of immigration (if any). Computer models that simulate these factors can be used to predict retention of rare alleles and guide management options toward reaching the desired probability of allele retention in managed populations.

The computer models currently available are not sufficient for many *in situ* conservation programmes, as each is subject to limitations. Some of these programmes are intended to maximise allele retention through controlled or manipulated breeding of captive populations (PMX [Ballou et al. 2011]; and MetaPop [Pérez-Figueroa et al. 2008]); require molecular data (BottleSIM [Kuo & Janzen 2003]; and MetaSIM [Richter et al. 2008]); cannot model a bottleneck and subsequent population growth (EasyPOP

[Balloux 2001]); or are intended to model a specific organism (mohuasim [Tracy et al. 2011]). VORTEX (Lacy et al. 2009) offers sufficient flexibility to model most real animal populations, but is intended to be used primarily for a population viability analysis and has some limitations relevant to modelling allele loss in reintroduced populations. VORTEX models retention of alleles that are present in the founding population, but this cannot be translated into retention of alleles from a source population. Simulation of management options is also limited, and the fates of animals from different origins (i.e. immigrants versus locally produced individuals) cannot be easily tracked. Manipulation of output is also limited within the programme, and use of the programme requires learning the unique graphical user interface. The last two shortcomings could be addressed with a programme that operates in a widely-used environment such as R (R Development Core Team 2013). Multiple scenarios with different input parameters can easily be set up and run as batches in R, and output can be manipulated in the same environment or exported. Such a programme would be extremely useful for assessing a variety of management options for a population of conservation interest.

A new model to simulate allele loss

I developed a new model, AlleleRetain, to address the limitations of programmes available for modelling retention of rare, selectively neutral alleles in bottlenecked populations. AlleleRetain is implemented in R and the source code is freely available, along with the R package installation file and detailed user guide, on its website (https://sites.google.com/site/alleleretain/) and on the Comprehensive R Archive Network (cran.r-project.org). AlleleRetain employs a series of user-specified parameters to realistically simulate demography, allele retention, and inbreeding accumulation in animals with overlapping generations and a wide variety of life-history traits under many management options. AlleleRetain is particularly useful for identifying management options that will maximise retention of allelic diversity of a population established by translocation (or bottlenecked for another reason), especially when the population is capped at a small size with no natural immigration and thus at risk of genetic drift. The model simulates top-up translocations (to supplement the initial founder population in subsequent years) and immigration (ongoing supplementation) according to flexible specifications, tracks the proportion of each group (founders, immigrants, locals) that recruits to breed, and estimates the number of effective immigrants (those that breed) each generation.

AlleleRetain can also track descendant pedigrees and output the mean inbreeding coefficient of the simulated population alongside the probability of retaining rare alleles at the specified initial frequency (the only free programme to do so, to my knowledge).

AlleleRetain is intended to simulate the probability of retaining a rare allele to the end of a specified period of time. It is not intended as a population viability analysis programme, so it does not include random effects such as environmental stochasticity, as those effects would make it more difficult to examine the consequences of different management options for allele retention. However, the wide variety of demographic options available in AlleleRetain, along with its implementation via R, make this programme the most flexible option currently available for modelling allele retention, accumulation of inbreeding, and basic demography of a single population, especially when the effects of immigration are of particular interest.

AlleleRetain structure and function

I used mohuasim, which was developed to simulate allele retention in translocated populations of mohua (Mohoua ochrocephala; Tracy et al. 2011), as the starting point to build AlleleRetain and dramatically expanded upon its original capabilities. I modified several parameters implemented by mohuasim and created others to enhance the model's flexibility so that it can be used to model species with a variety of life-history traits under a range of management schemes. I developed and tested AlleleRetain in versions 2.12 - 3.0 of R on a Windows platform. Detailed information on the structure and function of the model are provided in Box 2.1, with a schematic of the overall structure in Figure 2.1.

The user of AlleleRetain specifies the demographic and management parameters affecting the population of interest (Box 2.2). This includes the number of individuals released to establish the population (bottleneck size), along with their age class (adult or juvenile), sex ratio, and post-release survival rate. These individuals can be released all at once or gradually over a period of years. Released individuals are assumed to be unrelated and are randomly assigned genotypes (zero, one, or two copies of a hypothetical neutral allele) according to a user-specified frequency of the allele in the source population. The simulated population can be held at the initial size to simulate a prolonged bottleneck (while reproduction occurs, by randomly removing surplus individuals), or the user can specify a post-release lag with no reproduction. Otherwise, released individuals mature and recruit according to user-specified parameters. AlleleRetain can model species with

delayed sexual maturity by preventing subadults from breeding until a user-specified age. Adults are randomly sorted into monogamous or polygamous breeding pairs, which re-form for multiple matings within one season (in polygynandry systems), remain together seasonally (in monogamous, polygynous, or polyandrous systems), or remain together lifelong (in monogamous systems) depending on user-specified settings. Reproduction occurs each year based on individual or population means (depending on settings for individual variation in reproductive output) for the number of offspring produced per pair per year, which can change with age of the parents. Sex is randomly assigned to each offspring according to the user-specified sex ratio for juveniles and offspring inherit alleles from their parents via Mendelian inheritance. When the simulated population reaches the specified carrying capacity, population growth ceases by either preventing recruitment of offspring or removing randomly selected individuals (as indicated by the user). When individuals are removed at random, the user can choose to give priority to remain in the population to established breeders and/or immigrants (if any). Annual survival of individuals is determined by user-specified probabilities for each age class, with optional sex-specific survival rates for adults. The user can choose to incorporate density dependence into juvenile and subadult/nonbreeder survival probabilities and/or to specify senescence effects in survival of adults beyond a certain age.

AlleleRetain runs the simulation over a specified number of replicates and averages the output. Because of the probabilistic nature of the model, running more replicates will improve the precision of predictions, but will also substantially increase the time needed to run the simulation. Each simulation may take several minutes to > 2 hr, depending on the computer system and the input parameters (running more replicates over a longer period or with a larger carrying capacity will noticeably increase run time). I recommend first testing the simulation on a small number of replicates (10-50) and moderate carrying capacity (< 500) to ensure the input will result in correct operation and expected demography, then running 1000 replicates to predict allele retention.

AlleleRetain simulates immigration at regular user-specified intervals by adding more individuals from the original source population (with the same initial allele frequency specified for founders). Immigrants can be prioritised over locally produced individuals to recruit into breeding vacancies. This scenario may be realistic when most locally produced juveniles would emigrate or when locally produced juveniles would be removed by managers. Emigration can be simulated by reducing survival of the relevant age class, assuming emigrants and their descendants do not return to the population.

The summary functions included in AlleleRetain provide both demographic and genetic output, averaged across replicates (Box 2.3). The user specifies whether to include replicates in which the population went extinct in the summaries. A population census is output for each year, along with the probability of the rare allele being retained until that year (with user-specified confidence limits) and the allele frequency (with standard error). The probability of retaining the rare allele is equivalent to the proportion of rare alleles which had occurred at the same initial frequency in the source population that would be retained in the simulated population (though the allele frequency may have changed). Additional information for each individual can be stored through the simulation; this requires more RAM and may considerably increase running time depending on demographic settings, but enables the user to call a summary of individual data (e.g. probability of breeding and average lifespan for individuals of each origin) and pedigree data. The output from these summaries can be used immediately or saved (e.g. in .csv format) for later manipulation in R or another programme of the user's choice (e.g. to create figures with the most relevant data). Figure 2.3 shows an example of the output obtained from simulated data based on a population of North Island robins (*Petroica longipes*) founded by 20 individuals. The output can be used to estimate (and compare across scenarios with different management options) the population growth rate, which indicates demographic viability of the population; and the probability of retaining rare alleles over time, and whether this achieves the intended goal. The model also outputs other information, such as mean inbreeding coefficient, which may be of interest for some populations.

Model validation

To assess whether my model performed as expected, I compared output from AlleleRetain with output from equivalent scenarios run in VORTEX (Lacy et al. 2009). With each programme, I tested one scenario with a stable population (100 individuals) and one with a bottlenecked population (30 individuals) that grew to carrying capacity (100 individuals) during the simulation, with simplified demographic parameters that could be held constant across the two programmes. The 95% CI for the probability of retaining a rare allele estimated over 1000 replicates by AlleleRetain (stable: 0.709-0.765; bottlenecked: 0.509-0.571) overlapped substantially with those estimated by VORTEX (stable: 0.675-0.733; bottlenecked: 0.523-0.586). Results from AlleleRetain also agree

with the theory of genetic drift, in that the allele frequency remains essentially constant (or drifts at a very slow rate such that the change is imperceptible over short periods) in the absence of migration, selection, and mutation, for reasonably large populations (>500 individuals) over 10 generations (data not shown). I am therefore confident that this model performs appropriately in simulating loss of rare alleles.

Although AlleleRetain simulates populations more realistically than many other programmes (relying on fewer simplifying assumptions), accuracy of the predictions could be compromised if assumptions are violated. For example, if some founders are related or if there is assortative mating, the new population will have lower genetic diversity (and a lower chance of retaining rare alleles) than predicted by the model. Likewise, accuracy of the predictions will depend on accuracy of the input values used. If demographic rates are not well known for a species of interest, multiple scenarios could be run to bracket the potential rates and which rare alleles will be lost and the range of management options that could mitigate allele loss. I further explore which demographic rates have the strongest influence on allele retention and effectiveness of management in Chapter 3.

Box 2.1. Model flow of AlleleRetain.

The following describes the major actions implemented by `aRetain`, the main function in AlleleRetain. Input and output (arguments, objects) and functions are in `Courier New font`. Internal objects (which are used by, but not output from, AlleleRetain or its functions) are in *italics*.

1. The model checks all input to ensure values are valid; if not, the simulation stops and outputs a warning message.
2. The model sets up empty matrices needed later (including *population*, which will contain adults that have recruited into the breeding population and individuals that have died if `trackall = TRUE`; *nonbreeders; juveniles; migrants*)
3. Initial population is formed with `addnew` and `newinfo`.
4. Each year (looped over 1:`nyears`):
   a. Starters are put into *nonbreeders* if they are adults (no breeding will occur this year), or into *juveniles* otherwise. This is year 1.
   b. *pairs* (if any) are also stored in *oldpairs* to later assess which ones were paired last year.
   c. The total number of adults (if `KAdults = TRUE`) or all individuals (if `KAdults = FALSE`) now present is recorded to be used later in density-dependent effects.
   d. Breeding occurs (unless in year 1 or within `reprolag` years of founding):
      i. # young/year for females in the first reproductive stage is adjusted by multiplying their individual means by `ypF1`.
      ii. Reproduction is simulated by running *pairs* through `breed`; offspring are put into *juveniles*.
      iii. If `nMatings` > 1, *pairs* are re-formed and breeding repeats until `nMatings` is achieved for females.
      iv. Information from `newinfo` and parent IDs are recorded for each offspring.
   e. Any supplementals are added with `addnew` and `newinfo`. The year in which they were added is recorded. If juvenile, they are added to *juveniles;* if adult, they are added to *nonbreeders.*
   f. Any immigrants are added, as for supplementals. If `removeL = TRUE`, the corresponding number of local adults is removed from *population*.
   g. Survival: individuals are randomly selected to survive or die, based on the appropriate survival probability:
      i. Adult: survival rate is adjusted for age (Figure 2.2a):

$$S_a = S_x - \frac{S_x}{\texttt{MaxAge} - \texttt{SenesAge}} * (a - \texttt{SenesAge})$$

      where S_a = survival at age a and S_x = `adsurvivalF` or `adsurvivalM`.
      ii. Nonbreeder: survival rate is density-dependent (Figure 2b):

$$S_{E_t} = \frac{S_0}{1 + \beta * E_t}$$

      where S_{E_t} is survival rate at population density E in year t, S_0 is survival when density is near 0, β is the decline in survival as density increases, and E_t is population density at time t (Morris & Doak 2002). "Density" is defined in this model as the proportion of `K` that has been filled. The model solves for β according to the user-specified values for `nonbrsurv` (S_0) and `nonbrsurvK` (S_1), then uses β and S_0 to calculate density-dependent survival probability in each year. Nonbreeder survival is applied to all individuals in *nonbreeders*, even those that are older than `mature` (they remain in *nonbreeders* until they recruit to breed).
      iii. Juvenile: density dependent as for nonbreeders, depending on `juvsurv` and `juvsurvK`.

h. *juveniles* are added to *nonbreeders*.
i. Maturing individuals are randomly selected to recruit into the breeding population (not all those selected may be able to pair, depending on the sex ratio). If `retainBreeders = "none"`, all maturing individuals recruit into *population*, which is later truncated to `K` by randomly removing individuals (new recruits have the same chance as established breeders to remain in the population). Otherwise, only the number of individuals needed to reach `K` (or `startN`, if within `Klag`) is recruited from nonbreeders that will be old enough to breed next year (depending on `mature`). If `mpriority = TRUE`, immigrants are selected first; then locals are randomly selected to fill any remaining spaces.
j. The population is truncated to `K` (or to `startN`, if within `Klag`), if necessary. This is not necessary if `KAdults = TRUE` and `retainBreeders ≠ "none"` because recruitment was limited to only filling vacancies; there are no surplus individuals.
    i. If `KAdults = FALSE`, *nonbreeders* are included in carrying capacity (`K`). Otherwise, only adults in *population* are included.
    ii. Individuals that have priority are retained through truncation:
        1. Retained breeding adults, if any (depending on `retainBreeders`). Only necessary if `KAdults = FALSE`, because if `KAdults = TRUE` and `retainBreeders ≠ "none"`, no surplus individuals are present (see 4.h.i).
        2. Immigrants, if `mpriority = TRUE`.
    iii. Then other available individuals are randomly selected until the population is at `K`. Dead nonbreeders are moved into *population*.
k. *pairs* are re-formed for next year (by `pairoff` if monogamy or `polypair` otherwise):
    i. If `matingLength = "seasonal"`, *pairs* are split up and all individuals are put into the *singles* pool. Otherwise, *pairs* with both members still alive remain intact.
    ii. If `matingSys = "monogamy"`, widowed or divorced individuals of the sex indicated by `retainBreeders` are guaranteed a new mate (if the opposite sex is available). Individuals of the non-retained sex(es) are returned to the *singles* pool.
    iii. If `matingSys = "polygyny"` or `"polygynandry"` and `matingLength = "lifelong"`, males will not receive a new mate if at least one of their previous mates is still alive (probably not realistic for most species).
    iv. Any remaining *singles* are paired (as the sex ratio allows).
l. # of years alive and # of breeding seasons is updated for all individuals.
m. The population is censused.

5. Steps 1-4 are repeated over the specified number of years. The censuses from all years are compiled into one matrix, and the list of individuals simulated in this replicate is saved.
6. Steps 1-5 are repeated over the specified number of replicates.

Box 2.2. User-specified parameters and options used in AlleleRetain.

Source population:

- Frequency of rare allele of interest
- Size of source population

Individuals to release into the new population:

- Number of individuals to initially release ("starters"); or bottleneck size
- Number of additional individuals to release in specified subsequent years ("additional releases"), if any
- Number of immigrants to release at regular intervals, if any
- Age class and sex ratio of released individuals
- Post-release survival rate (not including annual mortality)

Characteristics of the new population:

- Number of years (if any) after establishment when breeding does not occur
- Length of bottleneck, if population is held at the starting size before growing (reproduction does occur)
- Carrying capacity of the established population, and whether this applies to adults or to all individuals (subadults and nonbreeding helpers)
- Whether migrants have priority over locally produced individuals to recruit into breeding vacancies

Life history traits of the simulated species:

- Age at sexual maturity
- Mating system: seasonal or lifelong monogamy, polygyny, or polygynandry
    - For polygynous/polygynandrous systems: Measures of male reproductive success
    - For monogamous systems: whether either sex (or both) retain their breeding status once established as breeders (are not replaced by maturing recruits)

Expected demography of the new population:

- Survival rates:
    - Adult (can be sex-specific); can be reduced by senescence after a certain age
    - Nonbreeder; can be density dependent
    - Juvenile (first winter); can be density dependent
    - Maximum lifespan
- Fecundity:
    - Mean, standard deviation, and maximum possible number of offspring produced per female each year
    - Can be lower for young females until a specified age
    - Sex ratio of offspring

Simulation and output specifications:

- Number of years to simulate
- Number of replicates to run
- How frequently to print a message to track simulation progress
- Whether to track individuals (to later summarise inbreeding coefficients, probability of breeding for founders vs. migrants vs. locals, etc.)
- Whether to count alleles in the whole population, or only in breeding adults

Box 2.3. Output from summary functions included in AlleleRetain.

The main summary outputs information for each year, averaged across replicates:

- Mean and SE of the # adults and # breeding pairs
- Mean # of breeding males, breeding females, and subadults
- Mean # of starters and immigrants in the population
- Mean age of breeding adults
- Probability that the population is extant (with user-specified confidence limits)
- Probability that the rare allele is present (with user-specified confidence limits)
- Frequency of the rare allele (with user-specified confidence limits)

The individual summary function groups individuals by origin (starters, additional releases, locals, and immigrants) and gives the following information for each group, excluding individuals added in the final generation and those that died immediately post-release (as they had an unequal chance of breeding):

- Total number
- Proportion that bred
- Mean # of breeding seasons per individual (including those that never bred)
- Mean # of breeding seasons per individual (only those that bred)
- Mean lifespan
- Mean # that bred per generation

The pedigree summary function averages inbreeding accumulation across replicates:

- Mean F, averaged across individuals and then across replicates
- Among-replicate variance in F
- Among-individual variance in F, averaged across replicates

Table 2.1. Parameter values used to simulate allele retention in North Island robins.

Parameter	Value	Reference
Carrying capacity (*K*)	108	Chosen to give an average of 50 breeding females at carrying capacity
Age at maturity	1 year	Armstrong et al. (2000)
Mean annual juveniles/female	3.19	Parlato and Armstrong (2012)
SD annual juveniles/female	1.23	Parlato and Armstrong (2012)
Max annual juveniles/ female	6	Armstrong et al. (2000)
Retain established breeders	males only	Armstrong et al. (2000)
Mating system	lifelong monogamy	Armstrong et al. (2000)
Juvenile survival	0.60	Parlato and Armstrong (2012)
Juvenile survival at *K*	0.30	Parlato and Armstrong (2012)
Adult female annual survival	0.77	Parlato and Armstrong (2012)
Adult male annual survival	0.77	Parlato and Armstrong (2012)
Age at onset of senescence	5 years	Generation interval (mean age of breeding adults in the simulated population)
Maximum lifespan	16 years	New Zealand Department of Conservation (unpubl. data)

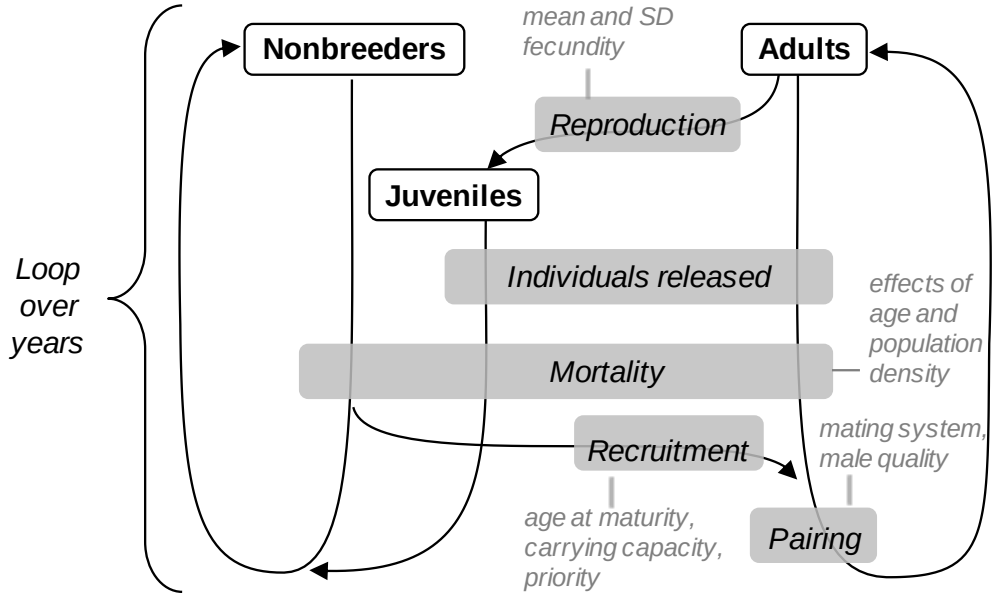

Figure 2.1. Flow of simulations implemented in AlleleRetain. Groups of individuals tracked by the model are shown in white boxes; life history or management events are in gray boxes; and user-specified life history traits that affect those events are in gray text. The population is established by releasing individuals after reproduction but before annual mortality; additional releases and immigrants are also introduced at that point in the cycle.

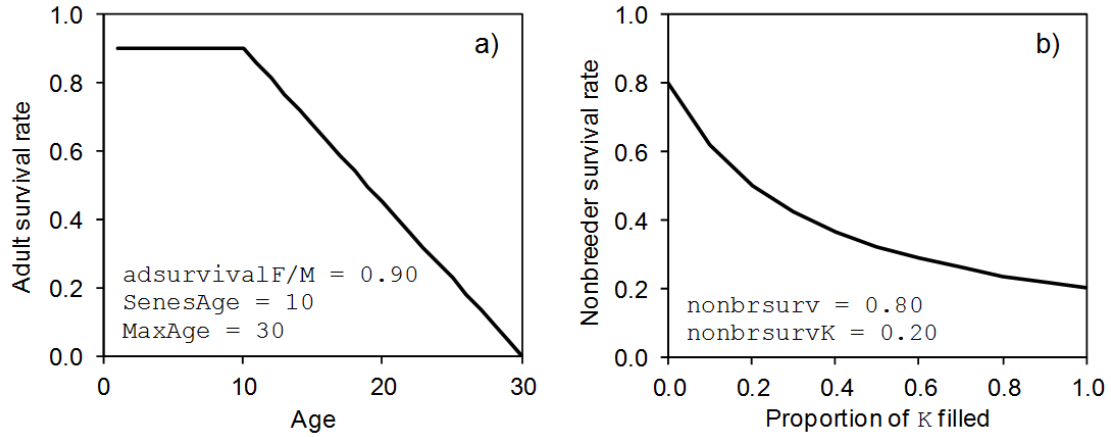

Figure 2.2. Adjustments to survival rates used in AlleleRetain. a) Adult survival declines linearly with age, from `SenesAge` to 0 at `MaxAge`. b) Nonbreeder survival declines with density (proportion of `K` filled), from near `nonbrsurv` at very low densities to `nonbrsurvK` at `K` (juvenile survival declines the same way, according to `juvsurv`/`juvsurvK`).

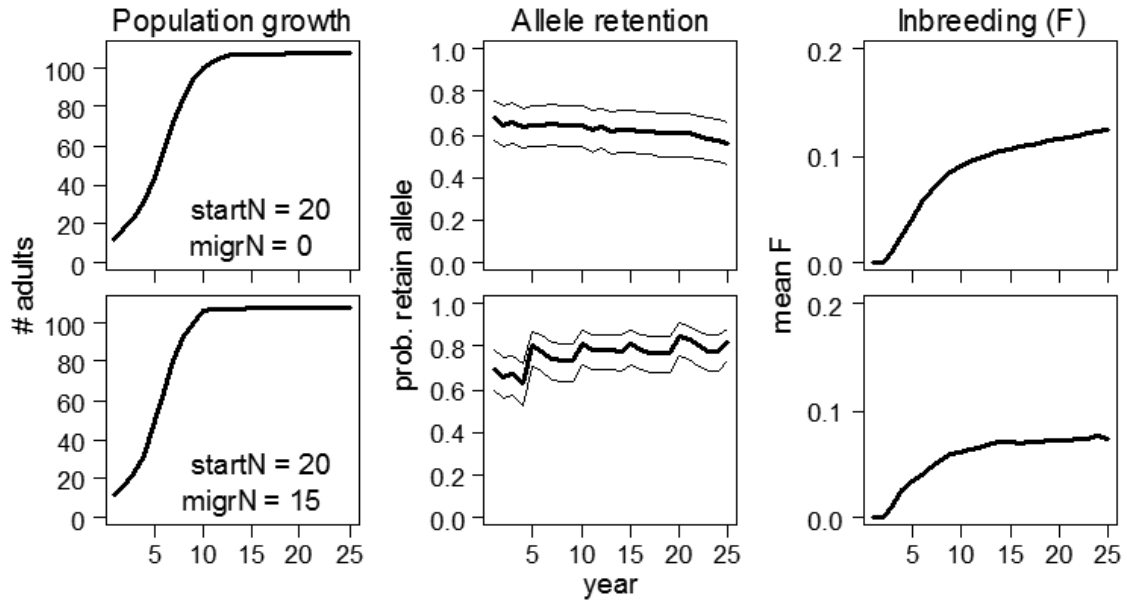

Figure 2.3. Plots of AlleleRetain output from the North Island robin example. Input parameters are provided in Table 2.1. startN = number of individuals released to establish the population; migrN = number of immigrants per generation (5 years).

Chapter 3. Effects of demography and life history traits on management options for retaining genetic diversity



Adult North Island brown kiwi in the captive breeding programme at Westshore Wildlife Reserve, Napier, New Zealand.

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Abstract

Allele loss is difficult to predict in animals with overlapping generations, so I used AlleleRetain (Chapter 2) to assess whether cross-species generalisations could be useful. I simulated retention of rare alleles in small populations of three species with contrasting life-history traits: North Island brown kiwi (monogamous, long-lived), North Island robins (monogamous, short-lived), and red deer (polygynous, moderate lifespan). I simulated closed populations under various demographic scenarios and assessed the amounts of artificial immigration needed to achieve a goal of retaining 90% of selectively neutral rare alleles (frequency in the source population = 0.05) after 10 generations. The number of immigrants per generation required to meet the genetic goal ranged from 11 to 30, and there were key similarities and differences among species. None of the species met the genetic goal without immigration, and red deer lost the most allelic diversity due to reproductive skew among polygynous males. However, red deer populations required only a moderate rate of immigration relative to the other species to meet the genetic goal because nonterritorial breeders had a high turnover. Conversely, North Island brown kiwi populations needed the most immigration because the long lifespan of locally produced territorial breeders prevented a large proportion of immigrants from recruiting. In all species, the amount of immigration needed generally decreased with an increase in carrying capacity, survival, or reproductive output and increased as individual variation in reproductive success increased, indicating the importance of accurately quantifying these parameters to predict the effects of management. Overall, retaining rare alleles in a small, isolated population requires substantial investment of management effort. Use of simulations to explore strategies optimised for the populations in question will help maximise the value of this effort.

Introduction

Allelic diversity is important to retain in managed populations because it defines the capacity for a population to adapt and survive in changing conditions (Allendorf & Luikart 2007; Markert et al. 2010). If expected allele loss can be predicted, management can be planned accordingly to minimise loss (e.g. Tracy et al. 2011). However, expected retention of allelic diversity is difficult to calculate beyond the first generation, especially for species with overlapping generations (Allendorf 1986). Conservation managers have

therefore often relied on generalised guidelines to plan management of a genetically robust population. For example, the one-migrant-per-generation rule, derived from work by Wright (1931) and others (e.g. Slatkin 1985; Spieth 1974), was developed to limit loss of heterozygosity in small populations. The premise was based on a series of simplifying assumptions that are typically not upheld by wild populations, and more recent work suggests that up to 10 effective immigrants/generation may be needed under varying circumstances (Mills & Allendorf 1996; Vucetich & Waite 2000; Wang 2004). Despite these recent findings, the one-migrant-per-generation rule has been widely applied in conservation plans (Mills & Allendorf 1996). This is a convenient rule-of-thumb, especially for data-deficient species; but applying it indiscriminately presents a risk of mismanaging a population of conservation concern.

I assessed whether generalised guidelines are useful in managing allelic diversity in small populations. Although the one-migrant-per-generation rule was developed to minimise loss of heterozygosity, allelic diversity is an arguably more important measure of genetic diversity for small populations of threatened species (Chapter 1). I therefore used AlleleRetain, the new model I presented in Chapter 2, to assess management options that would maximise retention of rare alleles in various species. Specifically, I assessed the amount of immigration (by translocation to an otherwise closed population) needed to retain rare alleles in three example species with contrasting life-history traits and under varying demographic scenarios. I compared the results across scenarios to examine overall patterns in the effects of key parameters on allele retention and the management effort consequently required to minimise allele loss. Finally, I used the results to assess whether it would be possible or useful to develop general rules-of-thumb for managing allelic diversity in small populations.

Methods

I used AlleleRetain to explore how the amount of immigration needed to reach a genetic goal would change with variation in demographic parameters. My goal was to retain 90% of rare alleles (frequency = 0.05 in the source population) to 10 generations. The model assumption is that these alleles are neutral throughout the simulated period, but such alleles are potentially important for future adaptation and may be feasible to conserve in small populations (Tracy et al. 2011). Alleles may occur at this low frequency even in

species that have been subjected to a prolonged bottleneck (e.g. the critically endangered takahe, Porphyrio hochstetteri; Grueber et al. 2008a).

I explored the effects of varying relevant demographic parameters on the amount of immigration necessary to achieve this goal in three species with contrasting life-history traits: North Island brown kiwi (*Apteryx mantelli*; hereafter "kiwi"), a long-lived, monogamous, flightless ratite; North Island robin (*Petroica longipes*; hereafter "robin"), a short-lived, monogamous, volant passerine; and red deer (*Cervus elaphus*), a polygynous ungulate with a moderate lifespan. Managers are currently reintroducing kiwi to a fenced reserve free of introduced mammalian predators (Rotokare Scenic Reserve, Taranaki, New Zealand). Project advisors requested this modelling exercise to estimate the optimal number of kiwi to release initially and as periodic immigrants to create and maintain a genetically robust population (J. Scrimgeour, personal communication). Juveniles produced in this population will be harvested to contribute to other populations, so maintaining genetic diversity and capacity for adaptation is a priority for this project. Kiwi are very long lived with fairly low fecundity (Robertson et al. 2010); they represent the slow end of the life-history continuum of birds that are the subjects of translocation projects. I chose the North Island robin as an example because this species has been part of several well-documented reintroductions in New Zealand and is at the faster end of the life-history continuum represented by birds of conservation concern (Armstrong & Ewen 2002). Kiwi and robins are monogamous, so I used red deer as the third example species. The demography of red deer, including reproductive skew among polygynous males, has been closely monitored on the Isle of Rum, Scotland (Clutton-Brock 1988; Coulson et al. 2004). From a practical perspective, these three species were chosen because they are well-studied, so life-history data used to parameterise the models could be readily obtained from the literature with a minimum of assumptions.

I used demographic parameter values appropriate for populations of these species that occur in isolated areas free from anthropogenic threats (Table 1). To explore the effects of demography on allele retention, I first simulated a mean-parameter scenario for each species; then simulated a set of alternative scenarios in which I varied relevant parameters one at a time for each species. I used the mean age of breeding adults in the simulated population, which is calculated by AlleleRetain, as the generation interval for each species. I set the number of starters (individuals released to establish the population) to the moderate value of 40 adults in the mean-parameter scenarios and to 20, 60, or 80 adults in alternative scenarios. I also simulated alternative scenarios in which starters

were released as juveniles rather than adults. I assumed all individuals survived the translocation and set carrying capacity for the mean scenario at 50 breeding females equal to the carrying capacity predicted for kiwi at Rotokare (J. Scrimgeour, personal communication), and varied this to 25 or 100 females in alternative scenarios. See Chapter 2 for details on the structure and function of AlleleRetain.

I input mean parameters for each species (Table 1) into AlleleRetain to determine the number of immigrants per generation needed to achieve the genetic goal. Immigrants had priority over locally produced individuals to recruit into breeding vacancies, as would be the case under strict management or if many locally produced individuals were harvested to contribute to other populations; although I also tested the impact of this assumption in alternative scenarios. I further examined the consequences of varying reproductive output, breeding system, and survival rates. While holding all other parameters at their mean values, I varied one parameter at a time across a range of possible values in alternative scenarios for each species (Table 1) to determine how these affected the amount of immigration needed and whether there were consistent patterns across the three species. Aside from artificial immigration, I assumed the managed populations were closed (no natural immigration or emigration). I ran 1000 replicates for each scenario over 10 generations for each species (410 years for kiwi, 50 for robins, and 80 for red deer). The source population was of infinite size and the average sex ratio was 0.5. I concluded that a scenario successfully achieved the genetic goal if the 95% CI for the probability of allele retention was > 0.90.

Results

Ninety percent retention of rare alleles required substantial management effort in the small, isolated populations I simulated. Without any immigration and with mean parameter values, the probability of retaining a rare allele declined over 10 generations for all three species, ultimately reaching 0.62 (95% CI 0.59-0.65) for kiwi, 0.55 (0.51-0.58) for robins, and 0.34 (0.30-0.37) for red deer (Figure 3.1). When I simulated the red deer population in a monogamous rather than polygynous system, the probability of allele retention with no immigration (mean = 0.63, 95% CI 0.60-0.66) was similar to that predicted for the mean scenarios for kiwi and robins (Figure 3.2). When kiwi and robin populations were simulated under polygyny with the same male reproductive skew used for red deer, the model predicted allele retention similar to that for the mean scenario for

red deer (kiwi: mean = 0.32, 95% CI 0.29-0.35; robins: mean = 0.31, 95% CI 0.29-0.34). The duration of the pair bond across years was not important, but higher variance in reproductive success further reduced the probability of allele retention in all three species under the polygynous scenario (Supporting Information).

The simulations indicated that 90% retention of rare alleles would be achieved with mean parameter values and 19 immigrants/generation (41 years) for kiwi, 12 immigrants/generation (five years) for robins, and 17 immigrants/generation (eight years) for red deer. Each immigration event caused an immediate increase in the probability of retaining the rare allele in the population (Figure 3.1). This increase was followed by a gradual decline until the next immigration event because the rare allele was increasingly likely to have been lost to genetic drift over time. I assumed that immigrants would recruit preferentially over locally produced birds; thus the simulations represented the best-case scenario for the effectiveness of each immigrant. When immigrants were not given priority to recruit, more immigrants per generation were needed to reach the genetic goal (65 for kiwi, 30 for robins, and 24 for red deer).

Even in simulations that gave priority to immigrants to recruit, not all immigrants were able to breed. The number of breeding vacancies was limited by the adult mortality rate, and the likelihood of an immigrant surviving until a breeding vacancy became available was limited by density-dependent mortality rates of juveniles and nonbreeders. When mean parameters were used, an average of 45%, 58%, and 68% of immigrants bred for at least one year in the simulated kiwi, robin, and red deer populations, respectively (Table 2). Even though many immigrants did not breed, giving priority to immigrants enabled them to be up to three times as likely to breed as locally produced individuals. When immigrants were not given priority, only 16%, 31%, and 54% bred in the respective simulated populations.

