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RESEARCH ARTICLE

# Genetic variation in the kakerori (*Pomarea dimidiata*), an endangered endemic bird successfully recovering in the Cook Islands

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**Abstract** The Cook Islands endemic kakerori (*Pomarea dimidiata*) underwent a severe population decline following the introduction of ship rats (*Rattus rattus*) in the late 1800s. By 1989, the sole population on Rarotonga consisted of 29 known birds. Subsequent intensive management efforts enabled this population to recover to around 250–300 birds in recent years. This study, using microsatellite and mitochondrial DNA markers, assesses the level of genetic diversity and the genetic structure of the contemporary kakerori population on Rarotonga. No mitochondrial control region and cytochrome *b* haplotype diversity was found in the 11 samples examined at each locus. In 81 samples genotyped at 7 polymorphic microsatellite loci, an average of 4 alleles per locus were found, with an average observed heterozygosity of 0.65. No subpopulation division was found in this population. There was no evidence of inbreeding, but genetic bottleneck tests showed that the population had indeed experienced a significant genetic bottleneck. Recovery of the kakerori was successful in the past two decades despite low genetic diversity in terms of allelic diversity. Our data suggested that low allelic diversity did not hamper population expansion and the continued survival of this species, however, longer-term effects are still possible.

**Keywords** *Pomarea dimidiata* · Genetic diversity · Microsatellites · Cytochrome *b* · Mitochondrial control region · Bottleneck

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## Introduction

Preserving genetic diversity is now one of the main objectives in wildlife conservation and management programmes. Populations with higher genetic diversity may have an advantage in adaptation to demographic changes, and lower extinction risk from inbreeding depression (Frankham 1996). However, it has not been established whether high levels of genetic diversity are essential for the long-term survival of a species. On one hand, it has been shown that extinction risk increases with inbreeding depression (Saccheri et al. 1998), but on the other hand, species with low genetic variation continue to survive, for example, the Przewalski's gazelle (*Procapra przewalskii*; Lei et al. 2003), and the Amsterdam albatross (*Diomedea amsterdamensis*; Milot et al. 2007). In the black robin (*Petroica traversi*), successful recovery to about 200 birds has been reported from a single breeding pair, suggesting that genetic diversity may not be essential for the survival of a species (Ardern and Lambert 1997). Experiments with *Drosophila* fruit flies have also shown that lowered resistance to toxins and diseases in inbred populations were caused by the presence of specific alleles rather than as a result of more general inbreeding effects (Spielman et al. 2004).

The kakerori (*Pomarea dimidiata*), also commonly known as the Rarotonga monarch or Rarotonga flycatcher,

