Broad-scale genetic population structure in blue duck *Hymenolaimus malacorhynchos*

Pilot study of mitochondrial genetic variation

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A B S T R A C T

Sequence data for the mitochondrial control region were used to investigate genetic distinction between North and South Island populations of blue duck *Hymenolaimus malacorhynchos*. Such information is crucial in assessing the appropriateness of inter-island translocations for supplementing managed populations. Using phylogenetic reconstruction, we found a monophyletic clade in the South Island, while levels of sequence variation were inadequate to resolve relationships among North Island individuals. Based on population genetic theory, we consider that the South Island clade is indicative of a lack of past inter-island connectivity. We suggest that additional sampling of blue duck populations is needed before inter-island translocation can be fully assessed as a management tool in blue duck conservation.

Keywords: blue duck, *Hymenolaimus malacorhynchos,* population structure, genetic differentiation, inter-island translocation, New Zealand.

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1. Introduction

The New Zealand blue duck *Hymenolaimus malacorhynchos* is an ancient form, having indeterminate taxonomic relationships within the waterfowl. Once widespread, the current distribution of blue duck is restricted and fragmented: it is now limited to a small number of river systems with minimal habitat modification in the North and South Islands (see Adams & Molloy 1997). As a whole, the species is not secure. Introduced predators and hydroelectric power generation are among the threats to the species (Adams & Molloy 1997). These threats are not uniform across the blue duck's distribution, resulting in varying productivity between river systems (Adams & Molloy 1997).

Studies of dispersal in blue duck are few. Available evidence suggests that individuals are occasionally exchanged between neighbouring river systems (Adams & Molloy 1997). Recently, a study by King et al. (2000), examining genetic variation in minisatellite DNA, concluded that productivity differences between rivers could result in populations of high productivity (sources) supplying recruits to populations of low productivity (sinks). For example, they suggest that low levels of genetic similarity coupled with the poor productivity of blue duck populations on the Tongariro River identify this river as a sink. The potential for a source/sink landscape across the blue duck's range raises the possibility of using translocations as a management measure to either supplement existing populations (i.e. sinks) or initiate new populations (i.e. Recovery Plan Objective 5: Adams & Molloy 1997). Indeed, previous attempts at translocation of wild and captive birds into the Mt Egmont National Park may have established a breeding population in that area (Adams & Molloy 1997).

As part of the current conservation management of the blue duck, the New Zealand Department of Conservation (DOC) approached us to examine the broad-scale genetic population structure of *H. malacorhynchos*. Specifically, DOC were interested in knowing whether the North and South Island blue duck populations are genetically distinct, so as to assess the appropriateness of inter-island translocations for supplementing managed populations, where the number of females is a limiting factor. We have examined sequence variation at the mitochondrial control region, using blue duck samples from river systems in the North and South Islands of New Zealand. The mitochondrial control region is the marker of choice for intra-specific genetic analyses because it is a fast evolving, maternally inherited marker, which is predicted to show marked differences between populations, especially where females are philopatric (e.g. Worthington Wilmer et al. 1994).

