**High Mitochondrial and Nuclear Genetic Diversity in One of
the World's Most Endangered Seabirds, the Chatham Island
Taiko (*Pterodroma magentae*)**

Running title:

High Genetic Diversity in an Endangered Seabird

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Abstract Interpreting the levels of genetic diversity in organisms with diverse life and population histories can be difficult. The processes and mechanisms regulating this diversity are complex and still poorly understood. However, endangered species typically have low genetic variation as a consequence of the effects of genetic drift in small populations. In this study we examine genetic variation in the critically endangered Chatham Island Taiko (Tchaik, *Pterodroma magentae*), one of the world's rarest seabirds. The Taiko has a very small population size of between 120-150 individuals, including just 8-15 breeding pairs. We report surprisingly high mitochondrial and nuclear genetic diversity in this critically endangered long-lived species. We hypothesise that the present Taiko population has retained a significant proportion of its past genetic diversity. However, it is also possible that undiscovered birds are breeding in unknown areas, which could increase the population size estimate. Importantly from a conservation perspective we show that the high level of variation is unlikely to be maintained in the future since chicks currently being born have only a limited number of the mitochondrial DNA haplotypes found in adults. Reduced genetic variation will mean that our ability to infer past events and the population history of Taiko using genetics could soon be lost and the power to determine for example parentage and other close order relationships will be diminished. Therefore the maintenance of genetic diversity in future generations is an important consideration for conservation management of the Taiko.

Introduction

Interpreting the levels of genetic diversity in organisms with diverse life and population histories can be difficult. This is because the mechanisms and processes that regulate this diversity are complex and still poorly understood. Therefore, determining the level of genetic variation in an endangered long-lived seabird and comparing this to other avian species is helpful especially in the context of known population history, demography and life history characteristics. From a conservation perspective, priority should be given to the quantification and characterisation of levels of genetic diversity for all endangered species. This assessment of diversity can assist us in evaluating the likely genetic effects of future population changes, and can be a guide in conservation management that aims to maintain current levels of diversity (Roques and Negro 2005).

Endangered species typically have low genetic variation, especially when compared to closely related taxa that are not threatened (Ardern and Lambert 1997; Spielman et al. 2004; for exceptions see Avise 2004). Reductions in genetic variation associated with population bottlenecks effectively cause genetic homogenisation that therefore limits our ability to recover historical information. Moreover, a reduction in genetic diversity will also reduce the utility of genetic markers in resolving contemporary conservation issues such as identifying parentage and more distant relationships. These can in turn be used to elucidate an endangered species' behaviour, mating systems, philopatry, spatial genetic structure and population history.

We have investigated genetic variation in a study of one of the world's most endangered seabirds, the Chatham Island Taiko (Tchaik, *Pterodroma magentae*;

IUCN 2006). This burrowing gadfly petrel is endemic to the main Chatham Island (Rekohu/Wharekauri) located 860km east of New Zealand (Aikman et al. 2001). The Taiko was considered extinct by western science until 1978, and is now ranked “critically endangered” (Crockett 1994; IUCN 2006). Presently there are only 8 to 15 Taiko pairs known to have bred in recent years. In addition, there are a number of non-breeding individuals and the entire species is thought to comprise only 120-150 birds (Scofield unpubl. data). Many of the life history characteristics of Taiko that are known are shared with other related seabird species. These include delayed breeding (seven years being the youngest recorded; Lawrence, Millar et al. 2007), low reproductive rate (only one egg is laid per year), social monogamy, long-term partnerships, and a long lifespan (perhaps 30-40 years; Aikman et al. 2001). However the Taiko is difficult to observe and study because it is rare, pelagic, and only comes to land during the breeding season where it is nocturnal and inhabits underground burrows in a single remote location (Aikman et al. 2001). Therefore, the relationship between individuals is very difficult to determine by observation alone. A variety of molecular methods can provide an alternative approach to this problem but require the presence of a degree of genetic variation in the population. Knowledge of relatedness is important in understanding mating systems and behaviour such as philopatry.