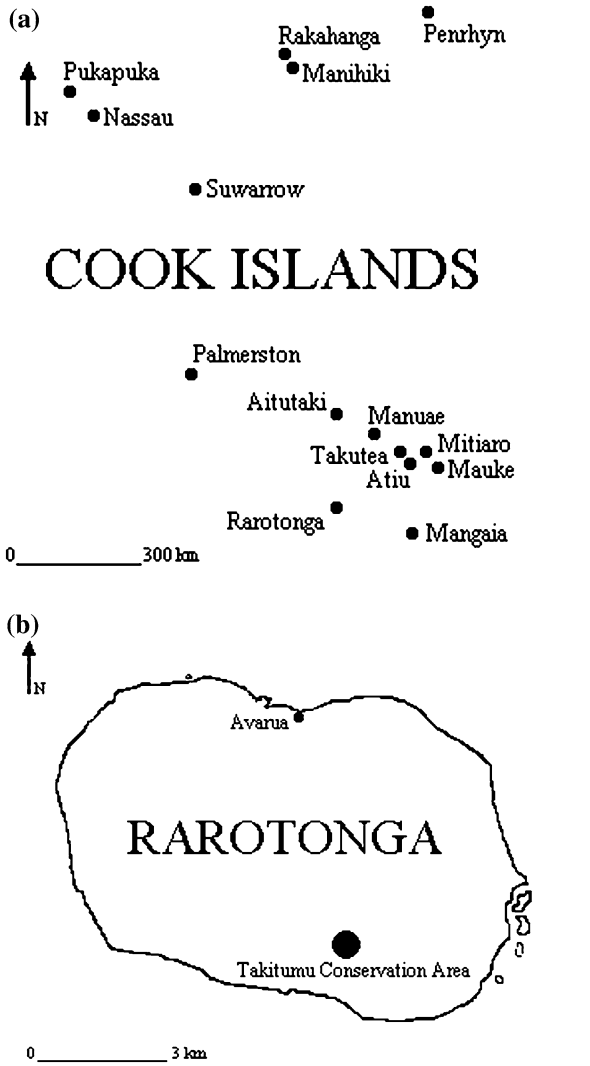


**Fig. 1** The kakerori is endemic to the Cook Islands: **a** the main population is on Rarotonga and an insurance population was set up on Atiu; **b** on Rarotonga, the kakerori is found in the Takitumu Conservation Area and surrounds, on the south-eastern part of the island

is a small passerine endemic to the island of Rarotonga in the Cook Islands (Fig. 1). Studies of the mitochondrial cytochrome *b* locus suggested that the kakerori is one of the basal species in the phylogenetic tree of *Pomarea* monarchs, which are endemic to the islands of eastern Polynesia in the Pacific (Cibois et al. 2004).

In the mid 1800s, the kakerori was widespread and common on Rarotonga, however, the arrival of ship rats (*Rattus rattus*) led to a serious population decline (Robertson et al. 1994). In 1989, the census size reached a low point of 29 known birds (including 13 females), but since then, intensive management based on rat poisoning in the 150 ha Takitumu Conservation Area, set up to protect this and other native species, has helped the kakerori population to recover rapidly to 250–300 at present (Fig. 2; Robertson

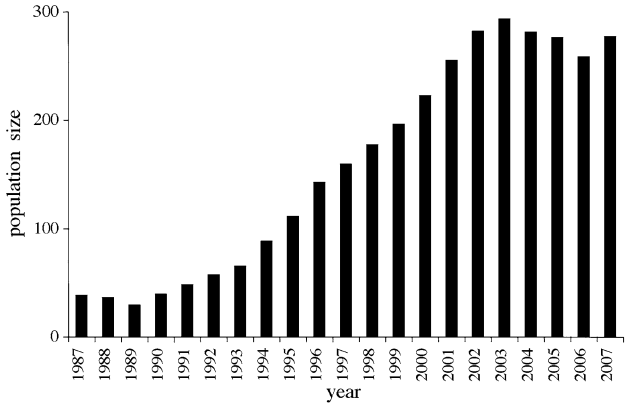


**Fig. 2** Kakerori population size on Rarotonga between 1987 and 2007

et al. 1994, 2009; Saul et al. 1998). Since 1987, the kakerori population on Rarotonga has been monitored closely. In August each year from 1987 to 2007, before the breeding season, birds were colour-banded and territories were mapped as part of an annual census (Robertson et al. 2009). To lower the extinction risk of this single-island endemic, especially from factors such as habitat destruction from a tropical cyclone or the accidental introduction of new predators or diseases, an insurance population was established on the island of Atiu by transferring 30 young birds between 2001 and 2003 (Robertson et al. 2006). Atiu is free of ship rats, and has a variety of forest habitats available for use by kakerori. The Atiu population is monitored annually and had grown to at least 43 birds in 2007, however, due to the difficult terrain, surveying of birds is not possible in many parts of Atiu (Robertson et al. 2009).

Island populations, in general, may have lower genetic diversity than mainland populations (Frankham 1997), and are thus more prone to extinction due to genetic effects, such as inbreeding (Frankham 1998). Reduction in genetic diversity is often a consequence of a population bottleneck. The severity of a bottleneck, in terms of the lowest number of breeding pairs, and the duration of a bottleneck (number of generations at small population size), determines the extent of loss of genetic variability. While a severe population contraction can lead to reduction of allelic diversity in subsequent generations, extreme population declines over a large number of generations are required to lower heterozygosity substantially (Amos and Balmford 2001; England et al. 2003; Milot et al. 2007).

