Taxonomic status of the brown teal *(Anas chlorotis)* in Fiordland

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Abstract

Mitochondrial 12S rRNA sequence data were used to examine the taxonomic status of the endemic brown, Auckland Island, and Campbell Island teal. Based on phylogenetic reconstructions using the 12S rRNA data we have found that the Great Barrier Island brown teal, and the Auckland and Campbell Island teals form a discrete clade, to the exclusion of the Fiordland brown teal. All of the Fiordland brown teal sequenced show striking similarity to grey duck and mallard sequences, with which they group. It therefore seems extremely likely that the Fiordland population of brown teal have at sometime in the past hybridised extensively with these invading species.

1. Introduction

Five extant teal species inhabit the Australasian region. Of these, three, the brown, Auckland Island, and Campbell Island teal are endemic to New Zealand and the subantarctic islands. The brown teal was once widespread, but in recent years the range of the brown teal has decreased dramatically and it is now found only in the north of the North Island, on Great Barrier Island, and in Fiordland. In contrast, self-introduced Australian and exotic duck species, such as the grey teal, grey duck and mallard, have become abundant and widely distributed.

Recently, Kennedy & Spencer (2000) identified two possible instances of hybridisation between Fiordland brown teal and mallard or grey ducks. If widespread, such inter-specific hybrids pose serious problems for managers engaged in the conservation of our rare endemic teals. Consequently, we were asked to extend the molecular genetic work of Kennedy & Spencer (2000) to examine further the taxonomy of New Zealand teals. Specifically, we have sought to confirm and gauge the extent of hybridisation between brown teal and exotic duck species, such as the mallard and grey duck.

2. Methods

2.1 SAMPLES

Total genomic DNA was obtained for each of the samples listed in Table 1 using a modification of the Chelex protocol (Walsh et al. 1991). Approximately 50 M1 of whole blood was suspended in 300 M1 of digestion buffer containing 5% Chelex. Proteinase K and RNase were added to final concentrations of 100 Mg/ml and the samples were incubated overnight at 37°C. The samples were centrifuged at 13 000 rpm to precipitate debris. The supernatant

was transferred to a fresh tube and an equal volume of 5% Chelex in TE added. The sample was centrifuged once more at 13 000 rpm, and the supernatant removed and stored at -80°C.

2.2 PCR AMPLIFICATION AND SEQUENCING

Amplification of mitochondrial 12S rRNA gene partial sequences was achieved by polymerase chain reaction (PCR) using "universal" primers modified from Kocher et al. (1989) to include T7 and T3 tails to enable direct fluorescent sequencing: 12S rRNA, L1091 + T7 5'-GTAATACGACT CACTATAGGGCAAACTGGGATTAGATACCCCACTAT 3', H1478 + T3 5'-AATTAACCCTCACTAAAGGGACAGAGGGTGACGGGCGGTGTGT 3'. PCRs were carried out in 50 Ml reaction mixtures containing 50 ng of template DNA, 10 pmol of each primer, 5 nmol of each dNTP, 5 ml of 10 x reaction buffer (500 mM KCI, 100 mM Tris-HCI, pH 9.0), 1.5 mM MgC1 ? and 1 unit of *Taq* polymerase (Roche). All reactions were denatured for 2 min at 95 °C prior to initiation of the PCR. For all 12S rRNA reactions, the cycling parameters were 35 cycles of 95°C/30 sec, 60°C/30 sec, and 72 °C/45 sec, followed by a final extension step of 72°C/4 min.

Following amplification, the integrity and size of PCR products were examined using agarose gel electrophoresis, and the products were purified by precipitation with isopropanol to remove residual primers and dNTPs. PCR products were sequenced using infrared labelled Licor IRD 800T7 or T3 promoter primers with a Thermosequenase cycle sequencing kit (Amersham Pharmacia Biotech). The reaction conditions consisted of an initial denaturation at 95' C for 5 minutes, followed by 30 cycles of 95 °C/30 sec, 55 °C/30 sec, 70°C/1 min, and 10 cycles of 95 °C/30 sec, 72°C/1 min. For each individual, several PCR products were sequenced in both directions to ensure sequence fidelity. All sequencing reactions were run on a Licor automated sequencer and analysed using the Base ImagIR software (Licor corporation).

2.3 PHYLOGENETIC ANALYSIS

Individual sequences were aligned using Clustal W (Thompson et al., 1994), then identical sequences were filtered and collapsed in MacClade v3.06 (Maddison & Maddison 1996). 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. A 12S rRNA sequence from Muscovy duck *(Cairina moschata)* was obtained from Genbank (accession number L16769) for use as an outgroup. Additional ingroup 12S sequences were obtained from Kennedy & Spencer (2000): mallard *(Anas platyrhynchos),* grey duck (*A. superciliosa),* grey teal (*A. gracilis),* chestnut teal (*A. castanea),* brown teal (*A. chlorotis),* Auckland Island teal (*A. aucklandica),* Campbell Island teal (*A. nesiotis).* For sequence details see Genbank accession numbers AF173480-AF173487. All other sequences used are from this study.

