Loss of genetic diversity with captive
breeding and re-introduction: a case study
on pateke/brown teal (*Anas chlorotis*)

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Quote from Williams (2001) on the decline of pateke:

'The retreat of this species, from ubiquity to remnant, during the past 130 years, has been, arguably, more dramatic than for any other of New Zealand's endemic birds'.

To my parents for everything you have done and still do

Abstract

Pateke/brown teal (*Anas chlorotis*) have experienced a severe population crash leaving only two remnant wild populations (at Great Barrier Island and Mimiwhangata, Northland). Recovery attempts over the last 35 years have focused on an intensive captive breeding programme which breeds pateke, sourced almost exclusively from Great Barrier Island, for release to establish re-introduced populations in areas occupied in the past. While this important conservation measure may have increased pateke numbers, it was unclear how much of their genetic diversity was being retained. The goal of this study was to determine current levels of genetic variation in the remnant, captive and re-introduced pateke populations using two types of molecular marker, mitochondrial DNA (mtDNA) and microsatellite DNA.

Feathers were collected from pateke at Great Barrier Island, Mimiwhangata, the captive breeding population and four re-introduced populations (at Moehau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island). DNA was extracted from the base of the feathers, the mitochondrial DNA control region was sequenced, and DNA microsatellite markers were used to genotype individuals.

The Great Barrier Island population was found to have only two haplotypes, one in very high abundance which may indicate that historically this population was very small. The captive breeding population and all four re-introduced populations were found to contain only the abundant Great Barrier Island haplotype as the vast majority of captive founders were sourced from this location. In contrast, the Mimiwhangata population contained genetic diversity and 11 haplotypes were found, including the Great Barrier Island haplotype which may have been introduced by captive-bred releases which occurred until the early 1990s.

From the microsatellite results, a loss of genetic diversity (measured as average alleles per locus, heterozygosity and allelic richness) was found from Great Barrier Island to captivity and from captivity to re-introduction. Overall genetic

diversity within the re-introduced populations (particularly the smaller re-introduced populations at Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island) was much reduced compared with the remnant populations, most probably as a result of small release numbers and small population size. Such loss of genetic diversity could render the re-introduced populations more susceptible to inbreeding depression in the future.

Suggested future genetic management options are included which aim for a broader representation of genetic diversity in the pateke captive breeding and release programme.

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Chapter 1

General introduction

1.1 Captive breeding

Captive breeding for re-introduction is the removal of animals from declining wild populations for retention and breeding in captivity followed by eventual release back into wild ranges. The goal of captive breeding for re-introduction is to increase demographic representation while maintaining genetic diversity (Allendorf & Luikart 2007). Over the past few decades, captive breeding has become an increasingly relied upon conservation technique (Ebenhard 1995; Rahbek 1993). High profile and successful captive breeding programmes include those of the peregrine falcon (*Falco peregrinus*), Arabian oryx (*Oryx leucoryx*) (Rahbek 1993), black footed ferret (*Mustela nigripes*) (Wisely et al. 2003), Guam rail (*Gallirullus owstoni*) (Haig et al. 1990) and the Californian condor (*Gymnogyps californianus*) (Miller 1995). Captive breeding has been particularly successfully in the conservation of waterfowl (Hayes 2002).

1.1.1 Background

The purpose of holding wildlife in captivity has changed dramatically over the last 100 years. In the late 1800s, wildlife was held in zoos for display purposes with no conservation intent (Balmford et al. 1996). But by the 1970s-1980s, realisation of the dire situation that many species faced in the wild led to increased conservation awareness in zoos, which subsequently housed more endangered species (Frankham et al 2002; Whitford & Young 2004). In order to establish viable captive populations, collaborative breeding programmes between zoos and other institutes began with the long-term goal of re-introduction to the wild (Frankham et al. 2002).

Captive breeding has been highly endorsed and recommended in the conservation of endangered species. According to Ebenhard (1995), captive breeding could become essential to the preservation of 3000 species of mammals and birds. In 1992 The Global Waterfowl Conservation Assessment and Management Plan recommended that 150 of the 234 waterfowl species worldwide (64%) needed some level of captive confinement and breeding (Ellis-Joseph et al. 1992; Williams 1994). In addition, the Conservation Assessment and Management Plans under the Conservation Breeding Specialist Group of the International Union for Conservation of Nature (IUCN) recommended captive breeding for 1193 (36%) of the 3,314 species considered (Seal et al. 1993). Furthermore, captive breeding has been recommended in 200 (64%) of 314 approved recovery plans for species listed under the Endangered Species Act in America (Tear et al. 1995).

1.1.2 Limitations

Despite captive breeding programmes being recommended for a large number of species, their limitations are often not addressed (Allendorf & Luikart 2007; Snyder et al. 1996). Few endangered taxa have actually established successful captive populations and been successfully re-introduced to the wild (Millar et al. 1997; Snyder et al. 1996). Limitations and negative impacts within captive breeding programmes can include a departure of focus from the initial causes of decline in the wild (i.e. exotic predators, habitat loss) (Bell & Merton 2002), inbreeding and genetic drift (Lacy 1987), high cost of housing animals in captivity, adaptation to the captive environment (Frankham 2008), and disease outbreaks (Snyder et al. 1996). Frankham (2008) noted that re-introductions from a captive source have failed more often than re-introductions from a wild source to establish a viable wild population, indicating captive breeding may not always be an effective conservation tool.

1.1.3 Captive breeding in New Zealand

Human arrival to New Zealand resulted in drastic decline of endemic species (Duncan & Blackburn 2007; Park 1995; Wilson 2004). Numerous species became completely extirpated from the mainland to offshore islands and a large

number of extinctions occurred (Atkinson 2001; Armstrong et al. 2002). The decline of endemics has been primarily attributed to population fragmentation as the landscape was modified and the introduction of exotic predators (Atkinson 2001; Clout 2001; Holdaway 1989; Sinclair et al. 1998). Pateke/brown teal (*Anas chlorotis*), is a prime example. Pateke was once an abundant species but are now restricted to two small remnant populations and numbers under 1000 individuals. Decline in endemic species, is still ongoing.

Conservation management of endangered species in New Zealand has tended to act in two ways, firstly to eliminate the factors involved in decline or restore the habitat and secondly to increase the size of populations of endangered species through captive breeding or translocation (Jamieson et al. 2008). In terms of increasing endangered species demographically, the technique of translocation to offshore islands ‘island marooning’ (Williams 1977) has been widely used. Captive breeding has also been used in New Zealand to aid the conservation of several endangered birds including the black stilt (*Himantopus novaezelandiae*), kaka (*Nestor meridionalis*), blue duck (*Hymenolaimus malacorhynchos*), pateke, Campbell Island teal (*Anas nesiotis*), shore plover (*Thinornis novaeseelandiae*) and a number of herpetofauna (e.g. the Otago skink (*Oligosoma otagense*)) (Reed 1993; Millar et al. 1997; Maxwell & Jamieson 1997; Wilson 2004).

One successful example of captive breeding in New Zealand was the recovery of the Campbell Island teal. From an initial captive population of eleven individuals, captured from Dent Island in the 1980s, over 150 Campbell Island teal have been bred in captivity and re-introduced to Campbell Island after a predator-eradication programme was completed (M. Williams pers. comm. 2007). Pateke have also been successfully bred in captivity in New Zealand’s longest ongoing captive programme (since 1973) (Dumbell 2000). New pateke populations have been successfully established on three offshore islands and at two mainland sites (Williams & Dumbell 1996).

1.1.3.1 Genetic diversity in New Zealand endemics

Debate over the importance of genetic factors (such as inbreeding depression) in conservation practice in New Zealand gained traction in the 1980s and 1990s,

along with the development of new technologies for measuring levels of genetic diversity (Jamieson 2007a, 2007b). A long-term threat to endangered endemics restricted to offshore islands and mainland islands is thought to be reduced evolutionary potential and inbreeding depression associated with the erosion of genetic diversity in small populations (Frankham 1998). Jamieson et al. (2006) noted the very low levels of genetic variation within a number of endangered birds (kakapo (*Strigops habroptilus*), takahe (*Porphyrio hochstetteri*), South Island saddleback (*Philesturnus carunculatus carunculatus*), South Island robin (*Petroica australis australis*), and kokako (*Callaeas cinerea*)) and highlighted the mounting evidence that many species are suffering a loss of fitness (Bossenkool et al. 2007; Briskie & Mackintosh 2004; Jamieson et al. 2007c; Jamieson & Ryan 2000; Jamieson et al. 2003; Mackintosh & Briskie 2004). Overall, the need to maintain genetic diversity (where possible) through the recovery of threatened endemic species is becoming more recognised, although this path has not been without its critics (Craig 1991; 1994).

1.2 Conservation genetics and captive breeding

Captive breeding programmes are often established with the goal of minimising loss of genetic variation and natural selection, and to avoid inbreeding where possible (Allendorf & Luikart 2007; Lacy 1994). The impacts of genetic adaptation to captivity and inbreeding on small captive and re-introduced populations can lead to lowered individual fitness which impact on population size (Caughley 1994). For example a genetically depauperate population could become less able to withstand disease causing pathogens (O'Brian et al. 1985) and lack the ability to adapt (Caro & Laurenson 1994). Loss of adaptability and adaptation to captivity are of particular concern when attempting to re-introduce animals back into the wild (Frankham et al. 2002; 2008). In New Zealand, the loss of genetic diversity through translocation of wild individuals to isolated islands has resulted in negative fitness consequences for New Zealand robins (Hale & Briskie 2007; Jamieson et al. 2007c; Mackintosh & Briskie 2004), takahe (Jamieson & Ryan 2000) and kakapo (Jamieson et al. 2006). However, the loss of genetic diversity in captive breeding operations in New Zealand has not been studied in any detail.

Two strategies to minimize inbreeding and loss of genetic diversity within captive breeding programmes are the use of pedigrees and examining molecular genetic loci (Allendorf & Luikart 2007). Pedigrees (the detailing of mating and offspring) can be extremely powerful in maintaining genetic diversity by evenly distributing founder representation and minimising matings between close relatives (Montgomery et al. 1997). However pedigree recording is sometimes not implemented until well after the species has been brought into captivity and a significant loss of the genetic diversity of the founders may have already occurred. In the absence of pedigrees, examination of genetic loci can give a good estimate of the level of genetic diversity within a population and information on relatedness of individuals (Fernandez et al. 2004; Jones et al. 2002; Sikino et al. 2004; Russello & Amato 2004). Levels of genetic diversity can be measured by allele frequencies and heterozygosity data based on nuclear microsatellite DNA and sequence data from mitochondrial DNA. Genetic data can also be used to select wild individuals which are most representative of remaining genetic diversity, for introduction into a captive population.

The use of genetic markers to measure diversity for conservation purposes (i.e. for measuring genetic diversity within captive breeding programmes) has only been readily available over the last few decades (Allendorf & Luikart 2007). The technique of protein electrophoresis, whereby variation in proteins can be measured by allozyme markers, was available from the 1970s (Lewontin 1991). The use of DNA markers became available with the discovery of the polymerase chain reaction (PCR) and the first studies of DNA variation examined mitochondrial DNA (Avice 1986). The use of mitochondrial DNA to study variation became more widely available from the 1980s (Allendorf & Luikart 2007). Microsatellite DNA markers (from nuclear DNA) were first discovered in the 1980s and became widely used for conservation studies in the 1990s (Schlotterer 1998).

1.2.1 What is missing?

The theoretical aspects of genetic management of captive breeding programmes has been well investigated over recent times (Rodriguez-Clark 1999). Such management has been investigated using model species and computer simulations. For example using drosophila (*Drosophila melanogaster*), Briscoe et al. (1992) found a rapid loss of genetic variation over time in captivity. Furthermore using housefly (*Musca domestica*), Backus et al. (1995) found immigration increased fitness of the model captive population, Bryant et al. (1986) found low-founder populations suffered higher extinction rates, and Bryant and Reed (1999) noted that the frequency of deleterious mutations increased in captivity in the absence of manipulation. Statistical and mathematical models have also been used to investigate the genetic management of captive populations (Eardhardt et al. 2004). However, very few empirical assessments of the genetic management of captive programmes and loss of diversity from source to captivity to re-introduction have taken place (Wisely et al. 2003).

1.3 Study species

Pateke or brown teal (*Anas chlorotis*) is a small dabbling duck, endemic to New Zealand, classified within the genus *Anas* and the family *Anatidae*. Pateke are recognized as a distinct species, initially described by Oliver (1955). Kennedy and Spencer (2000) include pateke in the Austral teal group. Pateke has a labile taxonomic history (Dumbell 1987) but is presently accorded separate species (*chlorotis*) status from that of two derivatives of sub-Antarctic teal, the Auckland Island teal (*Anas aucklandica*) and the Campbell Island teal (Marchant & Higgins 1990). Pateke is currently one of the world's rarest waterfowl (Dumbell 1987) and recognised as endangered (Moore & Battley 2003).

Figure 1.1: Pateke on Great Barrier Island (photo by J. Sim, 2007).

1.3.1 Past distribution

Pateke were once widespread throughout New Zealand, including Stewart Island and the Chatham Islands (Buller 1888; Ferreria & Taylor 2003; Hayes & Williams 1982; Williams 2001; Worthy 2002) (Figure 1.2, left). They were possibly more widespread and more abundant than any other endemic waterfowl, and, at the time of European settlement were extremely abundant on lakes and in swamp forests (Potts 1972; Hayes & Williams 1982). Historically, pateke had a wide variety of habitat including; podocarp-broadleaf forests, kahikatea forests, estuaries, tidal flats, beaches, rivers, hill swamps, gully-heads and mountain lakes (Marchant & Higgins 1990; Worthy 2002).

Figure 1.2: Maps indicating the past and current distribution of pateke. The first map (on the left) shows distribution at the time of European arrival to New Zealand (reproduced from Hayes 2002, Hayes & Williams 1982 and Worthy 2002). The second map shows the current distribution of remnant pateke populations (indicated with arrows).

1.3.2 Population crash

The decline in pateke abundance was first remarked upon by the late 19th century (e.g. Buller 1888; Potts 1972). Williams (2001) described the decline in pateke as ‘arguably, more dramatic than any other of New Zealand’s endemic birds’. The decline was attributed to loss of habitat (Oliver 1955), massive wetland drainage and forest clearance which took place from the late 1800s and early 1900s. Further contributors to the decline included hunting pressure, exotic predators (mustelids, hedgehogs, feral cats, feral and domestic dogs and rats), pukeko (*Porphyrio porphyrio*) (pukeko prey on pateke ducklings) and waterways pollution (Williams 2001).

Pateke quickly disappeared from the Chatham Islands (1920s), Stewart Island (1972) and the South Island (2002). A small remnant population at Fiordland was recently declared extinct following hybridization with mallard (*Anas*

platyrhynchos) (Barton 2003; Gemmell & Flint 2000). Pateke declined rapidly in the Northland area, the final mainland stronghold from the late 1960s and by the late 1980s only a few declining populations remained (Hayes & Williams 1982; Parrish & Williams 2001). Today, only two remnant pateke populations survive; one in Northland at Teal Bay in Mimiwhangata (on Northland's East coast, latitude/longitude, 35.43°S/174.21°E) and one on Great Barrier Island (latitude/longitude, 36.11°S/175.25°E). According to the latest population estimate (2004) there are around 600 individuals on Great Barrier Island and an estimated 300 at Mimiwhangata.

1.3.3 Captive breeding

As a response to the dramatic decline in pateke a captive breeding programme was set up in 1973 (M. Williams pers. comm. 2007). Since this time the programme has undergone a number of changes and management techniques. Up until the mid 1990s a total of 76 pateke were sourced from Great Barrier Island as the captive founders (Dumbell 2000). During this time, no pedigree records or parentage information was systematically recorded (Dumbell 2000; K. Evans pers. comm. 2007). In the mid 1990s, a new management technique was implemented, whereby new wild origin pateke were brought into the programme to replace the unknown origin birds (Dumbell 2000; Hayes 2002). Since 2001, some wild pateke have been added to the captive programme including a number of birds from Great Barrier Island (comprising 13 new bloodlines), two bloodlines from Mimiwhangata, two from elsewhere in Northland and one bloodline from Little Barrier Island (K. Evans pers. comm. 2007). Today the captive population is made up of 29 breeding pairs and run by 22 captive holder volunteers who each house one or two breeding pairs. Overall, the programme has been highly successful as pateke readily adapt to captivity and a large number of captive offspring have been released (Hayes 1981, 2002).

1.3.4 Re-introduction

Offspring from the captive population have been used to attempt the re-establishment of new populations in the wild and to boost numbers at Mimiwhangata (Williams & Dumbell 1996). Captive offspring have been

successfully established in the Moehau area (at Port Charles from 2003-2007), supplementing a remnant population remaining in Moehau¹ (J. Roxburgh pers. comm. 2006). Captive offspring have also been introduced and established on Tiritiri Matangi Island (1987,1990), Kapiti Island (1968 and 2000-2001), Mana Island (2000-2001) and Karori Wildlife Sanctuary (2000-2001). Other releases have occurred on Moturoa Island (1985, 1986, and 1994) and Urupukapuka Island (1988, 1990, 1992 and 1994). A plethora of releases in Northland in the 1980s (Williams & Dumbell 1996) failed to establish populations, and there were earlier failures at Manawatu (1977–1983), Kaihoka in Nelson (1978), and on Matakana Island (1982) (Hayes & Williams 1982). Current releases have concentrated on the Northland area.

1.3.5 Genetic diversity in pateke

This history of pateke conservation and its reliance on captive propagation makes this species a good case example for examining the maintenance of genetic diversity within a captive breeding programme; a process which, as mentioned previously, has not been studied empirically (Wisely et al. 2003). Initially, the captive programme was founded by 76 pateke from Great Barrier Island. No pedigree or studbook information was tracked and within captivity there was ‘artificial’ selection in favour of highly productive birds (Dumbell 2000). Recent additions of pateke from the wild to the captive programme have been opportunistic and justified on the basis of conferring better representation of wild genetic diversity. However, there has never been any appraisal of genetic diversity of pateke in the wild (other than studies using allozyme markers on Great Barrier Island: Dumbell 1986; Daugherty et al. 1999) and the extent to which the captive breeding programme constituted a ‘genetic bottleneck’ has never been explored (Dumbell 2000). Therefore this research aims to examine genetic diversity within the remnant, captive and re-introduced pateke populations with the aim of aiding and informing pateke recovery

¹ The pateke population at Moehau is noted here as possibly being partially a remnant population. However, as a large number of captive releases have occurred at this site, Moehau is referred to as one of the ‘re-introduced populations’ throughout this thesis.

1.4 Population locations and sampling

For this study, DNA was extracted from feather samples provided from Great Barrier Island, Mimiwhangata, the captive breeding population, and four of the re-introduced populations (at Moechau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island). Three samples from historical locations of pateke habitation were also provided (Table 1.1).

1.4.1 Samples

Table 1.1: Sample table detailing sample location, the overall number of feather samples obtained², the total number of feather samples used³ and the sources from which samples were obtained for this research (see Appendix 1 and 2 for detailed sample information, refer to acknowledgements section for individuals involved in sampling from each location). Samples were obtained from the pateke populations at; Mimiwhangata (Mimi), Great Barrier Island (GBI), Moechau (Moe), the captive breeding population (CB), Karori Wildlife Sanctuary (KWS), Tiritiri Matangi Island (Tiri) and Mana Island (Mana).

Extant locations	lat/long	Samples obtained	Samples used	Samples provided by	Collection dates
Mimi	35.43S/174.21E	286	51	DoC	2001-2007
GBI	36.11S/175.25E	134	44	DoC	2002-2007
CB	Many locations	43 ⁴	25	Captive Breeding Network	2007
Moe	36.45S/175.31E	54	25	DoC	2007
KWS	41.19S/174.46E	12	12	Karori Wildlife Sanctuary	2004-2007
Tiritiri	36.52S/174.46E	8	8	DoC	2006
Mana	40.57S/175.03E	3	3	DoC	2006
Total		540	168		
Historical locations					
Fiordland	-				
Loch Maree	45.15S/170.0E	1	1	M. Williams	1977
Helena Bay	35.43S/174.21E	1	1	Te Papa	1978
Waipu Gorge	36.54S/174.47E	3	1	Te Papa	1935
Total		5	3		

² A large number of feathers were provided in total some of which included several samples from the same bird (often taken on separate dates). Therefore careful identification of samples beforehand was carried out and only samples from different birds used.