In this study, we examine the genetic effects of the historical population bottleneck to the kakerori using mitochondrial and microsatellite DNA markers, and discuss management implications from the new genetic information for the present day population.

## Methods

### Sample collection and DNA extraction

Breast feathers were collected from individually colour-banded kakerori caught by mist netting during successive field seasons on Rarotonga in 2006 (12 samples), 2007 (30 samples), and 2008 (39 samples). DNA was extracted from the quills of 6 feathers from each individual, using High Pure PCR Template Preparation Kit (Roche).

### Mitochondrial DNA sequence diversity

The cytochrome *b* locus in the mitochondria was PCR amplified and sequenced from 11 randomly chosen samples from the 2008 collection. About 10 ng of DNA templates were amplified in 25 µl reactions containing 1 × *Ex Taq* buffer (Takara), 2 mM $MgCl_2$, 0.1 mM dNTPs, 0.4 µM of each of the primers L14990 and H15916 (Cibois et al. 2004), and 0.625 U *Ex Taq* polymerase (Takara). The reactions were run on an Eppendorf Mastercycler ep thermocycler for 95°C—5 min, 30 × (95°C—30 s, 50°C—30 s, 72°C—90 s), 72°C—10 min. The control region from 11 other individuals was amplified using reaction mixtures with same ingredients as for cytochrome *b* amplifications, except that primers L16743 and H1248 (Tarr 1995) were used instead. Cycling parameters for the control region target were 94°C—3 min, 30 × (94°C—15 s, 51°C—15 s, 72°C—60 s), 72°C—7 min. Amplification products were sequenced with primers L14990 and H15916 (cytochrome *b*; Cibois et al. 2004), L16743, H1248, L437 and H417 (control region; Tarr 1995) using the DNA sequencing services provided by the Allan Wilson Centre Genome Service at Massey University, Palmerston North, New Zealand. Sequences at these loci were combined for each individual and compared between individuals using the LASERGENE software package (version 4; DNASTAR Inc. http://www.dnastar.com).

### Microsatellite DNA genotypic diversity

All 81 individual samples were genotyped at polymorphic microsatellite loci *Pdim2021*, *Pdim2829*, *Pdim3031*, *Pdim3233*, *Pdim3435*, *Pdim3637* and *Pdim5657* as described in Chan et al. (2008). For each locus, the number of alleles, observed and expected heterozygosity values, and allele frequencies were calculated using the MICROSATELLITE ANALYSER software (version M3.15; Dieringer and Schlötterer 2002). Deviations from Hardy–Weinberg equilibrium (HWE) were tested by the Markov chain method (Guo and Thompson 1992) implemented in the GENEPOP software (version 3.4; Raymond and Rousset 1995).

### Genetic structure of the Rarotonga population

The number of genetic clusters or subpopulations ($K$) in the Rarotonga kakerori population was calculated from the microsatellite data using the STRUCTURE software (version 2.1; Pritchard et al. 2000). This software implements a Bayesian clustering technique which finds individual combinations within a population that maximize HWE at a pre-determined value of $K$. Simulations were run with $10^6$ replications after a 30,000 step burn-in period at $K = 1$–10. Posterior probabilities for $K$ were determined based on log-likelihood.

### Effective population size

The effective population size ($N_e$) of the population was computed with the ONeSAMP estimator (version 1.1; Tallmon et al. 2008), which estimated $N_e$ by approximate Bayesian computation (Beaumont et al. 2002; Tallmon et al. 2004). The estimator created 50,000 simulated populations based on the microsatellite genotypic data provided, and the effective size of each of these simulated populations were drawn from a random number between the lower and upper $N_e$ at 2 to 300 a priori. After the simulated populations reproduced for 2–8 generations following a Wright-Fisher model, 81 samples were drawn and $N_e$ was calculated as described in Tallmon et al. (2008).

