Loss of genetic diversity and inbreeding in New Zealand's threatened bird species

Ian G. Jamieson

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ABSTRACT

This report summarises findings from a 5-year research project (2003–2007) investigating the extent of loss of genetic diversity and inbreeding across various New Zealand threatened birds. Introduced predators and habitat loss are impacting on many New Zealand native species, but many species also have exceptionally low genetic diversity as a consequence of persisting in small and isolated populations. Research indicated that temporary bottlenecks associated with founder events during translocations do not contribute as much to loss of genetic variation as the small, finite population sizes of island sites. The build-up of inbreeding within closed island populations can result in further reductions in individual fitness. There is evidence of moderate inbreeding depression in a reintroduced population of North Island robins (*Petroica australis longipes*) on Tiritiri Matangi, and weak inbreeding depression in takahe (*Porphyrio mantelli*) translocated to offshore islands. To what extent reduced individual fitness translates to reduced population growth rates depends on the frequency of close inbreeding, the magnitude of inbreeding depression and which life history traits (i.e. fecundity versus survival) are most affected. Genetic management of New Zealand threatened species should not take priority over other management concerns such as controlling predators or improving habitat quality, but it does need more attention than it currently receives. Recommendations for genetic management emulating from this research should not be viewed in isolation, but considered alongside other contributing factors to help inform management decisions. Moreover, the maintenance of genetic diversity should become a fundamental component in long-term management strategies for threatened species in New Zealand.

Keywords: bottlenecks, disease, extinctions, genetic drift, inbreeding depression, translocations

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1. Introduction

It is now generally accepted that the factors that cause populations to decline (e.g. habitat loss, over-exploitation, introduced predators) and the processes that become amplified in small populations (e.g. demographic and environmental variation, catastrophic events, genetic drift and inbreeding) play a combined role in increasing the risk of extinction of threatened species (Hedrick et al. 1996). Ever since Frankel & Soulé's (1981) seminal textbook on conservation biology, conservation biologists have become increasingly aware that small populations are particularly vulnerable to loss of genetic diversity through processes such as genetic drift (the random loss of alleles due to mortality or failed breeding), while the negative consequences of inbreeding depression (the reduction in fitness in inbred relative to outbred individuals or populations) have been known since Darwin's time (Keller & Waller 2002). The loss of genetic diversity can lead to a reduced ability to adapt to changing environments, lowering the chances of long-term persistence, whereas inbreeding depression can directly affect population growth rates, ultimately increasing extinction risk (Frankham 2005). The importance of distinguishing between the two main genetic consequences of small population sizes—loss of genetic diversity and inbreeding depression—is emphasised throughout this paper. Their different properties and consequences are described in further detail in Appendix 1.

The primary goal of any conservation programme is to identify and remove the agents of decline (Caughley 1994). Historically, populations of endemic birds in New Zealand declined as a result of both human hunting pressure and deforestation (Worthy & Holdaway 2002), while current populations continue to decline as a result of introduced predators. Focussing on the maintenance of genetic diversity in management programmes is of little value when populations are in severe decline due to extrinsic factors. Therefore, there has been little need historically in New Zealand to focus on the role of genetic diversity in the viability of non-captive populations. However, more recently, the immediate threat of introduced predators has been reduced for many of our threatened populations through eradication programmes on offshore islands or through intensive predator control or creation of fenced sanctuaries on the mainland. In the process of reintroducing threatened species to these protected sites, relatively small numbers of individuals are released and small populations are usually established due to the limited areas involved. Ultimately, small population sizes bring into play the potential genetic issues mentioned above.

When researchers outside New Zealand first started to address the management issues surrounding small population sizes, they recommended that reintroduction programmes should aim to establish a minimum population of 500 individuals to ensure genetic viability in the longer term (Franklin 1980). John Craig of Auckland University was the first to recognise that strict adherence to this '500 rule' would rule out the reintroduction of many of our threatened bird species to small, 'predator-free' island sanctuaries such as Tiritiri Matangi (Craig 1991, 1994). Craig, as well as many other New Zealand conservation biologists, realised that the establishment of these 'predator-free' islands, no matter how small, was essential for ensuring the immediate survival of many of

our declining mainland populations. Craig also proposed that, although normally harmful in outbred populations, inbreeding depression would be less evident in historically inbred populations like those that presumably occur in New Zealand, as such populations would have purged the deleterious recessive alleles normally associated with inbreeding depression through the process of natural selection (Craig 1991, 1994). This hypothesis and reasoning became widely accepted among many New Zealand conservation managers and scientists (see Wallis 1994), who believed our indigenous avifauna was already inbred and hence immune to the effects of inbreeding depression relative to outbred species in large continental habitats[1].

It has subsequently been shown that purging, while effective at removing lethal alleles, is much less effective at removing weaker deleterious alleles that can affect factors such as reproductive performance (Keller & Waller 2002). Furthermore, the purging argument only focussed on the consequences of inbreeding depression and not on the loss of evolutionary responsiveness that is associated with loss of genetic diversity. In light of these findings, in 2002 the New Zealand Department of Conservation (DOC) identified genetic diversity and its role in maintaining long-term viability of threatened species as a national research priority (DOC 2002/2003).

This report summarises the main findings of the first stage of a 10-year research programme investigating the effect of inbreeding and loss of genetic diversity on the survival and reproductive parameters of threatened bird populations. A thorough justification for the proposed research was initially required and laid down in a series of review-type papers that addressed the general neglect in New Zealand of the importance of inbreeding and inbreeding depression in recovery programmes (Jamieson et al. 2006); the relative importance of genetics versus introduced predators in increasing extinction risk of island endemics (Jamieson 2007a, b); and the general importance of managing genetic diversity in threatened populations in New Zealand (Jamieson et al. 2008). It was argued that intrinsic factors such as genetic drift and inbreeding are potentially impacting many of our locally threatened species, but their effects tend to occur over a considerably broader timescale than extrinsic factors such as introduced predators (Jamieson et al. 2006, 2008; Jamieson 2007a, b). Consequently, they are much more difficult to detect and, ultimately, to justify investing additional resource towards. Genetic management of New Zealand threatened species should not take priority over other management concerns such as controlling predators or improving habitat quality, but it does need more attention than it currently receives and should be considered a fundamental component in long-term management strategies for threatened species (Frankham et al. 2002; Allendorf & Luikart 2007).

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[1] It should be noted that while Craig (1991, 1994) did present some observations on the frequency of inbreeding in some New Zealand bird populations, he provided no evidence or data on inbreeding depression.

2. Aims and objectives

The goal of this research programme was to determine the level of genetic diversity and the frequency and extent of inbreeding and inbreeding depression in New Zealand's avifauna. Specifically, the research aimed to:

- Determine the degree of loss of genetic variation in several bottlenecked bird populations using microsatellite DNA markers, and compare historical (pre-bottlenecked) versus contemporary populations, mainland versus island populations, and serial bottlenecked/translocated island populations.
- Examine the frequency and pattern of inbreeding in small island populations using pedigree data.
- Determine the magnitude of inbreeding depression in small island populations.
- Review the relationship between loss of genetic diversity and risk of disease.

In this report, I outline the study species, study sites and general methods used, and report on various methodological issues that arose during the course of the research (e.g. can feather samples substitute for blood for DNA analysis?) (section 3). I then report the results of preliminary analyses that tested critical assumptions (e.g. confirming that pedigrees are derived from genetically monogamous species) (section 4). The remaining sections (sections 5–9) cover the results of the specific objectives listed above plus related work on population bottlenecks and disease risk conducted by Katrina Hale as part of her PhD research, supervised by Dr Jim Briskie, University of Canterbury, Christchurch (Hale 2007; Hale & Briskie 2007).

Most of the research referred to in this report has already been peer-reviewed and published, mainly in specialised international journals. A small fraction of the work will refer to unpublished data or unpublished reports and theses. Rather than repeat the details of methods and results that are available from other sources, this report will attempt to specify the particular problem that the research was addressing, summarise the findings, and provide specific recommendations and guidelines where appropriate.

3. Study species, study areas and general methods

3.1 STUDY SPECIES AND AREAS

Twenty-five DOC recovery groups dealing with issues involving inbreeding and/or genetic diversity were initially contacted. The majority of these were involved with bird species (14 groups, 52%), with bats (2), fish (2), frogs (1), insects (2) and plants (4) making up the remainder (unpubl. data). Therefore, this study focussed on three species of bird: takahe (*Porphyrio mantelli*), South Island (SI) saddleback (*Philesturnus carunculatus carunculatus*) and New Zealand robin—both South Island (SI) (*Petroica australis australis*) and North Island (*Petroica australis longipes*) subspecies[2].

Birds are ideal subjects for the study of genetic variation and inbreeding, as bird blood is rich in DNA and avian microsatellite markers are readily available. Constructing pedigrees is also relatively straightforward in birds because offspring can be colour-banded in the parents' nest or on their territory, and subsequently subjected to DNA testing to ensure the social parents are also the genetic parents (see below). In addition, fitness across a number of life history parameters can be readily estimated in terms of egg fertility, hatching success, fledging success, juvenile and adult survival, and recruitment into the breeding population.

Island populations that are the result of translocations are also ideal for this sort of study because pedigrees can be constructed and maintained when monitoring starts soon after the birds are released. Furthermore, because the species used in this study are either flightless (takahe), poor flyers (saddleback) or unwilling to fly (robins) and do not occur on the adjacent mainland, these islands effectively harbour closed populations, so there are no unknown birds immigrating into the populations and birds that disappear can be assumed to have died.

As part of this study and related research (Steffans et al. 2005; Michel 2006), the founders and virtually all offspring from seven cohorts of SI saddlebacks and SI robins reintroduced to Ulva Island in 2000 were studied. Birds were colour-banded, blood samples were collected, and survival and breeding success were recorded. Similar data were also collected over a shorter period of time (5 years) for SI robins released on the Doubtful Islands, Lake Te Anau, where they co-exist with kiore (*Rattus exulans*) and transient stoats (*Mustela erminea*). Collaboration with Doug Armstrong (Massey University, Palmerston North) provided access to 13 years of banding and monitoring data for NI robins reintroduced to Tiritiri Matangi. The Takahe Recovery Group provided 20 years of banding and monitoring data to allow the construction of pedigrees extending back seven generations for takahe introduced to four offshore islands in the mid-1980s. A banding and monitoring study of SI saddlebacks on Motuara Island (Marlborough Sounds) was also initiated (Michel et al. 2008), but there

[2] Holdaway et al. (2001) considered the subspecies of saddlebacks and robins on the North and South Islands of New Zealand as separate species, but we treat these as subspecies here, following Higgins & Peter (2002) and Higgins et al. (2006).

were too many unbanded adult breeders to complete the pedigrees (although blood samples were collected and used as part of other studies; see below).

Blood samples were collected from seven SI saddleback and four SI robin populations to analyse patterns of loss of genetic variation. Additional robin blood samples were obtained from three sites in the Nelson and Marlborough region, which were collected for a previous study by Jim Briskie, Bruce Waldman and Ian McLean of the University of Canterbury. Surplus feather and DNA samples used for sexing takahe were obtained from Ian Anderson (Massey University), as well as blood collected during an earlier genetic study on takahe by Marieke Lettink in David Lambert's lab (Massey University) (Lettink et al. 2002). Tissue samples were also obtained from museum specimens of SI saddlebacks, SI robins and takahe collected from various South Island sites in the 1800s–1900s to determine genetic diversity in historical populations.

3.2 GENERAL METHODS

All birds were colour-banded as part of the original translocations, and their nests were subsequently found and monitored. Offspring were also banded and blood samples taken, and resightings of banded birds were recorded. In addition, blood and faecal samples were collected for disease screening, habitat types were mapped, and food availability was sampled (Ulva and Motuara Islands), saddleback calls were recorded to assess mechanisms of inbreeding avoidance (Ulva Island), and weather patterns were recorded (Ulva Island). Pedigrees were constructed using the software program PedSys (www.sfbr.org/pedsys/; viewed August 2007) and drawn using the program PedDraw (www.pedigree-draw.com; viewed August 2007). For further details on constructing and analysing pedigrees, see Jamieson et al. (2007) and Grueber & Jamieson (2008).

DNA was extracted from blood and tissue samples, and microsatellite markers were developed to estimate levels of genetic variation (allelic richness and heterozygosity) and for use in parentage analysis to confirm pedigrees. Markers were optimised either from primers developed for other (normally closely related) species or from species-specific microsatellite libraries that were developed at the University of Otago, Dunedin. Considerable effort had to be spent to find informative markers (i.e. polymorphic loci with two or more alleles) in SI saddlebacks and takahe because both species had very low levels of genetic variation, with the majority of loci being monomorphic. For further details on methods for extracting DNA, marker development and genetic analysis, see Boessenkool et al. (2007), Taylor et al. (2007, 2008), Taylor & Jamieson (2008), and Grueber et al. (2008a).

Details about the number of birds translocated and how many survived to breed on the various study islands are given in section 7.

3.3 METHODOLOGICAL ISSUES

3.3.1 Does collection of blood for genetic analysis affect the bird's behaviour or subsequent survival?

During the course of the project, difficulties were occasionally encountered in obtaining permission to take blood samples, especially as part of a translocation. The reason generally given was to avoid stressing the birds more than necessary. However, studies on captive and wild bird populations have shown that collecting blood has no significant effect on changes in body weight, loss of territories or annual survival of males, or rates of nest abandonment, nest success, fledging rate or annual return rates of females (Hoysak & Weatherhead 1991).