³ The exact samples used for Chapters 2 and 3 differed slightly, therefore the ‘Sample used’ for Table 1.1 does not match exactly with the total number of samples used for Chapters 2 and 3. This was because from some samples DNA was unable to be exacted and/or amplification of specific samples was unable to be achieved.

⁴ The samples for the captive breeding population were made up from captive breed individuals and a number of captive offspring (pre-release). The samples used for this study were made up of

1.4.2 Population locations

Figure 1.3: Map of population locations. Red stars and bold font indicate the extant remnant pteke populations (Mimiwhangata and Great Barrier Island), and the four re-introduced populations (Moehau, Tiritiri Matangi Island, Mana Island and Karori Wildlife Sanctuary). Purple stars and italic font indicate the now extinct areas from which samples were obtained, Loch Maree in Fiordland, Waipu Gorge and Helena Bay (once an extension of the Mimiwhangata population).

captive breed individuals. If captive individuals were not sampled they were replaced with their progeny.

1.5 Aims, predictions and thesis structure

1.5.1 Justification of aims and predictions

The history of pateke recovery is summarised below (Figure 1.4). Ideally, the captive population should be a good representation of the wild diversity identified on Great Barrier Island and furthermore each re-introduced population should be a good representation of the diversity found in the captive population. However, if significant loss of genetic diversity has occurred then it would be expected that the Great Barrier Island population would have higher genetic diversity than the captive population and the captive population would have higher genetic diversity than each re-introduced population.

Furthermore, as a result of isolation and distance between the two remnant populations it is expected that each contains unique genetic diversity. However, as the captive breeding programme has been fuelled almost entirely by stock from Great Barrier Island, it is possible that some of the genetic diversity still remaining in the wild (at Mimiwhangata) has not been brought into the captive programme.

Figure 1.4: Diagram showing pateke captive breeding and re-introduction. The captive population has been founded almost entirely from the Great Barrier Island remnant population. Each re-introduced population has been founded from captive offspring. Captive offspring have also been released into Mimiwhangata to boost population size.

1.5.2 Aims and predictions

The first aim of this research was to determine levels of genetic diversity within and among the two remnant populations (Mimiwhangata and Great Barrier Island), the captive population and four re-introduced populations (Moehau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island) using a mtDNA marker.

The first two predictions erected to guide this research follow the premise that loss of genetic diversity will have occurred due to the process of captive breeding and re-introduction.

1. If genetic diversity is lost as a consequence of creating a captive breeding programme, then the Great Barrier Island population will have a greater diversity (measured as number of haplotypes, haplotype diversity, and nucleotide diversity) compared with the captive breeding population.
2. If genetic diversity is lost within the captive breeding population or as a consequence of bottlenecks expected in the release group, then the captive breeding population will have a greater diversity (measured as number of haplotypes, haplotype diversity, and nucleotide diversity) when compared with each individual re-introduced population.

The third prediction is that, due to isolation and distance, the two remnant populations are expected to each possess a degree of unique genetic diversity.

3. Mimiwhangata and Great Barrier Island will each possess unique haplotypes

The second aim was to complete the analysis again using microsatellite DNA markers. The predictions forming the basis of this research were as follows;

1. If genetic diversity is lost as a consequence of creating a captive breeding programme, then the Great Barrier Island population will contain greater

genetic diversity (measured as average alleles per locus, heterozygosity and allelic richness), when compared with the captive population.

2. If genetic diversity is lost within the captive breeding population or as a consequence of bottlenecks expected in the release group, then the captive population will contain greater genetic diversity (measured as average alleles per locus, heterozygosity and allelic richness) compared with each individual re-introduced population.
3. Mimiwhangata and Great Barrier Island will each possess unique alleles.

The third aim of this thesis was to inform the Pateke Recovery Group of the current genetic situation in pateke, and, if genetic diversity has been lost, include suggestions as to potential methods of increasing and maintaining genetic diversity through long-term recovery.

1.6.3 Thesis structure

This thesis is structured as two individual papers (Chapters 2 and 3), Chapter 2 presents the mtDNA results (aim one) and Chapter 3 the microsatellite results (aim two). A brief discussion is included within Chapters 2 and 3. Further discussion is presented in Chapter 4 which also examines the final aim. A degree of repetition is therefore incorporated within this thesis.

Chapter 2

Low genetic diversity found within the captive breeding programme and re-introduced populations using mitochondrial DNA

2.1 Introduction

Captive breeding for re-introduction is becoming more relied upon to save threatened species from extinction in the wild (Allendorf & Luikart 2007; Ebenhard 1995). However, captive and re-introduced populations face the negative genetic consequences of inbreeding and genetic drift associated with creating small populations (Frankham 1995a, 1995b; Lacy 1987), which often remain unaddressed (Snyder et al. 1996). Inbreeding and genetic drift can lead to inbreeding depression, as demonstrated by recent high profile studies on the Greater Prairie chicken (*Tympanuchus cupido*) (Westemeier et al. 1998) and song sparrow (*Melospiza melodia*) (Keller et al. 1994).

Captive breeding has been implemented for recovery of a number of threatened species within New Zealand. A typical example is the captive breeding programme of the pateke/brown teal (*Anas chlorotis*). This captive breeding programme was set up in 1973, founded initially and subsequently from Great Barrier Island (the larger of the two remaining remnant populations of pateke). Release of captive-bred pateke has met with varying success as many releases have failed to establish self-sufficient populations (Williams & Dumbell 1996). A number of successful re-introductions have occurred to offshore Islands (Tiritiri Matangi, Kapiti and Mana) and to Karori

Wildlife Sanctuary and Moehau. Furthermore, a number of releases into the smaller remnant population at Mimiwhangata have occurred (see Figure 1.4).

One of the objectives of the pateke recovery effort is to retain genetic diversity through the captive process (Dumbell 2000; O'Conner et al. 2007). However to date no assessment of genetic diversity in the pateke captive breeding population has been undertaken. Accurate pedigree records for the entire captive period are not available and the extent of which pateke has undergone a bottleneck from population remnant to captivity to re-introduction, is unknown. This research aims to examine the outcome of captive breeding on genetic diversity in pateke by comparing genetic diversity remaining within the two remnant populations with that present in the captive and the re-introduced populations.

To measure genetic diversity within a species, mitochondrial DNA (mtDNA) is one molecular tool which can be used (Allendorf & Luikart 2007). As mtDNA is maternally inherited it does not undergo meiosis and recombination, therefore it can be considered a single non-recombining genealogical unit with multiple haplotypes (Avice 1994, 2004). Furthermore mtDNA is polymorphic and has a wide range of applications including population genetics, DNA bar-coding and constructing phylogenies (Moritz 1994, 2002). Previous studies exploring conservation issues have used mtDNA markers to assess population structure (Encalada et al. 1996), species geographic structure (FitzSimmons et al. 1997), and measure genetic diversity loss in endangered species (Blois & Arbogast 2006; Ciborowski et al. 2007; Munoz-Fuentes et al. 2005; Wilmer et al. 1993).

2.1.1 Aims and predictions

The aim of the research reported in this chapter is to determine whether genetic diversity has been lost as a consequence of creating the pateke captive breeding programme, using mtDNA. The following aims and predictions were erected to structure this research:

1. If genetic diversity is lost as a consequence of creating a captive breeding programme, then the Great Barrier Island population will have greater diversity (measured as number of haplotypes, haplotype diversity and nucleotide diversity) compared with the captive breeding population.
2. If genetic diversity is lost within the captive breeding population or as a consequence of bottlenecks expected in the release group, then the captive breeding population will have greater diversity (measured as number of haplotypes, haplotype diversity, and nucleotide diversity) when compared with each individual re-introduced population.

As a result of isolation and distance, the two remnant populations are expected to each possess a degree of unique genetic diversity. This could mean genetic diversity present in Mimiwhangata has not been brought into the captive programme. The third prediction to guide research is therefore:

3. Mimiwhangata and Great Barrier Island will each possess unique haplotypes

2.2 Methodology

2.2.1 Study sites and feather collection

For this study, feather samples from 150 pateke were collected from Great Barrier Island, Mimiwhangata, the captive breeding population and four of the re-established populations (Moechau, Tiritiri Matangi Island, Karori Wildlife Sanctuary and Mana Island) (see Table 2.1 and Figure 2.1).¹ In addition, three samples (one blood and two tissue) was provided from three now extinct locations; Loch Maree in Fiordland, Helena Bay (an area which once formed an extension of the Mimiwhangata population), and Waipu Gorge (Bell 1959).

Table 2.1: Summary of pateke feather samples successfully used for the study from the pateke populations (at Mimiwhangata, Great Barrier Island, the captive breeding population, Moechau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island) and three museum specimens from Loch Maree/Fiordland, Helena Bay and Waipu Gorge (see Appendix 1 for detailed sample table including band number, sex, age, and location details of each bird sampled).

Extant population location	lat/long	Feather sample	
		no.	Collection date
Mimi	35.43S/174.21E	49	2001-2007
GBI	36.11S/175.25E	39	2002-2007
CB	Many locations	20	2007
Moe	36.45S/175.31E	19	2007
KWS	41.19S/174.46E	12	2004-2007
Tiritiri	36.52S/174.46E	8	2006
Mana	40.57S/175.03E	3	2006
Total		150	
Museum specimens location			
<i>Loch Maree, Fiordland</i>	45.15S/170.0E	1	1977
<i>Helena Bay</i>	35.43S/174.21E	1	1978
<i>Waipu Gorge</i>	36.54S/174.47E	1	1935
Total		3	

All feather samples were stored in small paper bags in a controlled temperature room (set at 4°C) throughout the duration of this study. At the end of this study all

¹ DNA was not obtained from 11 samples; two from the captive breeding programme, four from Great Barrier Island, three from Mimiwhangata and two Waipu Gorge museum specimens (these samples have not been included in Table 2.1).

remaining feathers were given to the Department of Conservation (DoC) as for many birds, more than one feather was taken.

Figure 2.1: Map of population locations. Red stars indicated locations and bold font indicates the extant remnant pateke populations, normal font the four re-introduced populations (Moehau, Tiritiri Matangi Island, Mana Island and Karori Wildlife Sanctuary) and italic font the museum samples.

2.2.2 DNA extraction, amplification and sequencing

The base of each feather (approximately 3mm) was excised with a sterilised scalpel and the standard phenol-chloroform DNA extraction procedure was carried out as follows. Firstly, the 3mm base section of the each feather was placed in 400µL of extraction buffer (10mM Tris pH 8.0, 50 mM NaCl, 10mM EDTA). Between 10 and 20µL of proteinase-K was added (depending on the size of the sample) and 10µL SDS (0.2%) was added. The samples were incubated overnight at 45°C. Following this digestion phase, DNA was extracted using phenol, then chloroform:isoamyl alcohol and precipitated by adding 1mL of 100% ethanol at -20°C for 2-3 hours. DNA was then pelleted and washed with 1mL 70% ethanol, dried, and re-suspended in TE buffer.

A fragment of the mitochondrial DNA control region (636bp) was amplified using primers developed from the congeneric mallard (*Anas platyrhynchos*). The forward primer, L78 (5' -GTTATTTGGTTATGCATATCGTG- 3') was taken from Sorenson and Fleischer (1996) and the reverse primer H774 (5'-CCATATACGCCAACCGTCTC -3') from Sorenson et al. (1999).

PCR amplifications using 1-2µL of DNA template were carried out in 25µL volumes with 10mM Tris pH 8.0, 50mM KCl, 1.5 mM MgCl₂, 0.4 µg/ml BSA, 0.4µL of each of the forward and reverse primer, 200µmol of each dNTP, and 0.5 to 1 units of Bioline DNA polymerase (Applied Biosystems Inc.). The thermal cycling was carried out using an Eppendorf Mastercycler for; 94°C 2min, (94°C 3min, 50-54°C 30sec, 72°C 2min), repeated for 30-40 cycles, and 72°C 3min.

PCR products were electrophoresised in agarose gel and a molecular weight standard was used to determine the size of amplified products. PCR products of the correct size were purified using column purification (Roche) or Exo-sapit (Applied Biosystems Inc.).

Following purification all samples were sequenced at the Allan Wilson Centre at Massey University, Palmerston North.

2.2.3 Sequence alignment and data analysis

DNA sequences were edited by eye and then aligned using ClustalW in MEGA v4.0 (Kumar et al. 2004). Homogeneity of base compositions was tested using PAUP 4.10b (Swofford 2002). Genetic distances were calculated in MEGA v4.0 and standard errors were estimated using a bootstrap method with 500 replicates. Base frequencies were calculated in Modeltest 3.7 (Prosada & 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). A network tree was created in TCS v1.21 (Clement et al. 2000) to identify haplotypes and a phylogram was constructed in PAUP v4.10b to illustrate a graphical representation of the haplotypes found. For the outgroup, a mallard sequence (Genbank accession number AY928900; Kulikova et al. 2005) was added to the data (see Appendix 5 for outgroup selection details).

2.2.4 Molecular diversity and test for neutrality

The number (*h*) and diversity (*H*) of the mtDNA haplotypes, mutation number (*n*), nucleotide diversity (*π*), and the number of polymorphic sites (*s*) were calculated using DnaSP v 4.10.9. Tajima's D-test (Tajima 1989), Fu and Li's D-test (Fu & Li 1993) and Fu's F-test (Fu 1997) were implemented in DnaSP v4.10.9 and were used to test for departure from neutrality for each populations (Table 2.3). Tajimas's D-test calculates the D-test statistic proposed by Tajima (1989) to test the neutral theory of molecular evolution; this test is based on the fact that under the neutral model, the number of segregated sites and the number of nucleotide differences are correlated. Fu and Li's D-test and Fu's F-test both test predictions about the neutral theory of molecular evolution.

2.3 Results

The final data set was comprised of 153 pateke sequences; 49 from Mimiwhangata, 39 from Great Barrier Island, 20 from the captive network, 19 from Moechau, 12 from Karori Wildlife Sanctuary, eight from Tiritiri Matangi Island and three from Mana Island. In addition, one sample from Loch Maree in Fiordland, one sample from Helena Bay, and one sample from the Waipu Gorge area were sequenced and added to the data set.

After aligning the sequences in MEGA 4.0 the sequence length was 636 bp long. The data set contained 80 variable sites (12.57%) and 50 were parsimony-informative (7.86%). When the outgroup and historical samples were removed, the data set contained only 18 variable sites (2.8%) and eight parsimony-informative sites (1.3%). The base frequencies were variable (A=29.8%, C=33.8%, G=12.4% and T=24.0%).

2.3.1 Summary statistics

2.3.1.1 Between population diversity

Between populations the average genetic distance (excluding the museum samples and the outgroup) was 0.0045 and for the entire data set was 0.0102.

From the grouped data set (excluding the museum samples and the outgroup), only the Mimiwhangata sample had variability between sequences. The within group mean genetic distance was found to be 0.0092 for the Mimiwhangata sample and 0.0000 for the Great Barrier Island, captive network, Moechau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island groups.

Table 2.2: Distance matrix for each pateke population showing between population genetic distances (using the nucleotide maximum composite likelihood model in MEGA 4.0). A mallard sequence is the outgroup (no. 11). Museum specimens are included in italics.

Location	1	2	3	4	5	6	7	8	9	10
1 Mana										
2 Tiri	0.0000									
3 KWS	0.0000	0.0000								
4 Moe	0.0000	0.0000	0.0000							
5 CB	0.0000	0.0000	0.0000	0.0000						
6 GBI	0.0000	0.0000	0.0000	0.0000	0.0000					
7 Mimi	0.0069	0.0069	0.0069	0.0069	0.0069	0.0069				
8 <i>Helena Bay</i>	0.0181	0.0181	0.0181	0.0181	0.0181	0.0181	0.0164			
9 <i>Waipu Gorge</i>	0.0226	0.0226	0.0226	0.0226	0.0226	0.0226	0.0206	0.0318		
10 <i>Fiordland</i>	0.2331	0.2331	0.2331	0.2331	0.2331	0.2331	0.2321	0.2263	0.2522	
11 Outgroup	0.1983	0.1983	0.1983	0.1983	0.1983	0.1983	0.1974	0.1920	0.2164	0.0325

Comparing the seven pateke populations, no genetic distances between populations, using a pair-wise nucleotide maximum composite likelihood model in MEGA 4.0 (Table 2.2) was found between Great Barrier Island, the captive population and the re-introduced populations. However a distance of 0.7% was found between the Mimiwhangata population and all other pateke populations (Table 2.2).

Comparing the populations with the museum samples, a distance of 1.8% was found between the Helena Bay sample and the Great Barrier Island, captive and re-introduced populations and the Waipu Gorge sample was 2.26% different (Table 2.2). In comparison, the Helena Bay sample was less different from the Mimiwhangata population (1.64%) as was the Waipu Gorge sample (2.06%). Between the Helena Bay and Waipu Gorge sample a distance of 3.2% was found.

The greatest genetic distance (22.63% - 25.22%) was found between the Fiordland sample and all other groups (Table 2.2), higher even than the distance between the outgroup (mallard) and the other groups (19.20 - 21.65%). The distance between the Fiordland sample and the outgroup (mallard) was relatively small (3.25%).

2.3.1.2 Within population diversity

Table 2.3: Summary statistics for the seven pateke populations showing diversity within and between populations. N is the number of sequences; *h* is the number of haplotypes; H is the haplotype diversity; S is the number of polymorphic sites; *II* is the nucleotide diversity; and n is the number of mutations. Also included are Tajima’s D statistic, Fu & Li’s D statistic and Fu’s F statistic.

Population	N	<i>h</i>	H	S	<i>II</i>	n	Tajima's D	Fu & Li's D	Fu's Fs
Mimi	49	11	0.6990	12	0.0088	12	-0.7155	-1.2112	-0.3790
GBI	39	2	0.0645	1	0.0003	1	-1.1447	-1.6930	-1.2390
CB	20	1	0	0	0	0	n/a	n/a	n/a
Moe	19	1	0	0	0	0	n/a	n/a	n/a
KWS	12	1	0	0	0	0	n/a	n/a	n/a
Tiri	8	1	0	0	0	0	n/a	n/a	n/a
Mana	3	1	0	0	0	0	n/a	n/a	n/a
Total	150	12	0.3900	12	0.0044	12	-1.3913	-1.8340	-1.7380

Overall the seven pateke populations contained 12 haplotyes and had a haplotype diversity (H) of 0.3900 and nucleotide diversity (*II*) of 0.0044 (Table 2.3). The total haplotype number identified over the combined data set was 15 (Table 2.4). Greatest diversity was recorded from the Mimiwhangata where a total of 11 haplotypes were found (10 unique) and nucleotide diversity (*II* = 0.0088) and haplotype diversity (H = 0.6990) were comparatively high. Within Great Barrier Island, one sample had a unique haplotype (L) and all other samples were haplotype A. Nucleotide diversity (*II* = 0.0003) and haplotype diversity (H = 0.0645) were low within the Great Barrier Island population compared to Mimiwhangata. Within the captive breeding population and all four re-introduced populations only one haplotype was found (A) and therefore no nucleotide or haplotype diversity was present.

The results from the test of neutrality (Tajima’s D-statistic, Fu & Li’s D-statistic and Fu’s F-statistic) were consistent with neutral evolution and non-significant results were observed for individual populations and the combined data set.

Of the museum specimens, the Loch Maree/Fiordland sample indicated a unique haplotype (M) as did the Waipu Gorge sample (N) (Table 2.4). The Helena Bay sample was found to have a haplotype C which was identified in the Mimiwhangata sample (Table 2.4).

Table 2.4: Haplotype distribution for the seven pateke populations and three museum samples. N is the sample size (total number of sequences) for each population and ‘Haplotypes’ shows the designation of the haplotypes within each population. Haplotypes identified in this study were given the letters A-N to distinguish between them.

Population	N	Haplotypes
Mimi	49	A,B,C,D,E,F,G,H,I,J,K
GBI	36	A,L
CB	20	A
Moe	19	A
KWS	12	A
Tiri	8	A
Mana	3	A
<i>Helena bay</i>	1	C
<i>Fiordland</i>	1	M
<i>Waipu gorge</i>	1	N

2.3.2 Network tree and phylogram

The haplotype network tree (Figure 2.2) developed in TCS v1.21, shows the relationship between the haplotypes identified. The most abundant haplotype from this analysis is A (n=118), followed by haplotype B (n=16) and haplotype C (n=5). The haplotype identified in the Loch Maree/Fiordland sample (M) had too many base pair changes from the extant pateke populations to be included in the network tree.

