Hybridisation in the last remaining individuals of the extinct Fiordland population of Brown Teal (*Anas chlorotis*)

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The New Zealand endemic Brown Teal (*Anas chlorotis* Gray, 1845) was once widespread on the three main islands of New Zealand, some offshore islands and the Chatham Islands. Hunting and drainage of wetlands during the early years of European colonisation, however, resulted in a severely reduced range for the species and by 1990, the last remaining wild populations were on Great Barrier Island, and in eastern Northland and Fiordland. However, by 2007, the Fiordland population of Brown Teal was assumed extinct. The potential role of hybridisation with Mallard (*Anas platyrhynchos* Linnaeus, 1758) and Grey Duck (*Anas superciliosa* Gmelin, 1789) in the decline of the Fiordland population of Brown Teal has previously been recognised, though specimen details and tissue voucher samples associated with the DNA sequences were not retained. Here, we provide new mitochondrial DNA sequences from four specimens of Fiordland Brown Teal registered in the collections of Canterbury Museum. The results provide evidence for hybridisation with Mallard/Grey Duck in all four individuals, and support previous suggestions that hybridisation could have played a role in the decline of the Fiordland Brown Teal population.

Keywords: Conservation, genetics, Mallard, museum, voucher specimens

Introduction

The New Zealand endemic Brown Teal (*Anas chlorotis* Gray, 1845), or pāteke, was formerly widespread on the three main islands of New Zealand, some offshore islands and the Chatham Islands (Milicich and Daugherty 2000; Worthy and Holdaway 2002). However, hunting and drainage of wetlands during the early years of European colonisation resulted in a severely reduced range for the species (Dumbell 1986; Ferreira and Taylor 2003). As a result Brown Teal were fully protected in 1921 (Dumbell 1986), although hunting may have continued for some time after (Hayes and Williams 1982; Dumbell 1986). More recently, predation and competition with exotic species have resulted in further declines in the range of Brown Teal (Hayes and Williams 1982; Ferreira and Taylor 2003), especially on the South Island. By 1990, the last remaining wild populations of Brown Teal were on Great Barrier Island, and in eastern Northland and Fiordland (Gemmell and Flint 2000). By 2007, the Fiordland population of Brown Teal was assumed extinct (O'Connor et al. 2007).

Remnant Brown Teal populations are still threatened by habitat modification, traffic and predation by introduced rats (*Rattus norvegicus* (Berkenhout, 1769)), stoats (*Mustela erminea* Linnaeus, 1758), possums (*Trichosurus vulpecula* (Kerr, 1792)), cats (*Felis catus* Linnaeus, 1758) and dogs (*Canis familiaris* Linnaeus, 1758) (Ferreira and Taylor 2003). Despite this, intensive management has halted the overall decline of the species. Some populations are now increasing and several new populations have been established (Hayes 2010). However, hybridisation with other *Anas* species, e.g. Grey Teal (*A. gracilis* Buller, 1869), Grey Duck

Table 1. Information associated with Campbell Island Teal (*A. nesiotis*), Auckland Island Teal (*A. aucklandica*) and Fiordland Brown Teal (*A. chlorotis*) tissue samples used in this study.

Museum	Museum Number	Species	Collection Location	Collection Date	Notes
Te Papa Tongarewa	OR.029911/b	*Anas nesiotis*	Beeman Wharf, Campbell Island	2005	
Te Papa Tongarewa	OR.029910/b	*Anas aucklandica*	Te Anau Wildlife Park	2010	
Canterbury	FTeal 1: 2017.4.1	*Anas chlorotis*	Lake Hakapoua, Fiordland	Egg collected 1999	Hatched at Burwood Bush, Duckling, M.J.W.
Canterbury	FTeal 2: 2017.4.2	*Anas chlorotis*	Lake Hakapoua/ Lake Poteriteri, Fiordland	Adult collected 1999	Leg Band – L-28690, M.J.W.
Canterbury	FTeal 3: 2017.4.4	*Anas chlorotis*	Lake Hakapoua, Fiordland	Adult collected 1999	White leg band, M.J.W.
Canterbury	FTeal 4: 2017.4.5	*Anas chlorotis*	Near Loch Maree, Fiordland	Early 1997	Suspected stoat predation
Canterbury	FTeal 5: 2017.4.6	*Anas chlorotis*	Near Loch Maree, Fiordland	Early 1997	Found 4 metres from FTeal 4, Decayed

(*A. superciliosa* Gmelin, 1789) and Mallard (*A. platyrhynchos* Linnaeus, 1758) is known to occur and may pose a risk to the persistence of the species (Gemmell and Flint 2000), yet is difficult to manage against.