3. Results and discussion

We have analysed the same 394 by fragment of the 12S rRNA gene as Kennedy & Spencer (2000), but we have extended their investigation by sequencing 13 additional individuals from the endemic teal species, including seven more Fiordland brown teal. We have found that based on this mitochondrial marker all Fiordland brown teal fall outside of a monophyletic clade consisting of the subantarctic island teal species and brown teal from Great Barrier Island (Figure 1). Indeed, based on the available sequence data the Fiordland brown teal show absolutely no affinity to any of the endemic duck species and instead form part of an incompletely resolved clade with the mallard and grey duck (Figure 1).

Our result is supported by approximately 270 by of sequence data obtained by Kennedy & Spencer (2000), who previously suggested hybridisation as a likely explanation of their data. However, those sequence data were obtained with difficulty and were incomplete. Consequently, Kennedy & Spencer (2000) were unable to rule out the possibility that their data were derived from sequences of nuclear origin, Numt (Lopez et at. 1997). However, we have had no problems amplifying the 12S rRNA gene region from the samples provided, have obtained full length sequences, and none of the sequences obtained appears to be pseudogene-derived because all inter-sequence comparisons show transition/transversion ratios consistent with those previously reported for mtDNA (Lopez et al. 1997). It therefore seems highly unlikely that the sequences we have obtained are nuclear in origin and the only possibility remaining to explain the current data is hybridisation.

The 12S rRNA gene is located on the maternally inherited mitochondrial DNA, so if hybridisation is occurring, it is the brown teal males that are driving this hybridisation by mating with mallard or grey duck females. This scenario seems likely since brown teal males are apparently highly aggressive during the mating season and may be able to assert dominance over competing mallard and grey duck males (Dumbell 1986). Unfortunately, because mallards and grey ducks hybridise extensively in the wild it is almost impossible to tell which is the most likely female partner for the male brown teal. Nor is it possible to tell when this hybridisation occurred. However, given that all seven Fiordland brown teal sequenced in this study show mallard or grey duck mitochondrial haplotypes it seems likely that the hybridisation occurred at least several generations ago. Indeed, it is possible that the initial hybridisation may have occurred prior to the description of the Fiordland population as a separate sub-species (Mathews 1937) and that the majority of the phenotypic differences that were used to elevate this population to subspecies are the result of that hybridisation. Further research is warranted if we wish to document more fully the nature and extent of this hybridisation. In particular, it will be vital to comprehensively examine the Fiordland population to determine whether any true-breeding individuals remain in this population.

Neither the subantarctic island teals, nor the Great Barrier Island brown teal show any evidence of hybridisation with exotic duck species. The sample

sizes taken from the subantarctic islands are currently quite small, but the Great Barrier Island sample is of adequate size to have been able to detect hybridisation if it were extensive in this population. The absence of mallard or grey duck mitochondrial sequences in these populations is encouraging, but may most likely be attributed to the fact that mallards and grey ducks have yet to reach these locales. If this is indeed the case, it is vital that a regular monitoring programme is undertaken on Great Barrier Island and the subantarctic islands to ensure that the genetic composition of those populations is not compromised by hybrids in the future.

4. Conclusion

Further research, using nuclear genetic markers is warranted if we wish to document more fully the nature and extent of the hybridisation in Fiordland brown teal. In particular, it will be vital to comprehensively examine the Fiordland brown teal population to determine whether any true breeding individuals remain. In addition, it would be extremely valuable if we could document when and where hybridisation began in the Fiordland population, and how quickly hybrids came to dominate the Fiordland brown teal population. Such information may be obtainable through the use of ancient DNA methodologies on the numerous samples of Fiordland brown teal that are available from museums throughout New Zealand and the world (Murray Willams, pers. comm.). Genetic analysis of these museum samples should be encouraged, as they might provide us with important new data on the hybridisation process, and enable us to more effectively develop management strategies to limit hybridisation in other endangered populations or species in the future.

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Table 1. Species, localities of origin, and numbers of individuals used in this study.