From the haplotype network tree (Figure 2.2) the base-pair changes between the haplotypes identified were small. Haplotypes H, I, J, K, G (all identified at Mimiwhangata) and haplotype L (identified on Great Barrier Island) had only one or

two base-pair change from haplotype A (Figure 2.2 and 2.3). Haplotypes D² and B (both identified at Mimiwhangata) were more distant from haplotype A with between three and four base-pair changes. Haplotypes C, F and E were between five and six base-pair changes from haplotype A. Haplotype N (identified in the Waipu Gorge sample) was found to be ten base-pair changes different from haplotype A.

The phylogram (Figure 2.3) developed in PAUP v4.10b shows the distribution of the different haplotypes, as in Figure 2.3, but includes the Loch Maree/Fiordland sample and the outgroup (mallard) sequence. The phylogram indicates that haplotypes (L, G, K, J, H and I) are all closely related to haplotype A and haplotypes B, C, D, E and F are less closely related to haplotype A. The phylogram also illustrates the large distance between haplotype M, identified in the Loch Maree/Fiordland sample, and the other pateke samples. Haplotype M is closely grouped to the mallard outgroup, in line with the genetic distance results (Table 2.2).

Finally, a distribution map (Figure 2.4) visually demonstrates the distribution of haplotype diversity over all the pateke populations sampled.

² NB: haplotype D was identified as two separate haplotypes in the TCS network analysis (Figure 2.2) but not in the phylogram (Figure 2.3).

Figure 2.2: TCS v1.21 network tree including 152 sequences (Loch Maree/Fiordland sample not included) from the seven extant pateke populations (Mimiwhangata, Great Barrier Island, captive breeding populations, Moehau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island) and two sequences from museum samples (from Helena Bay and Waipu Gorge). Each node represents one base-pair change. The number of samples showing each haplotype is included in the ovals. Haplotype letters are included (A-L).

Figure 2.3: Phylogram of the mtDNA haplotypes showing relationships between haplotypes. Haplotypes A-K were found in the Mimiwhangata population. Haplotypes A and L were found in the Great Barrier Island population. Only haplotype A was found in the captive breeding population and all four re-introduced populations. The Helena bay museum sample had haplotype C and the Waipu gorge sample had haplotype N. The Loch Maree/Fiordland sample had haplotype M. The outgroup (mallard) is included. Bootstrap values are not included as they add little value to phylogram.

Figure 2.4: Distribution map of pateke haplotypes. This chart demonstrates the high diversity of the Mimiwhangata population compared with the low diversity of the Great Barrier Island, captive and re-introduced populations.

2.4 Discussion

2.4.1 Loss of mtDNA diversity from remnant to captivity to re-introduction

This study undertook an assessment of genetic diversity from remnant to captivity to re-introduction, a rarely empirically studied process (Wisely et al. 2003). The history of pateke captive breeding provides a good opportunity to examine genetic diversity loss from remnant to captivity to re-introduction, to date such a study has not been undertaken on a captive breeding programme in New Zealand.

Genetic diversity at the mitochondrial level (haplotype number, haplotype diversity and nucleotide diversity) is essentially non-existent within the Great Barrier Island remnant population and there is no diversity at this level identified within the captive or re-introduced populations (Table 2.2, Figure 2.4). Never the less there is loss of mtDNA diversity from remnant source (Great Barrier Island), because haplotype L, present at a very low frequency on Great Barrier Island, is not captured in the captive population. Obviously there is no loss of diversity at the mtDNA level from captivity to re-introduction (prediction two) since no mtDNA diversity was identified within the captive or re-introduced populations.

2.4.2 Is there evidence for a past bottleneck or founder effects on Great Barrier Island?

The dominance of haplotype A within the Great Barrier Island population could be the result of founder effects or an historical bottleneck at this location. A bottleneck is viewed as the process whereby a population experiences a loss of genetic diversity by temporarily going through a reduction in effective population size (Allendorf & Luikart 2007; Chakraborty & Nei 1977). In comparison, founder effects are the loss of genetic diversity in a population established by a small number of founders that carry only a small amount of the genetic diversity from a larger population (Hedrick et al. 2001).

Previous studies have indicated that low haplotype number and diversity could signify a bottleneck or founder effects have occurred within a population (Arrendal et al. 2004; Fedorov et al. 1996; Leonard et al. 2005; Le Page et al. 2000; Munoz-Fuentes et al. 2005; Ogden et al. 2005). For example, in a study on the white-headed duck (*Oxyura leucocephala*), three haplotypes were found within contemporary samples compared to 10 in historical samples (Munoz-Fuentes et al. 2005). The white-headed duck underwent a dramatic historical decline followed by recent recovery and the conclusion from this study was that low contemporary haplotype number was the result of the bottleneck (Munoz-Fuentes et al. 2005).

Previous studies on Great Barrier Island pateke have also found low genetic diversity (Daugherty et al. 1999; Dumbell 1987). From a total of 58 pateke, Daugherty et al. (1999) found no allozyme diversity at 14 loci and suggested a significant genetic bottleneck resulting in a period of small population size may have occurred. Moreover, Dumbell (1987) investigated pateke genetic diversity from three areas on Great Barrier Island using 15 allozyme makers and found very little differentiation. Dumbell (1987) noted that low genetic differentiation between sites was unexpected due to ecological findings that pateke on Great Barrier Island were segregated into areas with little dispersal even between valleys.

Early observations of pateke in New Zealand also indicate that historically they may have been scarce on Great Barrier Island. Sightings and surveys prior to 1950 record pateke on Great Barrier Island only being in low numbers due possibly to extensive hunting from the mid 1800s until the early 1900s (Bell & Braithwaite 1964; Dumbell 1987; Turbott 1967). Furthermore, a bird survey on Great Barrier Island in the mid 1800s failed to record pateke altogether over a period of three months (Hutton 1868). Historical accounts corroborated with genetic findings from this study and past studies (Daugherty et al. 1999; Dumbell 1987) substantiates the conclusion that pateke were either not historically present on Great Barrier Island and their contemporary abundance a recent phenomenon, or the historical population

underwent a significant decline 120-150 years ago resulting in a genetic bottleneck. Analysis of historic museum specimens would help resolve this.

2.4.3 mtDNA diversity at Mimiwhangata

Within the Mimiwhangata remnant population diversity at the mitochondrial level (haplotype number, haplotype diversity and nucleotide diversity) was much higher than in the other pateke populations studied (Table 2.3 and 2.4, Figure 2.2, 2.3 and 2.4).

The pateke population at Mimiwhangata is the last remaining mainland population, the final stronghold from the once highly populated Northland area (Hayes & Williams 1982; Parrish & Williams 2001; Worthy 2002). Historical mtDNA diversity within and between pateke populations in New Zealand is likely to have once been high due to the non-migratory nature of this species and high population segregation into small areas within ranges (Dumbell 1987; M. Williams pers. comm. 2007). This pattern is evident from the museum specimens sampled. The museum specimen from Helena Bay was found to have a haplotype identified within the Mimiwhangata sample; Helena Bay is the next bay north of Mimiwhangata and was once the site for an extension of the Mimiwhangata population. In comparison, the Waipu Gorge museum sample had a unique haplotype which is consistent with the distance between Mimiwhangata and Waipu Gorge which is likely to have precluded migration between these sites³. That there was such high mtDNA diversity remaining within the Mimiwhangata population possibly indicates that historical population genetic diversity in pateke was higher than the low diversity found on Great Barrier Island. Therefore the low genetic diversity identified on Great Barrier Island could be possibly an historical abbreviation. Future protection of Mimiwhangata is vital to ensuring survival of genetic diversity within this species.

³ Access was sought for further Northland sourced museum specimens from Auckland Museum to evaluate haplotype diversity beyond Mimiwhangata and to seek that past presence of haplotype A in Northland, but access to these specimens was denied.

2.4.3.1 Genetic ‘swamping’ of Mimiwhangata?

The high frequency haplotype found on Great Barrier Island (A) was also found within 33% of the Mimiwhangata samples (Table 2.4, Figure 2.4) and is possibly a result of past captive releases into Mimiwhangata. A total of 282 captive offspring (from Great Barrier Island captive founders) have been released in eight separate releases between 1985 and 1991 into Mimiwhangata (Dumbell 2000; Williams & Dumbell 1996) (Figure 1.4). This could have resulted in the relatively high frequency of haplotype A within the highly diverse Mimiwhangata population. An alternative possibility is that haplotype A was historically present within the Northland region and occurs naturally within the Mimiwhangata population. Further sequencing of museum specimens is required to examine whether the Great Barrier Island haplotype was historically present within the Northland region or now occurs within Mimiwhangata as a result of captive releases. Whatever its origin, haplotype A is common at Mimiwhangata and further release of captive (Great Barrier Island sourced) offspring into Mimiwhangata could result in possible ‘genetic swamping’ of the highly diverse Mimiwhangata population.

2.4.3.2 Current Mimiwhangata pateke in captivity

Initially the captive pateke population was founded by 76 pateke from Great Barrier Island (Dumbell 2000). However, recently new Great Barrier Island pateke (n=21) and two sets of sibling from Mimiwhangata (n=9) have been brought into the captive population to replace the old Great Barrier Island founders (K. Evans. pers. comm. 2007). It is likely that the Mimiwhangata pateke currently in captivity also have haplotype A as this was the single haplotype identified in the captive sample. Not all birds at Mimiwhangata are of equal value to the captive population if an expansion of its genetic diversity is sought.

2.4.4 Implications of low mtDNA diversity

Loss of genetic diversity associated with founder effects and bottlenecks has been shown to have negative fitness consequences, in particular within small populations (Briskie & Mackintosh 2004; Keller et al. 1994; Packer et al. 1991; Westemeiser et

al. 1998). It has long been recognized that genetic diversity is a prerequisite for a population to be able to face future environmental changes and ensure long-term response to selection (Amos & Balmford 2001; Ballou & Lacy 1995; Caro & Laurenson 1994).

Within this study, genetic diversity at the mtDNA level was used as a proxy for diversity within other areas of the genome. In particular for areas associated with function, for example the MHC complex which is associated with disease resistance (Hess & Edwards 2002). Low diversity within the MHC complex has been implicated in population decline (Sommer 2005). For example, a study on the black robin (*Petroica traversi*), found low diversity within the MHC complex after a genetic bottleneck and concluded the population would be highly vulnerable to future pathogen exposure (Millar & Lambert 2004). Furthermore, Hale and Briskie (2007) found a decrease in immune response in severely bottlenecked populations of the New Zealand robin (*Petroica australis*) compared to a population which had not undergone a bottleneck.

In addition to use as a proxy for diversity in other areas of the genome, diversity within the mitochondria has been associated with fitness (Ciborowski et al. 2007). Ciborowski et al. (2007) examined Atlantic salmon (*Salmo salar*) stocking and found increased fitness (disease resistance) associated with higher haplotype diversity. Within pateke, higher productivity rates have been observed in Northland when comparing past monitoring of Northland pateke with Great Barrier Island pateke (using the results of Dumbell 1987 and Barker & Williams 2002). However future monitoring is required to determine if this difference is substantiated when other impacts are taken into consideration (i.e. sampling technique, sample sizes and environmental and biological differences between sites).

2.4.5 Fiordland hybridization

The one pateke sample from Fiordland taken at Loch Maree in 1977 was found to have a haplotype (haplotype M) that grouped closely with the mallard sequence

(genetic distance 3.71%) compared with the other pateke samples from Great Barrier Island (genetic distance 21.44%) and Mimiwhangata (genetic distance 21.38%) (Table 2.2). Kennedy & Spencer (2000) were the first to identify hybridization of pateke and mallards at Fiordland and more recent studies (Barton 2003; Gemmell & Flint 2000) have verified that extensive hybridization occurred at this site. Hybridization is also occurring, although rarely, within Mimiwhangata (S. Moore pers. comm. 2007; K. Evans pers. comm. 2007) and is an issue of concern for future management. Future genetic monitoring of the pateke populations is important to ensuring hybridization is not occurring and to ensure hybrids are not incorporated into the captive breeding population.

2.4.6 Conclusions

1. mtDNA diversity was found to be non-existent within the pateke captive breeding population and the re-introduced populations due to sourcing of captive founders from a genetically impoverished remnant population (Great Barrier Island) which had likely undergone a genetic bottleneck or founder effects.
2. The Mimiwhangata population was found to be highly genetically diverse.

Strategies can now be put in place to increase genetic diversity in the captive and re-introduced pateke populations. This study has highlighted the need for similar assessment of other endangered species captive breeding programmes to ensure re-introduced populations are genetically representative, where possible, of wild remnant populations.

Chapter 3

Investigating loss of microsatellite diversity from population remnant to captivity to re-introduction

3.1 Introduction

One method used to measure genetic diversity in natural populations is to examine allele frequencies and heterozygosity using microsatellite DNA markers (Balloux & Lugon-Moulin 2002). Microsatellites are tandem repeats of a short sequence motif made up of one to six nucleotides found within the nuclear genome of most species (Allendorf & Luikart 2007). The application of microsatellite markers includes population genetics and parentage analysis (Hedrick 1999; Selkoe & Toonen 2006).

As microsatellite markers are generally highly polymorphic and evolve rapidly, they can be used to study population processes such as migration, inbreeding, founder effects and dispersal (Selkoe & Toonen 2006). The usefulness of microsatellite markers is enhanced as primer pairs developed for one species can often amplify the same loci in closely related species (Baratti et al. 2001). However pitfalls in using these markers include; difficulty (and high costs) involved in isolating species-specific makers, difficulty in identifying the mutational model that should underlie the analysis, and problems with generating good amplified products for analysis (Ellengren 2004).

Neutral molecular markers, such as microsatellites, can be used to give an indication of the level of genetic diversity throughout the genome (Haig 1998). Genetic variation at neutral loci has been shown, in simulation studies, to correlate with genetic variation at functional loci which are under selection

(Batallion, 1996). Therefore if genetic diversity within a threatened population is found to be low at nuclear microsatellites loci, there could be also low diversity at function loci (e.g. loci involved in disease resistance) which could render the species vulnerable to new pathogens (Hale & Briskie 2007).

There are a number of differences between mitochondrial DNA (mtDNA) and microsatellite DNA. Firstly, mtDNA is maternally inherited whereas microsatellite DNA is bi-parentally inherited. Secondly, microsatellite DNA has a faster mutation rate compared with mtDNA, due to different mutational mechanisms (Allendorf & Luikart 2007). These differences denote slightly different applications of these two types of DNA markers as molecular tools. The use of mtDNA sequence data is more applicable to studies concerning historical divergence between populations, whereas data from microsatellite DNA markers can indicate recent genetic changes (e.g. associated with migration, inbreeding, founder effects and dispersal) (Selkoe & Toonen 2006).

Microsatellite markers have been widely used in many studies of the ecology and conservation of threatened species (Arrendal et al. 2004; Bellinger et al. 2003; Moritz et al. 2002; Munoz-Fuentes et al. 2006; Ogden et al. 2005; Paetkau et al. 1998; Sigg 2006). For example, loss of genetic diversity following translocation of South Island robins (*Petroica australis australis*) (Bossenkool et al. 2007) and South Island saddleback (*Philesturnus carunculatus carunculatus*) (Taylor & Jamieson 2008) was identified using microsatellites. Additionally a study on the koala (*Phascolarctos cinereus*) in Australia used microsatellite markers to identify levels of genetic diversity within populations and highlight populations with low genetic diversity which were of conservation concern (Houlden et al. 1996).

Furthermore, microsatellite markers are used to assess genetic diversity within captive programmes to aid management (Russello & Amato 2004). For example, microsatellite markers were used to assess and maximise genetic diversity within the captive and re-introduction programmes for the black footed ferret (*Mustela nigripes*) (Wisely et al. 2003), the Persian wild ass (*Equus hemionus onager*)

(Nielsen et al. 2007), the whooping crane (*Grus americana*) (Jones et al. 2002) and the bearded vulture (*Gypaetus barbatus*) (Gautschi et al. 2003).

3.1.1 Aims and predictions

The aim of the research described in this chapter is to use microsatellite DNA makers to determine whether genetic diversity has been lost as a consequence of creating a captive breeding programme. The aims and predictions are given as follows:

1. If genetic diversity is lost as a consequence of creating a captive breeding programme, then the Great Barrier Island population will contain greater genetic diversity (measured as average alleles per locus, heterozygosity and allelic richness), when compared with the captive population.
2. If genetic diversity is lost within the captive breeding population or as a consequence of bottlenecks expected in the release group, then the captive population will contain greater genetic diversity (measured as average alleles per locus, heterozygosity and allelic richness) compared with each individual re-introduced population.

Given the results of Chapter 2, the Mimiwhangata population is expected to possess unique genetic diversity. Therefore it is predicted that:

3. The Mimiwhangata population will possess unique alleles

3.2 Methodology

3.2.1 Sampling procedures and locations

For this study, feather samples from 150 pateke were obtained from the two extant remnant populations (Great Barrier Island and Mimiwhangata), the captive breeding population and four of the re-introduced populations (Moehau, Tiritiri Matangi Island, Karori Wildlife Sanctuary and Mana Island)¹.

Sample sizes for this study were slightly different to those in Chapter 2 as some of the DNA extractions used for Chapter 2 had degraded before the microsatellite laboratory work began and a number of new samples became available. Furthermore, the samples from historical pateke populations were not included in the sampling in this chapter.

Table 3.1: Summary of pateke feather samples from the seven pateke populations (Mimiwhangata, Great Barrier Island, the captive breeding population, Moehau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island) successfully used for this study (see Appendix 2 for detailed sample table including band number, sex, age, and location details of each bird sampled).

Location	lat/long	Feather Samples	Collection dates
Mimi	35.43S/174.21E	39	2001-2007
GBI	36.11S/175.25E	28	2002-2007
CB	Many locations	36	2007
Moe	36.45S/175.31E	24	2007
KWS	41.19S/174.46E	12	2004-2007
Tiri	36.52S/174.46E	8	2006
Mana	40.57S/175.03E	3	2006
Total		150	

All feather samples were stored in small paper bags in a controlled temperature room (set at 4°C) throughout the duration of this study. At the end of this study all feathers not used (from many birds, more than one feather was collected) were given to the Department of Conservation (DoC).

¹ DNA could not be obtained from a number of feather samples. These included four feather samples from Mimiwhangata, five from Great Barrier Island, five from the captive breeding programme and one from Karori Wildlife Sanctuary. These samples are not included in Table 3.1.

Figure 3.1: Map of sample locations including the two remnant populations at Mimiwhangata and Great Barrier Island and four re-introduced populations at Moehau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island.

3.2.2 DNA extraction, amplification and genotyping

Standard phenol-chloroform extraction procedure was carried out for all DNA extractions. The base of each feather (approximately 3mm) was excised, using a sterilised scalpel and placed in 400 μ L of extraction buffer (10mM Tris pH 8.0, 50 mM NaCl, 10mM EDTA). Between 10 and 20 μ L of proteinase-K was added (depending on the size of the sample) and 10 μ L SDS (0.2%) and the sample was incubated overnight (approximately 15 hours) at 45°C. Following this digestion phase, DNA was extracted using phenol and then chloroform:isoamyl alcohol,

and precipitated by adding 1mL of 100% ethanol at -20°C for 2-3 hours. DNA was then pelleted and washed with 1mL 70% ethanol, dried and re-suspended in TE buffer.

For this study, microsatellite markers developed for the congeneric mallard (*Anas platyrhynchos*) were used for pateke as no species-specific markers have been developed for pateke. Microsatellite primers for the mallard were selected from the literature (Table 3.2). The microsatellite primers chosen displayed a high level of observed heterozygosity (0.68 to 0.97) and allele number (5-13) in the mallard and also amplified in other species. Five microsatellites were chosen, *caudo24*, *caudo19*, *caudo13*, *caudo1* (Huang et al. 2005) and *adph13* (Maak et al. 2003).

Table 3.2: Microsatellite primer sequences, optimal annealing temperatures (°C) and size of fragment (bp) for the five microsatellite loci used in this study.

Primer	Primer sequence (5'-3')	Annealing temperature	Size (bp)	Reference
		(°C)		
<i>CAUDO24</i>	TCGCATTAAGCTCTGATCT ATCAACAGAATCCAAAATATG	55.5	270-340	Huang et al. (2005)
<i>CAUDO19</i>	CTTAGCCCAAGTGAAGCATG GCAGACTTTTACTTATCACTC	58.1	132-213	Huang et al. (2005)
<i>CAUDO13</i>	ACAATAGATTCCAGATGCTGAA ATGTCTGAGTCCTCGGAGC	58.1	85-113	Huang et al. (2005)
<i>CAUDO1</i>	ACAGCTTCAGCAGACTTAGA GCAGAAAGTGTATTAAGGAAG	55.5	315-331	Huang et al. (2005)
<i>ADPH13</i>	CAACGAGTGACAATGATAAAA CAATGATCTCACTCCCAATAG	52.0	179	Maak et al. (2003)

The M13 sequence method was used for labelling the microsatellite primers (Schuelke 2000). The fluorescent markers 6FAM© and VIC© were used (Applied Biosystems).

