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RESEARCH ARTICLE

Genetic diversity and population differentiation within and between island populations of two sympatric *Petroica* robins, the Chatham Island black robin and tomtit

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Abstract Small island populations are particularly prone to extinction due to the effects of genetic drift and inbreeding reducing genetic variation and fitness of such populations. Furthermore, isolated island populations may experience population divergence due to drift or divergent selection. Reciprocal translocations of individuals between populations may be used to stimulate gene flow between such isolated populations. To determine whether populations of the endangered Chatham Island black robin *Petroica traversi* may benefit from such translocations, we compared levels of genetic diversity and differentiation within and among populations of the black robin and its sympatric sister-species, the Chatham Island tomtit *Petroica macrocephala chathamensis*. Although the black robin has recovered following a severe population bottleneck, the bottleneck and subsequent intense inbreeding experienced by the black robin have likely had long-term consequences affecting the viability of this endangered species. We analysed the genetic diversity and population structure of the black robin at 15 polymorphic microsatellite loci, and compared this to the level and pattern of genetic diversity from 17 polymorphic loci for the tomtit, which comprises three larger island populations. The black robin displayed a lower number of alleles and expected heterozygosity than the Chatham Island tomtit. We also found that island populations of both species have differentiated from one another, likely due to strong genetic drift acting independently on these populations over a period of isolation. Reciprocal translocations of black robins between islands are recommended to prevent further loss of diversity through drift, and so to improve the probability of species persistence.

Keywords Black robin · Chatham Island tomtit · Genetic diversity · Conservation

Introduction

Small populations are inherently at a higher risk of extinction than larger populations due to demographic, environmental, and genetic stochasticity (Caughley 1994; Lande 1993). Strong genetic drift in small populations may overwhelm the effects of natural selection, and may result in deleterious alleles becoming fixed in the population, reducing fitness, and driving the population towards extinction (Lynch et al. 1995). In addition, in extremely small populations, related individuals may have no alternative but to mate with each other, resulting in inbred offspring, and reducing fitness through inbreeding depression (Charlesworth and Charlesworth 1999; Grueber et al. 2010; Keller and Waller 2002; Wright et al. 2008). As a population decreases in size, the combined effects of genetic drift and high rates of inbreeding accelerate the extinction process. Moreover, low genetic variation limits the adaptive potential of small populations, reducing their ability to adapt and survive if environmental conditions

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change, thus further increasing their vulnerability to extinction (Caballero and García-Dorado 2013).

Strong genetic drift and/or divergent selection acting upon small isolated populations can also lead to rapid population differentiation (Funk et al. 2016). Populations of dispersal-limited species, such as those on islands or in remnants of habitat surrounded by inhospitable environments may be trapped in their habitat patches with little or no gene flow occurring that could increase genetic diversity of these populations (Frankham 1997; Slatkin 1987). Identifying genetic diversity and potential differences in diversity between populations of a threatened species may be used to determine appropriate conservation management strategies. Translocation, the intentional movement of individuals from one area to another (IUCN 1987), is a commonly-used conservation strategy to establish additional populations of threatened species. Translocations may also be used to move individuals between existing populations, as a means of genetic rescue or restoration (Weeks et al. 2011). Population fitness and persistence can be improved through the introduction of unrelated, outbred individuals into an existing population, resulting in reductions in the genetic load and the frequency of inbreeding (Hedrick et al. 2014; Ingvarsson 2001; Vilà et al. 2003; Vucetich et al. 2005; Weeks et al. 2011). It has recently been shown that, for species where outbred, non-bottlenecked populations are not available, translocations between inbred source populations can effectively improve fitness within each recipient population (Heber et al. 2013).

The Chatham Island black robin (*Petroica traversi*) is one such species that may benefit from translocations between populations. The black robin is an endangered passerine endemic to the Chatham Islands, an archipelago 800 km east of New Zealand (BirdLife International 2016; Massaro et al. 2013). Approximately 35 individuals survived for over eighty years on a single small island (Little Mangere Island), but the black robin became known as the world's most endangered bird when in 1980 the population was further reduced to include only a single breeding pair (Butler and Merton 1992; Massaro et al. 2013). Conservation management averted extinction of the species by cross-fostering black robin eggs and nestlings to its sympatric sister-species, the Chatham Island tomtit (*Petroica macrocephala chathamensis*), stimulating increased egg laying by the black robin (Butler and Merton 1992). Translocations during 1982–1990 relocated an estimated 23 birds and 53 eggs from Mangere to Rangatira (Hokorereora) Island (~11 km south-east of Mangere Island) to establish a second population (Butler and Merton 1992; Kennedy 2009). Currently the black robin numbers around 291 individuals; with 246 robins on Rangatira (M. Massaro, personal communication) and 43 on Mangere (Department of Conservation, pers. comm.).

