Captive breeding and release diminishes genetic diversity in Brown Teal *Anas chlorotis*, an endangered New Zealand duck

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Abstract

We examined levels of mitochondrial DNA (mtDNA) and DNA microsatellite genetic variation in Brown Teal (*Anas chlorotis*), mainland New Zealand's rarest waterfowl. Samples were taken from the two remaining wild populations, a captive population, and four new populations established by released captive offspring. The larger wild population on Great Barrier Island (GBI) had two mtDNA haplotypes, one very rare, perhaps indicative of a historic bottleneck. The captive population was founded exclusively from GBI individuals; it and all new populations contained only the common GBI haplotype. In contrast, the smaller wild population at Mimiwhangata (MIW), Northland, contained 11 mtDNA haplotypes, including the common GBI haplotype which was probably introduced by captive-sourced releases 18–20 years ago. Microsatellite allelic richness was high in wild populations compared with captive and new populations. We suggest that genetic supplementation should be considered for the captive and new populations, and that the long-term goals of the Brown Teal recovery programme would benefit from assiduous and persistent genetic management and monitoring.

Key words: *Anas chlorotis*, Brown Teal, captive breeding, genetic diversity, reintroduction.

Captive breeding programmes are invariably crisis responses to extensive population declines and fragmentation, and to threatening circumstances in the wild. They are used to maintain the demographic viability of endangered populations and, to a lesser extent, to counteract the loss of genetic diversity, which is a feature of small remnant or recently founded populations. Captive breeding contributes to on-going conservation when the progeny of any successful breeding are released into the wild, either to establish a new population or to supplement an existing one. Well-

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publicised captive breeding successes for Peregrine Falcon *Falco peregrinus* (Cade & Burnham 2003), Nene *Branta sandvicensis* (Black 1995; Blanco *et al.* 1999) and Arabian Oryx *Oryx leucoryx* (Rahbek 1993) among many others, raise the profile and publicly reinforce the value of this conservation technique.

Captive breeding programmes are often established with low levels of genetic variation because they are a crisis response to a seriously depleted population. The captive populations are typically small and have restricted gene flow with any other populations, conditions that cause strong genetic drift and which eventually erode genetic diversity. Genetic issues associated with captive breeding are now better understood (Ebenhard 1995; Allendorf & Luikart 2007) and include the possibility of inbreeding depression, long-term captivity causing undesirable adaptation (Frankham 2008), and concerns for the long-term viability of populations founded or supplemented from genetically depauperate captive-raised progeny (O'Brian *et al.* 1985; Caro & Laurenson 1994; Frankham *et al.* 2002). A long-term management perspective is necessary in captive breeding programmes to avoid these unintended genetic consequences for small and confined populations.

Captive breeding programmes have featured in historic and contemporary responses to the conservation plight of many New Zealand birds including Takahe *Porphyrio hochstetteri* (Lee & Jamieson 2001), Kiwi *Apteryx* sp. (Anon 2004), Black Stilt *Himantopus novaezeelandiae* (Reed *et al.* 1993) and Brown Teal *Anas chlorotis* (Williams & Dumbell 1996; O'Connor *et al.* 2007). While modern management guidelines for these programmes include statements about the management of genetic diversity among captive animals (Dumbell 2000; Anon 2004), limited consideration is given to how representative released individuals are of the genetic diversity remaining in the wild populations.

Historically the Brown Teal was distributed throughout lowland forests and on the freshwaters of New Zealand's three main islands, and on several near-shore islands (Worthy 2002). It is now the rarest and most endangered waterfowl on mainland New Zealand (Miskelly 2008) with just two natural populations remaining, on Great Barrier Island (GBI) and surrounding Mimiwhangata (MIW) on Northland's east coast (Fig. 1).