During the course of this research, the minimal processing of a bird, which included taking its weight, applying one metal and three colour bands, taking a < 0.1 mL blood sample, and measuring the tarsus, could take an experienced handler approximately 10 minutes. This represents a small fraction of the time a bird would be held during a translocation (from capture in a mist net to release). Furthermore, although additional handling of the bird is likely to be stressful for the bird in the short term, it is unlikely to add significantly to the already high stress levels caused by earlier handling and captivity.

As part of this research, a study was carried out on Ulva Island on the effects of mist-netting and handling (including taking blood) of SI saddlebacks immediately before the egg-laying period. These activities were found to have no measurable short-term effects on timing of egg laying or nesting success (Jamieson et al. 2005).

As part of a larger study, measurements and blood samples were taken from SI saddlebacks on Breaksea Island before they were placed in an aviary for up to 5 days and then recaptured and translocated by helicopter to Erin Island in Lake Te Anau (Taylor & Jamieson 2007b). SI saddlebacks were mist-netted at two sites on Breaksea: one within 20 m of the hut and aviary where all processing occurred, and another approximately 1 hour away from the hut by foot. Six of the 46 birds caught died in the aviary before they were transferred. The initial analysis indicated that neither age (adult versus yearling), sex (determined using morphometrics and discriminant functions of saddlebacks of known sex from Ulva Island; see Taylor & Jamieson 2007a), nor time held in the aviary had a significant effect on survival, but capture location did: all six birds that died came from the further site. After excluding all birds that were caught away from the hut ($n = 21$), the analysis indicated that poor body condition and presence of ecto-parasites had significant effects on short-term survival (first 2 weeks post-translocation) for the remaining birds released on Erin Island. There was no relationship between genetic variation and survival or between genetic variation and parasite load, possibly because genetic variation is relatively low in saddlebacks. Other studies have found such relationships, however (e.g. Whiteman et al. 2006). It was concluded that excessive distances between the mist net and the banding station should be avoided, but otherwise short-term survival is more likely affected by the birds' condition and health rather than handling and the taking of blood samples per se (Taylor & Jamieson 2007b).

3.3.2 Can feathers substitute for blood in genetic analyses?

Although there was a general reluctance to take blood, there was a general willingness to take several feathers plucked from the breast area of a bird for subsequent genetic analysis, based on the belief that this was less stressful on the bird. There is little doubt that plucking feathers is technically an easier task to perform than vein puncture and blood collection with a small capillary tube. However, if done properly by someone with experience, it is questionable whether blood collection is more stressful on the birds than feather plucking for two reasons. First, a bird's general stress levels may reach a maximum when caught in a mist net, removed by hand and then further handled, irrespective of whether the bird has its feathers or blood removed (Romero & Romero 2002). Second, plucked feathers take several weeks to be replaced, and their loss and re-growth are energetically expensive (Waite 1990); indeed feather re-growth is likely to be more energetically costly than replacing <0.1 mL of red blood cells. In addition, as noted above (section 3.3.1), studies have failed to find an effect of blood collection on changes in body weight, loss of territories, rates of nest abandonment, nest success, fledging rate or annual return rates (Hoysak & Weatherhead 1991), and thus have concluded that blood sampling is not obviously harmful to wild birds.

There is a substantial difference in the quality and quantity of DNA obtained from blood versus feather pulp (Harvey et al. 2006). Avian red blood cells are extremely rich in nuclear DNA, so that very little blood is required per extraction to provide high product yield. In contrast, feather samples contain poorer quality DNA and yield smaller amounts of product, which can lead to higher rates of failed amplification and genotyping errors (Segelbacher 2002)—although use of the blood clot located at the superior umbilicus of the feather shaft rather than the shaft tip can produce better results (Horvath et al. 2005). Furthermore, although feather samples can produce adequate results for DNA sexing techniques (Harvey et al. 2006), they may not be adequate when additional types of molecular analyses are required (Taberlet et al. 1999). There may also be a greater chance of contamination of feather samples either from other birds' feathers or from humans.

In conclusion, if the only reason for collecting samples is to carry out DNA sexing, then feather samples will be more than adequate. However, if the samples are to be used for additional molecular analysis (sometimes unforeseen at the time of sampling), then blood is preferable to feather samples. If individuals involved in banding or translocating birds are inexperienced in collecting blood samples, then feather samples should be taken; otherwise, it is recommended that blood samples be taken over feather samples when there is a choice, assuming animal ethics permits are obtained. Given the ease of the field technique and the vast potential for information to be gained, the inclusion of blood analyses in research programmes should not be precluded because of concerns over negatively affecting the birds' health or behaviour, as long as animal ethics approval is sought and proper precautions are taken.

4. Testing of critical assumptions and preliminary analyses

4.1 ARE SADDLEBACKS, ROBINS AND TAKAHE GENETICALLY MONOGAMOUS?

In order to use field observations of breeding pairs at the nest site to accurately construct pedigrees and estimate reproductive success, we need to know whether the species in question is genetically monogamous. High rates of extra-pair paternity (EPP) can be relatively common in passerines, whereas low rates or absence of EPP are often associated with taxa that are long-lived and exhibit obligatory paternal care, such as seabirds (Arnold & Owens 2002; Griffith et al. 2002). As part of this research programme, microsatellite DNA analysis was used to examine EPP in two passerine species (SI saddleback and SI robin), and one non-passerine species (takahe).

4.1.1 Saddlebacks and robins

To examine rates of EPP, individuals were organised into family groups consisting of parents and all offspring produced by the pair across all years. EPP was considered to have occurred if offspring had alleles not present in their parents at two or more loci. To account for null alleles/mutations and prevent overestimation of EPP, EPP was not counted if discrepancies between parent and offspring genotypes occurred at a single locus. All mismatches were checked by re-amplifying DNA from the parents and offspring and running the samples in adjacent lanes. For each species, EPP rates were calculated by dividing the number of pairs in which EPP occurred by all pairs.

No EPP (0%) was detected in SI saddlebacks (39 pairs, 202 offspring), and only one case (1.9%) in SI robins (54 pairs, 198 offspring) (Taylor et al. 2008). A previous study on SI and NI robins using minisatellite DNA also found no evidence of EPP (Ardern et al. 1997b), so as a rule it can be concluded that SI saddlebacks and SI robins are likely to be genetically monogamous.

Both SI saddlebacks and SI robins have relatively low annual mortality rates (6.5%–11% and 10%–20%, respectively) in their natural environment (i.e. without introduced predators) (Taylor et al. 2008). Therefore, genetic monogamy in these passerine species supports the hypothesis that low annual mortality rates play an important role in explaining variation in rates of EPP across species. In addition, it is reasonable to assume the breeding pairs seen at their nest site are the genetic parents of the offspring, making the pedigrees obtained highly accurate.

4.1.2 Takahe

Takahe are free to form natural pairings in island sanctuaries, and breeding is closely monitored by DOC staff; every offspring is colour-banded on its natal territory, given a name and entered into the studbook. Previous research using minisatellite DNA indicated that breeding pairs of takahe were genetically monogamous (Lettink et al. 2002). This pattern was confirmed in a study using

microsatellite DNA (Grueber 2005). In a few instances, takahe on islands also bred in groups of two to three males and/or two females, and in all these cases DNA analysis was used to resolve which birds were the genetic parents (C. Grueber and I. Jamieson, University of Otago, unpubl. data).

4.2 USING DNA ANALYSIS TO RECONSTRUCT MISSING PEDIGREE DATA

Although all nesting attempts of robins on Ulva Island were monitored since they were released, monitoring of saddleback nests did not commence until part way through the 2001 breeding season. Hence there were gaps at the base of the saddleback pedigree. Therefore, DNA analysis was used to reconstruct the missing parentage data. In April 2000, 30 SI saddlebacks were translocated from Big Island (off Stewart Island/Rakiura) to Ulva Island. Of these, 23 survived to the first breeding season in October 2000. Although there was no extensive nest monitoring in the first breeding season, it is now known that 12 of the 16 birds for which DNA samples were available were breeding pairs and the remaining 4 were single females based on both observations and subsequent parentage analysis (see below).

All saddleback adults and offspring were genotyped with 12 polymorphic loci consisting of 2–6 alleles each. Ken Dodds of AgResearch, Invermay, assigned offspring to unknown parents using a computer program designed to include known male and female pair combinations (Dodds et al. 2005). In this case, it was initially assumed that six pairs that were either seen together in 2000 or were known to have paired together in 2001 also paired together in 2000. Subsequent analysis indicated that this assumption was likely to be correct, as two to five offspring from the 2000 cohort were assigned to five different pairs with a high degree of probability (> 90%). Furthermore, nine offspring from the 2002 cohort for which the parents were known were all assigned correctly in a test sample, using the same procedure.

Of the original 17 juvenile SI saddlebacks of unknown parentage that fledged and survived from the 2000/01 cohort, 12 could be assigned to six breeding pairs with high probability (> 90% difference between two known pairs), 3 with moderate probability (70% difference) and 2 with lower probability (< 30% difference) (S. Taylor and I. Jamieson, University of Otago, unpubl. data). Similarly, of 28 juvenile SI saddlebacks from the 2001/02 cohort that were of unknown parentage, 18 could be assigned among 14 breeding pairs; the remaining 10 juveniles that could not be confidently assigned were left out of the analysis. When two datasets were used to analyse inbreeding data for SI saddlebacks on Ulva Island (one where parentage was assigned with high confidence (> 90%), and one that also included assignments of moderate confidence (35%–90%)), there was very little difference in the calculation of overall rates of inbreeding or percentage of closely related pairs.

4.3 DO SADDLEBACK CALLS CONTAIN KINSHIP RECOGNITION SIGNALS?

Bird song carries information and is known to influence mate choice. In certain situations, two opposing selection pressures may affect bird song: males gain advantages by sharing song types with their neighbours, thus reducing territorial disputes (Beecher & Brenowitz 2005), but it could also be advantageous for young males to share song types with their father so that their sisters can avoid mating with them (Grant & Grant 1996). Given that populations of SI saddleback occur on small, isolated islands, it was expected that selection pressures would favour relatedness cues in male song so that females could recognise kin and avoid inbreeding (Jenkins 1978).

Two different measures were used to investigate phrase type similarity between fathers, sons and the sons' post-dispersal neighbours, and both methods produced very similar results (Ludwig 2007; Ludwig & Jamieson 2007; K. Ludwig, University of Otago, L. Molles, Lincoln University, Christchurch, and I. Jamieson, unpubl. data). On a large scale, an investigation of shared phrase types among all possible tutors indicated that fathers contributed an average of 19% of their son's repertoire while all neighbours combined contributed 72% of the focal bird's repertoire. On a finer scale, visual comparisons of shared phrases between father, son and the son's neighbour suggested that, on average, shared phrases were matched to the bird's father 26% of the time and to the neighbour 73% of the time (K. Ludwig, L. Molles and I. Jamieson, unpubl. data). These results suggest that whole phrases and the singing of phrases in SI saddlebacks resulted in the retention of a paternal signature in the son's repertoire. However, despite this, paternal kinship cues are relatively weak and may not provide a strong enough signal for females to recognise and avoid pairing with sibling males before they settle in a territory. The next section investigates whether there is evidence that saddlebacks actively avoid pairing with close kin.

4.4 DO SADDLEBACKS AND ROBINS SHOW INBREEDING AVOIDANCE?

Mating with a close relative (i.e. inbreeding) generally results in reduced survival and/or reproductive success (i.e. inbreeding depression). Therefore, natural selection should favour behaviour that reduces the occurrence of inbreeding (Pusey & Wolf 1996). The most commonly cited form of inbreeding avoidance is dispersal (Pusey 1987). However, if dispersal is not an option and encounters between kin are likely to occur, individuals need to recognise their close relatives and avoid pairing with them (Pusey & Wolf 1996). New Zealand robins and saddlebacks possess life history traits (such as being relatively long-lived, having life-long pair bonds, and being socially and genetically monogamous) that indicate inbreeding would lead to a prolonged and substantial reduction in fitness for the breeding pair. Studies of robins have indeed shown that substantial inbreeding depression can occur (Briskie & Mackintosh 2004; Jamieson et al. 2007). Based on this information, current theory predicts that both species should show evidence of inbreeding avoidance by either avoiding breeding with close relatives (as determined from pedigree data) or by choosing genetically

dissimilar mates (as determined from microsatellite DNA data). Jamieson et al. (in press) undertook a study to document the level of inbreeding in one island population of saddlebacks and two island populations of robins based on 5–6 years of banding and nest monitoring data in each case. A randomisation approach was used to test whether the frequency of close inbreeding was less than would be expected from a random mating model. The hypothesis of inbreeding avoidance was further tested by determining whether saddlebacks and robins were more likely to choose genetically dissimilar mates relative to the average genotype available at the time of pairing based on microsatellite DNA.

Results indicated that out of 11 population-years of pedigree data for both species, there was evidence of inbreeding avoidance in 1 year, inbreeding preference in 1 year, and random mating in the other 9 years (Jamieson et al. in press). There was also no evidence that incestuous pairings were actively avoided or that individuals were choosing genetically dissimilar mates based on microsatellite DNA analysis. A review of the literature revealed that although inbreeding avoidance is common in cooperatively breeding birds, it is much less common in singular-pair breeding birds, which tend to mate randomly with respect to relatedness. A simple quantitative model was developed that incorporated encounter rates with close kin for various degrees of mate-searching effort. The results of the model showed that inbreeding avoidance is beneficial at intermediate levels of encounter rates with close kin (as found in cooperative breeders), but that random mating is more beneficial otherwise. From this, it can be concluded that random mating normally results in such low rates of close inbreeding in natural populations (i.e. pre-bottleneck) that it exerts negligible selection pressure to evolve kin recognition, when kin recognition plays no part in other aspects of social behaviour (Jamieson et al. in press).

