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# High genetic diversity in the remnant island population of hihi and the genetic consequences of re-introduction

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## Abstract

**The maintenance of genetic diversity is thought to be fundamental for the conservation of threatened species. It is therefore important to understand how genetic diversity is affected by the re-introduction of threatened species. We use establishment history and genetic data from the remnant and re-introduced populations of a New Zealand endemic bird, the hihi *Notiomystis cincta*, to understand genetic diversity loss and quantify the genetic effects of re-introduction. Our data do not support any recent bottleneck events in the remnant population. Furthermore, all genetic diversity measures indicate the remnant hihi population has retained high levels of genetic diversity relative to other New Zealand avifauna with similar histories of decline. Genetic diversity ($N_A$, alleles per locus, allelic richness, $F_{IS}$ and $H_S$) did not significantly decrease in new hihi populations founded through re-introduction when compared to their source populations, except in the Kapiti Island population (allelic richness and $H_S$) which had very slow post-re-introduction population growth. The $N_e/N_c$ ratio in the remnant population was high, but decreased in first-level re-introductions, which together with significant genetic differentiation between populations ($F_{ST}$ & Fisher's exact tests) suggest that extant populations are diverging as a result of founder effects and drift. Importantly, simulations of future allele loss predict that the number of alleles lost will be higher in populations with a slow population growth, fewer founding individuals and with nonrandom mating. Interestingly, this species has very high levels of extra-pair paternity which may reduce reproductive variance by allowing social and floater males to reproduce a life history trait that together with a large remnant population size may help maintain higher levels of genetic diversity than expected.**

*Keywords*: bottlenecks, genetic diversity loss, island species, stitchbird, translocation

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## Introduction

Maintaining the genetic diversity of endangered species is critical to their long-term survival (Hedrick & Kalinowski 2000; Frankham *et al.* 2002; Spielman *et al.* 2004). Conservation efforts for island species, however, often use the re-introduction of a few individuals to initiate new populations, thus creating both founding (Groombridge *et al.* 2000; Briskie & Mackintosh 2004; Jamieson 2009) and sequential bottleneck events (Pruett & Winker 2005; Taylor & Jamieson 2008) that can erode genetic diversity. Bottlenecks typically lead to the loss of standing genetic diversity because of the population existing at a small population size for multiple generations (Frankham *et al.* 2002).

Populations that are small and isolated for prolonged periods encounter a number of genetic risks. First, the loss or fixation of alleles as a result of genetic drift reduces genetic variation and therefore the population's adaptive potential (Keller & Waller 2002). Second, mutation accumulation increases as the efficiency of selection processes become weaker (Lynch *et al.* 1995).

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Third, limited mating opportunities lead to an increased frequency of matings between relatives reducing the population's mean fitness as a result of inbreeding (Briskie & Mackintosh 2004). Finally, where multiple small populations of a species exist with no gene flow, as is often the case in re-introduction programmes (Frankham 2009), divergence in terms of allelic diversity, heterozygosity and allelic fixation can occur (Frankham *et al.* 2002). Therefore, even if the total genetic diversity across populations does not necessary decline, individual local populations may face the problems aforementioned.

Current models predicting the rate of genetic diversity loss and its impacts stem largely from theoretical consideration and analysis of model systems, often in laboratory settings (England *et al.* 1996; Montgomery *et al.* 2000; Bijlsma *et al.* 2000). Recently, more emphasis has been placed on trying to understand the effect of genetic diversity loss in wild populations of endangered species (Groombridge *et al.* 2000; Jamieson *et al.* 2006; Jamieson 2009). Studying endangered populations in the wild provides a clearer picture of the interaction between environmental effects and the rates and consequences of genetic diversity loss, which is often lacking in laboratory settings (Frankham 2000). By studying wild populations, we will therefore better understand the evolutionary processes connected to founding bottlenecks (Grant *et al.* 2001; Clegg *et al.* 2002) and be able to produce more integrated conservation management strategies, particularly involving re-introduction (Jamieson 2009).

Re-introduction is a widely used conservation tool for the preservation of species that have been extirpated from their historical range (Jamieson 2009). There are a number of studies that seek to understand the effect of re-introduction on the genetic diversity of these populations (Hudson *et al.* 2000; Lambert *et al.* 2005; Biebach & Keller 2009a; Jamieson 2009). Many of the threatened species in New Zealand that have been through re-introductions have been found to have very low levels of genetic diversity (Hudson *et al.* 2000; Lambert *et al.* 2005; Jamieson 2009; Robertson *et al.* 2009). Until the last decade it was believed that New Zealand's native avifauna had a low genetic load, purged over a prolonged history of isolation and small population size because of their confinement to small islands (Craig 1991; Caughley 1994; Jamieson *et al.* 2006). However, the extent to which purging is effective at relieving the effect of genetic diversity loss and inbreeding depression in the wild is still uncertain (Boakes *et al.* 2007).

The hihi is a medium sized, phylogenetically distinct and endemic New Zealand passerine (Ewen *et al.* 2006; Driskell *et al.* 2007) that has been the focus of re-introduction management. Hihi were once found throughout the North Island mainland and the northern offshore islands. Following European colonization, however, they declined to a single remnant population on Little Barrier Island (~3083 ha) with the last recorded mainland sighting in 1883 (Taylor *et al.* 2005). Beginning in the early 1980s, conservation management has involved re-introducing small groups of hihi to additional predator-free reserves. To date, there have been eighteen translocations of wild-caught hihi to seven additional sites [Fig. 1: Cuvier (181 ha); Hen (718 ha); Kapiti (1963 ha); Mokoia (263 ha) and Tiritiri Matangi (220 ha) islands; Karori Wildlife sanctuary (225 ha) and Waitakere ranges (>1100 ha) mainland sites and a captive breeding population in Mt Bruce]. The most recent attempts have been sourcing hihi from a population established through re-introduction (Tiritiri Matangi Island), resulting in the progression from first- to second-order re-introductions (Figs 1 and 2a).

In this study, we quantify the remaining genetic diversity within the remnant population and use the establishment history of the hihi to understand the genetic consequences of re-introduction management. Our aims are to quantify the level of genetic diversity in all existing populations (remnant and re-introduced) and to quantify the genetic consequences of re-introduction. Furthermore, we evaluate the longer-term consequences of re-introduction by simulating genetic diversity loss under different possible demographic scenarios. Finally, based on these findings we provide conservation management recommendations.

## Materials and methods

### *Study species and sampling*

Hihi have an average life expectancy of 4 years, but can live up to 9 years (Low & Pärt 2009). Males and females reach sexual maturity at 1 year and breed annually in the Austral summer producing up to two clutches per season with between three to five eggs per clutch (Taylor *et al.* 2005). Uncharacteristically for a New Zealand species and island species in general (Griffith 2000), the hihi is highly sexually dimorphic in size and coloration and has a promiscuous mating system (Castro *et al.* 1996). Males display two different, but not mutually exclusive, reproductive strategies where they can be territorial or unpaired floaters. Territorial males defend their territory, mate-guard their paired female during egg laying and search for extra-pair copulations in other territories (Ewen *et al.* 2004). Floater males do not hold a territory, but search for copulations from paired females. Extra-pair copulations in this species are frequent and result in high levels of mixed paternity (Ewen *et al.* 1999; Castro *et al.* 2004).

