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# Genetic similarity of Hutton's shearwaters (*Puffinus huttoni*) from two relict breeding populations

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**Abstract** Hutton's shearwater (*Puffinus huttoni*) currently breeds only in 2 colonies in the Seaward Kaikoura mountains, South Island, New Zealand. Conservation measures now include re-locating young to establish a new low altitude colony. To assess the genetic similarity of birds breeding in the 2 colonies as a basis for decisions on sourcing recruits to the present and potentially other new colonies, we genotyped 9 microsatellite loci, with 3-13 alleles, in 30 birds from the Kowhai River catchment colony and 29 from Shearwater Stream. There was no significant population genetic differentiation between the 2 sampling locations. Our results suggest that there would be little genetic risk to mixing birds from both relict colonies in newly established colonies. Future analyses of the former distributions of Hutton's shearwater, the fluttering shearwater (*P. gavia*), and the extinct Scarlett's shearwater (*P. spelaeus*) will require an analysis of the levels of genetic similarity between birds from the relict colonies and those of former, widely separated colonies.

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**Keywords** genetic similarity; Hutton's shearwater; relict populations; New Zealand; *Puffinus huttoni*

## INTRODUCTION

Hutton's shearwater (*Puffinus huttoni*) was described in 1912 but its breeding colonies high in the Seaward Kaikoura Range were not mapped until the middle of the 20th century (Harrow 1965). Surveys conducted from 1965 onwards (Harrow 1965, 1976; Sherley 1992) confirmed the presence of colonies in 2 catchments (Kowhai River and Shearwater Stream) on the eastern side of the mountains, and the likelihood that colonies were formerly present in other catchments of both the Seaward and Inland Kaikoura Ranges within the previous century.

The apparent decline in distribution – and therefore in numbers – of the species led to further analysis of possible causes (*e.g.,* Sherley 1992; Sommer *et al*. 2009), and to active conservation measures. These included trapping of stoats

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(*Mustela erminea*) and shooting feral pigs (*Sus scrofa*) to protect the birds in their natural colonies, and from 2005, the translocation of chicks to establish a new colony at low altitude on Kaikoura Peninsula, *c.* 20 km south of the Kowhai River colonies. The establishment of this colony raised the question as to the genetic similarity of birds in the 2 remaining natural colony areas, which are < 20 km apart. In this study, we used 9 microsatellite loci to determine whether individuals sampled in the Kowhai River and Shearwater Stream colonies were drawn from a single panmictic population, or from 2 genetically distinct populations.

## METHODS

*Sample collection*

Blood was collected from individuals caught in the Kowhai River catchment ($n$ = 30) and Shearwater Stream ($n$ = 29) via brachial venipuncture, with *c.* 1 – 2 drops of blood spotted onto filter paper. Blood spots were dried and stored in 1.5 mL microtubes at -20°C until DNA extraction.

*Microsatellite genotyping*

DNA was extracted from the dried blood spots on filter paper using a PureLink Genomic DNA kit (Invitrogen) following manufacturer's instructions. Six microsatellite loci originally developed for the Balearic shearwater (*Puffinus mauretanicus*) (Puff4B, PuffG2C, PuffG11F, PuffG2F, PuffPM2 and PuffC5D; Gonzalez *et al.* 2009) and 3 microsatellite loci originally developed for the flesh-footed shearwater (*Puffinus carneipes*) (PcD109, PcD103 and PcD3; Hardesty *et al.* 2013) were amplified for each individual using the following PCR conditions. Each locus was amplified singly in a total reaction volume of 15 μL consisting of: 1x *Taq* buffer (Bioline), 2 mM $MgCl_2$, 0.08 mM each dNTP, 0.33 μM each primer, 0.6 U BioTaq (Bioline) and 0.5 μL DNA extract (containing 5 ng – 120 ng DNA). The forward primer of each locus was labelled with a fluorescent dye: 6-FAM, VIC, Ned or PET (Applied Biosystems; Table 1).