These results have implications for the conservation and management of endangered species such as saddlebacks and robins. Most inbreeding in threatened wild populations is assumed to arise as an inevitable consequence of small population size. Population viability models usually ignore inbreeding avoidance, instead employing the simpler assumption of random mating. If species show variation in inbreeding avoidance, then the accuracy of these assumptions will depend on the mating system of the endangered species in question. The results suggest that at least for socially monogamous birds, many threatened species are unlikely to have a natural 'built-in' mechanism for avoiding inbreeding by kin discrimination, and the assumption of random mating may be appropriate and accurately reflect the true rate of inbreeding in small populations (Jamieson et al. in press).

5. Loss of genetic diversity in New Zealand endemics

Genetic diversity allows a population to genetically adapt to a changing environment or to be buffered against stochastic events such as harsh weather or disease outbreaks. Genetic diversity has been an important consideration in the development of management strategies for threatened populations around the world. In New Zealand, however, species recovery programmes have tended to focus on increasing population size at the expense of decreasing genetic diversity (e.g. over-represented founders) (Jamieson et al. 2008). If New Zealand's threatened species have relatively low genetic variation, then even if the effects of introduced predators are eliminated, they may still be at risk in the long term due to reduced resilience. The three main factors affecting genetic diversity—genetic drift, inbreeding and population fragmentation—potentially impact many of our locally threatened species, but because their effects tend to occur over a considerably broader time scale than the effects of predation, they are difficult to detect and ultimately to justify additional resource spending (Jamieson et al. 2008).

The aim of this section is to report the extent of loss of genetic diversity in threatened New Zealand birds relative to threatened species elsewhere, describe the pattern of loss over time and in different geographical contexts (e.g. mainland versus islands), and provide examples of how genetic diversity can be managed and maintained.

5.1 GENETIC DIVERSITY IN THREATENED BIRDS

Although one needs to exercise caution hen comparing microsatellite data across species, threatened endemic birds from isolated island populations, including those in New Zealand, generally have lower genetic variation than threatened species from mainland areas (Table 1). Furthermore, low levels of minisatellite variation (as measured by high band-sharing coefficients) are also evident in a number of other threatened New Zealand endemics (Robertson 2006: table 2). For example, Campbell Island teal (*Anas nesiotis*) has the highest band-sharing coefficient reported for any bird (0.87), and similar high values are evident in black robin (*Petroica traversi*; 0.84), kakapo (*Strigops habroptilus*; 0.71), Auckland Island teal (*Anas aucklandica*; 0.71), and shore plover (*Thinornis novaeseelandiae*; 0.56–0.68) (Robertson 2006). Therefore, the molecular evidence is consistent with the view that many of New Zealand's threatened endemic birds have gone through a relatively long period of small population size and subsequent inbreeding, and thus have low genetic variation.

TABLE 1. LEVELS OF MICROSATELLITE GENETIC DIVERSITY IN THREATENED BIRD SPECIES.

Species are categorised as either isolated island (I) or mainland (M) populations, and ranked according to expected heterozygosity (H_e) for polymorphic loci; average number of alleles per locus (A) is also reported. Means are presented for genetic data, and ranges for the number of individuals sampled when data were available from two or more populations. Only data from wild populations or unrelated founders held in captivity are included. New Zealand species are given in bold.

THREATENED SPECIES*		ISLAND/ MAINLAND	NO. INDIVIDUALS SAMPLED	NO. POLYMORPHIC LOCI	H_e	A
Mariana crow	*Corvus kubaryi*	I	16	6	0.24†,‡	2.2†
NZ snipe (1)	***Coenocorypha* undescribed sp.**	**I**	**9–33**	**9**	**0.25**	**2.2**
Great bustard	*Otis tarda*	M	52	6	0.35	4.8
Takahe (2)	***Porphyrio hochstetteri***	**M/I**	**225**	**24**	**0.33**	**2.3**
SI robin (3)	***Petroica a. australis***	**M/I**	**12–170**	**10**	**0.36**	**2.8**
Seychelles kestrel	*Falco araea*	I	4	2	0.38†	2.5†
San Clemente I. loggerhead shrike	*Lanius ludovicianus mearnsi*	I	26	6	0.40	2.3
Mauritius kestrel	*Falco punctatus*	I	75	3	0.42†	2.3†
SI saddleback (4)	***Philesturnus c. carunculatus***	**I**	**15–190**	**6**	**0.45**	**2.7**
Kakapo (5)	***Strigops habroptilus***	**I**	**90**	**30§**	**0.47§**	**3.3§**
Seychelles warbler	*Acrocephalus sechellensis*	I	25	30	0.48	2.8
Peregrine falcon	*Falco peregrinus*	M	22–28	12	0.49	4.1
Laysan finch	*Telespiza cantans*	I	44	9	0.51	3.1
Spanish imperial eagle	*Aquila adalberti*	M	38	18	0.52	4.0
Black stilt/kaki (6)	***Himantopus novaezelandiae***	**M**	**20**	**8**	**0.54**	**3.2**
Kokako	***Callaeas cinerea***	**M**	**8–24**	**4**	**0.56**	**3.8**
Greater prairie chicken	*Tympanuchus cupido*	M	18	21	0.56	4.5
Houbara bustard	*Chlamydotis undulata undulata*	M	87	6	0.56	7.0
Eagle owl	*Bubo bubo*	M	66	7	0.59	5.3
Florida scrub jay	*Aphelocoma coerulescens*	M	24–248	31	0.59	6.0
Taita thrush	*Turdus helleri*	M	17–80	7	0.59	5.2
NI brown teal (7)	***Anas chlorotis***	**M**	**117**	**2**	**0.59**	**6.2**
Eurasian vulture	*Gyps fulvus*	M	10	5	0.65	5.0
Mexican spotted owl	*Strix occidentalis lucida*	M	82–127	7	0.79	9.9
Corn crake	*Crex crex*	M	15	9	0.90	12.9

* Numbers in brackets refer to the following references: 1—A. Baker, University of Toronto, C. Miskelly, DOC, and O. Haddrath, University of Toronto, unpubl. data; 2—C. Grueber and I. Jamieson, University of Otago, unpubl. data; 3—Boessenkool et al. (2007); 4—Taylor & Jamieson (2007); 5—B. Robertson, University of Otago, unpubl. data; 6—Steeves et al. (2008); 7—G. Bowker-Wright, Victoria University of Wellington, unpubl. data. References for species without numbers can be found in Jamieson et al. (2006: table 1).

† Values have been recalculated based on polymorphic loci only.

‡ Only observed heterozygosity was reported.

§ Loci were chosen based on known polymorphism; therefore, H_e and A are likely to be high relative to estimates for other species.

5.2 HISTORICAL AND CONTEMPORARY LEVELS OF GENETIC DIVERSITY

Many studies use contemporary levels of genetic variation to evaluate the effects of recent bottlenecks, population fragmentation or genetic drift, and then assess extinction risk and management options for threatened species. However, contemporary patterns of genetic variation may not be recent in origin. Therefore, where possible, recovery programmes should try to include analysis of their species' genetic history to better understand loss of genetic variation over time and clarify relationships among fragmented populations.

Many studies that have used ancient/historical DNA to examine loss of genetic variation have found that heterozygosity, haplotype variation and allelic diversity have decreased following population bottlenecks (Bouzat et al. 1998; Groombridge et al. 2000). Although such reductions are consistent with theoretical expectations, low genetic variation in contemporary populations can also result from ancient events such as post-glacial re-colonisation or bottlenecks caused by prehistoric human activity. Similarly, long-term drift and ancient founder events may cause genetic invariance in small, isolated populations. Small, historically isolated populations may be more likely to survive current species declines because their isolation protects them from threats such as loss of habitat through agriculture and urbanisation, exotic predators, and infection from pathogens.

Using DNA extracted from museum skins (referred to here as 'historic' samples), the link between past and present genetic variation was examined in three New Zealand bird species, SI saddleback, SI robin and takahe, all of which have different dispersal capabilities and histories of decline (Taylor et al. 2007; Boessenkool et al. 2007; C. Grueber and I. Jamieson, University of Otago, unpubl. data).

5.2.1 South Island saddleback

Historic samples of SI saddlebacks came from several locations on mainland South Island (1877–1898; $n = 24$) and from the remnant population on Big South Cape, Solomon and Pukeweka Islands (1931–1965; $n = 20$). Contemporary SI saddlebacks were sampled from Big and Kaimohu Islands in 2005. These islands are home to the source populations of all extant saddlebacks, which presumably possess the greatest genetic variation of the 15 translocated island populations (Taylor et al. 2007).

Genetic analyses based on microsatellite DNA showed that the extinction of SI saddlebacks on the mainland resulted in the loss of substantial genetic diversity. At least 108 of 143 (75.5%) alleles present in the historic mainland samples were not detected in the historic remnant population on Big South Cape Island (Fig. 1), and all other measures of genetic diversity show a similar pattern. Furthermore, there was no significant difference between the two translocated populations on Kaimohu and Big Islands (contemporary samples) and their source, Big South Cape Island (historic samples) (Fig. 1A). These results suggest that the low genetic variation observed in contemporary SI saddleback populations is a consequence of past bottleneck(s) or historic drift rather than the recent decline caused by the rat plague in 1964, or founder effects and subsequent drift since the translocated populations were established (Taylor et al. 2007).

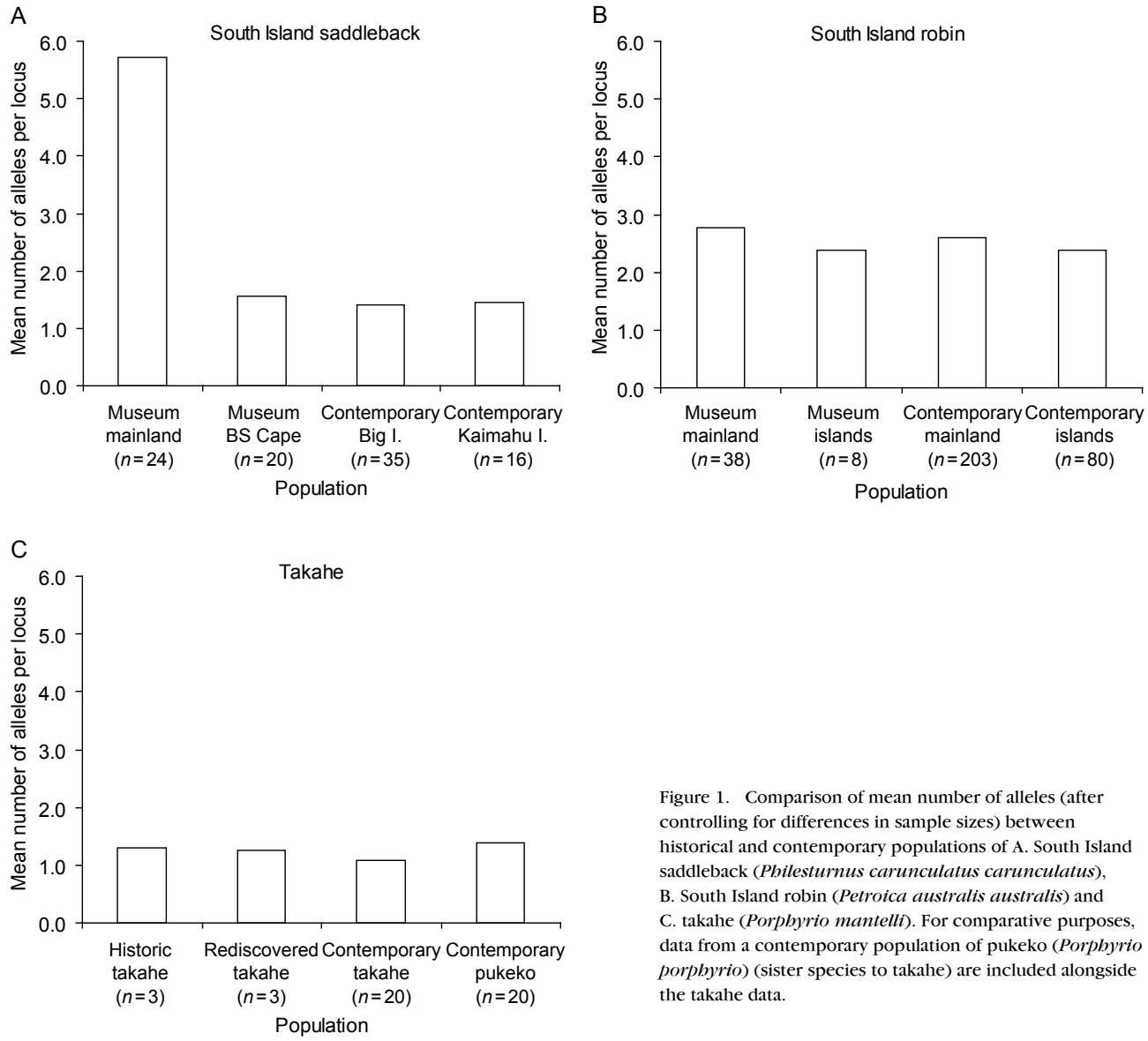

Figure 1. Comparison of mean number of alleles (after controlling for differences in sample sizes) between historical and contemporary populations of A. South Island saddleback (*Philesturnus carunculatus carunculatus*), B. South Island robin (*Petroica australis australis*) and C. takahe (*Porphyrio mantelli*). For comparative purposes, data from a contemporary population of pukeko (*Porphyrio porphyrio*) (sister species to takahe) are included alongside the takahe data.