A captive breeding programme commenced in 1973, founded by 22 birds then in captivity, to which a further 42 were added within three years (Williams & Dumbell 1996). An additional 15 wild birds entered the captive population in 1987–1988; they, like all others, were sourced from GBI, the larger of two remaining wild populations. By 2000 approximately 1,700 captive-raised birds had been dispersed as 66 releases to 15 regional sites, including supplementing the smaller wild MIW population (Dumbell 2000). At only one site, mammal-free Tiritiri Matangi Island, was a new population successfully established. Between 2000–2008 another 16 birds from GBI were added opportunistically to the captive population and 474 captive-raised birds released at seven sites (K. Evans, pers. comm.).

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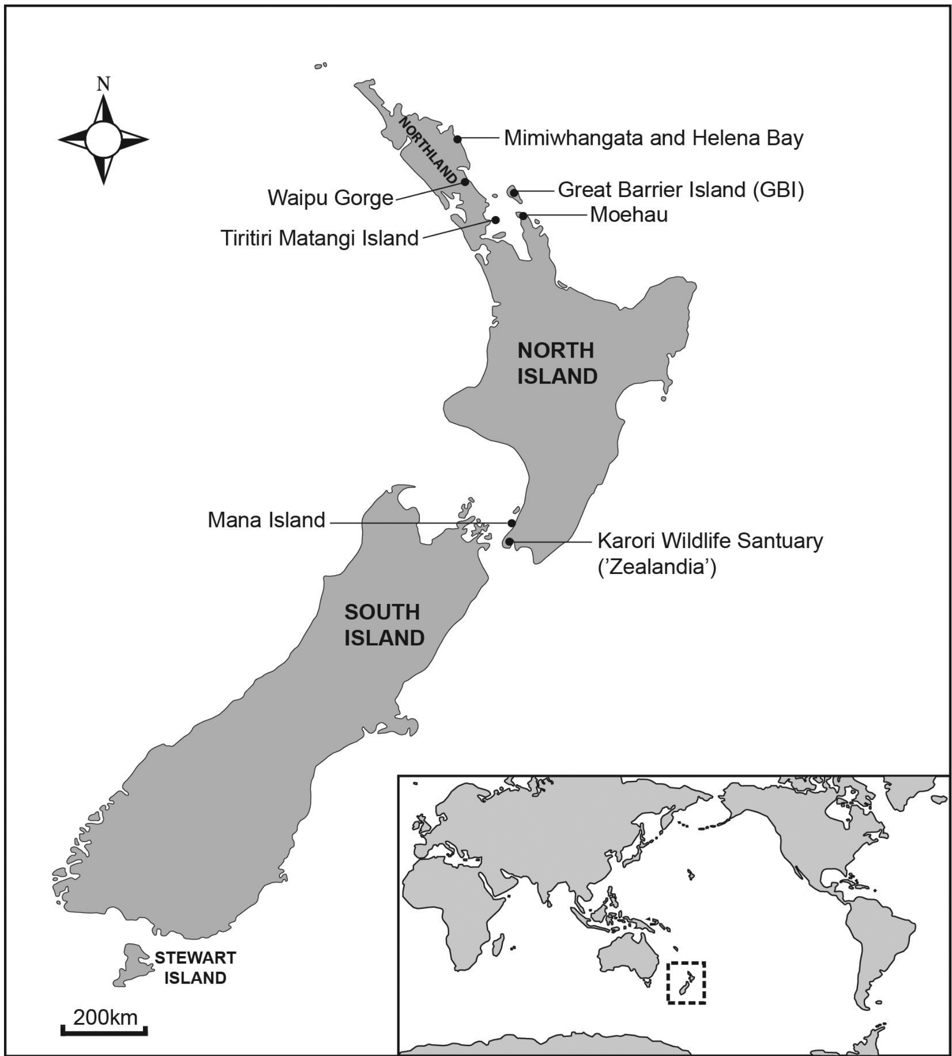

Figure 1. Map of New Zealand and the locations of Brown Teal populations that were sampled. Waipu Gorge and Helena Bay were the locations at which the sampled museum specimens were collected.