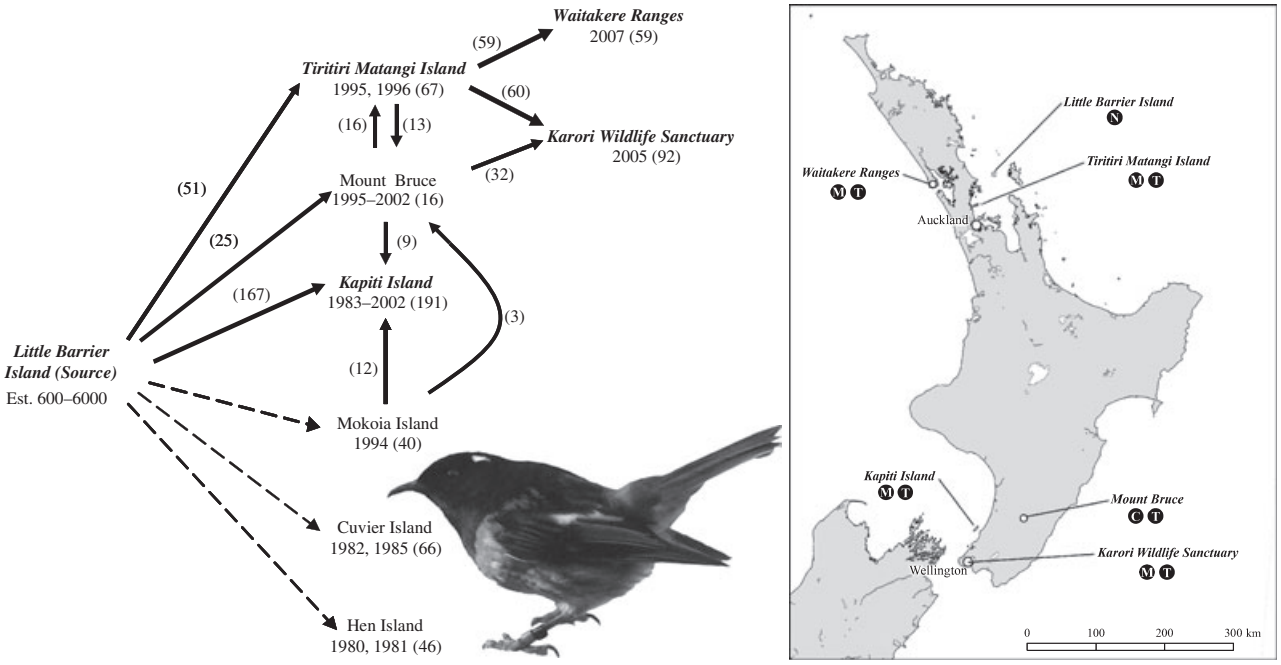


**Fig. 1** Summary of hihi re-introductions from the remnant source population on Little Barrier Island. Arrows indicate source and release locations. The number (in parentheses) under each island represents the total number of individuals translocated to that site. The numbers associated with the arrows indicate how many individuals were re-introduced from a particular source. The years under each island represent the major release events from the wild source populations. Timing of translocations to and from the small captive population at Mt Bruce is more varied. Extant populations are highlighted by bold lettering and bold arrows whereas those populations that failed to establish are highlighted by normal lettering and dashed arrows. The inset map shows the location of all extant populations (including the captive population at Mt Bruce; C) and identifies where management (M) occurs (food and/or nesting boxes provided) and how the population was established, either natural (N) or by translocation (T). Map modified from Taylor *et al.* (2005); hihi image modified from Buller (1888).

Free-flying adult and juvenile hihi were sampled during the Austral summers from September 2004 through February 2007 from three extant island populations, including the natural remnant (Little Barrier) and the descendants of two re-introduced (Tiritiri Matangi and Kapiti) (Figs 1 and 2a, b). In addition, 42 of the 60 wild-caught founders of the 2005 re-introduction of hihi to Karori Wildlife Sanctuary and 54 of 59 individuals from the 2007 re-introduction to the Waitakere Ranges were sampled at their source (Tiritiri Matangi; Figs 1 and 2a). In total, 269 hihi were caught in mist nets or in feeding cage traps across the three populations. Each bird was identifiable by a unique numbered leg-band. Immediately after capture, blood samples were collected via brachial venipuncture (approximately 70 μl) and stored in 95% ethanol for subsequent analyses. Genomic DNA was extracted from blood using the ammonium acetate precipitation method following protocols detailed in Nicholls *et al.* (2000). All samples were screened at 19 microsatellite loci. Four of these loci were identified by testing loci originally isolated in other avian species and a further 15 by isolating new microsatellites from a hihi-specific genomic library. These loci were characterized in unrelated hihi from the Tiritiri Matangi Island population. Each locus displayed between two and 10 alleles, and the observed heterozygosities ranged between 0.29 and 0.91. PCR conditions follow those detailed in Brekke *et al.* (2009). Departure from Hardy–Weinberg equilibrium and linkage disequilibrium (HWE & LD; Fisher's exact test) was assessed for each population using Genopop v3.4 with Benjamini-Yekutieli corrections for multiple tests (Benjamini & Yekutieli 2001; Narum 2006).

## Genetic diversity in the remnant and re-introduced populations

### *Genetic diversity*

To determine the level of genetic diversity, we measured the allelic frequency, number of alleles per locus and allelic richness (corrected for sample size; El Mousadik & Petit 1996). Observed ($H_O$) and expected heterozygosity ($H_E$), the average genetic diversity per locus for subdivided populations ($H_S$; Nei 1987) and the proportion of the variance in the subpopulation

contained in an individual ($F_{IS}$; Wright 1951; Nei 1977) were also calculated using FSTAT version 2.9.3 (Goudet 2001). The total number of alleles ($N_A$) and the number of private alleles ($N_{PA}$) in each population were estimated using GenAlEx 6.1 (Peakall & Smouse 2006).

### *Bottleneck events in the remnant population*

Understanding the historical patterns of genetic diversity in the remnant population, for example, because of oscillations in population size, is critical to interpret its current genetic status and that of all the re-introduced populations. To check for the genetic footprint of a bottleneck in the remnant population of Little Barrier Island, we ran the program Bottleneck (Piry *et al.* 1999). Very limited population and demographic data were available for this population because of its remoteness. Therefore, genetic data prove very valuable. Bottleneck tests for heterozygosity excess based on the theoretical expectation of a more rapid loss of alleles than heterozygosity in declining populations (Cornuet & Luikart 1996) which was examined under the infinite alleles model (IAM), stepwise mutation model (SMM) and two-phased model (TPM) of mutation, with TPM characteristics set as suggested by Piry *et al.* (1999) (95% single-step mutations with variance among multiple steps of 12). The TPM method is believed to be better suited to microsatellite data than the IAM or SMM models (Piry *et al.* 1999). Three different tests were performed using the allele frequency data under the different models: standardized differences test, Wilcoxon signed-rank test and also qualitative test of mode-shift.

### *Effective population size ($N_e$)*

As demographic data were not available for all populations sampled, we used genotypic data to estimate $N_e$ (Wright 1931). We estimated $N_e$ using two methods: (i) sibship assignment, dependant on the frequencies of full- and half-sib dyads (Wang 2009) using the program COLONY V 2.0.0.1 (Wang 2009); and (ii) linkage disequilibrium, in the program LDNe (Waples & Do 2008). The linkage disequilibrium method assumes isolated populations without immigration or emigration while the sibship assignment method assumes that a sample of individuals is taken at random (with respect to kinship) from a single cohort of the population. Therefore, we used only individuals known to be juveniles from plumage morphology in the years in which they were genetically sampled (Cohort year sampled: Little Barrier 2004 $n = 28$; Tiritiri Matangi 2005 $n = 22$; Kapiti 2004 $n = 14$). In nonequilibrium populations (such as those recently established by re-introduction), linkage disequilibrium is influenced by the last few generations, as it takes some generations to reach a new asymptotic linkage disequilibrium (Waples 2005). Therefore, in re-introduced populations that are generally growing, this method reflects the harmonic $N_e$ of the last few generations (Waples 2005). We used two methods as they have different assumptions that are complementary. However, these alternative measures used only a single sampling event and may result in varied estimates that provide only an approximation of the true $N_e$ [see Wang (2005) for a critical review of alternative approaches]. This is particularly important in nonequilibrium populations, and our results must therefore be treated as trends only.

## Consequences of re-introduction

### *Long-term consequences of re-introduction*

Extensive monitoring of the Tiritiri Matangi hihi population allowed us to understand the long-term ramifications of re-introduction, by providing the details on (i) known size and timing of founding bottleneck; (ii) number of generations (4.9) and population growth rate since the founding bottleneck; (iii) adult sex ratio; and (iv) current managed carrying capacity. The carrying capacity of Tiritiri Matangi is currently managed at ~150 individuals as a number of juveniles are removed yearly to supply new re-introductions and translocations (D. P. Armstrong & J. G. Ewen unpublished data; Fig. 2a). We were therefore able to forecast the change in the average number of alleles per locus of the Tiritiri Matangi population through time under different founding bottleneck and life history scenarios and compare the average number of alleles in the Tiritiri Matangi population at our sampling point (13 years after re-introduction) to the simulated expected average number of alleles found under each of the scenarios below.

