Genetic diversity and population viability in translocated North Island saddleback (*Philesturnus rufusater*) populations at Zealandia Karori Sanctuary and Kapiti Island

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Abstract: Genetic variation in two translocated populations of North Island saddleback (*Philesturnus rufusater*) on Kapiti Island and at Zealandia was investigated using five microsatellite loci and compared with the source populations in the Hauraki Gulf. Although the absolute number of alleles in the two populations was low (3 alleles per locus), both populations carried all the alleles found in their immediate source populations, but lacked one rare allele found in only one individual from the original remnant population on Hen Island. Overall heterozygosity was high and inbreeding coefficients were low. Population viability analyses showed that these populations will likely reach carrying capacity by the middle of this decade, and genetic simulations predicted that they should retain between 90% (Kapiti) and 95% (Zealandia) of the heterozygosity of their sources. The difference between the two populations is most likely due to the prolonged post-translocation bottleneck on Kapiti when rats were still present on the island. While our results suggest that additional top-up translocations would be unnecessary and unwarranted at this time, further work on potentially selected loci or inbreeding depression could justify this decision to be revisited.

Keywords: genetic variation; microsatellites; serial bottlenecks

Introduction

Conservation-motivated reintroductions have been undertaken for over 100 years (Kleiman 1989), but the last few decades have seen a dramatic increase in the use of translocation as a conservation tool to safeguard vulnerable species from extinction (Seddon et al. 2007). Although translocations quickly gained in popularity during the 1970s and 1980s, the majority of attempted translocations of threatened or sensitive species failed during that time (Griffith et al. 1989). These failures led to the emergence of the field of reintroduction biology and a more rigorous framework to maximise the chances of translocations leading to the establishment of viable populations (Seddon et al. 2007; Armstrong & Seddon 2008).

In New Zealand, where the avifauna evolved in the absence of terrestrial mammals, wildlife managers are mainly concerned with limiting predation from introduced mammals (Clout 2001). Thus, many predator-free offshore islands have served as refugia to establish successful new populations of threatened birds (Armstrong & McLean 1995). With the improvement in mammalian predator control, a number of vulnerable native species, including the North Island saddleback (*Philesturnus rufusater)*, have now also been translocated to 'mainland islands' – areas of mainland habitat in which introduced predators have been excluded by fencing or continued predator control.

The North Island saddleback was once widespread throughout the North Island as well as on several offshore islands (Hooson & Jamieson 2003), but by the late 1800s had been reduced to just one remnant population of c. 500 birds on Hen Island (484 ha) in the Hauraki Gulf (Merton 1975; Williams 1976). This only surviving natural population subsequently served as the basis of an extensive, well-documented and successful translocation programme. From the 1960s, translocations resulted in the establishment of 10 populations on other islands in the Hauraki Gulf (Fig. 1). From the 1980s onwards, birds were translocated to found new populations on islands outside the Hauraki Gulf such as Mokoia Island (1992) and Kapiti Island (beginning in 1981) (Lovegrove 1996). Due to the success of this translocation programme, the North Island saddleback is currently considered as 'Recovering' (Miskelly et al. 2008) within the 'At Risk' category defined in the most recent revision of the New Zealand Threat Classification System (Townsend et al. 2008). Although some authors (BirdLife International 2008) still classify the North Island saddleback as a subspecies (*P. carunculatus rufusater*), here it is treated as a distinct species from the South Island saddleback (*P. carunculatus*), following the nomenclature of Holdaway et al. (2001).

Increasing the population size or the number of populations should not be the sole objective of translocations. Management guidelines should also aim to prevent inbreeding and maintain genetic diversity (Jamieson et al. 2006, 2008). Therefore, translocations should attempt to capture the total genetic diversity still present in the source population(s). However, this is often difficult to accomplish as the number of founders is typically small, leading to a possible genetic bottleneck. The loss of genetic diversity associated with a bottleneck may lead to a short-term decrease in fitness through inbreeding depression (Keller & Waller 2002; Jamieson et al. 2006) and long-term reduction of evolutionary potential (Milligan et al. 1994; Frankham 2005). Several factors may affect the severity of a genetic bottleneck; for instance, if populations expand rapidly after reintroduction, the loss of genetic diversity through drift is minimised (Allendorf & Luikart 2007). In addition, top-up translocations can take place once a viable population is

