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ORIGINAL PAPER

Large mainland populations of South Island robins retain greater genetic diversity than offshore island refuges

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Abstract For conservation purposes islands are considered safe refuges for many species, particularly in regions where introduced predators form a major threat to the native fauna, but island populations are also known to possess low levels of genetic diversity. The New Zealand archipelago provides an ideal system to compare genetic diversity of large mainland populations where introduced predators are common, to that of smaller offshore islands, which serve as predator-free refuges. We assessed microsatellite variation in South Island robins (*Petroica australis australis*), and compared large mainland, small mainland, natural island and translocated island populations. Large mainland populations exhibited more polymorphic loci and higher number of alleles than small mainland and natural island populations. Genetic variation did not differ between natural and translocated island populations, even though one of the translocated populations was established with five individuals. Hatching failure was recorded in a subset of the populations and found to be significantly higher in translocated populations than in a large mainland population. Significant population differentiation was largely based on heterogeneity in allele frequencies (including fixation of alleles), as few unique alleles were observed. This study shows that large mainland populations retain higher levels of genetic diversity than natural and translocated island populations. It highlights the importance of protecting these mainland populations and using them as a source for new translocations. In the future, these populations may become extremely valuable for species conservation if existing island populations become adversely affected by low levels of genetic variation and do not persist.

Keywords Genetic variation · New Zealand · Bottleneck · Population differentiation · *Petroica australis australis*

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Introduction

Loss of genetic diversity can have consequences for a population's viability by lowering the population's average fitness and its adaptive potential in a changing environment (Frankham 1995; Lande and Shannon 1996; Amos and Harwood 1998). Population bottlenecks are predicted to lead to a reduction of genetic diversity, with the degree of loss dependent on both bottleneck size and rate of post-bottleneck population growth (Nei et al. 1975). A population that remains small over a long period of time will lose additional genetic variation by the process of random genetic drift (Templeton and Read 1994). The effects of genetic loss can be gradual and therefore only become apparent after many generations (Amos and Balmford 2001; Keller and Waller 2002). In addition to loss of genetic variation, small populations have increased levels of inbreeding, resulting in lowered fitness of inbred individuals compared to their non-inbred counterparts (reviews by Crnokrak and Roff 1999; Hedrick and

Kalinowski 2000; Keller and Waller 2002). In a geographically isolated population (i.e., no gene flow) replenishment of any lost genetic variation depends entirely on the process of mutation, as there will be no influx of new genetic material through migration.

In New Zealand, loss of natural habitat and the introduction of exotic flora and fauna following Polynesian and European settlement have had a strong detrimental impact on the native avifauna (Bell 1991; Clout 2001), resulting in scattered and isolated populations on the mainland (i.e., the North and South Island). Small offshore islands that were either never colonised by introduced mammals or were later restored (including eradication of mammals) represent natural sanctuaries for many bird species (Clout 2001). In the past decades, translocation of endangered species to these islands has been one of New Zealand's most common and successful conservation tools (Armstrong and Mclean 1995; Taylor et al. 2005; Jamieson et al. 2006). Most of New Zealand's translocated island populations thrive in the absence of mammalian predators, even though they were typically founded by a small number of individuals and are isolated by large water barriers that prevent immigration (Taylor et al. 2005). The level of genetic diversity in newly founded populations is strongly influenced by the genetic variation of the source population from which individuals were translocated, with the most appropriate source material having high levels of genetic variability. However, genetic variation of source populations has rarely been considered in the design of translocations.