In all simulations, we used BottleSim version 2.6 (Kuo & Janzen 2003) to quantify the process of genetic drift by predicting the loss of genetic diversity (we chose average observed number of alleles per locus as a proxy) over 100 years. This analysis utilized genotype frequency data from the source population (Little Barrier) and used the source population estimate [estimated census population size ($N_c$) = 600–6000; Taylor *et al.* 2005; ] as the prebottleneck population size. The sex ratio parameter was male-biased. Sex ratio post-re-introduction was strongly male-biased (Ewen *et al.* 1999), but it is known to have fluctuated from year to year in the Tiritiri Matangi population (Ewen *et al.* 2010). We therefore used the average sex ratio across the 13 years since founding (60% males:40% females). Simulations used 1000 iterations with generational

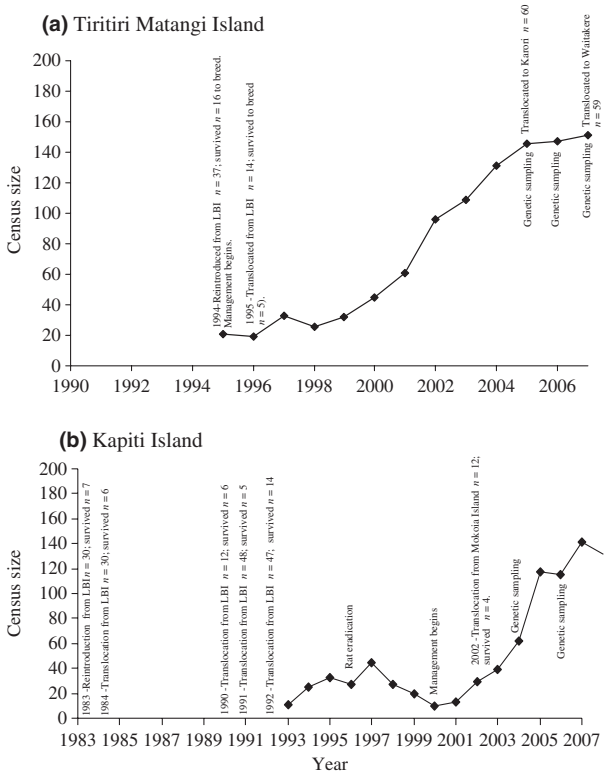


**Fig. 2** Population growth graphs from yearly censuses taken in September and October for the (a) Tiritiri Matangi Island and (b) Kapiti Island populations. No demographic/monitoring information is available for the populations in Kapiti between 1983 and 1993 and Little Barrier Island (LBI) (graphs modified from Taylor *et al.* 2005). 'Survived' refers to birds seen to be alive in the following year after re-introduction/translocation (Rasch *et al.* 1996).

overlap and set as dioecy with random mating [except in scenario 3, (see details of hihi life history above)]. As our results were not sensitive to extreme ranges of estimated prebottleneck population size, we present outputs for a prebottleneck population of 600 individuals (data not shown).

### *Scenario 1: varying founding bottleneck size*

We modelled changing average number of alleles under three founding bottleneck scenarios based on previous hihi conservation management and given the subsequent growth and carrying capacity on Tiritiri Matangi (as detailed above). Prior to 2005, most founding population sizes for re-introduction were of around 40 individuals (20 male:20 female; Taylor *et al.* 2005). However, postrelease monitoring on Tiritiri Matangi revealed low survival of founders to the first breeding season (21 individuals; Armstrong *et al.* 2002) and hence the proposed founding population size was not achieved. More recently, founding population sizes have increased to ~60 individuals given the sustainability of cropping such numbers from the remnant source population (D. P. Armstrong & J. G. Ewen unpublished). Therefore, scenario (1a) aimed at forecasting changes in the number of alleles with the observed number of individuals that survived to breed on Tiritiri Matangi ($n = 21$), scenario (1b) forecasts the changes in the average number of alleles with double the founding bottleneck size $n = 40$ individuals, and scenario (1c) with three times the founding bottleneck size $n = 60$ individuals.

### *Scenario 2: varying post-re-introduction population growth rate*

The Tiritiri Matangi population is unique in hihi re-introductions for its rapid population growth to a managed carrying capacity (Fig. 2a). Although most previously re-introduced populations declined to extinction (Fig. 1), the Kapiti population has maintained a low number of individuals since its foundation (9.1 generations, founded in 1983; albeit with a number of translocation events, Fig. 2b) until recent changes in management (in 2003) resulted in a substantial population increase (Fig. 2b). Scenario two therefore forecasts the average number of alleles had the Tiritiri Matangi population suffered the same limited population growth observed in the Kapiti population (Fig. 2b). Therefore, the population growth was maintained at a constant of $n = 21$ individuals for 18 years and then allowed to grow until carrying capacity (see above).

### *Scenario 3: varying mating system*

Hihi also have unusually high levels of promiscuity (see above). To understand whether the type of mating system may influence average number of alleles lost, we simulated a bottleneck event using the observed Tiritiri Matangi population scenario (1a) under two alternative mating systems available in BottleSim (Kuo & Janzen 2003). Scenario (3a) used a polygynous mating system where a single male gained most of the reproductive success and scenario (3b) used a cooperative mating system where a single pair generally reproduces with the assistance of helpers. This simulation includes a number of assumptions for the hihi mating system (e.g. equal mating opportunities and comparable fitness between territorial vs. floater males), and therefore the results should be interpreted as trends only.

### *Distinguishing genetic effects of founding bottleneck events and drift*

The present levels of genetic diversity in the first- and second-order translocated populations are the result of both the initial founding bottleneck event and the subsequent genetic drift. To examine the impact of an initial founder event and to differentiate it from the effect of drift, we simulated the movement of 21 individuals from a large population, similar to the initial founding bottleneck event of Tiritiri Matangi from individuals on Little Barrier in 1995 and 1996 (Figs 1 and 2a). We assumed that mutation has been negligible and that the total number of alleles observed in the five populations currently (116 alleles across 19 loci) was present at randomly assigned frequencies in the source population (Little Barrier). We repeated the random sampling of allele frequencies, followed by selection of individual genotypes, 5000 times (macro produced in Microsoft Excel) to estimate the total number of alleles expected to be lost as a result of the initial founding bottleneck.

### *Population differentiation*

Pairwise comparisons of allele frequencies were carried out in Genepop v3.4 utilizing Fisher's method for genic differentiation between population pairs (Raymond & Rousset 1995). $F_{ST}$ values (Wright 1951; Nei 1977) were calculated using the $\Theta$ estimator (Weir & Cockerham 1984) in pairwise comparisons after Bonferroni corrections for multiple tests in FSTAT version 2.9.3 (Goudet 2001). Differences in genetic diversity measures ($N_A$, alleles per locus, allelic richness, $F_{IS}$ and $H_S$) between populations were tested using Wilcoxon signed-rank tests conducted in R (R Development Core Team 2007) using a sequential Benjamini-Yekutieli correction for multiple tests (Benjamini & Yekutieli 2001; Narum 2006).

## Re-introduction management

### *Capturing genetic diversity*

A randomization function was developed to estimate the number of individuals needed to capture the total number of alleles observed in the source population and provides a guidance tool for managers to determine appropriate founding population sizes from a genetic perspective (macro produced in Microsoft Excel). The recent re-introductions from Tiritiri Matangi allow us to compare the results from these simulations with empirical data. This approach included all the alleles found in the individuals sampled from the source population and randomly subsampled this pool of individuals in incremental groups of five without replacement. This method determines the total number of alleles 'captured' in each group of individuals (5, 10, 15 etc.) until the maximum number of alleles from the source population is captured. For each group size, an average was calculated from 5000 repeated random sampling runs. The pool of individuals available from the source populations were $n = 56$ in Little Barrier and $n = 88$ in Tiritiri Matangi Island. In addition, observed capture of alleles was calculated from sampled founding individuals in second-order re-introductions. This includes a more accurate representation of founders of the Karori Wildlife Sanctuary population where postrelease mortality is quantified and $N_c$ is known at the start of the first breeding season ($n = 29$), of which genotypic data were available for $n = 22$ individuals.