PCR amplifications using 1-2µL of template DNA were carried out in 15µL volumes with 10mM Tris pH 8.0, 50mM KCl, 1.5 mM MgCl₂, 0.4 µg/ml BSA,

0.6µL of the reverse primer and 0.15µL of the forward primer, 200µmol of each dNTP, and 0.5 to 1 units of Bioline DNA polymerase (Applied Biosystems). Thermal cycling was carried out using an Eppendorf Mastercycler for; 94°C 5min, (94°C 30sec, 50-58°C² 45sec, 72°C 45sec) repeated for 30 cycles, (94°C 30sec, 53°C 45sec, 72°C 45sec) repeated for 8 cycles, followed by 72°C for 10min. The final phase of the PCR cycle was the attaching phase of the M13 sequence.

PCR products were electrophoresed in 1.5% agarose gel and a molecular weight standard was used to determine the size of the amplified products. PCR products of the correct size were diluted depending on brightness of the gel.

All samples were genotyped at the Allan Wilson Centre at Massey University, Palmerston North.

3.2.3 Analysis

3.2.3.1 Scoring method

Allele frequencies were scored using GeneMapper® v4.0 software (Applied Biosystems). The binning mode was implemented to ensure accuracy in scoring between batches of samples. All samples were scored twice on two consecutive days to ensure accuracy in scoring (see Appendix 7 for further details).

3.2.3.2 Analysis overview and considerations

For this study, tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were performed. Observed heterozygosity (H_O), expected heterozygosity (H_E) and allelic richness were then calculated to measure diversity within and between populations. Simulations of future genetic diversity loss were also calculated.

The number of small sample sizes (when $n < 20$) and the small number of loci which were amplified for this study resulted in a limited number of analyses being performed. As only two loci were amplified, population differentiation

² The annealing temperature was specific to the primers (see Table 3.2)

(F_{ST}) could not be calculated and the results of Hardy-Weinberg are tentative. However, where possible, the differences in sample size have been accounted for where necessary (see 3.2.3.6).

3.2.3.3 Hardy-Weinberg equilibrium and linkage disequilibrium

Tests for HWE and linkage disequilibrium were calculated using GENEPOP v4.0³ (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). Tests for null alleles were implemented in HP-RARE 1.0 (Kalinowski 2005). Linkage disequilibrium, which tests for genotypes at one locus being independent of genotypes at another locus, was tested for using Fisher's exact test.

3.2.3.4 Observed and expected heterozygosity, allelic richness and inbreeding coefficient

Observed (H_O) and expected (H_E) heterozygosities were calculated in GENEPOP v4.0. H_E was calculated using Nei's estimation of heterozygosity. Allelic richness (the number of alleles per population corrected for sample size) was calculated in HP-RARE 1.0. Statistical tests for differences in H_O , H_E and allelic richness between populations were tested for at the 95% confidence level in FSTAT v1.2 (Goudet 1995) using one sided probability (i.e. Population1>Population2) and 1000 permutations (Bonferroni adjusted). The inbreeding coefficient F_{IS} was calculated in FSTAT v1.2 and significance from 0 was assessed at the 95% confidence level.

3.2.3.5 Simulations of future diversity changes

Simulations of heterozygosity and allele loss over the next 50 pateke generations (approximately 110 years) were implemented in GeneLoss (England & Osler 2001) and graphs of the simulation results were plotted using Microsoft Excel.

³ See Appendix 6 for information on computer software packages used for analysis.

3.2.3.6 Differences in sample size

Although allelic richness accounts for sample size, other measures of diversity (H_E , H_O and mean alleles per locus) can depend on sample size. Therefore, a correction for differences in sample size was made following (but not exactly) the methods of Arrendal (2004) and Walker et al. (2001). To account for differences in sample size, 50 random draws of eight individuals (the sample size of Tiritiri Matangi Island) were made from the Mimiwhangata, Great Barrier Island, captive, and the Moehau sample (all with sample size $n \geq 20$). Calculations for the average values of H_O , H_E and mean alleles per locus were made from these random draws and 95% confidence intervals calculated. These values were then compared to the smaller re-introduced populations from which only small sample sizes ($n < 20$) were obtained (Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island).

3.3 Results

Of the five loci tested for this study, only two were found to be polymorphic (*caudo24* and *caudo13*). The other three loci were monomorphic (*caudo19*, *caudo1* and *adph13*). A total of 14 alleles were identified at the *caudo24* locus and eight at the *caudo13* locus.

3.3.1 Hardy-Weinberg equilibrium and linkage disequilibrium

Mimiwhangata appeared to be in HWE for both loci ($P > 0.05$). Within the other populations, deviations from HWE were found at either one or both loci ($P < 0.05$). The departure from HWE observed in the Great Barrier Island sample 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 $P < 0.05$). Within the re-introduced populations, significant HWE departures were found due to heterozygote deficit at both loci (global $P < 0.001$). The frequency of null alleles was non-significant over both loci for all populations. Because of small sample size, tests for HWE could not be performed on the Mana Island sample.

The inbreeding coefficient (F_{IS}) was positive and significant within the Great Barrier Island, the captive population, and re-introduced populations (Table 3.3) but not within the Mimiwhangata population. A positive and significant inbreeding coefficient indicates a heterozygote deficit.

Linkage disequilibrium was not found within any of the pateke populations ($P > 0.05$), indicating the two loci were not linked.

3.3.2 Levels of genetic diversity

Overall the remnant populations (Mimiwhangata and Great Barrier Island) had greater genetic diversity compared with the captive and re-introduced populations (Table 3.3). The highest level of observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness, and the highest number of alleles per locus were detected in the Mimiwhangata population (Table 3.3) and these values were lowest in the Mana Island population.

Differences in H_O , H_E and allelic richness were not significant between Mimiwhangata and Great Barrier Island. Between each remnant population and the captive population, significant differences were found only between Mimiwhangata and the captive population regarding H_O ($P < 0.01$) and allelic richness ($P = 0.03$).

Between the captive population and the pooled re-introduced populations, a significant difference was only found in allelic richness ($P = 0.04$). However comparing the captive population and each re-introduced population, differences were found between allelic richness ($P = 0.01$), H_E ($P = 0.03$) and H_O ($P < 0.01$) at Tiritiri Matangi Island and between allelic richness ($P < 0.01$), H_E , ($P < 0.01$), and H_O ($P < 0.01$) at Mana Island. However, significant differences were not found between either Moehau and the captive population or Karori Wildlife Sanctuary and the captive population.

Significant differences in allelic richness ($P = 0.01$), H_O ($P = 0.02$), and H_E ($P = 0.01$) were observed when comparing the Great Barrier Island population to the

pooled re-introduced populations and to each individual re-introduced population.

Table 3.3: Summary statistics including; sample size (n) number of polymorphic loci, total number of alleles, mean alleles per locus, mean allelic richness, private allele number, observed (H_O) heterozygosity, and expected heterozygosity (H_E) and the Inbreeding coefficient (F_{IS}) for the seven pateke populations (Mimiwhangata, Great Barrier Island, captive breeding population, Moehau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island).

Population	n	Polymorphic loci	no. of alleles	Mean alleles per locus	Mean allelic richness	Private alleles	H_O	H_E	F_{IS}
Mimi	39	2	22	11	4.366	2	0.782	0.833	0.019
GBI	28	2	18	9	4.222	1	0.773	0.788	0.279*
CB	36	2	18	9	4.075	1	0.571	0.751	0.163*
Moe	24	2	15	7.5	3.322	0	0.521	0.637	0.193*
KWS	12	2	12	6	3.369	0	0.500	0.745	0.339*
Tiri	8	2	6	3	2.441	0	0.313	0.515	0.422*
Mana	3	0	2	1	1.000	0	n/a	n/a	n/a

*Significantly different from 0 at $P < 0.05$

For this study, it appears the population with the highest variability, corresponds to the one with the highest sample size (Table 3.3). However, when sample size was corrected for within the larger samples (Mimiwhangata, Great Barrier Island, the captive population, and Moehau), the highest values for H_O , H_E and mean alleles per locus were still obtained from Mimiwhangata, followed by Great Barrier Island, the captive population, and the Moehau population respectively (Table 3.4).

Table 3.4: Sample size (n), mean alleles per locus, observed heterozygosity (H_O) and expected heterozygosity (H_E). Figures in bold are mean values from 50 random samples of eight individuals (sample size for Tiritiri Matangi Island) and their 95% confidence intervals for Mimiwhangata (Mimi), Great Barrier Island (GBI), captive breeding population (CB), and Moehau (Moe).

Population	n	Mean alleles per locus	95% CI	H_O	95% CI	H_E	95% CI
Mimi	39	11		0.782		0.796	
		10.34	9.80-10.69	0.743	0.722-0.804	0.757	0.735-0.843
GBI	28	9		0.773		0.788	
		8.01	7.91-9.83	0.610	0.511-0.713	0.776	0.719-0.832
CB	36	9		0.571		0.751	
		7.45	6.98-8.98	0.554	0.500-0.615	0.648	0.599-0.792
Moe	24	7.5		0.521		0.637	
		6.56	6.01-7.24	0.480	0.400-0.600	0.570	0.504-0.702

When H_O , H_E and mean alleles per locus found within the small re-introduced populations (Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island) (Table 3.3) were compared with the equivalent values obtained through random draws from Mimiwhangata, Great Barrier Island, the captive population, and Moehau (Table 3.4), it appears, even when sample size is accounted for, the values of H_O , H_E and mean alleles per locus are smaller within the re-introduced populations. However, the value of H_E obtained for the Karori Wildlife Sanctuary sample ($H_E = 0.745$) appears to be higher than this value obtained from random draws of the captive population ($H_E = 0.648$) and the Moehau population ($H_E = 0.570$).

3.3.3 Allele frequency differences between populations

Allele frequencies differed considerably between populations (Figure 3.2) with the highest number of alleles being detected in the Mimiwhangata population. Unique alleles were identified within the Mimiwhangata population (two), the Great Barrier Island population (one) and the captive population (one). The unique allele detected on the *caudo24* locus within the captive population (not found in either remnant population) is possibly due to sampling and if a larger sample from Great Barrier Island was taken, this allele might be expected to occur at this location or within the Mimiwhangata population.

Figure 3.2: Allele frequencies at two loci, *caudo24* and *caudo13* showing the different frequencies found in each pateke population.

3.3.5 Loss of genetic diversity modelled in GeneLoss

To model the predicted loss of heterozygosity and alleles over time, simulations were run using the GeneLoss computer software program (England & Osler 2001). The results from simulations of remaining heterozygosity (Figure 3.3 a) and remaining average alleles per locus (Figure 3.3 b) after one (~ 2.2 years), five (~ 11 years), ten (~ 22 years), 25 (~ 55 years) and 50 generations (~ 110 years) show a greater decline in heterozygosity and allele number in the re-introduced and captive population compared with the remnant populations (Mimiwhangata and Great Barrier Island). Each pateke generation is estimated at 2.2 years (Williams & Dumbell 1996).

The greatest loss of genetic diversity (heterozygosity and allele number) is predicted within the captive population (Figure 3.3) and the smaller re-introduced populations (Tiritiri Matangi Island and Karori Wildlife Sanctuary). Comparatively, the Moehau population was found to lose less genetic diversity.

The genotypes identified within the re-introduced populations were then pooled in order to simulate the effect of increasing the effective population size (N_e) to imitate meta-population dynamics. When the re-introduced populations were pooled the collective loss of average alleles per locus was from 9 to 6.56 (Figure 3.4 b) and heterozygosity decreased from 0.78 to 0.72 (Figure 3.4 a) which was much reduced from the decreases observed when these populations were not pooled (Figure 3.3 a, b).

The decrease observed within the non-pooled re-introduced populations concerning average alleles per locus was 1.43 (Tiritiri Matangi Island) to 1.59 (Karori Wildlife Sanctuary) and to 4.63 (Moehau). Furthermore, loss of heterozygosity was 0.54 to 0.15 (Tiritiri Matangi Island) 0.74 to 0.19 (Karori Wildlife Sanctuary) 0.72 to 0.60 (Moehau) (Figure 3.3 a, b).

Figure 3.3: GeneLoss computer simulation of (a) the average expected heterozygosity and (b) number of alleles remaining at one, five, ten, 25 and 50 generations for each pateke population. This simulation assumes no migration or translocation between populations. The number of breeding pairs was obtained from current estimates from each of the populations. Each simulation was performed with 1000 iterations. The error bars are the standard errors (SE) which increase with increasing number of generations. The Mana Island sample was too small for simulations to be run ($n=3$).

Figure 3.4: GeneLoss computer simulation of (a) the average expected heterozygosity and (b) average number of alleles remaining at one, five, ten, 25 and 50 generations for the combined allele frequencies of the re-introduced pateke population. The number of breeding pairs was obtained from current estimates from each of the populations. Each simulation was performed with 1000 iterations. The error bars are the standard errors (SE) which increase with increasing number of generations. This simulation shows the potential impacts of translocation between the re-introduced populations if they were managed as a meta-population with translocation between each population.

3.4 Discussion

3.4.1 Loss of genetic diversity from remnant to captivity to re-introduction

Genetic diversity (mean alleles per locus, heterozygosity and allelic richness) within the remnant source population (Great Barrier Island) was found to be greater than that found within the captive population (Table 3.3). This followed the prediction that greater genetic diversity would be present within the source population.

A decrease in genetic diversity (mean alleles per locus, heterozygosity and allelic richness) was found from the captive to the re-introduced pateke populations providing evidence for the second prediction of a reduction in genetic diversity from captivity to re-introduction (Table 3.3). These differences in diversity still remained when sample size was corrected for (Table 3.4). The decrease in genetic diversity from the captive to the Moehau population was less pronounced.

3.4.2 Genetic diversity among the pateke populations

3.4.2.1 The remnant populations

Of all the pateke populations, Mimiwhangata had the highest genetic diversity (mean alleles per locus, heterozygosity and allelic richness). This finding is important as captive founders have been almost exclusively sourced from Great Barrier Island (Dumbell 2000; K. Evans pers. comm. 2007).

Only two alleles were unique to Mimiwhangata (Figure 3.2). However, Chapter 2 indicated that Mimiwhangata possessed a large number of unique haplotypes. As only two polymorphic loci were sampled for this study, results are tentative and more markers are needed to draw conclusions.

3.4.2.2 The re-introduced populations

Low genetic diversity was observed within the re-introduced populations, especially the smaller populations at Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island (Table 3.3). This could be the result of founding effects

and/or genetic drift as the re-introduced populations have been characterized by small release numbers and the populations persist in small numbers (with the exception of Moehau).

Small release numbers and small population size are a concern within the Karori Wildlife Sanctuary population. This population was founded, between 2000 and 2001, by a total of 18 birds (nine females and nine males) from the captive population over two release events (Anderson 2005). So far no new individuals have been added to the Karori Wildlife Sanctuary population. Furthermore, the effective population size of this population has been severely reduced by social processes as one dominant pair has breed profusely. Pedigree records, kept by colour banding birds, show the parentage of the current stock are almost entirely descended from the one very fecund pair (Anderson 2005).

A similar situation has also occurred within the Tiritiri Matangi Island population. This population was founded initially in 1987 when six pateke from the captive network were released, a further release of six individuals occurred in 1990 followed by a further 11 in 2002 (Rimmer 2004). However the population was reduced to six individuals in 2002 due to predation by the Australasian harrier (*Circus approximans*) and short finned eel (*Anguilla australis*). The current population has therefore been subject to several founding events and been through a recent bottleneck.

The re-introduced pateke population on Kapiti Island, although not sampled for this study, has also been founded by few individuals and characterized by small population size. The first release of pateke onto Kapiti Island occurred in 1968 when ten pateke were released from an undisclosed source (Dumbell 2000; Williams 1969). Due to the rat eradication on Kapiti Island in 1996, all pateke on the island at this time were brought into captivity and later re-released following successful eradication of rats (nine were found in total) (Dumbell 2000). In 2001 ten individuals from the captive population were released onto Kapiti Island.

A larger number of pateke have been released at Port Charles in Moehau. The population in this area is thought to be partially a remnant population and it is

possible that some immigration may have occurred from the nearby Great Barrier Island population (J. Roxburgh pers. comm. 2007). Release numbers have been large as a total of 38 birds were released in 2003, 42 in 2004, 62 in 2005 and 72 in 2006 and 50 in 2007, all in a 1:1 sex ratio (J. Roxburgh pers. comm. 2007). The large size of the Moehau population and the repeat release of large numbers of pateke could account for the higher genetic diversity found within the sample for this site.

A significant heterozygote deficit and a large inbreeding coefficient were present within the re-introduced pateke populations⁴, possibly as a result of non-random mating due to small population size and inbreeding (Hartl & Clark 1997). Other potential causes of the significant heterozygote deficit, null alleles and/or population fragmentation (the Wahlund effect), are less likely as the frequency of null alleles was found to be non-significant within all pateke population samples in this study and there are no known breeding barriers within the re-introduced populations which could result in an effect of fragmentation.

Inbreeding is a definite concern facing the smaller re-introduced populations. Documentation of inbreeding by pedigree charts has only been recorded from the Karori Wildlife Sanctuary population, however, although not documented, the small population sizes and small release numbers at Tiritiri Matangi Island and Mana Island would invariably have led to similar inbreeding events.

3.4.3 Departure from random mating on Great Barrier Island: is the source important?

A high inbreeding coefficient within the Great Barrier Island sample ($F_{IS}=0.279$) indicated a departure from random mating due to a heterozygote deficit. Although a tentative result, it is possible that inbreeding or a degree of population fragmentation (the Wahlund effect) is occurring within the pateke population on Great Barrier Island.

⁴ The departure from random mating due to a heterozygote deficit observed within the Great Barrier Island, captive and re-introduced population are subjective due to the small number of loci used and small sample sizes (particularly within the smaller re-introduced populations). Further research is needed with larger sample sizes and a larger number of loci before these trends can be verified. These results therefore are tentative.

A previous study on Great Barrier Island pateke by Dumbell (1987) revealed that the birds were divided into several functionally discrete populations, each confined to one of the island's valleys and a single flock site. Furthermore, any migration between valleys was found to be extremely low. This study was conducted over 27 months (1984 to 1987) in which time 335 pateke were trapped and banded. Although Dumbell (1987) did not find genetic differentiation between valleys (using 15 protein allozyme makers), conclusions were drawn that very little movement between valleys (and flock sites) occurred.

The majority of the pateke feather samples used for this study (and the majority of the pateke currently in the captive programme) were sourced from Okiwi. The Okiwi flock site, the largest on Great Barrier Island, makes up one of the discrete entities referred to by Dumbell (1987). Therefore relatedness within the Okiwi area could signify the sample obtained for this study showed greater relatedness than would a sample made up of birds from a wider number of locations on Great Barrier Island. Furthermore, the possibility of an historical bottleneck within the Great Barrier Island population could have contributed to overall lowered genetic diversity and increased relatedness (see section 2.4.2). The source of captive birds from Great Barrier Island, therefore, is relevant to the captive programme.

A similar situation was encountered by Sigg (2006) within bridled nailtail wallaby (*Onychogalea fraenata*) recovery. Using microsatellite markers Sigg (2006) genetically tested the wild remnant bridled nailtail wallaby population and nine individuals which were used for a wild-bred translocation. It was found that the wild remnant population was made up of nine distinct subpopulations between which matings were infrequent yet eight of the nine individuals used for the translocation were sourced from a single subpopulation, thus explaining the high degree of relatedness found within the individuals selected for translocation.

3.4.4 Future genetic diversity loss in pateke

Comparing the remnant pateke populations, a faster rate of genetic diversity loss (mean alleles per locus and heterozygosity) was predicted from the smaller Mimiwhangata population, which, within approximately 100 years, was

predicted to be less genetically diverse than the Great Barrier Island population (Figure 3.3). This could have devastating impacts for pateke recovery as the Mimiwhangata population currently has the highest genetic diversity and is the last remaining mainland population (Parrish & Williams 2001; Williams & Dumbell 1996).