Fledgling survival is critical in order to prevent the extinction of the Taiko. Taiko chicks are vulnerable to predation so intense predator control is maintained around burrows and fledgling flight paths during the breeding season (Aikman et al. 2001). A predator exclusion fence has been built around the site of an extinct Taiko colony and management plans for Taiko include the translocation of chicks to artificial burrows within this safer area (Aikman et al. 2001). Such a transfer would occur before chicks emerge from burrows so that they will potentially become imprinted on the new site

(as with other petrels; Gummer 2003). It is anticipated that the birds' philopatric behaviour will result in Taiko returning to the predator-excluded area after they return from the sea to breed. In addition, Taiko will be attracted to the new colony from flight by broadcasting of vocalisations (Aikman et al. 2001).

Mitochondrial DNA (mtDNA) is a useful marker to evaluate genetic diversity in Taiko because it has been used extensively in studies of birds thereby enabling comparison to other avian species. Mitochondrial DNA is haploid and generally maternally inherited so is more susceptible to genetic drift and bottlenecks (Avice 2004). Therefore, although useful, mtDNA variation can underestimate overall genomic variation and is not necessarily representative of nuclear diversity (Zhang and Hewitt 2003). Furthermore, Bazin et al. (2006) have also recently questioned the link between mtDNA diversity, population size and history in a study in which they compared a very diverse array of animal taxa. However, in contrast, when more closely related species are compared a positive correlation between population size, history and mtDNA diversity was found (e.g. Hughes and Hughes 2007) indicating the continued utility of the marker.

The different population history, life history and reproductive ecology of animals can affect genetic diversity and rates of decline (Kuo and Janzen 2004). To contribute to our understanding of this, we evaluated genetic diversity in a sample comprising almost the entire known Taiko population. To assess genetic diversity in the Taiko we have used mtDNA sequencing of the cytochrome *b* gene and both copies of a fragment of the duplicated control region domain I, and multilocus minisatellite DNA techniques. These genetic markers could be used to aid management initiatives important for the conservation management of the critically endangered Taiko.

Methods

Taiko breeding pairs, chicks, many unpaired male Taiko and a few unpaired females were caught on the ground. In addition, Taiko were caught in flight (using spotlighting techniques; Crockett 1994). Blood samples were collected from almost every Taiko caught since 1996 ($N = 142$; $N = 117$ were used in this study). DNA was extracted by proteinase K digestion and a modified version of the phenol/chloroform method (Sambrook et al. 1989).

The complete mitochondrial cytochrome *b* gene was amplified using the polymerase chain reaction (PCR) with primers L14863 (Nunn et al. 1996) and HTaikoThr2 5'-GGTTTACAAGACCAATGTT-3' (designed by Leon Huynen; for PCR conditions, product purification and sequencing information see Lawrence, Taylor et al. 2007). Sequencing primers included L14863, HTaikoThr2 and internal primers LCytB432 5'-TGAGGACAAATATCATTCTGAGG-3' and HCytB571 5'-GGAAGGTGAGGTGGATTAAGG-3'.

Duplication of the mitochondrial control region is known in Procellariiform species (Abbott et al. 2005). We sequenced domain I of the control region in Taiko and found double peaks in sequencing electropherograms at some nucleotide sites, suggesting duplication also exists in Taiko. The possible existence of an amplified nuclear pseudogene was tested for and excluded (for details see Lawrence, Taylor et al. 2007). Both copies of a 315bp region in domain I were amplified and sequenced (for PCR conditions, product purification and sequencing information see Lawrence, Taylor et al. 2007). These regions were designated fragments 1 and 2. Fragment 1 was amplified by PCR with primers F1AF 5'- AATGGCCCATGTGCGGTTGT-3' and

HCRPtb 5'-CTAGGGGTGTAGGGGAAAG-3', fragment 2 with F2AF 5'-AATGGTCTATGTGTGGGTGC-3' and HCRPtb. Sequences were deposited in the National Institutes of Health (U.S.A) genetic sequence database GenBank®. Accession numbers are XXXXXX. Sequences were aligned using Sequencher™ version 4.2.2 (GeneCodes®). We tested for selective neutrality of mtDNA haplotypes using Tajima's *D* calculated in Arlequin version 3.1 (Tajima 1989; Excoffier et al. 2005). Nucleotide and haplotype diversities were calculated according to Nei (1987) using Arlequin version 3.1 (Excoffier et al. 2005).