## Results

### *Genetic diversity in the remnant and re-introduced populations*

*Genetic marker assessment.* No locus departed from HWE or displayed a high null allele frequency (above 10%) after sequential Benjamini-Yekutieli corrections. Evidence for gametic linkage disequilibrium was found between fourteen pairs of loci ($P < 0.01$, Wilcoxon signed-rank statistic with Benjamini-Yekutieli correction), but disequilibria were not consistent across all populations for any of the locus pairs. This is unlikely to be the result of physical linkage (Brekke *et al.* 2009). Removal of loci with strongest evidence ($P < 0.001$) of gametic disequilibrium did not change the outcome of our results on genetic diversity within and between populations, and these loci were therefore retained in all analyses. There appears to be substantial genetic diversity remaining in all the hihi populations (Table 1). However, there was a reduction in a number of genetic diversity measures following re-introduction (e.g. a loss of 9% and 28% of alleles in re-introductions to Tiritiri Matangi and Kapiti, respectively; Table 1, Appendix I). There were no significant differences in the mean number of alleles per locus between populations ($P > 0.01$, Wilcoxon signed-rank test with sequential Benjamini-Yekutieli correction), but allelic richness (adjusted for sample size) differed significantly between Kapiti and all other populations ($P < 0.01$, Wilcoxon signed-rank test with sequential Benjamini-Yekutieli correction). $H_S$ (Nei 1987) was also significantly different between Tiritiri Matangi and Kapiti Islands ($P < 0.01$, Wilcoxon signed-rank test with sequential Benjamini-Yekutieli correction). A number of private alleles ($N_{PA}$) were detected, with the remnant population containing the majority (Table 1, Appendix I),

which decreased substantially from the remnant to re-introduced populations. $F_{IS}$ values for each of the populations did not differ from zero ($P > 0.05$ Wilcoxon signed-rank test with sequential Bonferroni correction).

*Bottleneck events in the remnant population.* We found a significant heterozygosity excess for all tests used under the expectations of IAM. Similarly, under SMM, the standardized differences and Wilcoxon signed-rank test also showed heterozygosity excess. However, under TPM, there was no significant excess using any of the tests. The mode-shift method was L-shaped, which does not indicate a bottleneck (Appendix II). Therefore, this analysis provides no clear support for recent bottleneck events in Little Barrier.

*Effective population size ($N_e$).* The methods used to estimate $N_e$ did not provide consistent measures for the remnant population (linkage disequilibrium: $N_e/N_c = 0.29$; sibship: $N_e/N_c = 0.014$; Table 2). The sibship estimate of $N_e$ may have been lower than the linkage disequilibrium estimate because individuals were all caught from the southwestern corner of Little Barrier Island. This potentially increased the likelihood of catching related individuals (this is also the area where all individuals have been caught for re-introduction to other islands). However, the estimates for the re-introduced populations were consistent and had smaller $N_e$ estimates. All estimates concur that $N_e$ is lower than $N_c$ leading to a low $N_e/N_c$ ratio in each of the populations (Tiritiri Matangi, linkage disequilibrium: $N_e/N_c = 0.27$; sibship: $N_e/N_c = 0.18$; Kapiti, linkage disequilibrium: $N_e/N_c = 0.08$; sibship: $N_e/N_c = 0.15$; Table 2).

### *Consequences of re-introduction*

*Long-term consequences of re-introduction.* The populations with larger founding bottlenecks retained a larger number of alleles. The observed average number of alleles per locus retained 13 years after the bottleneck event lies above the mean and 95% confidence interval predicted by the forecast under observed conditions (scenario 1a) (observed allele retention = 96.2%, predicted allele retention = 83%; Fig. 3a and Appendix III). The most severe genetic erosion is predicted to occur when population growth rate post-re-introduction is limited over a number of years (scenario 2; Fig. 3a; Appendix III). Finally, simulations under different mating systems suggest that allelic loss is lower under a promiscuous mating system (albeit with a number of assumptions) than under a polygynous or cooperative mating system (Fig. 3b; Appendix III). Number of alleles lost was highest under a cooperative mating

**Table 2** Alternative estimates of effective population size ($N_e$) and census size ($N_c$) for the natural remnant (Little Barrier) and two re-introduced hihi populations (Tiritiri Matangi and Kapiti). Confidence intervals (CI) shown in parentheses

| Population | $N_e$ (±95% CI) using linkage disequilibrium | $N_e$ (±95% CI) using sibship | $N_c$ |
|---|---|---|---|
| Little Barrier | 876 (186–∞) | 43 (27–78) | 600–6000 |
| Tiritiri Matangi | 40 (31–50) | 27 (15–49) | 150 |
| Kapiti | 12 (9–17) | 22 (12–41) | 144 |

**Table 1** Genetic diversity in five hihi populations. (L) refers to re-introduction level, (S) refers to source population and (T) indicates re-introduced population and whether it was a first-order (1) or a second-order (2) re-introduction. Number of generations was calculated using the average age at which females produce offspring, (NS) represents the number of individuals genotyped in each population, (NA) is the observed total number of alleles and (NPA) is the number of observed private alleles. Allelic diversity, observed (HO) and expected (HE) heterozygosity, average genetic diversity per locus for subdivided populations (HS) and the inbreeding co-efficient (the proportion of the variance in the subpopulation contained in an individual; FIS), are shown for the natural remnant (Little Barrier), two re-introduced populations (Tiritiri Matangi and Kapiti, established from Little Barrier) and the wild-caught founders of 2 second-order re-introductions (from Tiritiri Matangi to Karori (pre) Wildlife Sanctuary and to the Waitakere Ranges). Genetic diversity is also shown for individuals in the Karori (post) population that survived to the first breeding season and represent the 'true' founders

| Population | L | Number of generations | $N_S$ | $N_A$ | $N_{PA}$ | Mean alleles per locus | Mean allelic richness | $H_O$ | $H_E$ | $H_S$ | $F_{IS}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Little Barrier | S | Unknown | 56 | 106 | 10 | 5.53 | 5.19 | 0.66 | 0.68 | 0.69 | 0.042 |
| Tiritiri Matangi | T1 | 4.9 | 88 | 97 | 1 | 5.11 | 4.71 | 0.66 | 0.64 | 0.64 | −0.028 |
| Kapiti | T1 | 9.1 | 29 | 76 | 1 | 4.00 | 3.88 | 0.61 | 0.58 | 0.57 | −0.051 |
| Karori-pre | T2 | Founders | 42 | 91 | 0 | 4.79 | 4.67 | 0.66 | 0.66 | 0.66 | NA |
| Karori-post- | T2 | 0.4 | 22 | 88 | 0 | 4.63 | 4.42 | 0.65 | 0.66 | 0.66 | 0 |
| Waitakere | T2 | Founders | 54 | 97 | 2 | 5.11 | 4.69 | 0.64 | 0.65 | 0.64 | −0.002 |

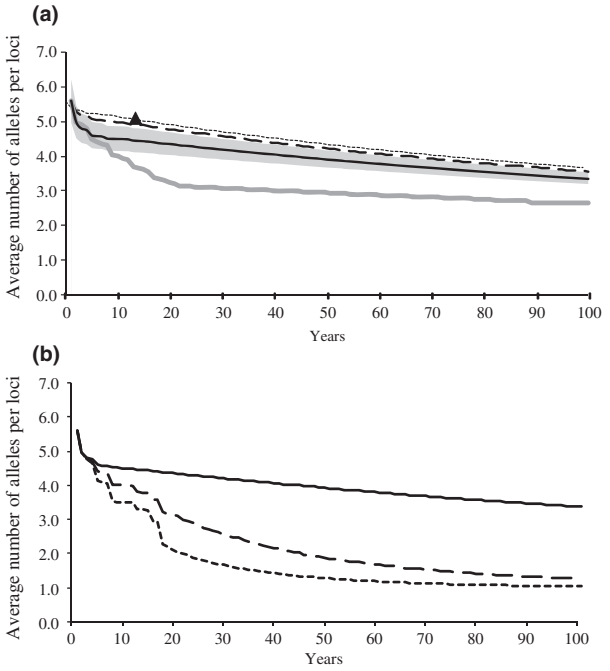


**Fig. 3** Simulated loss of average number of alleles per locus over 100 years in the Tiritiri Matangi hihi population under different management (3a) and mating system (3b) scenarios. In 3a; the solid black line represents the predicted loss of alleles under scenario (1a) (founder size = 21) with the grey area indicating the 95% confidence intervals, the large dashed line represents the predicted loss of alleles under scenario (1b) (founder size = 40), the small dashed line under scenario (1c) (founder size = 60) and the checked line under scenario (2) (reduced population growth rate). The solid triangle indicates the observed number of alleles present 13 years after the founding of the population (*x*-axis as below). In 3b; the solid black line represents the predicted loss of alleles under scenario (1a) (promiscuous mating system), the large dashed line under scenario (3a) (polygynous mating system) and the small dashed under scenario (3b) (cooperative mating system). For details of simulations and scenarios see Methods.

