Geographic differentiation in the Australasian great crested grebe (*Podiceps cristatus australis*)

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ABSTRACT

Great crested grebes are designated as a single subspecies in Australia and New Zealand (*Podiceps cristatus australis*). The presence of sub-fossil remains found in the Taupo region indicates that the species has been in New Zealand since the Holocene (> 10 000 years BP), and it is possible that subsequent gene flow into New Zealand from Australia has occurred via the prevailing westerly wind patterns. There has been a dramatic decline in the New Zealand crested grebe population, and in order to assign a conservation priority to it, it was necessary to find out how genetically distinct it is from the Australian population. We examined sequence variation in a 265 base pair fragment of the mitochondrial cytochrome b gene using samples from the South Island of New Zealand (11 localities) and south-east Australia (7 localities). We found only one haplotype present in New Zealand, and this was also the dominant haplotype in Australia. We conclude that the New Zealand form of great crested grebe is not a distinct subspecies of *P. cristatus*; it is a population of the Pan-Australasian subspecies *P. c. australis*.

Keywords: great crested grebe, *Podiceps cristatus*, genetic differentiation, distribution, haplotype.

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1. Introduction

The great crested grebe, *Podiceps cristatus*, is a polytypic species with a widespread distribution, ranging from the Palaearctic (*P. c. cristatus*) and Africa (*P. c. infuscatus*) to Australia and New Zealand (*P. c. australis*). The Australian and New Zealand forms have been suggested to be two distinct subspecies based on subtle plumage and size differences, but little other evidence exists to support this suggestion (Rogers 1990).

Great crested grebes have clearly been in New Zealand since the Holocene (> 10 000 ybp) based on the presence of sub-fossil remains found in the Taupo region (Turbott 1990). Furthermore, the discovery of crested grebe bones in Maori middens in both the North and South Island (Turbott 1990) suggests that the species may also have been common.

New Zealand's avifauna possesses a distinct Australian element, which is a consequence of natural self-introductions of Australian species via the prevailing westerly wind patterns (Baker 1991). In the last 40 years alone, two species of Australian grebe have colonised New Zealand (Turbott 1990). Whether the New Zealand form of great crested grebe is genetically distinct from those found in Australia then will rest on the level of trans-Tasman migration of the Australian form, which could in theory result in a lack of genetic divergence between the New Zealand and Australian populations.

Over the last century, great crested grebes in New Zealand have disappeared from many lakes where previously they were 'numerous' (i.e. Lake Te Anau, Henry 1903). Once found on lakes of both the North and South Island, they are now restricted to a small number of alpine and sub-alpine lakes of the main range of the South Island. A census in 1980 suggested that as few as 250 birds survive, with the majority on the lakes of the Canterbury region (Sagar 1981). Stoat predation, water fluctuations associated with hydroelectric power generation, and an increase in recreational use of lakes, have been implicated in the species' decline (Westerskov 1971).

As part of the current conservation management of the New Zealand great crested grebe population, the New Zealand Department of Conservation (DOC) approached us to undertake a genetic analysis to examine the genetic structure of *P. c. australis*. Specifically, DOC were interested in whether the New Zealand population is genetically distinct from the Australian population, information necessary to assign a management priority for this species. To address this issue, we have examined sequence variation at the mitochondrial cytochrome b gene using samples from New Zealand and Australian great crested grebe populations. Considerable information is available regarding the evolution of this gene in a range of avian species, and this has helped make cytochrome b the marker of choice for resolving recent evolutionary phylogeny in birds (see review in Moore and DeFilippis 1997).