2. Methods

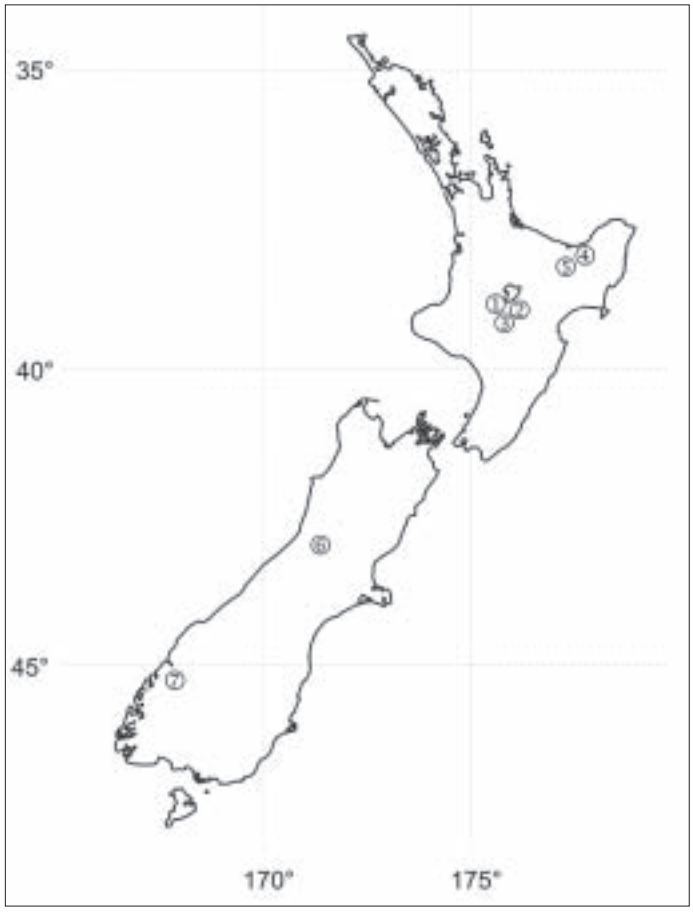
2.1 SAMPLES

Blood samples were obtained from blue ducks from four broad regions (Bay of Plenty, central North Island, central South Island, and Fiordland): we sequenced 10 individuals from seven separate localities within these regions (Fig. 1). Genomic DNA was extracted using a 5% chelex protocol (adapted from Walsh et al. 1991), as described in Robertson & Gemmell (2002).

2.2 PCR AMPLIFICATION AND SEQUENCING

Amplification of a partial section of the 5′ region of the mitochondrial control region was achieved by polymerase chain reaction (PCR) using primers designed from a Clustal alignment of Anatidae control region sequences obtained from GenBank: L-duck tRNA-glu, 5′-CTA CCC GAG ACC TAC GGC TCG AA-3′; H-Dbox-duck, 5′-ATA AAA GGA ACC AGA GGC GC-3′. PCRs were carried out in 25 µL reaction mixtures containing 2 µL template DNA, 10 pmol of each primer, 5 nmol of each dNTP, 500mM KCl, 100mM Tris-HCl, pH 9.0, 2 mg/mL BSA, 1.5mM $MgCl_2$ and 1 unit of *Taq* polymerase. For all reactions, the cycling parameters were an initial 3-minute denaturation of 94°C, followed by 35 cycles of 94°C/30 sec, 55°C/30 sec and 72°C/40 sec.

Figure 1. Localities of blue ducks examined.



REGION	LOCATION	NO. OF SAMPLES
Central Plateau	1. Whakapapa	3
	2. Tongariro	
	3. Mangatepopo	
Bay of Plenty	4. Whitikau	3
	5. Waioeka	
Arthur's Pass	6. Otira	2
Fiordland	7. Clinton	2
Total		10

Following amplification, the integrity and size of PCR products were examined using agarose gel electrophoresis and then the remaining products were electrophoresed in a 2% agarose gel. Bands were excised under low-intensity UV, soaked overnight in 0.5M ammonium acetate, phenol/chloroform extracted and finally precipitated with isopropanol/5mM LiCl. PCR products were sequenced using the H-Dbox-duck primer with an *Ampli*cycle cycle-sequencing kit (PE Applied Biosystems). The reaction conditions consisted of an initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C/30 sec, 64°C/30 sec, 72°C/1 min, and 1 cycle of 72°C/10 min. Sequencing reactions were run on 6% denaturing PAGE gels, exposed to Biomax MR autoradiography film (Kodak), and scored manually.

2.3 PHYLOGENETIC ANALYSIS

Individual sequences were aligned using Clustal W (Thompson et al. 1994), and then identical sequences were filtered and collapsed in the program BioEdit (Hall 1999). Maximum parsimony analyses were performed using the PAUP* package (Swofford 2000). Bootstrap analyses (Felsenstein 1985), based on 1000 replications, were performed within PAUP* to provide an estimate of the statistical significance of the maximum-parsimony tree topology.

3. Results and discussion

We have analysed 255 base pairs (bp) of nucleotide sequence located in the 3′-end of the first domain of the blue duck mitochondrial control region. Secondary DNA structures at the 5′ end of the control region prevented us from obtaining the complete sequence for the 577 bp fragment amplified. Persistent attempts to sequence through this region failed to obtain further readable sequence. Regardless, the sequence that we have obtained is sufficient to address the question posed in this study, that is, whether the North and South Island blue duck populations are genetically distinct.