**Table 3** Pairwise $F_{ST}$ values between the natural remnant (Little Barrier) and two first-order re-introduced populations (Tiritiri Matangi and Kapiti). *Comparisons significant at $P < 0.001$

| Populations | | |
|---|---|---|
| Populations | Little Barrier | Kapiti |
| Kapiti | 0.085* | |
| Tiritiri | 0.035* | 0.126* |

system, as is expected as most individuals in the population will not leave descendants causing $N_e$ to be much lower than $N_c$.

*Distinguishing genetic effects of founding bottleneck events from drift.* The total number of alleles lost from the Tiritiri Matangi population was 19 (assuming Little Barrier Island contained the alleles found across all populations). Across the randomizations simulating the initial founding bottleneck event of Tiritiri Matangi, the mean loss of alleles was 10.3, with a 95% confidence interval of (5, 16). This result indicates that the initial founding bottleneck event of Tiritiri Matangi may have accounted for the loss of between five and 16 alleles, meaning that subsequent drift in the population over a period of 13 years has led to the loss of a further three to 14 alleles.

*Population differentiation.* Pairwise comparisons showed significant genic differentiation between the remnant source population and all re-introduced populations (Fisher's method: $P < 0.0001$). Similarly, $F_{ST}$ comparisons between each population pair (not including second-order re-introductions) revealed significant differences between the remnant source and re-introduced populations (Table 3). Kapiti Island and Tiritiri Matangi had the highest pairwise $F_{ST}$ value (Fig. 2). The size of islands or sites available for hihi re-introduction are typically small and can only thus maintain small population sizes, exacerbating loss of potentially beneficial alleles through drift.

### *Re-introduction management*

*Capturing available genetic diversity.* Our simulations indicated that about 30 breeding individuals would be sufficient to capture 95% of the maximum number of alleles when translocating individuals from either Little Barrier or Tiritiri Matangi Islands (Fig. 4). Interestingly, the founders of the two observed re-introductions from Tiritiri Matangi to Karori Wildlife Sanctuary ($n = 42$ birds genotyped) and the Waitakere Ranges ($n = 54$ birds genotyped) captured 97 (100%) and 91 (94%) alleles, respectively. Postrelease survival of the first transfer on Karori Wildlife sanctuary was 55% ($n = 29$ birds), which captured 88 alleles (91%).

## Discussion

### *Genetic diversity in the remnant and re-introduced populations*

This study has found that the remnant population of the nationally endangered hihi has high levels of genetic diversity in terms of $N_e$, $N_A$, $N_{PA}$, alleles per locus, allelic richness, $H_E$, $H_O$ and $H_S$ when compared to other threatened New Zealand avifauna (Hudson *et al.* 2000; Boessenkool *et al.* 2007; Taylor & Jamieson 2008; Jamieson 2009; Robertson *et al.* 2009) and threatened avifauna at a global level (Evans & Sheldon

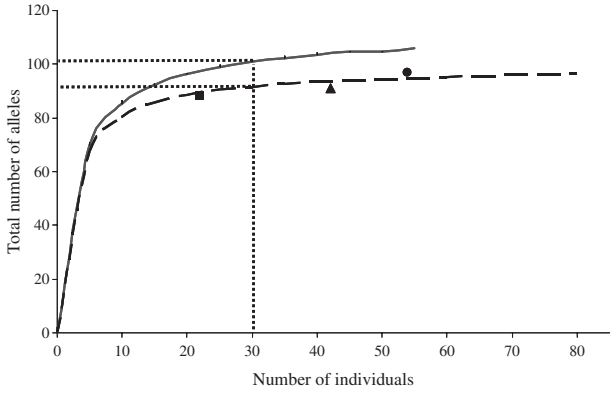


**Fig. 4** Simulation of number of the individuals needed to capture available genetic diversity. Simulation shows the total number of alleles captured against the number of individuals sampled from the Little Barrier population (solid line; up to $n = 56$ sampled individuals and 106 possible alleles) and Tiritiri Matangi population (dashed line; up to $n = 88$ sampled individuals and 97 possible alleles). The dashed horizontal and vertical lines identify the numbers of individuals that would need to be re-introduced to sample 95% of the available alleles in each source population (101 alleles from Little Barrier and 92 from Tiritiri Matangi). The solid triangle indicates the observed total number of alleles transferred with the 42 genotyped hihi from Tiritiri Matangi to Karori Wildlife Sanctuary, the solid circle indicates the observed total number of alleles transferred with the 54 genotyped hihi from Tiritiri Matangi to the Waitakere Ranges and finally the solid square indicates the observed total number of alleles seen after postrelease survival in 22 genotyped hihi from Karori Wildlife Sanctuary that remained alive by the first breeding season.

2008). These genetic diversity measures remain high in re-introduced populations when compared to those reported for species with similar re-introduction bottleneck history (Hudson *et al.* 2000; Lambert *et al.* 2005; Boessenkool *et al.* 2007; Taylor & Jamieson 2008; Biebach & Keller 2009a) or small $N_e$ (Tarr *et al.* 1998; Biebach & Keller 2009b), but decline when compared to the remnant population. Reductions in $N_A$ measures are particularly large when re-introduced populations suffer slow post-re-introduction population growth.

There are a number of hypotheses that could explain higher levels of genetic diversity in hihi than in other species, including, (i) the species historic levels of genetic diversity and decline; (ii) a larger remnant population size; (iii) longer generation time; and/or (iv) random mating (no reproductive skew) within the subpopulations than other species with a similar population bottleneck history.

Contemporary genetic diversity in a species is known to be dependent on the characteristics of the remnant population and its genetic history (Taylor *et al.* 2007). The hihi's remnant population size is thought to have experienced fluctuations in size (Reischek 1885; Rasch *et al.* 1996; Angehr 1984). However, this study did not detect any recent bottleneck events, and the Little Barrier Island population appears to have a relatively large $N_e$. Hihi is known to have been a fairly common species in the northern regions of New Zealand up until at least after European colonization in the 1800s (del Hoyo *et al.* 2009), and this may have allowed gene flow between previously neighbouring populations. An ongoing part of our research is trying to understand historical patterns of genetic diversity by incorporating museum samples from the hihi's historical range.

Other New Zealand avifauna, with similar remnant population size, show lower levels of genetic diversity. For example, kokako ($H_E = 0.56$, mean alleles per locus = 3.8; Hudson *et al.* 2000) and South Island robin [*Petroica a. australis* ($H_E = 0.51$, mean alleles per locus = 4.2; Taylor & Jamieson 2008)] have remnant populations of approximately 1400 (Rasch 1992; Basse *et al.* 2003) and >1000 individuals, respectively (Boessenkool *et al.* 2007). Generation time and longevity in hihi are comparable to most of the New Zealand's native passerines (del Hoyo *et al.* 2009). However, saddleback ($H_E = 0.48$, mean alleles per locus = 2.9; Lambert *et al.* 2005) and kokako and kakapo ($H_E = 0.47$, mean alleles per locus = 3.3; Robertson *et al.* 2009) are longer-lived species than hihi (del Hoyo *et al.* 2009), but seem to have retained lower levels of genetic diversity.