Genetic diversity loss (mean alleles per locus and heterozygosity) from the captive pateke population was predicted to be rapid over the next 100 years (approximately) (Figure 3.3). The captive pateke population is currently carefully managed to ensure matings do not occur between individuals known to be related and new individuals are periodically brought into the captive population (K. Evans pers. comm. 2007).

A high rate of genetic diversity loss was predicted within the small re-introduced populations (Figure 3.3). Increasing gene-flow through translocation could help to increase genetic diversity within the re-introduced populations (Figure 3.4).

3.4.5 Conclusions

1. A loss of genetic diversity was found from remnant source (Great Barrier Island) to captivity.
2. A loss of genetic diversity was found from captivity to re-introduction. This effect was less noticeable in the re-introduced population at Moehau (possibly due to larger population size and continued large releases). The smaller re-introduced populations were found to possess low genetic diversity.
3. Only two alleles were unique to Mimiwhangata however a large percentage of unique genetic diversity was observed using mtDNA (Chapter 2).

Results of Pairwise Population PhiPT Analysis
Input as Codominant Genotypic Distance Matrix for Calculation of PhiPT
Data Sheet Whitakeri data
Data Title Codominant data template

No. Samples 76
No. Pops 3
No. Regions 1
No. Permutations 999 No. PW Perms 999

Pairwise Population PhiPT Values

	Middle	Founders	Korapuki	
Middle	0.000	0.441	0.007	Middle
Founders	0.000	0.000	0.225	Founders
Korapuki	0.022	0.008	0.000	Korapuki
	Middle	Founders	Korapuki	

PhiPT Values below diagonal. P-values based on 999 permuts are shown above diagonal.

Warning! Negative PhiPT of -0.005 between pops Middle and Founders converted to zero!

Chapter 4

General discussion

4.1 Loss of genetic diversity within threatened species recovery: a global issue?

'Conservation can be viewed as an attempt to protect the genetic diversity that has been produced by evolution over the previous 3.5 billion years' (Eisner et al. 1995).

Many species have suffered decline in the wild due to a range of factors. Most prominent are habitat loss and introduction of exotic predators (Caughley 1994). Conservation management of endangered species can act in two ways, firstly to eliminate the factors involved in decline or restore the habitat, and secondly to restore the size of the populations to self-sustaining levels through captive breeding, translocation or supplementation¹ (Jamieson et al. 2008).

A major concern surrounding conservation action is the retention of genetic diversity throughout long-term recovery programmes. A large theoretical knowledge base and many empirical studies support the importance of considering genetic factors within threatened species recovery (Allendorf & Luikart 2007; Amos & Balmford 2001; Caro & Laurensen 1994; Frankham 1995a, 1996).

¹ Captive breeding for re-introduction (as defined in Chapter 1) is *ex situ* breeding of a species for re-introduction to wild ranges or introduction to new ranges. Translocation refers to the conservation practice of moving wild-born animals from one place to another and supplementation or supportive breeding is the practice of removing a subset of individuals from a wild population for captive breeding and releasing the captive-born offspring back into the wild population (Frankham et al. 2002).

The small population size characteristic of most species recovery programmes increases the frequency of inbreeding and the strength of genetic drift (Frankham 1995b). This can give rise to loss of alleles and reduce heterozygosity. Furthermore, inbreeding can result in inbreeding depression which can manifest as reduced fertility, lowered hatchling success and shorter life span (Charlesworth & Charlesworth 1987, 1999; Hedrick & Kalinowski 2000). Overall loss of genetic diversity (alleles and heterozygosity) can reduce the ability of a population to adapt in the long term (Amos & Balmford 2001).

Recent advances in molecular genetics have enabled conservation managers to collect more detailed data on the genetic constitution of a population. For example, molecular genetic techniques and pedigree analysis have allowed conservation managers to better manage captive breeding and translocation programmes to minimise inbreeding and maximise genetic diversity (DeSalle & Amato 2004; Haig 1998; Hedrick 2001).

This thesis research highlights the loss of genetic diversity that can result during a species recovery programme if genetic considerations are not taken into account. This section will illustrate, using examples from the wider literature, loss of genetic diversity has occurred within many endangered species recovery programmes, and is a topical issue.

4.1.1 Loss of genetic diversity within translocation programmes

Translocations can result in a loss of genetic diversity, initially as a result of founder effects but also over time as a result of strong genetic drift associated with small population size (Frankham 1998). Loss of genetic variation and inbreeding depression can also conspire to increase the chance of population extinction (Frankham 1998).

Many empirical studies have illustrated these concerns. For example, Sigg (2006) tested microsatellite diversity within the wild bridled nailtail wallaby (*Onychogalea*

fraenata) population and nine individuals translocated to establish a new population. The wild remnant population comprised of nine genetically distinct subpopulations but eight of the nine individuals used for the translocation were sourced from the same subpopulation.

Further examples are the translocation programmes of the koala (*Phascolarctos cinereus*) and Laysan finch (*Telespiza canatans*). Within the koala translocation programme (Australia), the translocated populations were found to have much reduced genetic diversity compared with the wild source population (Haig 1998; Houlden et al. 1996). In addition, Tarr et al. (1998) found that a loss of genetic diversity had occurred following translocations of the Laysan finch in the Hawaiian Islands from a single source (Laysan Island) to three other islands (Southeast, North, and Grass).

Similarly, Arrendal et al. (2004) identified a loss of genetic diversity between the source and translocated populations of the Eurasian sea otter (*Lutra lutra*). The smallest of the translocated populations was found to contain the lowest level of genetic diversity and was significantly differentiated from the source population; the authors concluded that this was the product of random genetic drift, induced by small population size.

4.1.2 Management of genetic diversity within captive populations

'For nearly 3,000 taxa of birds and mammals, conservation [captive] breeding may be the only possible way to avoid extinction' (Ebenhard 1995).

The number of endangered species currently held in captivity for conservation purposes is increasing (Whitford & Young 2004). The zoo industry has become more focused on conservation, often working in conjunction with endangered species recovery programmes, to breed individuals for re-introduction to the wild (Gippoliti & Carpaneto 1997). A key issue within captive programmes for re-

introduction, and any captive population, is the retention of genetic diversity and minimizing of inbreeding (Allendorf & Luikart 2007).

Genetic concerns are a fundamental component of the guidelines to captive breeding (Captive Breeding Specialist Group, IUCN). The current object for management of captive breeding populations over successive generations is to maintain 90% of wild genetic diversity and a concomitant increase in inbreeding coefficient of no more than 10% over 100 years (Frankham et al. 2002).

Low founder number and genetic diversity of founders can dictate to some extent how much genetic diversity can be maintained (Frankham et al. 2002). However, even when a large number of founders are available, management practice can result in failure to retain genetic diversity.

4.1.2.1 Management to retain genetic diversity

There are a number of examples within the wider literature of captive breeding programmes that have succeeded in retaining genetic diversity over successive generations. The application of molecular techniques have allowed for more accurate genetic testing and genetic management (DeSalle & Amato 2004). Examples of captive breeding programmes which have succeeded in retaining genetic diversity are those on the black footed ferret (*Mustela nigripes*), whooping crane (*Grus americana*), Persian wild ass (*Equus hermonus onager*), and the bearded vulture (*Gypaetus barbatus*).

Despite a small founder number (n=18), the genetic objective of the black footed ferret captive breeding programme, to maintain 80% of the genetic diversity of the founder population, has been achieved for 25 years (Wisely et al. 2003). Furthermore, within both the whooping crane (Jones et al. 2002) and the Persian wild ass (Nielsen et al. 2007) programmes, DNA analysis has been used to identify genetic relationships and measure relatedness. Founder representation has also been controlled. Individuals for translocation were selected to represent full genetic

diversity of the captive population (Jones et al. 2002). In addition, the bearded vulture captive programme has maintained genetic diversity and minimized inbreeding using DNA analysis and pedigree records (Gautschi et al. 2003).

4.1.2.2 When genetic diversity is lost in captive populations

If individuals within captive populations are allowed to reproduce freely or artificial selection is applied (i.e. high output breeders are favoured), the population can quickly become dominated by a few individuals resulting in rapid loss of genetic diversity (Montgomery et al. 1997). For example, Haig et al. (1990), used simulations of selection criteria to choose captive founders of Guam rail (*Rallus owstoni*) for release into the wild and found that selecting the most fecund breeders resulted in loss of unique or rare alleles, whereas simulations which selected for equal founder representation retained the greatest amount of genetic diversity.

Another example of captive management which failed to equalise founder contribution by letting the captive population reproduce unmanaged is the recovery programme of the golden lion tamarin (*Leontopithecus rosalia*) (Frankham et al. 2002). From the original 242 captive founders only 48 individuals have contributed to the current population, two-thirds of which were derived from one (very fecund!) breeding pair (Ballou & Lacy 1995; Frankham et al. 2002).

There has been a similar outcome with the captive breeding programme of the wolf (*Canis lupus*), bred in a number of Scandinavian zoos (Laikre & Ryman 1991). This programme was initiated in the early 1950s, and, at the time of study a total of 43 breeding pairs had produced 113 litters in captivity. However, a rapid loss of genetic diversity as well as extensive inbreeding was found to have resulted from management practice which allowed only a small fraction of the animals to reproduce (Laikre & Ryman 1991).

A further example is a study on the Elliot's pheasant (*Syrnaticus ellioti*) captive programme using mtDNA, which identified only three haplotypes within the captive

breeding population of 36, whereas 16 haplotypes were identified within the wild population from sampling of 17 individuals (Ping-ping et al. 2005).

Selection for highly productive individuals over less productive individuals has often been applied to captive programmes (Haig et al. 1990). However, this can result not only in loss of genetic diversity, but also in genetic adaptation to the captive environment (Frankham 2008). Such adaptation can ultimately result in low success of re-introductions as the captive-bred individuals are unable to adapt to wild conditions (Frankham 2008).

4.1.4 Genetic impacts of supplementation

Supplementation is another species recovery strategy that can lead to loss of genetic diversity in the absence of careful genetic management. Supplementation of a wild population by captive-bred individuals can act to halt decline within a wild population if the agents of decline have been identified and dealt with (Hedrick et al. 2000). However, while supplementation may temporarily solve demographic problems, it may cause genetic changes which could lead to an increased risk of extinction once the supplementation ends (Reisenbichler & Rubin 1999).

Genetic changes to wild population, as a consequence of supplementation have also been demonstrated in salmon recovery programmes (Hedrick et al. 2000; Reisenbichler & Rubin 1999). For example, Reisenbichler and Rubin (1999) argued that supplementation of three Pacific salmon species (*Oncorhynchus mykiss*, *O. tshawytscha* and *O. kisutch*) by hatchery-bred individuals for conservation purposes, has resulted in significant genetic changes as the addition of hatchery-bred individuals lowered genetic diversity. Moreover, lowered overall fitness within the natural populations was found following supplementation (Reisenbichler & Rubin 1999).

Additionally, genetic changes associated with supplementation have been found within the Atlantic salmon (*Salmo salar*) recovery programme (Tessier et al. 1997).

In a comparison of wild salmon and captive-bred salmon (using microsatellites and mtDNA), Tessier et al. (1997) found that significant genetic changes had occurred and low-frequency alleles were not present within the captive-bred salmon. They concluded that susceptibility to inbreeding and genetic drift would increase within natural wild populations after supplementation.

4.2 Is New Zealand any different?

'New Zealand's wildlife might be less susceptible to inbreeding depression than species elsewhere' (Craig et al. 2000).

'...in general, genetic issues have had little influence on New Zealand translocation strategies' (Armstrong & McLean 1995).

Within New Zealand, the large number of endangered species, vulnerable to the presence of introduced predators and continued habitat loss, render conservation a high priority (Duncan & Blackburn 2007; Holdaway 1989; Wilson 2004; Parks 1995). Conservation actions, both to eliminate the factors causing decline and to increase the number of an endangered species, are widely used within New Zealand. However, recent reviews (Jamieson et al. 2006, 2008) have highlighted that genetic factors have yet to receive sufficient emphasis within long term conservation planning in New Zealand. This case study on captive breeding of pateke emphasises such concerns.

This apparent lack emphasis on genetic factors within species recovery programmes may be because of the perception that New Zealand's endemic wildlife is less susceptible to inbreeding depression (Craig 1991, 1994). The reasoning supporting these earlier views is that New Zealand's endemic wildlife have undergone periods of small population size and frequent inbreeding events which may have purged deleterious alleles (Craig 1991, 1994). However, Jamieson et al. (2006) strongly

argues against this pretence as the conditions for purging are very restricted, and, according to Keller and Waller (2002) unlikely to be common in natural populations.

Mounting evidence has indicated that a number of New Zealand's endemic birds have suffered from inbreeding depression following prolonged inbreeding (Hale & Briskie 2007; Briskie & Mackintosh 2004; Jamieson et al. 2003, 2006; Jamieson & Ryan 2000; Lettink et al. 2002; Mackintosh & Briskie 2004). Two compelling examples of this are studies looking at loss of genetic diversity following bottlenecks of the New Zealand robin (*Petroica australis*) (Hale and Briskie 2007) and the black robin (*Petroica traversi*) (Miller & Lambert 2004) and a study which identified hatchling failure within a number of endemic avifauna (Briskie & Mackintosh 2004).

Loss of disease resistance can occur following a bottleneck event (Spielman et al. 2004). Loss of immunocompetency in the New Zealand robin following a translocation to an offshore island was identified by Hale and Briskie (2007). This finding is predicted to lower the population's ability to withstand future disease outbreaks (Hale & Briskie 2007). Furthermore, recent research on genetic variation within the major histocompatibility complex (MHC), which plays a key role in disease resistance (Sommer 2005), has indicated that the black robin has very low diversity within MHC genes (Miller & Lambert 2004). Although black robins were not found to suffer from increased susceptibility to pathogens in a preliminary study, the population could be highly vulnerable to new diseases (Miller & Lambert 2004).

Lowered hatchling success, a well identified symptom of inbreeding depression, has also been correlated with bottleneck events in some endemic avifauna in New Zealand. Hatchling failure was found to increase with the severity of population bottlenecks within 22 native New Zealand species; this effect was most obvious when the size of the bottleneck through which the species had passed was less than 150 individuals (Briskie & Mackintosh 2004). Additionally, Mackintosh and Briskie (2004) found a translocated population of the South Island robin (*Petroica a.*

australis), founded by five individuals, displayed hatchling failure three times that of mainland populations.

4.2.1 Genetic management of translocations in New Zealand

Translocations are a common conservation practice in New Zealand (Armstrong & McLean 1995). The practice was coined ‘island marooning’ by Williams (1977) as translocations were (and are now) most commonly to offshore predator-free islands and are often one-off events involving little further management (Armstrong & McLean 1995).

Overall genetic management of translocations within New Zealand has been minimal with genetic considerations having little influence over practice (Armstrong & McLean 1995). However many case-studies within New Zealand have recently highlighted the loss of genetic diversity associated with the establishment of small populations on offshore islands (Bossenkool et al. 2007; Briskie & Mackintosh 2004; Jamieson et al. 2003, Jamieson 2007c; Jamieson & Ryan 2000; Lambert et al. 2005; Mackintosh & Briskie 2004). Nevertheless in contradiction to the argument that translocations always result in loss of genetic diversity, a recent study (Taylor & Jamieson 2008) found that sequential translocations to offshore islands (following the primary translocations) did not result in further loss of genetic diversity in the South Island saddleback (*Philesturnus carunculatus carunculatus*).

An example where genetic factors are well integrated into an endangered species management plan based on translocation is the management of the takahe (*Porphyrio hochstetteri*) (Jamieson et al. 2008). The takahe was translocated, during the 1980s and 1990s from the sole remnant population in Fiordland to four offshore islands (Grueber & Jamieson 2008). While genetic diversity has been lost within the island populations, migration from the Fiordland population could allow for 90% of genetic diversity to be maintained over the next 100 years (Grueber & Jamieson 2008).

4.2.2 Genetic management of captive breeding in New Zealand

Captive breeding has been widely used within endangered species recovery in New Zealand. Management of captive breeding programmes in New Zealand have often left genetic issues unaddressed in favour of maximising productivity and population growth; often at the expense of retaining genetic diversity and minimizing inbreeding (Jamieson et al. 2006).

For example, within the management of the pateke captive breeding programme, the consideration of genetic factors has not been a high priority. According to Hayes (2002) the long-term success of pateke captive breeding is not thought to rely heavily on a wide gene pool as the Laysan teal (*Anas laysanensis*) and the Hawaiian goose (*Branta sandvicensis*) have undergone severe genetic bottlenecks and yet recovered numbers rapidly.

This research on pateke has highlighted the negative genetic impacts arising when genetic factors are not well integrated in this type of species recovery. The captive programme for pateke is typical of most captive operations in New Zealand. Currently similar programmes are in use for the black stilt (*Himantopus novaeseelandiae*), blue duck (*Hymenolaimus malacorhynchos*) shore plover (*Thinornis novaeseelandiae*), kaka (*Nestor meridionalis*) and Otago skink (*Oligosoma otagense*).

The captive programme of the Campbell Island teal is a well noted success story of New Zealand conservation. A total of eight birds (one female and seven male) were brought into captivity initially from a tiny remnant population. All the captive birds were DNA fingerprinted to assess relatedness and all the seven male birds were represented in the release population (M. Williams pers. comm. 2007). The release population persists on Campbell Island.

A captive programme for the threatened blue duck was initiated in 1989 and there are currently 18 pairs of blue duck held in captivity, but so far breeding success has

been low (Adams et al. 1997). Captive bred blue ducks have been used as the principal source of birds for population establishment attempts on Mt. Egmont/Taranaki. However, survival of the released ducks was low with many of the captive-bred birds dying of starvation, possibly due to lack of foraging/feeding skills as a product of adaptation to captivity (Holmes & Caskey 2001). Management of inbreeding has not been closely monitored or managed within the blue duck captive population (J.Wilckman and I. Fraser unpublished data; *in* Jamieson et al. 2008).

A further example of a captive programme in New Zealand in which inbreeding has not been closely managed is that of the Otago skink (Jamieson et al. 2008). Close monitoring has indicated that a large number of the captive population is highly inbred (Connolly 2005 *in* Jamieson et al. 2008). High inbreeding of the captive founders could inhibit the success of re-introduction of the Otago skink.

Furthermore, genetic factors have not been addressed within the captive programmes of the black stilt, shore plover, or kaka. Concern around inbreeding depression is noted within the black stilt recovery plan only in association with the wild population and high levels of philopatry which could result in inbreeding (Reed & Murray 1993). Assessment of genetic diversity is however one of the eventual research aims of management of the captive kaka population (Moorehouse & Greene 1998).

4.2.2 Conclusion

A large theoretical framework and many case studies emphasize the importance of retaining genetic diversity and minimizing inbreeding within threatened species recovery programmes. However, on a global scale, conservation practice involved in increasing the numbers of many threatened species often emphasises increasing demographic representation, sometimes at the expense of genetic concerns. Conservation practice within New Zealand is essentially a microcosm of a global issue with a greater importance placed on quantity of individuals within recovery

programmes rather than quality. Greater emphasis needs to be placed on the retention of genetic diversity and minimizing inbreeding within recovery for long-term persistence of threatened species rather than just short term increases (Allendorf & Luikart 2007; Jamieson et al. 2006, 2008).

4.3 Pateke recovery: where to from here?

4.3.1 Summary of the main findings

The introduction chapter of this thesis (Chapter 1) posed the use of pateke as a model species for examining the extent of genetic diversity loss through captive breeding and re-introduction. The pateke captive breeding programme, initiated in 1973, sourced individuals (predominantly from Great Barrier Island) for captive propagation and subsequent re-introduction into a number of locations, with varying success. My research aimed to assess and compare genetic diversity within and between the two remaining remnant populations (Great Barrier Island and Mimiwhangata), the captive breeding population, and four re-introduced populations (Moechau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island), using two neutral molecular markers, mitochondrial DNA (mtDNA) and microsatellite DNA.

The predictions erected to guide this research premised firstly on the notion that a loss of genetic diversity was expected from the remnant source population (Great Barrier Island) to captivity and from captivity to re-introduction. Secondly, that some genetic differences were to be expected between the two remnant populations (Great Barrier Island and Mimiwhangata). The former prediction is well supported in Chapter 3 and the latter in Chapter 2.

Chapter 2 indicated a wide difference in mtDNA diversity between the two remnant populations, Great Barrier Island and Mimiwhangata (Figure 2.5). I argued that this was the result of an historical bottleneck or founder effects within the Great Barrier Island population. Furthermore, the single abundant haplotype found within the

Great Barrier Island population was found to be the single haplotype within the captive and re-introduced populations. I argued that the presence of this Great Barrier Island haplotype within the Mimiwhangata population is possibly the result of supplementation by captive offspring or that supplementation may have increased the frequency of this haplotype within the Mimiwhangata population.