Big South Cape Island may have been colonised during a glacial period when the continental shelf was exposed and land was continuous between Big South Cape Island, the South Island and Stewart Island/Rakiura. When the last inter-glacial period began 10 000–15 000 years ago, sea levels rose, separating Big South Cape Island from Stewart Island/Rakiura by approximately 2 km of open water (McGlone et al. 2003). Any population die-off/recovery on Big South Cape Island during an inter-glacial period when gene flow with Stewart Island/Rakiura was limited or non-existent would lead to the low genetic variation seen in the Big South Cape Island saddleback museum samples. Similarly, genetic drift occurring over 1200 generations (or 9600 years) would produce the ratio of genetic variation observed between a mainland population (e.g. Taramakau) and Big South Cape Island, assuming an effective population size of 500 individuals on Big South Cape Island (Taylor et al. 2007).

The low historic genetic variation of SI saddlebacks on Big South Cape Island and some minor loss of diversity following the translocations to Big and Kaimohu Islands have resulted in two contemporary populations with very low expected heterozygosity (0.10 and 0.13; based on polymorphic loci from museum samples).

These measures are similar to that found for two kestrel species found on the islands of Mauritius and Seychelles (0.10 and 0.12, respectively), which were also bottlenecked to very small population sizes (Groombridge et al. 2000).

Low levels of genetic diversity can reduce a population's ability to evolve new adaptations and reduce resistance to disease and parasites, thereby increasing the probability of extinction. Consequently, the low levels of genetic variation observed in the remaining SI saddlebacks may be a cause for concern. However, SI saddlebacks have likely persisted in isolation for thousands of years on Big South Cape Island with extremely low genetic variation. Such long-term persistence coupled with low genetic variation has also been reported in other species (Hitchings & Beebee 1996; Visscher et al. 2001; Hadly et al. 2003) and questions the view that low genetic variation necessarily threatens fitness and population persistence. Small populations may maintain fitness through back-mutations of deleterious alleles to the original functional alleles (Lande 1998) and new mutations that compensate for the negative fitness effects of fixed deleterious alleles (Whitlock & Otto 1999). However, the relationship between fitness and genetic variation at neutral markers such as micosatellites is still unclear. Until the function and effectiveness of mechanisms that may maintain fitness are confirmed, it would be prudent to protect genetic variation in species such as saddlebacks, which may have had a relatively constant environment in the past, but could show little resilience to human-driven environmental changes including new pathogens.

These results illustrate the importance of dispersal capability and island/mainland sites for the maintenance of genetic variation. By the early 1900s, genetically diverse SI saddlebacks from the mainland were virtually extinct. The saddlebacks that remained were confined to one small, historically bottlenecked island, which has resulted in a permanent loss of allelic diversity in the species. Had SI saddlebacks on the mainland passed through and survived a bottleneck of similar size to the rat-induced bottleneck on Big South Cape Island, they would have retained more genetic variation than that observed on Kaimohu and Big Islands: each of the three historic mainland sub-populations (Taramakau, Otago, Fiordland) with sample sizes of just 5–6 birds showed far more genetic variation than the entire sample ($n = 20$) from the Big South Cape group. Furthermore, individuals from the mainland typically had higher levels of heterozygosity than individuals from Big South Cape Island or contemporary populations. A similar pattern may also exist in kakapo, where Fiordland birds appear to be more genetically diverse than Stewart Island birds (B. Robertson, University of Otago, pers. comm.).

Arguably, many species that have been reduced from large mainland populations to small, relict island populations may have exceptionally low genetic variation as a consequence of historical events, especially species with poor mobility. In New Zealand, saddlebacks, takahe, Stewart Island kakapo, and two frog, two tuatara and several lizard species were once widespread on the mainland but are now only present on small islands and/or in isolated montane areas. This situation has potentially exacerbated loss of genetic variation and contributed significantly to the very low genetic variation now observed in these species (Towns & Daugherty 1994; Bell et al. 1998; Holyoake et al. 2001; Hay et al. 2003; Miller et al. 2003; Lambert et al. 2005; Jamieson et al. 2006). Retaining allelic diversity may be crucial to evolutionary potential because single alleles are often important for disease

resistance (Fuerst & Maruyama 1986). Therefore, every effort should be made to protect diverse mainland populations, particularly for species that do not readily disperse.

5.2.2 South Island robin

Historic samples of robins from 1873–1955 were from several sites on mainland South Island (including Stewart Island/Rakiura; $n = 34$) and islets off Stewart Island/Rakiura and Queen Charlotte Islands ($n = 8$). Contemporary robin samples were collected between 2000 and 2005 as part of a larger study, which included two naturally colonised island and three mainland populations from a variety of South Island locations. Sample sizes of museum specimens from specific locations were very small; therefore, samples were combined into two groups: small islands (excluding Stewart Island/Rakiura; $n = 8$) and large mainland (including Stewart Island/Rakiura; $n = 32$). Similarly, contemporary SI robin data for Nukuwaiata ($n = 25$) and Breaksea ($n = 55$) Islands were combined to create an island sample ($n = 80$), and data for the Eglinton Valley ($n = 170$), Flagstaff Point ($n = 12$) and Nelson Lakes ($n = 21$) were combined to produce a mainland sample ($n = 203$), to allow comparisons with historic samples (Taylor et al. 2007).

Using microsatellite DNA, there were small but significant differences between historic and contemporary mainland robin samples in allelic richness (which corrects for sample size; 2.77 historic v. 2.59 contemporary) and expected heterozygosity (0.39 historic v. 0.34 contemporary). In contrast, there was no significant difference in allelic richness or expected heterozygosity between historic and contemporary island robin samples (Fig. 1B; Taylor et al. 2007).

The overall loss of genetic diversity between historic and contemporary populations of SI robins has been considerably less than that of SI saddlebacks (Fig. 1A & B). Although the distribution of SI robins has been negatively affected by introduced predators, this has not been to the same extent as SI saddlebacks, as robins continue to exist in low numbers on the mainland. The small temporal loss of genetic variation in mainland robins that was detected is attributable to habitat fragmentation and subsequent genetic drift as mainland populations are geographically isolated from each other.

Island robin populations appeared to have similar levels of genetic variation as mainland populations within both the historic and contemporary samples (Taylor et al. 2007). The natural island populations on Nukuwaiata and Breaksea Islands are adjacent to large mainland populations of SI robins (northern South Island and Fiordland, respectively). If SI robins are able to disperse from mainland populations to nearby islands, but are unable to reach other more distant mainland populations (e.g. Dunedin/Flagstaff area), then loss of genetic variation may occur for fragmented populations on the mainland but not between adjacent mainland and island sites. Although some contemporary robin populations have individually lost alleles and show reduced heterozygosity (see below), this loss is not necessarily permanent because managers could potentially reintroduce missing alleles and increase heterozygosity through translocations among the remaining mainland populations—something that is not an option for saddlebacks.

A finer scale comparison of genetic diversity of SI robins, using polymorphic loci from contemporary populations only (including translocated populations), was

also carried out (Boessenkool et al. 2007). The large mainland robin populations of Nelson Lakes and Eglinton Valley were found to have retained the highest number of alleles of all populations sampled. Both of these populations were polymorphic for all the analysed loci and rare alleles were detected frequently. Moreover, since only a small proportion of these populations was sampled (e.g. approximately 2% for Nelson Lakes), it is possible that the observed genetic variation is an underestimate of the actual variation. In contrast, the small mainland robin population at Flagstaff near Dunedin had very little genetic variation, with five out of ten loci monomorphic, low expected heterozygosity and relatively low allelic richness (a measure adjusted for differences in sample size). The Flagstaff robin population has probably been isolated since the late 1800s when the forests around the city of Dunedin were clear-felled (A. Mark, University of Otago, pers. comm.). Since immigration from the nearest robin population 120 km away is unlikely, the Flagstaff population may have been small for many generations. Such a long-term bottleneck can cause reductions in levels of genetic variation and heterozygosity due to random genetic drift and inbreeding (Boessenkool et al. 2007).

Even where island populations are relatively large, they typically still have less genetic variation than mainland populations, probably due to a combination of factors including past bottlenecks (possibly during original colonisation), genetic drift and isolation. Surprisingly, genetic variation did not differ between natural and translocated island populations, even though one of the translocated populations (Motuara Island) was established with five individuals, and possibly one pair. Although four out of the ten loci were monomorphic, overall levels of genetic diversity were similar to those found on the other islands, including Nukuwaiata Island from where the robins were sourced (Boessenkool et al. 2007). The reduction in heterozygosity following a bottleneck largely depends on both the bottleneck size and the rate of population growth following the bottleneck, while the loss of alleles is primarily dependent on bottleneck size alone (Nei et al. 1975). The robin's high reproductive potential has enabled the Motuara Island population to grow from 5 to 600 individuals in just 30 years or about ten generations, a rate of population growth that may explain the maintenance of heterozygosity observed on the island (Boessenkool et al. 2007).

For conservation purposes, islands are considered safe refuges for many species in New Zealand. However, small island populations have lower levels of genetic variation and higher levels of inbreeding than mainland populations. Although this may not pose an immediate threat to the viability of these populations, it could become increasingly important in the long term with possible changes in habitat, climate or the introduction of disease. Small island populations therefore provide a valuable solution in the short term, but their persistence over the long term may be at risk unless they are periodically augmented with new genetic stock from mainland sources. The ability of large mainland populations to retain high levels of genetic variation demonstrates the importance of also protecting and maintaining these populations. Such protection requires ongoing management, which can be logistically difficult and expensive. It is important to realise, however, that not all mainland populations will necessarily have high levels of genetic diversity; prolonged periods of population decline in these populations can cause similar dramatic reductions in genetic diversity as was measured in the robins at Flagstaff (Boessenkool et al. 2007).

5.2.3 Takahe

A preliminary analysis of historical versus contemporary genetic diversity in Fiordland takahe indicated a different pattern again (Fig. 1C). DNA was extracted from study skins of six museum specimens grouped as 'European settlement' samples (one each collected in 1856, 1863 and 1889) and 'rediscovered' samples (one collected in 1949 and two from 1958). DNA was also extracted from blood samples from 20 contemporary Fiordland birds (collected 2000–2004). For comparative purposes, blood samples were also taken from 20 pukeko (*Porphyrio porphyrio*) (sister species to takahe) from near Dunedin. Twenty-one microsatellite loci that had been developed specifically for takahe (Grueber et al. 2008a) were assessed. This included six polymorphic and 15 monomorphic loci found in modern takahe.

A trend of decreasing allelic diversity in takahe was observed over time ($P = 0.059$) (Fig. 1C). Genetic diversity in the historic Fiordland samples was low to start with relative to historic SI saddleback and SI robin samples, but similar to contemporary population of pukeko in Otago (Fig. 1). Genetic diversity in historic Fiordland takahe appears to be low even relative to Fiordland kakapo (B. Robertson, University of Otago, pers. comm.). These differences are perhaps not too surprising when we consider that takahe were virtually extinct by the time Europeans arrived in New Zealand, while kakapo, saddlebacks and robins were relatively common. The results suggest that takahe had experienced a genetic bottleneck much earlier than the 1800s. Since sub-fossil evidence indicates that takahe were relatively common and widespread throughout the Holocene, 1000–5000 years ago, this bottleneck may go back to population declines and range contraction associated with hunting pressure from early Polynesians (Trewick & Worthy 2001).

The low levels of genetic diversity in extant takahe means that significant loss of genetic diversity in the future might be minimal since there is relatively little genetic diversity left to lose. However, despite this prediction, an earlier study using five polymorphic microsatellite loci found that introduced island populations of takahe had significantly lower levels of genetic variation than the main Fiordland population, as well as significantly different gene frequencies, with some alleles going to fixation (i.e. no variability) on the islands (Grueber 2005).

In addition to the molecular data, a gene-drop analysis was conducted based on the established pedigree of introduced island takahe, from which it was estimated that island takahe had lost 7.5% of allelic diversity relative to the founding population (Grueber & Jamieson 2008). Founders are used here to imply 'genetic founders', which are individuals that are at the base of the pedigree and have produced at least one descendant, as opposed to simply birds that were released onto the islands; 8 of the 25 introduced takahe from Fiordland either did not breed on the islands or left no descendants in the current population, and thus are not considered genetic founders. It is also not required that genetic founders were ever part of the island population, only that they have at least one living descendant so that a portion of their genome is represented in the current island population.

The gene-drop analysis indicated that a significant part of the loss of genetic diversity was due to unequal founder representation in the descendant population

(Grueber & Jamieson 2008). Three pedigree-based statistics can be used to evaluate the structure within a pedigree:

1. **The number of founders**: This is simply the number of individuals at the top of the pedigree, for which no further parental information is available. These individuals are presumed to be unrelated.
2. **Founder equivalents (f_e)**: This represents the expected number of equally-contributing unrelated founders that would give rise to the observed level of genetic diversity in the study population, thus providing a relative measure of the variance in founder representation. An f_e value that is close to the number of actual founders suggests proportional representation from each founder in the descendant population.
3. **Founder genome equivalents (f_g)**: This represents the expected number of unrelated founder genomes that would contain the observed level of genetic diversity in the study population and is generally estimated by gene-drop simulation (Lacy 1989; Haig & Ballou 2002). As f_g increases towards f_e, the proportion of founder alleles that are retained in the descendant population also increases, thus reducing the amount of genetic diversity that has been lost since founding (Lacy 1989). Values of f_e or f_g that are much lower than the total number of founders normally indicate a small number of founders producing disproportionately large numbers of offspring. In addition, an f_g value that is much lower than f_e for a given population suggests that some alleles have been lost due to genetic 'bottlenecks' or other structuring within some founder lineages, e.g. if only one or a few offspring were produced shortly after founding, despite subsequent descendants in that lineage breeding well (for further details see Lacy 1989; Haig & Ballou 2002).