We performed a multilocus DNA profiling pilot study to determine which restriction enzyme / probe combination to use to achieve the optimal resolution of fragments. Subsequent minisatellite DNA profiling was performed as in Millar et al. (1994), with *Alu* I digestions in combination with the probe (CA)_n (Ellegren 1991).

Results

Cytochrome *b* and domain I control region haplotypes were determined for 90 Taiko adults and 66 chicks born since intense predator control and monitoring began in 1993. Ten individuals fall into both categories since they were caught as chicks and returned as adults. In total, 117 individuals were sequenced and haplotypes for others were inferred from known maternal relationships.

A total of 12 polymorphic sites defined 10 unique haplotypes in Taiko for cytochrome *b* (Table 1). Fragment 1 contained 32 polymorphic sites defining 20 unique haplotypes. Fragment 2 contained 21 polymorphic sites defining 19 haplotypes. When the cytochrome *b*, fragment 1 and fragment 2 sequences are

combined, 21 haplotypes are apparent (Table 1). Only 11 of these haplotypes were recorded among the 66 chicks (Table 1).

There is one nucleotide site that has ‘true’ heteroplasmy in haplotype AY (Table 1). There is a double G/A peak at this site, whereas in fragment 1 haplotype AX at this site has a definite G. This is the only difference between these two fragment 1 haplotypes, but the uniqueness of these haplotypes is reinforced by fragment 2 haplotypes (Table 1).

Haplotype and nucleotide diversities are presented in Table 2. Standard error for haplotype diversities were as follows: for cytochrome *b* ± 0.0481 , control region F1 ± 0.0140 , control region F2 ± 0.0142 . Standard error for nucleotide diversities were: for cytochrome *b* ± 0.000872 , control region F1 ± 0.011704 , control region F2 ± 0.009644 .

Mitochondrial DNA diversity was compared between Taiko, seabird and other avian species, with respect to phylogeny and conservation status (Table 2). The number of cytochrome *b* and control region domain I (CR I) haplotypes were divided by the number of individuals included in the study, and number of variable nucleotides per 100bp were calculated to enable ease of comparison with mitochondrial DNA diversity in Taiko. For DNA samples collected prior to 1998, levels of minisatellite DNA bandsharing were calculated between pairwise combinations of Taiko adults (mean 0.17 ± 0.032 SE, range 0 - 0.25, $N=19$).

Discussion

Mitochondrial DNA diversity in Taiko can be usefully compared to that in other seabirds and in other avian groups. These comparisons include species with different degrees of phylogenetic relatedness to Taiko and varying conservation status. In the case of cytochrome *b*, Taiko had only slightly less variation than that found in the two other Procellariiformes (tube-nosed seabirds), but more than some Charadriiform seabirds and most other avian species (Table 2). The conservation status of Charadriiform and other avian species having less variation than Taiko included species that are endangered, vulnerable and in the least concern category (as ranked by the World Conservation Union, IUCN 2006).

The number of control region domain I (CR I) haplotypes in Taiko was less than that recorded for most other seabirds analysed. However the number of variable sites in CR I in Taiko is more than that found in two other seabirds (Table 2). In addition, Taiko had more CR I variation than most of the non-seabird avian species (Table 2). The species of Procellariiformes (albatross) we compared with Taiko were either endangered or vulnerable (IUCN 2006). However these albatross species are comprised of more than one population and have many thousands of breeding pairs (e.g. Burg and Croxall 2001; compared to the Taiko which has 8-15). The Taiko did have an equivalent number of CR I haplotypes, and many more variable sites than one non-threatened seabird (the Ancient Murrelet; Table 2). Moreover, Taiko had more CR I diversity than many threatened or near threatened avian species and most of the avian species of 'least concern' used for comparison (Table 2). Therefore, mitochondrial genetic variation in Taiko is relatively high especially considering the Taiko has extremely low numbers (estimated at 120-150 birds). Furthermore the

extensive sampling of Taiko provided a rare opportunity to obtain a very robust measure of mitochondrial DNA diversity in a vertebrate species.