In Chapter 3, a loss of genetic diversity from the remnant source population (Great Barrier Island), to captivity to re-introduction was identified (Table 3.3). The larger re-introduced population at Moehau retained greater genetic diversity compared with the smaller re-introduced populations, possibly the result of multiple releases and a larger effective population size.

4.3.2 Limitations of this research

The main factors limiting this research were difficulties in obtaining feather samples, extracting DNA, and identifying polymorphic microsatellite markers. For this study the majority of feather samples were provided by the Department of Conservation (DoC). Large sample sizes were obtained from Mimiwhangata and Great Barrier Island. However, at Moehau, Karori Wildlife Sanctuary, Tiritiri Matangi Island and Mana Island, access issues, cost, staff availability, difficult terrain, and the small size of the populations involved limited the number of feather samples which could be obtained. Furthermore, feathers from some of the birds within the captive breeding programme could not be collected as the birds were nesting and sampling could have been detrimental and disruptive. Where possible, sample size was accounted for within analyses and trends have been discussed with reference to sample size (this was particularly as issue for the analysis in Chapter 3).

4.3.2.1 DNA extraction

Extracting DNA from some feathers proved difficult as feathers varied according to type (wing, body, tail), age and/or state. From a number of feathers, DNA could not be extracted (these samples are included in footnote 1 in Chapters 2 and 3). Furthermore, DNA was unable to be extracted from two museum feather samples

provided from Auckland museum and two tissue sample provided from Te Papa, due to the degraded nature of the samples.

4.3.2.2 Identifying polymorphic markers

A further difficulty was identifying and amplifying polymorphic microsatellite markers. All microsatellite markers used in Chapter 3 were developed for the mallard. A total of five markers were chosen from the literature but only two were polymorphic for pateke. Due to cost and time limitations, further markers could not be tested to identify if they were polymorphic for pateke. Funding for this research did not extend to developing microsatellite markers specific to pateke. This however is one of the recommendations of future research (see section 4.7).

4.3.2.3 Cost limitations

The samples provided from Mimiwhangata and Great Barrier Island were larger than those used for this study. However cost limitations dictated the number of samples which could be used.

4.3.3 Future genetic management and conservation of pateke

The key needs concerning increasing and maintaining genetic diversity within the pateke recovery programme which have arise from this research are:

- To increase representation of wild genetic diversity within captive breeding and re-introduction
- To increase and maintain genetic diversity over the long-term, within re-introduction

To achieve these genetic goals for future genetic management there are two possible strategies that could be executed. The first strategy outlines re-modeling the current pateke captive breeding programme so that genetic diversity is increased and maintained (4.3.4, Figure 4.1). The second strategy summarizes a method by which pateke recovery could be conducted by translocation rather than captive breeding and an argument for this strategy is outlined (4.3.5, Figure 4.2).

4.3.4 Re-modeling the pateke recovery programme

Concerning future genetic management of the current pateke captive breeding programme recommendations include; sourcing future captive individuals from the Mimiwhangata population for captive breeding that broadly represent the allelic and haplotypic diversity, equalising the founder contribution of captive individuals to the re-introduced populations, decreasing the strength of founder events within releases, and increasing effective population size within the re-introduced populations.

4.3.4.1 Sourcing future captive founders from Mimiwhangata

The original captive programme was founded entirely by Great Barrier Island founder stock (Dumbell 2000) and the majority of current founders are also of Great Barrier Island origin (K. Evans pers. comm. 2007). The current Mimiwhangata birds within the captive population appear to contain the Great Barrier Island haplotype as no other haplotypes were identified within the captive population in this study. It is therefore recommended that the majority of future captive founders should be from Mimiwhangata with its unique haplotypes. However, the Mimiwhangata population is small (n~200-300) and vulnerable to exotic predators present on the mainland (Williams & Dumbell 1996), so the impact of removing individuals for captive breeding purposes should be carefully assessed to ensure no detrimental impact to the source population.

4.3.4.2 Equalising founder representation within re-introduction

At the beginning of the pateke captive breeding programme, artificial selection for high reproductive output individuals was applied (Dumbell 2000). Such selection can result in the production of individuals better adapted to captivity rather than to wild conditions (Frankham 2008) (also see section 4.1.2). Currently artificial selection is not applied to the captive population; however ensuring equal founder representation within birds for release to re-introduced populations would be highly beneficial towards increasing genetic diversity

4.3.4.3 Decreasing founder events and increasing effective population size within re-introduction

In terms of increasing genetic diversity within the re-introduced pateke populations, decreasing the strength of founder events and increasing effective population size (decreasing the strength of genetic drift) are important. Increasing gene-flow within subpopulations of threatened species has long been cited as effective in terms of maintaining genetic diversity (Ingvarsson 2001; Mills & Allendorf 1996; Newman & Tallmon 2001; Vila 2002).

Decreasing the strength of founder events could be achieved by:

- Including more individuals in releases and transfers
- Direct transfers from the remnant populations

Increasing the effective population size of the re-introduced populations could be achieved by:

- Making each re-introduced population larger
- Managing the re-introduced populations as one meta-population by actively rotating individuals between the new sub-populations

Figure 4.1: Proposed management to maintain genetic diversity in the pateke captive breeding programme using both Mimiwhangata founders (with unique haplotypes) and Great Barrier Island founders, repeated re-introductions from captivity, direct translocations from the remnant populations (denoted by the large arrows) to the re-introduced populations, and translocations between the re-introduction populations.

4.3.4.4 Possible negative implications

There are two main potential negative implications of the proposed management to maintain genetic diversity (Figure 4.1). Firstly, it is possible that removing individuals from the small Mimiwhangata population could be detrimental to this population. Therefore it is recommended that if such a strategy be put in place, individuals are removed slowly from Mimiwhangata and monitoring is included.

A second potential negative implication is an increase in the chance of disease transmission through the movement of individuals between the pateke populations.

4.3.5 Translocation for pateke recovery: is captive breeding the most effective option?

The results from this research call into question the overall benefits of conducting pateke recovery through a captive breeding programme. Although the pateke recovery programme has been hugely successful in producing a large quantity of captive offspring for re-introduction, the success of re-introduction has been limited (Williams & Dumbell 1996). In addition, the results from this research call into question the overall genetic impacts of the pateke captive programme.

4.3.5.1 The limitations of captive breeding

The dependence on captive breeding is increasing worldwide and the practice has been recommended for an increasing number of endangered species (Ellis-Joseph et al. 1992; Seal et al. 1993; Tear et al. 1995). Waterfowl conservation, in particular, has a history of dependence on captive breeding (Hayes 2002). For example, captive breeding has been used for conservation of many endangered waterfowl overseas (i.e. the Hawaiian goose, Laysan teal, Aleutian Canada goose (*Branta canadensis leucoparia*) and the trumpeter swan (*Cygnus c. buccinators*)) and within New Zealand (pateke, Campbell Island teal, blue duck). Waterfowl have been shown to readily adapt to the captive environment with a high level of reproductive success compared to wild environments (Hayes 2002).

However, as seen within the pateke recovery programme, high reproductive success within captive breeding does not necessarily equate to successful re-introduction. This finding is not unique, for in the overall literature there are few examples of successful re-introductions of captive-bred founders into the wild to create self-sustaining populations (Cade 1988; Imoden 1987; Rahbek 1993). Snyder et al. (1996) noted in a review of 145 re-introduction programmes of captive-bred animals, only 15 cases (11%) were successful at establishing wild populations.

Furthermore, although some high profile captive breeding programmes appear to be successful at breeding threatened animals in captivity and raising the profile of the

species, the actual success rate of re-introduction is low (Rahbek 1993). For example, only half of the re-introductions from the black footed ferret (*Mustela nigripes*) captive programme, a well cited ‘successful’ example of captive breeding, have persisted (Russel et al. 1994; Wisely et al. 2003)

In addition to low success of re-introductions, evidence for the limitations of captive breeding as a conservation tool has been building (Snyder et al. 1996). As well as the negative genetic implications and low rate of re-introduction success, captive programmes are costly to run, have a high frequency of disease outbreaks, and can lead to a removal of focus from the agents of decline in the wild (Snyder et al. 1996).

Rahbek (1993) concluded that captive breeding is important within conservation, but only as a last resort when all other avenues have been explored. Furthermore, when captive breeding is applied, it should only be seen as a short-term option and with considerable attention paid to genetic factors, continued attention to agents of decline in the wild, careful monitoring of re-introduction, and consideration of the costs involved.

4.3.5.2 Translocations for pateke recovery

The success of translocation in establishing a self-sufficient population in the wild is higher than captive breeding followed by re-introduction (Armstrong & McLean 1995; Griffith et al. 1989). The underlying reason, according to Frankham (2008), is again that genetic adaptation to the captive environment occurs during captive breeding.

An example of a species currently well managed though translocation in New Zealand is the takahe (Jamieson et al. 2008). By tracking pedigree records on the offshore islands to which takahe have been translocated and continuing immigration from Fiordland by transfers, Grueber and Jamieson (2008) show how genetic diversity could be maintained in the long-term. This example moves away from the

one-off translocation method as it includes continued management to maintain genetic diversity.

A proposed model for pateke recovery via translocations is outlined in Figure 4.2. This model proposes direct translocation from the remnant populations combined with translocation between the re-introduced populations.

Figure 4.2: Proposed management of the pateke recovery programme using translocations. Direct translocations of individuals from the Mimiwhangata and Great Barrier Island populations are proposed initially followed by translocations between the re-introduced populations.

4.3.6 Other factors for consideration in pateke conservation

This research has also highlighted the importance of two other conservation issues that need to be addressed for pateke. Firstly the ever present issue of dealing with continued decline of pateke in the wild, and secondly, the issue of addressing the conservation status and monitoring practice around hybrids.

4.3.6.1 Greater focus on the agents of decline in the wild

A point strongly made by Williams and Dumbell (1996) is that decline of pateke is still occurring within wild ranges, despite huge effort being directed towards captive breeding. Pateke are particularly vulnerable to introduced predators, especially mustelids, hedgehogs, feral cats, feral and domestic dogs and rats (Williams 2001).

Further decline within the genetically diverse Mimiwhangata population, in particular, could have devastating long-term implications for the preservation of genetic diversity in pateke. Decline is occurring within the Mimiwhangata population due to the presence of introduced predators on the mainland (Norway rat and mustelids) which are not present on Great Barrier Island (Williams & Dumbell 1996). Introduced predators have also resulted in the failure of past re-introductions (Williams & Dumbell 1996). Overall, a continued focus on agents of decline in the wild, particularly within the Mimiwhangata area and areas where future releases are planned, is a priority for future pateke conservation.

4.3.6.2 Genetic monitoring for hybrids and assigning conservation status

The small genetic distance identified between mallard and the Loch Maree/Fiordland pateke sample, indicated the presence of hybridization in the now extinct Fiordland population (Table 2.2). Previous studies have identified and documented this hybridization (Barton 2003; Gemmell & Flint 2000; Kennedy & Spencer 2000).

Hybridization between pateke and mallard has also been found at Mimiwhangata, elsewhere in Northland, and on Great Barrier Island (K. Evans. pers. comm. 2007; S. Moore pers. comm. 2007). Any hybridization poses a serious threat to the future recovery of pateke especially given the large presence of mallards in areas of pateke distribution.

The conservation status of future hybrids needs to be addressed within pateke recovery. Allendorf et al. (2001) notes the increasing prevalence of hybrids and the necessity to assign a conservation status. The remnant pateke population in

Fiordland was dismissed from conservation concern because of the hybridization. Currently hybrids are removed from pateke populations (S. Moore pers. comm. 2007), however if hybridization increases, removing further hybrid individuals could be very detrimental to the population. The issue of assigning some conservation status to future hybrids therefore needs to be addressed.

4.4 Suggestions for future research

There are several avenues for future research on pateke following this project and there are also avenues for similar research on other species in New Zealand and elsewhere.

4.4.1 Future pateke research

4.4.1.1 Historical genetic diversity in pateke

Future research to examine historical genetic diversity present in pateke (though the extraction of DNA from museum specimens) is important. Such research would help to determine whether the presence of the Great Barrier Island haplotype within Mimiwhangata was due to historical presence of that haplotype in Northland or whether its presence can be attributed to past captive releases. In addition, a representation of historical genetic diversity within pateke could be ascertained. A number of pateke specimens are held within museums around New Zealand.

4.4.1.2 Monitoring

Future monitoring of all wild pateke populations would help determine agents of decline in the wild, which are currently not fully understood (O'Connor et al. 2005). Monitoring of future introductions is particularly important as many re-introductions have been unsuccessful (Williams & Dumbell 1996). Occurrence of inbreeding depression within the small re-introduced populations could jeopardize long-term survival therefore monitoring of hatchling success, clutch size and juvenile survival, within the re-introduced populations could aid in long-term recovery.

4.4.1.3 Population Viability Analysis

Within the addition of demographic data from all pateke populations, a Population Viability Analysis (PVA) could be run as a future research project. Using a PVA, with demographic and genetic data, can help to assess which factors will have an impact on a population's persistence and will inform management strategies (Lacy 1993). A successful example of using a PVA is the recovery programme for the plains zebra (*Equus quagga*) in South Africa which resulted in the formulation of management strategies to best retain genetic diversity in the long-term (Bowland et al. 2001).

4.4.1.4 Useful loci: Example - Major Histocompatibility Complex (MHC)

A relatively new genetic system important for conservation research is the major histocompatibility complex (MHC) which is involved in disease resistance (Hedrick 1994). High diversity within the MHC complex has been correlated with increased resistance to pathogens (Sommer 2005). Suggestions that captive programmes should focus on retaining diversity within the MHC complex are a current yet controversial issue (Hughes 1991; Millar & Hedrick 1991). Future research on isolating the MHC complex and examining diversity within the pateke remnant, captive and re-introduced populations would be helpful in establishing future recovery options for pateke, and the wisdom of persisting with captive breeding as the lead recovery tool.

4.4.2 Research on other species

This research provides a model for studying genetic diversity within captive breeding programmes for other species undergoing similar recovery. Within New Zealand, genetic testing could be applied to such captive programmes as the kaka, blue duck, shore plover, and black stilt.

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Appendices

Appendix 1 - Sample table for Chapter 2

Table (a): Detailed sample table for Chapter 2 including sample number, sample code, band number, location and location details.

No.	Sample code	Band number	Location	Location details
1	MANA_1	no band	Mana Is.	
2	MANA_2	S73241	Mana Is.	
3	MANA_3	S73239	Mana Is.	
4	KWS_1	S81163	KWS	
5	KWS_2	S72087	KWS	Tailors wetland
6	KWS_3	YM-YO	KWS	Upper dam - north end
7	KWS_4	S72064	KWS	Outside sanctuary
8	KWS_5	L32983	KWS	Upper dam - south end
9	KWS_6	L32986	KWS	Upper dam - south end
10	KWS_7	S72082	KWS	
11	KWS_8	S72068	KWS	
12	KWS_9	L15237 M-WO	KWS	
13	KWS_10	S72079	KWS	
14	KWS_11	S72077	KWS	
15	KWS_12	S72076	KWS	
16	TIRI_1	BGR/ S78765	Tiritiri Matangi Is.	Emergency landing
17	TIRI_2	S84051/B	Tiritiri Matangi Is.	Emergency landing
18	TIRI_3	B/S84061	Tiritiri Matangi Is.	Emergency landing
19	TIRI_4	S84062/Y	Tiritiri Matangi Is.	Emergency landing
20	TIRI_5	S80523/-	Tiritiri Matangi Is.	Bunkhouse pond
21	TIRI_6	/S84063	Tiritiri Matangi Is.	Bunkhouse pond
22	TIRI_7	BWY/S78767	Tiritiri Matangi Is.	Landing Pond
23	TIRI_8	S78782/W	Tiritiri Matangi Is.	Wattle Gully
24	MOE_1	S81312	Mochau release	Port Charles
25	MOE_2	S83733	Mochau release	Port Charles
26	MOE_3	S81305	Mochau release	Port Charles
27	MOE_4	S83734	Mochau release	Port Charles
28	MOE_5	S81302	Mochau release	Port Charles
29	MOE_6	S81306	Mochau release	Port Charles
30	MOE_7	S81307	Mochau release	Port Charles
31	MOE_8	S81313	Mochau release	Port Charles
32	MOE_9	S81304	Mochau release	Port Charles
33	MOE_10	S83735	Mochau release	Port Charles
34	MOE_11	S81309	Mochau release	Port Charles
35	MOE_12	S84003	Mochau release	Port Charles
36	MOE_13	S84007	Mochau release	Port Charles
37	MOE_14	S84002	Mochau release	Port Charles
38	MOE_15	S84004	Mochau release	Port Charles
39	MOE_16	S81640	Mochau release	Port Charles
40	MOE_17	S83738	Mochau release	Port Charles
41	MOE_18	S81634	Mochau release	Port Charles
42	MOE_19	S83739	Mochau release	Port Charles
43	GBI_1	S70550	Great Barrier Island	Okiwi
44	GBI_2	S83366	Great Barrier Island	Okiwi
45	GBI_3	S70411	Great Barrier Island	Okiwi- barn wetlands
46	GBI_4	L33898	Great Barrier Island	Okiwi - barn wetlands
47	GBI_5	S83357	Great Barrier Island	Okiwi- sheepyard RA

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48	GBI_6	S80167	Great Barrier Island	Okiwi - paddock 6
49	GBI_7	S80163	Great Barrier Island	Okiwi - translator wetlands
50	GBI_8	S80171	Great Barrier Island	Okiwi - orchard drain
51	GBI_9	S83355	Great Barrier Island	Okiwi - paddock 36
52	GBI_10	S80108 TX59	Great Barrier Island	Okiwi - paddock 31
53	GBI_11	S80194 TX85	Great Barrier Island	Okiwi - lower okiwi stream
54	GBI_12	S80147	Great Barrier Island	Okiwi stn - bush pond
55	GBI_13	S80154	Great Barrier Island	Okiwi stn - campground/P21
56	GBI_14	S80155	Great Barrier Island	Okiwi stn - skeleton ridgewetland E of skeleton ridge
57	GBI_15	S70502	Great Barrier Island	Okiwi
58	GBI_16	S70430	Great Barrier Island	Okiwi - P8 near woolshed
59	GBI_17	S70451	Great Barrier Island	Okiwi - below haybald P32
60	GBI_18	S70425	Great Barrier Island	Okiwi - wetland east edge P15
61	GBI_19	S70423	Great Barrier Island	Okiwi - in reserve opp Okivi cattle yards
62	GBI_20	S70490	Great Barrier Island	Okiwi - Pond at cattle yards
63	GBI_21	S70475	Great Barrier Island	Okiwi - Pond 3
64	GBI_22	S70491	Great Barrier Island	Okiwi - Whangapoua stream headwaters
65	GBI_23	S80104	Great Barrier Island	Okiwi - Entrance okiwi station
66	GBI_24	S80129	Great Barrier Island	Okiwi - RA S of P31
67	GBI_25	S80118	Great Barrier Island	Okiwi - woolshed #2
68	Gbi_26	S80111	Great Barrier Island	Okiwi - bush wetland opp. Coopers
69	GBI_27	S83320	Great Barrier Island	Medlands
70	GBI_28	S83368	Great Barrier Island	Okiwi
71	GBI_29	unbanded	Great Barrier Island	Shoal Bay
72	GBI_30	unbanded	Great Barrier Island	Claris
73	GBI_31	S80176	Great Barrier Island	Awana
74	GBI_32	S70502	Great Barrier Island	okiwi
75	GBI_33	S70428	Great Barrier Island	okiwi - unfenced wet area P1
76	GBI_34	S70418 TX 4	Great Barrier Island	okiwi - brent barkers property
77	GBI_35	S70420	Great Barrier Island	okiwi - stream btwn P15 & 4
78	GBI_36	S75158	Great Barrier Island	okiwi - paddock 8 RA
79	GBI_37	S75110	Great Barrier Island	okiwi - unfenced wet area p5
80	GBI_38	S70403	Great Barrier Island	okiwi - wetland east edge P15
81	GBI_39	S70484	Great Barrier Island	okiwi - RA in P5
82	NTH_1	S83518	Mimiwhangata	Queens Corner Pond
83	NTH_2	S83517	Mimiwhangata	Lake Surprise
84	NTH_3	S81782	Mimiwhangata	Te Rewa Stream
85	NTH_4	S83514	Mimiwhangata	Y-gully culvert
86	NTH_5	S83523	Mimiwhangata	Cattleyard Pond
87	NTH_6	S83540	Mimiwhangata	Trials flats
88	NTH_7	S83518	Mimiwhangata	Queens Corner Pond
89	NTH_8	S83524	Mimiwhangata	Pohutukawa over Puddle
90	NTH_9	S80546	Mimiwhangata	Dune Lake
91	NTH_10	S78786	Mimiwhangata	Cat Cage Corner
92	NTH_11	S83510	Mimiwhangata	Lower Cattleyard Pond
93	NTH_12	S78795	Mimiwhangata	South Boundary wetland
94	NTH_13	S83504	Mimiwhangata	Scotty's Pond
95	NTH_14	S78800	Mimiwhangata	Cattleyard Delta
96	NTH_15	S78794	Mimiwhangata	True Right Swamp
97	NTH_16	L32896	Mimiwhangata	20 kph
98	NTH_17	S78790	Mimiwhangata	Cat Cage Corner
99	NTH_18	S80536	Mimiwhangata	Trials flats
100	NTH_19	S80534	Mimiwhangata	Silage Pit Pond
101	NTH_20	L32892	Mimiwhangata	F-drain
102	NTH_21	L32895	Mimiwhangata	2nd top crossing