### Genetic bottleneck effects and inbreeding

Reduction in allelic diversity (number of alleles) and heterozygosity can be observed in a genetically bottlenecked population, and allelic diversity is reduced at a higher rate than heterozygosity. Therefore, recently bottlenecked populations may be expected to show higher heterozygosity values than expected from the number of alleles observed in the population (Cornuet and Luikart 1996). We tested for recent genetic bottlenecks in the kakerori population based on the microsatellite genotypic data using the BOTTLENECK software (version 1.2.02; Cornuet and Luikart 1996). The software implements a Wilcoxon sign-rank test for heterozygosity excess under the infinite allele model (IAM; Kimura and Crow 1964), stepwise mutation model (SMM; Ohta and Kimura 1973), and the two-phase mutation model (TPM; Di Rienzo et al. 1994) for microsatellites. All tests were run for $10^6$ replications, and the TPM tests were run twice, assuming 95% single-step mutations with 5% multi-step mutations in the first run, and 70% single-step mutation with 30% multi-step mutations in the second run. To test for the possibility of inbreeding in the Rarotonga population, we calculated the estimator of $F_{IS}$ (Weir and Cockerham 1984) using the software GENODIVE (version 2.0b12; Meirmans and van Tienderen 2004).

## Results

### Mitochondrial DNA haplotypic diversity in the kakerori

DNA sequences from cytochrome *b* did not show any variation in 11 individuals. The sequence obtained is identical to that reported by Cibois et al. (2004) who used a different sample to those used here. The control region sequences also did not show variation in a further 11 birds. The results suggested that only one mitochondrial haplotype may be present in the current kakerori population. The control region sequence obtained is deposited in DDBJ under accession number AB548658.

### Allelic diversity at microsatellite loci

Between 3 and 5 alleles were observed at each microsatellite locus genotyped (Fig. 3; Table 1). The observed heterozygosities ranged from 0.54 to 0.73, and expected heterozygosities were between 0.51 and 0.72 (Table 1). No significant deviation from HWE was detected ($P > 0.05$).

### Genetic status of the Rarotonga population

Bayesian clustering analysis with the software STRUCTURE suggested there was genetically no subpopulation division in the Rarotonga population ($K = 1$). The mean effective population size of this population was estimated to be 32.58, with a 95% credible limit between 25.57 and 54.32. The estimated mean effective population size was reasonably close to 29, the number of birds found in the 1989 census when the population was at the lowest size. No significant inbreeding was detected in this population ($F_{IS} = -0.027$, $P = 0.171$), however BOTTLENECK tests suggested the population had experienced a significant genetic bottleneck under the IAM ($P = 0.004$), SMM ($P = 0.008$), and TPM ($P = 0.004$). TPM tests with 70% single-step mutations gave the same probability as with 95% single-step mutations.

## Discussion

### Population genetics of the kakerori on Rarotonga

Despite a rapid recovery from 29 to over 250 birds in the past 20 years, the contemporary kakerori population on Rarotonga shows low levels of allelic diversity at nuclear microsatellites, and no haplotypic diversity at the two mitochondrial loci examined in this study. Sequences from the mitochondrial cytochrome *b* and control region were identical between all the individuals tested, suggesting all living kakerori may be descendants from one maternal lineage. Microsatellite analyses of the kakerori population showed generally low numbers of alleles, averaging four alleles per locus, and moderate observed heterozygosity values (about 0.65; Table 1). The average number of alleles and heterozygosity in the kakerori are similar to those reported in some other threatened avian island endemics (Jamieson et al. 2006). Our analysis also confirmed that a genetic bottleneck had indeed occurred in this population.