The Chatham Island tomtit co-exists with the black robin on Mangere and Rangatira islands (Department of Conservation 2001). The tomtit has not experienced such a severe bottleneck as the black robin, and is estimated to number around 1000 individuals on three islands (Department of Conservation 2001). Thus, these larger populations are likely to have maintained greater genetic diversity than those of the black robin. It is not known whether tomtit individuals disperse between the three island populations.

Although the black robin population size has recovered from the severe population bottleneck, high levels of inbreeding when the population was extremely small (Ardern and Lambert 1997) may have contributed to increased population vulnerability, as some individuals are estimated to have inbreeding coefficients that are higher than those in selfing populations (Kennedy et al. 2014). Severe inbreeding has led to reduced fitness, such as reduced juvenile survival (estimated at 6.85 lethal equivalents; Kennedy et al. 2014) and the impact of strong drift is exhibited in the spread of a mal-adaptive trait, whereby females lay eggs on the rim of their nests, which then fail to hatch as they are not incubated (Massaro et al. 2013). No dispersal of black robins between Mangere and Rangatira has ever been observed, with the limited dispersal capability of the black robin across open areas preventing movement between these two islands (Butler and Merton 1992). Hence both populations on Rangatira and Mangere islands will have independently experienced genetic drift during the 26 years of isolation, and so may have differentiated from one another. If there has been some genetic differentiation between these populations, it may be beneficial to conduct reciprocal translocations between island populations to reinforce standing genetic diversity.

On Rangatira Island, the black robin is hypothesised to exist in two populations, each inhabiting a distinct bush area; Woolshed Bush to the north, and Top Bush to the south, separated by Skua Gully (Kennedy 2009; Weiser et al. 2016). Dispersal may have been limited between forest patches (Butler and Merton 1992; Kennedy 2009), and so there may be some level of differentiation between robins in these two distinct habitat areas. Differentiation within the Rangatira Island population may have implications for the selection of individuals for translocations.

To assess the levels of genetic diversity and differentiation within and among the black robin populations on Mangere and Rangatira islands, we use polymorphic microsatellite loci developed via next-generation Illumina sequencing from the Chatham Island black robin genome (Almojil et al. 2016), in addition to loci developed for other species that were previously found to be polymorphic in the black robin (Cubrinovska et al. 2016). Using these markers, we compare levels of diversity within the two island populations (Mangere and Rangatira), and examine

whether these populations have differentiated over the past 26 years of isolation. Additionally, we determine whether there is genetic differentiation between robins occupying the two distinct forest patches on Rangatira Island. A number of the markers developed for the black robin amplify and are polymorphic in the Chatham Island tomtit (Almojil et al. 2016). Measuring diversity in the tomtit will allow comparison of genetic diversity with a similar species that has not experienced such an extreme bottleneck and severe inbreeding as the black robin. We intend to use the results of this study to determine whether translocations of individuals between populations have the potential to improve population viability of this endangered species.

Materials and methods

Sample collection and DNA extraction

Black robin and tomtit samples were collected from Mangere and Rangatira each breeding season from 2008 to 2011, using mistnets or drop traps to capture birds. Brachial venepuncture was used to collect blood samples that were stored in ethanol, lysis buffer, or dried on filter paper. Alternatively, feather samples were collected from some birds. DNA was extracted from blood spots or feathers of black robin and tomtit individuals using an Invitrogen PureLink™ Genomic DNA MiniKit. Thirty black robin individuals from Mangere were preferentially selected for genotyping based on DNA quality at extraction, to ensure amplification of loci. From the Rangatira black robin population, 174 individuals were genotyped in total. However, closely related individuals are more likely to have similar allele frequencies than the true population mean, and so only a single individual from each known family group (including observed parents, offspring, or siblings) was included in the analyses of diversity and differentiation to limit bias in mean allele frequencies ($n = 115$). All 204 individuals genotyped (Mangere $n = 30$, Rangatira $n = 174$) were included in testing for evidence of dispersal between islands. Tomtit individuals from both Mangere ($n = 22$) and Rangatira ($n = 30$) were selected for genotyping based on DNA quality to allow for comparisons of diversity with black robins.

To investigate population subdivision within Rangatira, individuals were separated into 'Woolshed Bush' and 'Top Bush' populations, based on GPS data (Garmin GPSMAP60CSx, <10 m) collected during banding and sampling of individuals. Of all samples available, 27 were determined to belong to the Top Bush population, 25 of which were genotyped and included in this analysis. These 25 were compared against a random subset of thirty individuals from the Woolshed population. As demonstrated in Hale et al. (2012), 25–30 individuals is sufficient to estimate the level of diversity within a population, as increasing costs outweigh the smaller gains in information.