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103	NTH_22	S70630	Mimiwhangata	Molly's Bush
104	NTH_23	S80518	Mimiwhangata	Scotty's Pond
105	NTH_24	S78832	Mimiwhangata	Kingfern Gully
106	NTH_25	S81774	Mimiwhangata	Cattleyard Wetland
107	NTH_26	S78816	Mimiwhangata	3rd boundary swamp
108	NTH_27	S70624	Mimiwhangata	True Right Swamp
109	NTH_28	S70641	Mimiwhangata	Trials flats
110	NTH_29	L33448 BWG	Mimiwhangata	Molly's Bush
111	NTH_30	S78801 BGG	Mimiwhangata	Blackberry and Bracken swamp
112	NTH_31	S70646	Mimiwhangata	Silage Wetland
113	NTH_32	S78803	Mimiwhangata	South Boundary dam
114	NTH_33	S81785	Mimiwhangata	Cattleyard Pond
115	NTH_34	S70635	Mimiwhangata	F-drain
116	NTH_35	S81758	Mimiwhangata	20 kph
117	NTH_36	S70632	Mimiwhangata	Trials Stock Pond
118	NTH_37	S81763	Mimiwhangata	Trials Stock Pond
119	NTH_38	S78844	Mimiwhangata	Pohutukawa Grove Pond
120	NTH_39	S78849	Mimiwhangata	Puriri Cave
121	NTH_40	S81765	Mimiwhangata	Scotty's Pond
122	NTH_41	S81767	Mimiwhangata	Scotty's Pond
123	NTH_42	L33441	Mimiwhangata	Creek by POP
124	NTH_43	S78805	Mimiwhangata	Lower 3 Pond
125	NTH_44	L32943	Mimiwhangata	Clay Track
126	NTH_45	S78835	Mimiwhangata	Pumphouse
127	NTH_46	S81281	Mimiwhangata	Molly's Bush
128	NTH_47	S81284	Mimiwhangata	F-drain
129	NTH_48	S81257	Mimiwhangata	Te Rewa Stream
130	NTH_49	unbanded	Northland	Whananaki south rd, 2640850-70 6631832-20
131	CB_1	S43889	captive breeding	
132	CB_2	S80241	captive breeding	
133	CB_3	S80311	captive breeding	Esplanade Aviary
134	CB_4	S80250	captive breeding	Orara Wildlife Park
135	CB_5	S80223	captive breeding	Orara Wildlife Park
136	CB_6	S80303	captive breeding	Peacock springs
137	CB_7	S54395	captive breeding	
138	CB_8	S80240	captive breeding	
139	CB_9	S80308	captive breeding	
140	CB_10	L31851	captive breeding	
141	CB_11	L29489	captive breeding	
142	CB_12	S70510	captive breeding	
143	CB_13	S70450	captive breeding	
144	CB_14	S80202	captive breeding	
145	CB_15	S83010	captive release 2007	otorohanga kiwi house
146	CB_16	S53633	captive release 2007	otorohanga kiwi house
147	CB_17	S70439	captive release 2007	
148	CB_18	S43477	captive release 2007	Orara Wildlife Park
149	CB_19	S43448	captive release 2007	
150	CB_20	S80305	captive release 2007	Peacock springs
151	FIO_1		Fiordland	Loch Maree
152	MU_1	ref no. 21490	Helena Bay	Te Papa collection
153	MU_2	ref no. 21491	Waipu Gorge	Te Papa collection
154	MU_3	ref no. 21492	Waipu Gorge	Te Papa collection

Table (b): Detailed sample table for Chapter 2 (cont.) including sample number, sample code, band number, date of collection, sex of bird, age of bird and collator (s).

No.	Sample code	Band number	Date of collection	Sex of bird	Age of bird	Collector(s)
1	Mana_1	no band	7/11/2006		J	Clare Duston & Grant Timlin
2	Mana_2	S73241	7/11/2006		A	Clare Duston & Grant Timlin
3	Mana_3	S73239	7/11/2006		A	Clare Duston & Grant Timlin
4	KWS_1	S81163	24/02/2006			Raewyn Empson
5	KWS_2	S72087				
6	KWS_3	YM-YO				
7	KWS_4	S72064				
8	KWS_5	L32983				
9	KWS_6	L32986				
10	KWS_7	S72082	24/02/2006			Raewyn Empson
11	KWS_8	S72068	24/12/2004			Raewyn Empson
12	KWS_9	L15237 M-WO	24/12/2004	M		Raewyn Empson
13	KWS_10	S72079	24/12/2004			Raewyn Empson
14	KWS_11	S72077	24/12/2004			Raewyn Empson
15	KWS_12	S72076				
16	TIRI_1	BGR/ S78765	28/12/2006	M	J	James Frazer
17	TIRI_2	S84051/B	28/12/2006	F	A	James Frazer
18	TIRI_3	B/S84061	28/12/2006	M	J	James Frazer
19	TIRI_4	S84062/Y	28/12/2006	F	J	James Frazer
20	TIRI_5	S80523/-	29/12/2006	F	A	James Frazer
21	TIRI_6	/S84063	29/12/2006	M	J	James Frazer
22	TIRI_7	BWY/S78767	29/12/2006	M	A	James Frazer
23	TIRI_8	S78782/W	29/12/2006	M	A	James Frazer
24	MOE_1	S81312				
25	MOE_2	S83733				
26	MOE_3	S81305				
27	MOE_4	S83734				
28	MOE_5	S81302				
29	MOE_6	S81306				
30	MOE_7	S81307				
31	MOE_8	S81313				
32	MOE_9	S81304				
33	MOE_10	S83735				
34	MOE_11	S81309				
35	MOE_12	S84003				
36	MOE_13	S84007				
37	MOE_14	S84002				
38	MOE_15	S84004				
39	MOE_16	S81640				
40	MOE_17	S83738				
41	MOE_18	S81634				
42	MOE_19	S83739				
43	GBI_1	S70550	8/03/2005	F	A	Simon Stevenson
44	GBI_2	S83366	5/03/2005	F	A	Simon Stevenson
45	GBI_3	S70411	5/03/2005			Simon Stevenson
46	GBI_4	L33898	5/03/2005			Simon Stevenson
47	GBI_5	S83357	5/03/2005			Simon Stevenson
48	GBI_6	S80167	2/11/2004	M	J	Simon Stevenson
49	GBI_7	S80163	2/11/2004	M	A	Simon Stevenson
50	GBI_8	S80171	2/11/2004	F	J	Simon Stevenson

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51	GBI_9	S83355	17/02/2005	F	A	Simon Stevenson
52	GBI_10	S80108 TX59	15/02/2005	F	A	Simon Stevenson
53	GBI_11	S80194 TX85	15/02/2005	F	A	Simon Stevenson
54	GBI_12	S80147	8/03/2004	M	A	Simon Stevenson
55	GBI_13	S80154	9/03/2004	F	A	Simon Stevenson
56	GBI_14	S80155	10/03/2004	F	A	Simon Stevenson
57	GBI_15	S70502	19/03/2002			
58	GBI_16	S70430				
59	GBI_17	S70451				
60	Gbi_18	S70425				
61	GBI_19	S70423				
62	GBI_20	S70490	21/10/2003			Shaun O'Connor
63	GBI_21	S70475	20/10/2003			Shaun O'Connor
64	GBI_22	S70491	22/10/2003			Shaun O'Connor
65	GBI_23	S80104	23/10/2003			Shaun O'Connor
66	GBI_24	S80129	3/12/2003			Shaun O'Connor
67	GBI_25	S80118				Shaun O'Connor
68	Gbi_26	S80111	1/12/2003			Shaun O'Connor
69	GBI_27	S83320	19/12/2006	M	A	Jo Sim
70	GBI_28	S83368	17/10/2005	F	J	
71	GBI_29	unbanded		M	A	
72	GBI_30	unbanded				
73	GBI_31	S80176				
74	GBI_32	S70502	19/03/2002			
75	GBI_33	S70428				
76	GBI_34	S70418 TX 4	17/10/2002			Brent Barker
77	GBI_35	S70420				
78	GBI_36	S75158	15/01/2003			
79	GBI_37	S75110				
80	GBI_38	S70403				
81	GBI_39	S70484	21/10/2003			Shaun O'Connor
82	NTH_1	S83518	18/10/2005			
83	NTH_2	S83517	2/03/2006			Emma Neill
84	NTH_3	S81782	22/02/2006			Emma Neill
85	NTH_4	S83514	22/02/2006			Emma Neill
86	NTH_5	S83523	27/02/2006			Emma Neill
87	NTH_6	S83540	29/11/2005			Emma Neill
88	NTH_7	S83518	18/10/2005			
89	NTH_8	S83524	27/10/2005			
90	NTH_9	S80546	16/03/2005			
91	NTH_10	S78786	14/03/2005			
92	NTH_11	S83510	23/02/2005			
93	NTH_12	S78795	1/03/2005			
94	NTH_13	S83504	2/12/2004			
95	NTH_14	S78800	29/11/2004			
96	NTH_15	S78794	8/11/2004			
97	NTH_16	L32896	14/10/2004			
98	NTH_17	S78790	14/10/2004			
99	NTH_18	S80536	23/02/2004			
100	NTH_19	S80534	18/02/2004			
101	NTH_20	L32892	17/02/2004			
102	NTH_21	L32895	17/02/2004			
103	NTH_22	S70630	8/12/2003			
104	NTH_23	S80518	28/10/2003			
105	NTH_24	S78832	26/09/2003			
106	NTH_25	S81774	23/09/2003			
107	NTH_26	S78816	11/11/2002			
108	NTH_27	S70624	11/11/2002			

109	NTH_28	S70641	23/01/2003			
110	NTH_29	L33448 BWG	6/03/2003			
111	NTH_30	S78801 BGG	4/03/2003			
112	NTH_31	S70646				
113	NTH_32	S78803	28/02/2003			
114	NTH_33	S81785	24/02/2003			
115	NTH_34	S70635	20/02/2003			
116	NTH_35	S81758	8/10/2002			
117	NTH_36	S70632	7/10/2002			
118	NTH_37	S81763	8/10/2002			
119	NTH_38	S78844	25/09/2002			
120	NTH_39	S78849	25/09/2002			
121	NTH_40	S81765	25/09/2002			
122	NTH_41	S81767	15/10/2002			
123	NTH_42	L33441	25/09/2002			
124	NTH_43	S78805	13/11/2001			
125	NTH_44	L32943	20/12/2001			
126	NTH_45	S78835	4/09/2002			
127	NTH_46	S81281	26/10/2006	F	J	Joe Altham
128	NTH_47	S81284	26/10/2006	M	J	James Frazer
129	NTH_48	S81257	7/12/2006	M	A	James Frazer
130	NTH_49	unbanded				
131	CB_1	S43889	22/04/2007	F	A	S.M. Jenkins
132	CB_2	S80241	22/04/2007	M	A	S.M. Jenkins
133	CB_3	S80311	28/05/2007	F	A	P.C. Russell
134	CB_4	S80250	12/06/2007	M	A	Tara Atkinson-Renton
135	CB_5	S80223	12/06/2007	F	A	Tara Atkinson-Renton
136	CB_6	S80303	30/07/2007	F	J	Carol and Margaret
137	CB_7	S54395	20/08/2007	M	A	R.Langdon
138	CB_8	S80240	20/08/2007	F	A	R.Langdon
139	CB_9	S80308	30/07/2007	F		Carol and Margaret
140	CB_10	L31851	23/07/2007	M		C.Dom
141	CB_11	L29489	23/07/2007	F		C.Dom
142	CB_12	S70510	23/07/2007	M		C.Dom
143	CB_13	S70450	23/07/2007	F		C.Dom
144	CB_14	S80202	10/08/2007	F		Rhys Mills
145	CB_15	S83010		F		
146	CB_16	S53633		F		
147	CB_17	S70439	30/07/2007	M		Carol and Margaret
148	CB_18	S43477		M		
149	CB_19	S43448		F		Chris Turner
150	CB_20	S80305	30/07/2007	M	J	Carol and Margaret
151	FIO_1					
152	MU_1	ref no. 21490				
153	MU_2	ref no. 21491				
154	MU_3	ref no. 21492				

Appendix 2 - Sample table Chapter 3

Table (a); Detailed sample table for Chapter 3 including sample number, sample code, band number, location and location details.

No.	Sample code	Band number	Location	Location details
1	Mana_1	no band	Mana	
2	Mana_2	S73241	Mana	
3	Mana_3	S73239	Mana	
4	TIRI_1	BGR/ S78765	Tiritiri Matangi Is.	emergency landing
5	TIRI_2	S84051/B	Tiritiri Matangi Is.	emergency landing
6	TIRI_3	B/S84061	Tiritiri Matangi Is.	emergency landing
7	TIRI_4	S84062/Y	Tiritiri Matangi Is.	emergency landing
8	TIRI_5	S80523/-	Tiritiri Matangi Is.	Bunkhouse pond
9	TIRI_6	/S84063	Tiritiri Matangi Is.	Bunkhouse pond
10	TIRI_7	BWY/S78767	Tiritiri Matangi Is.	Landing Pond
11	TIRI_8	S78782/W	Tiritiri Matangi Is.	Wattle Gully
12	KWS_1	S81163	KWS	
13	KWS_2	S72087	KWS	tailors wetland
14	KWS_3	YM-YO (used to be YM-YG)	KWS	upper dam - north end
15	KWS_4	S72064	KWS	outside sanctuary
16	KWS_5	L32983	KWS	upper dam - south end
17	KWS_6	L32986	KWS	upper dam - south end
18	KWS_7	S72082	KWS	
19	KWS_8	S72068	KWS	
20	KWS_9	L15237 M-WO	KWS	
21	KWS_10	S72079	KWS	
22	KWS_11	S72077	KWS	
23	KWS_12	S72076	KWS	
24	MOE_3	S81305	Moehau	
25	MOE_4	S83734	Moehau	
26	MOE_5	S81302	Moehau	
27	MOE_6	S81306	Moehau	
28	MOE_7	S81307	Moehau	
29	MOE_8	S81313	Moehau	
30	MOE_9	S81304	Moehau	
31	MOE_10	S83735	Moehau	
32	MOE_11	S81320	Moehau	
33	MOE_12	S81309	Moehau	
34	MOE_13	S84003	Moehau	
35	MOE_14	S84007	Moehau	
36	MOE_15	S84002	Moehau	
37	MOE_16	S84004	Moehau	
38	MOE_17	S81640	Moehau	
39	MOE_18	S83738	Moehau	
40	MOE_19	S81634	Moehau	
41	MOE_20	S83739	Moehau	
42	MOE_21	S83740	Moehau	
43	MOE_22	S83742	Moehau	
44	MOE_23	S83743	Moehau	
45	MOE_24	S83744	Moehau	
46	MOE_25	S83746	Moehau	
47	MOE_26	S843457	Moehau	
48	MOE_27	S43458	Moehau	
49	MOE_28	S43454	Moehau	
50	MOE_29	S83749	Moehau	

51	GBI_2	S83366	GBI	okiwi
52	GBI_3	S70411	GBI	okiwi- barn wetlands
53	GBI_4	L33898	GBI	okiwi - barn wetlands
54	GBI_5	S83357	GBI	Okiwi - sheepyard RA Sheep yards RA
55	GBI_6	S80167	GBI	okiwi - P6
56	GBI_7	S80163	GBI	okiwi - translator wetlandtranslator wetland
57	GBI_8	S80171	GBI	okiwi - orchard drainorchard drain
58	GBI_9	S83355	GBI	okiwi - P36
59	GBI_10	S80108 TX59	GBI	okiwi - Paddock 31
60	GBI_11	S80194 TX85	GBI	okiwi - lower okiwi stream
61	GBI_12	S80147	GBI	okiwi stn - Bush pond
62	GBI_13	S80154	GBI	okiwi stn - Campground/P21
63	GBI_14	S80155	GBI	okiwi stn - skeleton ridgewetland E of skeleton ridge
64	GBI_15	S70502	GBI	okiwi
65	GBI_16	S70430	GBI	okiwi - P8 near woolshed
66	GBI_17	S70451	GBI	okiwi - below haybard P32
67	GBI_18	S70425	GBI	wetland east edge P15
68	GBI_19	S70423	GBI	okiwi - in reserve opp Okivi cattle yards
69	GBI_20	S70490	GBI	okiwi - Pond at cattle yards
70	GBI_22	S70491	GBI	okiwi - Whangapoua stream headwaters
71	GBI_23	S80104	GBI	okiwi - Entrance okiwi station
72	GBI_25	S80118	GBI	okiwi - woolshed #2
73	GBI_26	S80111	GBI	okiwi - bush wetland opp. Coopers
74	GBI_27	S83320	GBI	Medlands
75	GBI_28	S83368	GBI	Okiwi
76	GBI_30	unbanded	GBI	Claris
77	GBI_31	S80176	GBI	Awana
78	GBI_32	S70434 TX 73	GBI	okiwi-maybe P.opp coopers
79	GBI_33	S70428	GBI	okiwi-unfenced wet area P1
80	GBI_34	S70460	GBI	okiwi-P2 pond near cattle yards
81	GBI_35	S704021	GBI	okiwi
82	GBI_36	S70418 TX 4	GBI	okiwi-brent barkers property
83	GBI_37	S70420	GBI	okiwi-stream btw P15 & 4
84	GBI_38	S80110	GBI	okiwi
85	GBI_39	unbanded	GBI	Shoal Bay
86	GBI_40	unbanded	GBI	Medlands
87	NTH_1	S83518	Mimiwhangata	Queens Corner Pond
88	NTH_2	S83517	Mimiwhangata	Lake Surprise
89	NTH_3	S81782	Mimiwhangata	Te Rewa Stream
90	NTH_4	S83514	Mimiwhangata	Y-gully culvert
91	NTH_6	S83540	Mimiwhangata	Trials flats
92	NTH_7			
93	NTH_8	S83524	Mimiwhangata	Pohutukawa over Puddle
94	NTH_10	S78786	Mimiwhangata	Cat Cage Corner
95	NTH_11	S83510	Mimiwhangata	Lower Cattleyard Pond
96	NTH_12	S78795	Mimiwhangata	South Boundary wetland
97	NTH_14	S78800	Mimiwhangata	Cattleyard Delta
98	NTH_13	S83504	Mimiwhangata	Scotty's Pond
99	NTH_15	S78794	Mimiwhangata	True Right Swamp
100	NTH_9	S80546	Mimiwhangata	Dune Lake
101	NTH_16	L32896	Mimiwhangata	20 kph
102	NTH_17	S78790	Mimiwhangata	Cat Cage Corner
103	NTH_18	S80536	Mimiwhangata	Trials flats
104	NTH_19	S80534	Mimiwhangata	Silage Pit Pond
105	NTH_20	L32892	Mimiwhangata	F-drain
106	NTH_21	L32895	Mimiwhangata	2nd top crossing