For island takahe, despite the relatively large number of genetic founders (31 birds originating from the Murchison Mountain population in Fiordland), the proportion of descendants each has contributed is highly skewed. Consequently, the number of founder equivalents ($f_e = 12.5$) and the number of founder genome equivalents ($f_g = 6.6$) are low relative to the number of genetic founders (31). The value for f_g is much lower than f_e, indicating that many of the genetic founders contributed only one or two offspring. For example, 42 of the 83 birds in the current population (51%) can trace their lineage back to a particular translocated pair named Squeak (female) and Taku (male) (Grueber & Jamieson 2008).

Without any management intervention, the population is projected to maintain only 76% of the original founding genetic diversity in the next 100 years. The loss of genetic diversity results primarily from unequal founder representation during the establishment phase of the population and from the limited carrying capacity of the islands, and does not include any additional decrease in heterozygosity due to substantial inbreeding (see section 8). Further losses of genetic diversity could be reduced either by expanding the population to include more islands, or by introducing two new breeding birds every 4–5 years (Grueber & Jamieson 2008). Unfortunately, no new islands are to be added to the Takahe Recovery Programme for the next 5 years and the current islands are at their carrying capacity, making successful introduction of new genetic stock less likely. Therefore, as a consequence of these findings, the Takahe Recovery Group has implemented a plan to reduce carrying capacities on islands by translocating young birds back into the Fiordland population (after going through an intensive

disease screening process) before introducing new Fiordland juveniles to the islands (P. Tisch, DOC, pers. comm.).

Managing this sort of metapopulation by artificially creating gene flow creates a possible trade-off between maintaining genetic diversity and slowing the rate of selection for local adaptations on the islands, but genetic drift is theoretically much stronger than natural selection in very small populations (Frankham et al. 2002). It is believed that a balance can be reached by drip-feeding Fiordland birds into the island sites. Furthermore, since the island population was originally seen as a back-up to the natural and more vulnerable mainland population in Fiordland, management has focussed on increasing growth in the Murchison Mountain population rather than in the apparently secure island sites (P. Tisch, pers. comm.). Thus, surplus island birds could also be translocated back into Fiordland, which will not only reduce densities on the islands, but also enhance the growth rate of the Murchison Mountain population.

5.2.4 Other New Zealand endemics

Two recent studies have show two extremes in loss of genetic diversity in two New Zealand endemic bird species.

A recently completed genetic study of New Zealand snipe (*Coenocoryph* spp.) (A. Baker, University of Toronto, C. Miskelly, DOC, and O. Haddrath, University of Toronto, unpubl. data) included the tiny populations that persisted on 218-ha Rangatira Island (Chatham Islands) and 19-ha Jacquemart Island (Campbell Island group). Using both microsatellite and mitochondrial DNA, the researchers reported the almost total loss of measurable genetic variation in both populations, relative to the genetic variation recorded in the much larger population on Adams Island. They argued that this low level of variation (perhaps the lowest recorded in a wild bird population) is likely to have implications for the long-term viability of this species in the face of global warming and introduction of avian diseases. On the other hand, it is interesting that such a small (approximately 20 birds) and presumably highly inbred population persisted on Jacquemart Island for 160 years without going extinct due to accumulated effects of inbreeding and inbreeding depression. It would be interesting to compare vital and fecundity rates between the larger Adams Island population and the much smaller Jacquemart Island population.

At the other extreme, a recent study on Chatham Island taiko (*Pterodroma magentae*), the world's most endangered seabird (120–150 individuals, 8–14 breeding pairs), reported surprisingly high levels of genetic diversity in mitochondrial and minisatellite DNA compared to other species of seabirds (Lawrence et al. 2008). The authors suggested that the taiko population retained a significant proportion of its past genetic diversity either because the severe decline in numbers occurred only recently, or because there could be other undiscovered population(s) that result in gene flow. This result bodes well for other threatened populations of New Zealand seabirds, but more studies are required.

5.3 RESTORING AND MAINTAINING GENETIC DIVERSITY

In true metapopulations, where exchange between sub-populations is minimal, human-assisted translocation can substitute for natural migration. Migration can aid the maintenance of genetic variation in the metapopulation, as it increases the effective population size by connecting sub-populations (Newman & Tallmon 2001). This can disperse rare or novel alleles throughout the population, increasing overall genetic diversity. Often very little migration is required for a significant increase in genetic diversity; one (reproducing) migrant per generation has been suggested as a rule-of-thumb for threatened population management (Mills & Allendorf 1996; Wang 2004). Such an approach may circumvent most effects of population subdivision, while minimising the stress and expense associated with translocation. Such a programme has been proposed for managing genetic diversity in island populations of takahe (see above).

At the other extreme, deliberate crosses between individuals from populations that have been separated for thousands of years can break down locally adapted gene complexes, resulting in outbreeding depression (Frankham et al. 2002; Edmands 2007). Therefore, unless a population is exhibiting severe inbreeding depression or is on the verge of extinction, crosses between strongly divergent populations or subspecies should be avoided.

6. Loss of genetic variation due to bottlenecks during serial translocations

Theory predicts that bottlenecks should reduce allelic diversity, initially through random sampling of individuals from the source population and subsequently through genetic drift. Rare alleles are most likely to be lost after bottlenecks, and genetic drift will most affect populations that are kept small for long periods (Nei et al. 1975; Allendorf 1986). Repeated population bottlenecks could therefore lead to loss of genetic variation and normally should be avoided in threatened species to preserve evolutionary potential. It has been suggested that the SI saddleback is a prime candidate for such losses due to the number of serial translocations it has experienced (J. Briskie, University of Canterbury, pers. comm.). Therefore, the effect of repeated bottlenecks, in the form of sequential translocations, on loss of genetic variation in the SI saddleback was examined using microsatellite DNA. The aim was to examine immediate loss of genetic variation associated with founder events, and to model the subsequent loss of genetic variation over a 100-year period in six contemporary populations (Taylor & Jamieson 2008).

6.1 LOSS OF GENETIC DIVERSITY IN RELATION TO TRANSLOCATION ORDER

Contemporary populations of SI saddlebacks appear to have low levels of genetic variation. Of 61 scoreable loci, 55 were monomorphic and two of the six polymorphic loci were diallelic (i.e. two alleles). Genetic variation in terms of allelic richness was very low across all six saddleback island populations (Taylor & Jamieson 2008).

Sequential translocations did not appear to reduce genetic diversity; there was no significant difference among populations for numbers of alleles, mean alleles per locus, allelic richness or heterozygosity, irrespective of their classification as source, 1st, 2nd or 3rd order translocations (all P values > 0.10; Taylor & Jamieson 2008). Sample size tended to increase with translocation order. However, there were no significant differences in allelic richness or expected heterozygosity, which are robust to changes in sample sizes. Although differences in the number of alleles among populations were not significant, the source island population (Big South Cape) had five alleles that were not present in contemporary populations and, similarly, contemporary populations had three alleles not present in the Big South Cape population. This suggests that 17 alleles remain in contemporary birds out of a possible total of 22, indicating some loss of alleles between the source and contemporary populations. Importantly, the number of alleles present in contemporary populations (i.e. the populations available for management) was virtually identical among 1st, 2nd and 3rd order translocations (Taylor & Jamieson 2008).

6.2 POPULATION DIFFERENTIATION

All six SI saddleback populations plus the original source population on Big South Cape Island showed different allele frequencies, and all pairwise population comparisons showed significant genic differentiation, with the exception of Ulva and Big Islands (Taylor & Jamieson 2008).

6.3 MAINTENANCE OF GENETIC VARIATION DURING TRANSLOCATIONS

Computer simulations indicated that translocations from populations with rare alleles (Big South Cape, Kaimohu, Putauhinu and Breaksea Islands) required a larger number of individuals to maintain genetic variation than translocations from populations with common alleles (Big, Ulva and Motuara Islands) (Taylor & Jamieson 2008). Translocation of ten breeding pairs (20 birds) appeared to be sufficient to maintain the genetic variation present in populations from Big, Ulva and Motuara Islands, and translocation of 15 breeding pairs (30 individuals) appeared to maintain the genetic variation present in all populations except Big South Cape Island. Even with the translocation of 30 breeding pairs (60 birds), any populations established with individuals from Big South Cape Island would have shown a loss of alleles, a result that agrees well with the loss of alleles between Big South Cape and Big and Kamaihu Islands noted above. Therefore, in terms of the planned reintroduction of SI saddlebacks back onto Big South Cape Island, a minimum of ten pairs from Big Island would appear to be adequate, assuming all ten pairs survived and bred; actual numbers translocated and released should be higher (Taylor & Jamieson 2008). Species with greater genetic diversity and hence more rare alleles than SI saddlebacks, such as mohua (*Mohoua ochrocephala*), may require double that number (L. Hegg and I. Jamieson, University of Otago, unpubl. data).

6.4 LOSS OF GENETIC VARIATION OVER TIME

Simulations using the software program BOTTLESIM showed considerable future loss of genetic diversity in two 1st order translocated populations on small islands (Big and Kaimohu Islands; 19%–54% loss of variation for observed number of alleles, effective number of alleles and expected heterozygosity), but little loss in 2nd and 3rd order translocated populations on larger islands (4%–17%) (Taylor & Jamieson 2008). Inclusion of three additional small saddleback islands (Betsy, North and Women's; all 2nd order translocations) also showed substantial future loss of genetic variation (32%–68%). Overall, these results indicate that carrying capacity (which is related to island area) predicts loss of genetic variation and allele fixation in extant populations of saddlebacks.

6.5 CONCLUSIONS

Most studies investigating sequential bottlenecks have found evidence that translocated populations have lower diversity than source populations and successive bottlenecks decrease genetic variation (Stockwell et al. 1996; Gautschi et al. 2002). Taylor & Jamieson's (2008) study of SI saddlebacks indicated that genetically depauperate species may be less sensitive to loss of genetic variation through founder events. This is presumably because the few remaining alleles are well represented in founding individuals, and the average number of SI saddlebacks transferred (29 ± 15.44 SD) was adequate to conserve these few alleles. Despite the lack of significant differences between populations, a few alleles ($n = 5$) were probably lost between the original source population (now extinct) and the six extant populations, which may have been biologically relevant. The number of alleles present among contemporary populations was virtually identical on each of the six islands; thus, even if differences between islands had been significant, the effect size was so small (1–2 alleles) that it would still have been concluded that translocations have not caused a biologically important loss of genetic diversity in the populations left to be managed. Contrary to these results, Lambert et al. (2005) reported that significant genetic changes occurred with sequential translocations in the NI saddleback. However, closer inspection of their data indicates that significant changes occurred for allele frequencies, not loss of alleles for six polymorphic microsatellite loci, a result that agrees well with the data reported for SI saddleback. Similar results were obtained using minisatellite data on translocated SI robins to Motuara and Allports Islands; there was a non-significant trend for a decrease in minisatellite variation in the two translocated populations relative to their sources, but the losses were not as great as expected given the severity of the bottleneck (five individuals) (Ardern et al. 1997a).

Although the six extant SI saddleback populations studied had similar levels of genetic variation, most populations showed significant differences in gene frequencies (Taylor & Jamieson 2008). This is a common outcome of translocations (Fuerst & Maruyama 1986; Williams et al. 2000). Population differentiation solely caused by differences in allele frequencies may not be an important management consideration in translocated species such as SI saddlebacks for two reasons. First, differences in allele frequencies would be recent (40 years or five generations at most in SI saddlebacks) and probably created by random sampling of individuals during translocations, not local adaptation to the new habitat. Second, the little allelic diversity that remains in SI saddlebacks appears to have been largely maintained within each of the six contemporary populations, suggesting that no population has superior potential to adapt to environmental change. Clearly, differences in allele frequencies among populations can be an important management consideration for other species, e.g. salmon (*Oncorhynchus tshawytscha*), where differences in allele frequencies among populations is a probable consequence of strong philopatry to natal streams that may reflect local adaptation worth conserving (Waples & Teel 1990). However, the transfer of SI saddlebacks among islands to ensure the presence of all alleles and identical allele frequencies is not only logistically impossible but also unnecessary.

Translocations of SI saddlebacks may have had little effect on current levels of genetic variation, but what about losses in the future? Of the six populations sampled, Big and Kaimohu Islands appear to be most at risk of losing substantial genetic variation over the next 100 years. By including three of the smallest saddleback islands in the modelling, it was shown that carrying capacity (determined by island area) appears to have the greatest impact on predicted loss of genetic variation (Taylor & Jamieson 2008). Small islands have limited carrying capacity, which curtails population size, prolongs bottleneck duration indefinitely, and increases the risk of allelic fixation via drift (Nei et al. 1975; Allendorf 1986). Additional SI saddleback populations that risk future loss of genetic variation due to drift include Jacky Lee (30 ha), Kundy (19 ha) and Pohowaitai (27 vegetated ha) Islands. Future SI saddleback translocations should be made to large islands, and existing populations on small islands may occasionally require new migrants (via translocations) to prevent long-term loss of genetic variation.

Neutral microsatellite loci, which presumably indicate diversity across the genome, were used in this study, but it might be useful in future studies to examine specific functional genes such as MHC loci (Westerdahl et al. 2000; Hansson & Richardson 2005). In the meantime, it seems that saddlebacks and other genetically depauperate species could endure sequential translocations and reintroductions, especially in cases where rapid establishment of new populations is required to minimise the risk of extinction due to stochastic demographic events (Taylor & Jamieson 2008). Clearly, however, sequential translocations are not ideal for more genetically variable species, which are likely to be more susceptible to loss of genetic variation through founder effects.