PCR cycling conditions were: 95°C for 12 min, followed by 10 cycles of 94°C for 15 s, $T_a$ °C (Table 1) for 30 s, 72°C for 30 s, followed by 30 cycles of 89°C for 15 s, $T_a$°C (Table 1) for 30 s, 72°C for 30 s, followed by a final extension of 72°C for 10 min. PCR products were combined into 2 sets for genotyping (Table 1) and 0.5 μL of each PCR product was added to 0.3 μL GS LIZ500 size standard (Applied Biosystems) and 12 μL HiDi formamide for each set, then denatured at 95°C for 5 min, prior to capillary separation on an ABI 3130xl Genetic Analyser. Alleles were visualised and sized using GeneMarker v2.6.1 (SoftGenetics LLC®).

**Table 1.** Microsatellite loci amplified and genotyped. $T_a$ = annealing temperature. Dye = fluorescent label on the 5′ end of the forward primer, 'Set' shows which loci were pooled together in the same well for genotyping. bp, base pairs.

| Locus | $T_a$ (°C) | Dye | Set | Allele size range (bp) |
|---|---|---|---|---|
| PcD109 | 56 | VIC | A | 132 – 148 |
| PuffPM2 | 56 | 6-FAM | A | 164 - 180 |
| Puff4B | 60 | NED | A | 160 - 202 |
| PcD3 | 60 | 6-FAM | A | 248 - 272 |
| PuffG2C | 56 | VIC | B | 148 - 174 |
| PuffC5D | 60 | 6-FAM | B | 168 - 198 |
| PuffG11F | 60 | NED | B | 170 - 192 |
| PuffG2F | 60 | PET | B | 210 - 227 |
| PcD103 | 56 | VIC | B | 178 - 202 |

*Data analysis*

Genetic diversity indices (the number of alleles per locus, $N_a$, and expected heterozygosity, $H_E$), the inbreeding coefficient ($F_{IS}$), and deviations from Hardy-Weinberg Equilibrium (HWE) were calculated for each sampling location using GenAlEx v.6.5 (Peakall & Smouse 2012). Genetic differentiation ($F_{ST}$) among the 2 sampling locations was calculated via Analysis of Molecular Variance (AMOVA) using GenAlEx v.6.5, from all 9 loci, and again after removal of loci that were significantly out of HWE in both sampling locations after Bonferroni correction for multiple comparisons (Rice 1989). The mean probability of all the genotype data comprising a single population (K = 1), as well as the mean probability of the genotype data representing 2 populations (K = 2), was calculated in Structure v 2.3.3 (Pritchard *et al.* 2000), using the admixture ancestry model, no prior population information and correlated allele frequencies, with a burn-in period of 5,000 reps, followed by 10,000 MCMC reps. Each scenario (K = 1 and K = 2) was run 5 times and the mean log probability of the data (ln P(D)) and standard error were then calculated.

## RESULTS

All 9 microsatellite loci were polymorphic in both sampling locations, with the number of alleles per locus ranging from 3 to 13 (Table 2). Two loci (PuffG11F and PuffPM2) were significantly out of HWE in both sampling locations after Bonferroni correction, with both loci showing a substantial deficit of heterozygotes in both locations (Table 2). However, as all individuals amplified a PCR product at both these loci, this deficit of

**Table 2.** Genetic diversity at 9 microsatellite loci within the 2 sampling locations (Kowhai and Shearwater Stream). *n* = number of individuals genotyped, $N_a$ = number of alleles, $H_O$ = observed heterozygosity, $H_E$ = heterozygosity expected under Hardy-Weinberg Equilibrium, $F_{IS}$ = the inbreeding coefficient. * = locus is significantly out of Hardy-Weinberg equilibrium after Bonferroni correction.