The low allelic and haplotypic diversities currently observed in the kakerori may be caused by low levels of ancestral polymorphism in the original founders of the Rarotonga population, or more recent genetic bottlenecks, or both. Our statistical tests showed that genetic bottleneck is likely to have played some roles in the reduction of genetic diversity in this population. However, we are unable to tell whether the population was originally founded by a small number of birds or established by multiple founders with low genetic diversity. Nevertheless, when compared to the heterozygosity levels in other birds (Evans and Sheldon 2008), the modern Rarotonga kakerori population still possesses reasonable genetic diversity in terms of heterozygosity, which is considered to be an important asset for the evolutionary potential and enduring fitness of any population (Amos and Harwood 1998; Hansson and Westerberg 2002).

Our estimate of effective population size at a mean of 32.58 suggested that genetic diversity of the population is very close to the level at 1989, when only 29 individuals were found. The kakerori population has most likely maintained close to its total genetic diversity in the recovery phase during the past two decades. The absence of genetic structure and inbreeding in the population also suggested that the formation of mating pairs is random or close to random, with a sufficient number of individuals that are genetically diverse in the present population.

### Genetic diversity and population recovery

While there are cases where association between loss of genetic diversity and genetic defects have been reported, such as in the cheetah (O'Brien et al. 1985) and the northern elephant seal (Hoelzel et al. 2002), other studies have reported survival and recovery of populations with low genetic diversity, such as in the black robin (Ardern and Lambert 1997), and Amsterdam albatross (Milot et al. 2007). These findings, and ours from kakerori, suggest that populations can persist and expand with low levels of genetic diversity as long as fitness-related genes are not seriously compromised. However, it is not known how well these populations can continue to persist under sudden environmental challenges or when new diseases arise. Although the neutral marker loci we used provide a good