7. Level of inbreeding during the establishment phase of island reintroductions

The above sections have dealt with the loss of genetic variation primarily as a result of genetic drift, which can lead to a reduced evolutionary potential even if populations recover. Inbreeding can also lead to loss of genetic diversity (i.e. increased homozygosity) and evolutionary potential, but in addition can result in an immediate loss of population fitness in the form of inbreeding depression, increasing the extinction risk of a population (Frankham et al. 2002). The release of a relatively small number of individuals during a translocation and reintroduction to an island or mainland-island sanctuary, and the subsequent immediate mortality that follows a release, could potentially lead to a small founder population, and thus high rates of both genetic drift and close inbreeding. Inbreeding can also result from differential breeding success, with the progeny of a few founders or breeding pairs becoming over-represented in the descendant population. The aim of this study was to examine the consequences of varying founder sizes of translocations on the rates of inbreeding for SI robins (Tiritiri Matangi, Ulva and Doubtful Islands), SI saddlebacks (Ulva Island) and takahe (Maud, Mana, Kapiti and Tiritiri Matangi Islands), and to determine the extent to which founders differentially contribute to the descendant population (I. Jamieson, unpubl. data).

Pedigrees of SI and NI robins, SI saddleback and takahe populations were constructed with the assumption that the adults attending a nest were the genetic parents, as molecular studies have indicated that extra-pair fertilisations are absent or extremely rare in these species (see section 4). The level of inbreeding for breeding pairs was calculated by constructing pedigrees using software programs. All inbreeding and kinship coefficients are relative to the founding birds, which are assumed to be unrelated ($F = 0$). It is important to remember that background levels of inbreeding continue to increase in closed populations, even when individuals pair randomly with respect to relatedness, but annual variation in mean inbreeding is most affected by the frequency of close inbreeding (i.e. normally between siblings, $F = 0.25$) (see Appendix 1).

7.1 SOUTH ISLAND AND NORTH ISLAND ROBINS

South Island robins were translocated from Freshwater Flats (Stewart Island/Rakiura) to Ulva Island on three separate occasions. Sixteen birds were initially released in September 2000, but five adult birds returned to territories at Freshwater Flats (Oppel & Beaven 2002) and six others died or disappeared before breeding. This was followed by a second release of four juveniles in January 2001 (one died) and a third release of five juveniles in November 2001 (one died). In total, 12 of the 25 released robins were present on the island in the breeding season after they were released. Nestlings have been banded and adult robins monitored since the first breeding season in 2000.

Twenty-one SI robins were released on Doubtful 2, Doubtful Islands in Lake Te Anau, in 2002 and a further 18 were released on Erin Island in September 2003. However, some of the released birds were subsequently seen on the nearby mainland (< 1 km away). Eight founding birds (four pairs) bred in 2002, and 13 founders plus one juvenile bred in 2003 (totalling seven pairs) (Taylor 2006; Martin 2008).

Forty-four NI robins were translocated from a large mainland population of robins near Rotorua to Tiritiri Matangi in April 1992, and a further 14 robins were translocated in June 1993 (Armstrong & Ewen 2001). The sex ratio in the first release was male-biased: only 7 of the 33 birds that survived to the start of the first breeding season (1992/93) were female. All seven females acquired mates but only two successfully fledged young (two each) in the first breeding season. A follow-up translocation in 1993 increased the number of released adults alive at the beginning of the second breeding season (1993/94) to 12 females and 21 males (Armstrong & Ewen 2001). The robin population has been monitored for 15 years and nestlings were banded in all years except 1997/98, creating a minor gap in the pedigree data. The breeding population on Tiritiri Matangi reached a carrying capacity of about 65 robins by 1996/97. Robins appear reluctant to fly across open water and there have been no known cases of banded birds from the island being sighted on the nearest point on the mainland, approximately 3.5 km away (Jamieson et al. 2007).

There was a striking difference in the pattern of inbreeding for NI and SI robins released on Tiritiri Matangi versus Ulva Island, respectively (Fig. 2A & B). Once first-generation birds started breeding, the mean level of inbreeding for new pairs gradually increased over 4 years for robins on Ulva Island but declined over 3 years for robins on Tiritiri Matangi (Fig. 2A & B). This was a result of a difference in the relative frequency of close inbreeding ($F = 0.25$) when the total number of breeding pairs was initially small. On Ulva Island, the background level of inbreeding slowly increased, but there were only 1–2 incidents of close inbreeding in any one season (Fig. 2A). By contrast, the frequency of close inbreeding on Tiritiri Matangi was initially high (3 of 13 new pairs in 1995), but declined thereafter, resulting in an overall decline in mean inbreeding levels for new pairs (Fig. 2B). Therefore, for Ulva Island the increase in the level of inbreeding over time is primarily a result of a build-up in the background level of inbreeding due to a small founding population of six pairs (compared to Tiritiri Matangi, which was founded by 12 pairs). Furthermore, there was unequal representation of the founding males and females on Ulva Island (Fig. 3), which contributed to the build-up in the background level of inbreeding.

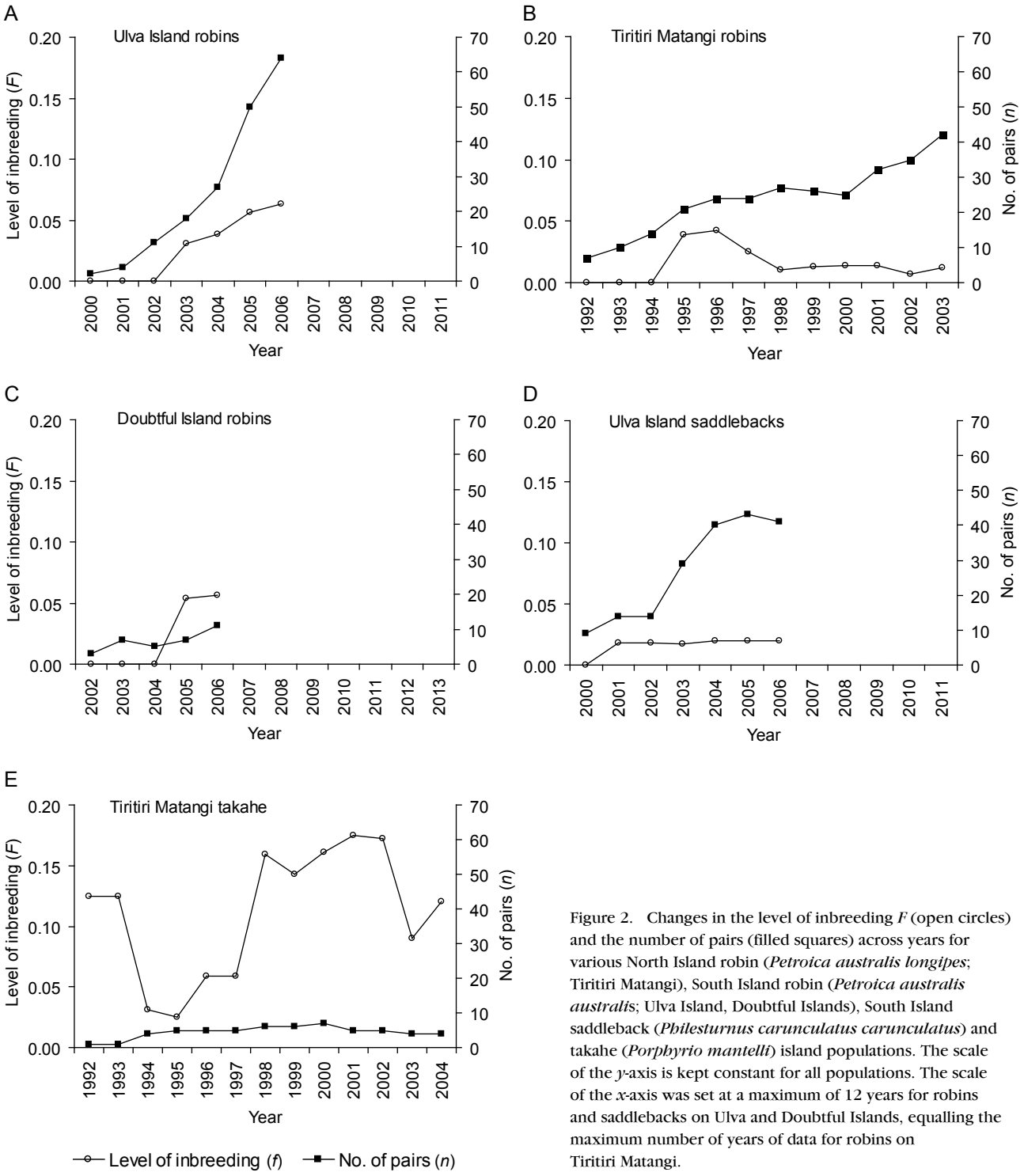

Figure 2. Changes in the level of inbreeding *F* (open circles) and the number of pairs (filled squares) across years for various North Island robin (*Petroica australis longipes*; Tiritiri Matangi), South Island robin (*Petroica australis australis*; Ulva Island, Doubtful Islands), South Island saddleback (*Philesturnus carunculatus carunculatus*) and takahe (*Porphyrio mantelli*) island populations. The scale of the *y*-axis is kept constant for all populations. The scale of the *x*-axis was set at a maximum of 12 years for robins and saddlebacks on Ulva and Doubtful Islands, equalling the maximum number of years of data for robins on Tiritiri Matangi.

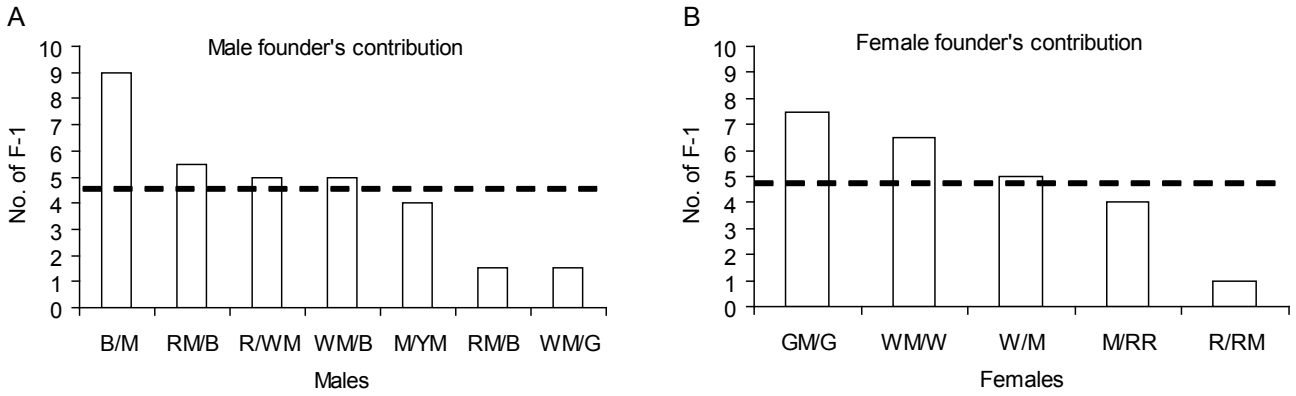

Figure 3. The number of first generation offspring (F – 1) recruited into the breeding population from seven male and five female South Island robin (*Petroica australis australis*) founders on Ulva Island. Each offspring contributed 0.5 toward the male/female's total. The expected number of descendants under a founder equalisation scenario (dashed line) was 4.5 per male and 4.8 per female.

By contrast, there has been a greater increase in inbreeding for SI robins on the Doubtful Islands (Fig. 2C). This is primarily due to a high frequency of close inbreeding ($F = 0.125–0.25$), which is likely a consequence of the small founding population (seven pairs). However, the number of years over which the data were collected ($n = 5$) was short relative to the other studies.

A more detailed analysis of the pattern of inbreeding for NI robins on Tiritiri Matangi is provided by Jamieson et al. (2007). There were 160 unique breeding pairs in the study, of which 21% were pairings between relatives ($F \geq 0$) and 3% were between close relatives ($F = 0.25$), yielding an average level of inbreeding of 0.014. Including only those individuals with four known grandparents, the number of pairs dropped to 82, of which 39% involved relatives and 6% involved close relatives, giving an average level of inbreeding of 0.027. As shown in other studies (e.g. Keller 1998), deriving mean inbreeding values from all pairs underestimated the true level of inbreeding because the ancestry of the founding birds was unknown but assigned $F = 0$. Because of this bias, the standard practice of including only those individuals for which all four grandparents were known was followed in the analyses.

Annual fluctuations in the annual level of inbreeding were primarily due to variation in the number of incidents of close inbreeding, as noted above (Jamieson et al. 2007). For example, the average level of inbreeding was highest in 1995, when three of nine pairs (with known grandparents) were brother–sister pairings. Thereafter, the number of closely related pairs declined to two in 1996 and one in 1997–2001. There were no closely related pairings in 2002–2003 and hence average inbreeding declined again, despite the overall frequency of related pairs increased from 18% in 2000 to 59% in 2003. Overall, although the frequency of inbreeding was high for a wild population, the initial translocation of a total of 19 females and 39 males, and the inevitable mortality that followed, did not result in exceptionally higher levels of inbreeding than would be expected in a natural island population of monogamous birds (Jamieson et al. 2007).

7.2 SOUTH ISLAND SADDLEBACK

Thirty SI saddlebacks were translocated from Big Island (off Stewart Island/ Rakiura) to Ulva Island in April 2000. Twenty-three survived to the first breeding season in October 2000. There was no extensive nest monitoring in the first breeding season, but DNA samples were obtained from 16 birds, 12 of which were identified as breeding pairs and 4 as single females based on both observations and subsequent parentage analysis (see section 3).

The overall level of inbreeding has increased relatively slowly for SI saddlebacks on Ulva Island (Fig. 2D), due to a relatively large number of founding pairs (12) and low frequency of close inbreeding.