107	NTH_22	S70630	Mimiwhangata	Molly's Bush
108	NTH_23	S80518	Mimiwhangata	Scotty's Pond
109	NTH_24	S78832	Mimiwhangata	Kingfern Gully
110	NTH_25	S81774	Mimiwhangata	Cattleyard Wetland
111	NTH_27	S70624	Mimiwhangata	True Right Swamp
112	NTH_28	S70641	Mimiwhangata	Trials flats
113	NTH_30	S78801 BGG	Mimiwhangata	Blackberry and Bracken swamp
114	NTH_31	S70646	Mimiwhangata	Silage Wetland
115	NTH_32	S78803	Mimiwhangata	South Boundary dam
116	NTH_33	S81785	Mimiwhangata	Cattleyard Pond
117	NTH_34	S70635	Mimiwhangata	F-drain
118	NTH_35	S81758	Mimiwhangata	20 kph
119	NTH_36	S70632	Mimiwhangata	Trials Stock Pond
120	NTH_37	S81763	Mimiwhangata	Trials Stock Pond
121	NTH_38	S78844	Mimiwhangata	Pohutukawa Grove Pond
122	NTH_39	S78849	Mimiwhangata	Puriri Cave
123	NTH_40	S81765	Mimiwhangata	Scotty's Pond
124	NTH_41	S81767	Mimiwhangata	Scotty's Pond
125	NTH_42	L33441	Mimiwhangata	Creek by POP
126	NTH_43	S78805	Mimiwhangata	Lower 3 Pond
127	NTH_26	S78816	Mimiwhangata	3rd boundary swamp
128	NTH_44	L32943	Mimiwhangata	Clay Track
129	NTH_45	S78835	Mimiwhangata	Pumphouse
130	NTH_46	S81281	Mimiwhangata	Molly's Bush
131	NTH_47	S81284	Mimiwhangata	F-drain
132	NTH_48	S81257	Mimiwhangata	Te Rewa Stream
133	NTH_50	L33892	Mimiwhangata	Clay Track
134	NTH_51	S78804	Mimiwhangata	Lower 3 Pond
135	CB_2	S80241	Captive breeding	
136	CB_3	S80311	Captive breeding	Esplanade Aviary
137	CB_4	S80250	Captive breeding	Orara Wildlife Park
138	CB_5	S80223	Captive breeding	Orara Wildlife Park
139	CB_6	S83010	Captive release 2007	otorohanga kiwi house
140	CB_7	S53633	captive release 2007	otorohanga kiwi house
141	CB_8	S43946	Captive breeding	Peacock springs
142	CB_9	S80242	Captive breeding	Peacock springs
143	CB_10	S54395	Captive breeding	
144	CB_11	S80240	Captive breeding	
145	CB_12	S70439	captive release 2007	
146	CB_13	S80308	captive breeding	
147	CB_14	S80303	captive breeding	peacock springs
148	CB_15	S80305	captive release 2007	peacock springs
149	CB_16	L31851	Captive breeding	
150	CB_17	L29489	captive breeding	
151	CB_18	S70510	captive breeding	
152	CB_19	S70450	captive breeding	
153	CB_20	S80202	captive breeding	
154	CB_21	S80207	Captive breeding	
155	CB_22	S80304	Captive breeding	Esplanade Aviary
156	CB_23	S78957	Captive release 2007	
157	CB_24	S78955	Captive release 2007	
158	CB_25	S78952	Captive release 2007	
159	CB_26	S83018	Captive release 2007	otorohanga kiwi house
160	CB_27	S83022	Captive release 2007	otorohanga kiwi house
161	CB_28	S43477	Captive release 2007	Orara Wildlife Park
162	CB_29	S43448	Captive release 2007	
163	CB_30	S81332	Captive release 2007	
164	CB_31	S82249	Captive release 2007	

165	CB_32	S43944	Captive release 2007	Te Anau Wildlife Park
166	CB_33	S82600	Captive release 2007	Queenstown Kiwi & Birdlife Park
167	CB_34	S43943	Captive release 2007	Te Anau Wildlife Park
168	CB_35	S82598	Captive release 2007	Queenstown Kiwi & Birdlife Park
169	CB_36	S43941	Captive release 2007	Te Anau Wildlife Park
170	CB_37	S53634	Captive release 2007	otorohanga kiwi house
171	CB_38	S83009	Captive release 2007	otorohanga kiwi house
172	CB_39	S83019	Captive release 2007	otorohanga kiwi house
173	CB_40	S53636	Captive breeding release 2007	otorohanga kiwi house

Table (b); Detailed sample table for Chapter 3 (cont.) including sample number, sample code, band number, date of collection, sex of bird, age of bird and collator (s).

No.	Sample code	Band number	date collection	of sex bird	of age bird	of collector(s)
1	Mana_1	no band	7/11/2006		J	Clare Duston & Grant Timlin
2	Mana_2	S73241	7/11/2006		A	Clare Duston & Grant Timlin
3	Mana_3	S73239	7/11/2006		A	Clare Duston & Grant Timlin
4	TIRI_1	BGR/ S78765	28/12/2006	M	J	James Frazer
5	TIRI_2	S84051/B	28/12/2006	F	A	James Frazer
6	TIRI_3	B/S84061	28/12/2006	M	J	James Frazer
7	TIRI_4	S84062/Y	28/12/2006	F	J	James Frazer
8	TIRI_5	S80523/-	29/12/2006	F	A	James Frazer
9	TIRI_6	/S84063	29/12/2006	M	J	James Frazer
10	TIRI_7	BWY/S78767	29/12/2006	M	A	James Frazer
11	TIRI_8	S78782/W	29/12/2006	M	A	James Frazer
12	KWS_1	S81163				
13	KWS_2	S72087				
14	KWS_3	YM-YO (used to be YM-YG)				
15	KWS_4	S72064				
16	KWS_5	L32983				
17	KWS_6	L32986	24/02/2006			Raewyn Empson
18	KWS_7	S72082	24/12/2004			Raewyn Empson
19	KWS_8	S72068	24/12/2004	M		Raewyn Empson
		L15237	M-			
20	KWS_9	WO	24/12/2004			Raewyn Empson
21	KWS_10	S72079	24/12/2004			Raewyn Empson
22	KWS_11	S72077				
23	KWS_12	S72076				
24	MOE_3	S81305				
25	MOE_4	S83734				
26	MOE_5	S81302				
27	MOE_6	S81306				
28	MOE_7	S81307				
29	MOE_8	S81313				
30	MOE_9	S81304				
31	MOE_10	S83735				
32	MOE_11	S81320				
33	MOE_12	S81309				
34	MOE_13	S84003				
35	MOE_14	S84007				
36	MOE_15	S84002				
37	MOE_16	S84004				
38	MOE_17	S81640				
39	MOE_18	S83738				
40	MOE_19	S81634				
41	MOE_20	S83739				
42	MOE_21	S83740				

43	MOE_22	S83742					
44	MOE_23	S83743					
45	MOE_24	S83744					
46	MOE_25	S83746					
47	MOE_26	S843457					
48	MOE_27	S43458					
49	MOE_28	S43454					
50	MOE_29	S83749					
51	GBI_2	S83366	5/03/2005	F	A	Simon Stevenson	
52	GBI_3	S70411	5/03/2005			Simon Stevenson	
53	GBI_4	L33898	5/03/2005			Simon Stevenson	
54	GBI_5	S83357	5/03/2005			Simon Stevenson	
55	GBI_6	S80167	2/11/2004	M	J	Simon Stevenson	
56	GBI_7	S80163	2/11/2004	M	A	Simon Stevenson	
57	GBI_8	S80171	2/11/2004	F	J	Simon Stevenson	
58	GBI_9	S83355	17/02/2005	F	A	Simon Stevenson	
59	GBI_10	S80108 TX59	15/02/2005	F	A	Simon Stevenson	
60	GBI_11	S80194 TX85	15/02/2005	F	A	Simon Stevenson	
61	GBI_12	S80147	8/03/2004	M	A	Simon Stevenson	
62	GBI_13	S80154	9/03/2004	F	A	Simon Stevenson	
63	GBI_14	S80155	10/03/2004	F	A	Simon Stevenson	
64	GBI_15	S70502	19/03/2002				
65	GBI_16	S70430					
66	GBI_17	S70451					
67	GBI_18	S70425					
68	GBI_19	S70423					
69	GBI_20	S70490	21/10/2003			Shaun O'Connor	
70	GBI_22	S70491	22/10/2003			Shaun O'Connor	
71	GBI_23	S80104	23/10/2003			Shaun O'Connor	
72	GBI_25	S80118				Shaun O'Connor	
73	GBI_26	S80111	1/12/2003			Shaun O'Connor	
74	GBI_27	S83320	19/12/2006	M	A	Jo Sim	
75	GBI_28	S83368	17/10/2005	F	J		
76	GBI_30	unbanded					
77	GBI_31	S80176					
		S70434 TX					
78	GBI_32	73					
79	GBI_33	S70428					
80	GBI_34	S70460					
81	GBI_35	S704021					
82	GBI_36	S70418 TX 4	17/10/2002			Brent Barker	
83	GBI_37	S70420					
84	GBI_38	S80110					
85	GBI_39	unbanded					
86	GBI_40	unbanded					
87	NTH_1	S83518	18/10/2005				
88	NTH_2	S83517	2/03/2006				
89	NTH_3	S81782	22/02/2006				
90	NTH_4	S83514	22/02/2006				
91	NTH_6	S83540	29/11/2005				
92	NTH_7						
93	NTH_8	S83524	27/10/2005				
94	NTH_10	S78786	14/03/2005				
95	NTH_11	S83510	23/02/2005				
96	NTH_12	S78795	1/03/2005				
97	NTH_14	S78800	29/11/2004				
98	NTH_13	S83504	2/12/2004				
99	NTH_15	S78794	8/11/2004				

100	NTH_9	S80546	16/03/2005			
101	NTH_16	L32896	14/10/2004			
102	NTH_17	S78790	14/10/2004			
103	NTH_18	S80536	23/02/2004			
104	NTH_19	S80534	18/02/2004			
105	NTH_20	L32892	17/02/2004			
106	NTH_21	L32895	17/02/2004			
107	NTH_22	S70630	8/12/2003			
108	NTH_23	S80518	28/10/2003			
109	NTH_24	S78832	26/09/2003			
110	NTH_25	S81774	23/09/2003			
111	NTH_27	S70624	11/11/2002			
112	NTH_28	S70641	23/01/2003			
113	NTH_30	S78801 BGG	4/03/2003			
114	NTH_31	S70646				
115	NTH_32	S78803	28/02/2003			
116	NTH_33	S81785	24/02/2003			
117	NTH_34	S70635	20/02/2003			
118	NTH_35	S81758	8/10/2002			
119	NTH_36	S70632	7/10/2002			
120	NTH_37	S81763	8/10/2002			
121	NTH_38	S78844	25/09/2002			
122	NTH_39	S78849	25/09/2002			
123	NTH_40	S81765	25/09/2002			
124	NTH_41	S81767	15/10/2002			
125	NTH_42	L33441	25/09/2002			
126	NTH_43	S78805	13/11/2001			
127	NTH_26	S78816				
128	NTH_44	L32943	20/12/2001			
129	NTH_45	S78835	4/09/2002			
130	NTH_46	S81281	26/10/2006	F	J	Joe Altham
131	NTH_47	S81284	26/10/2006	M	J	James Frazer
132	NTH_48	S81257	7/12/2006	M	A	James Frazer
133	NTH_50	L33892				
134	NTH_51	S78804				
135	CB_2	S80241	22/04/2007	M	A	S.M. Jenkins
136	CB_3	S80311	28/05/2007	F	A	P.C. Russell
137	CB_4	S80250	12/06/2007	M	A	Tara Atkinson-Renton
138	CB_5	S80223	12/06/2007	F	A	Tara Atkinson-Renton
139	CB_6	S83010		F		
140	CB_7	S53633		F		
141	CB_8	S43946				
142	CB_9	S80242				
143	CB_10	S54395	20/08/2007	M	A	
144	CB_11	S80240	20/08/2007	F	A	
145	CB_12	S70439	30/07/2007	M		
146	CB_13	S80308	30/07/2007	F		
147	CB_14	S80303	30/07/2007	F	J	
148	CB_15	S80305	30/07/2007	M	J	
149	CB_16	L31851	23/07/2007	M		C.Dom
150	CB_17	L29489	23/07/2007	F		C.Dom
151	CB_18	S70510	23/07/2007	M		C.Dom
152	CB_19	S70450	23/07/2007	F		C.Dom
153	CB_20	S80202	10/08/2007	F		Rhys Mills
154	CB_21	S80207				
155	CB_22	S80304				
156	CB_23	S78957		F		Ron Munro
157	CB_24	S78955		M		Ron Munro

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158	CB_25	S78952	M	Ron Munro
159	CB_26	S83018	M	
160	CB_27	S83022		
161	CB_28	S43477	M	
162	CB_29	S43448	F	Chris Turner
163	CB_30	S81332	M	Chris Turner
164	CB_31	S82249	M	Chris Turner
165	CB_32	S43944	F	
166	CB_33	S82600	F	
167	CB_34	S43943	M	
168	CB_35	S82598	F	
169	CB_36	S43941	M	
170	CB_37	S53634	M	
171	CB_38	S83009	F	
172	CB_39	S83019	M	
173	CB_40	S53636	M	

Appendix 3 – Haplotype table for Chapter 2

Table (a): Haplotypes identified at Mimiwhangata and corresponding sample information.

Sample no.	Band no.	Location	Haplotype
NTH_1	S83517	Lake Surprise	J
NTH_2	S81782	Te Rewa Stream	A
NTH_3	S83514	Y-gully culvert	A
NTH_4	S83523	Cattleyard Pond	A
NTH_5	S83540	Trials flats	D
NTH_6	S83518	Queens Corner Pond	A
NTH_7	S83524	Pohutukawa over Puddle	A
NTH_8	S80546	Dune Lake	C
NTH_9	S78786	Cat Cage Corner	C
NTH_10	S83510	Lower Cattleyard Pond	C
NTH_11	S78795	South Boundary wetland	H
NTH_12	S83504	Scotty's Pond	I
NTH_13	S78800	Cattleyard Delta	B
NTH_14	S78794	True Right Swamp	B
NTH_15	L32896	20 kph	A
NTH_16	S78790	Cat Cage Corner	C
NTH_17	S80536	Trials flats	B
NTH_18	S80534	Silage Pit Pond	B
NTH_19	L32892	F-drain	B
NTH_20	L32895	2nd top crossing	B
NTH_21	S70630	Molly's Bush	B
NTH_22	S80518	Scotty's Pond	A
NTH_23	S78832	Kingfern Gully	B
NTH_24	S81774	Cattleyard Wetland	B
NTH_25	S78816	3rd boundary swamp	A
NTH_26	S70624	True Right Swamp	B
NTH_27	S70641	Trials flats	B
NTH_28	L33448 BWG	Molly's Bush	F
NTH_29	S78801 BGG	Blackberry and Bracken swamp	A
NTH_30	S70646	Silage Wetland	A
NTH_31	S78803	South Boundary dam	A
NTH_32	S81785	Cattleyard Pond	A
NTH_33	S70635	F-drain	B
NTH_34	S81758	20 kph	E
NTH_35	S70632	Trials Stock Pond	A
NTH_36	S81763	Trials Stock Pond	B
NTH_37	S78844	Pohutukawa Grove Pond	B
NTH_38	S78849	Puriri Cave	D
NTH_39	S81765	Scotty's Pond	A
NTH_40	S81767	Scotty's Pond	A
NTH_41	L33441	Creek by POP	B
NTH_42	S78805	Lower 3 Pond	A
NTH_43	L32943	Clay Track	K
NTH_44	S78835	Pumphouse	E
NTH_45	S81281	Molly's Bush	G
NTH_46	S81284	F-drain	A
NTH_47	S81257	Te Rewa Stream	D
NTH_48	unbanded	Whananaki south rd, 2640850-70 6631832-20	B

Appendix 4 - Permits

Permits were obtained from each Department of Conservation (DoC) conservancy for the use of the feather samples from each pateke population. From the Auckland DoC conservancy a permit was obtained for the collection and use of feathers from Great Barrier Island and Tiritiri Matangi Island. This permit was issued by Darcy Liddell (Auckland Conservancy Office). From the Northland DoC conservancy a permit was granted from Donna Stuthridge (Northland Conservancy Office) for the use of up to 40 feathers from the Mimiwhangata population. A variation on the permit from Northland to use an additional 20 feathers (if required) was granted by Donna Stuthridge during 2007. A permit for the use of feathers from Karori Wildlife Sanctuary and Mana Island was granted by Colin Miskelly (Wellington Conservancy Office, Head Office). For the Moehau population, a permit was obtained from Jason Roxborough (Hauraki Area Office) for the use of feathers from this location. Consultation with local Iwi was carried out prior to permits being granted. Permits were valid for the thesis duration. Permits for this study are currently held at Victoria University.

Appendix 5 – Further information on mitochondrial DNA analysis (Chapter 2)

Selecting the outgroup

For the outgroup to the pateke mtDNA sequence data the mallard (*Anas platyrhynchos*) was chosen. This was because the mallard is within the same genus as pateke and has been well studied and mtDNA sequences are available on Genbank. Initially a five separate mallard sequences were added to the alignment. However, as there were only slight differences between the mallard sequences, only one sequence was used as the out group for the final phylogeny (accession number AY928900; Kulikova et al. 2005). This sequence was chosen as the primers used for this study were taken from within this reference. If this study was concerned with phylogeography or looking at evolution then selection

of the outgroup would have been more thorough and other *Anas* species would have been added to the alignment.

Appendix 6 – Further information on microsatellite analysis (Chapter 3)

Software packages used

A difficulty encountered when analysing microsatellite data was the abundance of software packages available. Outlined is a brief description of each software package used for this study, the type of data that can be analysed, and what calculations can be performed.

1. FSTAT v2.9.3.2 (Goudet 1995)

FSTAT tests gene diversities and differentiation for microsatellite data. It can be used to compute Nei and Weir & Cockerham estimators of gene diversities and F-statistics and uses a randomisation method. FSTAT calculates;

- number of individuals per sample and loci
- allele frequency per sample and overall
- observed and expected number of each genotype
- unbiased gene diversity per sample and locus
- allelic richness
- F_{IS} , F_{ST} , relatedness
- Testing of HWE

2. GeneMapper® v4.0 (Applied Biosystems)

Genemapper software is specifically for scoring of microsatellite data.

3. GENEPOP v4.0 (Raymond & Rousset 1995)

From this software package, computations can be made for (1) exact tests for Hardy-Weinberg equilibrium, population differentiation and genotypic disequilibrium among pairs of loci, (2) estimates of F-statistics, null allele frequencies, allele size-based statistics and the number of immigrants and (3) it performs analysis of isolation by distance from pairwise comparisons of

individuals or population samples. Data files from GENEPOP can be converted within this program to formats compatible with other software packages (i.e. FSTAT). GENEPOP v4.0 is available on the web.

4. GENELOSS (England & Osler 2001)

Simulations of the effects of population bottlenecks on genetic diversity can be performed from this software package (England & Osler 2001). These simulations show how much heterozygosity and allelic diversity remain after a specified number of generations. Microsatellite data (allele number and frequency at each loci) is required as well as the number of breeding pairs within the population. Loci need to be unlinked for these simulations. GeneLoss is not currently available on the web but can be obtained by emailing the author.

5. HP-RARE 1.0 (Kalinowski 2005b)

This computer package calculates estimates of allelic richness and private allelic richness for hierarchical study designs. HP-RARE also outputs the size of each sample, the number of alleles observed in each sample, and the expected heterozygosity for each sample and the frequency of null alleles. It uses genotypic data in the format of GENEPOP (Raymond & Rousset 1995). This program is available from the web.

Appendix 7 – Additional methodological considerations

A number of problems were encountered with lab work. How these difficulties were dealt with is outlined.

Mitochondrial DNA data (Chapter 2)

A problem with mtDNA data was editing sequences for alignment. In order to do achieve this accurately sequences were edited all at once and this was done twice. This was to ensure all sequences were edited in the same way and the cut

off points were the same for all sequences. Sequences that were difficult to read or had multiple misread base-pairs were re-sequenced.

Microsatellite DNA data (Chapter 3)

The main difficulty with the microsatellite data was ensuring consistency between batches of samples that were sent off for genotyping and equal scoring of samples. To overcome the former issues, two samples which worked well (one from Mimiwhangata and one from Great Barrier Island) were sent off with each batch of genotyping. This gave a base-line for each batch of samples if one batch was slightly different to another. The latter issue was dealt with by firstly using the 'binning mode' in GeneMapper v4.0. Secondly, the entire data set was scored twice on two consecutive days to ensure accuracy. Scoring error can be a problem in microsatellite data sets (Selkoe & Toonen 2006).