Presently, "new" populations derived from these releases persist at Mana Island, Kapiti Island, Tiritiri Matangi Island, Karori Wildlife Sanctuary (now called "Zealandia") and Moehau (Fig. 1), although the Moehau population originates from a tiny remnant wild population supplemented by releases of captive-raised teal.

Little was known about the genetic diversity remaining in the wild populations (Dumbell 1987) and nothing of that within the captive and new populations. This

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study sought to use mitochondrial and microsatellite DNA markers to determine the levels of genetic diversity in the two remaining wild Brown Teal populations, the present captive breeding stock, and in four populations newly established by releases of captive-raised teal. We use the results of our genetic analysis to make several recommendations for the future genetic management of the Brown Teal captive breeding and recovery programme (O'Connor *et al.* 2007).

Methods

Brown Teal feathers were collected from GBI (36°11'S, 175°25'E), MIW (35°43'S, 174°21'E), the captive breeding population, and new populations at Moehau (36°45'S, 175°31'E), Karori Wildlife Sanctuary (41°19'S, 174°46'E), Tiritiri Matangi Island (36°52'S, 174°46'E) and Mana Island (40°57'S, 175°03'E). Feathers were taken between 2001 and 2007 inclusive and stored individually in dry paper envelopes labelled with the banding code of the bird and the area caught. In addition, we obtained foot pad tissue from two museum skins from the Museum of New Zealand and collected in Northland at locations where Brown Teal no longer occur; Helena Bay in 1978 (35°43'S, 174°21'E) adjacent to the MIW population, and Waipu River gorge in 1935 (36°54'S, 174°47'E) (Bell 1959).

The base of each feather (approximately 3 mm) was excised with a sterilised scalpel and DNA was purified using the standard phenol-chloroform extraction procedure followed by ethanol precipitation (Sambrook *et al.* 1989). A portion of the mitochondrial DNA (mtDNA) control region (636 bp) was amplified using polymerase chain reaction (PCR) and the primers L78 (5'-GTTATTTGGTTAT-GCATATCGTG-3') and H774 (5'-CCATATACGCCAACCGTCTC-3'), taken from Sorenson *et al.* (1999). Five microsatellite DNA markers were selected from the studies of Huang *et al.* 2005 (*caudo24*, *caudo19*, *caudo13*, and *caudo1*) and Maak *et al.* 2003 (*adph13*) based on their high levels of observed heterozygosity (H_O ranged from 0.68 to 0.97) and allelic diversity (5–13 alleles per locus) in Mallard *Anas platyrhynchos*. The M13 sequence method (Schuelke 2000) was used to label one of the microsatellite primers in each pair with the fluorescent marker 6FAM or VIC (Applied Biosystems).

PCR amplifications were performed in 25μL volumes using 1–2 μL of DNA template, 10 mM Tris pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.4 μg/ml BSA, 0.4 μM of each of the forward and reverse primer, 200μM of each dNTP, and 0.5–1 units of BIOTAQ DNA polymerase (Bioline). For the mtDNA control region, thermal cycling was carried out for 30 s at 94°C, 30 s at 54°C, and 60 s at 72°C, each repeated for 35 cycles. For the microsatellite markers, the same conditions were used with the addition of an M13 attachment cycle added to the end of the thermal cycling conditions.

The resultant PCR products were electrophoresised in agarose gel and a molecular weight standard was used to determine the size of the amplified DNA fragments. For the mtDNA control region, PCR products were purified using either column purification (Roche) or ExoSAP-IT (GE Healthcare Lifesciences), and their

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DNA sequence was determined using dye-terminator reaction chemistry and analysed on an Applied Biosystems 3730 Genetic Analyzer. For the microsatellite markers, PCR products of the correct size were diluted depending on their concentration and the allele sizes were determined using an Applied Biosystems 3730 Genetic Analyzer.