2. Methods

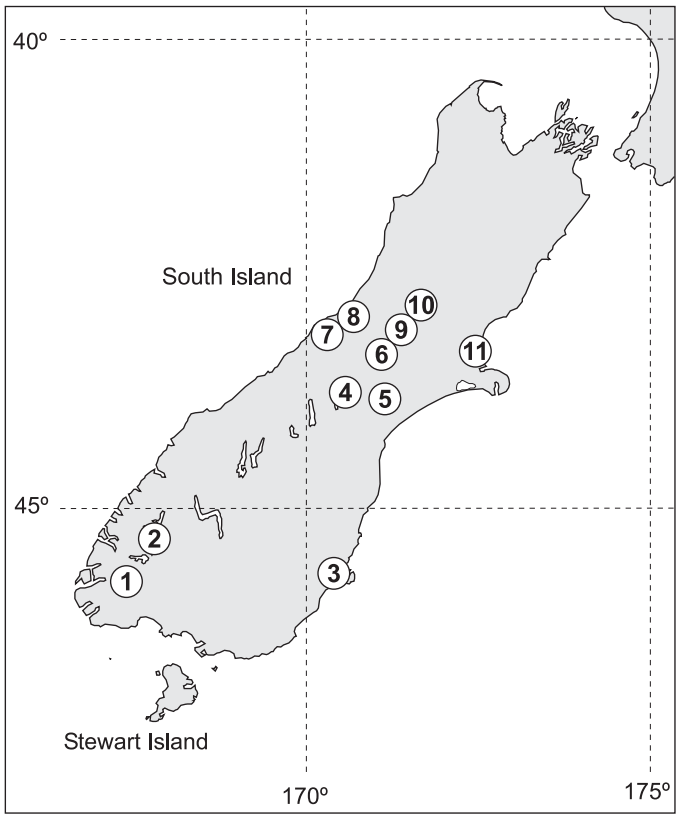
2.1 SAMPLES

Obtaining genetic samples from live grebes is difficult, as individuals are aquatic and, hence, difficult to capture. Owing to the time constraint on this study we have obtained most samples from museum skins collected over the last century (i.e. feathers and feet tissue). A single tissue sample from the extant New Zealand birds was obtained from an abandoned nest on Lake Pearson, in the form of a near-term embryo (Glen Newton, pers. comm.).

Overall we sequenced samples from 18 distinct localities within Australia and New Zealand. We obtained samples from 11 separate localities from within New Zealand (Fig. 1), including at least a single individual from all groups of lakes (except the Lake Sumner Group and Lake Guyon) on which grebes have previously been recorded (see Sagar 1981). In Australia, most grebes are in the south-east, centred on the Murray–Darling River system. Given the sampling constraints on the present study, we have attempted to sample the genetic diversity present among Australian individuals by examining geographically dispersed localities. One individual from each of seven localities representing the breadth of the grebe distribution was sequenced (Fig. 2).

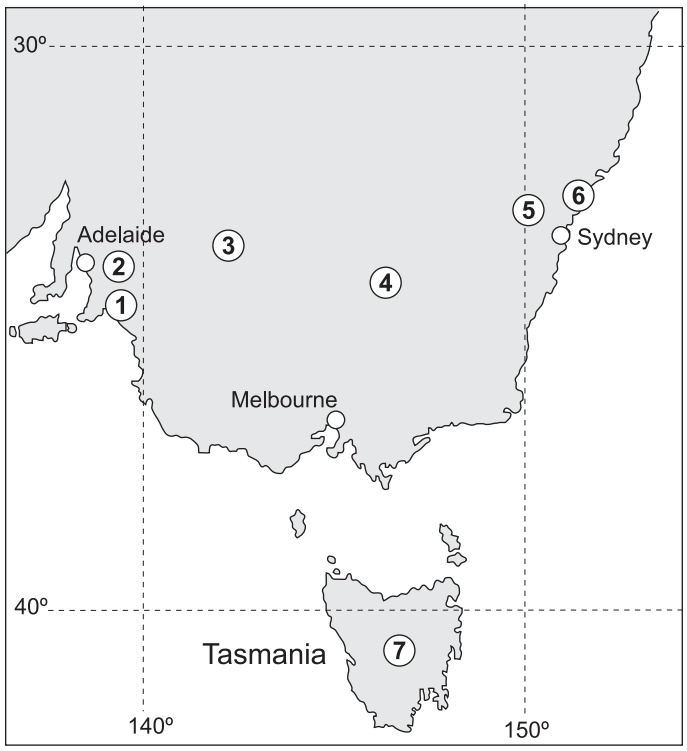
Genomic DNA was extraction from the recently deceased embryo (Lake Pearson), using a 5% chelex protocol (adapted from Walsh et al. 1991), and from museum skins, using a commercial kit (Qiagen DNeasy), as previously described (Robertson et al. 2000a; Robertson et al. 2000b). All extractions were

Figure 1. Localities of the New Zealand great crested grebe samples examined.