Mating system and in particular random mating has been previously found to be an important factor in the retention of genetic diversity by increasing $N_e$ (i.e. decreasing the variance in reproductive success) (Sugg & Cheeser 1994; Pearse & Anderson 2009). Low estimates of $N_e$ in the re-introduced populations suggest that this may not be a crucial factor in the maintenance of genetic diversity, but basic simulations under different mating systems found that a larger number of alleles can be retained under a promiscuous mating system than under a cooperative or lek mating systems. Furthermore, detailed examination of $N_e$ within one well-studied hihi population (Tiritiri Matangi) that accounted for both demographic and genetic data reported an $N_e/N_c$ ratio of 0.68 (Wang *et al.* 2010), revealing that the mating patterns of hihi are strongly reducing variance in reproductive success. In a previous study of the Mokoia Island hihi population, Castro *et al.* (2004) showed that mating system was relatively stable with respect to $N_e$, as reproductive skew towards dominant males was compensated by extra-pair paternity, except in a male-biased population, when $N_e$ may still be increased by extra-pair matings. This could provide some support for the conservation management decisions to make genetically under-represented males the focus of breeding programmes such as that of the lek mating New Zealand kakapo (*Strigops habroptilus*)

(Robertson 2006). These comparisons, albeit qualitative, are suggestive of the importance of a species' life history and historical background in explaining current levels of genetic diversity.

Generally, lower microsatellite diversity is found in studies which use cross-utility microsatellite loci compared with species-specific microsatellite loci (Evans & Sheldon 2008). Therefore, one possible reason for the high diversity ($N_A$, alleles per locus, allelic richness, $H_E$, $H_O$) we see is that our data set suffers from ascertainment bias. This study utilized 15 polymorphic species-specific and four cross-utility loci, which is a higher number of polymorphic species-specific loci than those developed for other New Zealand passerines in which the level of genetic diversity has been measured (Hudson *et al.* 2000; Lambert *et al.* 2005; Jamieson 2009). However, when compared to other vulnerable bird species where similarly large numbers of species-specific loci have been developed, hihi still display a high level of genetic diversity in terms of mean number of alleles per locus and heterozygosity (for example, 30 species-specific polymorphic loci developed in *Acrocephalus sechellensis*, $H_E = 0.48$; Richardson *et al.* 2000 and *S. habroptilus*, $H_E = 0.47$; Robertson *et al.* 2009; for further comparisons see Evans & Sheldon 2008), suggesting ascertainment bias is not the only explanation.

### *Consequences of re-introduction*

Despite the high levels of genetic diversity we have encountered in hihi populations, we have also shown that genetic diversity has been lost and that there has been divergence in re-introduced hihi populations when compared to the remnant population. This is revealed by lower $N_e$ and $N_{PA}$ in the remnant than re-introduced populations, shifts in allele frequency and high and significant $F_{ST}$ values.

*Genetic diversity loss.* The remnant population had the highest level of genetic diversity ($N_A$, $N_{PA}$, alleles per locus, allelic richness, $H_E$, $H_O$ and $H_S$), which systematically decreased through re-introduction. The decreases in $H_S$ and allelic richness, however, were only significant in comparisons with Kapiti Island. Single founding bottleneck events are generally expected to have a limited immediate effect on genetic diversity and to lead to modest differentiation between populations under laboratory (Charlesworth & Charlesworth 1987; Moya 1995) and wild conditions (Taylor & Jamieson 2008), but their impact is thought to become stronger through sequential re-introductions (Clegg *et al.* 2002; Mock *et al.* 2004).

In theory, heterozygosity is less sensitive to recent bottleneck events than allelic diversity (Nei 1975; Chakraborty & Nei 1977) and hence the latter is a better indicator of genetic diversity loss as rare alleles can be lost more easily through random sampling and/or long-term genetic drift (Nei 1975; England *et al.* 1996; Grant *et al.* 2001). Our results support this, as a number of alleles were found exclusively in the remnant source population and a considerable number of alleles were progressively lost from the source through single and successive founder events. This loss was more marked in the population that had undergone a long-term bottleneck, i.e. small population size for approximately 20 years (Kapiti lost up to 30 alleles). The Kapiti population has had multiple translocation events, the most recent of which involved the translocation of 12 individuals from Mokoia Island. Of these individuals, only four are known to have survived to the breeding season and seem to have had a limited impact on the number of alleles, allelic richness or heterozygosity of this population. This suggests that the retention of rare alleles is strongly impaired by re-introduction and that rapid post-re-introduction population growth is essential in maintaining genetic diversity.

*Founder vs. drift effects.* Unfortunately, it is not generally possible to disentangle the impact of the initial founder event from the subsequent action of drift in recently re-introduced populations when comparing observed genetic variation to that in the source population. However, a number of results indicate that genetic drift is having an impact on current genetic diversity and will continue to impact genetic diversity over time without appropriate management. First, our results show that in the hihi, founders of second-order re-introductions captured the majority of alleles present in the source population. Second, under realistic founder group sizes for endangered species, simulations indicate that in hihi the rate of population growth has a stronger effect on genetic erosion than the number of founders (hence directly related to drift). Third, a randomization approach suggests that approximately half of the alleles missing in the Tiritiri Matangi population were lost in the initial founding bottleneck event of 21 individuals with the remaining losses attributable to drift.

*Population differentiation.* Unlike most previous studies on threatened New Zealand species, we find the hihi's remnant source population is genetically diverse and the effects of re-introduction have not significantly eroded genetic diversity (but see Taylor & Jamieson 2008). However, the remnant and re-introduced populations are diverging (as shown by significant genic differentiation and $F_{ST}$ values). These two sets of analyses reflect important yet different aspects of the genetic consequences of re-introduction. Our results indicate

the bottlenecks through founding have little effect on $N_A$, $N_{PA}$, alleles per locus, $H_E$ and $H_O$, especially if there is rapid postrelease population growth (as was the case on Tiritiri Matangi). Considerable divergence, however, is continuing to alter the frequency of these alleles between populations. As such, population divergence in re-introduction biology should not be ignored when interpreting genetic data.

Taylor & Jamieson (2008), for example, recently reported that sequential re-introductions of genetically depauperate endangered species could be a valid conservation strategy that had little risk of eroding genetic variation (based on allelic diversity). Their study (on New Zealand's South Island saddleback) also reports significant $F_{ST}$ values between populations, and we suggest that their results, as in our own study, indicate substantial changes in allelic frequency (acknowledged by Taylor & Jamieson 2008) and hence differences in eventual fixation and loss of alleles across populations. Continuing genetic drift and other contributing factors like founder effect will therefore have significant impact on the genetic constitution of these populations and this is not escaped in more genetically depauperate systems. A recent example of this was found in the genetically depauperate Alpine ibex where re-introduction history determined contemporary genetic structure and the levels of inbreeding of the re-introduced populations (Biebach & Keller 2009a,b).

*Long-term consequences of re-introduction.* Simulations of future genetic erosion suggest that genetic decay is accelerated in populations that are chronically small. Fast population growth post-re-introduction aids retention of genetic diversity ($N_A$) as long-term bottlenecked populations have a higher risk of allelic fixation through genetic drift (Nei 1975; Allendorf 1986; Table 1). This is confirmed by a previous study on the importance of mortality rate in limiting population growth and therefore the $N_e/N_c$ ratio of hihi populations (Castro *et al.* 2004). Our simulations indicate that founder population size has a limited effect on genetic erosion as realistic founder group sizes for endangered species are generally low. Interestingly, the current $N_A$ found on Tiritiri Matangi resembles that of a population simulated to have been founded by a population size of 60 individuals rather than the 21 individuals it is derived from (Armstrong *et al.* 2002).

### *Re-introduction management recommendations*

**1** Appropriate site selection and management that facilitates rapid postrelease population growth. Particularly beneficial would be if larger release sites were available, as larger sites may provide a suitable opportunity for local adaptation, another important consideration in re-introductions. The use of food supplements would also be beneficial as it has been shown to assist population growth in this species (Armstrong & Ewen 2001; Armstrong *et al.* 2002; Castro *et al.* 2003; Taylor *et al.* 2005).

**2** Re-introduce a minimum number of birds to capture the maximum genetic diversity available and account for postrelease mortality. On average 40–50% of released birds survive to the breeding season (J. G. Ewen & D. P. Armstrong unpublished). This suggests enough birds should be released to form around ~30–38 pairs during the initial breeding season and account for postrelease mortality.

**3** Initiate artificial gene-flow. As hihi occur mainly in small isolated islands which constrain population growth and expansion, population subdivision and drift will lead to the loss/fixation of alleles in different populations (assuming a mutation rate too low to counter fixation). In addition, isolated populations can also suffer from the accumulation of mildly deleterious mutations on fitness-related traits and inbreeding depression (Brekke *et al.* 2010); the effects of which have been found to be relieved by the addition of immigrants (Vilá *et al.* 2003; Tallmon *et al.* 2004).