Mitochondrial DNA sequences were edited by eye and then aligned using Clustal W in MEGA v4.0 (Kumar *et al.* 2004). Overall levels of nucleotide diversity (π) for each population were calculated in MEGA v4.0 (Kumar *et al.* 2004) and standard errors were estimated using a bootstrap method with 500 replicates. DNA base frequencies and the appropriate model of sequence evolution were estimated using Modeltest 3.7 (Posada & Crandall 1998). The level of sequence divergence within and between populations was determined using a pair-wise analysis in DnaSP v 4.10.9 (Rozas *et al.* 2003). TCS v1.21 (Clement *et al.* 2000) was used to estimate a phylogenetic network of haplotypes using statistical parsimony. A homogolous DNA sequence from Mallard was retrieved from Genbank (accession number AY928900; Kulikova *et al.* 2005) and added to the data set as the outgroup taxon.

Microsatellite allele sizes were determined using the GeneMapper® v4.0 software (Applied Biosystems). Calculations of allelic richness and tests for null alleles were implemented in HP-RARE 1.0 (Kalinowksi 2005) using the rarefraction method to standardise for sample size. Allele and genotype frequencies, Observed (H_O) and Expected (H_E) heterozygosities, F_{IS}, tests for Hardy-Weinberg Equilibrium (HWE), and linkage disequilibrium (LD) were calculated using GENEPOP v4.0 (Raymond & Rousset 1995). Deviations from HWE were tested using global tests for heterozygosity deficit and excess within each population by means of the exact probability test with Markov chain parameters (with Bonferroni correction). Linkage disequilibrium was tested for using Fisher's exact test.

Results

Mitochondrial haplotype diversity

The DNA sequence of the mtDNA control region was determined for a total of 150 individual Brown Teal: 49 from MIW, 39 from GBI, 20 from the captive population, 19 from Moehau, 12 from Karori Wildlife Sanctuary, eight from Tiritiri Matangi Island and three from Mana Island (Table 1). The aligned sequence data set was 636 base pairs long, and contained 18 variable sites (2.8%) of which eight were parsimony-informative (1.3%). The pooled sample contained 12 haplotypes (labelled A to L), with a haplotype diversity (h) of 0.3900 and nucleotide diversity (π) of 0.0044 (Table 1). The highest level of mtDNA diversity was found within the MIW population, which contained 11 haplotypes (10 unique), with h = 0.6990 and π = 0.0088. Within the GBI population, only one sample had a unique haplotype (labelled L) and all other samples were haplotype A. Haplotype (h = 0.0645) and nucleotide (π = 0.0003) diversity were low within the GBI sample compared with MIW. Within the captive and all new populations only a single haplotype (A) was found. Considering all populations together, the most common haplotypes were A

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Table 1. The sampled Brown Teal populations and summary statistics of genetic diversity. Column headings: n_{ms} = microsatellite sample size; A = mean number of alleles per locus; *Arich* = mean allelic richness; H_O = observed heterozygosity; H_E = expected heterozygosity; n_{mt} = mtDNA sample size; H = the number of haplotypes (H); h = haplotypic diversity; S = number of segregating sites; π = nucleotide diversity; and the haplotypes present.

	Microsatellite loci							*Mitochondrial DNA*					
Population	n_{ms}	A	***Arich***	***Ho***	H_E	**Private alleles**	F_{IS}	n_{mt}	**H**	h	S	π	**Haplotype**
Mimiwhangata (MIW)	39	11	4.366	0.782	0.833	2	0.0177	49	11	0.6990	12	0.0088	A, B, C, D, E, F, G, H, I, J, K
Great Barrier Island (GBI)	28	9	4.222	0.773	0.788	1	0.2498	39	2	0.0645	1	0.0003	A, L
Captive breeding	36	9	4.075	0.571	0.751	1	0.1629	20	1	0	0	0	A
Moehau	24	7.5	3.322	0.521	0.637	0	0.1864	19	1	0	0	0	A
Karori Wildlife Sanctuary	12	6	3.369	0.500	0.745	0	0.3393	12	1	0	0	0	A
Tiritiri Matangi Island	8	3	2.441	0.313	0.515	0	0.4118	8	1	0	0	0	A
Mana Island	3	1	1.000	n/a	n/a	0	–	3	1	0	0	0	A
Pooled samples	150							150	12	0.3900	12	0.0044	

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(n = 118; 79%), B (n = 16; 11%) and C (n = 5; 3%). No DNA sequence differences were found between the GBI, captive and new populations. However, a genetic distance of 0.7% was found between the MIW population and all other populations. The network of mtDNA haplotypes is presented in Fig. 2.