No.	MUSEUM	LABEL NO.	DATE	LOCALITY
1	Te Papa	21556/1	1977	Lake Monowai
2		21078	?	Fiordland
3		25795	1984	Dunedin
4	Canterbury	AV37021	1995	L. Alexandrina
5	Te Papa	11847	1922	Sth Canterbury
6		19435	1976	Lake Heron
7		22734	1976	Okarito Lagoon
8		11337	1963	Lake Ianthe
9	Canterbury	AV36785	1989	Lake Selfe
10	–	–	2001	Lake Pearson
11	Canterbury	AV371	1929	Riccarton

Figure 2. Localities of the Australian great crested grebe samples examined.



No.	MUSEUM	LABEL NO.	DATE	LOCALITY
1	Sth Austr.	B33743	1980	Lake Alexandrina, SA
2		B26701	1964	Blanchetown, SA
3		B38094	1978	Mildura, NSW
4	Australian	54061	1980	Barren Box Swamp, NSW
5		4696	1892	Mudgee, NSW
6		46921	1978	Lake Myall, NSW
7	Sth Austr.	B9197	?	Tasmania

done in a UV-laminar flow hood using aerosol-resistant pipette tips (ART tips) and extraction negatives.

2.2 PCR AMPLIFICATION AND SEQUENCING

Amplification of mitochondrial cytochrome b gene partial sequences was achieved by Polymerase Chain Reaction (PCR) using 'universal' primers from Kocher et al. (1989): H15149 (5′-CCC CTC AGA ATG ATA TTT GTC CTC A-3′) and L14841 (5′-CCA TCC AAC ATC TCA GCA TGA TGA AA-3′). PCRs were carried out in 25 µL reaction mixtures containing 5 µL of template DNA, 10 pmol of each primer, 5 nmol of each dNTP, 500 m*M* KCl, 100 m*M* Tris-HCl, pH 9.0, 2 mg/mL BSA, 1.5 m*M* $MgCl_2$, and 1 unit of *Taq* polymerase (Roche). For all cytochrome b reactions, the cycling parameters were an initial five-minute denaturation of 94°C, followed by 40 cycles of 94°C/40 sec, 55°C/1 min and 72°C/1 min and then a final extension step of 72°C/10 min.

Following amplification, the integrity and size of PCR products were examined using agarose gel electrophoresis, and then the remaining products were electrophoresed in a 5% PAGE gel. Bands were excised under low-intensity UV, soaked overnight in 0.5*M* ammonium acetate, phenol/chloroform extracted and finally precipitated with isopropanol/5m*M* LiCl. PCR products were sequenced using both the H15149 and L14841 primers (γP^{33}-ATP or αP^{33}-dCTP) with an *Ampli*cycle cycle-sequencing kit (PE Applied Biosystems). The reaction conditions consisted of an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of 94°C/30 sec, 62°C/30 sec, 72°C/1 min, and 1 cycle of 72°C/10 min. All sequencing reactions were run on 6% denaturing PAGE gels, exposed to Biomax MR autoradiography film (Kodak) and scored manually.

Museum samples typically produce low yields of DNA; hence the risk of PCR contamination is high (Cooper 1994). In the present study, we addressed this risk by using a number of counter-measures (Newton & Graham 1994) including: extraction negatives; performing extractions in a UV laminar flow hood; using ART tips at every step; using PCR negatives and a PCR UV-hood; and exposing all previously autoclaved PCR reagents and consumables to short-wave UV before addition of template and *Taq* polymerase. Also, at least three individuals from New Zealand and Australia were extracted, PCR-amplified, and sequenced on two separate occasions.