7.3 TAKAHE

A total of 25 Fiordland takahe (mostly juveniles from banded territorial pairs) were introduced gradually to four islands (Tiritiri Matangi, Maud, Mana and Kapiti) between 1984 and 1999. Because each island and its available habitat are relatively small, takahe have been managed as a single population with transfers occurring between islands to balance the sex ratio and to occasionally break up closely related pairs. Takahe are free to form 'natural' pairings on the islands, and breeding is closely monitored by DOC staff; every offspring is colour-banded on its natal territory, given a name and entered into the studbook (Grueber & Jamieson 2008).

There has been a much more dramatic increase in the mean level of inbreeding over the short-term for takahe on Tiritiri Matangi (Fig. 2E), due to both close inbreeding and high background levels of inbreeding as a result of the tiny population size and unequal founder representation for the entire island population (see section 5).

The large annual variation in mean levels of inbreeding in takahe (Fig. 2E) is due to sibling pairs forming and later being separated by translocating one of the birds to another island. The recovery plan for island takahe indicates that they should be managed as a metapopulation, so that close inbreeding can be avoided through inter-island translocation (D. Crouchley, DOC, pers. comm.). However, although this would reduce the frequency of close inbreeding, it would not reduce the level of background inbreeding, which builds up rapidly due to the strong pattern of unequal founder representation as well as the relatively low effective population sizes on all four islands. When considering all islands as a single population, inbreeding was common in the takahe pedigree: 36 of 83 (43%) living birds were inbred, and 23 of these (64%) had $F \geq 0.125$ (i.e. close inbreeding). The overall mean level of inbreeding among the current breeding population is 0.089 (SE = 0.016), one of the highest levels for a bird population in the wild (Jamieson et al. 2007).

7.4 INCREMENTAL INCREASE IN INBREEDING

The incremental increase in the mean level of inbreeding in a closed population can be calculated by:

$$F_t = 1 / (2N) + [1 - 1 (2N)] F_{t-1}$$

where F_t is the inbreeding coefficient at generation t, N is the effective population size (estimated here as the number of breeding pairs), and F_{t-1} is the inbreeding coefficient in the previous generation. This means that the incremental increase in the level of inbreeding is primarily a function of population size or the island's carrying capacity.

When we project the incremental increase in average inbreeding coefficients of robin and saddleback pairs on Ulva Island and Tiritiri Matangi, assuming the populations have reached their estimated carrying capacities, inbreeding increases steadily but slowly as a consequence of the relatively large population sizes on each of the islands (Fig. 4A). It could take between approximately 150 and 450 years for the mean level of inbreeding to reach $F = 0.25$ (i.e. pairs related to each other at the sibling level), where a significant decline in fitness might be expected (see section 8). It is also clear that the overall difference in the rate of inbreeding between NI robins on Tiritiri Matangi and SI robins on Ulva Island is more a result of differences in the carrying capacity between the islands than differences in founder sizes that resulted in initial high levels of inbreeding on Ulva Island (Fig. 4A).

The incremental increase per generation time is much greater for takahe on Tiritiri Matangi than for NI robins because of the takahe's small population size, which in itself is a function of its larger breeding territories and the island's lower carrying capacity (Fig. 4A). As a consequence, takahe reach a mean level of inbreeding of $F = 0.25$ in approximately 25 years. This illustrates that birds with larger territories and hence lower carrying capacities would require much more ongoing management to minimise the effects of inbreeding.

These estimates are optimistic in the sense that they assume no variation around the carrying capacity, no catastrophes or population crashes, and no harvesting for translocations, as any decrease in the population size at the carrying capacity, even if temporary, would increase the incremental rate of inbreeding. For example, harvesting 30 robins or 30 saddlebacks and allowing the population to recover to its original carrying capacity before the next harvesting event would result in slight population declines approximately every 3 years. Harvesting either 30 juveniles or 30 breeding adults would increase the incremental rate of inbreeding substantially. This effect is especially large for adults, as by harvesting 30 adults the total population is reduced by 30, whereas by harvesting 30 juveniles the population is reduced by a fraction of the juvenile recruitment rate (Fig. 4B).

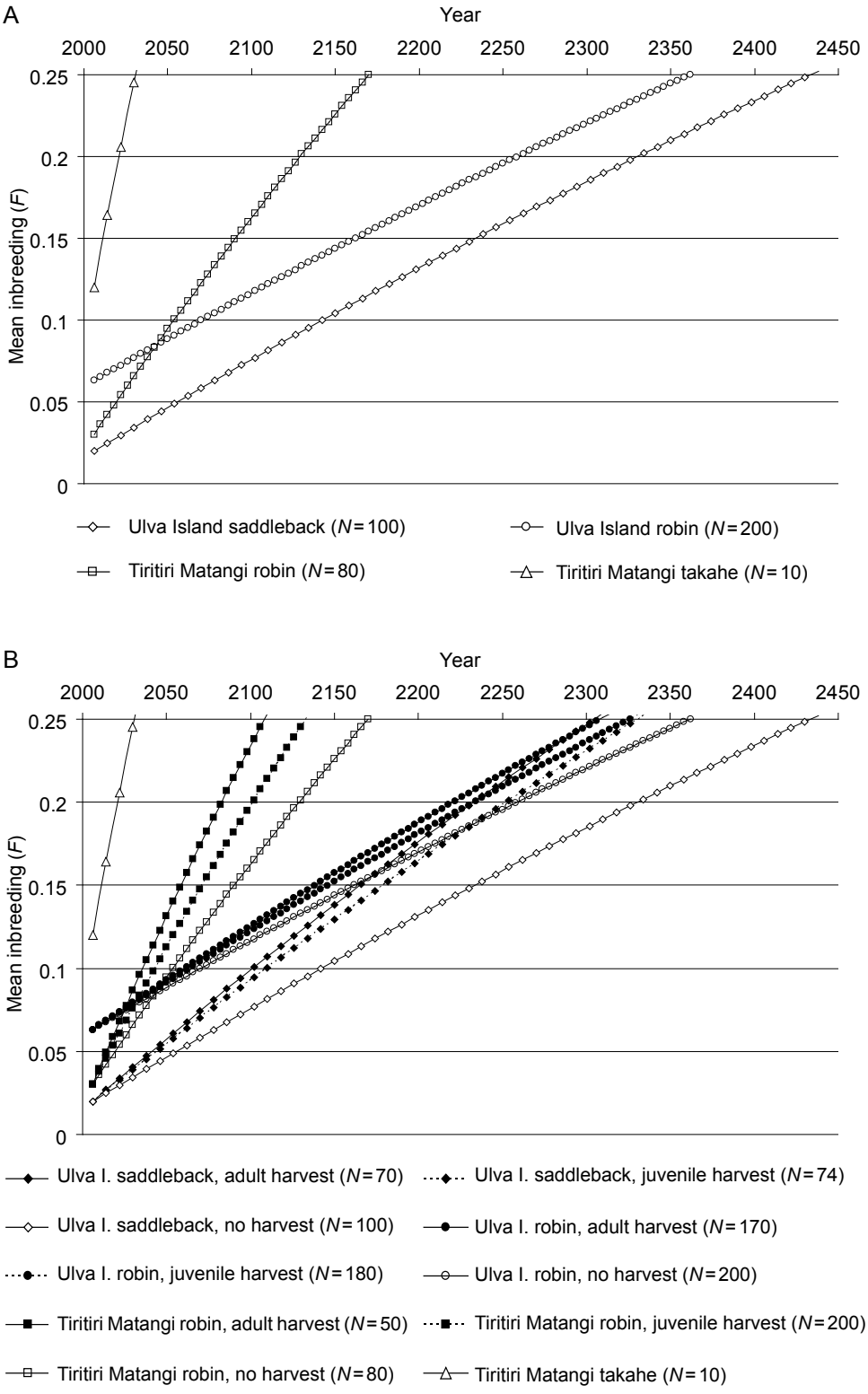

Figure 4. The projected rate of increase in mean inbreeding coefficients (up to a maximum of $F = 0.25$) for breeding pairs of South Island saddleback (*Philesturnus carunculatus carunculatus*), South Island and North Island robins (*Petroica australis australis* and *Petroica australis longipes*, respectively), and takahe (*Porphyrio mantelli*) on Ulva Island and Tiritiri Matangi, assuming the populations have reached their estimated carrying capacities. Two scenarios are provided: A. without harvesting birds; and B. with harvesting of 30 juveniles and 30 adults. Initial inbreeding levels were calculated from pedigree data. See section 7.4 for formula for predicting incremental increase in F per generation. Takahe and saddlebacks were estimated to have a generation time of 8 years and robins 4 years, based on mean survival rates of adults.

7.5 CONCLUSIONS

As a consequence of this research, takahe on islands are now being managed in a way that prevents populations from reaching high levels of inbreeding by translocating some juveniles back into the Murchison Mountains and by introducing new juveniles from Fiordland. However, the high frequency of translocation that is now required for takahe on DOC-managed islands does not bode well for takahe or other large avian species on small islands or in fenced reserves run by community trusts or private organisations. Inbreeding can be managed through inter-site transfers, but these may need to be frequent and it is not clear whether this additional level of management has been accounted for in the budgets and long-term management plans, particularly for fenced reserves.

The incremental increase in rate of inbreeding for robins and saddlebacks, both of which are smaller bird species, was slow relative to takahe, but inbreeding did increase nevertheless, and will eventually reach very high levels. The rate of increase is governed by the ultimate size of the population and the degree to which it fluctuates over time. Periodic decreases in the population caused by catastrophes or by harvesting juveniles and adults for translocation will increase the rate of inbreeding substantially. This report presents the number of years it would take to reach an average level of inbreeding of $F = 0.25$. However, it might be prudent to manage populations to reduce inbreeding before such high levels are reached.

Before introduced predators had extirpated populations on the mainland, many of these island populations would have maintained a certain level of gene-flow with the mainland, hence modulating the rate of inbreeding. Therefore, translocations are substituting for former natural processes such as gene-flow. In addition to the loss of genetic diversity, high levels of inbreeding can result in inbreeding depression and loss of fitness at the population level, the extent and consequence of which are examined in the next section.

8. Quantifying inbreeding depression

Inbreeding depression is defined as the loss of fitness in an inbred individual or related pair relative to an outbred individual or unrelated pair. To quantify the extent of inbreeding depression within a population, one not only needs an extensive pedigree but also a relatively high frequency of close inbreeding (sib–sib or parent–offspring pairings) to provide enough power in the sample to detect inbreeding depression.

Using long-term banding studies of bird populations, one can accumulate a significant number of incidents of close inbreeding, even when the frequency of such incidents in the population at any one time is very low. For example, in an 18-year study of pied flycatchers *Ficedula albicollis*, only 1% of 2139 matings resulted in offspring with a non-zero inbreeding coefficient (in this case, $F \geq 0.125$) (Kruuk et al. 2002). Similarly, in a 44-year study of great tits *Parus major*, consisting of 71 008 individuals and an average pedigree depth of 7.7 generations, close inbreeding ($F \geq 0.125$) was again very rare, occurring in only 1.0% of 5517 breeding events, 45 of which were at $F = 0.25$ and comprised 27 brother–sister, 6 father–daughter and 12 mother–son matings (Szulkin et al. 2007). The point is that for most large populations you need a very long study to detect enough close inbreeding events to measure the fitness effects of inbreeding.

Pedigree studies can also be conducted on small island populations of banded birds, which can yield higher frequencies of inbreeding (van Noordwijk & Scharloo 1981; Gibbs & Grant 1989; Keller 1998; Grant et al. 2001; Keller et al. 2002). In all of these cited studies, observations were initiated after the population had become established on the islands, so that the true level of inbreeding in the population is unknown. Nevertheless, pedigree studies in small island populations make the analysis of inbreeding depression much more tractable.

The aim of this section is to report data on inbreeding depression from two long-term banding and monitoring studies of New Zealand birds on islands: 18 years of takahe data since their introduction to four offshore islands (C. Gruber and I. Jamieson, University of Otago, unpubl. data) and 13 years of NI robin data since their reintroduction to Tiritiri Matangi (Jamieson et al. 2007).

8.1 TAKAHE

The first ever study of inbreeding depression using pedigree analysis of a wild population of New Zealand birds was conducted by Jamieson et al. (2003), and consisted of 14 years of data from island takahe. The only inbreeding depression that was detected was that inbred adult females had reduced fitness in the form of significantly lower fledging success; neither inbred males nor related pairs showed any indication of inbreeding depression based on the pedigrees constructed. Using a larger dataset of 19 years and more powerful statistical techniques, there

was again a strong trend that inbred females had lower fledgling success, but no trend for inbred males or related pairs (C. Grueber and I. Jamieson, University of Otago, unpubl. data). Egg fertility rates also decreased as the level of inbreeding in males increased.

Overall, the results indicated that recent inbreeding in an ancestrally inbred population can still result in inbreeding depression in the form of reduced egg fertility and fledging success, although the effects of recent inbreeding were weak relative to what was found for NI robins on Tiritiri Matangi, which were sourced from an outbred population (see below). Inbred male takahe had similar fledging success to non-inbred males, suggesting that there are likely to be differences in the costs of reproduction between males and females. More surprisingly, closely related pairs of takahe did not show any immediate signs of inbreeding depression, at least until their male offspring attempt to fertilise eggs and their female offspring attempt to fledge chicks. It needs to be remembered that egg fertility rates and hatching success are generally poor in island takahe (Jamieson & Ryan 2000), making detecting differences between related and unrelated pairs more difficult. An alternative explanation for the lack of difference between related and unrelated pairs is that most island pairs had become fixed for deleterious alleles that affect these early life history traits (Jamieson et al. 2003); this is considered a form of inbreeding depression at the population level (Keller & Waller 2002). Future research will test whether there is a correlation between reproductive success and heterzygosity based on microsatellite markers. The introduction of new birds from Fiordland to the islands over the next few years will also provide an opportunity to test these particular hypotheses.