When the DNA sequences of the outgroup and two museum samples were included, the data set contained 80 variable sites (12.8%) and 50 parsimony-informative sites (7.8%). The Waipu River gorge sample had a haplotype (labelled M) that was not found in any of the contemporary samples, whereas the Helena Bay sample had

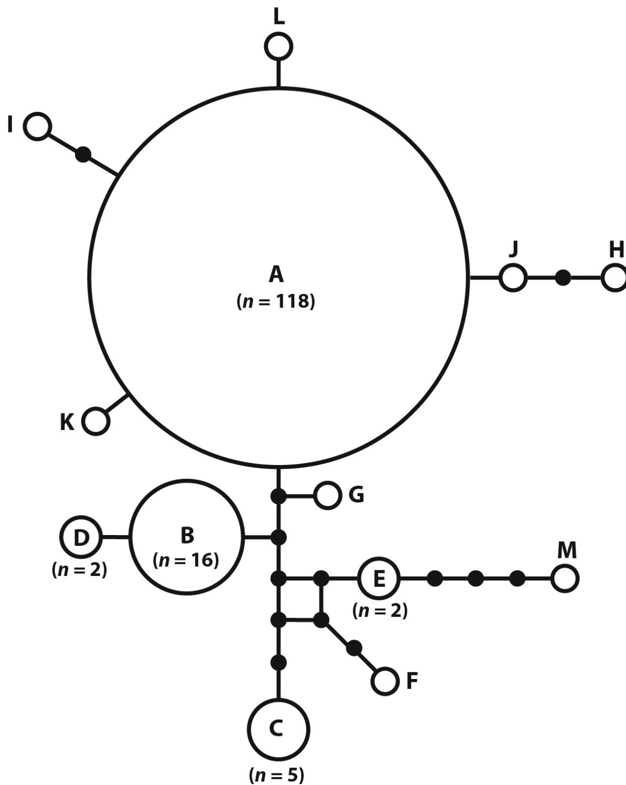

Figure 2. A phylogenetic network showing evolutionary relationships among the Brown Teal mtDNA haplotypes found in the seven extant populations (Mimiwhangata, Great Barrier Island, the captive breeding population, Moehau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island) and the two sequences from museum samples (Helena Bay and Waipu River gorge). Letters denote the haplotype identifier for samples (see Table 1 for the populations that contained haplotypes A–L). Circles denote the relative number of samples represented in each haplotype, and when more than one individual had a haplotype the total number is shown. The lines between haplotypes denote mutational steps between each DNA sequence, and closed circles indicate the inferred, unsampled haplotypes.

haplotype C that was present in the contemporary MIW population. The genetic distance between the Helena Bay museum sample and the GBI, captive and new populations was 1.8% whereas that between the Waipu River gorge museum sample and the GBI, captive and new populations was 2.3%. The genetic distance between the Helena Bay and Waipu River gorge samples was 3.2%.

Microsatellite diversity

One hundred and fifty samples were genotyped for five microsatellite loci, 39 were from MIW, 28 from GBI, 36 from the captive population, 24 from Moehau, 12 from Karori Wildlife Sanctuary, eight from Tiritiri Matangi Island and three from Mana Island (Table 1). Of the five loci used, two were polymorphic (*caudo24* and *caudo13*) and three monomorphic (*caudo19*, *caudo1* and *adph13*). A total of 14 alleles were identified at the *caudo24* locus and eight at the *caudo13* locus. The test for null alleles was non-significant over both polymorphic loci. The inbreeding coefficient (F_{IS}) was positive and significant within GBI, the captive population, and new populations but not within the MIW population. No evidence of linkage disequilibrium was found within any population ($P > 0.05$).