The values of bandsharing between the multilocus profiles of Taiko individuals are generally very low in comparison to similar studies of New Zealand birds (Lambert and Millar 1995 Table 2) and low in comparison to other petrels (e.g. Storm-Petrels *Oceanodroma leucorhoa* 0.58 ± 0.059 SD Mauk et al. 1995; Short-tailed Shearwaters *Puffinus tenuirostris* 0.298 ± 0.017 SE Austin et al. 1993). Hence, the low levels of bandsharing between Taiko indicate that this endangered species has large amounts of nuclear genetic diversity. Therefore, our findings indicate that both mitochondrial and nuclear genetic diversity are reasonably high in the Taiko compared to other avian species, including non-threatened birds. This is surprising since endangered species typically have low genetic variation (e.g. Arden and Lambert 1997; Spielman et al. 2004; for exceptions see Avise 2004).

When assessing genetic variation it is important to examine both mitochondrial and nuclear diversity for the same species. In the case of Taiko, mitochondrial DNA markers are useful because they enable a direct comparison with many other avian species. Evaluating diversity using nuclear genetic markers is also important so that both genomes are represented (Zhang and Hewitt 2003). The level of genetic variation in Taiko was higher in multilocus DNA profiles than in the mitochondrial DNA (as measured by comparison with other avian species).

Selection may occasionally favour the retention of high genetic diversity in small and isolated populations (Kaeuffer et al. 2007). We found no evidence for selection on mtDNA haplotypes (Tajima's D , $P > 0.05$ for each region). Another potential explanation for the reasonably high level of genetic variation in the critically endangered Taiko is the real possibility that undiscovered birds are breeding

elsewhere, this could increase the population size estimate. The mitochondrial diversity may be useful to detect the existence of any undiscovered birds. For example, if unique haplotypes are detected in birds caught in flight and not found in those caught on the ground this would suggest there are more Taiko breeding in undiscovered areas.

Another hypothesis for the high genetic diversity observed in Taiko could be that the current Taiko population retains a significant proportion of past genetic diversity. Relatively high genetic diversity in a rare species can indicate that decline in numbers is recent (Moritz 1994). Population contraction over a large number of generations may be required for loss of genetic variation, but this effect is influenced by a species' life history traits (Lippe et al. 2006). Long generation times and delayed sexual maturity can slow the loss of genetic variation (Kuo and Janzen 2004). Taiko are long-lived, reaching approximately 30-40 years, become sexually mature at a minimum of seven years, and have overlapping generations (Aikman et al. 2001; Lawrence, Millar et al. 2007). Furthermore, past demography could also be a contributing factor in the loss of diversity (Goossens et al. 2005). The Taiko was previously very numerous, once the most abundant burrowing seabird on Chatham Island (Aikman et al. 2001).

The speed of demographic fluctuation can also have an effect on genetic variation, e.g. more genetic diversity may be retained when a decline in population numbers is gradual (Lippe et al. 2006). It seems likely that the decline in Taiko numbers was initially gradual and probably the result of the arrival of the first humans on Chatham Island. However, the population probably underwent a steep decline in the early parts of the last century as predation and habitat clearance intensified. Knowledge of the history of the Taiko population allows inferences to be made from these genetic data.

Mori (the indigenous people) discovered the Chatham Islands/Rekohu around 1100-1500 AD (King 2000). These people harvested Taiko/Tchika for food and introduced Kio (Pacific Rat, *Rattus exulans*), which may have negatively impacted Taiko numbers (Crockett 1994). Europeans arrived in the early nineteenth century and introduced many animals that are known predators of Taiko (Crockett 1994). Also, by the late 1930s, large areas of Taiko habitat were cleared for farming (Begg 1977). Records indicate that Taiko were still reasonably abundant at the beginning of the 20th century (Crockett 1994). For example, in 1903 1000 Taiko chicks were harvested (King 2000). Since only a proportion of the population breed, there still would have been a significant population of Taiko at this time, around a century ago.