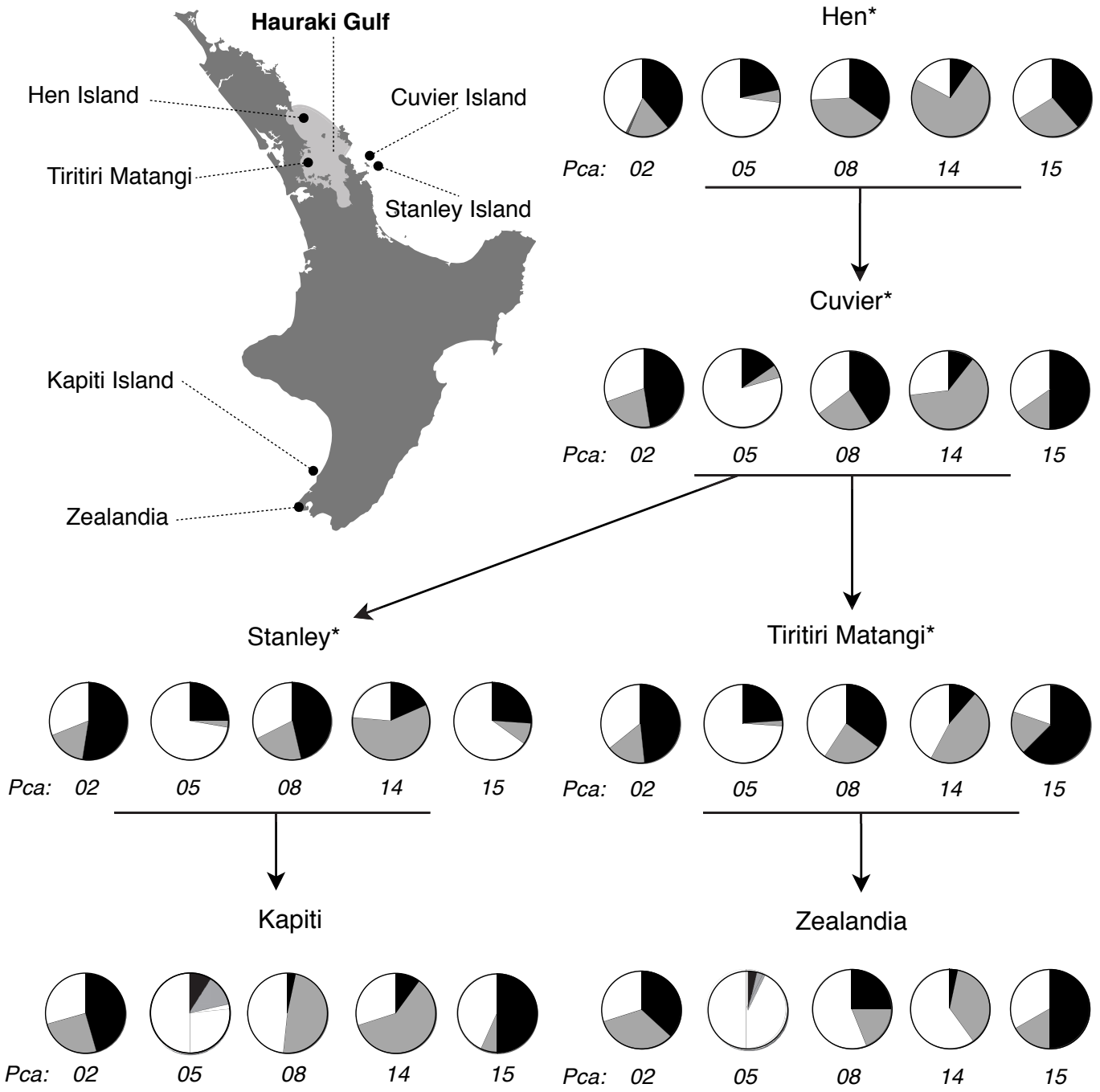

Figure 1. Map showing the location of the North Island saddleback populations mentioned in this article. The Hauraki Gulf area is slightly shaded. Arrows indicate the translocation history of each population and pie diagrams graphically illustrate the allele frequencies at all five microsatellite loci. Black refers to the shortest allele, light grey refers to the medium-sized allele and white refers to the longest allele (in number of base pairs (bp); see Table 1). For *Pca02* the fourth allele, only present in Hen Island, is dark grey (122 bp). Data for populations with asterisks (*) are from Lambert et al. (2005).

established. These top-ups may reduce the impact of the initial bottleneck and help ensure that long-term genetic diversity is maintained at the highest possible level (Boessenkool et al. 2006; Frankham 2009).