This study evaluates levels of microsatellite genetic diversity and population differentiation within and among mainland and island populations of two subspecies of New Zealand robin, the South Island robin (*Petroica australis australis*) and the Stewart Island robin (*P. a. rakiura*). Although South Island and Stewart Island robins were formerly described as separate subspecies (Heather and Robertson 1996), recent analysis using mitochondrial DNA does not support this classification (Miller 2003). For the purpose of this study, we treat South Island and Stewart Island robins as part of the same taxonomic unit, and refer to it as South Island robin. These birds were once distributed throughout South and Stewart Island forests, but are currently limited to several offshore islands and numerous patchy populations on the mainland (Bull et al. 1985; Heather and Robertson 1996). Robins are sedentary birds, highly intolerant of large treeless areas and reluctant to cross even 100 m of open ground or water (Flack 1979). The species has been successfully translocated to more than 7 offshore islands around South and Stewart Island, but recent studies have found that some island populations have reduced hatching rates and low levels of genetic variation (Ardern et al. 1997; Miller and Lambert 2004; Mackintosh and Briskie 2005; I. Jamieson, unpublished data). A total of seven isolated robin populations were sampled for this study and subsequently grouped into four categories: large mainland, small mainland, natural island and translocated island populations. We compared genetic variation in large mainland versus small mainland and natural island populations, and natural versus translocated island populations. We expect (1) large mainland populations to retain the highest levels of genetic diversity as a results of their large and stable population sizes, and (2) natural island populations to have more genetic diversity than isolated translocated island populations because of potential gene-flow from adjacent mainland populations and because translocated populations are often founded by small numbers of individuals. However, if natural island populations have become isolated from adjacent mainland populations and/or were originally colonised by a small number of individuals, then natural and translocated island populations may have similar levels of genetic variation. To investigate if populations that were established by small numbers of individuals show signs of reduced fitness, hatching failure rates were compared between two translocated and a large mainland population. Results are discussed with respect to long-term conservation management of island versus mainland populations.

Methods

Sampling and study sites

Robins were sampled throughout the South Island of New Zealand, including two large mainland populations, one small and isolated mainland population and four offshore islands (Fig. 1 and Table 1). The two large mainland populations, Eglinton Valley (>1,000 birds) and Nelson Lakes (>1,000 birds), are considered to be the remaining strongholds of robins on the South Island (Heather and Robertson 1996). The small, isolated Flagstaff population (<50 birds) is located in an exotic plantation and a small area of native forest surrounded by farmland. Breaksea Island (>1,000 birds) and Nukuwaiata Island (~400 birds) are both natural robin populations whose founding histories are unknown. Both islands had rats before their eradication in the 1980s and 1990s, and it is suspected that current robin numbers are higher than before the

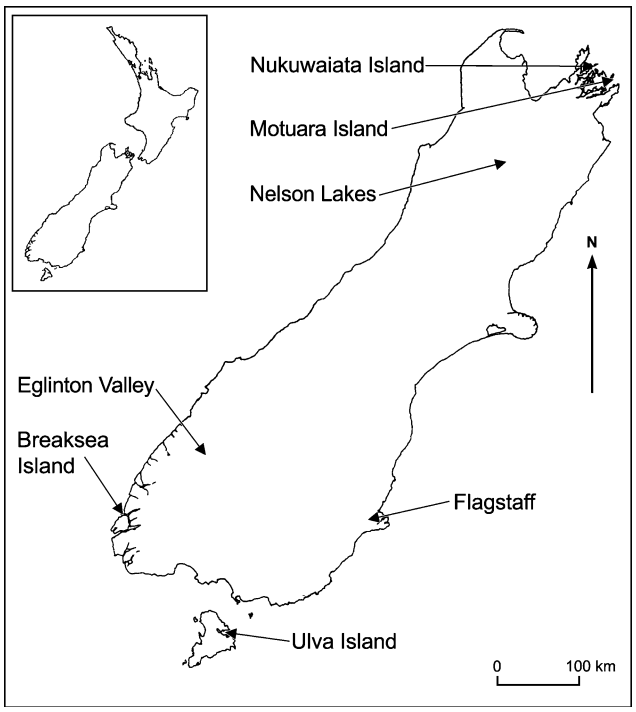

Fig. 1 Map of New Zealand (insert) with geographical locations of the sampled robin populations on South Island and Stewart Island

eradication. Two translocated robin populations were sampled for this study. The currently large Motuara Island population (~600 birds, Flack 1974) was established by transfer of five birds from Nukuwaiata Island in 1973. Although robins were not closely monitored on Motuara Island post-release, it is known that two pairs formed and both fledged young in the first season (Flack 1974). The Ulva Island population (124 birds) was established between 2000 and 2002 with 25 birds translocated from a declining, remnant population on Stewart Island (estimated to have 300 breeding pairs at the time; B. Beaven, pers. comm.). Of the 25 robins released, 12 are known to have survived and bred (Oppel 2000; Oppel and Beaven 2002; I. Jamieson, unpublished data).