Microsatellite genotyping

All 11 loci polymorphic in black robins and 17 loci polymorphic in tomtits developed in Almojil et al. (2016) were used here, using the PCR amplification protocols described therein. In addition, one locus (PAU26) developed for the South Island robin (Townsend et al. 2012) was included as it was found to amplify and be polymorphic in black robins (Forsdick 2016). This locus was amplified in 15 µl reactions containing 0.5 µl of genomic DNA, 1× NH_4 reaction buffer (Bioline), 2 mM $MgCl_2$, 0.08 mM dNTPs, 0.083 µM of forward primer, 0.33 µM reverse primer and 0.33 µM fluorescently labelled M13 primer (one of 6-FAM, NED, VIC, or PET, Applied Biosystems), and 0.6 U BIOTAQ DNA polymerase (Bioline), and the thermocycling protocol consisted of: 95 °C for 12 min, 10 cycles of 94 °C for 15 s, annealing temperature (48 °C) for 30 s, 72 °C for 30 s, followed by 30 cycles of 89 °C for 15 s, annealing temperature (48 °C) for 30 s, 72 °C for 30 s, with a final extension of 72 °C for 10 min. Samples were prepared for genotyping by adding 0.5 µl PCR product to 0.3 µl Genescan 500LIZ size standard (Applied Biosystems) and 12 µl HiDi formamide. These were then denatured at 95 °C for 5 min. A further three polymorphic loci, TG02-088, PCA12, and PGM1, that were previously found to be polymorphic in black robins were also included in this study for black robin genotyping (Cubrinovska et al. 2016; Dawson et al. 2010; Dowling et al. 2003; Lambert et al. 2005). Forward primers were labelled with a fluorescent dye (6-FAM, NED, or PET, Applied Biosystems). Loci were amplified in 15 µl reactions containing 0.5 µl of genomic DNA, 1× NH_4 reaction buffer (Bioline), 2 mM $MgCl_2$ (decreased to 0.167 mM for PGM1 to improve annealing), 0.08 mM dNTPs, 0.33 µM of forward primer, 0.33 µM reverse primer, and 0.6 U BIOTAQ DNA polymerase (Bioline). These three loci were amplified using the standard three-step PCR protocol described previously (Table 1). Genotyping was performed on an ABI Prism® 3130xl Genetic Analyser (Applied Biosystems). Allele sizes were scored visually using GeneMarker (v.2.20; SoftGenetics).

To measure genotyping error that may result in incorrect identification of genotypes and estimates of allele frequencies (Bonin et al. 2004; Broquet and Petit 2004), at least 20 samples per locus (for a total of 14.2% of all samples per locus) were amplified, genotyped, and scored twice. The error rate was calculated by dividing the number of errors by the total number of samples repeated (Hoffman and Amos 2005).

Table 1 List of microsatellite loci used for genotyping in each species

Locus	T_A (°C)	Black robins	Tomtits
PT1	TD1	✔	
PT10	54	✔	✔
PT12	TD1		✔
PT18	56	✔	✔
PT19	56		✔
PT2	56	✔	✔
PT24	60		✔
PT25	58		✔
PT26	TD1	✔	✔
PT27	60	✔	✔
PT35	56		✔
PT37	58	✔	✔
PT38	TD1	✔	
PT39	48	✔	✔
PT40	54	✔	
PT4	48		✔
PT5	54		✔
PT6	54		✔
PT7	54	✔	✔
PT9	TD1		✔
PAU26	48	✔	
PCA12	64	✔	
PGM1	56	✔	
TG02-088	50	✔	

T_A final annealing temperature used, ✔ indicates this locus was used in genotyping of black robins and/or tomtits in this study. TD1 refers to the touchdown thermocycling protocol described in Almojil et al. (2016)

Data analyses

STRUCTURE ver. 2.3.4 (Pritchard et al. 2000) was used to estimate the number of true genetic clusters, firstly to clarify differentiation between Mangere and Rangatira populations for both species, and secondly to investigate fine-scale structuring within the different forest areas in the Rangatira black robin population. Using the given data, K, the true number of genetic clusters, was estimated using Bayesian clustering (Pritchard et al. 2000). A burn-in length of 10,000 followed by 100,000 iterations was used to produce consistent results in replicate runs. The admixture model using LOCPRIOR and correlated allele frequencies was used, taking into account the sampling locations (either Mangere or Rangatira, or Woolshed or Top Bush). This is a more informative method when weak population structuring is expected (Porras-Hurtado et al. 2013), which is likely given the history of small population size, intense inbreeding, and translocations experienced by the black robin. Each of the tomtit (n = 52) and black robin data sets (n = 145) were analysed with K = 1–3 to test for between-island differentiation, allowing identification of potential localised structuring within islands. The subset of black robins from separate bush areas on Rangatira was run separately with K = 1–2 to determine the presence of any substructuring between individuals in the two forest areas. Analysis for each value of K was repeated twenty times to obtain means and standard errors. Results were visualised using STRUCTUREHARVESTER ver. 0.6.94 (Earl and von Holdt 2012). Comparison of mean log-likelihoods and variance of the range of K values was used to determine the most likely number of clusters present, with the highest value indicating the most likely K. Independent runs were combined using CLUMPP ver. 1.1.2 (Jakobsson and Rosenberg 2007) and visualised with DISTRUCT ver. 1.1 (Rosenberg 2004).