Research on genetic diversity in North Island saddleback populations from the Hauraki Gulf has been extensive (Livingston 1994; Lambert et al. 2005). In contrast, no similar study has yet been undertaken for the Kapiti Island and Zealandia Karori Sanctuary populations. Kapiti Island (1965 ha; 40°51′ S, 174°55′ E) was farmed until the late 1800s. Most of the island became a nature reserve in 1897, stock was taken off or culled, and the land was left to regenerate back to forest (MacLean 1999). Several saddleback reintroduction attempts were made before mammalian predator eradication, but these were mostly a failure (Lovegrove 1996). Only four birds out

of a total of almost 250 individuals translocated between 1981 and 1983 were confirmed alive in 1986, the year possums (*Trichosurus vulpecula)* were eradicated (Hooson & Jamieson 2003). An additional 122 birds were introduced between 1987 and 1989 (Lovegrove 1991). By 1996, when two species of rats (*Rattus exulans* and *R. norvegicus*) were eradicated, only nine pairs and 13 single males were reported to still be alive (Empson & Miskelly 1999). However, since 1997, the saddleback population on Kapiti Island has thrived, the number of pairs increased by 120% from 1997 to 1998 (Empson & Miskelly 1999) with a mean annual population growth rate of 33% since (Hooson & Jamieson 2003). Zealandia (225 ha; 41°29′ S, 174°75′ E) is a 'mainland island' sanctuary located in suburban Wellington. An 8.6-km-long fence helps keep this sanctuary free from all introduced mammalian predators except mice. In June 2002, 39 birds were translocated to found the first North Island saddleback population on the North Island mainland for over 100 years.

Both the Kapiti Island and Zealandia populations are third-order translocated populations (see Fig. 1); they were respectively founded with individuals from Stanley Island and Tiritiri Matangi. Those two source populations were themselves founded with individuals from Cuvier Island, which in turn was the result of the translocation of individuals from Hen Island, the only remnant 'natural' population of North Island saddleback. Thus both Kapiti Island and Zealandia populations ultimately arise from the same source, but have been through different bottleneck histories and have been isolated from each other since 1977.

Putting in place management practices that will at least maintain current levels of genetic diversity is important. The aim of this study was to provide the genetic data to help decide whether top-ups are required to supplement the genetic pool of these two populations. To achieve this aim, we used microsatellite markers to investigate the levels of heterozygosity and allelic diversity in the Kapiti Island and Zealandia populations, and then compared results with published data from the relevant source populations in the Hauraki Gulf (Lambert et al. 2005). As there was no population size estimate for either population, we also used population viability analysis (PVA) to model the population size of both populations. These models were computed to indicate when both populations are likely to reach carrying capacity. Saddlebacks are territorial birds all year round, and once carrying capacity is reached, new immigrants would presumably have greater difficulty establishing territories and becoming part of the breeding population.

Methods

Sampling

A total of 31 Kapiti Island samples and 16 Zealandia samples were analysed. Of the Kapiti Island samples 28 were blood samples collected in 2003 (Hale 2007). The remaining three samples were feather samples, collected using mistnets in 2008. For the Zealandia samples, 10 were feather samples collected using mistnets in 2008, while the remaining six samples came from toepads of deceased individuals collected by Zealandia staff.

DNA extraction and PCR

DNA from both feather and blood samples was isolated using a standard protocol of proteinase K digestion and phenol-chloroform extraction (Sambrook et al. 1989). All samples were genotyped at five microsatellite loci (*Pca02, Pca05, Pca08, Pca14* and *Pca15*). All PCR (polymerase chain reaction) were carried out in a total volume of 15 µl (14 µl PCR mix and 1 µl DNA template) and all primer working stock solutions had a concentration of 10 µM. Each PCR reaction contained: 1.5 µl buffer; 0.6 µl each of BSA, dNTP, reverse primer, and fluorescent-labelled M13 primer; 0.15 µl forward primer; 0.6 U of *Taq* DNA polymerase. $MgCl_2$ concentration differed for several loci (Lambert et al. 2005): *Pca02* was 0.5 mM, *Pca05* was 0.8 mM, and *Pca08, 14, 15* were 1.5 mM. The PCR regime was modified so that the last eight cycles of each PCR were performed with a lower annealing temperature (53°C) to favour annealing of the fluorescent-labelled M13 primer that matched an M13 tail on the 5' end of the forward primer (Schuelke 2000). The M13 primer used was 5'-TGTAAACG ACG GCC AGT-3'. FAM and VIC were the fluorescent labels used. Samples were cycled at 96ºC for 240 s, followed by 35 cycles of 96ºC for 30 s, locus-specific annealing temperature (55–58ºC from Lambert et al. 2005) for 30 s and 72ºC for 60 s. Then, eight cycles at 96ºC for 30 s, 53ºC for 30 s, 72ºC for 40 s and a final extension step of 72ºC for 60 s. Each PCR reaction included a positive and a negative control. All PCR products were analysed on a 3730 DNA analyser (Applied Biosystems). The ladder used for sizing was LIZ. Allele sizes were scored manually using GeneMapper 3.7 (Applied Biosystems) and scoring of each genotype was carried out at least twice. To ensure consistency with previously published data (Lambert et al. 2005), 30 blood samples (10 each from Hen Island, Stanley Island and Tiritiri Matangi Island) that were used in 2005 (but collected between 1990 and 1992) were obtained and reanalysed alongside the samples collected in the present study to ensure consistency in allele scoring.