We found a total of six haplotypes among the 10 individuals sequenced: two haplotypes were confined to the South Island sample and the remaining four haplotypes were only found in the North Island. The two haplotypes found in the South Island ducks differed by one nucleotide, but importantly both displayed 10 fixed nucleotide substitutions (Fig. 2) compared with all North Island halpotypes. Based on this variation, all South Island blue ducks formed a monophyletic clade with high bootstrap support (Fig. 3). Sequence variation among the North Island blue ducks was insufficient to resolve relationships: all individuals form an incompletely resolved clade. It is worth noting that one haplotype was present in both the Tongariro and Whanganui Rivers, which is consistent with the finding by King et al. (2000) that the Tongariro River is a sink population.

```
Whitikau BOP       AACGTATGGG CCTNAAGCTA GTCACATCGG ACATTATGTG CAAGGATTGC TGATTTC
Whitikau BOP       .......... .......... .......... .......... .......... .......
Waioeka BOP        .......... .......... .......... .......... .......... .......
Tongariro CP       .......... .......... .......... .......... .......... .......
Whanganui CP       .......... .......... .......... .......... .......... .......
Whakapapa CP       .......... .......... .......... .......... .......... .......
Arthur's Pass AP   .......... .......... .......... .......A.. .......... .......
Arthur's Pass AP   .......... .......... .......... .......A.. .......... .......
Clinton River FI   .......... .......... .......... .......A.. .......... .......
Clinton River FI   .......... .......... .......... .......A.. .......... .......

Whitikau BOP       TGAGGTGTAC GGCTAATAAA TCCATCTGGT ACGGAGCTTC ATGAGTTATG GGTAGGA
Whitikau BOP       .......... .......... .......... .......... .......... .......
Waioeka BOP        .......... .......... .......... .......... .......... .......
Tongariro CP       .......... .......... .......... .......... .......... .......
Whanganui CP       .......... .......... .......... .......... .......... .......
Whakapapa CP       .......... .......... .......... .......... .......... .......
Arthur's Pass AP   .......... .A........ .......... .......... .......... .......
Arthur's Pass AP   .......... .A........ .......... .......... .......... .......
Clinton River FI   .......... .A........ .......... .......... .......... .......
Clinton River FI   .......... .A........ .......... .......... .......... .......

Whitikau BOP       AGTAGTAGTT AGGGTATGTC CATCAAGCAT
Whitikau BOP       .......... .......... ..........
Waioeka BOP        .......... .......... ..........
Tongariro CP       .......... .......... ..........
Whanganui CP       .......... .......... ..........
Whakapapa CP       .......... .......... ..........
Arthur's Pass AP   .......... .......... ....G.....
Arthur's Pass AP   .......... .......... ....G.....
Clinton River FI   .......... .......... ....G.....
Clinton River FI   .......... .......... ....G.....
```

Figure 2. Representative DNA sequences from the 5′-end of *Hymenolaimus malacorhynchos* mitochondrial DNA control region (i.e. partial first domain). A dot refers to a similar nucleotide to one shown in the first sequence, whereas a letter refers to a nucleotide difference. Note the three nucleotide differences shared by all South Island ducks (i.e. fixed differences).

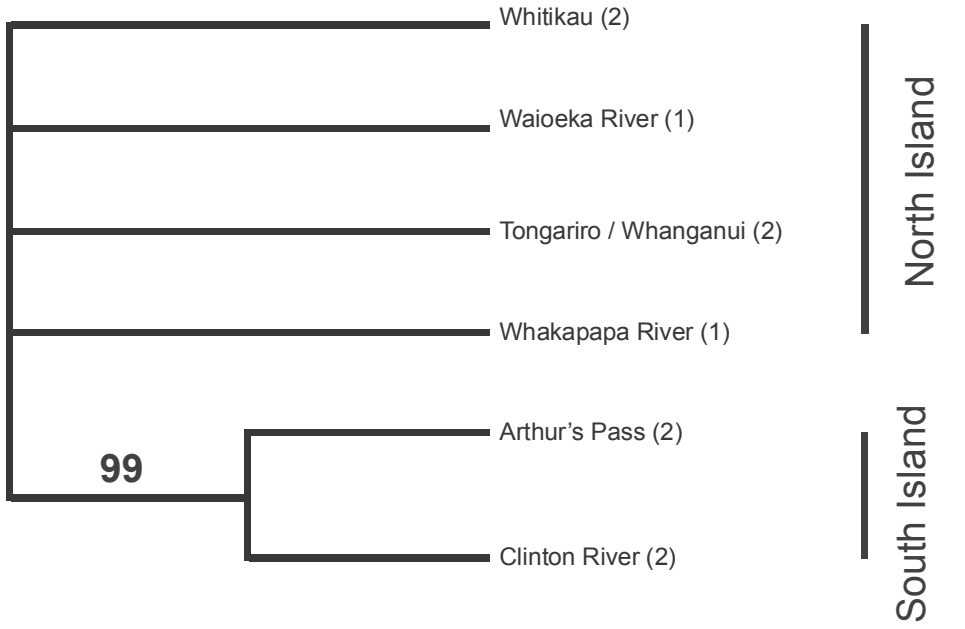


Figure 3. A maximum parsimony phylogram based on DNA haplotypes present in the 5′ end of *Hymenolaimus malacorhynchos* mitochondrial DNA control region (i.e. partial first domain). The number above the branch represents the percentage of bootstrap replicates (1000) where the same branching order was recovered. Branches with bootstrap values of less than 50% were collapsed. Numbers in brackets refer to the number of samples represented by each haplotype.