| | Location of colony | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Kowhai Stream | | | | | Shearwater Stream | | | | |
| Locus | *n* | $N_a$ | $H_O$ | $H_E$ | $F_{IS}$ | *n* | $N_a$ | $H_O$ | $H_E$ | $F_{IS}$ |
| PcD109 | 30 | 4 | 0.500 | 0.562 | 0.110 | 29 | 5 | 0.379 | 0.379 | 0.000 |
| Puff4B | 30 | 5 | 0.133 | 0.128 | -0.043 | 29 | 6 | 0.207 | 0.194 | -0.067 |
| PuffG2C | 29 | 13 | 0.759 | 0.853 | 0.111 | 29 | 12 | 0.828 | 0.875 | 0.054 |
| PuffG11F | 30 | 6 | 0.233 | 0.609* | 0.617 | 29 | 5 | 0.241 | 0.517* | 0.533 |
| PuffG2F | 30 | 8 | 0.833 | 0.799 | -0.043 | 29 | 10 | 0.759 | 0.779 | 0.026 |
| PcD103 | 30 | 8 | 0.867 | 0.793 | -0.092 | 29 | 7 | 0.655 | 0.771* | 0.150 |
| PuffPM2 | 30 | 5 | 0.167 | 0.346* | 0.518 | 29 | 6 | 0.069 | 0.402* | 0.829 |
| PcD3 | 30 | 3 | 0.067 | 0.065 | -0.026 | 29 | 5 | 0.138 | 0.163* | 0.153 |
| PuffC5D | 30 | 10 | 0.867 | 0.844 | -0.027 | 29 | 10 | 0.759 | 0.822 | 0.077 |
| Mean | | 6.89 | 0.492 | 0.555 | 0.125 | | 7.33 | 0.448 | 0.545 | 0.195 |

**Table 3.** Proportion of membership of each sampling location in each of the 2 inferred clusters, averaged over 5 runs for K = 2.

| Location | Cluster 1 | Cluster 2 |
|---|---|---|
| Kowhai | 0.504 | 0.496 |
| Shearwater Stream | 0.513 | 0.487 |

heterozygotes is unlikely to result from null alleles. The deficit is also unlikely to result from population sub-structuring within the sampling locations as most loci genotyped were in HWE, suggesting these 2 loci may possibly be linked to loci under selection.

There was no significant population genetic differentiation between the 2 sampling locations ($F_{ST}$ = -0.003, $P$ = 0.783). This result did not change when the 2 loci out of HWE (PuffG11F and PuffPM2) were excluded from the analysis ($F_{ST}$ = -0.002, $P$ = 0.604). The mean probability of the data comprising a single population (K = 1) was higher than the mean probability of the data comprising 2 populations (K = 2) (K = 1: mean Ln P(D) ± SE = -1390.64 ± 0.234; K = 2: -1398.74 ± 0.508). This result did not change when the 2 loci out of HWE (PuffG11F and PuffPM2) were excluded from the analysis (K = 1: mean Ln P(D) ± SE = -1160.38 ± 0.109; K = 2: -1166.50 ± 0.572). Assignment of individual genotypes to the 2 inferred clusters when K = 2 did not provide support for any genetic differentiation between the 2 sampling locations, as individuals from each sampling location were equally likely to be assigned to either of the 2 inferred clusters (Table 3, Fig. 1).

## DISCUSSION

The lack of any genetic differentiation between the 2 sampling locations, despite substantial variation at these 9 loci within locations, demonstrates that individuals breeding in the Kowhai River and Shearwater Stream colonies are effectively members of a single panmictic population. This conclusion is supported by the Structure analyses in which K = 1 was more probable than K = 2, and individuals in each sampling location were equally assigned to each cluster when K = 2. These results suggest that individuals from both sites could be transferred to the same artificial colony should that be necessary, without compromising the present genetic diversity of the species. However, as there may be differences not accessed by present methods between the 2 populations, introducing new stock from both colonies should not be undertaken as a matter of course.