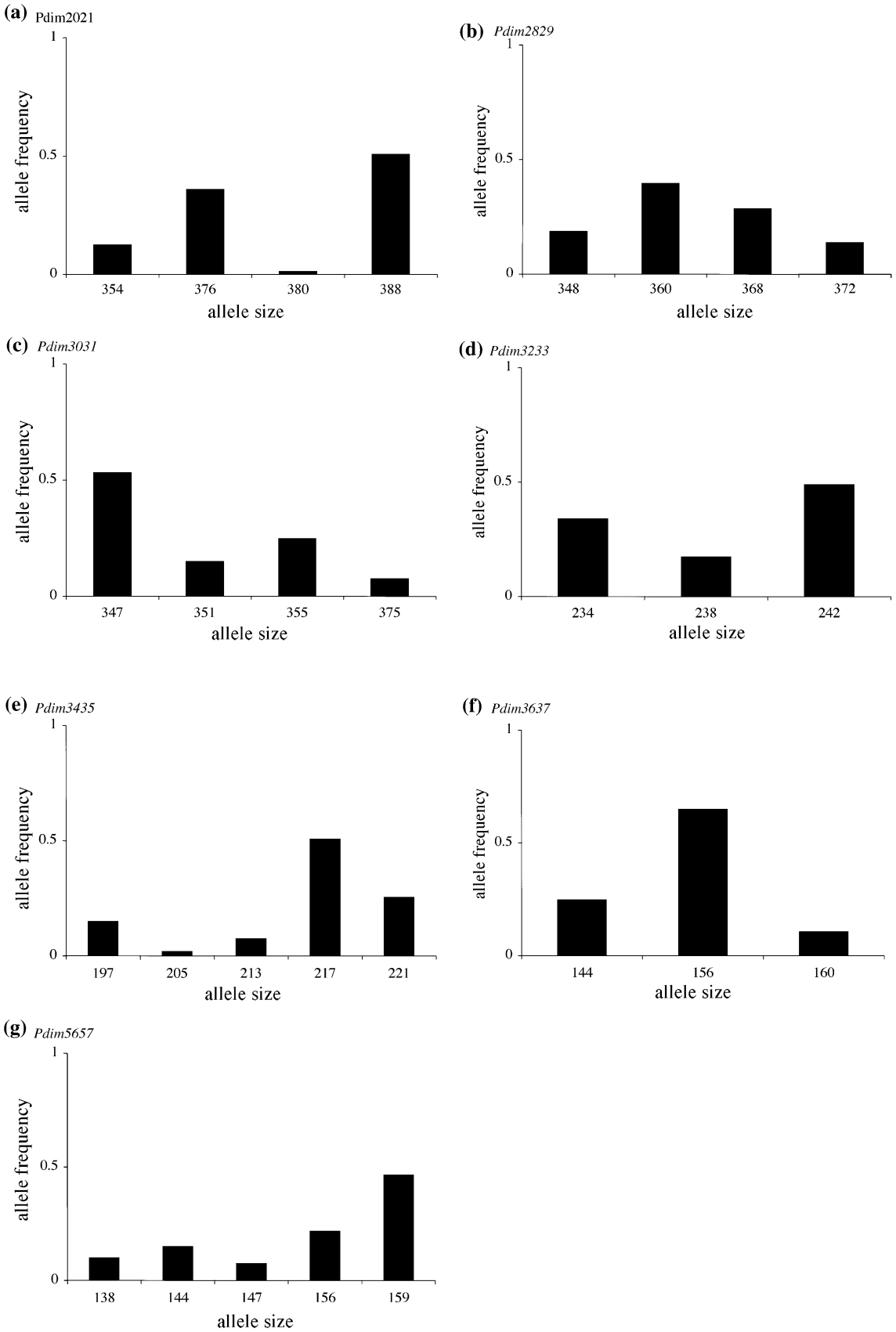


**Fig. 3** Allele frequency distributions at 7 microsatellite loci in the Rarotonga kakerori population

**Table 1** Allelic diversity and heterozygosities at 7 microsatellites in the 81 Rarotonga population samples

| Locus | $N$ | $H_O$ | $H_E$ |
|---|---|---|---|
| *Pdim2021* | 4 | 0.69 | 0.60 |
| *Pdim2829* | 4 | 0.73 | 0.71 |
| *Pdim3031* | 4 | 0.60 | 0.63 |
| *Pdim3233* | 3 | 0.65 | 0.62 |
| *Pdim3435* | 5 | 0.67 | 0.66 |
| *Pdim3637* | 3 | 0.54 | 0.51 |
| *Pdim5657* | 5 | 0.68 | 0.71 |
| Average | 4 | 0.65 | 0.63 |

Number of alleles is denoted by $N$, observed heterozygosity by $H_O$, and expected heterozygosity by $H_E$. Average heterozygosities between loci are also shown

indication of genome-wide genetic diversity, they may not be ideal indicators of fitness-related diversity as only a weak correlation between molecular and quantitative measures of genetic variation was found (Reed and Frankham 2001). Continued careful conservation management will certainly be crucial to the long term survival of such species.

## Conservation implications

Presently, ship rats pose the main existing threat to the survival of kakerori (Robertson et al. 2009), and other *Pomarea* species elsewhere in the South Pacific (Thibault et al. 2002). Continued control of the rat population in the Takitumu Conservation Area is important to the survival of the kakerori population, hence in preserving the population's genetic diversity. Should there be a direct relationship between a loss of genetic diversity and decreased disease resistance as suggested by Spielman et al. (2004), we would predict that the kakerori population might be highly susceptible to introduced avian diseases, and so great care must be taken to ensure that any pet birds imported to the Cook Islands are free of diseases.

The Atiu population was founded by translocation of 30 individuals from Rarotonga (Robertson et al. 2006). This is close to the mean effective population size of the Rarotonga population we estimated in this study. To ensure the Atiu population represents as much of the genetic diversity of the source population as possible, we recommend that genetic and genealogical monitoring should be prioritised in translocated birds to avoid loss of genetic diversity and inbreeding in this population.

Survival of the kakerori will depend on ongoing rat control on Rarotonga, detection and management of possible new threats, and the long-term monitoring of the two populations.

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