All the demographic variables I tested had some effect on the amount of immigration needed to maintain rare alleles in all three species (Figure 3.3). The number of starters released had a relatively minor effect on the amount of subsequent immigration required (Figure 3.3a). The age of the starters had a large effect, however. When individuals were released as juveniles, the amount of immigration needed at each bottleneck size increased by 62-191% over the number required when adults were released (data not shown). Carrying capacity was one of the most influential parameters I examined; smaller populations required much more immigration to retain rare alleles (Figure 3.3b). Reducing carrying capacity to 25 breeding females prevented the genetic

goal from being reached for kiwi regardless of the number of immigrants added. Reducing adult or nonbreeder and juvenile annual survival rates increased the number of immigrants required for all species, except juvenile survival had no effect in red deer (Figure 3.3c, d). Increasing mean reproductive output reduced the required amount of immigration to a certain point, beyond which increasing reproductive output increased the required amount of immigration in kiwi but had no additional effect in robins (Figure 3.3e). The simulated red deer population did not reach carrying capacity when productivity per female was reduced by 1 SD. Furthermore, increasing red deer reproductive output by 2 SD would have resulted in fecundity beyond the specified maximum of two young per female per year. Therefore I did not estimate the amount of immigration needed for those scenarios. Increasing variance in reproductive output sharply increased the number of immigrants needed for kiwi but had more moderate effects for robins and red deer (Figure 3.3f). The consequences of running the simulations without retaining established breeders across years (i.e. allowing younger individuals to replace older ones as breeders) depended on the species in question. The number of immigrants needed to retain 90% of rare alleles decreased for kiwi (14 per generation) but slightly increased for robins (to 13 per generation). I did not implement the option to retain breeders in the red deer models.

Discussion

Loss of alleles versus heterozygosity

Maintaining allelic diversity is important for preserving the evolutionary potential of populations of threatened species. The simulations showed that the amount of immigration needed to minimise genetic drift and retain 90% of neutral rare alleles in an otherwise isolated population is substantial and strongly dependent on a variety of demographic parameters. Without immigration, the probability of retaining a rare allele depended strongly on the mating system and associated variance in reproductive success. When immigration was facilitated, the amount needed to minimise allele loss depended on other demographic parameters, primarily those that affected the ability of immigrants to recruit to breed. Eleven to 30 immigrants/generation were required to conserve rare alleles in moderately small populations. These results indicate that one to 10 migrants per generation, which may be sufficient to minimise loss of heterozygosity (Mills & Allendorf

1996; Vucetich & Waite 2000; Wang 2004), would likely be insufficient to conserve rare, potentially important alleles in a small population.

The one-migrant-per-generation rule and subsequent modifications are thought to apply regardless of population size (Mills & Allendorf 1996; Vucetich & Waite 2000; Wang 2004; Wright 1931). In contrast, I found that the chance of allele loss was strongly dependent on carrying capacity. This was not surprising, as small populations are expected to lose alleles at a higher rate than larger populations due to genetic drift (Allendorf & Luikart 2007). Bottleneck size had a relatively small effect on allele retention in my simulations, indicating genetic drift was a more important driver of allele loss than the founder effect after 10 generations. Shorter-term simulations may find a stronger effect of bottleneck size (e.g. Tracy et al. 2011).

Importance of life history and demography

Long-lived monogamous kiwi had the highest probability of retaining rare alleles without immigration and highly polygynous red deer the lowest. In contrast, kiwi required the largest number of immigrants per generation to achieve the goal for allele retention, followed by red deer and then short-lived monogamous robins. This contrast indicates that the relative genetic contribution of each immigrant varied among species due to their differing life histories, as indicated by the respective proportions of immigrants that were able to breed. The ability of immigrants to breed successfully within a population determines their genetic contribution to the population (Mills & Allendorf 1996; Vucetich & Waite 2000; Wang 2004). Indeed, giving priority to immigrants over locals to recruit to breed dramatically reduced the number of immigrants needed to achieve the genetic goal in the simulations for all three species. Even with density dependence in juvenile and nonbreeder survival, many more individuals were available to recruit than there were breeding vacancies in each year of the simulations. Therefore, when immigrants were not given priority, many more were needed to ensure that some would be randomly selected to recruit.

Even with priority to recruit, only a fraction of immigrants ultimately bred because a substantial proportion died before sufficient breeding vacancies became available for the entire immigrant cohort to recruit, most notably in territorial species. Nonbreeders were subject to higher mortality than established breeders and were thus lost at a faster rate. Age of individuals released had a strong effect on the amount of immigration needed in the simulations, particularly for kiwi, which have the longest subadult period. This was

because juvenile starters and immigrants were subject to high mortality their first year and moderate mortality each year until they achieved breeding status, especially when density-dependent effects occurred. Consequently, many more immigrants were needed when released as juveniles rather than as adults to achieve the same genetic contribution to the population. When juvenile and nonbreeder survival rates were overestimated, which I simulated by reducing these values in the models, the proportion of individuals that contributed alleles to the population was further reduced, especially in kiwi. This effect was apparent even when immigrants were released as adults and the reduction in survival affected them only as nonbreeders. These results highlight the importance of continuing to monitor demographic parameters after the population has established (Sutherland et al. 2010) and updating models and management plans as needed.

Once individuals have achieved breeding status, the mating system will affect the proportion of individuals that contribute their alleles to the next generation. Polygyny is often accompanied by high individual variance in male reproductive success, which reduces the effective population size and thus increases allele loss due to genetic drift (Lacy 1989; Miller et al. 2009; Pérez-Figueroa et al. 2012). Results of my simulations upheld this expectation: polygyny and the associated reproductive skew greatly reduced the probability of allele retention in the absence of immigration. Similarly, inter-pair variation in reproductive output in monogamous systems strongly affected allele retention and the immigration rate needed to compensate. Lower reproductive rates overall also reduced the probability of allele retention because populations that grow slowly retain less allelic diversity than those that grow more quickly (Allendorf & Luikart 2007).

Turnover of breeding adults across years (i.e., whether established breeders were retained to breed the next year rather than competing annually with former nonbreeders to make up the new breeding population) strongly affected the number of immigrants needed. Turnover rate especially affected kiwi, the longest-lived of the three species. With no immigration, the kiwi population showed a much lower probability of allele retention with high turnover of breeders (0.42) than with low turnover (0.62). This indicates that retaining breeders is beneficial in the absence of immigration, probably because individuals born earlier in the simulation had higher allelic diversity than those born later, after alleles had been lost due to drift. Allowing those early individuals to breed for their whole lifespan, thus leaving a large number of descendants, was therefore beneficial for allele retention. Nevertheless, immigrants would be even more likely than early, locally produced individuals to possess the rare allele of interest. Preventing immigrants from

breeding in favour of retaining established local breeders (e.g., via territorial behaviour) thus reduced the proportion of immigrants that survived until breeding vacancies became available and required more immigration to compensate. One might therefore expect that reduced adult survival rates would improve allele retention by increasing turnover. However, my simulations showed that because reducing adult survival shortened breeding lifespans and limited reproductive output of all individuals (including immigrants), any benefit of higher turnover was negated and even more immigration was needed to retain rare alleles.

I assumed that turnover and adult survival rates remained constant over the simulated period. However, when populations are to be used as a source for additional new populations, managers may be able to influence these parameters in the intermediate population by selectively removing individuals to maximise allele retention, especially for territorial species. In the early years of population establishment, locally produced juveniles or nonbreeders may be the most suitable candidates for translocation out of the population. The absence of these individuals will have little effect on the productivity of the population because established breeders will remain. In later years and when immigrants are added, it may be preferable to harvest locally produced breeders in addition to nonbreeders to allow immigrants to recruit and improve allelic diversity. Simulations such as ours can identify the best strategy to maximise allele retention at a particular time for a particular population.

Management implications

These simulations indicated that periodic immigration is essential to retaining allelic diversity in small, closed populations and that the amount of immigration needed depends strongly on carrying capacity, survival, and reproductive rates. These findings have important implications for management projects that establish new populations by translocating individuals because many of these projects are intended to be one-time translocation events and aim to establish a population with the goal of long-term viability. Many such populations have little or no chance of natural immigration (e.g. on offshore islands or in isolated patches of habitat; Saunders & Norton 2001). It is important that the carrying capacity of the site and the life-history traits of the species are taken into account when designing translocation projects or managing small populations to minimise allele loss. The availability of potential immigrants and financial or logistic constraints may limit the number of individuals that can be translocated on a regular basis; thus, the

measures that can be taken to improve allele retention, especially for very small populations, are also limited. Increasing carrying capacity and equalising reproductive success would be the most effective means of increasing allele retention. Some of the other key parameters might be manipulated by managers (e.g. survival or productivity could be improved by supplementary feeding; Castro et al. 2003). Alternatively, managers could assess the value of a small population with lower allelic diversity. For populations that could readily be supplemented or replaced if they became threatened by changing conditions or where multiple such populations could be founded, a lower level of allele retention in a population may present an acceptable risk. Targeting a lower threshold of the proportion of rare alleles to retain would also reduce the number of immigrants needed: 80% of rare alleles (source frequency = 0.05) would be retained with seven, six, and nine immigrants per generation in the mean scenarios for kiwi, robin, and red deer, respectively. Alternatively, more common alleles are much easier to retain in small populations than the rare alleles I assessed. For example, 90% of moderately rare alleles that occur at a frequency of 0.10 in the source population would be retained with only three, three, and six immigrants per generation for the mean scenarios for kiwi, robin, and red deer, respectively.

These findings indicate that appropriate management options for a particular population will depend on management goals, life-history traits, and demographic parameters of the species of interest. General patterns are apparent, but are not sufficient to inform specific management action. Conservation plans for small populations must be addressed on a case-by-case basis whenever possible, rather than attempting to apply a broad rule of thumb to determine appropriate management.

Table 3.1. Parameter values used to simulate allele retention in small, bottlenecked populations of three species.

	North Island brown kiwi		North Island robin		Red deer	
Parameter	Mean[a]	Alternative[b]	Mean	Alternative[b]	Mean	Alternative[b]
Carrying capacity (*K*) [c]	108 (1)	57, 212	108	57, 212	100	50, 200
Age at maturity	4 (2)		1 (3)		3 (4)	
Age at which males breed	n/a		n/a		5-13 (5)	
Age when adult fecundity achieved	4		1		4 (4)	
Mean annual juveniles/adult female	0.70 (1,2)	0.45, 0.95, 1.2	3.19 (6)	1.96, 4.42, 5.65	1.25[d]	2.0
Mean annual juveniles/young female	n/a		n/a		0.4 · adult rate (4)	
SD annual juveniles/female	0.25 (1)	0, 0.5, 0.75, 1.0	1.23 (6)	0, 2.46, 3.69, 4.92	0.75[d]	0, 1.5, 2.25, 3.0
Maximum annual juveniles/ female	2 (2)		6 (3)		2[d]	
Retain established breeders[e]	both sexes (7)	none	males only (3)	none	none	
Mating system	lifelong monogamy (7)	seasonal monogamy; polygyny	lifelong monogamy (3)	seasonal monogamy; polygyny	annual polygyny (5)	lifelong monogamy; seasonal monogamy
Mean/SD male LRS[f]	n/a	1.04/2.35 (5)	n/a	1.04/2.35 (5)	1.04/2.35 (5)	
Juvenile survival	0.90 (2)	0.855, 0.81	0.60 (6)	0.57, 0.54	0.88[g]	0.84, 0.79
Juvenile survival at *K*	0.45[h]	0.43, 0.41	0.30 (6)	0.285, 0.27	0.44[i]	0.38, 0.36
Nonbreeder annual survival[j]	0.95 (2)	0.90, 0.86	0.60	0.665, 0.63	0.93[g]	0.88, 0.83
Nonbreeder annual survival at *K*	0.75[h]	0.71, 0.675	0.30	0.57, 0.54	0.93[g]	0.88, 0.83
Adult female annual survival[k]	0.98 (2)	0.93, 0.88	0.77 (6)	0.73, 0.69	0.96 (4)	0.91, 0.86
Adult male annual survival[k]	0.98 (2)	0.93, 0.88	0.77 (6)	0.73, 0.69	0.93 (9)	0.88, 0.83
Maximum lifespan	100 (10)		16 (10)		19 (4)	
Generation interval[l]	41		5		8	

[a] Numbers in parentheses are source codes: 1, J. Scrimgeour, personal communication (estimates for kiwi at Rotokare Scenic Reserve, Taranaki, New Zealand); 2, Robertson et al. (2010), corrected for predation; 3, Armstrong et al. (2000); 4, Benton et al. (1995); 5, Clutton-Brock (1988); 6, Parlato and Armstrong (2012), drawn from a normal distribution with mean = 1.29 (SD 0.21) and then back-transformed from the natural log scale; 7, McLennan (1988); 8, Rose et al. (1998); 9, Coulson et al. (2004); 10, New Zealand Department of Conservation (unpubl. data).

Table 3.1, continued

[b] All other parameters were held at mean values for each species when assessing the effects of varying each parameter; except juvenile and nonbreeder survival rates varied together.

[c] Test simulations were used to determine the number of individuals that would result in the desired average number of breeding females for each species: 50 females for mean scenarios and 25 or 100 females for alternative scenarios.

[d] Published estimates of fecundity for red deer on Isle of Rum are highly variable depending on rainfall. Annual variability is not incorporated in the model, and use of mean reproductive values did not allow the simulated population to reach carrying capacity (*K*) within 10 generations. I therefore increased reproductive values so the simulated population would reach *K* in 3 generations, and it would be more comparable to the North Island brown kiwi and North Island robin populations. This required increasing the maximum of 1 juvenile · female^{-1} · year^{-1} to 2, as shown (red deer on Isle of Rum do not twin [Clutton-Brock et al. 1988]).

[e] With this option, individuals that recruit to breed once are given priority to breed in following years until their death (i.e., nonbreeders or immigrant cannot replace them in the breeding pool [Chapter 2]).

[f] LRS: lifetime reproductive success. The relation between age and annual reproductive success in red deer was described as $success_{age} = -5.435 + 1.536 \cdot age - 0.084 \cdot age^2$, estimated from Clutton-Brock et al. (1988).

[g] Juvenile and nonbreeder (subadult) survival rates were calculated by averaging survival rates for male (Rose et al. 1998) and female (Benton et al. 1995) juveniles and subadults.

[h] No data on density dependence in survival or recruitment were available for kiwi, so I assumed that as for North Island robins, North Island brown kiwi juvenile survival would halve at carrying capacity.

[i] Half of nonbreeder survival at low densities, as for North Island brown kiwi and North Island robins.

[j] Nonbreeders include all subadults and adults that are not currently breeding.

[k] Reducing adult survival rates also reduced generation time from the value for the mean scenario, but I ran the models with the same immigration frequency regardless of changes in adult survival rates. Adult survival rates are reduced as an individual ages; survival declines from the rate given here at the age of senescence (generation length) to zero at the maximum age (defined by maximum lifespan).

[l] Generation interval, mean age of breeding adults in the simulated population. This value was also used for the age at onset of senescence in survival rate (see Chapter 2 for more details).

Table 3.2. Comparison of recruitment rates for individuals of each origin.

Species[a]	Origin[b]	Total per replicate[c]	% that bred (95% CI) [c,d]	# that bred per generation[d,e]	Mean # years bred[d,f]
Kiwi	Starters	40	93 (79-98)	-	36
	Locals	11634	8 (7-8)	87.9	40
	Immigrants	173	45 (38-53)	7.8	35
Robin	Starters	40	67 (50-80)	-	3.9
	Locals	6108	16 (15-17)	98.3	4.2
	Immigrants	110	58 (48-67)	6.4	4.0
Red deer	Starters	40	67 (51-81)	-	5.3
	Locals	2765	24 (22-25)	65.7	5.2
	Immigrants	159	68 (60-75)	10.7	5.6

[a] Populations were modelled with mean parameter values given in Table 1 and enough immigration to reach the goal of 90% retention of rare alleles (19 immigrants/generation for kiwi, 12 for robins, and 17 for red deer).

[b] Starters are individuals released to establish the population; locals are born locally; immigrants are added at regular intervals by translocation.

[c] Averaged across 1000 replicates for each simulation.

[d] Does not include individuals added or born during the last generation because they would have had an unequal chance to breed before the end of the simulation.

[e] Not calculated for starters because they were present only at the beginning of the simulated period.

[f] Averaged across replicates and then across individuals that recruited to breed within each group.

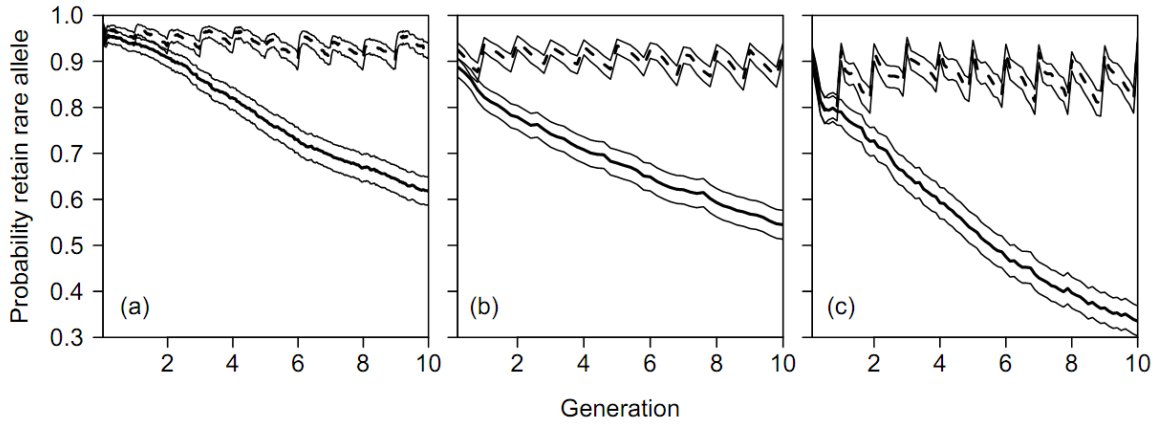

Figure 3.1. Probability of retaining a rare allele (frequency = 0.05 in the source population) in small, bottlenecked populations. (a) North Island brown kiwi, (b) North Island robins, and (c) red deer were simulated with mean demographic values (Table 3.1) (solid bold line, allele retention for a population with no immigration; dashed bold line, a population with sufficient immigration to achieve a 90% probability of rare allele retention after 10 generations [obtained by averaging over 1000 replicates]; fine lines, 95% CI around mean probability in each year). One generation equals 41, five, and eight years for kiwi, robins, and red deer, respectively.

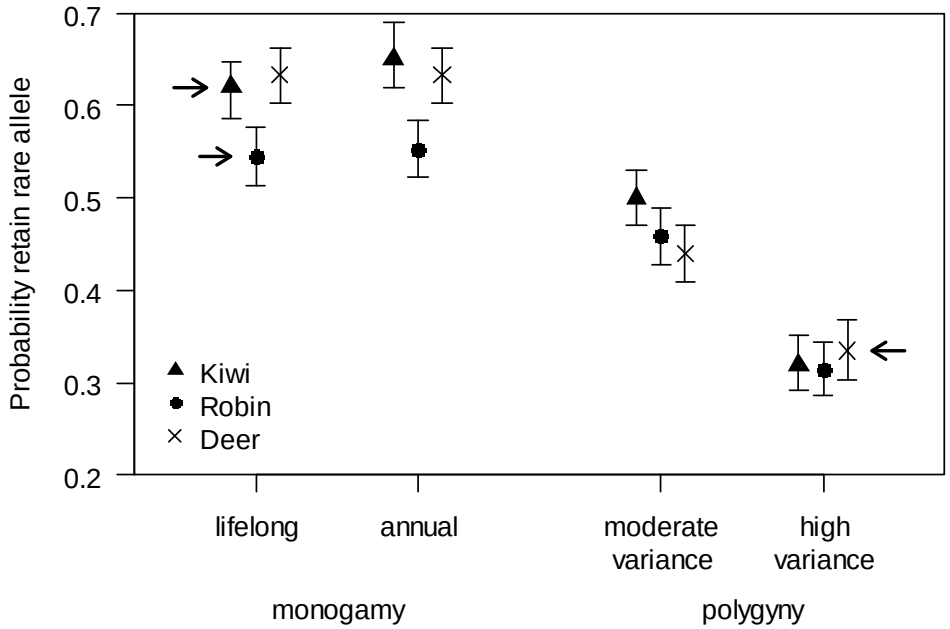

Figure 3.2. Probability of retaining rare alleles under various mating systems. Each scenario was simulated with no immigration; other demographic parameters were set at mean values given in Table 1, with variance in reproductive success (moderate: among-male SD = 1.175; high: SD = 2.35) for polygynous systems and lifelong or annual monogamy as indicated. Each point represents the mean probability averaged over 1000 replicates, with the 95% CI indicated by error bars. The expected mating system for each species is indicated by an arrow.

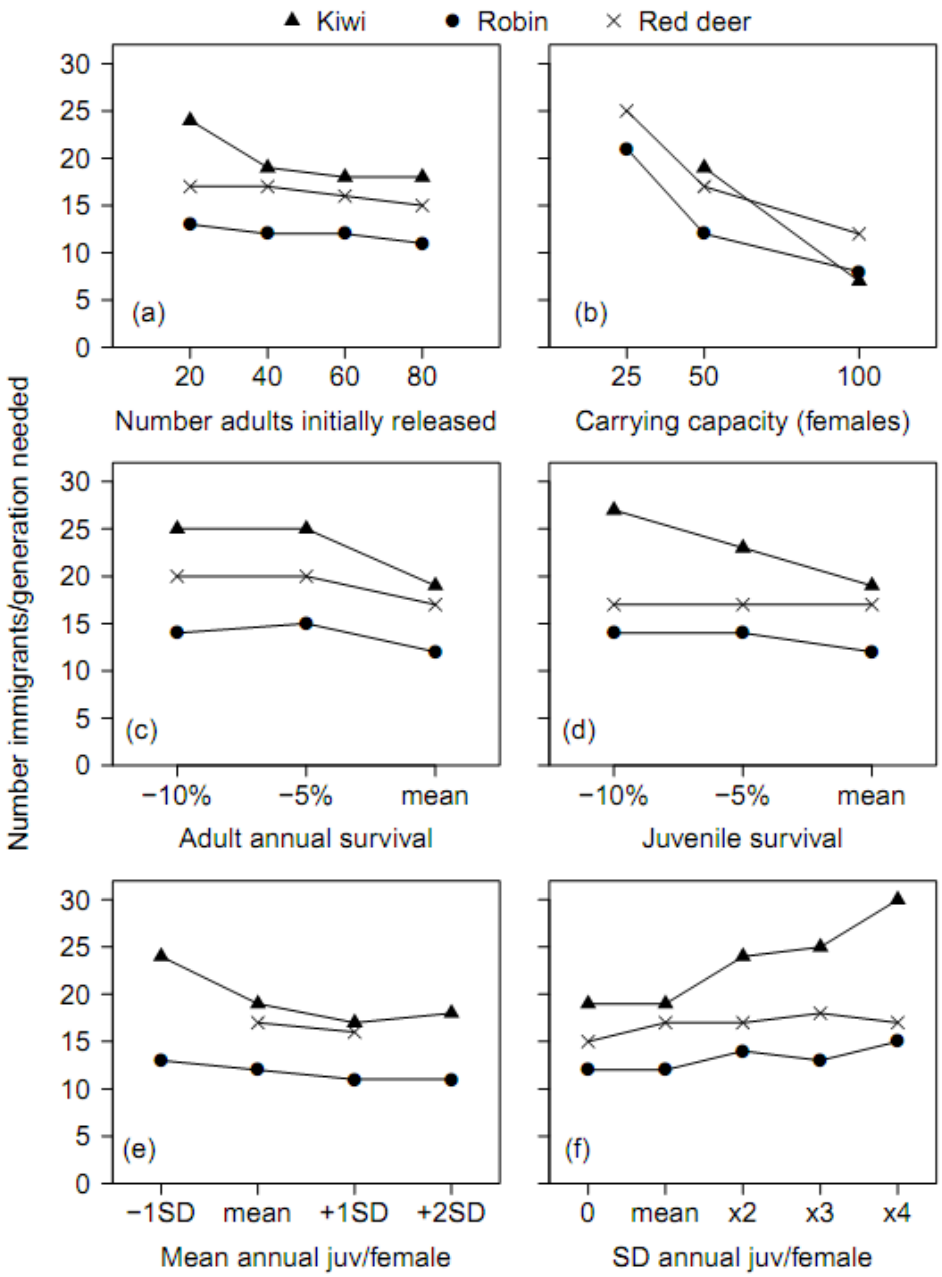

Figure 3.3. Number of immigrants per generation needed to retain 90% of rare alleles to 10 generations in small, bottlenecked populations. One parameter was varied in each scenario as indicated on the y-axis. Other parameters were held at mean values given in Table 3.1. Generation interval was 41 years for North Island brown kiwi, five years for North Island robins, and eight years for red deer. Rare alleles occurred at frequency = 0.05 in the source population.

Chapter 4. Assessing strategies to manage genetic viability of multiple fragmented populations



Adult North Island kokako at Pureora Forest, New Zealand.

This chapter has been modified from an unpublished report commissioned by the Kokako Recovery Group to advise management strategies and prioritisation for extant and potential kokako populations.

Abstract

As demonstrated in Chapter 3, management actions needed to retain allelic diversity vary among species and populations. Evaluation of management strategies, e.g. with predictive models such as AlleleRetain (Chapter 2), should therefore be conducted for each population of interest. Genetic management of species that occur in metapopulations may be more complicated than implied by the simple reintroduction examples presented in Chapter 3. In this chapter, I demonstrate the application of AlleleRetain to a situation with multiple existing populations of various sizes and bottleneck histories, using a case study of North Island kokako (*Callaeas wilsoni*). I evaluated a suite of management options (initial top-ups, periodic supplementation, and increasing carrying capacity) for improving genetic viability of each extant kokako population. The simulations indicated that supplementation can bolster the allelic diversity of all but the smallest populations, greatly improving the conservation value of each managed site. Even populations that had previously undergone small bottlenecks or those that had already lost a great deal of diversity to genetic drift over time could be successfully supplemented to restore at least 80% (and often 90%) of rare alleles. Larger populations could be supplemented immediately and then self-sustain allelic diversity, but smaller populations required ongoing supplementation to counteract genetic drift. The models also elucidated the number of birds that could safely be harvested from potential source populations without jeopardising the genetic viability of the source; these birds could be used to supplement other extant populations or to conduct further reintroductions. This case study demonstrates a wide variety of management options and genetic principles that are relevant to wild metapopulations with a previous history of decline and management.

Introduction

Reintroduction of a species to areas from which it had been extirpated has become an integral tool for conservation (Seddon et al. 2012). Because many reintroduced populations are small and have often been established with few founders (Griffith et al. 1989; Taylor et al. 2005), maintenance of genetic diversity becomes an important management consideration (Allendorf & Luikart 2007; Markert et al. 2010; Chapter 1). Simulation models such as AlleleRetain (Chapter 2) are intended to inform management

strategies to maximise retention of genetic diversity in small populations. Given that broad rules of thumb cannot be applied across species or even across different populations of the same species (Chapter 3), such models will need to be applied specifically to each case to produce useful recommendations. The case studies I examined in Chapter 3 (kiwi, robins, and red deer) provided several examples of basic reintroduction planning. However, the populations in Chapter 3 were hypothetical examples of single reintroductions, and were modelled under the assumption that existing source populations of each species were genetically robust and able to sustain harvest for translocations. More complex scenarios will become increasingly difficult to model.

In contrast to the pre-reintroduction planning scenarios addressed in Chapter 3, often the genetic health of a previously reintroduced population is assessed only after the population is established (e.g. Biebach & Keller 2012; Jamieson 2011; Miller et al. 2009; Vonholdt et al. 2008). Some existing populations would benefit from further management to improve their genetic viability. In these cases, models to assess management options should incorporate information about the history of each population, e.g. number and sex ratio of founders and population growth rate. Furthermore, many species exist in multiple, fragmented populations rather than large or interconnected, genetically robust populations. Management of such species should consider which population(s) would be the best source for birds to supplement or establish other populations, in terms of both existing genetic diversity and the ability of potential source populations to safely sustain harvest.

Other management options besides translocation may also be feasible, and predicting the effects of those options would be useful. For example, if translocating many individuals is recommended, populations could instead be connected by expanding the area of protected habitat to enable natural connectivity. Alternatively, as implied by the results of Chapter 3, retention of genetic diversity could be improved without translocation by changing the demography of a population (i.e. increasing growth rate). This could be accomplished by improving predator control (e.g. Innes et al. 1999; Robertson et al. 2010) or providing supplementary food (e.g. Chauvenet et al. 2012).

In this chapter, I use an example of the North Island kokako (hereafter "kokako") to demonstrate the evaluation of management options under realistic and complex circumstances. The kokako is listed as endangered by the IUCN (2013), but the New Zealand threat classification was downgraded from nationally endangered to nationally vulnerable following increases in the total kokako population (Innes et al. 2013; Miskelly et al. 2008). However, there is ongoing concern regarding the long-term viability of

kokako. Like many threatened species, this large, forest-dwelling songbird exists only in fragmented, protected reserves, e.g. mainland sanctuaries with effective predator control and offshore islands that are free of introduced predators (Figure 4.1; Innes et al. 2013). Each population has either been established by translocation (eight populations, typically with established with < 20 founders), or recovered from a relict population that had declined over >100 years (eleven populations). Relict populations had typically dwindled to a few dozen individuals and often experienced biased sex ratios and a skewed age distribution, because nesting females were the most susceptible to predation (mainly by rats and mustelids; Innes et al. 2013). Following these bottlenecks, most populations have been limited to tracts of habitat that can support ≤ 150 breeding pairs (Innes & Flux 1999; Innes et al. 2013). Productivity of many populations has been limited by continuing low levels of predation, even where predator control is implemented, so population growth rates are often slow. Both translocated and relict populations have therefore likely experienced substantial loss of genetic diversity. Genetic management of this species therefore must be assessed from a metapopulation perspective.

The Kokako Recovery Group (KRG), an advisory group composed of kokako managers and scientists, commissioned the modelling work that I present here to identify strategies for maximising genetic viability in existing populations. Molecular data from extant populations are scant (Murphy et al. 2006), so computer simulations are the best approach to assess the genetic viability of kokako populations. As with my previous work, I focused on retaining allelic diversity using AlleleRetain, the model I developed in Chapter 2, to assess management for long-term persistence of this threatened species. This work focuses on genetic viability, and recommendations provided here do not reflect financial, logistical, or other considerations that may influence management decisions.

The following questions were identified by the Kokako Recovery Group (KRG) to be addressed in this study:

- What actions (e.g. adding individuals or increasing carrying capacity [*K*]) could be implemented to secure the genetic viability of extant populations?
- Which populations are the best sources from which to take birds to supplement others? Could they sustain the necessary amount of harvest?
- How can new populations (with varying expected growth rates) be established to retain allelic diversity without further supplementation?

The kokako example thus demonstrates a wide variety of issues of interest to genetic management of wild populations. These issues are particularly relevant to species that currently exist in multiple small, fragmented populations.

Methods

I used AlleleRetain (Chapter 2) to assess retention of rare alleles in existing and (hypothetical) new kokako populations over 100 years (about 10 kokako generations), starting in 2013 (the year of the analysis). Of the 22 extant kokako populations (Innes et al. 2013), I examined all but three: one (Tiritiri Matangi Island) that is extremely small and already managed to reduce inbreeding, one (Puketi) that is in the process of establishment and can be treated as a new population, and one (Mokoia) that has been established as a male-only population for public advocacy. I also did not assess a recently introduced population that is presumed failed (Secretary Island). See Innes and Flux (1999) and Innes et al. (2013) for more information on the excluded populations.

AlleleRetain relies on demographic information about the population to be simulated, including reproductive and survival rates (Chapter 2). Because various kokako populations have shown different rates of growth (resulting partly, but perhaps not entirely, from varying effectiveness of predator control), I assigned each population to a growth rate category: low, moderate, and high. Exact growth rates of each population have varied from year to year, so assigning populations to broad categories was more useful than attempting to assess the specific rate of recent growth for each population. I defined the three growth-rate categories by assessing recent population growth, as measured by censuses of breeding pairs from 2000-2012 (KRG unpubl. data), and dividing populations into three annual growth rate categories: low ($r < 0.1$), moderate ($r = 0.1$ - 0.2), and high ($r > 0.2$). Assessments of recent growth were adjusted based on information about previous management activities (harvest from and supplementation into each population); i.e. the growth rate assigned to each population was corrected for any birds that were removed or added (and their potential descendents) each year.

Three populations (Ngapukeariki, Boundary Stream, and Pukaha) were originally evaluated to show high productivity, but were assigned to the low-growth category following discussion with the KRG because of uncertainty in what growth would have been without the large amounts of supplementation these populations received. Growth of populations established recently could not be accurately assessed, so these populations

were assigned to the moderate rate. Once a population had been assigned to a growth rate category, I assumed that it would remain in that category over the course of the simulations (with density dependence affecting juvenile and subadult survival as shown in Table 4.1). The results of these simulations are therefore based on the assumption that predator control regimes will continue as they have (on average) over the past 13 years for each population.

With assistance from the KRG (especially John Innes and Ian Flux), I then obtained demographic rates from published and unpublished studies of kokako (Basse et al. 2003; Flux et al. 2006; Higgins et al. 2006; Innes et al. 1996; Innes et al. 1999; KRG unpubl.) to use in the modelling. Values were not available for all populations, so I used rates estimated from a few well-studied populations under varying levels of predator control (ranging from no control to predator-free; Table 4.1). Based on available information about the growth rate of each population at the time that the demographic rates were measured, I used demographic rates appropriate to each population growth rate category. I assumed that adult female, subadult, and juvenile survival (but not adult male survival, which is relatively insensitive to predation; KRG unpubl.) would vary across categories (as per sources cited in Table 4.1), and then adjusted reproductive rates to achieve the targeted growth rate for each category.

The KRG provided information on how each population had been established (for reintroduced populations), or the smallest known modern size (for relict populations; the first available population estimate, typically made in the last few decades), as well the managed area of each population (Table 4.2). Average territory size on Hauturu (the only population currently at *K*) has recently been estimated at 6.6 ha (KRG unpubl.). There is some concern that territories in less productive habitat may be larger (I. Flux, pers. comm.) and Hauturu densities may overestimate *K* at other sites. I therefore estimated *K* of all other populations from the currently managed area by conservatively assuming an average of 8 ha per breeding pair.