The potential role of hybridisation in the decline of Brown Teal was demonstrated by the genetic study of Kennedy and Spencer (2000), who found evidence for hybridisation between Fiordland Brown Teal and Mallard or Grey Duck. Using mitochondrial 12S rRNA sequences, Gemmell and Flint (2000) identified a discrete clade of New Zealand Brown Teals, comprising the Great Barrier Island Brown Teal population, Auckland Island Teal (*A. aucklandica* (G.R. Gray, 1849)) and Campbell Island Teal (*A. nesiotis* (J.H. Fleming, 1935)) (as previously reported by Daugherty et al. 1999, and later confirmed by Mitchell et al. 2014), but also found that Fiordland Brown Teal fell separately with Grey Duck and Mallard, supporting the findings of Kennedy and Spencer (2000). Based on the details provided in these two studies, it appears they examined eight Fiordland Brown Teal specimens derived from three localities. However, the details given are insufficient to identify exactly which specimens were used, and this creates difficulties for replicability of results or performing additional analyses. To help rectify this, we report on mitochondrial DNA analysis of four adult specimens identified as Brown Teal based on morphology and plumage and a chick that was considered to be a Brown Teal based on the label on the specimen. We believe at least one (possibly two) may have been reported on by the previous studies; at least one (possibly two) may be new, with the status of the other/s being uncertain.

Methods

Study specimens: Four specimens identified as Brown Teal based on morphology and plumage and a chick that was considered to be a Brown Teal based on the label on the specimen, (DNA sample numbers FTeal 1–FTeal 5; corresponding to Canterbury Museum accession numbers: 2017.4.1, 2017.4.2, 2017.4.4–2017.4.6, see Table 1), were rediscovered during the cleaning of a freezer at the Department of Conservation,

Te Anau, in 2014. Labels with the specimens provided little detail on when and where they had been collected. However, some details have been ascertained after enquiries with the relevant authorities (Table 1).

FTeal 1–3: Specimens FTeal 1–3 most likely came from Lake Poteriteri and/or Lake Hakapoua in Fiordland (Murray Willans pers. comm. 2016). On 6–7 December 1999 one live adult was collected from Lake Poteriteri, and five adults and a clutch of seven eggs were collected live from Lake Hakapoua. DNA from these adults was reported on by Gemmell and Flint (2000) (Murray Williams pers. comm. 2015). Although it was not known whether the clutch from Lake Hakapoua was associated with any of the adults collected, five of the eggs hatched and the chicks were raised in captivity. With the exception of two individuals that died, all these birds were later re-released at Lake Hakapoua (Murray Willans pers. comm. 2015). We are unsure whether FTeal 1 (a duckling) represents a member of this clutch, or a duckling collected on a different occasion, but irrespective it seems that DNA from this specimen has probably not been previously reported. FTeal 2 and FTeal 3 are both adults with leg bands. There appears to be no records associated with the numbered metal leg band on FTeal 2, suggesting that this is the bird captured at Lake Poteriteri (the band number of this bird was not recorded at the time, Murray Williams pers. comm. 2015). Therefore, FTeal 2 appears to be one of the original wild captured adults reported on by Gemmell and Flint (2000). FTeal 3 only has a colour band (no metal band), and its association with previously reported specimens is uncertain.

FTeal 4–5: Labels with specimens FTeal 4–5 indicate that they were collected near Loch Maree, Fiordland, on 9 December 1996. Records indicate that a Brown Teal was captured, bled and radio-tagged at Loch Maree on 16 October 1996, and its remains (presumably after being predated by a stoat) were retrieved some months later (Murray Willans pers. comm. 2015). DNA from this bird was reported on by Gemmell and Flint (2000) and Kennedy and Spencer (2000) (Murray Williams pers. comm. 2015) and we suggest that either FTeal 4 or 5 could be the same bird (but that the other is likely to be a previously unstudied specimen).