All loci were tested for the presence of null alleles using MICROCHECKER ver. 2.2.3 (Van Oosterhout et al. 2004), with a confidence interval of 95% and 10,000 randomisations. Tests for deviations from Hardy–Weinberg equilibrium (HWE) and significant linkage disequilibrium (LD) were performed for each island population using ARLEQUIN version 3.5 (Excoffier and Lischer 2010). ARLEQUIN default parameters of 1,000,000 steps in the Markov Chain and 100,000 dememorisation steps were used for HWE, and 10,000 permutations for LD. These tests were corrected for multiple comparisons using the Benjamini–Yekutieli (B–Y) correction to provide a more conservative Type I error rate that is more appropriate for conservation genetic studies (Narum 2006).

Genetic diversity was quantified by calculating the allele frequencies using GENALEX ver. 6.5 (Peakall and Smouse 2006, 2012), rarefied number of alleles using HP-RARE version 1.0 (Kalinowski 2005) to standardise for variation in sample sizes (Leberg 2002), and expected and observed heterozygosities using GENALEX (Peakall and Smouse 2006, 2012). Population differentiation within and among island populations was analysed by calculating F_{ST} and F'_{ST} (F_{ST} standardised for within-population variance; Hedrick 2005) in GENALEX (Peakall and Smouse 2006, 2012) using 9999 permutations.

STRUCTURE ver. 2.3.4 (Pritchard et al. 2000) was used to test for the presence of dispersers in both tomtit and robin populations, with island populations predefined as independent clusters (K = 2), using a burn-in of 10,000 followed by 100,000 iterations. This was repeated ten times for each species to obtain mean values. Although no black robin dispersers have been detected from observations, it is important to determine whether any dispersal events have occurred that may allow natural gene flow between these islands. While hatching locations of black robins are available for some individuals from Rangatira Island during the 2007–2011 breeding seasons, all individuals (204

black robins, 52 tomtits) were included here regardless of known hatching location. Only a subset of individuals were sampled in each year, so inclusion of individuals regardless of hatching location may allow detection of individuals descended from dispersing individuals when samples from parents were unavailable. STRUCTURE identifies dispersers or their descendants as individuals having a low (<50 %) probability of being from the assumed population.

Results

The final set of loci used comprised 15 loci in black robins and 17 in tomtits (Table 1). The genotyping error rate was estimated at 1.42%. The Bayesian STRUCTURE analysis identified the presence of two distinct clusters among both black robins and tomtits (Table 2; Figs. 1, 2). In both species, all Mangere individuals formed one cluster, and all Rangatira individuals formed a second cluster. No evidence of structuring was detected within the Rangatira robin population (most likely K = 1), with all robins clustering as a single group, irrespective of the bush areas they inhabited (Table 2).

Following B–Y correction, one locus significantly deviated from HWE in the Rangatira black robin population (Table 3). No loci showed significant deviation in the Mangere black robin population. In tomtits, two loci significantly deviated from HWE in the Rangatira population, but none deviated from HWE in the Mangere population (Table 4). In black robins, 14 pairs of loci exhibited significant LD on Rangatira, while eight pairs showed LD on Mangere. However, only one pair appeared significantly linked in both populations (Supplementary Table S1). In the tomtit populations, significant LD was found in three pairs of loci in the Rangatira population, and in nine pairs in the Mangere population. Two of these pairs appeared to be significantly linked in both populations (Supplementary Table S1). While a substantial number of loci appeared linked in the black robin, the very low number that appeared linked in both populations or both species indicates that there are few pairs likely to be physically linked, with many likely to appear linked by chance. AMOVA analyses were carried out excluding the combinations of linked loci or those out of HWE. There was no clear difference in results, and all loci were used in the subsequent analyses to increase statistical power.

Comparisons of allelic diversity and heterozygosity show that tomtits have greater genetic diversity than black robins. The number of alleles in black robins ranged from two to five across all 15 polymorphic loci (average = 2.53 alleles per locus; Table 3), while in tomtits this ranged from two to ten across 17 polymorphic loci (average = 4.47 alleles per locus; Table 4). Tomtits displayed substantially higher variation than black robin for all measures at the eight shared polymorphic loci (Table 5). In tomtits, 14 of 17 loci contained more than two alleles, compared to the black robin where six of 15 loci had more than two alleles (Tables 3, 4). The Mangere populations of both species displayed generally lower diversity compared to the Rangatira populations in terms of number of alleles and expected heterozygosity for most loci (Tables 3, 4). A random sample of thirty individuals from Rangatira produced the same results (Supplementary Table S2). In the Rangatira black robin population, five alleles were present at locus PT1.