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The authors research interests are in conservation genetics, population genetics, behavioural ecology and molecular evolution.

## Appendix I

Allele frequencies and number of individuals sampled (N) at each locus and in each.

| Population | | | | | | |
|---|---|---|---|---|---|---|
| Locus | Allele/N | Tiritiri Matangi | Little Barrier | Kapiti | Karori | Waitakere |
| *Nci001* | N | 86 | 49 | 29 | 41 | 54 |
| | 189 | 0.20 | 0.08 | 0.03 | 0.22 | 0.19 |
| | 200 | 0.10 | 0.32 | 0.24 | 0.10 | 0.15 |
| | 202 | 0.16 | 0.04 | 0.00 | 0.09 | 0.19 |
| | 204 | 0.55 | 0.56 | 0.72 | 0.60 | 0.46 |
| *Nci002* | N | 84 | 53 | 29 | 40 | 53 |
| | 224 | 0.21 | 0.44 | 0.64 | 0.30 | 0.20 |
| | 239 | 0.79 | 0.56 | 0.36 | 0.70 | 0.80 |
| *Nci003* | N | 84 | 54 | 29 | 40 | 54 |
| | 266 | 0.26 | 0.17 | 0.10 | 0.28 | 0.23 |
| | 271 | 0.32 | 0.52 | 0.43 | 0.36 | 0.32 |
| | 273 | 0.42 | 0.32 | 0.47 | 0.36 | 0.44 |
| *Nci004* | N | 84 | 54 | 27 | 38 | 45 |
| | 145 | 0.48 | 0.52 | 0.24 | 0.51 | 0.52 |
| | 148 | 0.20 | 0.14 | 0.07 | 0.16 | 0.14 |
| | 152 | 0.32 | 0.34 | 0.69 | 0.33 | 0.33 |
| *Nci005* | N | 87 | 52 | 28 | 40 | 53 |
| | 282 | 0.21 | 0.17 | 0.30 | 0.25 | 0.20 |
| | 288 | 0.32 | 0.21 | 0.43 | 0.35 | 0.30 |
| | 292 | 0.39 | 0.35 | 0.21 | 0.34 | 0.43 |
| | 301 | 0.08 | 0.27 | 0.05 | 0.06 | 0.08 |
| *Nci006* | N | 87 | 56 | 29 | 41 | 54 |
| | 156 | 0.15 | 0.24 | 0.21 | 0.18 | 0.21 |
| | 164 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 |
| | 167 | 0.51 | 0.23 | 0.48 | 0.52 | 0.44 |
| | 169 | 0.10 | 0.05 | 0.00 | 0.04 | 0.10 |
| | 175 | 0.15 | 0.18 | 0.21 | 0.17 | 0.12 |
| | 202 | 0.09 | 0.30 | 0.07 | 0.09 | 0.12 |
| *Nci007* | N | 83 | 52 | 28 | 38 | 51 |
| | 290 | 0.35 | 0.26 | 0.05 | 0.37 | 0.29 |
| | 294 | 0.15 | 0.22 | 0.14 | 0.17 | 0.24 |
| | 296 | 0.15 | 0.16 | 0.32 | 0.15 | 0.12 |
| | 300 | 0.27 | 0.26 | 0.48 | 0.24 | 0.28 |
| | 304 | 0.01 | 0.05 | 0.00 | 0.01 | 0.01 |
| | 308 | 0.08 | 0.05 | 0.00 | 0.07 | 0.06 |
| *Nci008* | N | 88 | 50 | 26 | 41 | 54 |
| | 215 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 |
| | 219 | 0.01 | 0.14 | 0.00 | 0.00 | 0.01 |
| | 223 | 0.42 | 0.17 | 0.19 | 0.42 | 0.48 |
| | 227 | 0.01 | 0.07 | 0.14 | 0.01 | 0.01 |
| | 231 | 0.07 | 0.07 | 0.17 | 0.04 | 0.07 |
| | 233 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 236 | 0.06 | 0.08 | 0.25 | 0.05 | 0.03 |
| | 239 | 0.11 | 0.04 | 0.02 | 0.12 | 0.07 |
| | 243 | 0.16 | 0.16 | 0.00 | 0.21 | 0.18 |

**Appendix I** (Continued)

| Population | | | | | | |
|---|---|---|---|---|---|---|
| Locus | Allele/N | Tiritiri Matangi | Little Barrier | Kapiti | Karori | Waitakere |
| | 248 | 0.16 | 0.14 | 0.00 | 0.16 | 0.14 |
| | 251 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 |
| | 256 | 0.00 | 0.06 | 0.23 | 0.00 | 0.00 |
| | 260 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 |
| *Nci009* | N | 73 | 48 | 28 | 39 | 49 |
| | 238 | 0.69 | 0.66 | 0.93 | 0.58 | 0.70 |
| | 242 | 0.23 | 0.21 | 0.02 | 0.26 | 0.18 |
| | 246 | 0.08 | 0.14 | 0.05 | 0.17 | 0.11 |
| *Nci010* | N | 86 | 52 | 28 | 39 | 54 |
| | 246 | 0.19 | 0.14 | 0.05 | 0.18 | 0.26 |
| | 250 | 0.06 | 0.14 | 0.13 | 0.08 | 0.08 |
| | 254 | 0.07 | 0.06 | 0.32 | 0.10 | 0.07 |
| | 258 | 0.56 | 0.42 | 0.04 | 0.47 | 0.51 |
| | 262 | 0.09 | 0.22 | 0.11 | 0.06 | 0.07 |
| | 266 | 0.03 | 0.02 | 0.36 | 0.10 | 0.01 |
| *Nci011* | N | 79 | 49 | 26 | 35 | 54 |
| | 290 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 |
| | 299 | 0.01 | 0.01 | 0.00 | 0.03 | 0.04 |
| | 303 | 0.01 | 0.02 | 0.00 | 0.00 | 0.00 |
| | 307 | 0.00 | 0.10 | 0.12 | 0.00 | 0.00 |
| | 311 | 0.22 | 0.15 | 0.12 | 0.16 | 0.19 |
| | 315 | 0.06 | 0.08 | 0.12 | 0.07 | 0.04 |
| | 320 | 0.13 | 0.06 | 0.04 | 0.17 | 0.17 |
| | 323 | 0.10 | 0.11 | 0.00 | 0.07 | 0.16 |
| | 327 | 0.34 | 0.24 | 0.56 | 0.36 | 0.26 |
| | 332 | 0.05 | 0.08 | 0.06 | 0.00 | 0.07 |
| | 335 | 0.04 | 0.06 | 0.00 | 0.07 | 0.05 |
| | 340 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 |
| | 348 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 |
| | 352 | 0.06 | 0.01 | 0.00 | 0.07 | 0.03 |
| *Nci012* | N | 81 | 47 | 26 | 38 | 54 |
| | 255 | 0.13 | 0.12 | 0.00 | 0.07 | 0.08 |
| | 264 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| | 267 | 0.12 | 0.02 | 0.00 | 0.13 | 0.10 |
| | 271 | 0.02 | 0.02 | 0.02 | 0.05 | 0.01 |
| | 276 | 0.02 | 0.12 | 0.23 | 0.04 | 0.04 |
| | 279 | 0.28 | 0.31 | 0.27 | 0.36 | 0.28 |
| | 283 | 0.19 | 0.26 | 0.35 | 0.18 | 0.32 |
| | 288 | 0.14 | 0.12 | 0.14 | 0.15 | 0.14 |
| | 292 | 0.10 | 0.03 | 0.00 | 0.03 | 0.04 |
| *Nci013* | N | 87 | 52 | 29 | 40 | 54 |
| | 222 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 |
| | 226 | 0.07 | 0.03 | 0.00 | 0.05 | 0.01 |
| | 230 | 0.08 | 0.43 | 0.50 | 0.05 | 0.08 |
| | 234 | 0.40 | 0.20 | 0.09 | 0.29 | 0.37 |
| | 238 | 0.01 | 0.08 | 0.00 | 0.00 | 0.01 |
| | 246 | 0.12 | 0.09 | 0.02 | 0.18 | 0.09 |
| | 250 | 0.13 | 0.08 | 0.22 | 0.23 | 0.18 |
| | 254 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 |
| | 258 | 0.06 | 0.00 | 0.00 | 0.08 | 0.07 |
| | 281 | 0.10 | 0.06 | 0.17 | 0.09 | 0.16 |
| | 285 | 0.03 | 0.00 | 0.00 | 0.05 | 0.03 |
| *Nci015* | N | 87 | 56 | 29 | 41 | 54 |
| | 206 | 0.08 | 0.03 | 0.00 | 0.09 | 0.05 |