Data analyses

The software Genepop 3.4 (Rousset 2008) was used to calculate allele frequencies, expected (H_E) and observed (H_O) heterozygosity, inbreeding coefficient (F_{IS}) and deviations from Hardy-Weinberg Equilibrium (HWE), for each locus as well as across all markers combined. Fisher's exact tests of differentiation were also computed among all populations for each marker in Genepop. Genetic differentiation values (F_{ST}) were calculated in Arlequin 3.0 (Excoffier et al. 2005) and their significance was tested with 1000 permutations. For multiple tests, significance was assumed if P was smaller than 0.05 after a sequential Bonferroni correction (Rice 1989). Previously published data (Lambert et al. 2005) for Hen Island (n = 41), Stanley Island (n = 40) and Tiritiri Matangi (n = 28) were included when calculating pairwise measures of differentiation.

Vortex 9.96 (Lacy et al. 2005) was used to model the size of both Kapiti Island and Zealandia saddleback populations through population viability analysis (PVA). Saddleback is one of a few bird species that have been shown to be strictly monogamous (Taylor et al. 2008). Under optimum breeding conditions they are prolific breeders, with two to three clutches, each of two to three young, per season (Lovegrove 1991). Individuals can start reproducing from one year of age, especially in low-density, recently translocated populations (Armstrong et al. 2005). They are also a long-lived species (up to 17 years), with overlapping generations (Merton 1975). The following settings were applied to all scenarios when running Vortex: 1000 iterations, breeding from age one for both sexes,

up to three clutches a year (10% have a single clutch, 70% have two clutches and 20% have three clutches), two chicks per clutch, 40% mortality in first year (Zealandia, unpubl. data) and 10% mortality from age one, maximum breeding age set at 10 years. Simulations were performed for 100 years and started with nine breeding pairs in 1997 (time of rat eradication) for Kapiti Island and 19 pairs in 2002 for Zealandia. Based on each saddleback requiring 0.36 ha (Hooson & Jamieson 2003), carrying capacity was set at 600 for Zealandia and at 5000 birds for Kapiti Island. Sensitivity testing was carried out to assess the importance of several parameters (maximum breeding age, mortality rate in first year, number of offspring per clutch) in modelling the growth of each population.

Encounter data from Zealandia were used to calculate a second population size estimate to cross-validate PVA models. The encounter data covered the period from June 2006 to November 2008 and consisted of all sightings of saddlebacks divided into two subsets comprising either sightings of banded birds or unbanded birds only. Each subset was then analysed separately. Data for banded birds with no sightings during this period were removed before computing the population size estimate. The size of the population of banded birds was estimated using the Jolly–Seber model (Jolly 1965; Seber 1965) as implemented in the computer program MARK (White & Burnham 1999). The Jolly–Seber model assumes an equal chance of encountering a banded versus an unbanded bird. This assumption enables the probability of sighting to be calculated for the banded birds and then applied to calculating the number of unbanded birds in the population. The population size estimate for the unbanded birds was calculated using a conservative ad hoc proportional method where sightings of unbanded birds in the same territory were regarded as being sightings of the same bird. No recent survey data were available for the Kapiti Island population.

Finally, the software BOTTLESIM (Kuo & Janzen 2003) was used to model the expected trends in genetic diversity (number of alleles and H_E) for 100 years after the founding event for Kapiti Island and Zealandia. This software allows simulation of the bottleneck caused by the founding of a new population, as well as the post-establishment growth of the population. Life-history parameters were set to the same values as those employed for the PVA, and the number of individuals at each year in the simulations was that calculated in the PVA, starting with 39 individuals for Zealandia in 2002. For Kapiti Island, to account for the extended duration of the bottleneck between 1989 and 1997 (after translocation but before rat eradication), simulations started in 1989 with 31 individuals (but only nine females), and population size was maintained until 1997, when it started to grow following PVA predictions. While single males were excluded from the PVA (because their presence cannot additionally increase demographic growth), they were included in BOTTLESIM calculations because they may increase the genetic effective size of the population. The initial allele frequencies were those from Stanley Island for Kapiti Island and Tiritiri Matangi for Zealandia (from Lambert et al. 2005) and 1000 replicates were computed. BOTTLESIM simulations were also employed to evaluate the error associated with small sample sizes when estimating allele frequencies and H_E in serially translocated populations like these. We did this only for Zealandia, which had the smallest sample size of the two populations ($n = 16$). We saved the data from 1000 simulated translocations from Tiritiri Matangi at year 2008, calculated the real allele frequencies and H_E, then sampled (without replacement) 16 individuals from each replicate, recalculated frequencies and H_E from these subsamples and compared results with the real values.