Between the two species, there was a very high and significant level of differentiation at the eight shared loci ($F_{ST} = 0.490$, $F'_{ST} = 0.938$, $P \leq 0.001$). There was a moderate level of differentiation between the tomtit populations on Rangatira and Mangere, $F_{ST} = 0.102$, $F'_{ST} = 0.228$, $P \leq 0.001$ (17 loci). For the Rangatira and Mangere black robin populations differentiation was moderate and significant $F_{ST} = 0.121$, $F'_{ST} = 0.205$, $P \leq 0.001$ (15 loci). These results support the presence of two independent clusters among both species as determined by cluster analysis. There was no significant genetic

Table 2 Results of cluster analysis

	K	Repeats	Mean LnP (K)	SD LnP (K)
Black robins (n = 145)	1	20	−2704.64	0.059
	2	**20**	**−2578.45**	**0.376**
	3	20	−2646.71	59.907
Rangatira black robins (n = 108)	**1**	**20**	**−1939.18**	**0.145**
	2	20	−2022.69	28.418
Tomtits (n = 52)	1	20	−1936.17	0.491
	2	**20**	**−1799.57**	**1.910**
	3	20	−1893.74	32.680

Means and standard deviations were calculated using STRUCTUREHARVESTER based on the results of STRUCTURE runs. Values in bold represent the most likely true K

K number of clusters

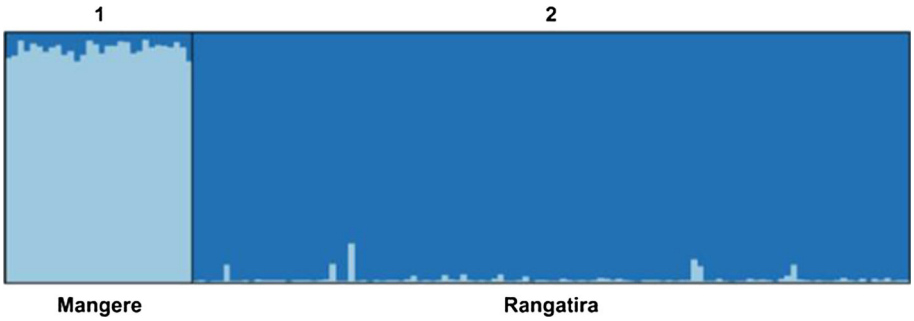

Fig. 1 DISTRUCT visualisation of clustering in black robins. Each individual (n = 145) is represented by a *vertical bar* partitioned into two *coloured segments* according to the proportion of membership in each cluster (K = 2)

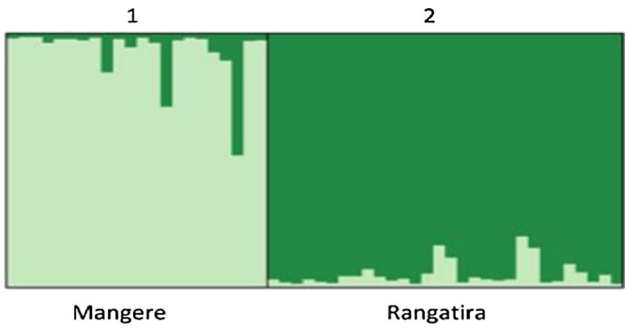

Fig. 2 DISTRUCT visualisation of clustering in tomtits. Each individual (n = 52) is represented by a *vertical bar* partitioned into two *coloured segments* according to the proportion of membership in each cluster (K = 2)

differentiation between Woolshed Bush and Top Bush black robins on Rangatira Island (F_{ST} = 0.003, P = 0.689).

Tests for the presence of dispersing individuals in STRUCTURE ver. 2.3.4 (Pritchard et al. 2000) identified one individual as a likely descendant of a disperser between island populations in the Mangere tomtit population (Table 6). This individual possesses alleles rare in the Mangere population but that are common in the Rangatira population. Four black robins were detected in the Rangatira black robin population as likely to have descended from dispersers (Table 6). This was due to all four individuals having an allele at locus PT26 that is only otherwise found in the Mangere population.

Table 3 Allelic diversity and heterozygosity of black robin populations on Rangatira and Mangere islands

Locus	Rangatira					Mangere				
	n	A	AR	H_O	H_E	n	A	AR	H_O	H_E
PT37	115	2	1.92	0.261	0.253	30	1	1.00	–	–
PT18	114	2	2.00	0.360	0.417	29	2	2.00	0.379	0.470
PT2	115	3	2.09	0.348	0.330	28	2	2.00	0.357	0.416
PT26	113	3	1.20	0.027	0.026	30	2	2.00	0.667	0.472
PT7	114	3	2.88	0.614	0.604	30	3	2.92	0.633	0.597
PT10	115	2	2.00	0.417	0.470	27	2	2.00	0.370	0.492
PT39	115	2	2.00	0.357	0.435	30	2	1.44	0.067	0.066
PT27	115	3	2.22	0.417	**0.382**	27	2	1.91	0.259	0.230
PT40	113	2	2.00	0.504	0.495	30	2	2.00	0.533	0.452
PT1	114	5	3.22	0.693	0.657	30	4	3.36	0.767	0.640
PT38	115	2	1.93	0.226	0.265	30	2	2.00	0.467	0.506
PAU26	115	2	1.99	0.391	0.365	30	2	1.70	0.133	0.127
TG-02	114	2	2.00	0.404	0.413	30	2	1.94	0.233	0.259
PCA12	88	2	2.00	0.466	0.492	30	2	2.00	0.400	0.472
PGM1	98	4	2.14	0.429	0.382	30	2	1.84	0.200	0.183
Average	111.5	2.60	2.10	0.394	0.399	29.4	2.13	2.01	0.390	0.384
SE	2.012	0.235	0.116	0.040	0.039	0.270	0.165	0.140	0.055	0.048