Data were collected during the 2004 breeding season at all sites. Populations on Ulva and Breaksea Islands were also sampled during the 2002 and 2003 breeding seasons. Samples from Nukuwaiata and Motuara Islands were kindly provided by L. Shorey and J. Briskie (University of Canterbury, New Zealand).

Adult robins were caught with handnets, clap traps or cage traps baited with mealworms (*Tenebrio molitor*). Blood samples (0.03–0.07 ml) were taken by wing venipuncture of the brachial vein and stored in 1 ml Queens lysis buffer (Seutin et al. 1991). For Eglinton Valley and Ulva Island, marked breeding pairs and their territories were monitored throughout the breeding season. Only nests of which the actual number of eggs and the number of nestlings could be determined were included in the analysis of hatching success.

Genetic methods

DNA was extracted and purified from blood samples using proteinase K (10 mg/ml) in a Chelex® 100 Resin solution (50 mg/ml). All robin samples were genotyped at 10 microsatellite loci, using primers that were originally developed for chicken (*Gallus gallus*; Gibbs et al. 1997), large crowned leaf warbler (*Phylloscopus occipitalis*; Bensch et al. 1997), New Zealand saddleback (*Philesturnus carunculatus rufusater*; Lambert et al. 2005), red-capped robin (*Petroica goodenovii*; Dowling et al. 2003) reed bunting (*Emberiza schoeniclus*; Hanotte et al. 1994), Seychelles warbler (*Acrocephalus sechellensis*; Richardson et al. 2000) and

Table 1 Characteristics of the seven robin populations sampled for this study

Population	Population type	Size (ha)	Current population size	References
Eglinton Valley	Large mainland	2000–4000	>1000	C. O'Donnell (pers. Comm.)
Nelson Lakes	Large mainland	>1,000,000	>1000	P. Gaze (pers. Comm.)
Flagstaff	Small mainland	~4400	<50	Duncan et al. (1999); P. Schweigman (pers. Comm.)
Breaksea Island	Natural island	170	>1000	H. Edmonds (pers. Comm.)
Nukuwaiata Island	Natural island	195	~400[a]	Byrne (1999)
Motuara Island	Translocated island (5 birds in 1973)	58	~600	Flack (1974); Byrne 1999
Ulva Island	Translocated island (25 birds in 2000–2002)	269	124	Oppel and Beaven (2002); I. Jamieson (unpublished data)

[a] Nukuwaiata Island is close to Te Kakaho Island and migration may occur, making the total population size an estimated 600 birds (J. Briskie, pers. comm.)

Table 2 Primer sequences and PCR characteristics of 10 microsatellite loci used in *Petroica australis* subspecies

Locus	Primer sequences (5′-3′)	T_a (°C)	$MgCl_2$ (mM)	A[a]	References
2F9	GCATTTCTGGGCTGTAACAT AAAGGACAATGTAATTGGTG	57	1.5	8	T. King (pers. comm.)
Ase18	ATCCAGTCTTCGCAAAAGCC TGCCCCAGAGGGAAGAAG	56	1.5	2	Richardson et al. (2000)
Ase64	CCACCTTTCATACTGGGGAG TTCAGCCAGTCAGTGTAGCC	57	1.5	9	Richardson et al. (2000)
Escμ6	CATAGTGATGCCCTGCTAGG GCAAGTGCTCCTTAATATTTGG	56	1.5	6	Hanotte et al. (1994)
GgaMu128	CAAAGTAATCAGCTTGTGCTAC CATTTCCACCGCATTGAGCAG	57	1.5	2	Gibbs et al. (1997)
Indigo28	CCCAGGAAGTATCCCAGAA CCTCCAATGCTTTAGTGACC	50	2.0	3	Sefc et al. (2001)
Pca12	TGTGGGAAACCAGAGGAAA CAGGGGAAAAATAGAGAGGG	56	1.5	5	Lambert et al. (2005)
Pca13	GCCTCGGTGTGAGCATCATT ACCCAAGCCCCATCCAAACA	56	1.5	2	Lambert et al. (2005)
Pocc6	TCACCCTCAAAAACACACACA ACTTCTCTCTGAAAAGGGGAGC	60	1.5	4	Bensch et al. (1997)
Pgm3	CACTGGGATGAAAAGACCTG TCTCCAGAGCTGGCTATAAAC	52	2.5	2	Dowling et al. (2003)