It is therefore likely that the surprisingly high level of genetic variation in the living Taiko population is at least in part due to the significant retention of past diversity. High genetic diversity despite substantial population decline has been observed in other long-lived species with delayed sexual maturity (e.g. the Ornate Box Turtle *Terrapene ornata*, Kuo and Janzen 2004; Orang-utan *Pongo pygmaeus*, Goossens et al. 2005; Copper Redhorse *Moxostoma hubbsi*, Lippe et al. 2006). This suggests that the life history traits of long-generation time and delayed maturity can buffer loss of genetic variation, especially when decline is recent. Hence our findings support the population genetic theory prediction that severe bottlenecks may not drastically reduce the genetic diversity when they last for a small number of generations (Amos and Balmford 2001).

However, low population growth rate can cause bottlenecks to persist, during which time genetic diversity will be further eroded (Allendorf and Luikart 2007). The Taiko has a long life span and slow reproduction that reduces the rate of genetic drift but also slows recovery from a small population size. The effects of the population

bottleneck have not yet been detected in the adult generation, but the current high level of genetic variation may be transient and is not likely to be observed in future generations. Intense predator control around and monitoring of Taiko began in 1993, as a result the number of Taiko chicks fledged has substantially increased. However, the mtDNA diversity in this next generation is severely reduced. Of the 21 mitochondrial DNA haplotypes identified in the adult population, only half (11) are represented in the next generation (i.e. chicks born since 1993). Six of the adult haplotypes are only found in male birds, so will not be passed to the next cohort. Four adult haplotypes are found in females not known to breed. Therefore from a scientific and management perspective it is important that genetic markers are used to study the Taiko immediately while the population still retains sufficient genetic variation. More of the genetic history will be lost in the next generation and the power to determine parentage and close order relationships might be very significantly diminished just one generation from now.

A valuable conservation management tactic is to lessen average kinship in order to maintain genetic diversity (Avisé 2004). This is relevant to a current conservation management initiative, i.e. the establishment of a new Taiko colony within a predator-excluded site. Chicks will be translocated so they will potentially become imprinted on the new site and return there to breed once mature. Chicks can be chosen in order to maximise the retention of genetic variation described in this study, and reduce founder effects in the future colony.

Table 1 Variable nucleotide sites of 21 mitochondrial DNA haplotypes in Taiko (*Pterodroma magentae*).

Dots indicate identity to the reference sequence.

Haplotype	Number of Taiko Adults	Number of Taiko Chicks ^a	Cytochrome b (1143bp)	Fragment 1 Control Region Domain I (315bp)	Fragment 2 Control Region Domain I (315bp)
			Nucleotide Position ^b	Nucleotide position ^c	Nucleotide position ^c
			2 4 5 7 7 7 7 8 8 9 1	1 1 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2	1 1 1 1 1 1 2 2 2 2 2 2 2 2
			4 0 3 7 1 2 3 4 1 4 1 2	1 1 5 6 6 6 7 7 7 9 9 9 9 0 0 1 1 3 4 5 6 6 6 9 0 0 2 2 3 3 5 7	5 5 7 7 7 8 9 0 3 4 5 6 9 0 0 2 2 3 3 5 7
			5 4 2 1 6 0 8 1 9 0 5 2	1 4 8 3 5 7 5 7 9 4 5 6 9 1 6 0 5 9 6 5 1 2 4 4 6 7 3 9 0 1 0 9	3 6 3 7 8 9 4 6 9 6 6 4 4 6 7 3 9 0 1 0 9
AZZ	3	0	C T G G C A C C A C T G	T T C A T C A T C C C T T G G G G C A C T C T C T C G C C A C	C C A C T G C G C A A C C T C C G C C A C
AYY	18 ^a	10	.	. T A . R T . A . T . T	. T . T . A T . A . T . T
AXX	1	0	.	. T A T . A . T . T	. . . T T . T A . T . T
AWW	6	0	.	. T C . . . A T . A . T G T	. . . T C A T . A . T G T
AVQ	10	18	.	. T T . . C A A T A . . T	. . . T T A . . T
AUU	2	0	.	. T C . . . A T . A . T . T	. . . T T . A . T . T
ATQ	1	1	.	 T . . C . A . A . . T A . . T	. . . T T A . . T
ATT	1 ^a	1	.	 T . . C . A . A . . T A . . T	. . . T . A T . . . A . . T
ASS	4	5	.	. T C . . . A . A T . . . T G .	. . . T . A T . . . T G .
ARR	2	0	.	 C A T . A . . T	. . . T T . A . . T
BBB	8	7	. . A	 A T . . . A . . T	. . . T T . . . A . . T
CCC	3	4	T T . G T C .	C C T . C . . C . . . A A A . . C T . T . . . A . G .	. . . T T T . . . A . G .
DDD	4	0	T T	. T T . . A A . T G A . .	. . . T . . T . T G A . .
EEE	6	2	T . . . T	. T T . C A A . . G A . .	. . . T . . T . G A . .
FFF	3	2	 T	. T G . . C A T . . . A . . T	T . . T . A G T T . . . A . . T
GKM	11	9	T A	. T . . T A A . . . G . . . T . A . .	. . . T . . T . G T . A . .
GLM	2	7	T A	. T T . . A . . G T . A . .	. . . T . . T . G T . A . .
HHH	1	0	T	. T T . . A . A . G A . .	. . G T . . T . G A . .
III	1	0	T C	. T T . . A A A . G . C T C . . . A . .	. . . T . . T A . .
JOO	2	0	. . . A	. T T . . A C T . . . T G .	. . . T . . . A C T . . . T G .
JPP	1	0	. . . A	. T A A C T . . . T G .	. . . T C T . . . T G .
TOTAL	21	90	66		