H_E values in bold represent significant deviation from HWE

n number of individuals sampled, *A* number of alleles per locus, *AR* allelic richness per locus (in terms of rarefied number of alleles), H_O observed heterozygosity, H_E heterozygosity expected under Hardy–Weinberg equilibrium, *SE* standard error

Table 4 Allelic diversity and heterozygosity of tomtit populations on Rangatira and Mangere islands

Locus	Rangatira					Mangere				
	n	nA	AR	H_O	H_E	n	nA	AR	H_O	H_E
PT37	30	2	2.00	0.400	0.506	22	2	1.63	0.000	0.089
PT18	30	3	3.00	0.600	0.677	22	3	2.93	0.636	0.601
PT2	30	6	4.48	0.700	0.754	22	4	3.87	0.591	0.730
PT26	30	4	3.82	0.467	0.681	22	4	3.02	0.591	0.574
PT7	30	5	4.25	0.800	0.755	22	4	3.62	0.773	0.665
PT10	30	2	1.99	0.400	0.325	22	2	2.00	0.500	0.485
PT39	30	2	1.99	0.367	0.345	22	2	2.00	0.364	0.406
PT27	28	7	5.28	0.714	0.690	22	2	2.00	0.318	0.426
PT19	30	7	5.58	0.833	0.812	22	5	4.79	0.818	0.745
PT5	30	3	2.64	0.433	0.515	22	2	1.39	0.045	0.045
PT9	30	4	3.62	0.300	**0.488**	22	2	2.00	0.409	0.426
PT35	30	3	2.89	0.467	0.453	22	2	1.87	0.182	0.169
PT6	30	5	3.52	0.467	0.563	22	3	2.39	0.500	0.475
PT12	30	4	3.60	0.567	0.610	22	3	2.87	0.545	0.506
PT25	29	10	6.10	0.586	0.727	22	5	3.77	0.591	0.691
PT4	29	6	4.50	0.897	0.753	21	6	4.79	0.857	0.688
PT24	30	3	2.99	0.200	**0.640**	22	3	2.39	0.364	0.489
Average	29.76	4.47	3.66	0.541	0.605	21.94	3.18	2.78	0.476	0.483
SE	0.136	0.529	0.303	0.047	0.036	0.059	0.312	0.256	0.060	0.052

H_E values in bold represent significant deviation from HWE

n number of individuals sampled, *A* number of alleles per locus, *AR* allelic richness per locus (in terms of rarefied number of alleles), H_O observed heterozygosity, H_E heterozygosity expected under Hardy–Weinberg equilibrium, *SE* standard error

Table 5 Direct comparison of diversity between the Chatham Island tomtit and the Chatham Island black robin, for eight loci that were polymorphic in both species, pooled across both island populations for each species

Locus	Tomtits					Black robins				
	n	A	AR	H_O	H_E	n	A	AR	H_O	H_E
PT37	52	2	1.94	0.231	**0.414**	145	2	1.64	0.207	0.208
PT18	52	3	2.85	0.615	0.656	143	2	1.95	0.364	0.428
PT2	52	6	3.59	0.654	0.740	143	3	1.91	0.350	0.348
PT26	52	4	3.29	0.519	**0.681**	143	3	1.73	0.161	**0.247**
PT7	52	5	3.61	0.788	0.747	144	3	2.75	0.618	0.621
PT10	52	2	1.93	0.442	0.406	143	2	1.99	0.406	0.486
PT39	52	2	1.90	0.365	0.369	145	2	1.91	0.297	**0.385**
PT27	50	7	3.26	0.540	0.599	142	3	1.96	0.387	**0.356**
Average	51.8	3.88	2.80	0.519	0.576	118.5	2.50	1.98	0.349	0.385
SE	0.250	0.693	0.268	0.062	0.055	0.567	0.189	0.118	0.049	0.046

H_E values in bold represent significant deviation from HWE

n number of individuals sampled, *A* number of alleles per locus, *AR* allelic richness per locus (in terms of rarefied number of alleles), H_O observed heterozygosity, H_E heterozygosity expected under Hardy–Weinberg equilibrium, *SE* standard error

Discussion

While the level of genetic diversity in terms of heterozygosity and number of alleles found in the Chatham Island black robin is lower than that of its sympatric sister-species, the Chatham Island tomtit, the level of microsatellite diversity in the black robin is higher than that reported in six other New Zealand species of birds (Supplementary Table S3). Given the extreme population bottleneck experienced by black robins, this result is