[a] A is the total number of alleles per locus

village indigobird (*Vidua chalybeate*; Sefc et al. 2001) (Table 2).

PCR reactions were carried out in 10 µl volumes containing 1 µl DNA, 0.5 µM of each primer, 0.8 µM dNTP, 1 µl buffer, 0.5 U *Taq* DNA polymerase (AB-Gene) and an optimised concentration of $MgCl_2$ (Table 2). For primers that produced several shadow bands, 2.2 µl betaine (5.0 M) and 0.2 µl DMSO were added to the PCR mix. PCR amplification was performed in a PTC-100^{TM} Programmable Thermal Controller (MJ Research Inc.) with the following profile: 3 min at 96°C, 35 cycles of 30 s at 96°C, 30 s at T_a (optimal annealing temperature) and 1 min at 72°C, followed by 2 min final extension at 72°C (Table 2). DNA fragments were visualised on 7–9% vertical, non-denaturing polyacrylamide gels (PROTEAN II xi Cell system from Bio-Rad). For three loci (Ase64, GgaMu128 and Pocc6), primers were radioactively labelled with γ^{33}P and PCR products were visualised on vertical denaturing acrylamide gels (6%). All gels were scored visually, by comparison to a ladder and to samples of known genotype. To ensure data accuracy all gels were scored by two individuals (S. Boessenkool and S. Taylor) independently.

Analysis of microsatellite data

Deviations from Hardy-Weinberg proportions and genotypic disequilibrium were calculated using GENEPOP v. 3.4 (Raymond and Rousset 1995). Deviations from Hardy-Weinberg were tested using the exact probability test (Guo and Thompson 1992), with markov chain parameters employing 10,000 dememorizations, 1,000 batches and 10,000 iterations per batch. Sequential Bonferroni correction (Rice 1989) for multiple comparisons was applied where necessary.

Calculations of allelic diversity, observed and expected heterozygosities (H_O and H_E; Nei 1978) and allele frequencies were performed in GENETIX v. 4.05 (Belkhir et al. 2004). Allelic richness (number of alleles per population corrected for sample size, El Mousadik and Petit 1996; Petit et al. 1998) was calculated in FSTAT v. 2.9.3 (Goudet 2002). Differences in number of alleles, allelic richness and expected heterozygosity per population type were tested using Wilcoxon signed rank tests. Statistical tests were done in SPSS v. 11.0 ($\alpha = 0.05$).

Population differentiation was measured using the θ estimator (Weir and Cockerham 1984) of Wright's F_{ST} (Wright 1969) in GENETIX. Significance levels were assessed using 1,000 permutations. A two-dimensional factorial correspondence analysis (FCA) was used to investigate the genetic similarity among individuals based on their allele frequencies and subsequent genotypes. FCA does not group individuals a priori and therefore provides an unbiased test of population structure. The relationship between genetic and geographical distance was explored using a Mantel test (Mantel 1967) in the ISOLDE program of GENEPOP. Genetic distance measured by $F_{ST}/(1–F_{ST})$ (Rousset 1997) was correlated to the natural logarithm of the geographical distance in kilometres. Significance of this relationship was assessed employing 1,000 permutations.