Results

There were three alleles observed at each microsatellite locus. Both the Zealandia and Kapiti Island populations lacked the fourth allele previously reported for *Pca02,* which was also absent in their source populations and found only in the remnant Hen Island population (Fig. 1). Allele frequencies in Zealandia, Kapiti Island, and all founding populations are graphically presented in Fig. 1. No significant deviations from HWE were detected among any population–marker combination. For Zealandia, mean $H_O = 0.602$ and mean $H_E = 0.533$, whereas for Kapiti Island, mean $H_O = 0.626$ and mean $H_E = 0.557$. Allele frequencies, H_E, H_O, and F_{IS} values are listed for each marker in Table 1. There were no signs of inbreeding in the two populations, with non-significant overall F_{IS} values of −0.019 ($P = 0.57$) in Zealandia and −0.049 ($P = 0.79$) in Kapiti Island. Genetic differentiation (F_{ST}) among populations across all loci was generally low but statistically significant, and marker-specific tests showed that this differentiation was in most cases driven by a single locus or two (Table 2).

From a remnant population of nine pairs, the PVA estimated there were 1294 (SD ± 709) saddlebacks on Kapiti Island in 2008 (13 breeding seasons since rat eradication). The population was predicted to reach carrying capacity in 2016. VORTEX estimated that the Zealandia population size in 2008 was 370 (SD ± 125) individuals. In 2015 (14 breeding seasons since the release date) this population is estimated by the PVA model to be at carrying capacity. A total of 203 birds were banded between 2002 and 2008 in Zealandia. Based on the encounter data, it was estimated that there were 40 (range is 30–65) banded birds in 2008. The estimate for unbanded birds was 210, giving a total population estimate of 250 individuals in 2008. This is within the size range obtained with the PVA, albeit at the lower end of the range. Sensitivity testing for the effect of maximum breeding age, the number of offspring per clutch and the survival rate into the second year shows that in the first 10 years of population expansion the number of offspring has the greatest impact on speed of recovery (Fig. 2). PVA models were not highly sensitive to changes in parameters and strongly suggest that both populations will reach carrying capacity around the middle of this decade.

BOTTLESIM simulations did not predict a loss of alleles in Zealandia for four of the loci (mean number of alleles between 2.985 and 3). However, for *Pca05*, the only locus with an allele at frequency < 0.1 in the source populations, that rare allele was lost in almost half of the replicates (460 replicates; mean number of alleles = 2.540), with 50% of these replicates losing the rare allele immediately after the translocation (allele absent from founders). For Kapiti Island, the final mean number of alleles for *Pca05* was 2.417, while it ranged from 2.785 to 2.985 for the other four markers. For all loci, the majority of lost alleles occurred in the first nine years after translocation, during the extended bottleneck due to rats still being on the island. Results for H_E are shown in Fig. 3.

The comparison of allele frequencies estimated from a subsample of 16 individuals out of 1000 simulated Zealandia populations of 370 individuals showed there was a very strong correlation between true population allele frequencies and those estimated from the subsamples ($r = 0.938$; $p > 0.001$). The mean difference between true and estimated frequencies

Table 1. Allele frequencies, sample sizes (*n*), observed (H_O) and expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}) for five microsatellite loci in the Zealandia and Kapiti Island saddleback populations. *Letters (A–D) refer to alleles as identified in Lambert et al. (2005).

Locus	Allele	Zealandia	Kapiti Island
Pca02	*n*	15	22
	104 (A) *	0.367	0.455
	118 (B)*	0.333	0.250
	122 (C)*	0	0
	126 (D)*	0.300	0.295
	H_O	0.467	0.727
	H_E	0.664	0.643
	F_{IS}	0.329	-0.107
Pca05	*n*	14	28
	152 (A)*	0.036	0.089
	154 (B)*	0.036	0.125
	160 (C)*	0.929	0.786
	H_O	0.143	0.393
	H_E	0.134	0.359
	F_{IS}	−0.020	-0.076
Pca08	*n*	16	30
	80 (A)*	0.250	0.033
	82 (B)*	0.188	0.483
	84 (C)*	0.562	0.483
	H_O	0.625	0.667
	H_E	0.586	0.532
	F_{IS}	−0.035	−0.238
Pca 14	*n*	15	30
	106 (A)*	0.033	0.100
	108 (B)*	0.367	0.600
	110 (C)*	0.600	0.300
	H_O	0.667	0.567
	H_E	0.504	0.540
	F_{IS}	−0.290	−0.033
Pca 15	*n*	15	30
	204 (A)*	0.500	0.500
	208 (B)*	0.167	0.067
	214 (C)*	0.333	0.433
	H_O	0.733	0.467
	H_E	0.611	0.558
	F_{IS}	−0.167	0.180