MIW was in Hardy-Weinberg equilibrium (HWE) for both loci ($P > 0.05$), but all other populations deviated from HWE at either one or both loci ($P < 0.05$). The departure from HWE observed in the GBI samples was due to a (non-significant) heterozygote deficit (global $P = 0.145$) at the *caudo13* locus. The captive population was in HWE for *caudo24* only and a heterozygote deficit was found at the *caudo13* locus (global $P < 0.05$). The new populations showed significant departures from HWE, due mainly to heterozygote deficit at both loci (global $P < 0.001$). Because of small sample size, tests for HWE could not be performed on the Mana Island sample.

Overall the two wild populations had greater genetic diversity than the captive and new populations (Table 1). Expected heterozygosity (H_E), allelic richness, and number of alleles per locus were highest within the MIW population and lowest in the Mana Island population. Two unique alleles were found within the MIW population, one in the GBI population and another in the captive population (Table 1). However, the unique allele found at the *caudo24* locus within the captive population (not found in either wild population) could be due to the limited sampling size, and if more samples from GBI were taken, this allele might be detected in the wild.

Discussion

Our study found different levels of genetic diversity among the populations of Brown Teal and the captive-raised birds had the lowest levels of diversity. The mtDNA analysis showed that there was a lack of genetic diversity within the GBI wild population, the source of all captive stock. Such low diversity indicates that the population may have experienced a historical bottleneck and/or founder effect (Arrendal *et al.* 2004; Leonard *et al.* 2005; Munoz-Fuentes *et al.* 2005; Ogden *et al.* 2005). A previous study on GBI Brown Teal using 14 allozyme markers also found no genetic diversity within 58 individuals

(Daugherty *et al.* 1999; Dumbell 1987). Furthermore, historic field observations suggest the species may once have been scarce on GBI (Bell & Braithwaite 1964; Dumbell 1987), and a mid-1800s survey of birds on the island failed to report Brown Teal (Hutton 1868).

In contrast to GBI, the samples from MIW revealed extensive mtDNA diversity. The high frequency of the common GBI haplotype A (33%) in the MIW population most likely arose from the releases of 321 captive-bred teal there between 1984–1991 (Dumbell 2000). Although more extensive DNA sampling of historic museum specimens might show haplotype A was once present elsewhere within the Northland region, it is now at a high frequency at MIW and further releases of captive-raised (GBI-sourced) birds into MIW, or elsewhere in Northland, will only result in raising its frequency and diluting the population's remaining genetic diversity. The MIW population, alone, represents almost all of the haplotype diversity now remaining in Brown Teal.

Low levels of microsatellite DNA variation and no mtDNA haplotype variation were found within the new populations at Moehau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island. The latter three populations were founded by small numbers of individuals (<20) and they have persisted thereafter in even smaller numbers (Anderson 2005; Dumbell 2000; G. Timlin, pers. comm.), which means the founder effect and/or genetic drift were likely to have been strong. Inbreeding is also a concern for the new populations; those at Karori Wildlife Sanctuary and Mana Island are now dominated by descendants of a single pair (Anderson 2005; R. Empson & G. Timlin, pers. comm.). We accept that having used only two microsatellite loci may lead to an under-estimate of the number of private alleles per population. When more loci become available for this species a re-analysis would be prudent.

Captive breeding and release has long been the sole pathway for advancing the numerical and range expansion of Brown Teal (Williams & Dumbell 1996; O'Connor *et al.* 2007). Brown Teal have struggled in the face of mammalian (especially mustelid) predation and, despite extensive releases (Dumbell 2000), new populations have been established only recently on mustelid-free islands (Tiritiri Matangi, Mana, Kapiti) or in mustelid-free enclaves (Karori Wildlife Sanctuary, Moehau). With the exception of Moehau, all new populations remain small (<20 birds) and are unlikely to persist without regular supplementation. The remaining wild populations have declined (between 1997–2007) despite concerted trapping of predatory mammals (Parrish & Williams 2001; Ferreira & Taylor 2003). Direct wild-to-wild transfers to establish new populations, or to augment the tenuous small populations, are not included in current management plans (O'Connor *et al.* 2007).