After parameterising the models with the above information, I simulated each population, starting from the known minimum population size for relict populations or from establishment for reintroduced populations. Information about each population's history to date is therefore incorporated into these simulations. I continued each simulation through to the year 2113 (100 years from the time of analysis; about 10 kokako generations, depending on the productivity of the site). After simulating each population in isolation to assess allele retention with no further supplementation, I then ran additional

simulations to determine the effects of connectivity among populations. For each population, I aimed to assess the number of additional birds required to either 1) initially secure the genetic health of a population by "topping up" the population over the next few years, after which the population would be genetically self-sustaining, or 2) periodically supplement a population that would not be genetically self-sustaining. I assumed that all birds were translocated as adults, as has been typical for kokako (KRG pers. comm.). Unlike the hypothetical cases in Chapter 3, for the kokako models I assumed that no priority would be given to immigrants over local birds to recruit into breeding vacancies. This is a more realistic scenario for most wild populations that are not intensively managed, and will result in only a proportion of immigrants recruiting to breed. I defined periodic supplementation in terms of the number of birds released once every 15 years.

I assessed genetic viability by evaluating the probability of retaining a rare allele (initial frequency 0.05). I aimed to achieve one of two goals for each population: 1) retaining 90% of rare alleles (where feasible), or 2) retaining 80% of rare alleles. In each case, I considered the goal to be achieved if the simulation predicted a 95% confidence interval that was fully above the goal; e.g. a confidence interval of 0.80-0.84 would meet the 80% retention goal. Although it is typically recommended that management actions aim to retain 90% of rare alleles over 10 generations (Tracy et al. 2011; Chapter 3), this goal can be very difficult to achieve in small populations (Chapter 3). In some cases, it may be necessary to set a lower goal, e.g. aiming to retain 80% of rare alleles. Although this compromise may reduce long-term genetic viability of a population, such a risk to each individual population may be acceptable for species like kokako that exist in a relatively large number of populations and are long-lived.

Finally, to examine patterns across populations in the context of results from Chapter 3, I used generalised linear models (Poisson distribution) to determine how predictors (bottleneck size, bottleneck year, *K*, and growth rate category) influenced the number of birds that needed to be released. For this analysis, I considered scenarios in which periodic supplementation achieved 80% retention of rare alleles, as this goal could be met by most kokako populations (see Results). After building the global model with all relevant covariates, I generated all possible submodels using the dredge function in R package MuMIn (Barton 2013). Inference was based on model averaging the top 2 ΔAIC_C (Burnham & Anderson 1998; Grueber et al. 2011) using the function model.avg in R package MuMIn. I used the averaged model and parametric bootstrapping to predict the amount of supplementation needed with each bottleneck size and each carrying capacity

(the most important predictors; see Results). For each predictor, I generated 1000 bootstrapped values of the effect size using the modelled estimate and its standard error as the mean and standard deviation of a normal distribution. I then held all other covariates in the final model at their mean values, and predicted the response variable at each level of the predictor of interest, using each bootstrapped effect size in turn. From this list of predictions, I took the mean and 95% quantiles as the fitted value and 95% confidence interval, respectively.

Results

Only one population (Pureora Forest) was expected to retain > 90% of its rare alleles over 100 years without supplementation (Table 4.3). Te Urewera was predicted self-sustain > 80%, but all other populations would require initial or ongoing supplementation to achieve either the 80% or 90% goal. Depending on population size and history, some populations could be topped up in coming years with enough individuals to meet either genetic goal (without further supplementation). Other populations, especially those small enough to rapidly lose rare alleles over time, would require periodic supplementation to achieve either genetic goal. No amount of supplementation could achieve the goal at Waikokopu, the smallest population included in this analysis (K = 94) unless K were increased.

Bottleneck size and K were the two most important predictors of the amount of supplementation needed (Table 4.4). Populations that had undergone a more restrictive bottleneck or those capped at a smaller population size required more supplementation to achieve 80% retention of rare alleles (Figure 4.2). The amount of supplementation required was further affected by factors such as growth rate and time to reach K that affected the proportion of immigrants expected to be able to breed. The expected proportion of released birds that would breed was generally between 0.20 and 0.50 (Table 4.3). Close monitoring of released birds could better elucidate the actual proportion that recruits to breed in each population and advise further translocations.

Although removing birds could reduce the allelic diversity of existing populations, I found that Pureora Forest (the only population expected to retain > 90% of rare alleles over 100 years without supplementation, and thus the best source population) could sustain a large amount of harvest every year without jeopardising its allelic diversity (Table 4.5). Other populations could also sustain some harvest of locally produced birds,

either after initial topping up or during ongoing periodic supplementation. These harvest levels would provide sufficient birds to both supplement existing populations and establish new populations. Although new populations would need to be relatively large to be genetically self-sustaining, feasible options for establishment were identified by the models, especially if productivity at new sites was high (Table 4.6).

Discussion

Despite the history of fragmentation and population bottlenecks, the models identified options that could achieve retention of at least 80% of rare alleles in all but one of the 19 kokako populations I examined. As predicted by Chapter 3, smaller populations were more difficult to supplement, and the smallest (K = 94) could not achieve even 80% retention of rare alleles in any scenario tested. Large populations at or near K, e.g. Hauturu, were also particularly difficult to supplement, as there were few breeding vacancies for immigrants to occupy. In contrast to findings in Chapter 3, however, bottleneck size was more important than K in predicting the amount of supplementation needed. This is likely because carrying capacities tested in this analysis (163-975 adults) were often larger than those tested in Chapter 3 (50-200 adults), and bottleneck size would become relatively more important for larger populations in which genetic drift would be somewhat less influential (Allendorf & Luikart 2007).

Kokako are very difficult and expensive to catch (Innes et al. 2013), so the amount of supplementation indicated for some scenarios may not be logistically or financially feasible, especially in the context of managing the species as a whole. However, some kokako populations are of higher conservation priority than others (Innes & Flux 1999); genetic management could focus on the populations that are most important for securing the species. Populations that remain unmanaged could lose genetic viability and conservation value, but potentially persist in the short term and provide other benefits such as public advocacy. Such prioritisation is in progress by the KRG, which will advise managers on the best national strategy for securing the kokako metapopulation.

Aside from financial or logistical constraints, there could be genetic reasons to forgo high rates of translocations among populations. In theory, several isolated populations may contain more total allelic diversity than one large, homogenous population (Allendorf & Luikart 2007). However, in practice, losing some diversity through homogenisation is generally considered to be less risky than allowing genetic drift

to continue in small, isolated populations (Weeks et al. 2011). Furthermore, homogenisation of subpopulations that are genetically differentiated may result in loss or prevention of local adaptation and reduce fitness, but usually not so much as to lead to extinction (Weeks et al. 2011). In contrast, small populations may face extinction if they remain isolated and lose the allelic diversity that they need to adapt to changing conditions. Thus, in a situation such as the kokako metapopulation, high connectivity would generally be a less risky strategy (for both individual populations and the species as a whole) than very limited or no connectivity. For populations larger than 1000 effective individuals, some separation may become desirable to allow local adaptation and genetic differentiation (Jamieson & Allendorf 2012; Weeks et al. 2011). Although effective population size is generally considered to be an order of magnitude lower than census population size as an average across all taxa (Frankham 1995), the ratio will be much larger in monogamous species. For example, the average ratio of effective to census population size for monogamous birds in Frankham's (1995) review is 0.496. This suggests that kokako populations > 2000 individuals could be sufficiently robust to genetic drift for isolation to be considered as a strategy for increasing diversity.

Even if populations are not fully isolated, there remains the potential for local adaptation and maintenance of any unique local characteristics. However, there is little to no spatial genetic structure among existing kokako populations, with no clear evidence of local adaptation or differentiation and likely only very recent (i.e. following human arrival in New Zealand) isolation among existing regions (Murphy et al. 2006). Mixing currently isolated kokako populations would therefore present very little risk of reducing local adaptations, and the risk would be far outweighed by the genetic benefits of connectivity. If kokako populations continue to grow, a conservative approach would be to eventually establish several large metapopulations (e.g. within geographic regions or other relevant boundaries), each with an effective population size > 1000. Once sufficient genetic diversity had been established within each population by transferring birds as outlined in Table 4.3, the metapopulations could then be isolated. Translocations would then occur within, but not among, metapopulations. This would allow the potential for future adaptation and differentiation among regions, potentially maintaining higher allelic diversity across the species as a whole. A similar strategy has been adopted to manage distinct provenances of North Island brown kiwi (Apteryx mantelli; Holzapfel et al. 2008).

To allow for some differentiation, Hedrick (1995) and Weeks et al. (2011) recommend that no more than 20% of a population should be composed of immigrants.

Even with the large amounts of translocation indicated by the kokako models, immigrants (including nonbreeders) were expected comprise < 10% of any kokako population in any given year (data not shown). The only exception was Boundary Stream, where immigrants were expected to comprise 56% of the population immediately after the first release (but only 7% on average over all simulated years) when managed to retain 90% of rare alleles (60 immigrants released once every 15 years). This represented only a temporary departure from the 20% guideline. The small size of this population (K = 200) indicates that rather than adapting to local conditions, Boundary Stream would rapidly lose genetic diversity to genetic drift, so connectivity with other populations is still recommended. Management for allele retention is therefore unlikely to present a threat of homogenisation to a species that persists in multiple fragmented populations.

Given the difficulty and expense of translocating kokako, other management options may be more feasible than moving large numbers of individuals as indicated by the models. In some cases, it may be possible to provide for natural gene flow among existing populations, e.g. by extending predator control and restoring habitat between two or more populations (Gilbern-Norton et al. 2010). Larger metapopulations may then be able to self-sustain genetic diversity and achieve the more stringent 90% goal (with or without supplementation) where individual smaller populations could not. Similarly, increasing the predator-controlled area (and thus K) of any individual population could reduce the amount of supplementation needed, as indicated in Table 4.3 for some populations where this has been identified as an option. Alternatively, improving predator control within existing managed areas would increase population growth rates. Such an increase would improve genetic viability of an isolated population, as the population would reach a larger size more quickly, reducing the influence of genetic drift. However, increasing growth rate would also reduce the effectiveness of supplementation, as the population would reach carrying capacity (decreasing the probability that immigrants would breed) sooner than with a lower growth rate. This trade-off suggests that the management strategy should be chosen based on the long-term plan for each population. For those that will not be supplemented (either because they are large enough to be self-sustaining or because supplementation is not feasible), improved predator control would increase growth and security of the population. For populations that will be periodically supplemented, improved predator control would be less useful or even counterproductive (unless the area of predator control were expanded to provide breeding habitat for newly released birds).

Reintroducing kokako to former areas of the species' range has been a key component of the kokako recovery strategy (Innes & Flux 1999). The KRG recommends that kokako be reintroduced only to areas with a long-term commitment to predator control of sufficient intensity to enable establishment and growth of a kokako population (Innes & Flux 1999). The importance of predator control is highlighted by the increased effort (e.g. doubling the number of birds released) needed to establish a genetically viable population in a low-productivity versus a high-productivity area. New populations that are intended to be genetically self-sustaining would be most successful (and feasible to establish) in high-productivity areas with very little or no predator activity.

As many kokako populations cannot be interconnected with others at the present time, genetic management of new and existing populations would require a great deal of translocation of individuals. Fortunately, the models predicted that Pureora Forest, the largest relict population, has retained sufficient allelic diversity to be a high-quality source population for translocations. This population is also large enough to sustain high levels of annual harvest: depending on the management strategies selected for each population, Pureora could therefore provide sufficient birds to supplement all other populations. The safe harvest levels estimated here emphasise the potential value of large populations as both genetic reservoirs and sources for future reintroductions.

The models also indicated that other populations could sustain lower levels of harvest, after topping up as appropriate. Because reintroduced populations have already been through a bottleneck, birds sourced from these populations (e.g. Ark in the Park, Hauturu) would not be genetically equivalent to those from relict populations. Reintroduced populations thus should not be the exclusive source for new populations; instead, using birds from a mix of source populations (including at least one relict) will provide sufficient genetic diversity.

Conclusions and implications

Genetic management of a metapopulation is inherently complex, with a wide range of factors to consider. Results from these kokako models indicate that predictive models can be successfully applied to address such diverse options as one-off or ongoing supplementation, predator management, establishment of new populations, and safe harvest of appropriate source populations. However, recommendations are only valid if the models were parameterised with appropriate input values. The influence of input

values such as demographic rates and population size, demonstrated both here and in Chapter 3, emphasises that success of management actions guided by these models will be secured only if the assumptions of the models hold true in each population. Post-release monitoring of supplemental birds or a new population will be crucial, not only to ensure the success of the translocation (Sutherland et al. 2010), but also to adjust input values and indicate whether models should be updated to provide more accurate genetic management recommendations for each population.

Table 4.1. Demographic rates used in models simulating retention of rare alleles in kokako populations.

Demographic parameter[a]	Growth rate			Reference
	Low	Moderate	High	
Age at first breeding: Female	2	2	2	Basse et al. (2003)
Age at first breeding: Male	3	3	3	Higgins et al. (2006)
Annual fledglings/female:				
Mean	0.70	1.21	1.86	Adjusted to achieve target growth, given all other rates. High rate from Innes et al. (1999)
SD	0.38	0.65	1.00	Proportional to SD = 1 for mean = 1.86 (Innes et al. 1999)
Maximum	6	6	6	Flux et al. (2006)
Maximum at *K*	3	3	3	Not known, but likely lower than at low densities (I. Flux pers. obs.)
Annual survival rates:				
Juvenile and subadult[b]	0.71	0.81	0.91	Low rate: Innes et al. (1996); high rate: Innes et al. (1999); moderate rate is midway between
Juvenile and subadult at *K*	0.50	0.60	0.70	Not known; values selected to produce reasonable population growth curves in the models
Adult female	0.85	0.90	0.96	Low and high rate: Innes et al. (1999); moderate rate is midway between
Adult male	0.97	0.97	0.97	I. Flux (unpubl.)
Generation length	10	11	11	Calculated from models (average age of simulated breeding adults)

[a] In all models, I assumed lifelong monogamy of pairs (though in reality there is some divorce; Flux et al. 2006) and that either member of a breeding pair would maintain its breeding status if its mate died (Flux et al. 2006). Maximum lifespan was set at 20 years (J. Innes and I. Flux unpubl.). *K* = carrying capacity of the population.

[b] Minimum estimates of first-year survival; some birds may have survived at least one year without ever being detected. Subadult annual survival rates were not known, so I used the same estimates as for juveniles.

Table 4.2. Summary of relevant historic information for each existing kokako population.

Population	Bottleneck[a]	Bottleneck year	Managed area (ha)[b]	Growth rate category[c]
Te Urewera	Relict (95)	1996	1180	Moderate
Pureora Forest	Relict (66)	1996	3900	High
Mapara	Relict (48)	1992	1300	Low
Mokaihaha	Relict (40)	2000	850	Low
Hauturu (Little Barrier Island)	32 over 14 yrs (9 bred; 94% male)	1981	2500	High
Kapiti Island	33 over 7 yrs (11 bred; 67% male)	1991	1000	Low
Opuiaki	Relict (26)	2005	900	Low
Waima/Mataraua	Relict (25)	2000	1070	Moderate
Kaharoa/Onaia	Relict (22)	1990	700	High
Rotoehu	Relict (50)	1990	650	Low
Hunua Ranges	Relict (2) + 35 over 14 yrs (17 bred)	1998	1200	Moderate
Manawahe	Relict (10)	2000	844	Moderate
Waikokopu	Relict (10)	1998	375	Low
Ngapukeariki (East Cape)	19 in 1 year	2005	800	Low
Otanewainuku	19 over 2 yrs	2010	925	Moderate
Ark in the Park (Waitakere Ranges)	22 over 2 yrs	2009	2500	Moderate
Whirinaki	20 in 1 yr (4 bred)	2009	1200	Moderate
Pukaha (Mt Bruce Forest)	16 over 8 yrs	2003	700[d]	Low
Boundary Stream	20 over 7 yrs (12 bred)	2001	800	Low

[a] For relict populations, minimum modern population size is given. This is the smallest known bottleneck size, i.e. the minimum recorded population in the last few decades. Most information for reintroduced populations is from Innes et al. (2013); all other information provided by KRG (unpubl.). Approximate sex ratio of released birds is shown here if known and otherwise assumed to be approximately even for reintroduced populations or 66% male for relict populations.

[b] Area in which predator control has been implemented in recent years (KRG unpubl.). Models assume that this area will stay constant over the next 100 years, except where effects of increasing carrying capacity were tested (see Table 3).

[c] Population growth rates were categorised as low (finite rate of population growth $r = 0.04$ on average), moderate ($r = 0.15$), and high ($r = 0.29$) based on recent population trends (KRG unpubl.). Recently established populations without an established population trend (Ark in the Park, Otanewainuku, and Whirinaki) were assigned moderate growth rates.

[d] Current managed area. Simulations assumed predator management would expand to 1400 ha as the kokako population grows, as indicated by the management plan for this site (KRG unpubl.).

Table 4.3. Potential genetic management actions for each kokako population.

		% rare alleles retained (no supplementation)		# of adults to release (# that breed in parentheses) to retain 80 or 90% of rare alleles[b]			
				Immediate top-ups		Periodic suppl.	
Population	*K*[a]	2013	100 yrs	80%	90%	80%	90%
Te Urewera	295	98-99	81-85	0	-	0	10 (4)
Pureora Forest	975	98-99	96-98	0	0	0	0
Mapara	325	88-92	71-77	45 (17), 5 yrs	170 (59), 15 yrs	9 (4)	18 (7)
	600	88-92	80-84	0		0	
Mokaihaha	212	80-85	59-65	-	-	8 (4)	18 (8)
Hauturu	844	74-80	70-76	400 (80), 10 yrs	-	20 (4)	75 (12)
Kapiti Island	250	44-51	35-42	300 (90), 10 yrs	-	25 (12)	55 (25)
Opuiaki	225	67-73	46-52	84 (52), 5 yrs	-	12 (6)	25 (11)
Rotoehu	163	80-85	56-62	-	-	10 (4)	30 (11)
	350	82-87	66-72	40 (24), 5 yrs	180 (108), 10 yrs	10 (4)	15 (6)
Waima/Mataraua	268	75-81	65-71	132 (59), 5 yrs	-	13 (6)	32 (8)
Kaharoa/Onaia	175	77-82	62-68	-	-	16 (2)	30 (4)
	350	78-83	70-76			8 (1)	24 (3)
Hunua Ranges[c]	300	53-59	45-51	(43), 10 yrs	-	27 (8)	-
Manawahe	211	41-47	34-40	-	-	17 (6)	
	350	42-48	38-44	-	-	16 (5)	
Waikokopu	94	26-31	16-20	-	-	-	-
	350	28-33	18-23	-	-	11 (6)	-
Ngapukeariki	200	62-68	42-48	-	-	11 (5)	20 (9)
	350	61-67	46-52			9 (4)	18 (8)
Otanewainuku	231	75-80	59-65	41 (33), 5 yrs	-	19 (5)	-
	350	73-78	63-69	21 (17), 5 yrs	-	16 (5)	
Ark in the Park	625	88-91	76-81	15 (12), 5 yrs	35 (27), 5 yrs	7 (2)	18 (5)
Whirinaki	300	25-31	12-17	240 (36), 10 yrs	-	11 (6)	-
	350	26-31	14-19	200 (30), 10 yrs	-	10 (6)	-
Pukaha	350	50-56	38-44	-	-	11 (5)	24 (12)
Boundary Stream	200	45-51	26-32	-	-	24 (11)	60 (24)

[a] Carrying capacity: number of territorial adults that can be supported within the managed area.
[b] Dash indicates a case where goal cannot be met; blank cell indicates a case that was not modelled. For each population, *either* immediate top-up (within the specified period) *or* periodic supplementation (once every 15 years, continuing indefinitely) should be chosen as the genetic management strategy to achieve the indicated goal at a given carrying capacity.
[c] Recruitment was very low for previous birds released, so the number to release was not estimated.

Table 4.4. Standardised effect sizes for predictors of the number of supplemental kokako to release.

Covariate[a]	Mean[b]	SE	Relative importance
Intercept	**2.443**	**0.085**	
Bottleneck size	**-1.616**	**0.271**	**1.00**
Carrying capacity	**-0.520**	**0.190**	**1.00**
Bottleneck year	-0.285	0.129	0.66
Growth rate	0.277	0.135	0.56

[a] Predictors were assessed across all 18 kokako populations for which the goal of retaining 80% of rare alleles could be achieved by periodic supplementation with current estimates of carrying capacity (i.e. excluding Waikokopu; Table 4.3).

[b] Estimates of effect sizes were obtained by averaging the subset of top models ($\Delta < 2$; Table 4.7). Bold values show effects significantly different from zero, as indicated by 95% CIs.

Table 4.5. Safe harvest levels from potential source populations of kokako[a].

Population	Number per year
Pureora Forest	70
Hauturu	20
Te Urewera National Park	5
Mapara	5
Kaharoa/Onaia (K = 350)	4
Rotoehu (K = 350)	4
Ark in the Park	3

[a] Harvest levels shown are the maximum that would still allow the population to achieve the goal for retaining 90% of rare alleles over the simulated period. Harvest should occur either after top-up (to the 90% goal) or while periodic supplementation is ongoing (removing only locally produced birds).

Table 4.6. Number of adults to release (number that should breed in parentheses) to establish new kokako populations.

	Low growth		Moderate growth		High growth	
K	80%[a]	90%	80%	90%	80%	90%
200	-	-	60 (48)	-	40 (36)	-
250	-	-	50 (40)	-	30 (27)	60 (53)
300	-	-	40 (32)	-	30 (27)	60 (53)
400	-	-	40 (32)	-	30 (27)	50 (45)
500+	60 (42)	-	35 (28)	60 (48)	30 (27)	40 (36)

[a] Strategies are shown to achieve retention of 80% or 90% of rare alleles (as indicated) over 100 years, with various estimated carrying capacities (K) for each growth rate category. Dash indicates a case in which the goal could not be achieved.

Table 4.7. Standardised effect sizes of covariates occurring in the top model set predicting the number of kokako to release to retain 80% of rare alleles over 100 years.

Model ID	Inter-cept	Bottleneck size	Bottleneck year	Growth rate	*K*	k[a]	Deviance	AIC_C[b]	Δ[c]	w_i[d]
1	2.437	-1.583	-0.3010	0.2866	-0.6125	5	-59.980	135.0	0	0.361
2	2.440	-1.679	-0.2638		-0.4978	4	-62.269	135.6	0.66	0.260
3	2.454	-1.556		0.2547	-0.4747	4	-62.724	136.5	1.56	0.165
4	2.453	-1.650			-0.3929	3	-64.463	136.6	1.68	0.156

[a] Number of estimable parameters.
[b] Akaike Information Criterion, small sample size correction.
[c] Change in AIC_C relative to the best model within each set.
[d] Relative weight of the model.

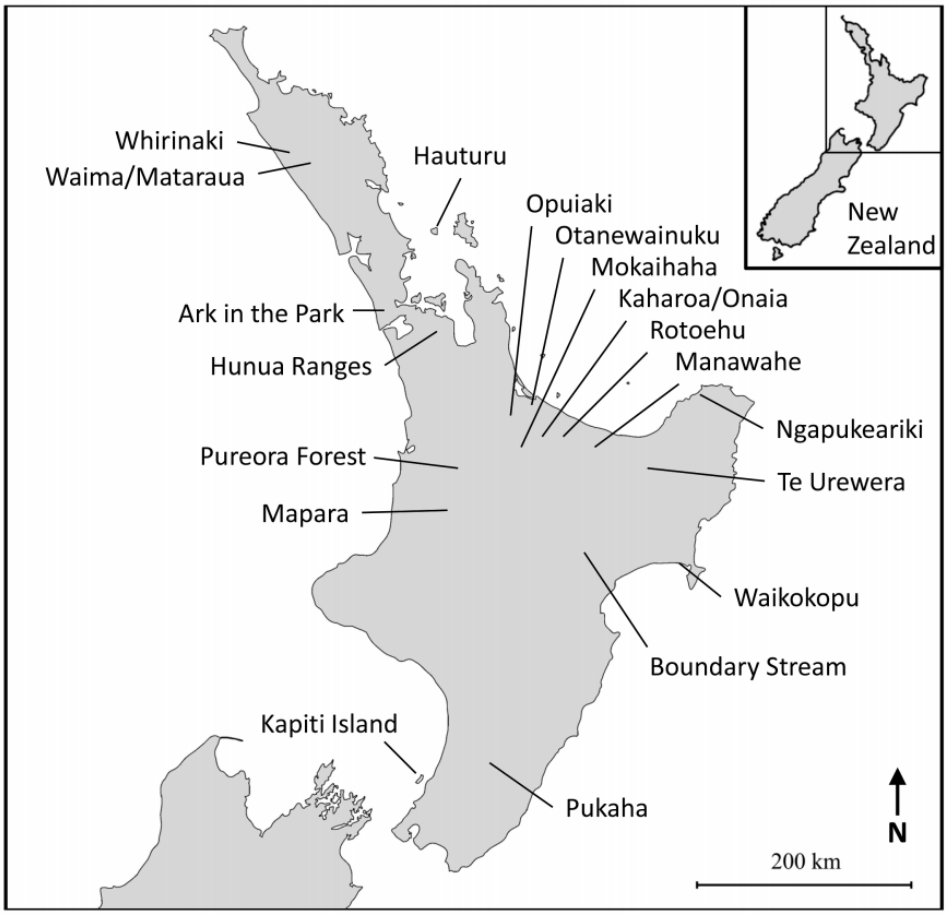

Figure 4.1. Locations of North Island kokako populations included in the analysis.

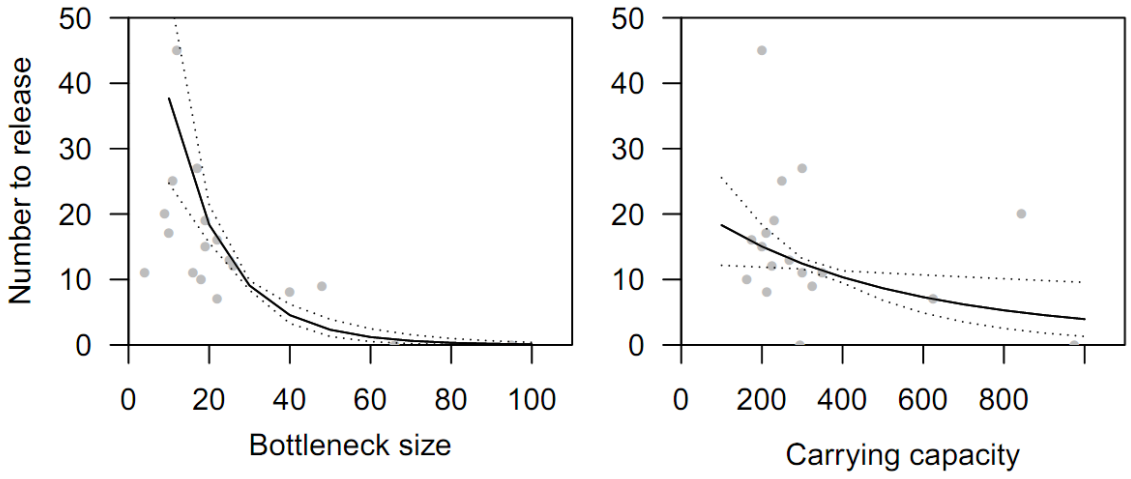

Figure 4.2. Predicted number of supplemental kokako to release to achieve 80% rare allele retention over 100 years. Estimates (solid line = mean, dotted lines = 95% CI) were made for values of the covariate shown on the horizontal axis with all other predictors (Table 4.4) held at their mean values. Grey points indicate data used to assess these relationships (Table 4.1, 4.3).

Chapter 5. Measuring fitness effects of inbreeding following a severe population bottleneck



Individually-marked adult black robin on Rangatira Island, New Zealand.

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Abstract

Inbreeding depression, the reduced fitness of offspring of related individuals, has become a central theme in evolutionary biology and has been well documented in wild populations. Fitness could also be affected by interactions between different measures of inbreeding, such as the relatedness of a breeding pair and the individual inbreeding coefficients of the members of the pair; but these interactions have not been examined. I found unexpectedly positive interactive effects of inbreeding in one of the world's most inbred wild species, the black robin. I show that high relatedness between members of a breeding pair improved survival of young black robins produced by the most-inbred mothers or fathers, but not those produced by the least-inbred mothers or fathers. This advantage could not be attributed to demographic or ecological factors, and appears to be a genetic effect. I propose that an inbreeding advantage arises when 1) the genotype of a highly homozygous (inbred) parent is proven successful by that individual surviving to breeding age, and 2) the offspring have a high probability of inheriting a very similar genotype (i.e. when parents are closely related to one another). This work provides the first indication that a genetic mechanism such as this "proven-homozygote advantage" may mitigate inbreeding depression.

Introduction

Although there are theoretical benefits for inbreeding, such as increasing inclusive fitness (Kokko & Ots 2006), the documented effects of inbreeding have been consistently negative (Charlesworth & Willis 2009; Crnokrak & Roff 1999; Keller & Waller 2002). Such inbreeding depression is generally thought to result from expression of harmful recessive alleles in inbred (more homozygous) individuals (Charlesworth & Willis 2009), and is more apparent in wild than captive conditions (Crnokrak & Roff 1999). Inbreeding depression may weaken in small populations when mildly deleterious alleles drift to fixation or are purged by natural selection (Waller 1993), although the latter process is unlikely to be efficient in most wild populations (Keller & Waller 2002). In the absence of fixation or purging, the viability of small populations can be threatened by negative fitness consequences of further inbreeding (Frankham et al. 2010; Saccheri et al. 1998). Interactive effects between a breeding pair's relatedness (i.e. the inbreeding level of the pair's offspring, F) and each parent's own F might also affect fitness, and could reveal

behavioural or genetic mechanisms underlying inbreeding depression. However, quantifying these interactions requires a large dataset with wide variance in inbreeding levels, so to my knowledge these potential effects have not previously been tested.

An ideal study species for examining the fitness effects of complex inbreeding interactions in a wild population is the black robin (*Petroica traversi*), a forest passerine endemic to the Chatham Islands, which lie 800 km east of New Zealand (Figure 5.1). Like many island species, the black robin has been subject to anthropogenic threats and is at risk of genetic problems that face small populations. Following habitat loss and the introduction of mammalian predators, the black robin was restricted for >80 years to a maximum of ~35 individuals, then famously declined further to five birds, including only one viable breeding pair, in 1979-1982 (Butler & Merton 1992; Kennedy 2009). Intensive management strategies, including translocating birds among islands and cross-fostering eggs and chicks to Chatham Island tomtits, *Petroica macrocephala chathamensis* (Kennedy 2009), enabled the species to increase to about 280 birds today. The black robin now persists in two isolated populations (on Rangatira and Mangere Islands; Figure 5.1), which were reintroduced from a remnant population on a small rock stack called Little Mangere Island (9 ha) (Butler & Merton 1992). Gene flow was enabled between the two reintroduced populations through the 1988-1989 breeding season by continuing translocation (Butler & Merton 1992). The two populations have been isolated from one another since then, as black robins are poor fliers and do not move independently between the two islands (Kennedy 2009).

The management period (1980-1989) was followed by close monitoring, but no management action, for a further 9 and 12 breeding seasons on Rangatira Island (1990-1998) and Mangere Island (1990-2001) respectively, providing highly detailed survival and breeding data for all individuals alive during this time (Kennedy 2009). These data include a complete species-wide pedigree recorded for ~20 years (six generations) following the single-pair bottleneck (Kennedy 2009; Kennedy et al. 2013). Although the black robin has experienced remarkable demographic recovery, it remains one of the most inbred wild species in the world. Inbreeding coefficients (F) ranged from 0.25 to 0.65 during the monitoring period (Figure 5.2). A previous analysis found inbreeding depression and no evidence for purging within the modern pedigree (Kennedy et al. 2013), but considered only one major fitness trait (juvenile survival) and did not test for interactive effects. Given the evidence of inbreeding depression found there, I expanded

upon this analysis by testing for inbreeding depression and interactive effects across all life-history stages of this species.

I tested two hypotheses: 1) multiple effects of inbreeding would still be evident for key demographic parameters (survival and reproductive success) even after the severe bottleneck experienced by this species, and 2) negative interactions between individual inbreeding coefficient (F; equivalent to the relatedness of the parents to one another) and father's F ($F_{♂}$) or mother's F ($F_{♀}$) would further reduce survival for offspring whose parents were both highly inbred and closely related to one another. This is the first time that such interactions of inbreeding have been tested in a wild population.

Methods

Dataset and pedigree

I considered the two birds comprising the single pair bottleneck (1979-1982) as the pedigree founders; the male's ancestry was known to grandparents, but the ancestry of the female and her relatedness to her partner were unknown. Estimates of inbreeding are relative to these two founders, and do not account for the likely extensive inbreeding in the remnant Little Mangere I. population prior to the single-pair bottleneck (Kennedy 2009). The behavioural pedigree of all descendants was recorded with minimal uncertainty in parentage through to 1998 (Rangatira Island) or 2001 (Mangere Island): parents are known for 98.4% of individuals and assigned with some uncertainty (based on breeding observations) for the remainder (Kennedy 2009). Genetic samples were not available for most individuals, so I could not confirm that genetic parentage corresponded with the behavioural pedigree. However, the incidence of extra-pair parentage is very low (0-2%) in moderately inbred populations of closely related species (Ardern & Lambert 1997; Taylor et al. 2008; Townsend & Jamieson 2013a) and extra-pair copulation was never recorded in black robins over 20 years of intensive observation (Kennedy 2009), so I expected little or no extra-pair paternity in the dataset. The absence of molecular data also meant that I examined pedigree inbreeding (expected identity-by-descent) rather than realised inbreeding (as measured by molecular markers), but both measures are typically good predictors for assessing inbreeding depression at the group level (Forstmeier et al. 2012; Pemberton 2004; Townsend & Jamieson 2013b). I used package pedigree (Coster 2011) in R (R Development Core Team 2013) to estimate inbreeding coefficients (F).

To avoid any confounding effects of management, I used fitness data only from the intensive monitoring period (Rangatira Island: 1990-1998; Mangere Island: 1990-2001). I included adults that hatched before 1990 if they were alive during the monitoring period. I omitted breeding records if one member of the breeding pair died during the breeding season, and I used a more restricted dataset for the analysis of lifetime reproductive success (see below). Two sites on Rangatira Island (Woolshed Bush and Top Bush) were connected by juvenile dispersal (~17% per year), but I assessed each site separately in this analysis because of their very different demographic rates (Kennedy 2009) and the potential for environment to interact with inbreeding (Armbruster & Reed 2005; Marr et al. 2006). I refer to breeding seasons by the year in which they began (e.g. the October 1990 - February 1991 breeding season is referred to as 1990).