Analysis of hatching failure data

Determining loss of reproductive potential due to genetic factors is difficult in mainland robin populations because of high rates of nest predation by introduced mammals (Etheridge and Powlesland 2001). We therefore followed Mackintosh and Briskie's (2005) definition of hatching failure as the proportion of eggs that failed to hatch relative to those surviving to the end of the incubation period, excluding nests that failed due to predation, desertion or accident. Breeding data were collected for the Eglinton Valley and Ulva Island populations and used to calculate hatching failure rates per clutch. As a result of limited accessibility of nests, only one clutch per breeding pair was available for analysis (i.e., all clutches were independent). Average hatching failure was previously reported for robins on Motuara Island (Mackintosh and Briskie 2005), but Mackintosh kindly provided hatching failure per clutch for the Motuara Island population to enable statistical comparisons of the three populations. To correct for restricted ranges of potential values and large numbers of zeros and ones, the proportion of eggs that failed to hatch per clutch (p) was transformed:

$$p' = 1/2\,[\arcsin\sqrt{(X\,/\,(n\,+\,1))} + \arcsin\sqrt{((X\,+\,1)\,/\,(n\,+\,1))}],$$

where p' is the transformed proportion of eggs that failed to hatch per clutch, X is the number of failed eggs and n is the clutch size (Zar 1996; Jamieson et al. 2003). Differences in hatching failure rates among populations were compared using a Kruskal-Wallis test followed by a Games-Howell test for multiple comparisons. Statistical analyses were performed in SPSS v. 11.0 ($\alpha = 0.05$).

Results

Disequilibria

Ten microsatellite loci were used to genotype DNA samples of 516 individual South Island robins from seven populations. All samples amplified successfully, with the exception of nine individuals at one locus each. A total of 43 alleles were identified ranging from two to nine alleles per locus, giving an average of 4.3 alleles/locus (Table 2).

Two significant departures from Hardy-Weinberg expectations due to an excess of heterozygotes were observed at loci Escμ6 and Ase64 in the Eglinton Valley population, and a deficiency of heterozygotes was observed at locus Pca 13 in the Ulva Island population ($P < 0.05$). However, these loci did not show any deviations from Hardy-Weinberg in any of the other populations, nor did any of the other loci in the Eglinton Valley or Ulva Island population. Genotypic disequilibrium was calculated in each population for all pairs of loci. Only 6 of the 208 combinations were significant (2.9%, $P < 0.05$) with 5 of these observed in the Ulva Island population.

Levels of genetic variation

All 10 loci were polymorphic in the two large mainland populations, Eglinton Valley and Nelson Lakes, and in the translocated Ulva Island population. All other island populations were monomorphic for two to four loci, and the small mainland population of Flagstaff was monomorphic for half of the loci tested (Table 3). Average number of alleles and allelic richness was significantly higher for large mainland populations compared to the small mainland population (Wilcoxon signed rank statistic; $Z = -2.7$ for both measures, $P < 0.01$) and compared to the natural island populations ($Z = -3.3$ and -3.2, respectively, $P < 0.005$). In fact, all but two alleles were found in the large mainland Eglinton Valley population. There were no significant differences in number of alleles or allelic richness between natural versus translocated island populations ($P > 0.05$). Expected heterozygosity averaged over all loci ranged from 0.199 (Flagstaff) to 0.483 (Ulva Island). When comparing expected heterozygosity of population types, only the difference between large versus small mainland populations was significant ($Z = -2.8$, $P < 0.01$).

Table 3 Genetic variation at 10 microsatellite loci in seven South Island robin populations

Population	n	Proportion of polymorphic loci	Alleles per locus	Allelic richness	H_o	H_E
Eglinton Valley	170	1.0	4.1	3.2	0.424	0.423
Nelson Lakes	21	1.0	3.4	3.2	0.401	0.392
Flagstaff	12	0.5	1.8	1.8	0.208	0.199
Breaksea	68	0.8	2.6	2.4	0.396	0.393
Nukuwaiata	25	0.7	2.5	2.4	0.382	0.322
Motuara	93	0.6	2.3	2.2	0.344	0.342
Ulva	127	1.0	3.0	2.6	0.485	0.483

Genetic differentiation among populations

Allele frequencies, including those of common alleles, differed considerably among populations. In more than half of the loci, the most common allele in one population was rare or absent in one or more of the other populations. Unique alleles were observed in the Eglinton Valley (four) and Nukuwaiata Island (one) populations only. In Eglinton Valley these unique alleles were all rare with frequencies of <0.060.