was relatively small, varying from ± 0.014 (for allele 154 at *Pca05*) to ± 0.073 (for allele 104 at *Pca02*), although the 95% confidence intervals (CIs) around the mean difference were much wider (from −0.181 to +0.175 for allele 104 of *Pca02*). The mean difference between true and estimated H_E ranged from ± 0.030 for *Pca08* to ± 0.060 for *Pca05*, but again with 95% CIs as large as (−0.125 to +0.185) for *Pca05*.

Discussion

This study shows that the Kapiti Island and Zealandia populations contain all alleles present in their immediate source populations, and all but one present in the original source population on Hen Island. This allele was found in a single individual sampled between 1990 and 1992, was not found in any surveyed first- or second-order translocated population (Lambert et al. 2005), and could have vanished from the Hen Island population in the past 20 years due to drift. Our results, although limited to a small number of loci, agree with previous work carried out on the South Island saddleback with a different suite of molecular markers that showed that even a small number of founders is often sufficient to capture all genetic diversity remaining in a genetically depauperate species (Taylor & Jamieson 2008; Taylor et al. 2007). The South Island saddleback was brought back from the brink of extinction, when only 36 individuals from a single island population were left, whereas the Hen Island population (North Island saddleback) did not fall below 500 individuals (Hooson & Jamieson 2003). Despite this difference, levels of genetic variation at microsatellite loci in both species are similar: microsatellite mean H_E in Hen Island (source of all North Island saddleback translocations) is 0.559 and mean number of alleles per locus is 2.8 (Lambert et al. 2005), while mean H_E in Big South Cape Island (source of all South Island saddleback translocations, now extirpated) was 0.516 and mean number of alleles was 3.2. It is likely that the Hen Island population itself originated from a very small number of colonisers from the mainland, which would explain the low levels of polymorphism (low number of alleles) harboured by this population, and consequently in all other North Island saddleback translocated populations.

Nonetheless, the Kapiti Island and Zealandia populations have experienced different post-translocation trajectories (see Methods), and the impact of the prolonged bottleneck in Kapiti Island on the expected preservation of heterozygosity can be seen in Fig. 3. Even though the carrying capacity in Zealandia is almost 10 times less than that of Kapiti Island, it is expected

Table 2. Pairwise F_{ST} values (above diagonal) among Kapiti Island, Zealandia, and their source populations, and number of significant exact tests of differentiation out of five loci (below diagonal). Asterisks (*) indicate significant F_{ST} values for $\alpha = 0.05$ following a sequential Bonferroni correction.

	Hen I.	Stanley I.	Tiritiri I.	Zealandia	Kapiti I.
Hen I.	-	0.025*	0.023*	0.064*	0.025*
Stanley I.	1/5	-	0.061*	0.085*	0.059*
Tiritiri I.	0/5	1/5	-	0.020	0.034*
Zealandia	2/5	1/5	0/5	-	0.037*
Kapiti I.	2/5	1/5	1/5	1/5	-