Statistical analyses of inbreeding effects

I used generalised linear mixed-effects models (function lmer in R package lme4; Bates & Maechler 2009) to test for effects of relevant covariates on each demographic rate of interest. I tested for an effect of site (Mangere Island, Woolshed Bush, or Top Bush) and individual, parental, and brood effects, as relevant, on the following demographic response variables: 1) clutch size (1-3 eggs); 2) survival probability of eggs (from the time they were first detected in the nest by field workers to hatching), nestlings (from hatching to fledging), and fledglings (from fledging to independence, when chicks were no longer provisioned by parents, as determined by field observations; typically 35-65 days after fledging; Kennedy 2009); 3) probability of a juvenile surviving to maturity (from independence to the beginning of observations in the following breeding season); 4) adult annual survival probability, from the beginning of one breeding season to the beginning of the next (prior to 1998, there was only one case in which a bird was not seen in one year but later found to be alive; so I assumed that birds died over the winter following the last breeding season in which they were recorded); 5) the probability of a female breeding in any given year (separated into yearlings and older females, as yearlings were less likely than older females to breed on Mangere Island; see Results); and 6) lifetime reproductive success (LRS; the total number of offspring produced over an individual's lifetime that survived to maturity, i.e. potential recruits). For the LRS analysis, I modelled males and females separately because different covariates affected reproductive success of each sex (see Results). To quantify LRS, I included only individuals that lived their entire lives within the monitoring period and did not disperse between sites as adults (74 males and 79

females). Age (mean = 3.09, SD = 1.91) and F (mean = 0.351, SD = 0.064) of birds in the LRS dataset were not different from those in the full dataset (mean age = 3.15, SD = 1.94; mean F = 0.341, SD = 0.060), so I was confident that restricting the dataset did not bias the effects found.

I included relevant random effects in each model to account for repeated measurements from individuals (individual effect for clutch size, adult survival, and probability of breeding for older females; parental pair effect on chick and juvenile survival) and annual variation (year effect in all models except LRS; and effect of hatch year in adult survival and probability of breeding for older females).

Before building the models, I used the function standardise in R package arm (Gelman & Su 2013) to centre the explanatory variables on their means and standardise over two standard deviations to facilitate direct comparison of effect sizes (Gelman 2008). I selected the error distribution for each model based on the data type and to remedy any overdispersion as indicated by residual deviance being much larger than residual degrees of freedom (Zuur et al. 2009). I used a Poisson distribution to model clutch size, a complementary log-log link function of the binomial distribution for all survival analyses (Agresti 2002; Keller 1998), and a logit link function of the binomial error distribution for probability of breeding. I found that a negative binomial distribution dealt with overdispersion better than a Poisson distribution for LRS of females; both distributions were equally appropriate for LRS of males, but I used the negative binomial distribution for both sexes for consistency.

After building the global model with all relevant covariates, I then tested for multicollinearity among predictors and sequentially removed variables with the highest condition index until the variance inflation factor was < 5.0 for all remaining variables in the model (Belsley et al. 1980). I then generated all possible submodels of the resulting adjusted global model using the dredge function in R package MuMIn (Barton 2013). Inference was based on model averaging the top 2 ΔAIC_C (Burnham & Anderson 1998; Grueber et al. 2011) using the function model.avg in MuMIn.

To interpret the biological effects of inbreeding on fitness in cases where F interacted with another covariate, I used parametric bootstrapping on the effect sizes to predict fitness at low (0.27), median (0.34), and high (0.54) F over the range of the interacting covariate. Although more extreme values of F were present in the dataset overall, these were the most extreme values common to all of the data subsets. As patterns were qualitatively similar across sites, I predicted overall effects by including a weighted

average for the site effect(s), if any. I generated 100,000 bootstrapped values using the model point estimate and its standard error as the mean and standard deviation of a normal distribution; the mean and 95% quantiles of these predictions were taken as the fitted effects of each variable of interest on the response variable at the high, median, and low values of F.

Further assessment of potentially confounding variables

I detected surprising positive effects of inbreeding on egg and chick survival (see Results), so I conducted further analyses to assess whether some other factor may explain the relationships. For these assessments, I focused on egg survival (for which there was a positive effect of F♀) and fledgling survival (for which the positive interaction between F and F♀ was strongest).

First, because inbreeding was accumulating over the duration of the dataset (Kennedy et al. 2013), a correlation between temporal trends and chick survival (which tended to increase over the management period) could have caused the apparent effect of inbreeding. To test for any temporal trends, I re-ran the egg and fledgling survival models with year included as a continuous variable rather than a random effect.

Second, nests laid early in the season are more successful than those laid late in the season for many bird species (Verhulst & Nilsson 2008). A positive effect of F might arise from closely related pairs laying eggs earlier (e.g. perhaps, being more "familiar" to each other, they were able to establish their pair bond quickly and start breeding earlier than less closely related pairs). For first-hatched broods of each pair (subsequent broods were typically hatched only by pairs whose first brood had failed), I assessed the effect of hatching date (consecutive day of breeding season, counted from 1 October) on fledgling survival and tested whether this changed the effects of inbreeding, relative to a fledgling survival model without hatching date for first broods only. Egg-laying dates were not known for many clutches, so I did not perform this assessment for egg survival.

Third, the number of years in which members of a pair have previously bred together can affect chick survival in monogamous bird species (Fowler 1995). If highly inbred females and/or closely related pairs were more likely to maintain pair bonds over multiple years (perhaps through higher similarity or compatibility of closely related individuals), the constancy of the pair bond could have driven the apparent positive effects of F and F♀ on egg and chick survival. Therefore, I examined the effect of adding pair-bond duration to the egg and fledgling survival models.

Fourth, I assessed whether associations with spatial factors might explain the apparent positive inbreeding effects. I tested spatial effects only for Woolshed Bush, the largest site, as habitat effects could vary unpredictably among sites. If birds dispersed shorter distances when they were hatched in good habitat (which improved chick survival), then they would be more likely to mate with closely related individuals, causing a heterogeneous distribution of inbreeding within the site and an apparent correlation between relatedness and chick survival. Territory density varied across Woolshed Bush, presumably reflecting habitat quality and potentially affecting local patterns of inbreeding; so I tested for an effect on egg and fledgling survival of latitude and longitude, as well as an effect of the distance (km) between the approximate centre of the breeding territory and that of the nearest neighbouring territory.

Fifth, I assessed whether a dominant allele that causes female black robins to lay eggs on the rim of the nest (where they are not incubated and therefore do not hatch if left in place; Massaro et al. 2013a) may have underlain the positive effect of $F_{♀}$ on egg survival. More-inbred females would be more likely to be homozygous at this locus, while less-inbred females would be more likely to be heterozygous. Only ~50% of homozygotes would carry the dominant allele (rim-layers), while all heterozygotes would express the dominant trait and be rim-layers. Thus, more-inbred females would be less likely to express the rim-layer trait than less-inbred females, and might experience higher hatching success for this reason. I tested for an effect of F on rim-laying in females with a generalised linear model with binomial distribution. I coded females as "rim-layers" if they had ever been known to lay eggs on the rim of a nest, and otherwise as "non-rim-layers," and included this binary variable in a new global model for egg survival.

All of these additional models were built by model-averaging as described for the main analyses, where the global model included all covariates from the top model set in the original egg and fledgling (respectively) survival analyses. If the effect sizes of $F_{♀}$ (for egg survival) or the interaction between the $F_{♀}$ and F (for fledgling survival) were qualitatively similar between the original models and the models including potentially confounding variables, I concluded that the potentially confounding variable was not the cause of the positive inbreeding effects on egg and fledgling survival.

I also used linear mixed-effects models to test for an effect of F on natal dispersal distance (distance between natal territory and first breeding territory) for females in Woolshed Bush, including hatch year as a random effect as habitat availability changed over time. If the parents of highly inbred females tolerated female offspring in or near

their territory more than the parents of less-inbred females, i.e. a form of kin selection, those more-inbred females might then have an advantage over less-inbred females (which would disperse farther and to unproven habitat) when they began breeding. Females dispersing shorter distances might also be more likely to breed with closely related males (males typically established breeding territories slightly nearer their natal site [mean = 341 m, SD = 451 m] than did females [mean = 598 m, SD = 393 m]). These factors would combine to result in highly inbred females with closely related mates having higher reproductive success as a function of habitat quality rather than inbreeding. Lack of correlation between dispersal distance and female F would indicate that this was unlikely.

Finally, I assessed whether the linear form of the $F_{♀}$·F interaction term in the fledgling survival model misrepresented the nature of the relationship. I tested two subsets of the data: chicks from the most inbred ($F > 0.40$) and those from the least inbred ($F < 0.30$) females. If the interaction term fit the overall dataset appropriately, I expected a positive relationship between F and chick survival for fledglings produced by the most-inbred females and a negative relationship for those produced by the least-inbred females.

Lethal equivalents

For comparison with other studies, I also calculated lethal equivalents (LE) for survival rates as -2β, where β is the effect size of F for each fitness trait (Keller & Waller 2002). Previous estimates of LE have not always considered covariates other than F, but interacting covariates can be included when relevant (Grueber et al. 2011). I used model averaging on centred but not standardised covariates, allowing assessment of effect sizes on the natural scale and facilitating comparison with LE reported previously (Keller 1998; Keller et al. 2002; Keller & Waller 2002; Kruuk et al. 2002; Townsend & Jamieson 2013b). In all other respects, model averaging for LE followed the methods described for the main analysis. When the averaged model included interactions between F and another covariate, I calculated total effects of F using the median of the interacting covariate (X); such that $\beta = \beta_F + \beta_{F \cdot X} \cdot \text{median}(X)$ and $SE(\beta) = SE(\beta_F) + SE(\beta_{F \cdot X}) \cdot \text{median}(X)$, with weighted mean site effects where F interacted with site. When the interacting covariate was $F_{♀}$, I also calculated LE at low (0.27) and high (0.54) values of $F_{♀}$ to fully elucidate the interactive effects; the low and high $F_{♀}$ values approximate first-order inbreeding and selfing, respectively, and fall within the range of observed values for each subset of the black robin data (Figure 5.2). Confidence intervals were obtained by parametric bootstrapping (as above) of -2β. Despite the absence of outbred birds in the dataset, my

LE estimates are still comparable to other studies as the slope (β) indicates the change in fitness with each unit increase in F (Keller & Waller 2002). Note that Kennedy et al. (Kennedy et al. 2013) defined juvenile survival from fledging to one calendar year of age, whereas I examined the somewhat shorter period of survival from juvenile independence to the beginning of the next breeding season (< 1 year of age), as I treated fledgling survival separately and some birds that died before reaching one calendar year of age may still have been able to breed successfully.

Results

Inbreeding coefficients (F) in the dataset ranged from 0.25 (equivalent to products of full-sib crossings in outbred populations) to 0.65 (more inbred than the product of selfing [F = 0.50] in outbred populations), with most individuals showing moderate to low levels within this range and only a few at the upper end of the range (Figure 5.2). A number of traits exhibited a typical pattern of inbreeding depression, including a negative effect of $F_{♂}$ on nestling survival, fledgling survival, and juvenile survival (Table 5.1, Figure 5.3). Close kinship of a breeding pair also reduced nestling and fledgling survival when the mother was less inbred than average (i.e. similar to inbred mothers in previous inbreeding studies; Figure 5.4), and had an overall negative effect on juvenile survival (Figure 5.3). I also observed interactions between F and age, where adult annual survival decreased with age for less-inbred individuals, presumably as a result of senescence (Table 5.2, Figure 5.4f). In contrast, annual survival increased with age for more-inbred adults, possibly because less-fit more-inbred individuals died at young ages, leaving only higher-quality inbred individuals at older ages. Three life-history traits (clutch size, probability of breeding, lifetime reproductive success) exhibited weak or neutral effects of further inbreeding (Table 5.1, 5.2, Figure 5.3).

Contrary to typical negative predictions for the effects of inbreeding, I found a positive relationship between $F_{♀}$ and egg survival (from discovery by field workers to hatching; Figure 5.3a, 5.4a). This effect was not explained by a suite of potentially confounding variables such as year effects, spatial structure, or pair bond duration (Table 5.3). I also found a surprising positive interaction between F and $F_{♀}$: a highly inbred female produced nestlings and fledglings with a higher chance of survival when she was more closely related to her mate than average, but with a lower chance of survival when she was less closely related to him (Figure 5.3b-c, 4b-c). Instead of further reducing

survival as I expected, this compound inbreeding conferred a strong advantage for both nestling and especially fledgling survival. The magnitude of the interaction between F and $F_{♀}$ was lower for the nestling stage than for the fledgling stage, potentially owing to the different lengths of each period; effects of inbreeding may have been consistent at both stages, but were more apparent over the longer fledgling period (mean = 42) than the shorter nestling period (~22 days; Kennedy 2009).

The positive effect of the interaction between F and $F_{♀}$ remained evident even after testing for effects of a large number of possible temporal and spatial confounders (inter-annual temporal trends, intra-seasonal temporal trends, length of pair bond, territory location) on fledgling survival (Table 5.4), the trait for which the effect was strongest. In addition, F was not negatively correlated with female natal dispersal distance (β_F = 1.19, 95% CI = -0.69, 3.07; n = 89 females), so the positive inbreeding interaction was unlikely to result from kin selection by parents of highly inbred females (e.g. tolerating female offspring that remained in or near the natal territory). Subsetting the data to the most-inbred ($F_{♀}$ > 0.40) and least-inbred ($F_{♀}$ < 0.30) females indicated that the interaction term in the main model fit the data appropriately, with a positive effect of F for most-inbred females (β_F = 1.55, 95% CI = 0.02, 3.08, n = 73 fledglings from 24 unique pairs) and negative for least-inbred females (β_F = -0.32, 95% CI = -0.77, 0.13, n = 156 fledglings from 55 unique pairs), as expected. The two inbreeding variables (F and $F_{♀}$) were weakly positively correlated (Figure 5.5), but tests for multicollinearity indicated that it was appropriate to keep both variables and their interaction in the model (variance inflation factors < 5; Belsley et al. 1980). I found a similar interactive effect between F and $F_{♀}$ for juvenile survival, which was also affected by a positive interaction between F and $F_{♂}$ (Figure 5.4d-e). However, the interaction did not appear to impact lifetime reproductive success of either females or males (Figure 5.3h; Table 5.2), probably because of the reduced sample sizes at this life-history stage (see Methods).

The positive interaction between F and $F_{♀}$ resulted in a very large advantage for offspring of highly inbred females that were closely related to their mates (Table 5.5, third column). Conversely, further inbreeding conferred a disadvantage for offspring of less-inbred females (Table 5.5, first column) that was moderate to strong relative to other wild species (Keller 1998; Keller et al. 2002; Keller & Waller 2002; Kruuk et al. 2002; Laws & Jamieson 2011; Townsend & Jamieson 2013b). Values of lethal equivalents for other fitness traits were not significantly different from zero, and represented neutral to mild effects of inbreeding relative to other studies (Table 5.5, middle column).

Discussion

Although all modern black robins are highly inbred, I found multiple fitness effects of further inbreeding in this species. These findings suggest that deleterious alleles have not been fully purged, contrary to theoretical predictions (Waller 1993) but in line with more recent suggestions that purging is unlikely to be efficient in wild populations (Keller & Waller 2002). The inbreeding effects found for the black robin also indicate that some functional allelic diversity remains even in this highly inbred species, despite little genetic variation being detectable with molecular markers (Ardern & Lambert 1997). Even more surprisingly, in addition to the neutral effects of inbreeding on some traits (clutch size, probability of breeding, lifetime reproductive success) and negative effects on others (adult survival, depending on age), I found some positive effects of further inbreeding on survival of young, especially those produced by highly inbred females.

The positive effect of $F_{♀}$ on egg survival, which was not affected by interactions with other measures of inbreeding, could indicate the presence of a beneficial recessive allele or dosage effect that improves hatching success. Either such genetic effect would be more likely expressed by more homozygous (more inbred) than more heterozygous (less inbred) individuals. For example, an allele that improved the mother's ability to provision nutrients during egg formation or to incubate eggs successfully could improve hatching success. Recent analyses have reported a dominant allele at a single locus that appears to be responsible for the maladaptive behaviour of some females laying eggs on the rim of the nest (Massaro et al. 2013a). As more-inbred individuals are generally more homozygous than less-inbred individuals, they are also more likely to be homozygous for the recessive, and in this case beneficial, allele for this trait. This pattern could provide a mechanism for the higher survival of eggs laid by more-inbred females. However, I found no relationship between F and rim-laying for females (β_F = -0.88, SE = 2.80, $p > 0.75$). Rim-laying (a binary trait assigned to females) was a significant predictor of egg survival, but including that covariate in the averaged model did not change the positive effect of $F_{♀}$ (Table 5.3). The rim-laying allele therefore does not explain the positive effect of $F_{♀}$ on egg survival that I detected; some other, as yet unidentified, allele may be responsible.

Unlike egg survival, nestling and fledgling survival were affected by the interaction between $F_{♀}$ and kinship in the model of egg survival. Different mechanisms may therefore underlie the patterns I found in nestling and fledgling survival than those for egg survival. Positive interactive effects of inbreeding, such as I describe here for chick

survival in the black robin, have not previously been documented. Observation of direct positive relationships between pairwise F and fitness have previously been attributed to: 1) more successful individuals leaving a larger number of related offspring that inherit traits that improve reproductive success (van Noordwijk & Scharloo 1981); 2) kin selection, whereby parental investment is increased for inbred offspring (Kokko & Ots 2006); or 3) increased extra-pair paternity with high-quality males when a female is socially paired with a related male (Blomqvist et al. 2002; Foerster et al. 2003). Any of these would affect less-inbred and more-inbred individual breeders to a similar extent, so these mechanisms are not relevant to the interactive effect I found here. For example, for extra-pair paternity to explain my results (including the positive interaction between F and $F_{♀}$ on nestling and fledgling survival), only more-inbred (and not less-inbred) females must have mated with extra-pair males when they were socially paired with closely related males. Although I did not have genetic data for most birds in the dataset, it seems unlikely that more-inbred females (which may be less fit than their less-inbred counterparts) would be better able to achieve extra-pair copulation, especially given the territorial nature of both sexes of this species and the fact that extra-pair copulation was never recorded in this species in 20 years of intensive observation (Kennedy 2009).

An apparent positive effect of inbreeding on survival probability is sometimes explained by early mortality of highly inbred offspring. If inbreeding depression reduces brood size and thus enables parents to invest more effort into surviving offspring, this would result in more-inbred broods showing higher survival than less-inbred broods (Rabon & Waddell 2010; Richardson et al. 2004). This was not the case in the dataset, as probability of breeding and clutch size were unaffected by inbreeding and brood size was actually increased by higher egg survival for highly inbred mothers. Trade-offs between fitness traits have also been reported, whereby a positive relationship between F and one trait (e.g. lifespan, immune competency) is offset by a negative effect of F on another trait (e.g. reproductive success) (Bilde et al. 2009; Gershman et al. 2010), but this analysis revealed no cost of inbreeding to lifetime reproductive success. A positive effect of inbreeding may also be seen when outbreeding depression occurs (Edmands 2007), but given the long and local nature of the historic bottleneck, outbreeding depression seems highly unlikely to affect black robins (Frankham et al. 2011).

I propose that these findings indicate a "proven-homozygote advantage" of inbreeding when the genotypes of offspring are likely to be very similar to those of one or both parents. This occurs when parent(s) are highly inbred (highly homozygous) as well

as closely related to one another: the particular combination of loci that show identity-by-descent (homozygosity) in the genotype inherited by the offspring has already been "proven" by the successful survival of the highly inbred parent(s) and is thus likely to confer a high survival probability. Offspring of closely-related pairs of less-inbred animals are more likely to inherit new, "untested" combinations of homozygous loci, resulting in increased expression of deleterious alleles and thus inbreeding depression. In the dataset, this effect was strongest for inbred females. The equivalent genetic benefit conferred by highly inbred fathers in closely related pairs may have been masked at the nestling and fledgling stage by some other fitness cost of the father's inbreeding level; for example, inability to establish or defend a high-quality territory (in which the male usually plays a greater role than the female; Kennedy 2009) or inability to provision chicks sufficiently (paternal provisioning is critical to offspring survival in black robins; Butler & Merton 1992; Kennedy 2009). However, I did observe a positive interaction between F and $F_{♂}$ at the juvenile stage, after the offspring had left their natal territory and parental care, further supporting a genetic basis for the observed benefits of inbreeding.

I expect that a proven-homozygote benefit of inbreeding will be most apparent in populations that are highly inbred, where the genotypes of offspring are very similar to those of their parents. Therefore it will be most evident in populations with low to moderate genetic load, resulting from a history of genetic purging, relaxed selection (e.g. captive environments), or chance (Bouzat 2010), so that expression of recessive alleles incurs only moderate fitness costs. Further work will be required to assess how generally this effect applies across species and contexts. However, even under these conditions, further inbreeding may benefit only a few of the most inbred individuals in a stable environment; the population may experience little net demographic benefit, and significant losses of genetic diversity may still impose costs over time (Ross-Gillespie et al. 2007). In Chapter 6, I evaluate the net effects of inbreeding for expected population growth and persistence in the black robin.

The surprising inbreeding effects found in the black robin emphasise that each species may present a unique case to conservation managers. Assumptions based on theoretical expectations, e.g. that the black robin would either suffer from inbreeding depression or be robust to further inbreeding as a result of purging, would have incompletely addressed points of potential concern for the continuing persistence of this iconic species.

Table 5.1. Standardised effect sizes estimated by model averaging for offspring survival. The top model set is provided in Table 5.6.

	Clutch size		Egg survival		Nestling survival		Fledgling survival		Juvenile survival	
Covariate[a]	Mean (SE)	RI[b]	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI
Intercept	**0.74 (0.03)**		0.05 (0.04)		0.07 (0.12)		**0.95 (0.13)**		**0.61 (0.11)**	
WSB	0	0	0.01 (0.12)	0.11	**0.44 (0.14)**	**1.00**	**-0.31 (0.16)**	**1.00**	**-0.30 (0.13)**	**1.00**
TPB	0	0	-0.15 (0.13)	0.11	0.21 (0.16)	1.00	**-0.91 (0.18)**	**1.00**	**-0.55 (0.16)**	**1.00**
F	-0.01 (0.06)	1.00	0.12 (0.09)	0.27	0.01 (0.12)	0.78	-0.01 (0.14)	1.00	-0.24 (0.13)	1.00
Age♀	0.02 (0.06)	0.16	-0.05 (0.08)	0.11	-0.10 (0.10)	0.16	0.07 (0.11)	0.19	0.09 (0.11)	0.28
F♀	0.01 (0.06)	0.15	**0.17 (0.09)**	**0.88**	-0.11 (0.24)	1.00	-0.17 (0.14)	1.00	0.01 (0.13)	0.16
F♂	-0.02 (0.06)	0.28	-0.03 (0.09)	0.10	-0.20 (0.11)	0.88	**-0.30 (0.12)**	**1.00**	-0.13 (0.12)	0.67
Brood size[c]			0	0	0.06 (0.09)	0.12	0.05 (0.11)	0.17		
F♀·F			0	0	**0.52 (0.19)**	**0.78**	**0.85 (0.25)**	**1.00**	0.36 (0.19)	0.16
F♂·F			0	0	-0.22 (0.21)	0.15	0	0	-0.34 (0.23)	0.41
F♀·F♂	0	0	0	0	-0.36 (0.19)	0.68	0.16 (0.24)	0.19	0	0
WSB·F♀	0	0	0	0	0.27 (0.27)	0.78	0	0		
TPB·F♀	0	0	0	0	**0.74 (0.29)**	**0.78**	0	0		
n	*641 clutches from 256 unique pairs*		*1346 eggs from 256 unique pairs*		*915 chicks from 259 unique pairs*		*673 chicks from 232 unique pairs*		*598 juveniles from 238 unique pairs*	

[a] Mangere Island (1990-2001) was used as the reference site; the Woolshed Bush (WSB) and Top Bush (TPB) sites were on Rangatira Island (1990-1998). Blank cells indicate that the covariate was not included in the global model (not relevant, or prevented convergence). A value of zero indicates that the covariate was tested but was not present in the top model set. Bold values show effects that were significant, based on 95% CIs that exclude zero. F = inbreeding coefficient (equivalent to kinship coefficient of parents); F♀ and F♂ are F of the mother and father, respectively, of each individual; ages of mother and father are denoted the same way.

[b] Relative importance of each covariate in the averaged model

[c] Clutch size for egg survival, number of hatched chicks for nestling survival, and number fledged for fledgling survival. Number of pairs is larger for nestling survival than for clutch size or egg survival because clutch size was unknown for some pairs, but number hatched was known.

Table 5.2. Standardised effect sizes estimated by model averaging for other fitness traits. See Table 5.1 for definitions of abbreviations. The top model set is provided in Table 5.7.

			Annual prob. breed (females)				Lifetime reproductive success (LRS)			
	Adult survival		Yearling		Older		Male		Female	
Covariate	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI
Intercept	**0.53 (0.08)**		**-1.01 (0.32)**		**4.47 (0.74)**		**0.96 (0.12)**		**0.63 (0.21)**	
WSB	-0.06 (0.08)	1.00	**2.32 (0.41)**	**1.00**	**-3.30 (0.45)**	**1.00**	0.08 (0.21)	0.29	0.45 (0.26)	0.72
TPB	**-0.52 (0.11)**	**1.00**	**1.96 (0.46)**	**1.00**	**-3.54 (0.57)**	**1.00**	-0.41 (0.27)	0.29	-0.27 (0.35)	0.72
Age	-0.12 (0.07)	0.79			-0.14 (0.33)	0.34				
Sex (male)[a]	0.07 (0.07)	0.35								
F	-0.07 (0.08)	0.50	0	0	0.35 (0.36)	0.40	-0.13 (0.19)	0.20	0.06 (0.44)	0.72
Age·F	0.26 (0.14)	0.36			1.21 (0.70)	0.15				
WSB·F	0	0	0	0			0	0	0.83 (0.58)	0.72
TPB·F	0	0	0	0			0	0	-0.98 (0.74)	0.72
Mean kinship[b]							0	0	0	0
# yrs bred							**1.36 (0.17)**	**1.00**	**1.75 (0.18)**	**1.00**
Mean kinship·F							0	0	0	0
F·# yrs bred							0	0	**-0.72 (0.30)**	**0.52**
n	*1290 records from 417 adults*		*184 yearling females*		*542 records from 170 females*		*74 males*		*79 females*	

[a] Female was the reference sex. Sex of most birds was not determined until they exhibited breeding behaviour as adults, so sex was unknown for most individuals that died as juveniles or before breeding. I therefore included sex only in the adult survival model.

[b] Weighted by the proportion of breeding lifespan with which the individual bred with each mate.

Table 5.3. Standardised effect sizes from models assessing effects of potentially confounding covariates on egg survival.

	Original model[b]		Year (continuous)[b]		Pair bond length[b]		Location[c]		Nearest neighbour[c]		Rim laying	
Covariate[a]	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI
Intercept	0.05 (0.04)		0.05 (0.05)	-	0.06 (0.06)	-	0.04 (0.06)	-	0.05 (0.06)	-	0.04 (0.04)	
WSB	0.01 (0.12)	0.11	0.01 (0.12)	0.11	0.00 (0.12)	0.22	0	0	0	0	0	0
TPB	-0.15 (0.13)	0.11	-0.15 (0.13)	0.11	-0.17 (0.14)	0.22	0	0	0	0	0	0
F	0.12 (0.09)	0.27	0.11 (0.09)	0.27	0.16 (0.09)	0.69	0.05 (0.12)	0.13	0	0	0.10 (0.09)	0.28
Age♀	-0.05 (0.08)	0.11	-0.05 (0.08)	0.12	-0.19 (0.10)	0.83	**-0.25 (0.11)**	**1.00**	**-0.28 (0.12)**	**1.00**	-0.04 (0.08)	0.13
F♀	**0.17 (0.09)**	**0.90**	0.17 (0.09)	0.88	0.15 (0.09)	0.67	0.20 (0.12)	0.75	0.20 (0.12)	0.63	**0.17 (0.09)**	**0.88**
F♂	-0.03 (0.09)	0.10	-0.03 (0.09)	0.10	-0.06 (0.09)	0.14	-0.06 (0.12)	0.14	-0.10 (0.13)	0.26	-0.03 (0.09)	0.12
Year			0.07 (0.09)	0.13								
Pair bond length					0.26 (0.10)	1.00						
Latitude							**0.25 (0.12)**	**1.00**				
Longitude							-0.03 (0.13)	0.13				
Distance									0	0		
Rim-laying mother											-0.17 (0.09)	0.84

[a] No covariate was sufficient to remove the inbreeding effect of interest (highlighted). Only covariates included in the top model set for the original analyses (Table 5.1) were included, plus each additional variable of interest as indicated. Bold values show effects that were significant, based on 95% CIs. See Table 5.1 for definitions of abbreviations.

[b] n = 1346 eggs from 256 unique pairs.

[c] Woolshed Bush only; n = 684 eggs from 129 unique pairs.

Table 5.4. Effect sizes from standardised models assessing effects of potentially confounding covariates on fledgling survival[a].

	Original model[b]		Year (continuous)[b]		First broods[c] Without hatch day		First broods[c] With hatch day		Pair bond length		Location[d]		Nearest neighbour[d]	
Covariate	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI
Intercept	**0.95 (0.13)**		**0.93 (0.13)**		**0.88 (0.15)**		**0.82 (0.16)**		**0.95 (0.13)**		**0.64 (0.09)**		**0.66 (0.08)**	
WSB	**-0.31 (0.16)**	**1.00**	-0.28 (0.16)	1.00	-0.11 (0.19)	1.00	-0.01 (0.19)	1.00	**-0.31 (0.16)**	**1.00**	0	0	0	0
TPB	**-0.91 (0.18)**	**1.00**	**-0.87 (0.18)**	**1.00**	**-1.04 (0.22)**	**1.00**	**-0.96 (0.23)**	**1.00**	**-0.90 (0.18)**	**1.00**	0	0	0	0
F	-0.01 (0.14)	1.00	-0.01 (0.14)	0.20	-0.04 (0.17)	1.00	-0.04 (0.17)	1.00	0.00 (0.14)	1.00	-0.07 (0.15)	0.70	-0.14 (0.14)	0.35
Age♀	0.07 (0.11)	0.19	0.06 (0.11)	1.00	0.14 (0.14)	0.22	0.16 (0.14)	0.35	0.07 (0.11)	0.15	0.06 (0.14)	0.05	0	0
F♀	-0.17 (0.14)	1.00	-0.18 (0.14)	1.00	-0.21 (0.17)	1.00	-0.2 (0.18)	1.00	-0.16 (0.14)	1.00	-0.08 (0.16)	0.65	-0.05 (0.15)	0.12
F♂	**-0.30 (0.12)**	**1.00**	**-0.32 (0.13)**	**0.20**	0	0	0	0	**-0.30 (0.12)**	**1.00**	-0.08 (0.15)	1.00	0	0
Brood size	0.05 (0.11)	0.17	0.05 (0.11)	0.56	0.13 (0.13)	0.22	0.14 (0.13)	0.34	0.05 (0.11)	0.14	-0.17 (0.14)	0.37	-0.15 (0.13)	0.24
F♀·F	**0.85 (0.25)**	**1.00**	0.85 (0.25)	1.00	**1.07 (0.32)**	**1.00**	**1.08 (0.33)**	**1.00**	**0.84 (0.24)**	**1.00**	**0.81 (0.37)**	**0.65**	0.59 (0.35)	0.12
F♀·F♂	0.16 (0.24)	0.19	**0.14 (0.24)**	**1.00**	0	0	0	0	0.16 (0.24)	0.15	0.29 (0.28)	0.20	0	0
Year			0.19 (0.13)	0.10										
Hatch day							**-0.35 (0.13)**	**1.00**						
Pair bond length									0.13 (0.12)	0.21				
Latitude											**0.34 (0.15)**	**1.00**		
Longitude											0.18 (0.15)	0.37		
Distance													-0.04 (0.14)	0.09

[a] Similar interactive effects were seen for nestling and juvenile survival; I chose to further examine fledgling survival as that parameter showed the strongest interactive effects. No covariate was sufficient to remove the inbreeding effect of interest (highlighted). See Table 5.1 for definitions of abbreviations.
[b] n = 673 fledglings from 232 unique pairs.
[c] First-hatched brood of the season for each pair; n = 555 fledglings from 231 unique pairs.
[d] Woolshed Bush site only; n = 365 fledglings from 120 unique pairs.

Table 5.5. Mean (95% CI) lethal equivalents[a] for black robin fitness traits.

Fitness trait	At low $F_{♀}$	Median values	At high $F_{♀}$
Clutch size		-0.13 (-1.03, 0.78)	
Egg survival		0.97 (-0.55, 2.49)	
Nestling survival	**3.79 (0.03, 7.57)**	-0.47 (-4.23, 3.30)	**-12.65 (-16.92, -8.37)**
Fledgling survival	**10.51 (6.11, 14.92)**	1.00 (-3.39, 5.40)	**-26.18 (-31.48, -20.90)**
Juvenile survival	**7.60 (3.67, 11.52)**	3.78 (-0.14, 7.70)	**-7.11 (-11.63, -2.63)**
Adult survival		1.49 (-1.37, 4.37)	
Annual prob. breed [b]		-1.05 (-16.69, 14.63)	
Male LRS [c]		-0.91 (-3.61, 1.78)	
Female LRS		6.30 (-13.83, 26.44)	

[a] Where lethal equivalents are given only at median values, inbreeding coefficient (F) did not interact with $F_{♀}$. Negative values indicate a benefit from further inbreeding. Bold values show lethal equivalents that were significantly different from zero, based on 95% CIs. Sample sizes are given in Table 5.1 and 5.2; effect sizes estimated by model averaging are provided in Table 5.8.

[b] For females > 1 year of age

[c] Lifetime reproductive success

Table 5.6. Standardised effect sizes of covariates occurring in the top model sets for offspring survival[a].