Significant population differentiation was indicated by an overall F_{ST} value of 0.205 and pairwise F_{ST} values ranging from 0.051 to 0.379 (Table 4). The highest F_{ST} values were observed between Flagstaff and the four island populations, while lower values were seen between Nelson Lakes and Eglinton Valley, and between Motuara Island and Nukuwaiata Island. Population differentiation increased significantly with geographical distance ($R^2 = 0.1873$, $P = 0.005$).

A two-dimensional FCA revealed genetic divergence among populations based on the individual genotypes (Fig. 2). Samples from the three northern populations (Nelson Lakes, Motuara Island and Nukuwaiata Island) clustered together, individuals from Flagstaff grouped within the Eglinton Valley population, and the Ulva Island population formed a clear separate group. Breaksea Island birds were separated from the other populations, although a few individuals were found within some of the other clusters. The placement of the populations along the first axis was consistent with a geographical north–south gradient.

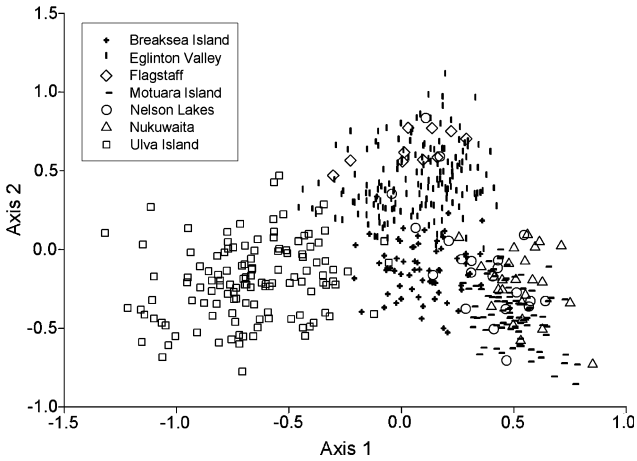

Fig. 2 Plot of the first two axes of a factorial correspondence analysis based on allelic variation at 10 microsatellite loci for 516 South Island robins. The axes explain 19.8% of the total variation

Hatching failure

Hatching failure per clutch ranged from 0% to 100% on Ulva Island (mean 15.6% ± 7.4 SE, $n = 16$) and Motuara Island (36.6% ± 8.7, $n = 16$), and from 0% to 33% in Eglinton Valley (4.2% ± 2.8, $n = 16$). There was a significant difference in hatching failure among the three populations ($\chi^2 = 32.0$, $P < 0.001$). Both Ulva and Motuara Island had significantly higher hatching failure rates than Eglinton Valley (Games-Howell posthoc test, $P < 0.001$), but differences between the two islands were non-significant ($P > 0.05$).

Discussion

Genetic variation in mainland and island populations

The large mainland robin populations of Nelson Lakes and Eglinton Valley retain the highest number of alleles compared to other populations that were sampled in this study. Both populations were polymorphic for all the analysed loci and rare alleles were detected frequently. 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 to these large mainland populations, the small mainland robin population in Flagstaff had very little genetic variation with 5 out 10 loci monomorphic, low expected heterozygosity and very few alleles per locus.

Table 4 Pairwise differentiation among South Island robin populations at 10 microsatellite loci measured by the θ estimator (Weir and Cockerham 1984) of F_{ST} (Wright 1969)

Population	Nelson Lakes	Flagstaff	Breaksea	Nukuwaiata	Motuara	Ulva
Eglinton Valley	0.075*	0.121*	0.184*	0.143*	0.169*	0.191*
Nelson Lakes		0.273*	0.142*	0.066*	0.051*	0.220*
Flagstaff			0.357*	0.298*	0.379*	0.313*
Breaksea				0.245*	0.210*	0.221*
Nukuwaiata					0.117*	0.293*
Motuara						0.265*

* Significantly different from 0 at $P < 0.01$

The Flagstaff population has probably been isolated since the late 1800s when the forests around the city of Dunedin were clear-felled (A. Mark, pers. comm.). Immigration from the nearest robin population is unlikely (120 km away, Bull et al. 1985), so the Flagstaff population may have been small for many generations. Such a long-term bottleneck can cause reductions in genetic variation due to random genetic drift and inbreeding (Nei et al. 1975; Merilä et al. 1996).