The level of diversity found is comparable to that in other *Puffinus* species that have been genotyped with these microsatellite loci (Gonzalez *et al.* 2009; Hardesty *et al.* 2013). Ascertainment bias is expected to result in lower diversity when using microsatellite primers developed for another species (Selkoe & Toonen 2006), yet the 2 sampling locations of Hutton's shearwaters contain greater levels of diversity than the range-restricted Balearic shearwater (*Puffinus mauretanicus*, $H_E$ = 0.543, $N_a$ = 6.33) and the Yelkouan shearwater (*Puffinus*

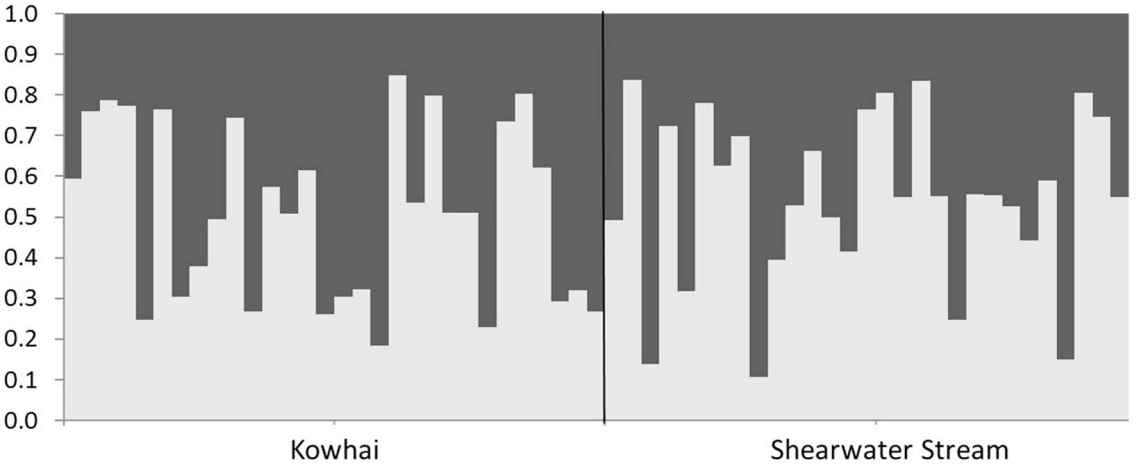


**Fig. 1.** Barplot of mean individual genotype assignments to each of 2 inferred clusters (dark grey, light grey) when K = 2. Each individual is represented by a single column broken into 2 shaded segments, with lengths proportional to the estimated membership coefficients for each of the 2 inferred clusters. Both sampling locations show a similar pattern, with all individuals being partially assigned to both inferred clusters. Thus the 2 inferred clusters do not represent the 2 sampling locations.

*yelkouan*, $H_E = 0.576$, $N_a = 5.17$) at loci developed for the Balearic shearwater (Gonzalez *et al.* 2009). The 2 Hutton's sampling locations showed only slightly lower diversity than the widespread flesh-footed shearwater (*Puffinus carneipes*, $H_E = 0.614$, $N_a = 6.33$) at loci developed for the flesh-footed shearwater (Hardesty *et al.* 2013). As a result there is no evidence that Hutton's shearwater populations have been through a strong genetic bottleneck.

Our analysis leaves open the question of possible genetic differences between birds from the known relict colonies and those in former colonies in both the Seaward and Inland Kaikoura Ranges. In the past, Hutton's shearwater may have enjoyed a wider breeding distribution in at least the South Island (Worthy & Holdaway 2002), but the slight morphological differences between their post-cranial skeletons makes it difficult to differentiate between fossil bones of Hutton's and fluttering (*P. gavia*) shearwaters, and often between bones of those and the extinct Scarlett's shearwater (*P. spelaeus*) (Holdaway & Worthy 1994). Advances in the extraction and analysis of genetic material from recent fossil bones could eventually clarify the former breeding distributions of these very similar species.

Hutton's shearwater was for many years taken to be a subspecies of the more widespread fluttering shearwater, which presently breeds on offshore islands from the Three Kings to the northwest of the North Island, south to Cook Strait (Heather & Robertson 1996). Analysis of former, mainland, breeding distributions of both Hutton's and fluttering shearwaters would require first an analysis of the levels of genetic similarity between birds from these widely separated colonies.

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