Fitness trait	Model ID	Intercept	Site	Age♀	F♀	Brood size	F	F♂	Site·F♀	F♀·F	F♀·F♂	F♂·F	k[b]	Deviance	AIC$_C$[c]	Δ[d]	w_i[e]
Clutch size	1	0.74					-0.01						4	-36.4	80.8	-	0.213
	2	0.74					-0.01	-0.02					5	-35.7	81.6	0.753	0.146
	3	0.74		0.02			-0.02						5	-36.3	82.7	1.925	0.081
	4	0.74			0.01		-0.02						5	-36.3	82.8	1.977	0.079
Egg survival	1	0.05			0.18								4	-873.5	1755.1	-	0.054
	2	0.04			0.18	-0.08							5	-873.1	1756.2	1.074	0.032
	3	0.04			0.15		0.09						5	-873.1	1756.2	1.081	0.032
	4	0.04					0.15						4	-874.3	1756.7	1.607	0.024
	5	0.04		-0.05	0.18								5	-873.4	1756.8	1.672	0.023
	6	0.08	+		0.16								6	-872.4	1756.8	1.700	0.023
	7	0.05			0.19			-0.03					5	-873.5	1757.0	1.937	0.021
Nestling survival	1	0.07	+		-0.18		0.02	-0.21	+	0.52	-0.37		12	-502.8	1030.0	-	0.060
	2	0.10	+	-0.10	-0.21		0.01	-0.21	+	0.52	-0.37		13	-502.3	1031.0	1.005	0.036
	3	0.08	+		-0.22		0.02	-0.19	+	0.63	-0.33	-0.22	13	-502.3	1031.0	1.065	0.035
	4	0.07	+		-0.18	0.06	0.01	-0.21	+	0.51	-0.36		13	-502.6	1031.6	1.609	0.027
	5	0.02	+		0.19								6	-509.7	1031.6	1.621	0.027
	6	0.03	+		0.20			-0.14					7	-508.8	1031.8	1.863	0.024
	7	0.08	+		-0.17		-0.03	-0.20	+	0.35			11	-504.8	1031.9	1.938	0.023
Fledgling survival	1	0.95	+		-0.17		0.00	-0.30		0.87			9	-281.8	582.0	-	0.159
	2	0.95	+		-0.17		-0.02	-0.29		0.80	0.16		10	-281.6	583.6	1.668	0.069
	3	0.94	+	0.07	-0.16		0.00	-0.31		0.87			10	-281.7	583.7	1.696	0.068
	4	0.96	+		-0.18	0.05	-0.01	-0.30		0.86			10	-281.8	583.8	1.877	0.062

Table 5.6, continued

Juvenile survival	1	0.60	+			-0.28				6	-329.7	671.5	-	0.108
	2	0.61	+			-0.23	-0.13			7	-328.9	672.0	0.491	0.085
	3	0.64	+			-0.20	-0.11		-0.25	8	-327.9	672.0	0.530	0.083
	4	0.61	+		0.01	-0.24	-0.16	0.36	-0.47	10	-325.9	672.2	0.701	0.076
	5	0.58	+	0.09		-0.27				7	-329.3	672.9	1.372	0.054
	6	0.59	+	0.09		-0.22	-0.13			8	-328.6	673.4	1.876	0.042
	7	0.62	+	0.09		-0.19	-0.11		-0.25	9	-327.6	673.5	1.948	0.041

[a] Year and pair ID were included as random effects in each model. $Age_{♂}$ and interactions between site and F, $F_{♂}$, and $Age_{♀}$ were excluded from the global models to reduce multicollinearity; $Age_{♀} \cdot F_{♀}$ was not present in the top model set for any response variable (thus not shown in table); + symbol indicates a categorical variable that was included in the model. Models within each set are sorted by w_i. See Table 5.1 for definitions of abbreviations not given here.

[b] Number of estimable parameters.

[c] Akaike Information Criterion, small sample size correction.

[d] Change in AIC_C relative to the best model within each set.

[e] Relative weight of the model.

Table 5.7. Standardised effect sizes of covariates occurring in the top model sets for other fitness traits[a].

Fitness trait	Model ID	Inter-cept	Site	F	Age	Sex	Site·F	Age·F	Sex·F	Site·age	Site·sex	# yrs bred	Mean kin-ship	F·# yrs bred	F·mean kin-ship	k	Deviance	AIC_C	Δ	w_i
Adult survival	1	0.60	+		-0.19							NA	NA	NA	NA	7	-658.4	1330.9	-	0.158
	2	0.61	+	-0.03	-0.19			0.27				NA	NA	NA	NA	9	-656.5	1331.1	0.220	0.142
	3	0.56	+		-0.19	+						NA	NA	NA	NA	8	-657.8	1331.7	0.862	0.103
	4	0.58	+	-0.03	-0.19	+		0.26				NA	NA	NA	NA	10	-656.0	1332.1	1.201	0.087
	5	0.60	+	-0.02	-0.19							NA	NA	NA	NA	8	-658.4	1332.9	1.985	0.059
Prob. breed (yearling females)	1	-1.01	+		NA	NA		NA	NA	NA	NA	NA	NA	NA	NA	4	-101.9	212.0	-	0.709
Annual prob. breed (older females)	1	4.47	+			NA	NA		NA	NA	NA	NA	NA	NA	NA	6	-177.7	367.5	-	0.378
	2	4.42	+	0.36		NA	NA		NA	NA	NA	NA	NA	NA	NA	7	-177.2	368.6	1.026	0.226
	3	4.52	+		-0.21	NA	NA		NA	NA	NA	NA	NA	NA	NA	7	-177.5	369.2	1.638	0.167
	4	4.49	+	0.33	-0.06	NA	NA	1.21	NA	NA	NA	NA	NA	NA	NA	9	-175.6	369.5	1.987	0.140
Male LRS	1	0.95			NA	NA		NA	NA	NA	NA	1.35				3	-150.9	308.2	-	0.236
	2	0.98	+		NA	NA		NA	NA	NA	NA	1.38				5	-149.2	309.2	1.089	0.137
	3	0.95		-0.13	NA	NA		NA	NA	NA	NA	1.34				4	-150.7	310.0	1.852	0.094
Female LRS	1	0.58	+	0.17	NA	NA	+	NA	NA	NA	NA	1.77		-0.72		9	-142.6	305.8	-	0.222
	2	0.74			NA	NA		NA	NA	NA	NA	1.72				3	-150.4	307.0	1.258	0.118
	3	0.60	+	-0.21	NA	NA	+	NA	NA	NA	NA	1.72				8	-144.8	307.6	1.839	0.089

[a] Year was included as a random effect in the adult survival and probability of breeding models; hatch year was included in the adult survival and probability of breeding (older females) models. Interactions between site and F and site and age were excluded from the adult survival model to reduce multicollinearity. NA indicates not tested for that fitness trait. See Table 5.1, 5.2 for definitions of abbreviations.

Table 5.8. Effect sizes (mean [SE]) from centred but not standardised models used to calculate lethal equivalents.

Covariate[a]	Clutch size	Egg survival	Nestling survival	Fledgling survival	Juvenile survival	Adult annual survival	Annual prob. breeding (females)		Male LRS	Female LRS
							Yearling	Older		
Intercept	**0.74 (0.03)**	0.05 (0.05)	-0.07 (0.12)	0.91 (0.15)	**0.61 (0.11)**	**0.59 (0.08)**	**-1.01 (0.32)**	**4.47 (0.74)**	**0.96 (0.12)**	**0.58 (0.22)**
WSB	0	0.01 (0.12)	**0.64 (0.14)**	-0.29 (0.17)	**-0.30 (0.13)**	-0.10 (0.08)	**2.32 (0.41)**	**-3.30 (0.45)**	0.08 (0.21)	0.49 (0.26)
TPB	0	-0.15 (0.13)	**0.32 (0.16)**	**-0.90 (0.20)**	**-0.55 (0.16)**	**-0.57 (0.11)**	**1.96 (0.46)**	**-3.54 (0.57)**	-0.41 (0.27)	-0.17 (0.37)
Age						**-0.04 (0.02)**		-0.03 (0.08)		
Sex (male)						0.07 (0.07)				
F	-0.13 (0.46)	0.97 (0.77)	0.25 (0.96)	-0.42 (1.13)	-1.89 (1.00)	-0.20 (0.65)	0	3.47 (3.58)	-0.91 (1.38)	0.88 (3.72)
Age·F						0.49 (0.27)		2.85 (1.66)		
Age♀	0.00 (0.01)	-0.01 (0.02)	-0.02 (0.03)	0.01 (0.03)	0.02 (0.03)					
F♀	0	**1.64 (0.82)**	1.95 (1.32)	-1.60 (1.31)	0.14 (1.19)					
F♂	-0.18 (0.41)	-0.19 (0.66)	-1.09 (0.81)	**-2.34 (1.01)**	-1.06 (0.93)					
Brood size		-0.08 (0.08)	0.09 (0.08)	0.04 (0.09)						
F♀·F		0	**30.44 (13.03)**	**67.95 (18.89)**	27.24 (13.95)					
F♂·F		0	0	0	-21.43 (14.61)					
F♀·F♂	0	0	-21.29 (14.51)	12.40 (17.79)	0					
WSB·F						0	0		0	6.58 (4.90)
TPB·F						0	0		0	-8.82 (6.29)
WSB·F♀	0	0	-0.01 (2.54)	0	0					
TPB·F♀	0	0	4.81 (2.77)	0	0					
# yrs bred									**0.42 (0.05)**	**0.57 (0.06)**
F·yrs bred									0	**-1.99 (0.85)**

[a] The same covariates were tested as for the standardised models (Table 5.1); if not given in this table, they were not present in the averaged nonstandardised model for any demographic rate. See Table 5.1 for definitions of abbreviations.

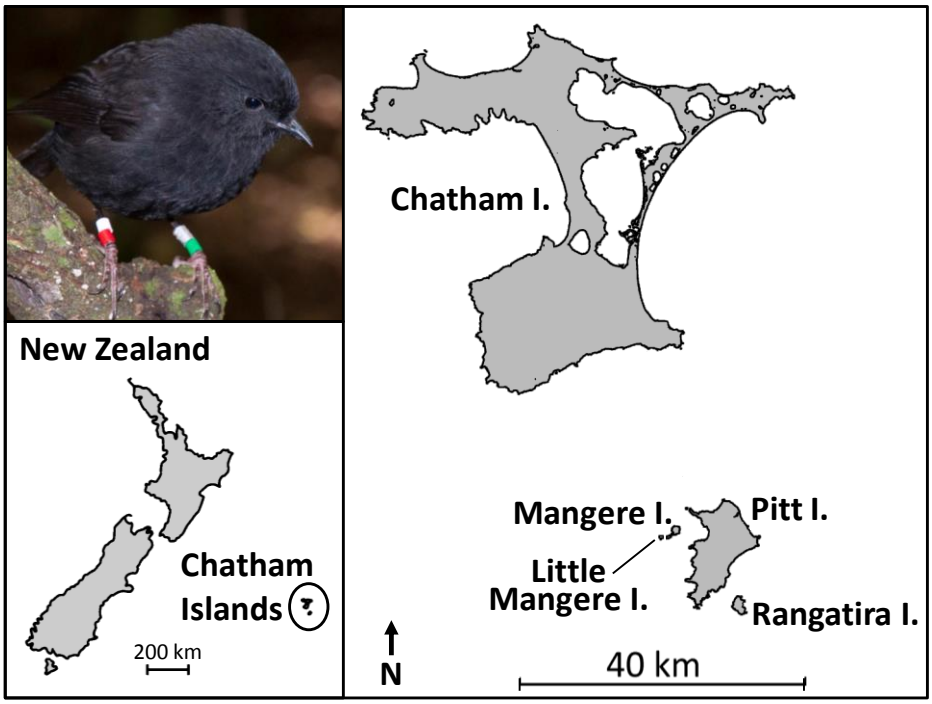

Figure 5.1. Location of the Chatham Islands and specific islands mentioned in text.

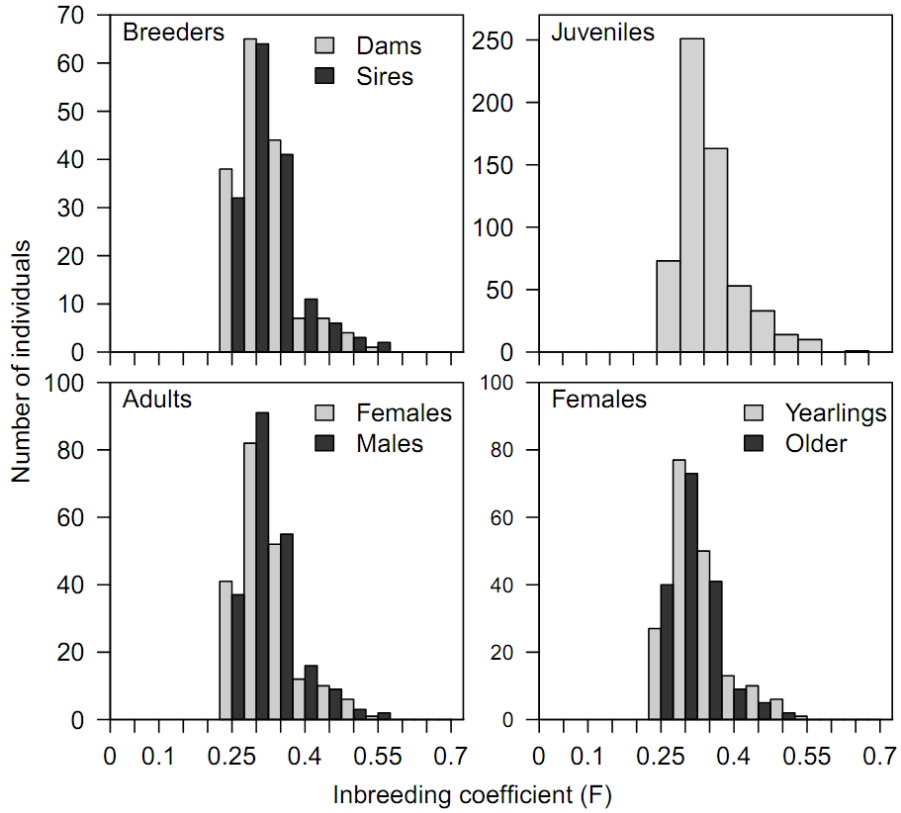

Figure 5.2. Distribution of inbreeding coefficients (F) in black robins. Data from 1990-2001 are shown for each subset of the main dataset (breeders: N = 166 females, 159 males; juveniles: N = 598; adults: N = 204 females, 213 males; females: N = 184 yearlings, 170 older).

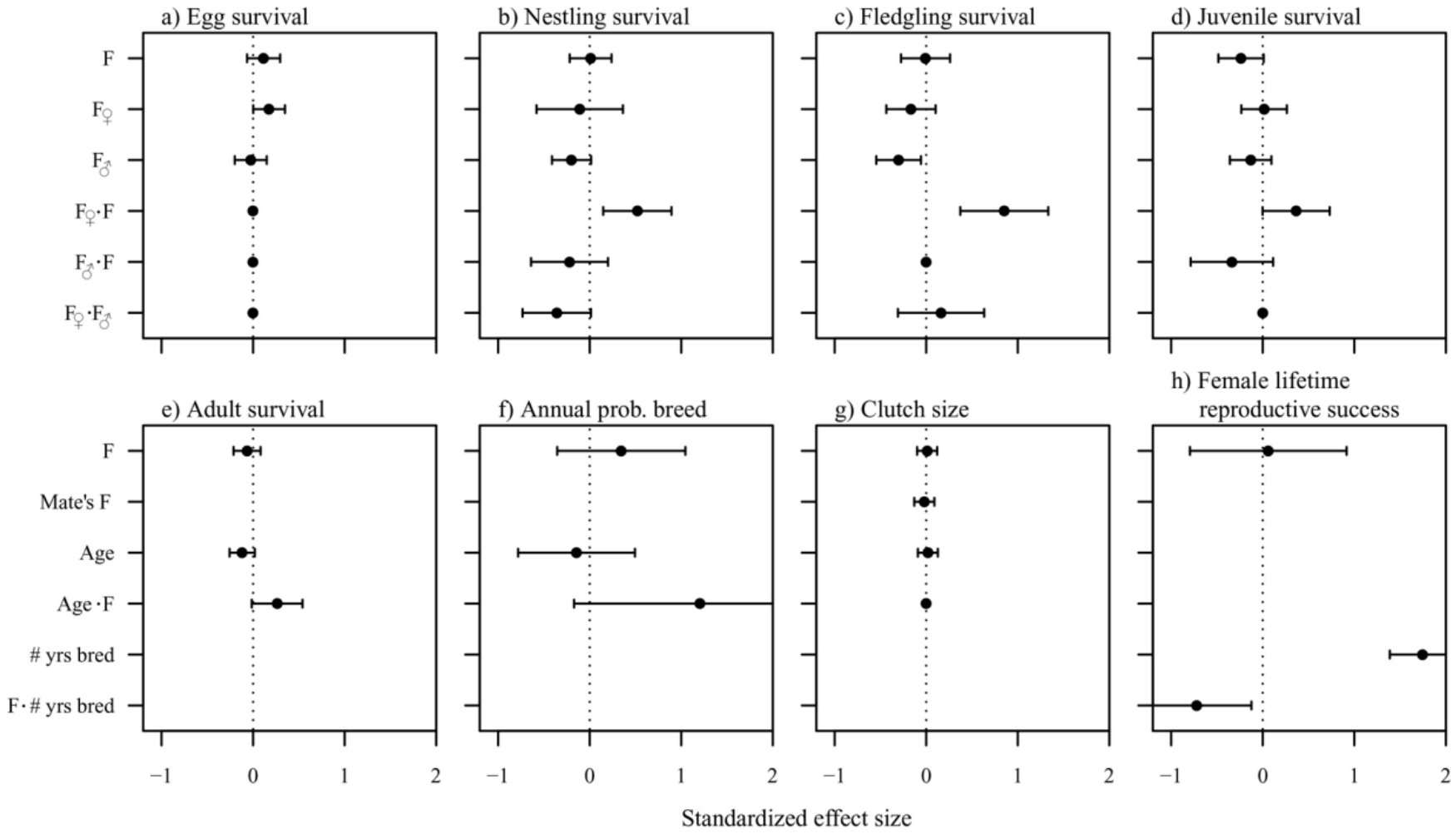

Figure 5.3. Standardised effect size and 95% CI (error bars) for each inbreeding (F) covariate of fitness traits for black robins. A point at zero with no error bars indicates a covariate that did not appear in the top model set; absence of a point indicates a covariate that was not tested for that fitness trait. Sample sizes and standardised effect sizes of other covariates are given in Table 5.1 and 5.2; top model sets used in model averaging are given in Table 5.6 and 5.8.

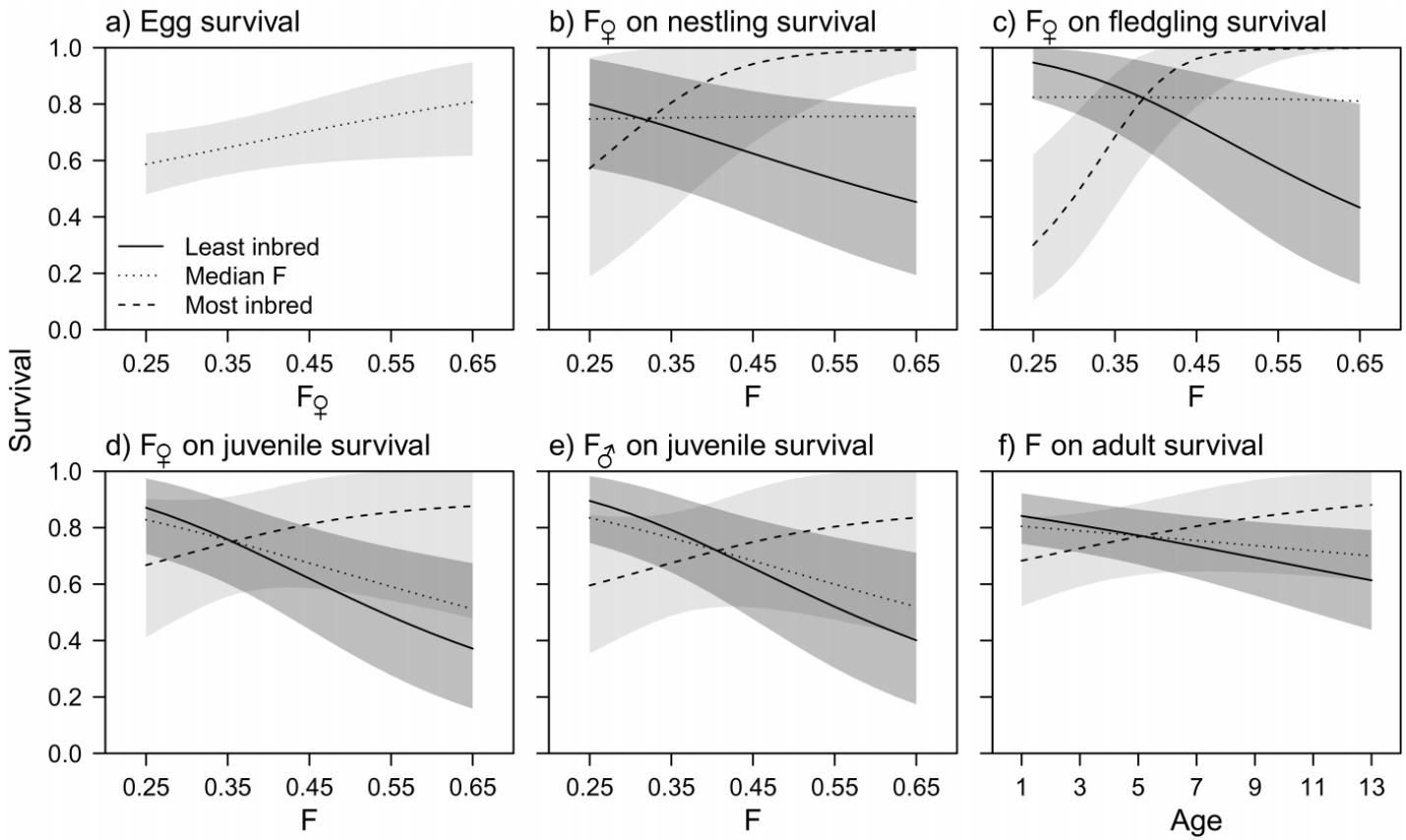

Figure 5.4. Effects of inbreeding and interacting covariates on fitness traits in the black robin. Predictions were generated by model averaging, and indicates a weighted average across sites. Each line on each plot indicates survival predicted at the low (0.27), median (0.34), or high (0.54) value of the inbreeding covariate indicated above each panel across the range of values of the covariate indicated on the horizontal axis. No covariates interacted with F♀ in the model for egg survival (a). Shaded bands indicate 95% confidence intervals. Sample sizes are given in Table 5.1.

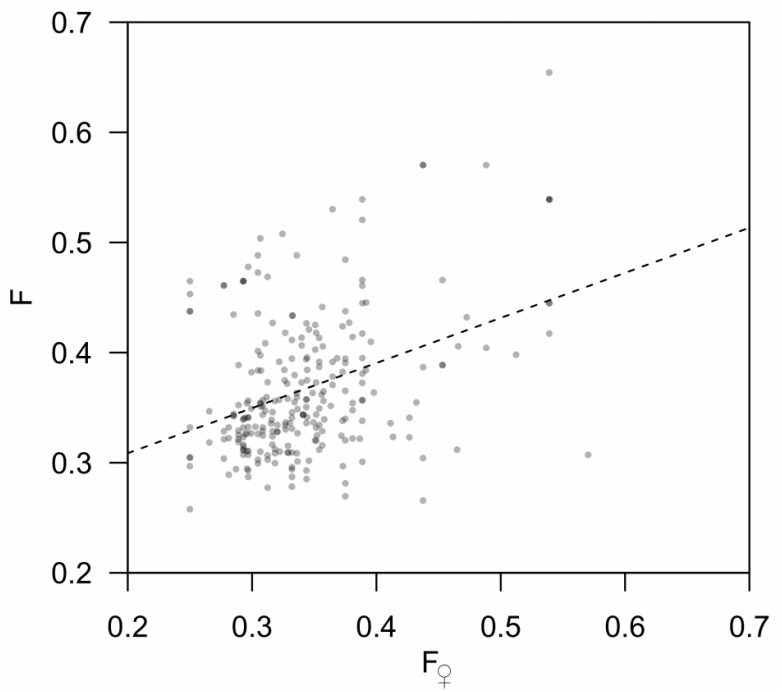

Figure 5.5. Linear relationship between F and $F_{♀}$ of breeding pairs. $R^2 = 0.1395$, $p < 0.001$, N = 256 unique pairs. Each grey point indicates one unique breeding pair.

Chapter 6. Integrating complex effects of inbreeding into population viability analysis



Juvenile (left) and adult (right) black robins on Rangatira Island, New Zealand.

A version of this chapter is being prepared for submission to Conservation Biology as: Weiser, E.L., C.E. Grueber, E.S. Kennedy, M.M. Massaro, and I.G. Jamieson. Will inbreeding save the black robin? Integrating complex inbreeding effects into population viability analysis.

Abstract

Although it is well known that inbreeding can jeopardize population viability, effects of inbreeding are only rarely fully incorporated into population viability analyses. Such effects are difficult to quantify, and thus not often described for threatened species that would benefit from predictions of population viability. I conducted a population viability analysis for the black robin, a well studied, highly inbred, endangered passerine endemic to the Chatham Islands, New Zealand. When I incorporated all available information for all fitness traits, including complex effects of inbreeding (Chapter 5), both existing black robin populations were expected to persist over the next 100 years. The strong effect of inbreeding on the prediction of viability was revealed when I simulated additional scenarios with fewer or no inbreeding effects: both populations showed slower growth and lower viability than when all inbreeding effects were incorporated. My analysis demonstrates that predictions of viability strongly depend upon estimated effects of covariates as well as demographic rates. Incomplete consideration of these effects could produce very misleading predictions for small, isolated populations, even when mean demographic rates are estimated correctly.

Introduction

Although maintaining allelic diversity is crucial to long-term viability of a population, inbreeding can be another important factor. This is especially true for populations that exist in small numbers or have experienced genetic bottlenecks (Madsen et al. 1999; Saccheri et al. 1998; Westemeier et al. 1998). Although negative effects of inbreeding on fitness (inbreeding depression) have been well documented in wild populations (Crnokrak & Roff 1999; Keller & Waller 2002), precise measurements of inbreeding effects are still only rarely included in predictive models used to guide management of populations of conservation value, e.g. population viability analysis (PVA; Beissinger & McCullough 2002).

One of the main reasons that inbreeding depression has not been better addressed in PVA is that inbreeding effects are difficult to quantify (Allendorf & Ryman 2002). Efforts to do so require detailed data on fitness, along with either a complete, accurate pedigree (e.g. Haig et al. 1993) or detailed genetic data (e.g. Johnson et al. 2011) to measure relatedness of individuals. These data are difficult to collect, and most studies of

inbreeding depression have used populations of non-threatened species as feasible case studies (e.g. >90% of taxa reviewed by Crnokrak and Roff [1999]). Because inbreeding effects vary among taxa (Keller & Waller 2002) and in response to population history (Bouzat 2010), information about inbreeding depression in large populations cannot be directly applied to small or bottlenecked populations (Grueber et al. 2008b) for which a PVA would be especially useful.

Another issue with incorporating inbreeding into PVAs is that studies of inbreeding have often focused on only one fitness trait (O'Grady et al. 2006). Such studies therefore cannot be used to assess inbreeding depression across the full lifecycle of a species (notable exceptions include Grueber et al. 2010; Keller 1998; Szulkin et al. 2007). Most PVAs that do incorporate inbreeding depression have simulated effects on juvenile survival only (Allendorf & Ryman 2002), and many of these have used a very general estimate of lethal equivalents (LE). However, the most commonly used value (LE = 3.14, derived from captive populations; Ralls et al. 1988) has been shown to underestimate inbreeding effects in most wild populations (Crnokrak & Roff 1999; O'Grady et al. 2006). Additionally, effects of inbreeding can interact in complex ways with other factors, such as environment (Armbruster & Reed 2005), age (Charlesworth & Hughes 1996), or sex (Coulson et al. 1999). Attempts to simplify such effects could misrepresent their net effects on population growth and persistence. Although it is well known that inaccurate demographic data produce PVAs that can be very misleading (Coulson et al. 2001; Patterson & Murray 2008), the implications of oversimplifying relationships among covariates, especially inbreeding coefficients, have not been well documented. I explore these implications with information from an unusually detailed study of a threatened species that has a history of isolation and inbreeding: the black robin. See Chapter 5 for relevant details of the history and current status of this species.

Although the black robin story demonstrates that intensive management can recover a species in even the most extreme cases (Butler & Merton 1992), there is concern about whether the black robin will continue to be self-sustaining. The species is currently listed as endangered by the IUCN because of its small population size and potential vulnerability to stochastic events (IUCN 2013). Moreover, although the Rangatira I. population has continued to grow, recent censuses have revealed that the Mangere I. population is smaller now than at the end of the intensive monitoring period and growing very slowly (see Results). This decrease occurred despite some habitat regeneration in the

meantime (Atkinson 2003) that should have increased the island's carrying capacity. The cause and exact timing of the decline and the following slow recovery are not known.

The extremely high level of relatedness in this species is also worrying. By the end of the intensive monitoring period in 1998, repeated inbreeding had resulted in mean inbreeding levels among the highest recorded for any wild population (Kennedy et al. 2013; Chapter 5). Inbreeding accumulates in small populations (Keller & Waller 2002), so any fitness effects of inbreeding for the black robin will likely become more pronounced over time and could threaten long-term persistence. My assessment of inbreeding effects across all life-history stages during the intensive monitoring period revealed a complex mix of negative, neutral, and unexpected positive effects of inbreeding on different measures of fitness in both black robin populations (Chapter 5). The implications of these diverse effects for the viability of the species were therefore unclear.

I incorporated these complex effects, along with information on population size and demographic rates from recent surveys, into a PVA to evaluate the probability of the black robin populations persisting over the next 100 years (> 30 black robin generations). I used the PVA to evaluate the effects of inbreeding on viability and assess sensitivity of the predictions to variation in key parameters and covariates. Although the bottleneck experienced by the black robin was extreme, many other threatened species share a similarly long history of isolation on offshore islands. The quality and quantity of data associated with long-term monitoring of this species provide a valuable opportunity to examine implications of inbreeding in threatened populations. My findings are especially useful in elucidating consequences of complex relationships between inbreeding covariates and demographic viability in small populations that have been isolated for many generations, and highlight the importance of including complete information about inbreeding effects in predictive models.

Methods

With this analysis, I was primarily interested in assessing the effects of inbreeding on population viability. To make an accurate assessment, I used all available information to describe every aspect of demography and other factors influencing population dynamics for the black robin. This information consisted of demographic rates recorded during the intensive monitoring period, including assessment of effects of covariates on these rates;

estimated carrying capacity; and documented population sizes and removal of birds over the past decade (all detailed below).

I also included information on coarse spatial structuring of the population on Rangatira I. Since reintroduction to the island, black robins have occupied two areas known as Woolshed Bush and Top Bush (Kennedy 2009). The border between these two sites has traditionally been delineated by an open strip of low vegetation known as "Skua Gully" which lies between the two forested areas. Black robins in Woolshed Bush have historically exhibited higher survival rates (for chicks, juveniles, and adults) than those in Top Bush (Table 6.1), perhaps owing to its lower altitude or an as-yet-undefined difference in habitat between the two areas (Kennedy 2009). Individuals move between Woolshed Bush and Top Bush, so the two areas comprise one population, but with different demography. I therefore assessed demographic rates separately in these two sites to inform the PVA, and also simulated dispersal of individuals between the two. Mangere I. was included in the PVA as an additional, fully isolated site.

Unless noted otherwise, all statistical analyses used to develop the input parameters were conducted in R 3.0.0 (R Development Core Team 2013). I conducted the PVA in VORTEX version 9.99c (Lacy et al. 2009).

Demographic rates

Methods for estimating demographic rates and effects of relevant covariates on each rate are described in detail in Chapter 5. Briefly, I used demographic data from the monitoring period (1990-2001) to assess the effects of predictors, including inbreeding coefficient and interactions as appropriate, on each demographic response variable with linear mixed-effects models and model averaging (following Grueber et al. 2011). This analysis differed from the one reported in Chapter 5 only in that I centred, but did not standardise, the predictors to quantify effects on the natural scale and enable them to be incorporated into the PVA. To facilitate input into VORTEX, I also examined total offspring survival from egg to independence from parents, in Chapter 5 I had examined egg, nestling, and fledgling survival separately.

Inbreeding effects (individual's inbreeding coefficient F, mother's F, father's F, and interactions among these) were the primary focus of this analysis, but I also incorporated other potential covariates (e.g. age and sex) to most accurately describe demography and account for any covariates that could confound inbreeding effects. I included effects of site (Mangere I., Woolshed Bush, and Top Bush) and relevant random

effects to account for repeated measurements from individuals, breeding pairs, and years. I derived the estimates of inbreeding from a complete social pedigree, assumed to accurately represent the genetic pedigree of this species (Kennedy 2009; Kennedy et al. 2013; Chapter 5). The response variables included all major fitness traits of this species: probability of breeding for females, clutch size, offspring survival (from the time that the egg was recorded by field workers to the time that the chick was independent of parental care, about 35-65 days after fledging [Kennedy 2009]), juvenile survival (from independence to the beginning of the next breeding season), and adult annual survival.

Carrying capacity

To estimate the initial (1998) carrying capacity of each site, I used WinBUGS (Lunn et al. 2000) via R package R2WinBUGS (Sturtz et al. 2005) to fit a Gompertz model to census counts from the intensive monitoring period. This analysis used the linear form of the discrete-time density-dependent Gompertz model, such that $\ln(N_t) = \alpha + \beta*\ln(N_{t-1})$ where N is population size at time t (Dennis et al. 2006), to fit the data on population sizes during the intensive monitoring period. I used the asymptote approached by the fitted line projected out 100 years as the initial carrying capacity in the PVA.

Black robins use forest habitat, which has been regenerating on both Rangatira and Mangere I. since livestock were removed in 1961 and 1968, respectively (Atkinson 2003; Nilsson et al. 1994). I therefore expected the carrying capacity of each site to increase over time from the 1998 estimate generated by the Gompertz model. I used historical observations from each island in conjunction with recent estimates to estimate the rate at which forest has regenerated (Table 6.2). I estimated the linear rate of increase as the average number of hectares regenerated per year between historic and recent observations. Although there is little evidence to support (or refute) that the rate of reforestation has followed a linear trend, and it is possible that regeneration may have been faster immediately following livestock removal, recent reforestation has been anecdotally observed to be slow. Reforestation on Rangatira I. has been hindered by activity of burrowing seabirds (Roberts et al. 2007) and also by *Muehlenbeckia australis*, a climbing vine that limits establishment of other species by blanketing open areas and young trees (C. Roberts, unpubl. data; E.S. Kennedy & M. Massaro, pers. comm.). Reforestation on Mangere I. has been aided by tree-planting efforts (Atkinson 2003), but regenerating habitat is mostly not yet suitable for black robins. If replanting increases the rate of

growth of carrying capacity on Mangere I., predictions based on the linear growth rate will be conservative.