Table 6 Identification of potential descendants of dispersing individuals from STRUCTURE analysis

Individual	Own
A104705	0.208 ± 0.002
B109427	0.330 ± 0.001
B98938	0.317 ± 0.001
B109665	0.297 ± 0.001
B81403	0.388 ± 0.001
Average A	0.936 ± 0.007
Average B	0.956 ± 0.021

Own = mean probability (±standard error) individual derived from the sampled population. All probabilities were averaged across ten repeats. Average A/B = mean probability individual derived from the sampled population across all black robin/tomtit individuals

B black robin individual, followed by individual band number, *A* tomtit individual

somewhat unexpected, but may be at least partially explained by the use of Illumina sequencing to develop markers (Almojil et al. 2016). This method allowed for a very large number of candidate microsatellites to be assessed, and for the preferential selection of loci that were likely to be highly variable, more so than is possible with the traditional enriched library method, or 454 sequencing (Castoe et al. 2012). Thus it is likely that the relatively high level of variation in the black robin is in part due to the ability to preferentially select for highly variable loci.

Five of the New Zealand species exhibiting lower heterozygosity than the black robin have experienced range reductions or population fragmentation, and conservation management has included translocations of small numbers to areas within the historic range or predator-free islands (Andrews et al. 2013; Baker et al. 2010; Boessenkool et al. 2007; Grueber et al. 2008; Tracy and Jamieson 2011). The black robin has the lowest mean number of alleles reported for any New Zealand bird species except the takahe (*Porphyrio hochstetteri*), which has experienced a severe bottleneck and exists as a highly fragmented population (Grueber et al. 2008), and the Chatham Island snipe (*Coenocorypha pusilla*) (Baker et al. 2010), which has recovered in size following substantial range contraction. Although the Chatham Island tomtit has an IUCN Red List ranking of Least Concern (BirdLife International 2016), it has similar levels of diversity when compared to species that are deemed to be more vulnerable among New Zealand birds, and other passerine species.

The reduction in population size and extirpation from parts of its historic range have likely reduced genetic diversity of the Chatham Island tomtit, though to a lesser degree than in the black robin. These markers were developed for black robins, so the ascertainment bias should result in a greater level of diversity at these loci in the black robin compared to the tomtit. However the results show that the strong impact of random genetic drift over the population history of the black robin has outweighed any effect of the ascertainment bias that would typically result in reduced diversity measured in non-target species. The lower variation in the smaller Mangere populations of both species clearly illustrates how the effects of drift are greatest in small populations, resulting in greater loss of alleles and reduced heterozygosity than in the larger Rangatira populations.

The presence of more than four alleles at one locus (PT1) in the black robin was not expected. At the time of the population bottleneck in 1980, there were five remaining individuals, only two of which were successful in raising offspring that survived and reproduced. The maximum possible number of alleles that can be passed on through a single-pair bottleneck in a diploid species is four, two from each parent. There are three possible scenarios that may explain the presence of more than four alleles. Firstly and most likely, is via mutation, as there were no other populations available to allow gene flow via dispersal. Secondly, there may have been unknown black robin individuals remaining on Mangere Island at the time of the translocation of the species from Little Mangere, and that may have bred with any of the five translocated individuals, and this could have resulted in more than four alleles being passed on in the population. However, this is extremely unlikely as no black robins were observed on Mangere at any time prior to the translocation, and no unbanded individuals were observed following the translocation of the species to Mangere. The third possibility is that there may have been a low level of extra-pair copulation occurring within the remnant population, where the sole breeding female, Old Blue, may have engaged in copulations with males other than her known partner. However, there was only one other male aside from the partner of Old Blue until the population began to grow and no evidence of extra-pair copulations has ever been observed in the black robin. Extra-pair copulations are common among many bird species (Griffith et al. 2002), and this is a possibility that needs further investigating in black robins.

The black robin population on Rangatira has been isolated for 26 years since establishment from a small subset of the Mangere population, which is derived from a single source population that survived for eighty years as a small population. In this study, we found that there is substantial differentiation between the two island populations. Similarly, substantial differentiation was found between tomtit populations on Rangatira and Mangere. This illustrates how strong genetic drift can lead to differentiation in a

relatively short time, even between populations with relatively low levels of genetic diversity. Sufficient variation was still present in the very small black robin population to allow such strong and rapid differentiation. While both island populations of robin remain small and will experience ongoing genetic drift, the lower level of diversity on Mangere indicates drift continues to have a stronger effect on the Mangere population, resulting in lower diversity than the Rangatira population, even though the Rangatira population was founded from a subset of the Mangere population. This may indicate that the Mangere population is particularly vulnerable to extinction due to continued loss of diversity and inbreeding in this closed population.