Molecular analysis

Genomic DNA was extracted from toe pads of the five Brown Teal specimens (or other soft tissues where toepads were not preserved), and from comparative specimens of Campbell Island Teal (Te Papa Tongarewa, OR.029911/b) and Auckland Island Teal (Te Papa Tongarewa, OR.029910/b) (Table 1), using the Qiagen DNeasy® Tissue Kit (Qiagen) following the tissue protocol. We amplified 598 bp and 730 bp (Fiordland Brown Teal/Campbell Island Teal and Auckland Island Teal, respectively) of the mitochondrial cytochrome c oxidase 1 (COI) gene (GenBank accession numbers MF469848–MF469853) using internal primers that contained five primer pairs (Patel et al. 2010). We performed polymerase chain reactions on a BIO-RAD MyCycler thermal cycler using Illustra™ PuReTaq Ready-To-Go Beads. The product was amplified from all specimens except FTeal 5, which was a highly decayed specimen with little remaining tissue. PCR products were sequenced using an Applied Biosystems 3500xL Genetic Analyzer. We used the programme Geneious R8 (Biomatters) to examine, edit and align forward and reverse consensus sequences for each specimen. We used MEGA v.6 to align sequences with the same region of CO1 for three additional species obtained from GenBank; Chatham Island Duck (*A. chathamica* Oliver, 1955), Grey Duck and Mallard (GenBank accession numbers: KF562761, JN801396, GU571240, respectively) and for the Australasian Shoveler (*A. rhynchotis* Latham, 1802) obtained from BOLD (Bold accession number: BROMB529-07). A maximum credibility phylogeny was created using BEAST, with the most appropriate model as determined using jModelTest 2 (Darriba et al. 2012) and the Akaike Information Critereon (Tamura-Nei with invariant sites), yule-speciation prior, and

MCMC chain length of 10,000,000 (recording every 1,000 states with a 10% burnin).

Results

Our results support recent findings of Mitchell et al. (2014) that the New Zealand Brown Teal and Auckland and Campbell Island teals form a distinct clade with the recently extinct Chatham Island Duck at the base (Fig. 1). Furthermore, the four Fiordland Brown Teal specimens that yielded mitochondrial DNA were all hybrids, falling within the clade containing Mallard and Grey Duck. The hybridising species (i.e. Mallard or Grey Duck) for each of the Fiordland Brown Teal is unclear given the low posterior values (<0.95) for branches within the clade. However, our results nevertheless support previous findings that hybridisation with Mallards/Grey Duck may have been widespread within the Fiordland Brown Teal population (Gemmell and Flint 2000; Kennedy and Spencer 2000). All specimens from the population now analysed have Mallard/Grey Duck mitochondrial DNA.