Based on plans for revegetating Mangere I. (Atkinson 2003) and personal observations on Rangatira I. (E.S.K. unpubl.), I estimated the maximum future extent of black robin habitat on the two islands as 29 and 161 ha, respectively. Given the linear rate of increase estimated from historic data, I projected the time to reach maximum forested extent on each island (Table 6.2). Mangere I. could potentially hold up to 81 ha of black robin habitat if revegetation work is extended (E.S.K. unpubl.), but I conservatively used the lower value of 29 ha to assess future population size under current management plans. For Rangatira I., I estimated the maximum extent of each area (Woolshed Bush and Top Bush) separately; but I assumed that they would share the same rate of growth, as the historical data was not separated by area (thus I could only estimate the rate of reforestation for the island as a whole). Woolshed Bush was expected to increase its area by 112% while Top Bush was expected to grow by only 26%, so the former was predicted to reach its maximum extent well after the latter (Table 6.2).

To inform the PVA, I translated the rate of increase in habitat into a rate of increase in carrying capacity. I assumed that the annual growth in carrying capacity was proportional to the annual increase in available habitat. That is, for Mangere I., initial habitat extent was 7.9 ha, increasing by 0.19 ha (2.4% of the initial extent) per year. Thus I assumed that carrying capacity would also increase by 2.4% of the initial carrying capacity (61 birds) per year, or a rate of 1.47 birds/year, until the year in which maximum habitat extent had been reached (Table 6.2). Black robin density at carrying capacity therefore remained constant as available habitat increased. I assumed no further change in habitat or carrying capacity once the maximum extent was reached.

Dispersal on Rangatira Island

Within the Rangatira I. population, black robins are known to move between Woolshed Bush and Top Bush (Kennedy 2009). I estimated dispersal rates as the average annual proportion of individuals from one area that moved to the other for the subsequent breeding season, separated by age class (juvenile vs. adult). I used centered linear mixed-effects models and model averaging as described above to test for effects of covariates on dispersal probabilities. I also tested for additional mortality during dispersal by comparing the 95% binomial confidence limits (Wilson score interval with continuity correction;

Newcombe 1998) for survival probability between dispersing and sedentary birds, for each age class and site.

Recent information

The PVA started in 1998, the last year in which a complete pedigree was recorded for the species. However, some information was available on demography and management actions in more recent years, so I also incorporated this information into the PVA.

First, 34 black robins were removed from Rangatira I. and 4 from Mangere I. in an effort to establish a third population on Pitt Island in 2002-2005 (Kennedy 2009). The effort was unsuccessful and all translocated birds died or disappeared. I included this event in the PVA, denoting each translocated bird as a loss to its respective source population in the corresponding year.

Second, after an unsuccessful attempt at mark-resight sample monitoring, managers reverted to individually marking and counting all birds in both populations in 2007 (Kennedy 2009). Most individuals on Mangere I. were banded by 2008 and on Rangatira I. by 2011 (Department of Conservation and M. Massaro, unpubl. data), enabling near-complete censuses to resume. I incorporated pre-breeding (October-November) census information from 2008-2012 for Mangere I. and from 2011-2012 for Rangatira I. into the PVA, limiting each simulated population to the recorded size in each year. Although a population census had also been conducted in 2010 for Rangatira I., many (at least 25) birds remained unmarked at that time, so there was some uncertainty around the count. Test simulations indicated that limiting the simulated population to the number (186) recorded in 2010 (Massaro et al. 2013b) prevented it from being able to reach the number (234) recorded in 2012, by which point nearly all birds were marked so the census count was likely close to accurate (Bliss 2013). This had a long-lasting negative effect on the simulated population (reduced size for >15 yrs), so I excluded the 2010 data from the model to ensure that the simulated population matched the observed 2012 census. I also did not include 2013 data because some birds may have been missed during that count (e.g. 24 birds were missed during the 2012 pre-breeding census but later observed during the post-breeding census; Bliss 2013), and the post-breeding census (scheduled for March 2014) had not yet occurred at the time of my analysis.

Finally, the surveys and censuses described above and a breeding study on Rangatira I. (Massaro et al. 2013a; M. Massaro unpubl.) provided some estimates of

survival and reproductive output in recent years. I estimated juvenile and annual adult survival for banded birds at all three sites in 2003-2012, and reproductive output (hatching and fledging rates) of banded breeding pairs in Woolshed Bush in 2008-2011. I compared these estimates to those from the earlier intensive monitoring period to assess whether demographic rates had changed over time.

Population viability analysis

I incorporated all of the above information into VORTEX (Lacy 2000; Lacy et al. 2009) for PVA over 115 years (starting in 1998 and predicting over 100 years from 2013, the date of the analysis), and simulated 1000 replicates. Appendix C provides detailed information about the specific methods used to input all information into my analyses using VORTEX's more sophisticated options. In brief, I implemented all covariate effects into functions describing each demographic rate, including uncertainty in effect sizes. I combined the output from the Woolshed Bush and Top Bush areas to generate overall trends for Rangatira I.

The main scenario represented my best estimate of the trajectory for each population, using all parameters described above and assuming no genetic or environmental change (aside from continuing habitat regeneration). In addition, I simulated several modified scenarios to examine causes of vulnerability.

I quantified the demographic effects of inbreeding by simulating several scenarios:

1. Excluding any effects of inbreeding.
2. Including all known effects of inbreeding on juvenile survival, but not on any other fitness trait. This model enabled us to assess the effects of using limited but species-specific information about inbreeding effects.
3. Excluding positive effects of inbreeding. The positive effects in this species are thought to result from a "proven-homozygote advantage," whereby offspring with genotypes very similar to those of their parent(s) have a fitness advantage (Chapter 5). Such an advantage has not previously been documented in wild populations, so I explored its effect by simulating a scenario that excluded positive effects of inbreeding, but included negative effects.

I simulated each of these alternative inbreeding scenarios after re-evaluating the generalised linear mixed-effects models for demographic parameters while excluding the indicated covariates.

Next, I assessed the sensitivity of the population viability predictions to variation in key demographic rates (probability of breeding and offspring, juvenile, and adult survival) under the main scenario. For each sensitivity analysis, I reduced one key rate by 10% (relative to the original rate; i.e. a survival probability of 0.80 was reduced to 0.72), and examined the resulting change in the probability of extinction of each population. Because there was uncertainty in whether I had accurately predicted habitat expansion, I also predicted population viability when the rate of increase in carrying capacity was halved or doubled relative to the best estimate. I additionally predicted viability when there was no further growth from the most recent estimates of population size (lower than predicted carrying capacity) as a "worst-case" scenario for carrying capacity.

Results

Demography and dispersal

Black robins at all three sites were extremely inbred at the beginning of the PVA (Table 6.1), and inbreeding and other covariates (and their interactions) affected a variety of demographic rates (Table 6.3) and dispersal rates (Table 6.4). None of the mean demographic values differed between the intensive monitoring period and recent years, on the basis of comparing 95% confidence intervals (Table 6.5). I therefore considered all demographic rates estimated from the intensive monitoring period to be representative of current and future demography at these sites. Juveniles often dispersed between Woolshed Bush and Top Bush during the intensive monitoring period, while adults were usually sedentary (Table 6.1, 6.4). Juveniles that dispersed from Top Bush to Woolshed Bush tended to have higher survival than those that remained in Top Bush (Table 6.6); this difference was not quite statistically significant, so I conservatively assumed that survival was the same (at the lower value calculated for sedentary juveniles) for both sedentary and dispersing birds originating in Top Bush.

Carrying capacity and habitat expansion

The current carrying capacity estimated for Top Bush (27 individuals) was lower than the observed census size in several years between 1990 and 1998, so I used the maximum pre-breeding census count from the 1990s (34 individuals; Kennedy 2009) as initial carrying capacity for Top Bush to better reflect known population trends in this area. Top Bush could be sustained above the Gompertz-estimated carrying capacity

because it is supplemented by immigration from Woolshed Bush, which compensates for the low survival in Top Bush (Table 6.1). The Gompertz model did not include information about immigration, so may have underestimated the maximum size of Top Bush. Given estimated habitat regeneration rates and the estimated maximum extent of forest, carrying capacity was expected to increase substantially over 111 years in Mangere I. and 143 years in Woolshed Bush, with a small increase over 34 years in Top Bush (Table 6.1).

Best predictions of viability

In the main scenario using all information and covariates, both populations were expected to grow to carrying capacity (Figure 6.1, dark red lines) and showed very low or no chance of extinction over the 115-year simulated period (Figure 6.2). Uncertainty was high in scenarios with slower population growth; the main scenario predicted the highest growth rates and populations consistently approached carrying capacity (Figure 6.3). Recent censuses showed that the Mangere I. population declined between 2001 and 2008, and Rangatira I. grew more slowly than expected; the PVA incorporated that information and therefore showed a decline in each population corresponding to the first year in which recent census information was available (Figure 6.1a, b).

Effects of inbreeding on predictions

Eliminating all demographic effects of inbreeding (Table 6.7, 6.8) resulted in moderately decreased predicted growth rates for both populations (Figure 6.1, dashed green lines), and increased the extinction probability of Mangere I. above my accepted threshold of 0.05 (Figure 6.2). Including inbreeding effects for only juvenile survival further decreased predicted growth of each population (Figure 6.1, dotted blue line), and predicted $PE > 0.05$ for both populations (Figure 6.2). Excluding positive effects of inbreeding (but including negative effects; Table 6.9) resulted in even more pessimistic predictions for growth (Figure 6.1, dashed orange lines) and persistence (Figure 6.2).

Sensitivity analysis

The sensitivity analysis revealed that some demographic rates were particularly important in determining predicted population growth and persistence. Adult survival was an important driver of extinction probability in both populations, but especially in Rangatira I. (Figure 6.2). In contrast, changes to probability of breeding or juvenile

survival had negligible effects on viability. Reducing offspring survival by 10% resulted in near certainty that Mangere I. would become extinct within the next 100 years, while having no effect on the extinction probability of Rangatira I. (Figure 6.2). The positive effect of mother's inbreeding level on offspring survival was stronger in both Woolshed Bush and Top Bush than in Mangere I. (Table 6.3), which may have helped to compensate for the reduction in offspring survival on Rangatira I. Moreover, the original offspring survival value for Mangere I. was low (relative to Woolshed Bush); my results suggest that the original survival rate was barely sufficient to sustain the population and a further reduction was not sustainable. Changing the rate of expansion in carrying capacity did not affect the predicted viability of either population; even with no further growth, both populations were expected to persist over the simulated period (Figure 6.2).

Discussion

Under current conditions, these analyses indicated that both black robin populations were expected to persist over the next 100 years - a remarkable prognosis for a species that underwent a single-pair genetic bottleneck. In contrast to the negative effects of inbreeding on demographic viability observed in most species (O'Grady et al. 2006), the complex effects of inbreeding on fitness produced a net positive effect on growth and demographic viability of both black robin populations. Continuation of this positive effect is critical to the persistence of the species: when positive effects of inbreeding on fitness were omitted in the simulations (so that only negative inbreeding effects remained), both populations showed a substantial chance of extinction within 100 years. Even when all inbreeding effects (both positive and negative) were omitted, the populations showed slower growth and a greater chance of extinction than currently predicted. Inbreeding therefore clearly has a net positive effect on viability of the species as a whole, even though only some individuals (i.e. the most inbred and closely related breeding pairs) experience the benefits directly (Chapter 5).

Crucially, my predictions rested on the assumption that conditions would not change over the next 100 years. Environmental change or introduction of a novel pathogen can present a serious threat to any species that has little allelic diversity (Allendorf & Luikart 2007; Markert et al. 2010). In severely bottlenecked species such as the black robin, whose persistence may not be threatened by inbreeding *per se*, genetic

management remains important for maximising genetic diversity and thus adaptive potential over the long term. I will further explore this consideration in Chapter 7.

To my knowledge, this analysis is the first to indicate that inbreeding can improve viability of a species in the wild, at least under specific (perhaps unusual) circumstances. Genetic purging has previously been suggested as a mechanism that may nullify negative effects of inbreeding, but this process is unlikely to be effective in wild populations (Keller & Waller 2002). If present, purging would result in a neutral effect of inbreeding on fitness (i.e. a lack of inbreeding depression) rather than the positive effects observed here. In addition, there was no evidence of purging in the black robin for the major life-history trait of juvenile survival (Kennedy et al. 2013). In Chapter 5, I suggested that the positive effects resulted from a "proven-homozygote advantage." Although this advantage may be particularly relevant to species like the black robin that have undergone severe bottlenecks and extreme inbreeding, it is also potentially relevant to other isolated species that have very little genetic diversity (i.e. are composed of highly homozygous individuals). Further investigation into the generality of the proven-homozygote advantage in wild populations will be especially useful for understanding how complex effects of inbreeding can impact population viability.

Limiting the amount of inbreeding data in the simulations had effects on the predictions that were sometimes even larger than the effects of changing mean demographic rates. If I had not fully assessed inbreeding effects on all life-history stages, I would have drawn very different (more pessimistic) conclusions about the viability of the black robin and the management effort required to sustain this species. It has long been recognised that parameterising a PVA with inappropriate demographic rates can severely affect the accuracy of the predictions (Coulson et al. 2001; Patterson & Murray 2008). Incomplete assessment of inbreeding effects has also been indicated as a source of error in PVAs (Allendorf & Ryman 2002), but is still widely implemented (e.g. Daleszczyk & Bunevich 2009; Hu et al. 2013; Miller et al. 2009; Thirstrup et al. 2009). My findings demonstrate that it is equally important to accurately assess both demographic rates and covariates of fitness. Relying upon more general estimates (from outbred or mildly inbred populations) could drastically misinform management efforts designed to conserve threatened species.

Table 6.1. Black robin demographic rates at three sites[a].

		Rangatira Island	
	Mangere Island	Woolshed Bush	Top Bush
Initial (1998) population[b]			
# pre-breeding adults	51	113	33
Inbreeding coefficient F	0.377 (0.057)	0.344 (0.043)	0.353 (0.049)
Carrying capacity[c]			
Initial (year 1998)	61	361	34
Maximum	224	763	43
Years until max. reached	111	143	34
Annual reproductive rates			
% yearling females breeding[d]	27 (9.6)	79 (9.5)	72 (9.6)
% females > 1 yr breeding[d]	99 (6.6)	76 (3.8)	72 (10.1)
Clutch size[d]	2.10 (0.106)	2.10 (0.070)	2.10 (0.128)
% breeding females producing[e]:			
1 clutch	60.0	45.9	34.3
2 clutches	36.3	45.8	53.6
3 clutches	3.7	7.0	11.2
4 clutches	0	1.3	0.85
Annual survival rates			
Offspring (egg to independence)	0.345 (0.159)	0.432 (0.090)	0.253 (0.108)
Juvenile	0.841 (0.112)	0.744 (0.059)	0.654 (0.131)
Adult	0.835 (0.090)	0.851 (0.076)	0.639 (0.122)
Annual dispersal rates			
Juvenile	0	0.165 (0.067)	0.166 (0.145)
Adult	0	0.015 (0.014)	0.008 (0.016)

[a] Values are given as mean[a] (SD, where relevant) and were estimated from generalised linear mixed effects models where indicated. These values are given here for the reader's reference; functions describing relationships with covariates were used to parameterise those values in the population viability analysis (see Appendix C and Figure 6.2).

[b] The full pedigree for the species, 1979-1998, was used to inform the simulation.

[c] Also adjusted for recent removal of birds and annual census information (see Methods).

[d] Percent of females producing each number of clutches per year, averaged over all years in the dataset.

[e] Percent of breeding females producing each number of clutches.

Table 6.2. Information used to assess habitat regeneration and growth in carrying capacity for three black robin sites.

		Rangatira I.		
	Mangere I.	Total	Woolshed Bush	Top Bush
Historic habitat extent (yr)	1.7 ha (1976)[a]	72 ha (1954)[b]		
1998 habitat extent[c]	7.9 ha	110 ha	26 ha	84 ha
Habitat growth: ha/yr	0.19	0.86		
% of 1998 extent/yr	2.4%	0.78%	0.78%	0.78%
Maximum habitat extent	29 ha[b]		55 ha[d]	106 ha[d]
Year max. extent reached	2109		2141	2032
K in 1998[e]	61		361	34
K at max. habitat	224		763	43

[a] Flack (1977).
[b] Atkinson (2003).
[c] Kennedy (2009).
[d] E.S. Kennedy (unpubl.).
[e] Gompertz estimate; this study. *K* = carrying capacity.

Table 6.3. Effect size and standard error estimated by model averaging for each centred covariate of black robin demographic rates.

	Prob. breed (yearling ♀)[b]		Annual prob. breed (older ♀)[b]		Clutch size[c]		Offspring survival[d]		Juvenile survival[d]		Adult annual survival[d]	
Covariate[a]	Mean (SE)	RI[e]	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI
Intercept	-1.01 (0.32)	-	4.47 (0.74)	-	0.74 (0.03)	-	-0.86 (0.14)	-	0.61 (0.11)	-	0.59 (0.08)	-
WSB	2.32 (0.41)	1.00	-3.30 (0.45)	1.00	0	0	0.29 (0.16)	1.00	-0.30 (0.13)	1.00	-0.10 (0.08)	1.00
TPB	1.96 (0.46)	1.00	-3.54 (0.57)	1.00	0	0	-0.37 (0.10)	1.00	-0.55 (0.16)	1.00	-0.57 (0.11)	1.00
Age			-0.03 (0.08)	0.34							-0.04 (0.02)	1.00
Sex (male)											0.07 (0.07)	0.40
F	0	0	3.47 (3.58)	0.40			-0.05 (1.09)	1.00	-1.89 (1.00)	1.00	-0.20 (0.65)	0.50
Age♀					0.00 (0.01)	0.22	-0.03 (0.03)	0.44	0.02 (0.03)	0.28		
F♀					0	0	0.21 (1.99)	1.00	0.14 (1.19)	0.16		
F♂							-1.97 (0.94)	1.00	-1.06 (0.93)	0.67		
Age·F			2.85 (1.66)	0.15							0.49 (0.27)	0.40
Age♀·F♀					0	0	0		0			
F♀·F							34.72 (12.88)	1.00	27.24 (13.95)	0.16		
F♂·F							0		-21.43 (14.61)	0.41		
F♀·F♂					0	0	-15.47 (15.05)	0.28	0			
WSB·F♀					0	0	4.38 (2.77)	0.25	0			
TPB·F♀					0	0	4.35 (3.00)	0.25	0			

[a] Effects were estimated by model averaging for each centred covariate for Mangere I. (reference population; 1990-2001) and for the Woolshed Bush (WSB) and Top Bush (TPB) areas within the Rangatira I. population (1990-1998). F is the inbreeding coefficient; F♀ and F♂ are F of the dam and sire, respectively; ages of dam and sire are denoted the same way. In addition to the covariates shown here, the interaction population·F was tested but not included in the averaged model for probability of breeding (yearling females) and adult annual survival; Age♀·F♀ for clutch size and offspring survival; and population·age and population·sex for adult annual survival. Blank cells indicate that the covariate was not included in global model for a particular fitness trait (not relevant, prevented convergence, or not accepted as input by VORTEX); a value of zero indicates that the covariate was tested but was not present in the top model set.

[b] Logit-transformed.

[c] Poisson-transformed.

[d] Binomial-transformed (complementary log-log link function).

[e] Relative importance of each covariate in the averaged model.

Table 6.4. Effect size and standard error estimated by model averaging for each centred covariate of black robin dispersal rates.

	Juvenile dispersal probabililty[b]		Adult dispersal probability[b]	
Covariate[a]	Mean (SE)	RI	Mean (SE)	RI
Intercept	-1.62 (0.14)	-	-4.20 (0.35)	-
TPB	-0.19 (0.32)	0.22	-0.29 (0.81)	0.17
Age			-0.18 (0.27)	0.20
F	-2.05 (2.41)	0.27	3.47 (5.12)	0.19

[a] Effects were estimated by model averaging for each centred covariate for the Woolshed Bush (WSB, reference site) and Top Bush (TPB) areas within the Rangatira I. population (1990-1998). See Table 6.3 for definitions of abbreviations.
[b] Logit-transformed.

Table 6.5. Survival rates[a] of dispersing and sedentary black robins on Rangatira I.

	Woolshed Bush		Top Bush	
	Juveniles	Adults	Juveniles	Adults
Sedentary	0.687-0.793 (273)	0.724-0.783 (844)	0.447-0.661 (88)	0.508-0.640 (226)
Dispersed[b]	0.692-0.907 (56)	0.202-0.882 (7)	0.660-0.997 (15)	0.018-0.875 (3)

[a] Given as 95% confidence limits (number of records).
[b] Birds that dispersed from Woolshed Bush moved to Top Bush, and vice versa.

Table 6.6. Comparison of recent black robin demographic rates with those from the main dataset.

		Hatching rate		Fledging rate		Juvenile survival[a]		Adult survival	
Location	Period	Mean (SD)[a]	*n*[b]	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*
Mangere I.	IM	0.612 (0.102)	140	0.619 (0.138)	101	0.828 (0.112)	165	0.817 (0.090)	489
	Recent	-	0	-	0	0.747 (0.164)	137	0.745 (0.198)	194
Woolshed Bush	IM	0.594 (0.089)	389	0.797 (0.047)	289	0.755 (0.059)	288	0.804 (0.076)	622
	Recent	0.644 (0.057)	214	0.737 (0.069)	162	0.720 (0.187)	56	0.807 (0.057)	506
Top Bush	IM	0.558 (0.100)	166	0.713 (0.146)	119	0.652 (0.131)	144	0.653 (0.122)	236
	Recent	0.792 (0.059)	28	0.653 (0.087)	22	-	0	0.722 (0.092)	116

[a] Mean (among-year SD) estimates of demographic rates were measured during the intensive monitoring (IM) period (1990-1998 for Woolshed and Top Bush, 1990-2001 for Mangere I.) and in recent years (2003-2012 for juvenile and adult survival; 2007-2011 for hatching and fledging success).
[b] Sample sizes (*n*) show the number of individual records (eggs, nestlings, juveniles, or adults) and do not account for do not account for data structure (i.e. pairs that bred over several years, or adults that survived multiple years).

Table 6.7. Effect size estimated by model averaging for each covariate of demographic rates when inbreeding effects were excluded. See Table 6.3 for definitions of abbreviations.

	Prob. breed (yearling females)		Annual prob. breed (older females)		Clutch size		Offspring survival		Juvenile survival		Adult annual survival	
Covariates	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI	Mean (SE)	RI
Intercept	-1.01 (0.32)	-	4.49 (0.73)	-	0.74 (0.03)	-	-0.78 (0.12)	-	0.51 (0.10)	-	0.58 (0.08)	-
WSB	2.32 (0.41)	1.00	-3.33 (0.45)	1.00			0.24 (0.14)	1.00	-0.17 (0.12)	1.00	-0.10 (0.08)	1.00
TPB	1.96 (0.46)	1.00	-3.58 (0.56)	1.00			-0.43 (0.17)	1.00	-0.43 (0.15)	1.00	-0.56 (0.11)	1.00
Sex											0.08 (0.07)	0.39
Age			-0.05 (0.07)	0.31	0.00 (0.01)	0.28					0	0
Age♀							-0.04 (0.03)	0.50	0.03 (0.03)	0.36		

Table 6.8. Effect size estimated by model averaging for each covariate of dispersal rates when inbreeding effects were excluded. See Table 6.3, 6.4 for definitions of abbreviations.

	Juvenile dispersal		Adult dispersal	
Covariates	Mean (SE)	RI	Mean (SE)	RI
Intercept	-1.61 (0.14)	-	-4.20 (0.35)	-
TPB	-0.19 (0.32)	0.30	-0.29 (0.81)	0.21
Age			-0.18 (0.27)	0.25

Table 6.9. Effect size estimated by model averaging for each covariate when positive inbreeding effects were excluded. Demographic rates that were not subject to inbreeding effects or those that were subject only to positive (no negative) inbreeding effects are not shown here. See Table 6.3 for definitions of abbreviations.

	Offspring survival		Juvenile survival	
Covariates	Mean (SE)	RI	Mean (SE)	RI
Intercept	-0.76 (0.12)	-	0.60 (0.10)	-
WSB	0.21 (0.14)	1.00	-0.29 (0.13)	1.00
TPB	-0.45 (0.18)	1.00	-0.55 (0.15)	1.00
F			-2.02 (0.96)	1.00
Age♀	-0.04 (0.03)	0.52	0.02 (0.03)	0.33
F♂	-1.29 (0.89)	0.50	-1.07 (0.90)	0.44

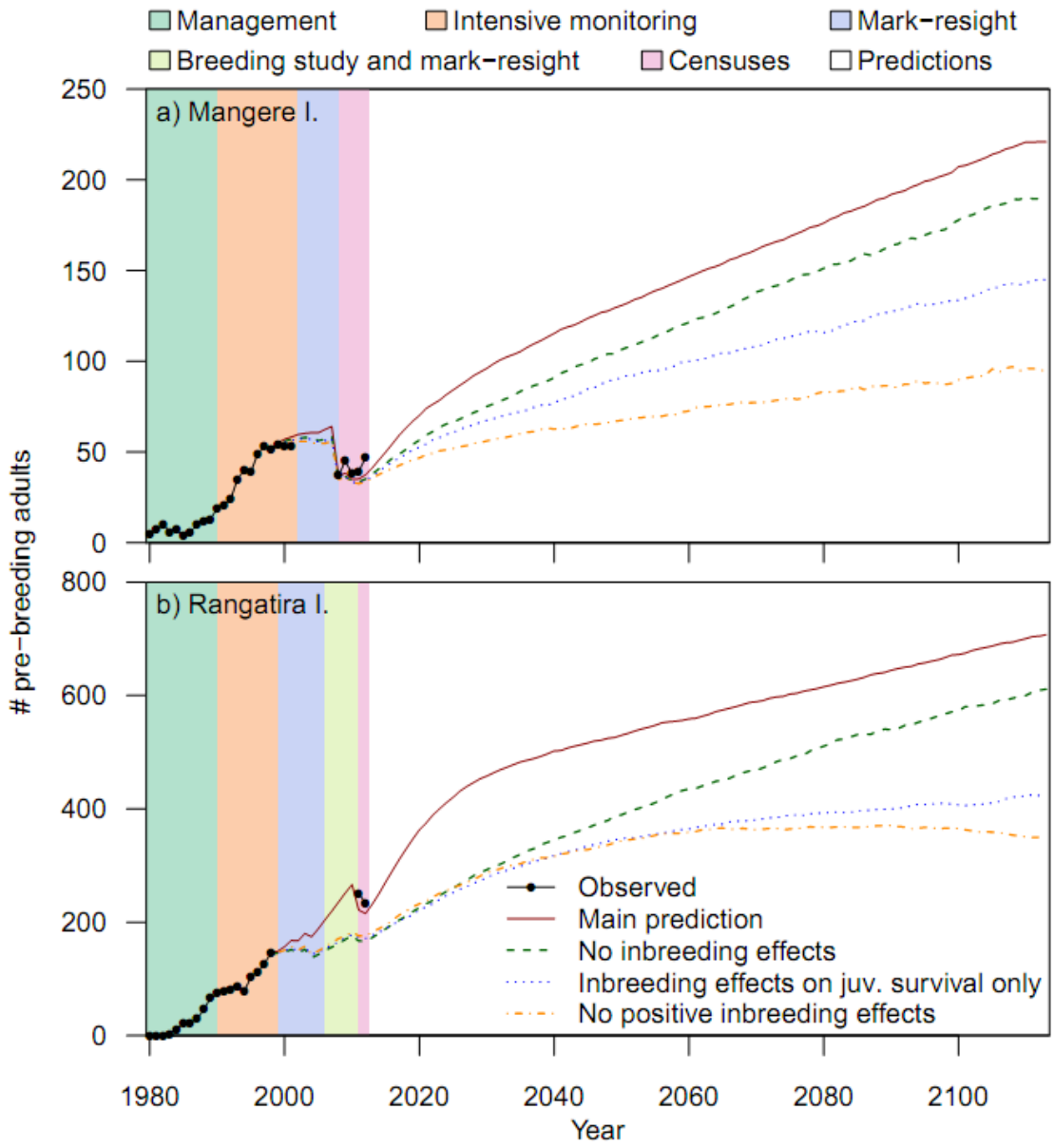

Figure 6.1. Population sizes and management regimes for two black robin populations. Birds that did not contribute genetically to the population (e.g. three of five birds alive in 1980) are included in the totals. Thirty-four birds were removed from Rangatira I. in 2002-2004 and four from Mangere I. in 2004-2005 in a failed reintroduction attempt (see Methods). Estimates of uncertainty around these predictions are displayed in Figure 6.3.

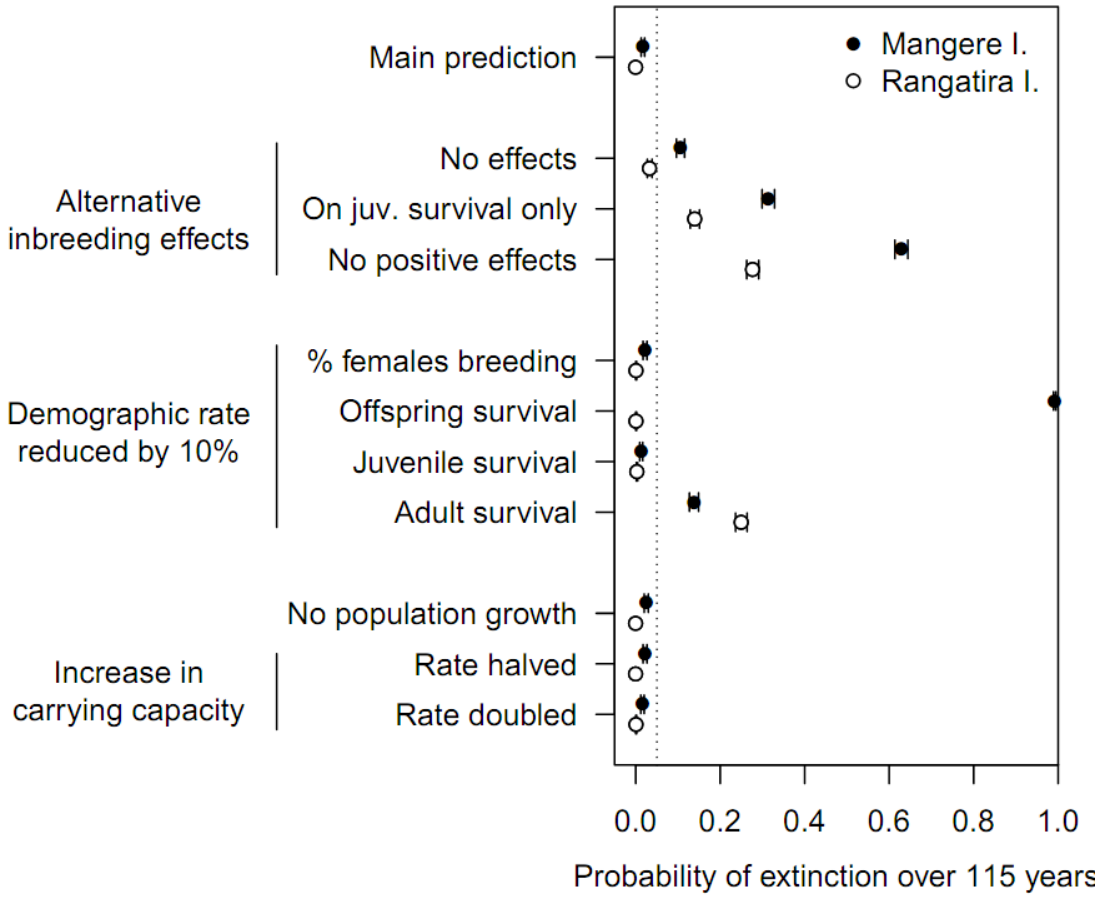

Figure 6.2. Probability of extinction (mean ± SE) over 115 years (from 1998) for each black robin population.

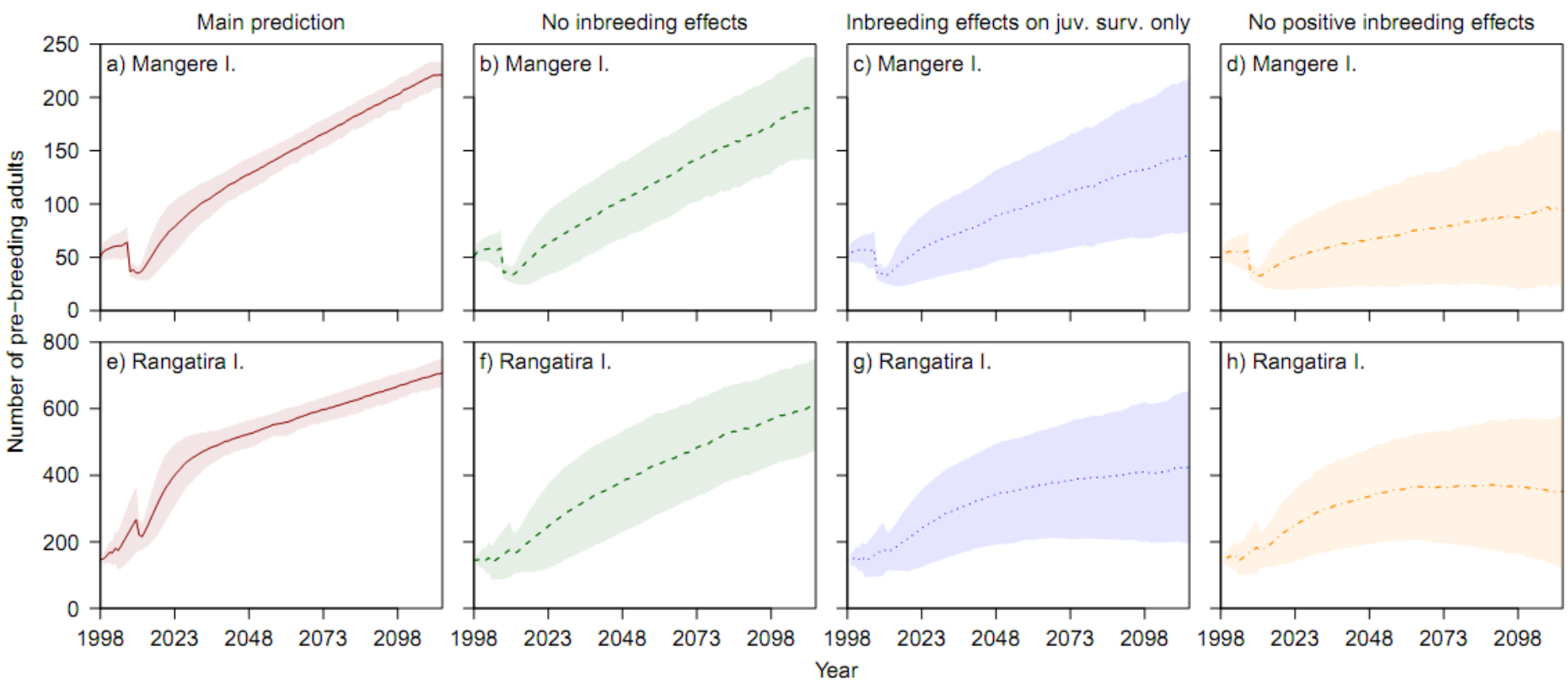

Figure 6.3. Uncertainty on estimates of predicted population size for each PVA scenario. Shaded bands indicate ± 1 SD from the mean prediction.