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). Limited variation between New Zealand and Australian samples negated the need for further phylogenetic analysis. A partial cytochrome b sequence from *Tachybapthus ruficollus* was obtained from Genbank (accession number: AF172370) for use as an outgroup. All other sequences used are from this study.

3. Results and discussion

We have analysed 265 base pairs of nucleotide sequence for the mitochondrial cytochrome b gene and found only one haplotype present among the 11 New Zealand great crested grebes. Alignment of this sequence to the outgroup *Tachybapthus ruficollis* revealed transition and transversion nucleotide substitutions (Table 1) consistent with the level of divergence expected of two closely related genera (Moore & DeFilippis 1997). The New Zealand haplotype was also present in six of the seven Australian grebe samples, with the seventh Australian individual (individual from Barren Box Swamp in New South Wales; Fig. 2) displaying a single nucleotide transition (i.e. T→C). Pair-wise comparison of the predominant Australasian haplotype with partial sequence from a single individual from Europe was consistent with the sub-specific distinction made between these two regions (Table 1).

TABLE 1. STATISTICS FOR CYTOCHROME B SEQUENCES USED ON THESE SAMPLES OF GREAT CRESTED GREBE MATERIAL.

Ts, nucleotide transition, C↔T or A↔G; Tv, nucleotide transversion, A↔C or G↔T.

PAIR	BASE PAIRS	Ts PER SITE	Tv PER SITE	Ts : Tv RATIO
NZ haplotype × Australian	265	0.004	0	
NZ haplotype × European	130	0.023	0.015	1.5
NZ haplotype × Outgroup	265	0.068	0.008	8.5

Our findings suggest that the New Zealand population of great crested grebes has originated from the Australian mainland, probably colonising New Zealand via the prevailing westerly wind patterns (e.g. Baker 1991). Sub-fossil bones of the species found in New Zealand date this colonisation event to at least since the Holocene (> 10 000 years BP), but crested grebes may have been present prior to this time. In the thousands of years since arriving in New Zealand, the population has not genetically diverged from that in Australia, indicating steady gene flow into New Zealand. The extent of this gene flow is unknown, but from a theoretical point of view, very few introductions are required to arrest genetic divergence (Hartl 1987). An equally plausible alternative, based on our data, is that the present New Zealand population of crested grebes may represent a more recent colonisation following a New Zealand-wide extinction. Genetic material from crested grebe sub-fossil bones discovered in the Taupo region might throw some light on these alternatives.

Surprisingly little population structure was noted in the Australian population. This finding, however, reconciles well with the species' nomadism. The aquatic habit of the crested grebe means that drought, a natural feature of the Australian environment, has considerable influence on the species' range and distribution. Australian great crested grebes also display considerable local movement, concentrating in non-breeding winter flocks, sometimes numbering > 800 birds, on saline lakes, estuaries and bays (Rogers 1990). In Tasmania, the small resident population, which was first noted to breed in 1971, can swell to over 200 in winter (Bolger 1964). Presumably these extra individuals are seasonal nomads from mainland Australia. In New Zealand, crested grebes are locally dispersive, moving between local lake systems (Sagar & O'Donnell 1982). Large-scale movements to coastal lakes or estuaries do occur, but are linked to periods of severe winter weather (Rogers 1990). Unfortunately, a lack of long-term population studies of marked individuals in both Australia and New Zealand means little is known of local population structure. What data are available, however, suggest that crested grebe populations are fluid, displaying little structure.

We conclude that the New Zealand form of great crested grebe is not a distinct subspecies of *P. cristatus*. It is a population of the Pan-Australasian subspecies *P. c. australis*. Previous suggestions that the New Zealand population be classified as a separate subspecies based on morphological traits (i.e. plumage coloration) are erroneous, as mentioned by Rogers (1990). The limited variation in the Australasian form warrants further research, particularly comparison with the European subspecies. European great crested grebes also display a nomadic habit, which suggests they may also be depauperate genetically.

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