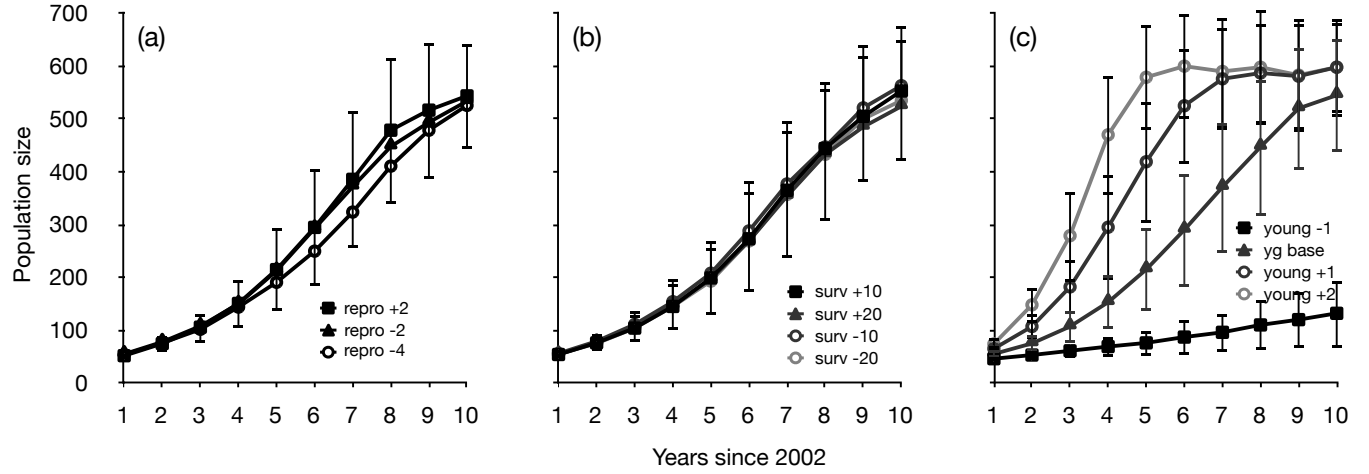

Figure 2. Graphic illustration of the effect of changing a range of PVA input parameters on speed at which the Zealandia population increases in the first 10 years since translocation: (a) varies the maximum breeding age with +2 (12 years), −2 (8 years) and −4 (6 years); (b) changes the percentage of survival in first year to +10 (70%), +20 (80%), −10 (50%) and −20 (40%); (c) changes the number of offspring per clutch with +1 (3 chicks), +2 (4 chicks), and −1 (1 chick).

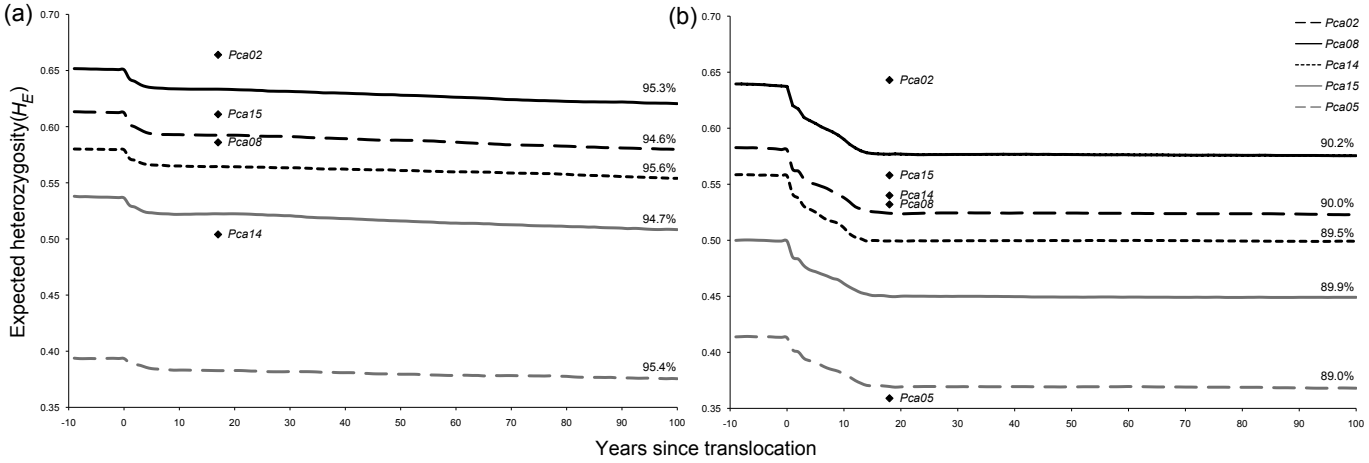

Figure 3. Simulation results showing the trends in expected heterozygosity (H_E) for five microsatellite markers in the Zealandia (a) and Kapiti Island (b) saddleback populations for 100 years after translocation (year 0). The plateaus preceding year 0 represent the source populations (Tiritiri Matangi for Zealandia and Stanley Island for Kapiti). ◆ symbols indicate heterozygosity measured from collected samples. (Note that the value for *Pca05* in Zealandia, 0.134, is not shown for graphing purposes). Percentage values at the end of curves indicate the proportion of heterozygosity from the source population retained after 100 years.

to maintain higher levels of heterozygosity and is more likely to retain all alleles, because of its rapid recovery following translocation. Taylor and Jamieson (2008) found that, for most South Island saddleback populations, 10 translocated pairs should suffice to ensure that the genetic variation (number of alleles and heterozygosity) will be maintained in the new population. However, our results for Kapiti Island emphasize that this is correct only if the newly established population quickly recovers.