Chapter 7. Managing for long-term viability in a severely bottlenecked species



Little Mangere Island was the last refuge for black robins from 1893-1976. Photo from March 2013.

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Abstract

Many species that have been through extreme bottlenecks appear to be recovering, with or without management intervention. However, such species have almost certainly lost a great deal of genetic diversity and thus could be vulnerable to new pathogens or changing environmental conditions. Any further loss of genetic diversity could further jeopardise population viability. For this chapter, I examined a well-studied example of a bird that underwent a genetic bottleneck of a single breeding pair ~10 generations ago: the black robin. After simulating the amount of allelic diversity expected to still be present in the species, I used a population viability analysis framework to assess management options to help retain that remaining diversity in both extant populations. I found that a very small amount of management effort (translocating one bird once every 2-10 years, on average) would restore allelic diversity already lost by the smaller of the two extant populations, and would maintain > 90% of any unique founder alleles in both populations over the next 100 years. I also evaluated options for establishing an additional population: very small populations would require modest supplementation (about 1-2 birds per year), but somewhat larger populations (established by releasing 40 birds and reaching a carrying capacity of 400) would self-sustain allelic diversity. My results show that species that have lost a great deal of genetic diversity may still benefit from genetic management. Modest levels of ongoing effort can help ensure long-term success of previous work to rescue species from the brink of extinction.

Introduction

Conservation genetic management generally aims to maximise viability of a species by preventing population bottlenecks and other causes of loss of genetic diversity. However, in some cases a species is reduced to very small numbers before conservation action is successful. Modern bottlenecks of just a few individuals have been documented for several species, including black robin (Butler & Merton 1992; Chapter 5, 6), Mauritius kestrel (*Falco punctatus*; Groombridge et al. 2001), black-footed ferret (*Mustela nigripes*; Wisely et al. 2002), and pink pigeon (*Columba mayeri*; Swinnerton et al. 2004). It has been suggested that management effort may not be cost-effective for species with fewer than 5000 individuals (an estimate of minimum viable population size generalised across all taxa), as these species may not be viable long-term (Clements et al. 2011). However,

short-term recovery and demographic viability of a number of such species, including those listed above, suggests a potential for long-term viability that could benefit from management. Management questions for these species then focus on how to maximise viability, mitigate any harmful effects of inbreeding, and prevent further loss of allelic diversity (Jamieson & Allendorf 2012).

Retaining rare alleles is more challenging than maximising demographic viability or minimising inbreeding and loss of heterozygosity, requiring less restrictive bottlenecks and larger population sizes (Allendorf 1986). Aiming to retain rare alleles is therefore a robust strategy for maximising long-term general viability of a species or population against the possibility of changing conditions. Allelic diversity is important for long-term viability because it defines the capacity for a population to adapt to change (Allendorf & Luikart 2007; Markert et al. 2010). Rare alleles that are currently selectively neutral may be especially important for adaptation to a changing environment, e.g. alleles that confer resistance to novel diseases (Slade & McCallum 1992). Thus, while it is possible to perform population viability analysis (Beissinger & McCullough 2002) to evaluate the demographic effects of inbreeding depression (e.g. Haig et al. 1993; Johnson et al. 2011), the long-term effects of genetic diversity loss are impossible to predict as it cannot be known which allelic variants will be favoured in future. Maintenance of maximal genetic diversity is therefore a central paradigm in conservation genetics management.

Here I examine the feasibility of management strategies to maintain allelic diversity in one of the world's most inbred wild bird species, the black robin. Although my analyses in Chapter 5 and 6 predicted that the black robin would persist, this will be true only if conditions remain stable. Any environmental change, novel disease, or catastrophe could jeopardise the species, which may not have the genetic capacity to adapt to change. Retaining any allelic diversity that the species still possesses may be critical to safeguarding the black robin against any future changes.

Thus there is clearly an opportunity for management actions to endeavour to further secure the long-term viability of this species. The detailed data available for the black robin also make it an excellent case-study for exploring management options for retaining genetic diversity in previously bottlenecked species. I built upon the population viability analysis (PVA) presented in Chapter 6 to assess management options for maximising genetic viability of the black robin.

In this analysis, I aimed to assess strategies that could improve the long-term viability of this species by maximising retention of allelic diversity and thus adaptability to

any changes. First, I simulated the prolonged bottleneck of the remnant Little Mangere I. population (1893-1979) to predict the allelic diversity that may have been present at the time of the single-pair bottleneck (1979-1982). Next, I evaluated management options (acceleration of habitat recovery or translocations between existing populations) that could prevent further loss of these alleles. I then identified options for establishing new populations that would retain modern allelic diversity, and predicted how much harvest (for translocations) could be sustained by existing populations without losing allelic diversity. With these aims, I specifically targeted strategies that would be directly relevant not only for advising black robin management, but also for other genetically depauperate populations. My findings emphasise that even species with low genetic diversity could be further safeguarded by management aimed toward long-term genetic viability.

Methods

Historic bottleneck

First, I simulated loss of allelic diversity during the prolonged 86-year bottleneck on Little Mangere I. Although little is known about the population during that period, records compiled by Kennedy (2009) suggest that the maximum population would have been ~35 birds, with gradual decline over much of that period followed by sharp decline in the last decade (Table 7.1). Nothing is known about demography or inbreeding effects during most of that period, so I simulated the population parameterised by mean demographic rates (without covariate effects) measured for modern-day Mangere I. (Chapter 6). I limited the carrying capacity of the simulated historic population to restrict it to the estimated sizes shown in Table 7.1. Because of the demographic stochasticity inherent in this individual-based model, the simulated population may have been below the recorded number in any given year.

VORTEX (Lacy et al. 2009) implements a gene-dropping procedure to simulate Mendelian inheritance of founder alleles through a simulated pedigree, providing an estimate of the proportion of unique founder alleles that remain in the population at the end of the simulated period. I used this capability to assess the allelic diversity that was likely present in 1979, at which time five black robins remained (Butler & Merton 1992; Kennedy 2009), assuming each of 35 founders started with two unique alleles in 1893. This is likely an optimistic scenario, as the small Little Mangere I. population was probably mostly composed of related individuals even when it was connected by

occasional dispersal to the Mangere I. population prior to 1893, so many of the founders would have shared alleles. I simulated 10 loci (to incorporate uncertainty in inheritance) in each of 1,000 replicates (to include uncertainty and individual variation in demography) of the PVA simulation. This equated to 10,000 replicates of the gene-drop. For each locus and each replicate, I recorded the number and frequencies of alleles remaining in the population at the end of the simulation. I then averaged the frequencies and numbers of alleles across all loci and replicates, excluding replicates in which the population ended with more or fewer than five individuals (as a result of demographic stochasticity inherent in the simulation).

Modern bottleneck and predictions for extant populations

In 1976, the remaining black robins were moved to Mangere I., where they were intensively managed to promote recovery of the species (Butler & Merton 1992; Kennedy 2009). Only two of these birds left descendants, for whom the full pedigree was recorded through 1998 (Kennedy 2009; Kennedy et al. 2013). Throughout this paper, I refer to those two individuals as the "founders" of the modern black robin population (i.e. I do not refer to the 35 birds presumed alive on Little Mangere I. in 1893 as such). The Rangatira I. population was established by translocating birds from Mangere I. during 1983-1988, and contains two demographically distinct areas, Woolshed Bush and Top Bush, that are linked by frequent dispersal (Kennedy 2009). As in Chapter 6, I simulated these areas separately (with dispersal) but combined the output for the whole Rangatira I. population.

I used the PVA presented in Chapter 6 to predict allele retention in the extant populations over the next 100 years. For this analysis, I first instructed VORTEX to randomly assign the genotypes of the two founders, given the mean frequencies estimated by the gene-dropping simulation from the modern bottleneck (above). VORTEX then used gene-dropping (for 10 loci in each of 1000 replicates of the PVA) to predict the genotypes of the individuals alive in 1998, at which time the predictive simulation was initiated. From the model output, I then calculated the mean number of alleles per locus, averaged across loci and then across replicates, present in each population at the end of the simulation (year 2113). I refer to this approach as the "simulated-founder-alleles scenario."

I then simulated a separate scenario the same way, except assigning two unique alleles to each founder. I refer to this simulation as the "four-founder-alleles scenario." This almost certainly overestimated the allelic diversity present at that time (Ardern &

Lambert 1997; Miller & Lambert 2004; see also Results), but allows for comparison with other gene-dropping simulations, which typically assign unique founder alleles to make predictions with maximum precision (MacCluer et al. 1986). This scenario could predict loss of a larger proportion of diversity than the simulated-founder-alleles scenario: because a minimum of one allele must be retained at each locus, up to 75% of alleles (three out of four) could be lost in the four-founder-allele scenario, while only 39% (0.64 out of the average of 1.64) could be lost in the simulated-founder-alleles scenario.

I set the goal of retaining 90% of founder alleles over the next 100 years. That is, when the historic bottleneck simulation predicted an average of 1.64 founder alleles per locus at the single-pair bottleneck (1979-1982; see Results), I aimed to retain a mean of at least 1.48 alleles per locus (90%) until the end of the predictive simulation (2113). I first assessed the mean proportion of founder alleles retained, $\bar{A}$, in the two extant populations without management intervention, assuming demographic rates, their relationships with covariates, and habitat regeneration continued as described in Chapter 6. I then expanded the PVA to simulate management alternatives that 1) increased growth in carrying capacity to double the expected rate (as in Chapter 6) for both populations; and 2) achieved the goal for allele retention by implementing a small amount of connectivity (by translocating a proportion of the juveniles produced by each population in each year) between the two extant populations, starting in 2014 (the year after this analysis). I explored these options for both the simulated-founder-alleles and the four-founder-alleles scenarios.

To ensure that meeting the goal for $\bar{A}$ was an indication of overall population viability and genetic health, I recorded the predicted probability of extinction (*PE*) and mean inbreeding coefficient (*F*) for each population under each management alternative in the simulated-founder-alleles scenario.

Finally, I assessed whether alleles from either founder were particularly vulnerable to being lost. The male founder bred with two daughters and one granddaughter (Butler & Merton 1992), so he was overrepresented in the pedigree relative to the female. Using the four-founder-alleles scenario for maximum precision, I compared expected frequencies from each founder at the beginning and end of the simulation to assess whether founder contributions changed over time.

Establishing a new population

Once I had identified management options that would allow both existing populations to retain 90% of founder alleles in the simulated-founder-alleles scenario, I added establishment of a new population to the simulation. Establishing a new population is a priority of the black robin recovery plan (Department of Conservation 2001), but a previous attempt was unsuccessful, possibly due to unsuitability of habitat or insufficient food (Kennedy 2009). Several candidate sites for a new population are now being evaluated, but likely still need time for habitat regeneration and removal of introduced mammalian predators before black robins can be reintroduced (Black Robin Recovery Group, pers. comm.). I therefore simulated a hypothetical new population under various options for establishment, all under the management alternative that resulted in $\bar{A} \geq 0.90$ for the two extant populations under the simulated-founder-alleles scenario.

First, I evaluated the minimum number of adults (with an even sex ratio) that would need to be translocated from Rangatira I. (the larger of the two extant populations) to ensure that at least 90% of simulated-founder-alleles would be transferred to the new population. Because the full pedigree is no longer known for birds in either extant population (Kennedy 2009), and the high degree of genetic similarity among individuals (Ardern & Lambert 1997) may preclude molecular analysis that could distinguish birds based on their genotype, I assumed that translocated birds would be randomly selected. I then varied carrying capacity of the recipient site to identify how large a new population would need to be so that it would not require ongoing connectivity to maintain $\bar{A} \geq 0.90$. Finally, I evaluated the amount of supplementation required to achieve $\bar{A} \geq 0.90$ in smaller populations that would not self-sustain allelic diversity. I simulated establishment of the new population in 2014 (the year following this analysis) for the above options. Because the timeframe in which suitable habitat may become available is uncertain, I also simulated models (40 birds translocated, K = 100) in which the new population was established in 2025 or 2050 instead of 2014.

Subtle differences in habitat may strongly affect the productivity of black robin populations. For example, black robins in the Top Bush area (on Rangatira I.) experience much lower survival and population density than those in the neighbouring Woolshed Bush area (Kennedy 2009). This is presumed to result from lower habitat quality or unfavourable environmental conditions in Top Bush, but the specific parameters of habitat that determine its quality for black robins have not been identified. Productivity of a new population thus cannot be accurately predicted at this time. I therefore built the models under the initial assumption that demography of the new population would mimic that

recorded in the Woolshed Bush area, which experienced the most growth during the intensive monitoring period that informed this analysis (Chapter 6). I examined the effects of this assumption by also simulating population establishment when a representative new population (40 birds translocated, K = 100) was established using demographic information from Mangere I. or Top Bush to parameterise the new population.

Sustainable harvest rates

Finally, I determined the number of birds that could be taken from Rangatira I. without jeopardising its genetic viability under the simulated-founder-alleles scenario. I assessed two possible scenarios for harvest (in which birds were removed from Rangatira I. but were not added to any other population): 1) the maximum number of sequential years in which all juveniles could be removed (one-off harvest), and 2) the proportion of each juvenile cohort that could be removed in every year of the simulation (ongoing harvest). In each case, I determined the maximum level of harvest that would result in $\bar{A} \geq 0.90$ for Rangatira I. This harvest was in addition to the translocations needed to implement the level of connectivity between Mangere I. and Woolshed Bush that would allow both of those populations to achieve $\bar{A} \geq 0.90$ (see Results).

Results

In the simulations of the historic prolonged bottleneck (Little Mangere I.), the number of alleles declined from 70 unique alleles per locus in 1893 to an average of 1.64 (SD = 0.624) in 1979 (Figure 7.1a-b). Most allele loss occurred early in the simulated period. Typically, one of the remaining alleles was common, while any other remaining alleles were rarer (Figure 7.1c).

After the period of intensive management, Mangere I. was expected to retain less diversity than Rangatira I. This was especially true in the four-founder-alleles scenario, and fell below the goal ($\bar{A} \geq 0.90$) by 2013 in both scenarios (Table 7.2). Increasing reforestation and thus the population growth rate improved allele retention in Mangere I. (Table 7.3), but because the population had already lost > 10% of founder alleles by 2013, further action would be needed to achieve the goal. Providing just a small amount of annual connectivity (translocating 0.2% in the simulated-founder-alleles scenario or 1% in the four-founder-alleles scenario of the juveniles between Rangatira I. and Mangere I.) was sufficient to achieve $\bar{A} \geq 0.90$ (Table 7.3). In March 2013, 92 juveniles were recorded

on Rangatira I. and 10 on Mangere I. (Bliss 2013), so these percentages would currently correspond to moving about one bird every 50 years from Mangere I. to Rangatira I. and one every 5 years from Rangatira I. to Mangere I. for the simulated-founder-alleles scenario, or one bird every 10 years from Mangere I. and about one bird per year from Rangatira I. for the four-founder-alleles scenario. As the populations continue to grow, the numbers of birds to move would increase; the PVA predicted that the maximum total number of birds to be moved would be one every second year (simulated-founder-alleles scenario) or 2.5 per year (four-founder-alleles scenario) when the populations reach their maximum expected sizes.

As expected, managing to prevent loss of unique founder alleles also mitigated inbreeding. Without management, mean inbreeding (F) was expected to increase substantially for Mangere I. and slightly for Rangatira I. (Figure 7.2). When 0.2% connectivity was implemented beginning in 2014, the increase over time in mean inbreeding for Mangere I. was somewhat reduced (Figure 7.2). Maintaining $\bar{A}$ also minimised the probability of extinction; $PE \leq 0.02$ was predicted for all of the above simulations.

Allele frequencies were robust to drift, changing very little (from a starting frequency of 0.25) for either extant population over the simulated period (Figure 7.3). Alleles that originated with the male founder were expected to be found in both populations at higher frequencies than alleles originating with the female founder, likely because of the male's disproportionate contribution that resulted from breeding with his descendants (see Discussion).

Establishing a new population

The simulations indicated that establishing a new population would be feasible. Establishment of a new population was demographically successful and started with $\bar{A} \geq 0.90$ (under the simulated-founder-alleles scenario) when at least 20 birds were translocated from Rangatira I. to the new site, assuming no dispersal from the release site and no mortality resulting from the translocation. However, because genetic drift quickly erodes allelic diversity in small populations, further supplementation would be needed if only 20 birds were initially released (Table 7.4). Alternatively, releasing at least 40 birds into a site with a carrying capacity of at least 400 would result in a population that would self-sustain $\bar{A} \geq 0.90$. Smaller populations established with 40 birds would not meet the goal for allele retention if isolated from the existing populations, but allelic diversity could

be sustained with a small amount of regular supplementation from Rangatira I. (Table 7.4).

The option that successfully retained allelic diversity in a new population established in 2014 (40 birds released, K = 400) was equally successful when I simulated establishment in 2025 or 2050 ($\bar{A}$ > 0.90, SE = 0.001 in each case). Demography of the new population had some influence on allele retention: the new population (40 birds released in 2014, K = 400) would achieve the goal with Mangere I. demographic rates ($\bar{A}$ = 0.902, SE = 0.001), but not with Top Bush demographic rates ($\bar{A}$ = 0.860, SE = 0.001). Moreover, when Top Bush values were used, the new population showed an extinction probability of 0.260 (SE = 0.014).

Sustainable harvest rates

Harvesting birds to establish the new population would jeopardise neither persistence nor allele retention of the Rangatira I. source population ($\bar{A} \geq 0.90$ and PE = 0 for all establishment options). Indeed, Rangatira I. could safely sustain harvest of the entire juvenile cohort for four successive years beginning in 2014, or for seven years beginning in either 2025 or 2050 ($\bar{A} \geq 0.90$, $PE < 0.02$; Figure 7.2). Alternatively, up to 25% of the juvenile cohort could be removed every year from 2013 to 2113 without jeopardising allele retention or persistence ($\bar{A} \geq 0.90$, $PE < 0.02$).

Discussion

Because the black robin underwent a prolonged and severe population bottleneck followed by a further single-pair genetic bottleneck, the amount of allelic diversity potentially present in the existing populations is extremely limited. The simulations indicated that an average of 1.64 alleles per locus may have been present in the five black robins remaining in 1979. Of these alleles, a mean of 94% per locus were expected to persist in the populations until 1998. This expectation of low diversity in the modern population agrees with both previous studies that found extremely low molecular diversity (Ardern & Lambert 1997; Miller & Lambert 2004) and those that found evidence of at least some diversity remaining (Massaro et al. 2013a; Chapter 5). If the allelic diversity remaining was higher than expected at the single-pair bottleneck (e.g. four alleles per locus), a greater proportion of that diversity has likely been lost since then (although a

greater-than-expected number of alleles per locus may remain relative to the simulated-founder-alleles scenario).

As predicted by genetic drift theory, the smaller Mangere I. population was found to be more vulnerable to allele loss than the larger Rangatira I. This was especially true under the assumption of higher founder diversity (four alleles per locus). Even when lower diversity (1.64 founder alleles per locus) was assumed, the Mangere I. population was expected to lose > 10% of founder alleles before the end of the simulated period, falling just below the threshold of retaining 90%. Even the larger Rangatira I. population is small enough to lose further diversity over time to genetic drift, so both populations could benefit from management. Further loss of diversity could be minimised by periodically translocating a small number of individuals (one every 2-10 years, or more if I assume maximum founder allelic diversity) between the two islands to provide gene flow and reduce drift. The simulations indicated that this would quickly restore genetic diversity to Mangere I. and maintain diversity in both populations above the levels that would be sustained without connectivity. This would ensure that either population would secure the future adaptive potential of the species even if one population were lost.

Any alleles from the female founder, "Old Blue," were more likely to be lost or decline in frequency than those of the male founder, "Old Yellow." This was because while Old Blue bred successfully only with Old Yellow, the latter also bred with two of his daughters and one granddaughter (Butler & Merton 1992). The two original founders therefore contributed disproportionately to the descendant population. Although Old Blue and Old Yellow were likely closely related, as their ancestors had interbred in a very small population for about 30 generations, each still could have carried unique alleles. For example, an allele coding for a maladaptive egg-laying trait was likely carried only by Old Yellow (Massaro et al. 2013a). The simulations of the Little Mangere I. population also suggested that in many cases, most loci would consist of one or more rare alleles (likely carried by only one founder) and one common allele (carried by both Old Blue and Old Yellow). Retention of potentially beneficial alleles from either founder could be important for future adaptation in this genetically depauperate species, e.g. for disease resistance (Edwards & Hedrick 1998).

Establishment of an additional population would help to secure the species in the face of environmental uncertainty. A genetically and demographically robust population could be feasibly established under the options I examined, assuming demographic rates were similar to those seen in Woolshed Bush or Mangere I. In some cases, the new

population needed supplementation to retain rare alleles, depending on both the size of the new population and the number of birds initially released. Large (K > 400) populations established by releasing at least 40 birds could self-sustain allelic diversity over the next 100 years. Such large populations would fall well short of some guidelines that recommend minimum viable population sizes on the order of a few thousand individuals (Clements et al. 2011; Reed et al. 2003; Traill et al. 2007; Traill et al. 2010). The simulations did not include potential threats such as environmental stochasticity or long-term change, and suggest only that such a population would be robust to demographic stochasticity given the inbreeding effects I quantified for this species. Maintaining multiple large populations would help to insulate the species from environmental stochasticity and other potential threats, which is why establishment of a third population is a priority of the black robin recovery plan (Department of Conservation 2001).

Sustainable harvest is an important consideration for translocations, as overharvesting can negatively impact the source population (Stevens & Goodson 1993). Despite the precarious history of the black robin, the simulations indicated that judicious harvest from Rangatira I. would not jeopardise that population. The birds needed to establish and maintain even a very small new population could be safely harvested, and surplus birds would be available to test additional options for establishing a new population (e.g. if habitat suitability were in question). The safe harvest rates indicated by the models would provide managers with important leeway for attempting to establish a viable third population without negatively impacting the existing populations. However, monitoring of the source population and re-evaluation of these predictions will be important when harvest commences, especially if key assumptions inherent in the model (e.g. expected growth rate) do not hold true (Dimond & Armstrong 2007).

Translocations among populations would themselves pose some risk to the species, as they would increase the chance of a disease spreading from one population to another (Cunningham 1996; Ewen et al. 2012; Viggers et al. 1993). For birds, translocation of eggs instead of juveniles or adults could reduce the risk of spreading a disease; quarantine and disease screening are also used for various taxa. Successful methods for translocating eggs and temporarily holding birds in aviaries were developed for black robins during the intensive management period (Butler & Merton 1992), so there are viable options for mitigating disease risk in this species.

This example of the well-studied black robin indicates that a small amount of management effort can help safeguard the long-term adaptive potential of even genetically

depauperate species. Given the amount of diversity already lost to bottlenecks and subsequent drift in these species, it is arguably even more important to preserve the remaining diversity. This analysis has identified feasible management strategies that could be used to further safeguard endangered species like the black robin that persist in small and extremely bottlenecked populations. Relative to the initial management effort invested into critically endangered species, the ongoing work needed to maximise long-term viability may require a modest level of management, and could be well worth the effort.

Table 7.1. Estimated size (number of pre-breeding adults) of the remnant black robin population during the historic prolonged bottleneck and decline.

Year	N	Source[a]
1893	35	1
1894-1972	Steady decline	1
1973	16	2, 3, 4, 5, 6
1974	11	3
1975	9	7
1976	7	3, 5, 7
1977	7	3
1978	7	3
1979	5	3

[a] 1) Estimated by Kennedy (2009) from historic records and modern population densities on Mangere I., 2) Atkinson et al. (1973), 3) Butler and Merton (1992), 4) Flack (1974), 5) Flack (1976), 6) R. Hay (unpubl. data), 7) R. Morris (unpubl. data). All sources were compiled by Kennedy (2009).

Table 7.2. Mean (SE) proportion of alleles per locus retained in simulated black robin populations.

Scenario	Year[a]	Mangere I.	Rangatira I.	Overall
Simulated founder alleles	1998	0.915 (0.098)	0.939 (0.098)	0.939 (0.098)
	2013	0.896 (0.098)	0.933 (0.098)	0.939 (0.098)
Four founder alleles	1998	0.882 (0.145)	0.962 (0.090)	0.975 (0.078)
	2013	0.822 (0.165)	0.948 (0.105)	0.962 (0.090)

[a] Estimates are given for the beginning of the simulation (1998, based on the species-wide pedigree recorded from 1979) and at the time of analysis (2013, predicted by the PVA). Values for the end of the simulated period (2113) are provided in Table 7.3.

Table 7.3. Mean proportion (SE) number of alleles retained until 2113 in both extant black robin populations under each management option. Mean reforestation rates were estimated in Chapter 6; faster reforestation was simulated at double the mean rate.

Scenario	Management	Mangere I.	Rangatira I.	Overall
Simulated founder alleles	No management	0.835 (0.098)	0.921 (0.098)	0.927 (0.098)
	Faster reforestation	0.848 (0.098)	0.921 (0.098)	0.933 (0.098)
	0.2% connectivity[a]	0.902 (0.098)	0.927 (0.104)	0.927 (0.098)
Four founder alleles	No management	0.662 (0.188)	0.902 (0.135)	0.932 (0.118)
	Faster reforestation	0.690 (0.192)	0.910 (0.130)	0.940 (0.112)
	1% connectivity[a]	0.920 (0.125)	0.928 (0.120)	0.935 (0.115)

[a] The indicated percent of locally produced juveniles was annually moved from Mangere I. to Rangatira I. and vice versa.

Table 7.4. Minimum amount of ongoing supplementation (% juveniles translocated from Rangatira I. each year) needed to maintain a new population with $\bar{A} \geq 0.90$ until 2113.

	Number initially released[a]	
K	20	40
100	1%	0.5%
200	0.05%	0.25%
300	0.025%	0.1%
400	0.1%	0
500	0.05%	0

[a] Establishment of the population was simulated in 2014 under the simulated-founder-alleles scenario. Each scenario shown here also includes 0.2% annual connectivity between Rangatira I. and Mangere I. to maintain $\bar{A} \geq 0.90$ in both those populations.

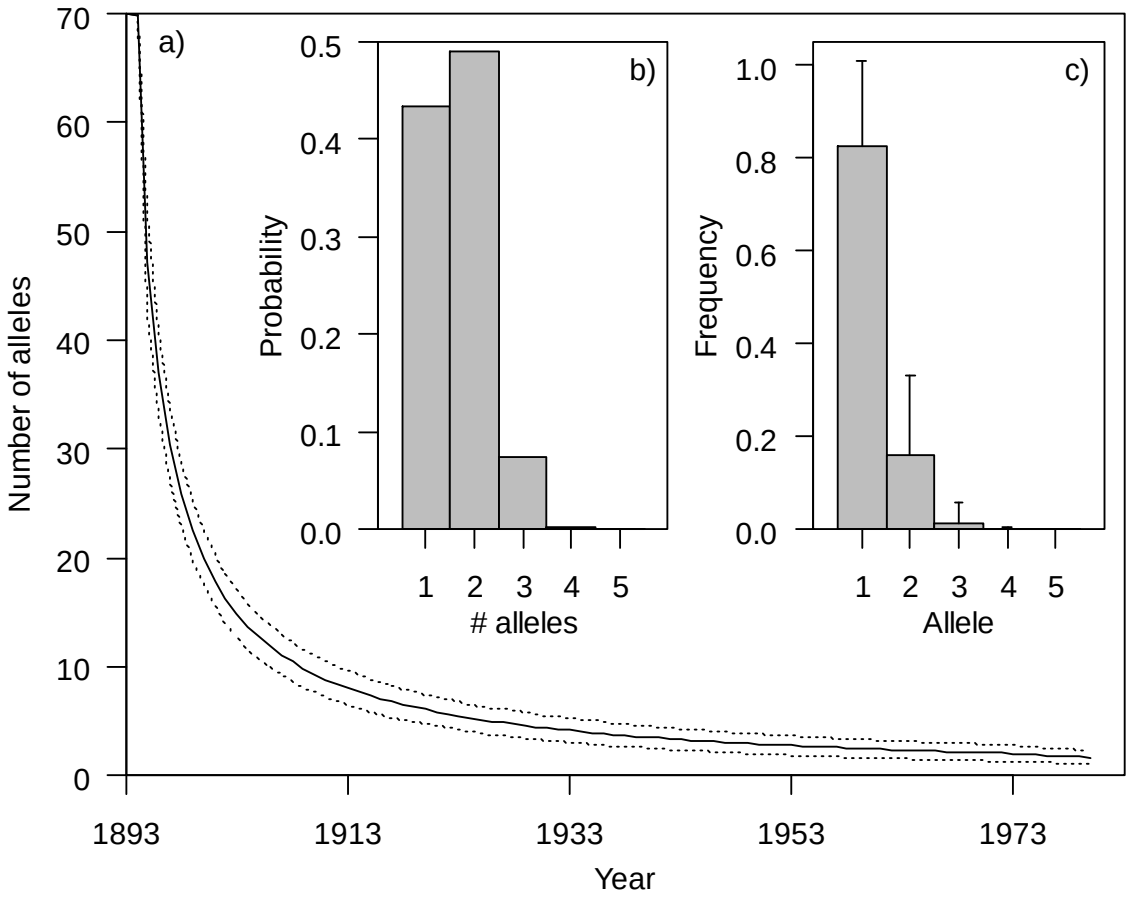

Figure 7.1. Simulated allele loss during the historic prolonged bottleneck (a), and expected number (b) and frequencies (c) of alleles remaining in 1979, for the black robin population on Little Mangere I. Dotted lines (a) and error bars (c) indicate 1 SD around the mean; probabilities in (b) were estimated as proportions of replicates, so variance is not shown. Data were generated from 10 loci in 2603 simulations in which the population ended with five individuals in 1979.

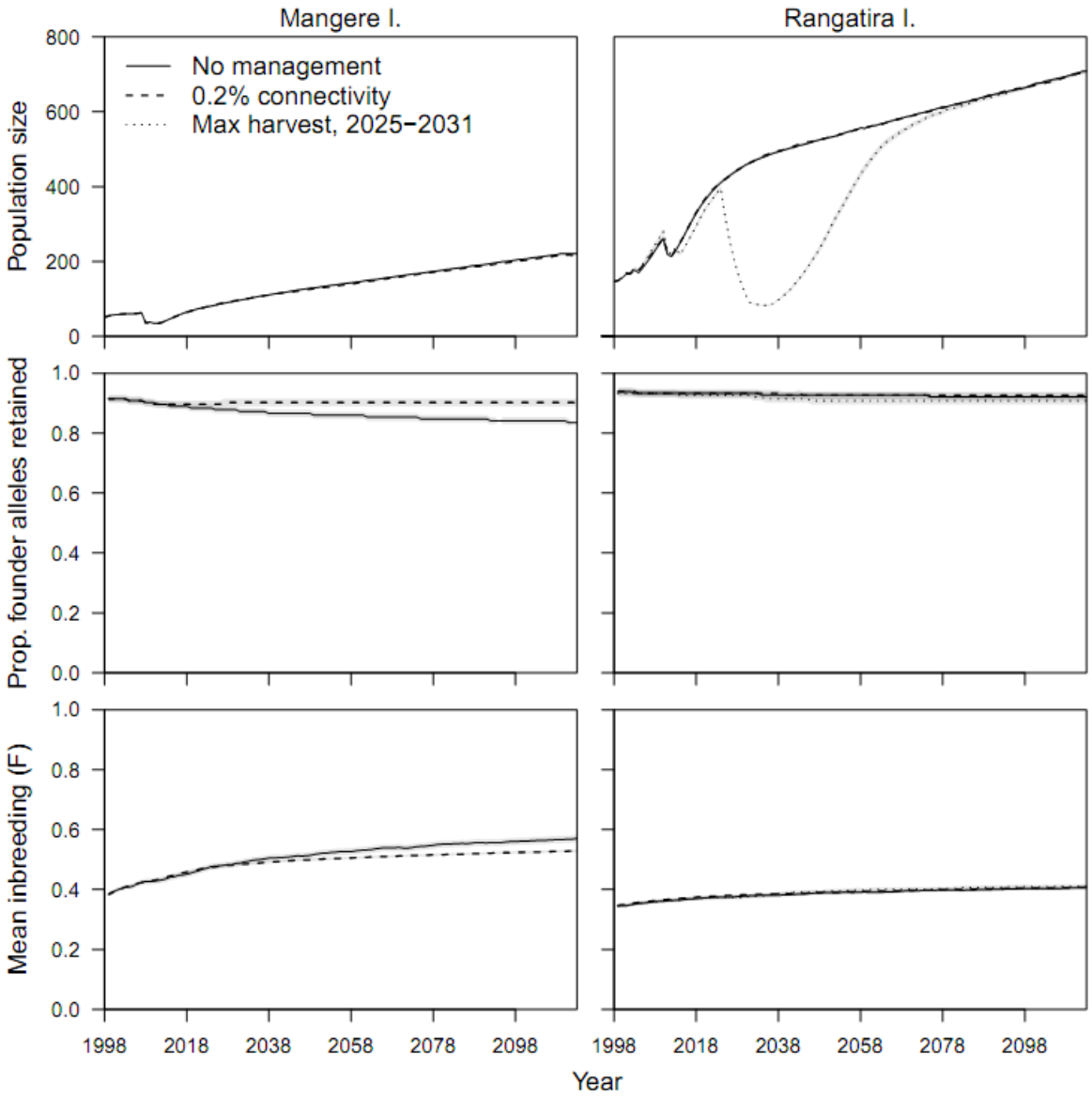

Figure 7.2. Predicted allele retention (mean proportion of founder alleles retained per locus, out of the expected mean of 1.64 alleles per locus) and accumulation of mean inbreeding in two black robin populations over the simulated period. In the connectivity scenario, 0.2% of juveniles produced by each population were moved to the other annually to achieve 90% retention of founder alleles. In the maximum harvest scenario, 0.2% connectivity was implemented, and all juveniles were removed from Rangatira I. in each of the indicated years. Shaded bands indicate 95% confidence intervals around the mean prediction (very narrow for most scenarios).