The presence of fixed differences in mtDNA haplotypes in the South Island blue duck suggests that female-mediated gene flow between the contemporary North and South Island populations (as sampled here) is negligible. Population genetic theory predicts that mtDNA haplotypes become monophyletic between $2N$ and $4N$ generations after a population has become genetically isolated, where N is the effective population size (Neigel & Avise 1986). Given this, two possibilities exists for blue duck: either the effective population size of blue ducks on both islands has been historically small (i.e. few females) or the South Island blue duck populations have been distinct from those on the North Island for a considerable period of time. Of these two scenarios, the latter seems more feasible, as available evidence suggest that blue duck were formerly more abundant (see Adams & Molloy 1997). The monophyly of blue ducks from Fiordland and Arthur's Pass suggests strong female-mediated gene flow through the river valleys of the Southern Alps. The similarity between these two locations may also be an example of a source/ sink dynamic, where the poor productivity of the Arthur's Pass population is a sink to the more productive river systems to the south.

A previous comparison of blue ducks from the North and South Islands using minisatellite DNA fingerprinting, a technique more suited to examining paternity and genealogy, found low levels of genetic similarity (i.e. akin to that found between unrelated individuals) at this broad scale of analysis (Triggs et al. 1992). Unfortunately, such levels of inter-island genetic similarity tell us little about inter-island distinctiveness; equivalent genetic similarity was noted between rivers within the same geographic region (see table 2 in Triggs et al. 1992). One point worth noting, however, is that Triggs et al. (1992) found that dispersal in the North Island appears to be confined mainly within river systems. Our finding of a monophyletic clade in the South Island suggests that dispersal, that is, female-mediated gene flow, may be dissimilar to what is occurring in the North Island. Perhaps this finding reflects less destruction of dispersal corridors in the South Island, particularly along the Southern Alps. Our finding of poorly resolved relationships among the North Island individuals may also be attributed to an inter-island difference in dispersal. This point, however, is speculative and requires further investigation.

The South Island individuals examined in this study fulfil the definition of an evolutionary significant unit (ESU), i.e. a historically isolated and independently evolving population (Moritz 1994). One school of thought when setting conservation priorities is to maintain ESUs, thereby maintaining evolutionary processes, not just phenotypic variants (e.g. Moritz 1999). Taking this process-orientated approach to conservation suggests that individuals should not be translocated between ESUs (Moritz 1999). If we take this stance for blue duck conservation, inter-island translocations should not be used to supplement managed blue duck populations. Indeed, there are potential costs associated with translocating individuals into historically isolated populations, including reduced viability due to disruption of local adaptation and genetic incompatibility. Current evidence, however, suggests that for the most part mixing of gene pools can have beneficial outcomes (Moritz 1999), including improved hybrid vigour and increased genetic diversity. Whether to use translocation as a conservation management tool, then, rests on the trade-off between the associated costs and benefits and should not be dismissed lightly

(Moritz 1999). In the case of a species that has a fragmented distribution and ongoing local extinctions—which is the case in the blue duck—the benefits of translocation *may* out weight the costs.

Although the South Island individuals examined represent a monophyletic clade, sampling limitations mean we cannot conclude that all blue duck in the South Island belong to this clade. Populations of blue duck exist in Golden Bay (i.e. Takaka and Motueka) and the Marlborough Sounds (i.e. Pelorus River) for which DNA samples are currently unavailable. The possibility of North Island haplotypes being present in these South Island locations cannot be ruled out. In this regard, the present study must be considered as preliminary only. Further research is needed to determine if the Golden Bay and Marlborough populations of blue duck are also distinct from the North Island duck populations. A further avenue of investigation worth pursuing, but beyond the scope of this study, is to focus on the complex relationships among the North Island populations (e.g. Triggs et al. 1992; King et al. 2000).

4. Acknowledgements

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