The natural robin populations on Breaksea and Nukuwaiata Islands have lower levels of genetic diversity than the larger mainland populations. Some alleles have been lost and several of the bi-allelic loci have become fixed, which is probably a consequence of past bottlenecks and continued isolation from the mainland. The observed trend of higher levels of genetic variation in large mainland populations is consistent with the general consensus that mainland populations retain more genetic variation than island populations, although most studies find a difference in allelic diversity as well as H_E, while we detected an effect on allelic diversity only (Frankham 1997; Eldridge et al. 2004; Mills et al. 2004). Even where island populations are relatively large, they typically 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 (e.g., Eldridge et al. 2004).

The number of individuals released during a translocation can be used as a proxy for bottleneck size, but similar information on founder size is not available for the natural island populations in our study. We compared genetic diversity between natural island robin populations and those that had been established by translocation, and observed no significant differences between the two groups. The Ulva Island population was the only island population in which all studied loci were polymorphic; evidently the island's 12 effective founders had substantial numbers of alleles and this may be an indication that high levels of diversity exist in the mainland Stewart Island population where these founders were sourced. Importantly, the Ulva Island population has been more extensively sampled (98% of the population) than the other studied populations, possibly resulting in an underestimation of the difference in genetic variation between Ulva Island and the large mainland populations.

In the robin population on Motuara Island four out of the 10 loci were monomorphic, but overall levels of genetic diversity were similar to those found on the other islands, including Nukuwaiata Island from where the robins were sourced. As only five robins were released on Motuara Island, with very low fertility recorded for one of two pairs that nested (Flack 1974), both reduced heterozygosity and loss of alleles were expected. The reduction in heterozygosity following a bottleneck depends on both bottleneck size and the rate of population growth following the bottleneck, while the loss of alleles is primarily dependent on bottleneck size only (Nei et al. 1975). High reproductive potential in robins (Powlesland 1983; Armstrong et al. 2000) has enabled the Motuara Island population to grow from 5 to 600 individuals in just 30 years or ~10 generations, a rate of population growth that may explain the maintenance of heterozygosity observed on the island. However, there were five alleles in Nukuwaiata which were absent in Motuara (allele frequencies not shown), indicating that these alleles were either not present in the Motuara founders or were subsequently lost due to random genetic drift. Interestingly, the Motuara Island sample also contained three alleles not detected in the Nukuwaiata sample. It is unlikely that the Nukuwaiata population lost these three alleles since the founding of the Motuara Island population, and thus we conclude that our sample size from Nukuwaiata Island was too small to detect all alleles (see Sjögren and Wyöni 1994). Consequently, we may have not been able to detect the full scope of alleles lost in the Motuara population.

The few studies that have compared genetic diversity of translocated island populations to that of natural island populations seem to find a consistent pattern in which the translocated island populations have lower genetic diversity than their source population. Two out of three first order translocated North Island saddleback populations show reduced minisatellite diversity compared to their source population, while no effect was observed in the third population (Lambert et al. 2005). Translocated Laysan finch populations have lower genetic diversity than their source populations, but the loss is less than was expected from the bottleneck sizes (Tarr et al. 1998). The finch source population was known to have gone through a bottleneck in the past, which may have led to a loss of rare alleles and thus may reduce the power to detect possible genetic consequences of the translocations (Tarr et al. 1998). Differences in the loss of genetic diversity of translocated versus source island populations is probably caused by a wide variety of factors including number, sex ratio and differential mortality of founders, mating system, breeding rates, habitat quality and levels of genetic variation in the source island population (see Lambert et al. 2005).