## Appendix I (Continued)

| Population | | | | | | |
|---|---|---|---|---|---|---|
| Locus | Allele/N | Tiritiri Matangi | Little Barrier | Kapiti | Karori | Waitakere |
| | 218 | 0.25 | 0.17 | 0.02 | 0.28 | 0.27 |
| | 222 | 0.10 | 0.27 | 0.14 | 0.12 | 0.07 |
| | 226 | 0.49 | 0.39 | 0.55 | 0.45 | 0.50 |
| | 230 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| | 234 | 0.08 | 0.13 | 0.29 | 0.06 | 0.11 |
| *Nci016* | N | 71 | 50 | 26 | 21 | 41 |
| | 197 | 0.27 | 0.11 | 0.00 | 0.21 | 0.22 |
| | 209 | 0.11 | 0.09 | 0.08 | 0.05 | 0.10 |
| | 213 | 0.12 | 0.09 | 0.02 | 0.12 | 0.11 |
| | 217 | 0.34 | 0.21 | 0.25 | 0.41 | 0.35 |
| | 225 | 0.16 | 0.22 | 0.46 | 0.21 | 0.22 |
| | 229 | 0.00 | 0.27 | 0.19 | 0.00 | 0.00 |
| | 233 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| *BMC4* | N | 87 | 53 | 29 | 41 | 54 |
| | 169 | 0.69 | 0.59 | 0.59 | 0.63 | 0.75 |
| | 171 | 0.09 | 0.18 | 0.07 | 0.10 | 0.10 |
| | 185 | 0.20 | 0.24 | 0.35 | 0.24 | 0.14 |
| | 187 | 0.02 | 0.00 | 0.00 | 0.02 | 0.01 |
| *Dpu16* | N | 88 | 56 | 29 | 41 | 53 |
| | 157 | 0.76 | 0.69 | 0.57 | 0.79 | 0.69 |
| | 159 | 0.03 | 0.13 | 0.02 | 0.07 | 0.06 |
| | 165 | 0.03 | 0.06 | 0.00 | 0.00 | 0.04 |
| | 167 | 0.18 | 0.12 | 0.41 | 0.13 | 0.22 |
| *Tgu-Gga-04-012* | N | 84 | 53 | 29 | 40 | 53 |
| | 129 | 0.36 | 0.31 | 0.12 | 0.34 | 0.40 |
| | 131 | 0.12 | 0.10 | 0.00 | 0.20 | 0.19 |
| | 133 | 0.17 | 0.36 | 0.36 | 0.20 | 0.11 |
| | 135 | 0.35 | 0.23 | 0.52 | 0.26 | 0.30 |
| *MSLP4-Tgu-EST* | N | 88 | 56 | 29 | 42 | 53 |
| | 155 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| | 158 | 0.28 | 0.22 | 0.16 | 0.35 | 0.42 |
| | 160 | 0.12 | 0.23 | 0.83 | 0.14 | 0.06 |
| | 162 | 0.60 | 0.54 | 0.02 | 0.51 | 0.53 |

## Appendix II

Different tests for mutation drift equilibrium in the remnant population of hihi on Little Barrier Island. Bold lettering refers to significant values. He refers to expected heterozygosity, infinite alleles model refers to the infinite allele model, stepwise mutation model refers to stepwise mutation model and two-phased model refers to the two-phased model.

| Test | | Infinite alleles model | Stepwise mutation model | Two-phased model with 95%SMM |
|---|---|---|---|---|
| Sign Test | Expected number of loci with He excess | 10.77 | 11.18 | 11.18 |
| | Observed number of loci with He excess | 19.00 | 14.00 | 12.00 |
| | *P*-value | **0.00** | 0.14 | 0.45 |
| Standardized Differences Test | T2 value | 4.74 | 2.07 | 1.48 |
| | *P*-value | **0.00** | **0.02** | 0.07 |
| Wilcoxon Sign Rank Test | One tail for He excess | | | |
| | *P*-value | 0.00 | **0.01** | 0.06 |
| Mode-shift | L-shaped distribution | | | |

## Appendix III

Simulated loss of observed alleles over $t = 100$ years in the Tiritiri Matangi hihi population under the observed and alternative theoretical bottleneck and demographic scenarios. In the simulated populations with 40 and 60 founders, all other parameters used are identical to those used to simulate the Tiritiri Matangi population. The ''reduced growth rate'' scenario takes the observed number of founders ($n = 21$) and constrains the population at this size for 18 years before allowing it to grow under the observed demographics. The polygynous and cooperative mating systems use the same parameters as those found in the observed simulation under different mating systems.

| Scenario | Observed (21 founders) | 40 Founders | 60 Founders | Reduced growth rate | Polygynous mating system | Cooperative mating system |
|---|---|---|---|---|---|---|
| (a) Observed number of alleles | | | | | | |
| $t = 0$ | 5.60 | 5.60 | 5.60 | 5.60 | 5.60 | 5.60 |
| $t = 100$ | 3.36 | 3.55 | 3.67 | 2.64 | 1.26 | 1.03 |
| % variation retained | 60.01 | 63.37 | 65.57 | 47.12 | 22.44 | 18.42 |
| (b) Effective number of alleles | | | | | | |
| $t = 0$ | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 | 3.80 |
| $t = 100$ | 2.33 | 2.45 | 2.51 | 1.93 | 1.12 | 1.02 |
| % variation retained | 61.46 | 64.44 | 66.14 | 50.72 | 29.60 | 26.87 |
| (c) Expected heterozygosity ($H_E$) | | | | | | |
| $t = 0$ | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 |
| $t = 100$ | 0.51 | 0.54 | 0.55 | 0.42 | 0.07 | 0.01 |
| % variation retained | 75.27 | 78.57 | 80.07 | 61.03 | 10.66 | 1.70 |
| (d) Fixation probability | | | | | | |
| *Nci001* | 0.01 | 0.01 | 0.00 | 0.11 | 0.77 | 0.98 |
| *Nci002* | 0.02 | 0.00 | 0.01 | 0.15 | 0.81 | 0.97 |
| *Nci003* | 0.00 | 0.00 | 0.00 | 0.09 | 0.74 | 0.98 |
| *Nci004* | 0.01 | 0.00 | 0.00 | 0.08 | 0.75 | 0.97 |
| *Nci005* | −0.01 | −0.01 | −0.01 | 0.03 | 0.74 | 0.96 |
| *Nci006* | −0.01 | −0.01 | −0.01 | 0.04 | 0.70 | 0.96 |
| *Nci007* | 0.00 | −0.01 | −0.01 | 0.01 | 0.69 | 0.95 |
| *Nci008* | −0.01 | −0.01 | −0.01 | 0.00 | 0.67 | 0.97 |
| *Nci009* | 0.04 | 0.02 | 0.03 | 0.18 | 0.81 | 0.98 |
| *Nci010* | 0.00 | −0.01 | −0.01 | 0.04 | 0.71 | 0.96 |
| *Nci011* | −0.01 | −0.01 | −0.01 | 0.00 | 0.66 | 0.95 |
| *Nci012* | −0.01 | −0.01 | −0.01 | 0.00 | 0.69 | 0.95 |
| *Nci013* | 0.00 | −0.01 | −0.01 | 0.05 | 0.72 | 0.96 |
| *Nci015* | −0.01 | −0.01 | −0.01 | 0.03 | 0.69 | 0.96 |
| *Nci016* | 0.00 | −0.01 | −0.01 | 0.02 | 0.68 | 0.95 |
| *BMC4* | 0.02 | 0.01 | 0.00 | 0.11 | 0.78 | 0.97 |
| *Dpu16* | 0.07 | 0.04 | 0.03 | 0.21 | 0.82 | 0.98 |
| *MSLP4-Tgu-04* | 0.01 | 0.00 | 0.00 | 0.08 | 0.77 | 0.97 |
| *Tgu-Gga-04-012* | 0.00 | −0.01 | −0.01 | 0.04 | 0.74 | 0.96 |