Species	Common Name	Locality	Number of Individuals
Anas chlorotis	Brown Teal	Fiordland	6
	Brown Teal	Fiordland	1*
	Brown Teal	ex. Great Barrier Island	2
	Brown Teal	ex. Great Barrier Island	8*
Anas aucklandica	Auckland Island Teal	In captivity	2
Anas nesiotis	Campbell Island Teal	ex. Dent Island 1990	2
Anas superciliosa	Grey Duck	Wellington and Taranaki	3
Anas platyrhynchos	Mallard	ex. Nga Manu Trust	1

* Samples previously analysed by Kennedy and Spencer (2000).

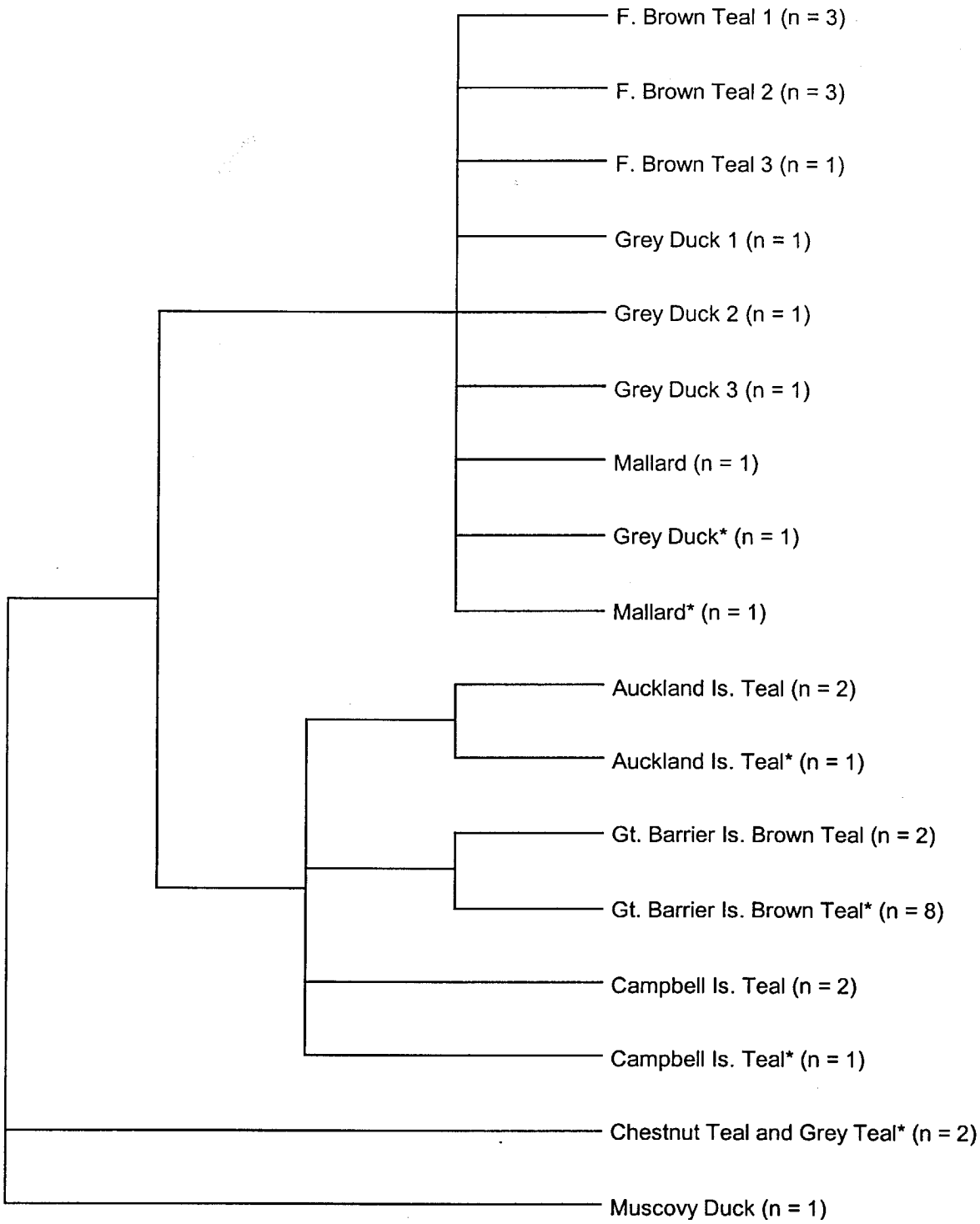

Figure 1. A cladogram illustrating the phylogenetic relationships of the New Zealand teals based on the 12S rRNA gene (394 by fragment). The tree was constructed in PAUP* (Swofford, 2000) using maximum parsimony analysis. Numbers above branches represent proportions of bootstrap replicates (1000) where the same branching order was recovered. Branches with bootstrap values of less than 50% were collapsed. The tree was rooted by outgroup using a Muscovy duck sequence.