8.2 NORTH ISLAND ROBIN

The translocation of a small subset of individuals from a genetically diverse source population could potentially lead to substantial inbreeding depression due to the high genetic load of the parent population. Jamieson et al. (2007) analysed 12 years of data from the reintroduced population of NI robins on Tiritiri Matangi to determine the frequency of inbreeding and magnitude of inbreeding depression. The initial breeding population consisted of 12 females and 21 males, which came from a large mainland population of robins. The frequency of matings between relatives ($F > 0$; 39%, $n = 82$ pairs) and close relatives ($F = 0.25$; 6.1%), and the average level of inbreeding ($F = 0.027$) were within the range reported for other small island populations of birds. The average level of inbreeding fluctuated from year to year, depending on the frequency of close inbreeding (e.g. sib–sib pairs).

Jamieson et al. (2007) detected significant inbreeding depression in the Tiritiri Matangi robin population in the form of lower probability of survival at the juvenile stage. Survival probability was estimated to drop from 31% among non-inbred birds ($F = 0$) to 11% in highly inbred juveniles ($F = 0.25$). The estimated number of lethal equivalents based on this relationship (4.14; –0.36 to 8.65, 95% confidence intervals) was moderate compared to values reported for other island populations of passerines (Jamieson et al. 2007). This reduction in juvenile survival resulted in lower offspring recruitment into the breeding population. Inbreeding had no significant effect on a pair's annual production

of fledglings (similar to result for takahe; see above), but no data were available on egg fertility or hatching success. There was insufficient power to detect the effect of inbreeding on the proportion of recruited fledglings.

It appears that a substantial portion of inbreeding depression in NI robins occurs at the juvenile life history stage, and the effects are strong. Closely inbred offspring ($F = 0.25$) were estimated to have a 65% lower probability of survival relative to non-inbred birds, but there was no evidence of significant effects at lower levels of inbreeding (Jamieson et al. 2007). Based on the estimated number of lethal equivalents reported for other island populations of passerines, inbreeding depression in the Tiritiri Matangi population of robins was moderate to high. Jamieson et al.'s (2007) study plus others (e.g. Briskie & Mackintosh 2004) also indicated that inbreeding depression in native New Zealand birds is more common than previously appreciated and, at least in this case, there was little evidence of purging of genetic load (see Jamieson et al. 2006).

Closely inbred birds made up a small fraction of the total population, however, and 70% of non-inbred juveniles also failed to survive to the first breeding season. Therefore, inbreeding appears to explain a relatively small proportion of the overall variation in juvenile survival. Analysis of a more extensive dataset (i.e. including birds lacking pedigree information) has shown that juvenile survival is density dependent and tightly constrained by the available habitat (Dimond & Armstrong 2007). The low survival and lack of apparent density dependence in the analysis reported here reflects the fact that most of the data were from years with relatively high density. No estimate of the negative effects of inbreeding on egg hatchability was available, although modelling has shown that lowered hatching success is likely to have only a slight effect on population growth rates in reintroduced robin populations (Taylor et al. 2005).

Continuing maturation of the replanted forest on Tiritiri Matangi should allow the robin population to expand over the next few decades (Dimond & Armstrong 2007). Simulations using VORTEX have predicted negligible chances of population extinction over the next 100 years under current conditions (Armstrong & Ewen 2002), although these projections did not take inbreeding depression into account. With no further releases, the overall proportion of inbred individuals should increase by $1/2N$ per generation (see section 7). Furthermore, the level of close inbreeding could increase substantially if the population were to go through a sudden crash, or if a portion of the birds was harvested for other translocations. Nevertheless, a single reintroduction of 10–20 new robins would presumably result in enough gene flow and heterosis (masking of recessive alleles by dominant alleles) to reverse any potential population decline due solely to inbreeding depression (e.g. Grant et al. 2001; Marr et al. 2002).

Although the future fitness consequences of any loss of genetic variation due to inbreeding are always uncertain, and the long-term consequences of loss of fitness due to inbreeding depression still require specific modelling, the immediate impact of inbreeding depression per se on the probability of establishment of reintroduced populations is likely to be low, as long as the population expands relatively quickly at low densities (Taylor et al. 2005). The impact of inbreeding depression on reintroduced populations with slower growth rates and lower carrying capacities (such as takahe and kakapo), or in more stressful environments, are likely to be greater (Jamieson et al. 2007).

8.3 CONCLUSIONS

Further research stills need to be carried out to determine how long it would take for the effects of inbreeding depression to cause negative population growth, and how this process could be ameliorated by introducing new genetic stock. The possible need for additional translocations not only to prevent loss of genetic diversity, but also to prevent populations from declining due to depressed fitness, may not have been fully accounted for in management plans for small mainland fenced sanctuaries. However, with the possible exception of kakapo, very few of New Zealand's threatened species appear to be suffering from reduced population growth rates and hence reduced viability due to inbreeding depression. Introduced island takahe (Jamieson & Ryan 2000) and fairy terns (*Sterna nereis*; Ferreira et al. 2005) have poor hatching success, which might be linked to inbreeding depression, and inbred blue ducks (*Hymenolaimus malacorhynchos*) have significantly lower survival rates than outbred individuals in captivity (J. Wilcken and I. Fraser, Auckland Zoo, unpubl. data), but in none of these cases is inbreeding depression known to be limiting population growth in their natural populations. Black robins are highly inbred and exhibited signs of inbreeding depression in the form of poor hatching success and abnormal nesting behaviour (see Jamieson et al. 2006), but the population was able to grow and recover as a result of intensive management. Since management ceased, however, the population has shown signs of decline and its overall viability is uncertain (E. Kennedy, Lincoln University, pers. comm.). Analysis of pedigree data to determine the effects of recent inbreeding on fecundity and vital rates for black robin is presently underway (E. Kennedy, DOC, pers. comm.).

9. Genetic diversity and disease risk management

One reason frequently given for managing genetic diversity in threatened species is to reduce the impact of disease, as levels of immunity may decline with inbreeding and loss of genetic variation (Frankham et al. 2002; Keller & Waller 2002). This relationship is firmly established in theory and has been supported by laboratory research, but its application to disease risk management of endangered species is often limited to recommendations that conservation managers minimise exposure of inbred or threatened populations to pathogens. Such recommendations add nothing new to current best practice in wildlife management; one can only hope that procedures for minimising the risk of exposing threatened species to infectious diseases, whether inbred or not, are already in place!

Susceptibility to disease is likely to be influenced by many factors other than just genetic variation, such as sociality, population density, climate and proximity to likely vectors. For example, avian malaria was introduced to Hawaii two centuries ago, but the devastating effect it had on Hawaii's endemic avifauna occurred primarily after the introduction of its main mosquito vector *Culex quinquefasciatus* (van Riper et al. 2002). Avian malaria can have disastrous effects on any naïve host it encounters, not just those with reduced genetic variation. It therefore makes sense that recent surveys of the prevalence of avian malaria in New Zealand have been undertaken with respect to the expanding distribution of *C. quinquefasciatus* (Tompkins & Gleeson 2006), and not necessarily with regard to threatened species with low genetic variation (Jamieson et al. 2008).

Nevertheless, the increased risk of extinction associated with disease agents and small populations appears to be real (de Castro & Bolker 2005), and the number of well-documented cases showing increased susceptibility to pathogens or decreased immune response with increased homozygosity is increasing (Coltman et al. 1999; Acevedo-Whitehouse et al. 2003; Pearman & Garner 2005; Tompkins et al. 2006; Whiteman et al. 2006). For example, island populations of the Galápagos hawk (*Buteo galapagoensis*) with low levels of genetic diversity had higher parasite abundances and lower antibody levels than island populations that were more genetically diverse (Whiteman et al. 2006).

The few New Zealand studies that have investigated the relationship between genetic diversity and disease risk have been reviewed by Jamieson et al. (2008). Tompkins et al. (2006) found that measures of immune functions were markedly higher in red-crowned parakeet (*Cyanoramphus novaezelandiae*) than in the endangered island endemic Forbes' parakeet (*C. forbesi*), as well as being higher in naturally occurring hybrids of the two species. Hale & Briskie (2007) compared external, blood and gastrointestinal parasite loads as well as blood cell counts, which are indicative of recent immune responses, in two populations of New Zealand robin to assess the immunocompetence of birds in a severely bottlenecked population (Motuara Island) relative to its presumably more genetically diverse source population (Nukuwaiata Island). They found that

despite both populations showing similar parasite loads, robins in the severely bottlenecked population showed lower counts of both total leucocyte and total lymphocyte numbers, but showed no difference in two other blood cell measures. When the immune system was experimentally challenged using the phytohaemagglutinin (PHA) skin test, robins in the severely bottlenecked population exhibited a significantly lower response than the source population in autumn but not in spring. Finally, neither leucocyte counts nor PHA responses correlated with ectoparasite loads.

Despite the mixed results, Hale & Briskie (2007) uncovered some evidence suggesting that birds passing through a severe bottleneck have a compromised immunocompetence. However, they and several other experts who commented on their study (Hawley 2007; Smits 2007; Tompkins 2007) agreed that the immune system of vertebrates is complex and interpretation of immune challenge experiments is rarely straightforward. Furthermore, Miller & Lambert (2004) found that the severely bottlenecked black robin populations had much lower variation in MHC genes (which play a major role in disease resistance in vertebrates) than SI robins on Motuara Island. Given that robins on Motuara Island had gone through a short but severe bottleneck, Miller & Lambert (2004) concluded that MHC variation may only be eroded when population size is at a low level for a substantial period of time, as is the case for black robins but not SI robins on Motuara Island (Ardern & Lambert 1997). Again, a preliminary survey did not find any evidence that black robins were suffering disproportionately from disease and pathogens, but they could be particularly vulnerable to new pathogens (Miller & Lambert 2004).

Hale (2007: chapter 3) examined reproductive success and parasite loads in the saddleback population on Motuara Island in the 3 years following the population crash in 2002, when the population decreased from approximately 140 birds to 50 birds. She found that reproductive success was similar across all 3 years following the crash, but by the time the population had recovered, adult saddlebacks had significantly higher feather mite loads and lower blood cell counts. These findings suggested that although reproductive success was not affected by changes in population density, there may be increasing risk of parasite transmission, as well as increasing levels of stress and immunosuppression, rendering high-density island populations more susceptible to disease outbreaks.

Hale (2007: chapter 4) also found that counts of several blood cell types and feather mite numbers varied significantly with both number of birds released and island area across 12 saddleback populations, but found no relationship between number of birds released, number of bottleneck events, island size or density of birds and either number of parasitic flies or coccidian loads. In addition, no blood parasites were detected in blood smears from any of the island populations. Many of the explanatory variables used were correlated, but island size was found to be the best overall predictor of parasite load. Hale (2007) argued that small islands tend to have higher bird densities leading to higher stress levels, although density was not as strong a predictor as island area, and several other measures of immunocompetence were significantly related to the number of birds originally released on the islands, suggesting that loss of genetic variation and inbreeding might be contributing to these relationships. However, the results from section 7 of this report show that the number of birds released does not necessarily relate closely to actual genetic founder number, and subsequent rapid increase

in the population leads to relatively low rates of inbreeding. Furthermore, smaller islands will lose significantly more genetic variation over the long term (e.g. > 100 years), but currently there is very little difference in (neutral) genetic variation between the saddleback populations that Hale (2007) sampled, with all of them showing extremely low levels of variation (Taylor et al. 2007; Taylor & Jamieson 2008). Therefore, it is possible that the significant correlations that Hale (2007) detected relate more generally to variables associated with the different islands, such as area or density of birds, rather than genetic variation per se.

Hale's (2007) results indicated that infection rates in SI and NI saddlebacks declined substantially in reintroduced island populations that were established with more than 90 individuals. However, this result needs to be treated with caution, as only two of the populations sampled were above this threshold and both were on the largest islands with the lowest densities of birds (indeed Kapiti Island is still growing). Furthermore, all translocated NI and SI saddleback populations come from two remnant populations, both of which have generally low genetic diversity, making it seem unlikely that relatively small variation in founder number and bottleneck size would have a sizeable effect on immune responses. There is little doubt that parasitic infestation and stress factors affecting the immune response will vary across islands, but Hale's (2007) study was only able to correlate these variables to the number of birds released, which for a variety of reasons may be a poor proxy for estimating overall variation in genetic diversity and intensity of inbreeding across saddleback populations.

These analytical issues aside, there is growing knowledge about the role of genetic variation in promoting disease resistance (for a recent review, see Sommer 2005). It also seems likely that additional intensive inbreeding as a consequence of further bottlenecks and small population sizes can increase susceptibility to disease above its baseline levels. This is perhaps most aptly shown by a recent study of the naked mole rat (*Heterocephalus glaber*), which is one of the most inbred free-living vertebrates due to its highly eusocial breeding system (Ross-Gillespie et al. 2007). The study investigated factors affecting mortality in a captive population of naked mole-rats struck by a spontaneous, lethal coronavirus outbreak, and found that closely inbred mole-rats ($F \geq 0.25$) were 300% more likely to die than their outbred counterparts (Ross-Gillespie et al. 2007). This example illustrates that loss of genetic diversity through inbreeding may render populations vulnerable to local extinction from emerging infectious diseases, even when other inbreeding depression symptoms are absent.

The fact that many of our threatened endemics are genetically depauparate and ancestrally inbred, and continue to be subject to high rates of inbreeding, does not bode well for their susceptibility to emerging diseases. It is for these reasons and the emerging evidence from studies elsewhere that we should pay greater attention to maximising or maintaining genetic variation and avoiding inbreeding in threatened populations.

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