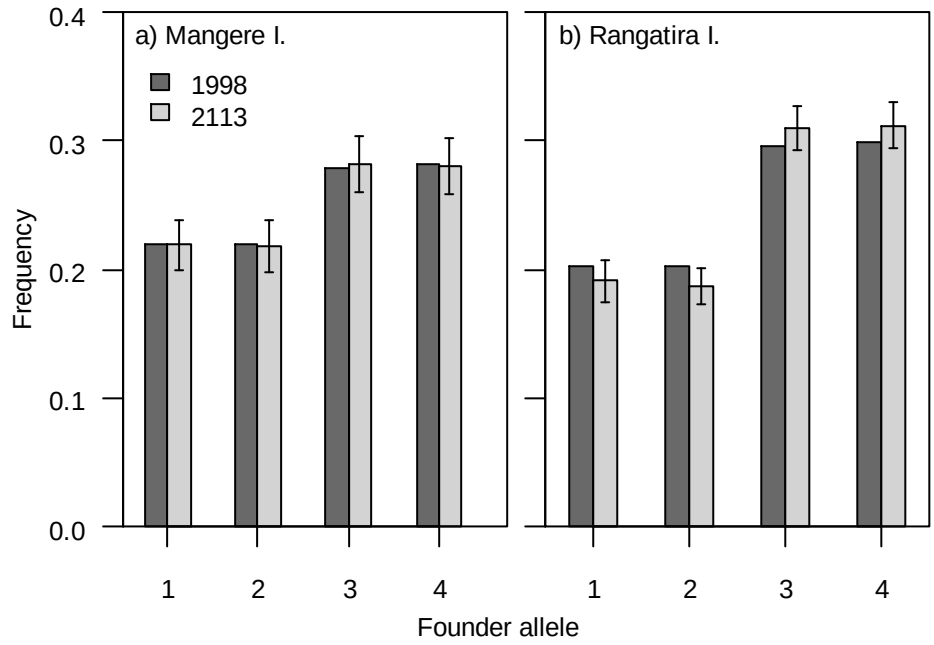

Figure 7.3. Mean frequency of four unique founder allele (1 and 2 from the founding female, 3 and 4 from the founding male, all starting at equal frequency at the single-pair bottleneck) in two extant black robin populations. Predicted allele frequencies are averaged over 10 loci and 1000 replicates and are given for the beginning (1998) and end (2113) of the simulated period. Error bars indicate 95% confidence intervals (negligible in 1998 as the pedigree was known until then).

Chapter 8. General discussion



View over Codfish Island, a predator-free sanctuary that is a key site for several critically endangered species, toward Stewart Island, for which plans to eradicate mammalian predators are under consideration.

Genetic considerations have been increasingly viewed as critical components of conservation management (Armstrong & Seddon 2008; Groombridge et al. 2012; Jamieson & Lacy 2012; Weeks et al. 2011), but methods for evaluating and planning management are still being developed. The primary aim of this thesis was to improve methods for assessing options to increase genetic viability. I focussed on the two main genetic considerations: allelic diversity and inbreeding. Both of these genetic factors are especially relevant to small populations, and thus many threatened species. Allelic diversity defines the capacity of a population to adapt to change, and is lost during population bottlenecks or to genetic drift in small populations (Allendorf 1986; Allendorf & Luikart 2007). Rare alleles are particularly difficult to retain, and management effort for retention of rare alleles will also be sufficient to minimise inbreeding and loss of heterozygosity (Allendorf 1986). In cases where inbreeding has already occurred, inbreeding depression may become a more immediate concern, as it can directly impact fitness and reduce population viability (e.g. Keller & Waller 2002; O'Grady et al. 2006; Saccheri et al. 1998). With this thesis, I demonstrated and developed methods for assessing these two considerations in bottlenecked populations, drawing on examples in which one or both of these factors were particularly relevant. I used various species of New Zealand birds, for which there is a long history of reintroduction for conservation purposes (Innes et al. 2010; Towns & Broome 2003), as relevant examples to explore the concepts and methods presented here.

Available models for assessing allele loss do not provide for all of the management options and predictions that would be useful to reintroduction projects (Chapter 2). I therefore first developed a new model, AlleleRetain, to facilitate assessment of various management options that will improve retention of rare alleles. This model and the included options are particularly relevant to small, bottlenecked populations, including reintroduced populations. AlleleRetain is highly flexible model and can simulate any sexually reproducing animal, and I have made it freely available for others to use. This flexibility is important given that the effectiveness of management options for allele retention depends upon life-history traits and demographic rates of population in question, as well as on population size and bottleneck size (Chapter 3). Although some broad patterns are evident across species (e.g. carrying capacity and mating system are particularly influential), each population of interest should be evaluated specifically. Models like AlleleRetain are particularly useful when assessing specific examples of real populations for which management planning is needed.

However, because it is implemented in R (R Development Core Team 2013), AlleleRetain is not readily accessible by conservation managers unfamiliar with the R computing environment. The National Office of New Zealand's Department of Conservation has agreed that AlleleRetain will be very helpful in managing small populations, including reintroductions. It is therefore currently developing a more user-friendly graphical interface for AlleleRetain so that predictions can be made by non-specialists. I have been involved with this project in an advisory capacity, though the bulk of the work is being spearheaded by Lynn Adams, Kate McInnes, Kevin Parker, and Barry Polley. The point-and-click interface, called "Conservation Supermodel," will be made publicly available for community groups and managers to assess options for genetic management that will be relevant to their particular populations of interest. The interface will enable users to make predictions for their species of choice, using demographic data compiled from published and unpublished sources for every species commonly translocated in New Zealand. This interface will be a valuable asset for assessing feasibility of long-term management of reintroduced populations, and will be a critical tool for community groups, which are becoming increasingly important in conservation management and may not have scientific expertise readily available.

Like AlleleRetain, Conservation Supermodel will be primarily intended to advise management of single reintroduced populations. However, my work with kokako (Chapter 4) demonstrates the extensive assessments that are possible in more complex situations. A metapopulation view is particularly important for species that persist in small, fragmented populations, and when potential source sites could be jeopardised by harvest. Such complex considerations would be difficult to assess with Conservation Supermodel, which will be aimed toward advising management of single populations; but a more in-depth analysis can be successfully used to prioritise options for a suite of populations. The public availability of AlleleRetain and the ease with which multiple scenarios can be batch-processed in R will facilitate such assessments by users willing to tackle the R interface.

Although retention of allelic diversity is an important and comprehensive aim for maximising genetic viability, in some cases a great deal of allelic diversity has already been lost. This is typically a result of prolonged or severe population bottlenecks. Inbreeding depression then becomes the primary concern for managers, as it has the potential to immediately impact fitness and threaten small populations (Keller & Waller 2002). When I assessed one extremely inbred species (Chapter 5), I found a surprising

mix of positive and negative effects of inbreeding on fitness, including important interactions between inbreeding covariates that had not previously been examined. These findings demonstrate the extent to which genetic effects can vary among and even within species. Although the black robin example provides the first evidence of an inbreeding advantage in wild populations, the mixed results are otherwise not surprising. A wide range of inbreeding effects, from neutral to severe, has been previously reported in the literature (Crnokrak & Roff 1999; Keller & Waller 2002). This variation is a result of chance playing a large role in how many deleterious recessive alleles (thought to be the main genetic cause underlying inbreeding depression) are present in any given population (Charlesworth & Willis 2009). The black robin example emphasises the importance of making individual species assessments rather than relying on broad assumptions of cross-taxa patterns.

The suggestion of an inbreeding advantage also raises the spectre of an idea that has recently fallen out of favour: that island species may be relatively robust to inbreeding (Craig 1991, 1994). However, my findings do not support that hypothesis, which was grounded on the assumption that most deleterious recessive alleles would have already been purged from populations that were historically limited to small sizes. Recent work has indicated that purging is unlikely to be efficient in small populations, and the black robin genome has clearly not been purged of all deleterious alleles (Kennedy et al. 2013; Massaro et al. 2013a; Chapter 5). Instead, I suggest that the black robin now experiences a net benefit from further inbreeding because it is so highly inbred that offspring, especially the more-inbred offspring, often share nearly the same genotype with their parents (especially the more-inbred parents), which have proven the success of the genotype by their own survival. This proven-homozygote advantage suggests that some highly inbred populations may become robust to the effects of further inbreeding, but only if they are not subject to strong effects of deleterious recessive alleles (which would substantially reduce fitness of homozygous individuals). Moreover, if conditions change, populations with high homozygosity and low allelic diversity would have a reduced capacity to adapt, likely compromising fitness and negating any homozygote advantage.

Most other species examined to date have exhibited negative effects of inbreeding on fitness corresponding to the presence of deleterious recessive alleles (Crnokrak & Roff 1999; Keller & Waller 2002). These effects can have important implications for population persistence (e.g. O'Grady et al. 2006; Saccheri et al. 1998), and must be included in any analysis attempting to predict population growth and viability. With

Chapter 6, I demonstrated the magnitude of the potential consequences of excluding inbreeding effects from a PVA; and with the accompanying material in Appendix C, I provided guidance for implementing such fitness effects in VORTEX, the most widely used PVA software. These materials add to previous calls for including genetic considerations in PVAs (Allendorf & Ryman 2002; Keller et al. 2007), and demonstrate how dramatically conclusions may differ with only partial inclusion of inbreeding effects in predictive models.

Both loss of allelic diversity and inbreeding depression feature prominently in suggestions that species with very small total population sizes may not be worthwhile to attempt to manage, as those species show a higher risk of extinction (Clements et al. 2011). However, I demonstrated in Chapter 6 that some such species may be robust to small-population problems such as inbreeding depression. Such genetic viability may be a result primarily of chance (i.e. if the genome contains few deleterious recessive alleles), and it may not be possible to predict which species will be more or less affected by inbreeding. However, my findings suggest that *any* species, even one that has been through an extreme and prolonged bottleneck, could become self-sustaining, thus justifying the management action initially needed to rescue the species from extinction.

Another major genetic factor that influences long-term viability is whether the species has the allelic diversity necessary to enable adaptation to changing conditions. This factor is particularly difficult to evaluate, as it is usually not known how conditions may change, nor which particular alleles will be necessary for successful adaptation. However, the black robin example demonstrates that even though it is "arguably the world's most inbred wild bird" (Kennedy et al. 2013) with genetic diversity "among the lowest reported" (Ardern & Lambert 1997), this species still retains enough diversity to show maladaptive traits (Massaro et al. 2013a) and both positive and negative fitness effects of further inbreeding (Chapter 5). If some diversity remains, then the species still has some adaptive potential; and management actions should strive to maximise retention of this diversity into the future. The same will be true for other species that have undergone severe bottlenecks or are currently limited to very small populations. My work in Chapter 7 demonstrated that options for genetic management remain for such species, and may involve very little effort relative to previous actions implemented to save the species.

All of the species and populations that I used as case studies in this thesis benefitted from a large amount of detailed data. Without those data, the analyses I have

presented would not have been possible. Simulation models incorporate detailed information about survival rates, reproductive rates, and life-history characteristics, and such information may not be available for many species of conservation concern. However, detailed monitoring is increasingly being seen as a critical component of conservation management, especially for reintroduced populations (Armstrong & Seddon 2008; Nichols & Armstrong 2012; Sutherland et al. 2010). As such monitoring becomes more common, more data will become available for a wide range of species, and can be used to update management plans in an adaptive management framework (McCarthy et al. 2012). When comprehensive data are unavailable, predictive models can still be used with an accompanying sensitivity analysis to assess the potential effects of uncertain variables (McCarthy et al. 1995). This strategy is useful not only for assessing the potential future trajectory of a species, but also for identifying highly influential demographic rates that would be the most important to monitor. For predictive planning, models can be conducted with the best available estimates of input variables and updated as needed following on-the-ground monitoring to better assess key variables.

With this thesis, I have not only explored principles and considerations of myriad aspects of demographic and genetic management, but also developed tools and provided practical advice for managers of real species and populations. These tools greatly improve conservation planning, providing justification and rationale for options to manage genetic viability in remnant or reintroduced populations. Though previously neglected, such genetic considerations are becoming increasingly important for conservation management in response to threats such as introduced predators and habitat loss.

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Appendix A. Recommended number of brown kiwi (*Apteryx mantelli*) needed to start a genetically robust population at Rotokare Scenic Reserve, Taranaki



Adult North Island brown kiwi in the captive breeding programme at Westshore Wildlife Reserve, Napier, New Zealand.

This appendix provides a report prepared for Jess Scrimgeour (Department of Conservation, Tongariro Whanganui Taranaki Conservancy) by E.L. Weiser, C.E. Grueber, and I.G. Jamieson on 6 April 2011.

Introduction

In reintroductions, the smaller the founding population size, the more the initial gene pool will deviate from that of the source population (i.e. founder effect). Genetic diversity is further reduced through genetic drift in subsequent generations when rare alleles are lost due to chance, especially if the population grows slowly or is limited by a low carrying capacity (Frankham et al. 2002; Allendorf & Luikart 2007).

Retaining 90% of genetic diversity is commonly used as a target for captive breeding programs, which are limited to fairly small numbers of individuals (Lacy 2000). The loss of 10% of the original diversity over 10 generations is considered acceptable because it corresponds with a low risk of inbreeding depression (Lacy 2000; Soulé et al. 1986). In these cases, genetic diversity is usually measured as heterozygosity, or the proportion of individuals who are heterozygous (have two different alleles) at a locus (Frankham et al. 2002). For this project, we looked instead at allelic diversity, or the number of alleles per locus across the population. This is an appropriate measure of genetic diversity when considering long-term effects, because loss of allelic diversity will affect the future adaptability and survival of species in the wild (Allendorf & Luikart 2007). Rare alleles are more vulnerable to genetic drift than common alleles and thus are more easily lost. Here, we consider a "rare" allele to be one that occurs at a frequency of 5% in the source population.

Allelic diversity is lost more quickly than heterozygosity (Allendorf 1986; Lacy 1989). For this project, we targeted 90% retention of rare alleles, meaning the population will retain more than 90% of heterozygosity. We explored setting the goal even higher (95% retention of rare alleles), but found that this would require numbers of kiwi that are probably unrealistic for this project (70-80 kiwi released in the first 10 years).

Methods, results, and discussion

We adapted a model previously used for mohua (Tracy et al. 2011) to assess the number of individual brown kiwi needed to be translocated and released inside a fenced reserve at Rotokare (230ha). We parameterised our model (AlleleRetain; Chapter 2) with published and unpublished values from existing brown kiwi populations (Table 1).

The numbers given in Table 2 will achieve at least a 90% probability of retaining rare, selectively neutral alleles after 100 years or ~8 generations. This probability is

equivalent to the percent of rare alleles that will be retained after 100 years. The estimates below take into account the uncertainty included in the models. In each case, the numbers listed provide 95% certainty of reaching the goal of 90% allele retention, assuming the parameters and assumptions we used were correct.

In each scenario in Table 2, individuals are introduced to the reserve either during the initial release (in year 0), in subsequent releases (annually for 5 or 10 years after the initial release), or as occasional new migrants (per generation, which is every 13 years). In all cases more than one migrant per generation is needed; migrants could be added in the same year or in different years within the same generation. The total number of individuals added to the reserve, in the first 10 years or the first 100 years, under each scenario is given in the last two columns for comparison of the demands on source populations. We assumed that the sex ratio of released individuals will be approximately equal, though not necessarily exact.

In each case, 40-45 individuals will need to be added in the first 5-10 years, and 16-24 additional migrants added over the first 100 years, for a total of 56-64 kiwi added to the reserve, to reach our genetic goal of 90% probability of retaining rare alleles. The number needed is slightly lower if adults are introduced rather than juveniles.

Table 1. Values used to predict allele retention in a population of brown kiwi at Rotokare Scenic Reserve.

Parameter	Value	Reference
Juveniles per pair per year	0.525-1.0	Robertson et al. (2010)
Probability of breeding	0.70	Robertson et al. (2010)
Annual adult survival	0.98	Robertson et al. (2010)
Annual subadult survival	0.95	Robertson et al. (2010)
Initial survival (after release)	0.90	Unpubl. data from Maungatautari Ecological Island (J. Scrimgeour)
Age at first breeding	3 years	Unpubl. data from Maungatautari Ecological Island (J. Scrimgeour)
Carrying capacity	50 adult pairs	Unpubl. data (J. Scrimgeour)

Table 2. Translocation strategies to establish and maintain a genetically robust brown kiwi population at Rotokare Scenic Reserve.

Age of birds released	# initially released	Additional releases	Migrants per generation	# added in first 10 years	# added over 100 years
Juvenile	20	2/yr for 10 yrs	3	40	64
	30	2/yr for 7 yrs	2	44	60
	40	1/yr for 5 yrs	2	45	61
Adult	20	2/yr for 10 yrs	2	40	56
	30	2/yr for 5 yrs	2	40	56
	40	0	2	40	56

Note that we have not tested all possible scenarios. For each number of initial releases, we have tested 0-2 additional released individuals for 5-10 years, with 0-3 migrants per generation. Other scenarios can be easily tested upon request.

The model assumes that adults maintain the same mate from year to year (if both survive), and that maturing subadults will not replace an adult that already has a territory (if that adult survives). It also assumes that all surplus juveniles/subadults are removed annually from the reserve once carrying capacity is reached. [N.B. In practice, they could be removed as eggs.] With the above high survival rates, only two maturing subadults are needed each year to replace the adults that are estimated to die. This translates to approximately three juveniles every year. Anything in excess of this could be removed without jeopardising the Rotokare population if individuals are needed to supplement other populations. This would allow migrants to recruit into the breeding population at Rotokare, especially if additional birds produced locally are removed when migrants are added (e.g. remove all but one local juvenile when releasing two juvenile migrants, or remove all local subadults of age 2 when releasing two migrant subadults of that age). The genetic diversity of the population will be improved by migrants only if they are able to breed successfully.

We recommend monitoring this population closely for at least the first five years after establishment to document vital rates, especially the parameters listed above. If any of the above parameters or assumptions turn out to be inaccurate for the Rotokare population, we can re-run the models with the observed parameters and determine whether more or less migration may be necessary to achieve the genetic goal.

Acknowledgements

Our research into the conservation genetics of New Zealand endemics is funded by Landcare Research (contract no. C09X0503), Marsden Fund and University of Otago.

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Appendix B. Recommended management strategies for maintaining genetically robust populations of Haast tokoeka (*Apteryx australis* 'Haast') in small, predator-free sanctuaries



Orokonui Ecosanctuary, Dunedin, New Zealand.

This appendix provides a report prepared for Hugh Robertson (Department of Conservation, National Office, Wellington) by E.L. Weiser and I.G. Jamieson on 21 November 2012.

Introduction

When it comes to reintroductions, the smaller the founding population size, the more the initial gene pool will deviate from that of the source population (i.e. founder effect). Genetic diversity is further reduced in the new population through genetic drift in subsequent generations when rare alleles are lost due to chance, especially if the population grows slowly or is limited by a low carrying capacity (Frankham et al. 2002; Allendorf & Luikart 2007).

Retaining 90% of genetic diversity is commonly used as a target for captive breeding programs, which are limited to fairly small numbers of individuals (Lacy 2000). The loss of 10% of the original diversity over 10 generations is considered acceptable because it corresponds with a low risk of inbreeding depression (Lacy 2000; Soulé et al. 1986). In these cases, genetic diversity is usually measured as heterozygosity, or the proportion of individuals who are heterozygous (have two different alleles) at a locus (Frankham et al. 2002). For this project, we used allelic diversity, or the number of alleles per locus across the population, as an appropriate measure of genetic diversity when considering long-term effects, because loss of allelic diversity will affect the future adaptability and survival of species in the wild (Allendorf & Luikart 2007). Rare alleles are more vulnerable to genetic drift than common alleles and thus are more easily lost. Here, we consider a "rare" allele to be one that occurs at a frequency of 5% in the source population.

Methods

We used a computer simulation model (Weiser et al. 2012) to assess the number of additional Haast tokoeka needed to establish genetically robust populations at each of three small sanctuaries (Orokonui Ecosanctuary [fenced], Pomona Island, Rarotoka Island) given the number of individuals that have already been released at each site, the estimated carrying capacity, and demographic parameters for Haast tokoeka (H. Robertson pers. comm.). The input parameters for the model are listed in Table 1. The model assumes that adults maintain the same mate from year to year (if both survive), and that maturing subadults will not replace an adult that already has a territory (if that adult survives).

Throughout this document, we refer to individuals initially released to establish the population as "starters." These starters are released within the first several years of population establishment, and may or may not become genetic founders of the population.

We use the term "immigrants" to refer to individuals released into the population in later years at regular intervals (e.g. 10 immigrants released once every 5 years). These immigrants also may or may not contribute to the population genetically, depending on their survival and ability to recruit into the breeding population.

We used the model to assess how many immigrants should be released at each site in order to achieve at least a 90% probability of retaining rare, selectively neutral alleles after 100 years. This probability is equivalent to the percent of rare alleles that will be retained after 100 years. The estimates below take into account the uncertainty included in the models. In each case, the estimates provide 95% certainty of reaching the goal of 90% allele retention, assuming the parameters and assumptions we used were correct.

In our simulations, we assumed that locally produced juveniles would be removed from each population to allow room for immigrants to recruit to breed. In some cases, we also simulated the removal of adults (the same number as the number of immigrants being introduced).

Table 1. Demographic parameters used in simulations of allele loss for populations of Haast tokoeka. Values were estimated by H. Robertson, assuming these sanctuaries remain free of introduced predators. We used the model to estimate the total carrying capacity that would result in the predicted number of territorial pairs at each site.

Parameter	Value used
Source population effective size	245
Initial starters (year 1)	Orokonui: 16, Pomona: 16, Rarotoka: 10
Age at release (starters and immigrants)	Juvenile
Carrying capacity: all (territorial pairs)	Orokonui: 54 (20), Pomona: 41 (15), Rarotoka: 20 (6)
Age at first breeding	4
Maximum age of reproduction	67
Mean hatched chicks per pair per year (SD)	0.7 (0.33)
Maximum number of chicks per year	2 (double-brooding possible but uncommon)
Juvenile survival (chick to 1 year)	0.56
Subadult annual survival	0.97
Adult annual survival (age 4 and older)	0.978

Results and discussion

Given the low numbers of individuals that have already been released at each of these sites, the probability of retaining rare alleles is quite low initially (< 0.6) and declines over time when there is no immigration in each of the three populations (Figure 1). Immigrants are needed to boost the proportion of rare alleles introduced to each population, and then to retain that proportion over 90% for the 100-year period. These immigrants must be added throughout the 100-year period, as the three populations are small enough that genetic drift will be too strong to allow 90% retention of rare alleles over time without sustained immigration.

No amount of immigration could achieve the genetic goal at Rarotoka; the population size is simply too small for 90% of rare alleles from the source population to be represented at Rarotoka, and the population is small enough that genetic drift rapidly erodes the diversity that is present. Four immigrants every 5 years achieved the genetic goal at Orokonui, and 6 every 5 years at Pomona (Figure 1). These numbers were estimated under the assumption that enough local juveniles will be removed to allow all surviving immigrants to recruit into breeding vacancies (i.e. they will not be outcompeted by local juveniles). If only enough local juveniles are left to compensate for adult mortality after the immigration event, all immigrants who survive to breeding age and find a mate will breed. Removing a few local adults did not change the amount of immigration necessary for Orokonui or Pomona.

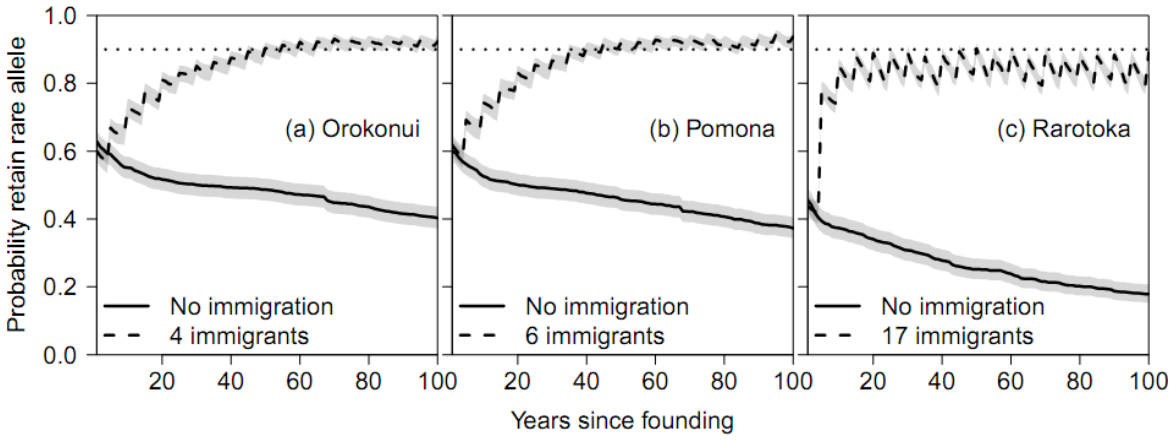

Figure 1. Expected retention of rare alleles in three populations of Haast tokoeka. Values were estimated with no immigrants (solid line) or with enough immigrants once every 5 years to achieve the goal of 90% retention of rare alleles over 100 years. Grey bands indicate 95% confidence intervals around the mean.

Because the goal for 90% retention of rare alleles cannot be achieved at Rarotoka, a less stringent goal could be considered. For example, management efforts could target retaining 80% of rare alleles, though this could result in a reduced chance that the population will persist long-term, from a genetic selection and future adaptability perspective. Adding 6 immigrants every 5 years would achieve this goal at Rarotoka. We recommend targeting the 90% goal for the other two populations, as this will maximize their chances of persisting; and the amount of immigration needed to achieve the 80% goal (2 immigrants every 5 years for Orokonui; 3 every 5 years for Pomona) is only slightly lower than that needed to achieve the 90% goal.

In most cases, the frequency of immigration is somewhat flexible; i.e. adding 8 immigrants every 10 years will have approximately the same genetic effect as adding 4 every 5 years. However, when larger groups of immigrants are released, it may become more important to remove locally produced adults in addition to juveniles to allow room for the immigrants to recruit.

If immigrants are released as adults rather than juveniles, slightly less immigration will be necessary, as those individuals will have a higher chance of breeding (contributing their alleles) before dying (i.e. not all immigrants released as juveniles will survive to adulthood). To achieve the genetic goal, the Orokonui population would need 4 adult immigrants (instead of 5 juveniles) every 5 years; Pomona would need 5 (instead of 6 juveniles); and the goal would still not be achieved at Rarotoka.

Not all of the immigrants released into each population need to breed in order to achieve the genetic goal. In our simulations, 49% of immigrants are expected to breed at Orokonui and Pomona, and 18% at Rarotoka. The necessary number of effective immigrants remains constant across scenarios (2.5 every 5 years at Orokonui and 2.9 at Pomona and Rarotoka); parameters such as the age of immigrants and whether or not local juveniles are removed will affect the proportion of immigrants that can breed, thus changing the total number that needs to be added to achieve these effective numbers.

The amount of immigration needed to reach the genetic goal indicates the difficulty of conserving allelic diversity in small, isolated populations. Because these small, isolated populations are so vulnerable to genetic drift, it is especially important to use accurate demographic parameters in the predictive model. We recommend monitoring these populations closely for at least the first five years after establishment to document vital rates, especially the parameters listed above. Monitoring the fate of immigrants would also allow assessment of whether the above assumptions about

recruitment of immigrants are valid. If any of the above parameters or assumptions turn out to be inaccurate for any of these populations, we can re-run the models with the observed parameters and determine whether more or less immigration may be necessary to achieve the genetic goal.

Acknowledgements

Our research into the conservation genetics of New Zealand endemics is funded by Landcare Research (contract no. C09X0503), Marsden Fund, Allan Wilson Centre for Molecular Ecology and Evolution, and University of Otago. Catherine Grueber, Murray Efford, Jess Scrimgeour, and Michelle Reynolds contributed to the development of the model we used for this analysis.

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Appendix C. Methods for incorporating complex covariate effects in VORTEX



Adult black robin on Rangatira Island, New Zealand.

This appendix has been prepared as supplementary material to be published with Chapter 6.

Here I provide a detailed account of my methods for predicting population viability of black robins, which can be used as a guide by others wishing to use VORTEX 9 (Lacy et al. 2009) to implement complex effects of inbreeding and other covariates in population viability analysis. Although many of my methods can be replicated by referring to the VORTEX 9 user manual (Miller & Lacy 2005), this guide provides specific information that will be particularly helpful for others aiming to incorporate complex inbreeding effects. Symbols and abbreviations used in this appendix are defined in Box C.1.

To account for the spatial structuring on Rangatira I. (the Top Bush and Woolshed Bush areas exhibit different demographic rates), I simulated Top Bush and Woolshed Bush as separate "populations" in VORTEX (though they are not distinct populations), connected by dispersal (mostly of juveniles) as recorded in the dataset. Mangere I. was the third population.

Box C.1. Definition of symbols and notations used in formulae input to VORTEX.

I	Inbreeding (defined by VORTEX on a scale from 0 to 100)
F	Inbreeding coefficient (on a scale from 0 to 1)
NRAND	Random number selected from a normal distribution (mean = 0, SD = 1)
A	Age of a simulated individual
S	Sex of a simulated individual (M = male, F = female)
P	Population (1 = Mangere I., 2 = Woolshed Bush, 3 = Top Bush)
Y	Year of the simulation
IS	Individual state variable
!=	≠

Tracking covariates

VORTEX records inbreeding (I) on a scale from 1 to 100, whereas I had used F values (range 0-1) to assess covariate effects; so I assigned Individual State Variable 1 (IS1) as I/100 to track each individual's F value. This was necessary to then track the F-value and age of each individual's mother and father with Individual State Variables. I designated IS2 as the father's F, IS3 as the mother's F, and IS4 as the mother's age (Box C.2). I did not assess effects of father's age because there was a strong correlation between mother's age and father's age in this dataset (Chapter 5).

Box C.2. Individual state variables used in VORTEX. "Init fn" (initialisation function, or the value assigned when the individual is created) is not important in these cases (can be any value). "Birth fn" is the value assigned to the individual at birth, and "Transition fn" indicates how the value changes from one year to the next (in each of these cases, the value remains constant from year to year). "IIS1(SIRE)" refers to the value of IS1 for the sire of the individual.

Individual State Parameter	Label	Init fn	Birth fn	Transition fn
IS1	F	=I/100	=I/100	=IS1
IS2	SireInbr	=1	=IIS1(SIRE)	=IS2
IS3	DamInbr	=1	=IIS1(DAM)	=IS3
IS4	DamAge	=1	=IIS3(DAM)	=IS4

Covariate effects

To incorporate information about relationships between covariates (inbreeding, age, sex) and demographic rates into the PVA, I used functions rather than constants to delineate demographic rates. Some input to VORTEX (e.g. distribution of clutch sizes among females) cannot be specified by functions. Use of these functions is described in the VORTEX user manual (Miller & Lacy 2005); I also solicited advice via the VORTEX listserv (https://listhost.uchicago.edu/mailman/listinfo/VORTEX). For example, in order to implement effects of mother's or father's inbreeding coefficient on egg or chick survival, Robert Lacy (primary author and maintainer of VORTEX) suggested that I would need to incorporate offspring survival (from egg to independence) into the "Juvenile Mortality" parameter. I did so by multiplying offspring survival by juvenile survival (then subtracting from 1 and multiplying by 100 to translate into percent mortality, as required by VORTEX).

Because the effect sizes calculated by the model-averaging approach were developed from centred covariates and generalised models, I had to back-transform the functions to the natural scale of each variable before implementing them in the PVA. I centred each individual's value for each covariate by subtracting the mean value of that covariate in the dataset used to develop the linear model (Box C.3). For example, the mean F in the adult survival dataset was 0.344; so for each adult, I subtracted 0.344 from their F value before applying the function to determine the individual's survival probability.

I used NRAND, a random number generator (mean = 0, SD = 1, normal distribution), to apply uncertainty around each effect size based on unconditional standard errors obtained from model averaging results (Box C.3), as described in the VORTEX user manual (Miller & Lacy 2005, pg. 102). I used NRAND rather than SRAND as the former incorporates among-iteration (as well as among-year and among-individual) variation, while the latter uses a seeded random number to keep the mean constant within each iteration. Finally, I applied the appropriate function to back-transform from the distribution I had used when developing the generalised linear model (e.g. binomial [logit] or Poisson [ln]; Box C.3).

Other information

The complete black robin pedigree was recorded through 1998, so I used this as the starting year of the PVA and uploaded the pedigree file as per instructions in the VORTEX user manual so that the simulations would start with birds known to be alive at the beginning of the 1998 breeding season.

Birds removed from Woolshed Bush and Mangere I. in the effort to establish a new population in Caravan Bush were designated with the "Harvest" option (Box C.4) to be removed from the simulations. Sex of most harvested birds was known, and I assumed an approximately even sex ratio for the remainder.

Information from recent censuses was incorporated by limiting carrying capacity to the recorded population size in each year (Box C.5). For the sensitivity analysis, I reduced each rate in turn by multiplying by 0.9.

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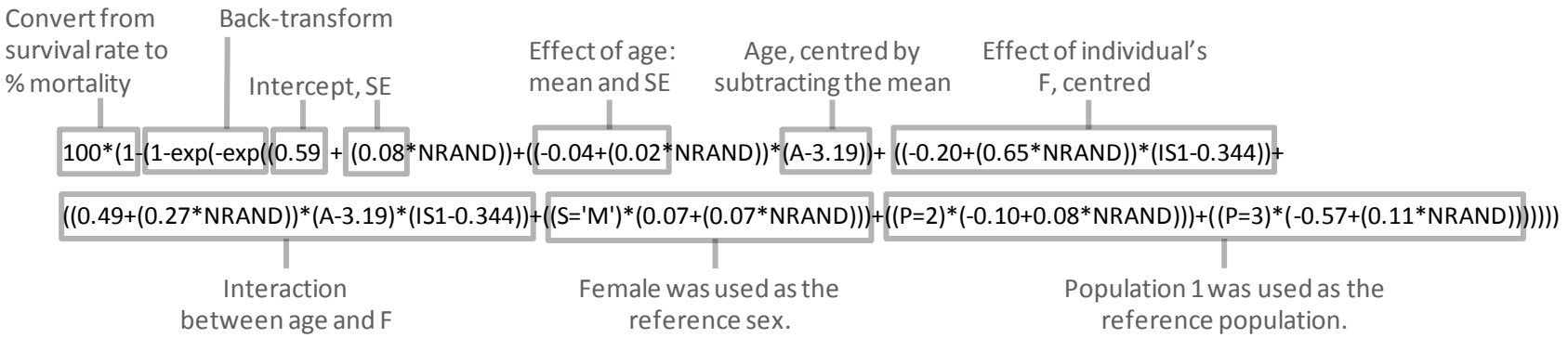
Box C.3. Formula used to specify mortality probability (calculated from survival probability and back-transformed from a clog-log distribution) for each adult in each year.



Box C.4. Formula used to specify harvest of females from Woolshed Bush. Harvest occurred in 2002 and 2004 (years 4 and 6 of the simulation).

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((Y=4)*6)+((Y=6)*7)
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Box C.5. Formula for carrying capacity of Mangere I.