Increased hatching failure in translocated island populations

Higher hatching failure rates were observed in the translocated island populations, Motuara and Ulva Island, compared to the large mainland Eglinton Valley population. Mackintosh and Briskie (2005) suggested that inbreeding depression was a likely explanation for the low hatching success of Motuara Island robins, because a food supplementation experiment had no effect on egg failure rates. There is little doubt that the Motuara Island population is inbred: it was founded by a maximum of five individuals at a time when there were no robins on the adjacent mainland, hence is a closed population. Inbreeding leads to increased levels of homozygosity and inbred populations are consequently expected to have lower levels of observed heterozygosity than outbred populations (Frankham 1998), but we observed no significant difference in heterozygosity between large mainland and translocated island populations.

Population genetic differentiation among mainland and island populations

Observed heterogeneity in allele frequencies and high overall and pairwise F_{ST} values indicate strong population differentiation among South Island robin populations, which is supported by the FCA. A north-south gradient can be seen along the first axis of the FCA, which coincides with significant isolation by distance based on F_{ST}. The clear separation of the Ulva Island population in the FCA supports the traditional subspecies classification of the Stewart and Island robins based on morphological features (Fleming 1950), but is not supported by mitochondrial data (Miller 2003).

A small number of founders can lead to rapid differentiation between source and founder populations due to changes in allele frequencies associated with founding events (Chakraborty and Nei 1977; Tarr et al. 1998). Changes in allele frequencies are caused by the differential survival of individuals during a bottleneck, or in the case of a founding bottleneck, the differential representation of alleles in the founding pool. Differentiation is further enhanced by random genetic drift leading to loss and fixation of alleles and increased rate of inbreeding (Suzuki et al. 1981). In this study, the strong differentiation among populations was caused by heterogeneity in allele frequencies, fixation of alleles at bi-allelic loci and loss of alleles at more variable loci. The northern populations of Nelson Lakes, Nukuwaiata and Motuara Island are the only populations that have alleles that are not found in the Eglinton Valley population, possibly due to an ancestral divergence between northern and southern populations. For the other populations, isolation may have been too recent for new alleles to originate by mutation, or genetic drift may have prevented retention of any new, low frequency alleles.

Management implications

For conservation purposes islands are considered safe refuges for many species, particularly in countries where introduced predators form a major threat to the native fauna (e.g., New Zealand). However, a conservation dilemma has arisen because an increasing number of studies have provided evidence that island populations have lower levels of genetic variation and higher levels of inbreeding than mainland populations (e.g., Eldridge et al. 2004; Mills et al. 2004; this study). Although lowered genetic diversity may not pose an immediate threat to the viability of these populations, it could become increasingly important in the long term if these populations are unable to adapt to changes in habitat, climate or novel pathogens. Island populations therefore provide a valuable solution in the short term, but their role may be limited for long-term conservation management of species, unless these populations 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 protecting and maintaining these populations. Such protection requires ongoing management, which can be logistically difficult and expensive. In New Zealand, introduced mammalian predators form a primary threat to avian populations and so predator trapping and poisoning have been implemented at various mainland sites, including the Eglinton Valley (Dilks et al. 2003) and the Lake Roitoiti area (~5,000 ha) in Nelson Lakes (Butler 2003). Although predator control is meant to protect more critically vulnerable species such as mohua (*Mohoua ochrocephala*), kaka (*Nestor meridionalis*), short-tailed bats (*Mystacina tuberculata*) and long-tailed bats (*Chalinolobus tuberculatus*; Hill 2005), other less endangered species living in the area, such as robins, clearly benefit. It is important to realise, however, that high levels of genetic diversity are not found in all mainland populations, and a prolonged period of population decline can also cause a dramatic reduction in genetic diversity in mainland populations such as that observed for robins at Flagstaff.

In summary, this study shows that large mainland populations retain higher levels of genetic diversity

than island populations. Although translocations to islands play a vital role in the recovery of threatened species, our results highlight the importance of conserving ancestral mainland populations for their high genetic diversity. Minimising loss of genetic variation should be incorporated into contemporary management programs whenever possible. Sourcing founders from large, genetically variable populations and using large founder numbers may reduce loss of genetic diversity following translocations, giving new populations greater potential for long-term persistence.

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