Our study highlights that: (i) the captive population does not represent the full range of genetic diversity present in the two wild populations, and (ii) the new populations do not encapsulate the full genetic diversity within the present captive-breeding population. Two serial genetic bottlenecks

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have been introduced by the establishment of the captive breeding programme and, again, during the release phase. There is mounting evidence that genetic bottlenecks are a concern for the long-term persistence of newly-established populations. Empirical studies have emphasised the negative consequences of low levels of genetic variation including inbreeding depression and an inability to respond to environmental challenges (*e.g.* disease or climate change) (Allendorf & Luikart 2007; Amos & Balmford 2001; Frankham 1995, 1996, 2005; Frankham *et al.* 2002).

The low levels of genetic diversity in populations associated with small founder sizes, whether established by wild-to-wild transfers or by captive-raised releases, as evidenced by inbreeding depression (*e.g.* Hendrik & Kalinowski 2000; Briskie & Macintosh 2004) or reduced immunocompetence (*e.g.* Hale & Briskie 2007), can be traced to the inadequate genetic sampling from the source population. From an evaluation of theory and case examples, Frankham (2005) considered the importance of genetic factors in the persistence of small isolated populations to be undeniable, a viewpoint re-examined and reinforced by Jamieson (2007). In effect, the debate has now largely moved on, to a consideration of how the genetic diversity remaining in small populations of threatened species can be managed and maintained, and to make "genetic diversity be a fundamental component in long-term management strategies for threatened species" (Jamieson *et al.* 2008). Management strategies that include a captive breeding component must take into account, in addition to genetic representation, the negative effects of adaptation to captivity and the accumulation of rare alleles which are deleterious but partially recessive in the wild (Frankham 2008). Furthermore, mixing genetically impoverished captive-raised stock with wild populations may also produce offspring of reduced fitness (Araki *et al.* 2007).

Without assiduous genetic management, conservation strategies which rely exclusively on captive breeding to establish new populations or to supplement declining ones, may well achieve short-term gain by establishing new populations, but over the long-term produce populations with limited evolutionary potential and which are vulnerable to any environmental change. If captive breeding and release has to remain the sole conservation pathway for Brown Teal, as O'Connor *et al.* (2007) outlined, we suggest that the present captive breeding stock should be augmented to capture the full genetic diversity remaining in the wild populations. By this means the adaptive potential within each wild population becomes available to all populations henceforth. However, because so many generations have elapsed since the captive population was established a complete renewal of the breeding stock should also be considered, especially while the more genetically diverse wild population at MIW remains (potentially) large enough to supply new captive recruits. Occasional or *ad hoc* additions to the existing breeding stock could simply perpetuate the existing genetic under-representation.

Furthermore, we suggest that additional genetic diversity be introduced to all new

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populations, but especially to the larger Moehau population, by wild-to-wild transfers from MIW or via a reconstituted captive breeding programme. These transfers may need to be repeated and certainly closely monitored, to ensure that those individuals added successfully reproduce. Protocols like those suggested by Haig *et al.* (1990) may be able to guide this approach. After genetic supplementation, the small populations established where habitat is limited and where single families have become dominant (*e.g.* Karori Wildlife Sanctuary, Mana, Kapiti, Tiritiri Matangi Islands) could be managed as a meta-population simply by rotating breeding individuals between them and monitoring the outcomes.

The conservation status of Brown Teal is still assessed solely on numbers (Hitchmough *et al.* 2007; Birdlife International 2008; Miskelly *et al.* 2008), but this perspective ignores the important long-term role of genetic diversity. By incorporating genetic information where available, conservation assessments could be viewed as less about number of populations or minimum overall numbers and more about "equipping populations" with the adaptive potential to respond to the future's inevitable environmental challenges.

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Photograph: Brown Teal with brood, courtesy of New Zealand Herald/Gerard Johnson.

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