Two recent studies (Kennedy et al. 2014; Weiser et al. 2016) have treated the Rangatira population as two separate populations inhabiting different forested areas. We found there is a lack of evidence for genetic differentiation between the two forest populations. Both cluster analysis and comparison of differentiation between individuals in these forest areas found no significant genetic differentiation between Woolshed and Top Bush. The suggestion was initially made due to the potential for the presence of the avian predator, the brown skua (*Catharacta skua lonnbergi*), and an aversion of flying across open spaces to limit dispersal, in addition to noticeable differences in forest type and black robin demography in terms of breeding success and population growth rates (Kennedy 2009). The genetic data presented here, in combination with evidence from a study of dispersal (Paris et al. 2016), show that Skua Gully is not a barrier to dispersal. Thus there is no need to distinguish between individuals inhabiting these forest patches for future analyses.

It was hypothesised that the tomtits may be able to use Pitt Island as a stepping stone allowing dispersal between all three islands, but the results here indicate a similar level of differentiation between the Mangere and Rangatira tomtit populations compared with that measured between the isolated black robin populations. The presence of only a single bird in the Mangere tomtit population that is identified as a potential descendant of a disperser indicates that while dispersal between the islands may be possible, it appears to have only occurred historically at a very low frequency. Future research including tomtit samples from Pitt Island would be beneficial to compare levels of diversity and differentiation between all three populations across the species range.

The results of analysis of dispersal for the black robin populations revealed four individuals with mixed ancestry in the Rangatira population, but no true dispersers. This provides evidence that there is no naturally occurring dispersal between the two island populations, as expected given that robins avoid flying across open areas, and there is substantial differentiation between these populations. Any gene flow between the populations would prevent populations from differentiating, as alleles that may drift to a low frequency in one population could be restored through dispersal of individuals from the second population. The four individuals indicated as descendants of dispersing birds were identified as such because they all share an allele that is only found in these four individuals on Rangatira, while this allele is common within the Mangere population. This allele is most likely a remnant from the initial population establishment on Rangatira, occurring at low frequency in the modern population. The presence of such an allele within the population is indicative of the strength and random nature of genetic drift, such that in one population an allele has drifted to a high frequency, yet in the other population, it has drifted to an extremely low frequency.

The presence of distinct alleles in one black robin population that do not occur in the other population (i.e., private alleles) indicates that sequential reciprocal translocations of individuals between islands may assist in reinforcing diversity within both populations. Individuals with low levels of relatedness should be preferentially selected for transfer. Such individuals are more likely to carry novel alleles at loci throughout the genome, including at loci associated with fitness, and so should improve average fitness as these alleles spread into the new population, and encourage population growth. These island habitats are similar and so it is unlikely that individuals have adaptive traits that are more advantageous on one island than the other.

However, the population on Mangere appears to be at carrying capacity (Kennedy 2009). This indicates the benefits of translocating individuals to Mangere may be limited, as insufficient habitat may limit population growth and the effective spread of new variation through the population. Therefore, in addition to reciprocal translocations, the establishment of a third population is recommended, using individuals sourced from both Rangatira and Mangere, and for repeated translocation events to maximise genetic variation in the new population. This third population would act as further insurance against catastrophic events, allow significant population growth beyond what is currently possible, and reduce extinction risk of the species. The larger combined source populations would allow a substantial number of individuals to be used for establishment while minimising risks to both existing populations. Further analyses will be required to determine sufficient numbers to establish a self-sustaining population, and the size and frequency of sequential translocations.

The potential for establishment of a third self-sustaining population on Pitt Island (6325 ha) was discussed in the 2001–2011 Black Robin Recovery Plan (Department of Conservation 2001), and an attempt was made to establish such a population by translocating individuals to a predator-

free fenced area (the Ellen Elizabeth Preece Conservation Covenant) between 2002 and 2004 (Kennedy 2009). However, all 34 robins translocated had died or disappeared by the end of 2007 with no clear cause, and no further translocations have been attempted (Kennedy 2009). Future attempts are dependent on the removal of invasive predators, most notably cats. Ultimately, establishment of an additional population would reduce the extinction risk of the Chatham Island black robin, as it would be more resilient in both the short-term where it will be less vulnerable to population fluctuations and stochastic events, and the long-term where maintaining diversity will ensure greater evolutionary potential to allow the species to adapt to future change.

In summary, the Chatham Island black robin has low genetic diversity due to its history as a small population, leading eventually to the extreme bottleneck event in 1980. The two island populations have low levels of genetic diversity, and have differentiated from one another over 26 years of isolation due to strong genetic drift acting independently on each population. The findings from this study will be used to assist the New Zealand Department of Conservation to develop future management plans for the iconic Chatham Island black robin. The best course of action to conserve the remaining genetic diversity and maximise evolutionary potential would be to establish a third population of Chatham Island black robins, to allow substantial population growth. At the minimum, reciprocal translocations of birds should be carried out between the two island populations to reinforce the standing level of genetic diversity, and reduce the extinction risk for this endangered species.

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