Note: Ten individuals fall into both adult and chick categories since they were first caught as chicks and have returned to the colony as adults

^aTwo of the adult haplotypes were inferred from their chick's haplotype, many chick haplotypes were inferred from their mother's haplotype

^brelative to the start of the gene.

^crelative to the 3' end of the light strand primer.

Table 2 Comparisons of Taiko (*Pterodroma magentae*) mitochondrial DNA diversity with Procellariiform, Charadriiform and other avian species of varying conservation status (continued on next page)

Phylogenetic Groupings	Common Name	Scientific Name	Conservation Status ^a	Sample Size (N)	Marker Sequenced ^b	Number of Sampled Haplotypes / N	Haplotype Diversity (h)	Number of Variable Sites per 100bp	Nucleotide Diversity (π , $\times 10^{-2}$)	Reference
Procellariiformes	Taiko	<i>Pterodroma magentae</i>	Critically Endangered	90	Cyt- <i>b</i> (1143bp)	0.11	0.68	1.05	0.13	
	Herald Petrel	<i>Pterodroma heraldica</i>	Least Concern	33	Cyt- <i>b</i> (307bp)	0.15	-	2.28	-	Brooke and Rowe 1996
	European Storm Petrel	<i>Hydrobates pelagicus</i>	Least Concern	65	Cyt- <i>b</i> (910bp)	0.12	0.70	1.32	0.40	Cagnon et al. 2004
Charadriiformes	Marbled Murrelet	<i>Brachyramphus marmoratus</i>	Endangered	47	Cyt- <i>b</i> (1045bp)	0.30	-	-	-	Friesen et al. 1996
	Least Tern	<i>Sterna antillarum</i>	Least Concern	51	Cyt- <i>b</i> (362bp)	0.06	-	0.55	-	Whittier et al. 2006
	Ancient Murrelet	<i>Synthliboramphus antiquus</i>	Least Concern	58	Cyt- <i>b</i> (306bp)	0.09	-	1.31	-	Pearce et al. 2002
Other Avian Orders	Black-faced Spoonbill	<i>Platalea minor</i>	Endangered	87	Cyt- <i>b</i> (548bp)	0.01	0	0	0	Yeung et al. 2006
	Great Bustard	<i>Otis tarda</i>	Vulnerable	66	Cyt- <i>b</i> (292bp)	0.03	-	0.34	0.13	Pitra et al. 2000
	Ferruginous Pygmy-Owl	<i>Glaucidium brasilianum</i>	Least Concern	103	Cyt- <i>b</i> (899bp)	0.29	-	3.34	0.43	Proudfoot et al. 2006
	Loggerhead Shrike	<i>Lanius ludovicianus</i>	Least Concern	72	Cyt- <i>b</i> (200bp)	0.04	-	1.00	-	Mundy et al. 1997

^a as recognised by the World Conservation Union (IUCN 2006)

^b Cyt-*b*: cytochrome *b*, CR I: control region domain I, CR II: control region domain II

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