The PVA models that we computed may slightly underestimate the time required for both populations to reach carrying capacity (as suggested by the comparison of the 2008 Zealandia size estimate derived from field data vs the one obtained with the PVA), but there is little doubt that newly established *Philesturnus rufusater* populations have the capacity to grow very quickly if the appropriate conditions are respected (i.e. no mammalian predators). There are two factors that could be partly responsible for the lower Zealandia population size estimate based on field data. Firstly, there have been saddlebacks that set up their territories outside, or straddling, the fence. Most of these birds eventually disappeared or were found dead outside the fence. This higher mortality among birds with territories bordering the perimeter fence was not accounted for in the PVA. A second factor may be the presence of mice in Zealandia.

Among the five loci we used, only *Pca05* had a rare allele, and this locus behaved somewhat differently than the other four in our analyses, as it was evidently more likely to lose an allele during and after translocation. The elimination of most rare alleles from serially bottlenecked populations makes it easier to capture the remnant genetic variation with a few founders, and a much greater number of individuals would

be required if many markers harboured rare alleles (Taylor & Jamieson 2008).

The heterozygosity values measured in Kapiti Island and Zealandia were generally high (see Table 1); for some markers they were higher than expectations obtained through simulations (Fig. 3) and even higher than those found in their source populations. Our analyses have shown that small sample sizes can, in general, allow a close estimation of allele frequencies and heterozygosity in genetically impoverished populations, but that extreme discrepancies are expected in some cases as well, so these figures should be regarded with caution. Nevertheless, no trend suggestive of a genome-wide effect on heterozygosity was noticed (see H_O values; Table 1), and we did not find evidence that the isolation of the Kapiti Island and Zealandia populations is leading to inbreeding. Genetic differentiation is emerging between these populations and their sources, but this is the result of the random sampling of alleles in the founders and of the subsequent stochastic drift, and therefore has little conservation significance (Taylor & Jamieson 2008).

Management guidelines for subdivided populations recommend the maintenance of low levels of gene flow to prevent local inbreeding (Caballero et al. 2009), but our results show no genetic inbreeding for the two populations. However, caution with the interpretation of inbreeding coefficients is necessary, especially when it is derived from few neutral loci, because it may not accurately reflect real inbreeding (e.g. from pedigree data) and the occurrence of inbreeding depression (Balloux et al. 2004). Further fieldwork would be required to conclusively evaluate the occurrence of inbreeding depression in these populations. Although their rapid growth suggests that traits such as hatching success and juvenile survival probably remain unaffected in the short term, work on other species emphasise that detrimental inbreeding effects can accumulate over life stages and thus be very difficult to measure (Grueber et al. 2008). Nevertheless, Jamieson (2010) identified carrying capacity as one of the key factors to avoid inbreeding in New Zealand bird species, including saddleback, and the speedy recovery of Kapiti Island and Zealandia populations coupled with their large carrying capacity should prevent inbreeding for at least the near future. Low levels of inbreeding were also found in another recently translocated saddleback population (Ulva Island, 30 birds translocated in 2000; Jamieson 2010).

It is widely accepted that the genetic management of species should aim at retaining 90% of the heterozygosity over 100–200 years (Soulé et al. 1986; Miller et al. 2009). Considering that (1) both Kapiti Island (only just) and Zealandia populations are expected to attain that target, (2) the totality of the species' remnant genetic diversity is found in the two populations, and (3) neither one is exhibiting genetic inbreeding, our study provides little evidence that would justify additional top-ups at this time. Furthermore, whereas genetic bottlenecks may lead to a reduction of the immune response in certain bird species (e.g. Hale & Briskie 2007), no relationship between microsatellite variation and either survivorship or parasite load was found in a translocated saddleback population (Taylor & Jamieson 2007), and another population founded with 26 individuals in 1994 was able to quickly recover from an outbreak of systemic coccidiosis in 2002 (Hale & Briskie 2009). Finally, taking into account the costs, the risks of transferring disease and the possible occurrence of yet unknown diseases in populations of endemic birds (e.g. Ortiz-Catedral et al. 2010), we cannot, at this moment, find any reason for top-ups.

Naturally, microsatellite variation is only a small aspect of genomic diversity that does not necessarily reflect adaptive variation (Väli et al. 2008). Thus, it is possible that the Hen Island population still harbours adaptive variation (e.g. at the major histocompatibility complex MHC) that may have been lost through translocations, as drift is likely to outweigh selection during bottlenecks (Miller & Lambert 2004). Further studies on this topic, along with careful evaluation of inbreeding depression, are required and could justify additional top-up translocations in the future, but successful translocations will become a more complicated endeavour if translocated birds are unable to establish territories as the populations get close to carrying capacity. Future translocation protocols may therefore need to allocate resources to monitor the speed at which newly translocated populations recover. If population growth is of concern, top-up translocations should occur as soon as practicable before numbers have reached carrying capacity. Additionally, top-ups could be performed by exchanging individuals between populations to facilitate successful integration into their new population.

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