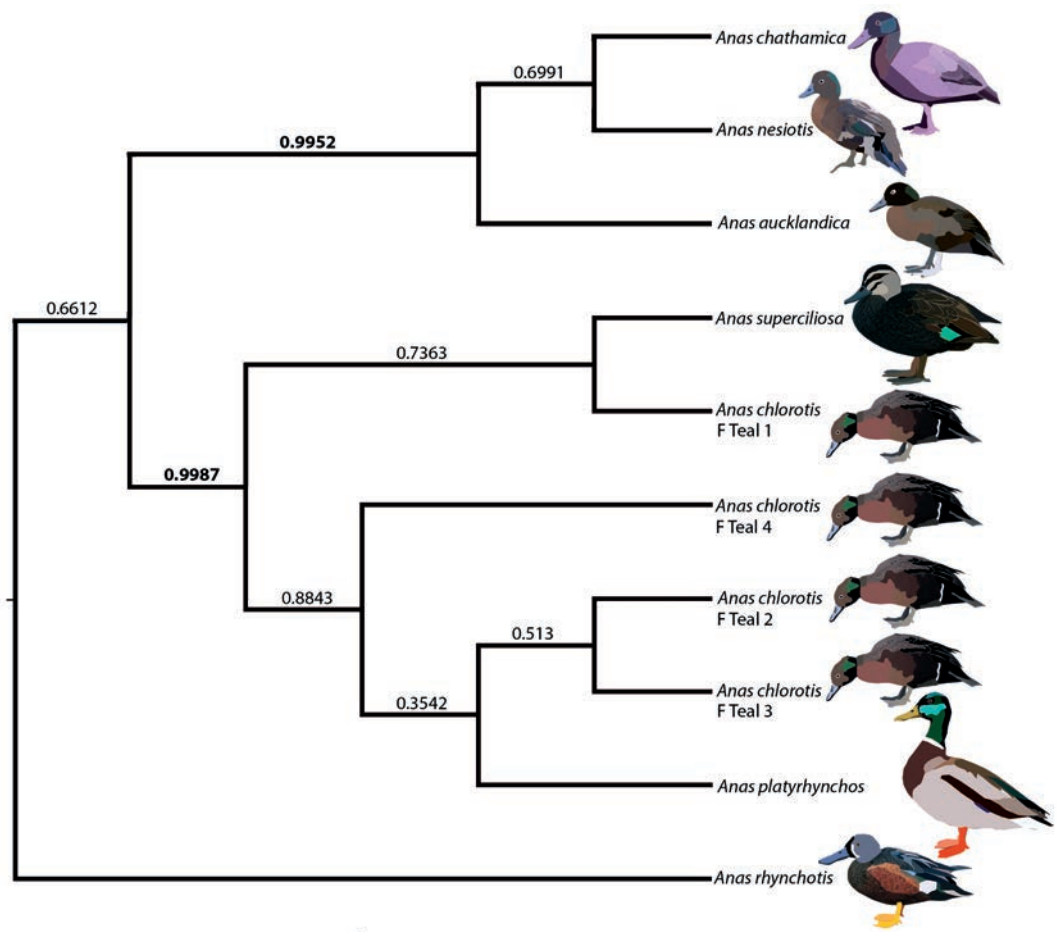

Figure 1. Phylogenetic tree showing our four Fiordland *A. chlorotis* specimens, and closely-related New Zealand teals (*A. chathamica*, *A. nesiotis* and *A. aucklandica*), two hybridisation-prone *Anas* species (*A. superciliosa* and *A. platyrhynchos*) and an outgroup (*A. rhynchotis*). Branch posterior values are shown above branches with bold text indicating statistically significant groupings (>0.95).

Discussion

Hybridisation is common within birds, particularly waterfowl (Grant and Grant 1992; McCarthy 2006). Almost 50% of Anseriforme species are known to hybridise, but this figure is likely an underestimate (Grant and Grant 1992). This high rate of hybridisation is probably due to the evolutionary history of Anseriformes, with relatively recent diversification events during the Miocene (23–5 million years ago) (Gonzalez et al. 2009), and very shallow divergence (Pleistocene, 1.20–3.48 million years ago) within the Mallard species-complex (Mitchell et al. 2014). It is no surprise therefore that the Mallard is one of the most hybridisation-prone waterfowl species (McCarthy 2006).

Hybridisation can influence evolution (Grant and Grant 1992; Barton 2001; Lancaster et al. 2007) and may be associated with diverse costs and benefits. For example, hybrids may have a higher fitness than true-breeding individuals (Grant and Grant 1992; Veen et al. 2001), and may result in speciation (Barton 2001). However, hybrids may also exhibit reduced fitness or fertility (Haldine 1922; Howard et al. 1998; Lancaster et al. 2007). Furthermore, hybridisation can be of great concern for conservation management, as hybrid genomes may spread throughout a population. This can result in the complete or local extinction of 'pure' genomes (Rhymer and Simberloff 1996; Allendorf et al. 2001), ultimately leading to local or global extinction(s) of the species. It is possible that lowered fitness associated with hybridisation may have partly contributed to the decline of the Fiordland population of Brown Teal, as there is no evidence that any true-breeding individuals remained in recent times.

In the absence of results from analysis of nuclear DNA we cannot rule out bi-directional hybridisation, yet our results corroborate the findings by Gemmell and Flint (2000), where male Fiordland Brown Teal were at least contributing to (if not driving) the hybridisation by mating with larger Mallard/Grey Duck females. It is also not possible to determine exactly when the hybridisation occurred, however, Gemmell and Flint (2000) suggested that it may have occurred several generations ago, given the wide geographical spread of where their specimens had been derived. Grey Duck is native and has been present in New Zealand since before human settlement (Holdaway et al. 2001). Mallard were first introduced in the late nineteenth and early twentieth centuries for recreational hunting (Dyer and Williams 2010; Guay et al. 2014), so if it was Mallard hybridising with Fiordland Brown Teal, it would have been a relatively recent event. It is also worth noting that Mallard and Grey Duck were present at Lake Hakapoua in December 1999 during the sampling expedition from which Gemmell and Flint's (2000) samples were derived, an observation that was further corroborated during a follow-up visit in January 2000 (Murray Williams pers. comm. 2015). Future examination of historical Brown Teal specimens housed in museums may provide further insights into the exact timing of hybridisation events in Fiordland.

Intensive conservation management efforts have halted the decline of remaining Brown Teal populations in New Zealand, and in 2015 the International Union for Conservation updated the status of Brown Teal from endangered to near threatened. Although the remaining populations have responded well to management and the species can become locally common when protected from predators, the risk of hybridisation between Brown Teal and other waterfowl species remains a credible threat that may lead to the decline of local populations, or indeed the entire species, as attested to by the Fiordland population.

Our study has again highlighted the importance of reporting details of specimens used in scientific studies and depositing vouchers of these specimens in museum collections. Inadequate reporting of specimen details (e.g. collection locations and dates, leg band numbers, museum registration numbers) can create difficulties in reproducing results, or interpreting previous results in the context of

new findings (Huber 1998; Pleijal et al. 2008). In this case, our results corroborate those of past studies on Fiordland Brown Teal, yet we are unsure whether this is because the specimens examined were actually those used in the previous work. Nevertheless, these specimens are now registered in the collections of Canterbury Museum for future research.

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