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Conservation Genetics of Island Takahe
(Porphyrio mantelli)
Conservation Genetics of
Island Takahe
(Porphyrio mantelli)

by
Marieke Lettink

A research report submitted in partial fulfilment of the requirements
For the Diploma in Wildlife Management

April 1999

University of Otago Wildlife Management
Report Number 96
EXECUTIVE SUMMARY

Investigation title
Conservation Genetics of Island Takahe (*Porphyrio porphyrio*)

Study venue
Molecular Ecology Laboratory, Department of Ecology, Massey University
Department of Zoology, University of Otago

Investigator
Marieke Lettink

Aim
To investigate genetic variation, mating system, and potential effects of inbreeding on the reproductive success of island takahe

Objectives
1. To determine whether or not takahe breeding on islands are genetically monogamous, using multilocus DNA profiling
2. To document levels of DNA minisatellite variation of island takahe and compare these to other New Zealand bird species
3. To determine whether or not inbreeding has adverse affects on the reproductive (hatching and fledging) success of island takahe

Scientific Methods
Multilocus DNA profiling was used to determine levels of genetic variation and paternity for 37 island takahe from 11 presumptive families in order to verify pedigree records. These records were subsequently used to investigate whether or not inbreeding affects the reproductive (hatching and fledging) success, using binomial general linear models.
Scientific Results

Paternity could be assigned to 27 chicks, and in all cases was found to be consistent with the resident male (i.e. presumed father). The paternity of 10 chicks could not be assigned, due to low levels of DNA minisatellite variation. Levels of genetic variation observed for island takahe fall within the range reported for other New Zealand bird species. Evidence that inbreeding affects the hatching success of pairs of island takahe with inbred females was found. In contrast, this effect was not evident for related pairs, or pairs with inbred males.

Conclusions

1. Island takahe appear to be genetically monogamous
2. Island takahe populations are genetically depauparate
3. Inbreeding depression may be a factor contributing to the low reproductive success of takahe on islands compared to the Fiordland population

Recommendations

1. Translocation frequency should not be increased solely for the purposes of “maximising genetic diversity”
2. A comparison of the levels of genetic variation for Fiordland vs island takahe should be performed
3. A cost-benefit analysis should be performed to determine the merits of preventing matings between closely related pairs

This research was supported by a Department of Conservation grant
General Introduction

Conservation of biodiversity is one the most important contemporary global problems faced by humanity. Human activity has caused the extinction of more than a thousand vertebrate species over the last century (Altukhov, 1994), with many more currently on the verge of extinction. As remaining natural areas continue to decline in size and become fragmented, an increasing number of species will require monitoring and management to ensure their persistence.

New Zealand contains 11% of the world’s endangered bird species (Reed and Merton, 1990). For many of these species, management options are limited to preserving relic populations and their habitats on the mainland, and/or maintaining a number of small populations on predator-free offshore islands. New Zealand has over 500 offshore islands, a large proportion of which are designated sanctuaries or nature reserves (Mortimer et al., 1996).

Over the last decade, awareness and concern over genetic problems potentially faced by small populations has grown considerably. Issues of particular concern are the maintenance of genetic variation and effects of inbreeding. This project constitutes an investigation into genetic issues related to the management of the takahe (Porphyrio mantelli), a large, flightless rail endemic to New Zealand. Presumed extinct until their “rediscovery” in 1948, just one relict population of ~120 individuals remains in the Murchison Mountains, Fiordland. In addition, four small populations have been established on predator-free islands since 1984 (Crouchley, 1994).

This project consists of two separate but inter-related chapters. Chapter One addresses the mating system and levels of genetic variation of takahe breeding on islands. Chapter Two assesses the effects of inbreeding on the reproductive success of island takahe. Conclusions and management recommendations are considered in the final part of this report. Some repetition is a consequence of this approach.
Literature cited


Chapter One

Mating System and Genetic Variation in the Takahe (*Porphyrio mantelli*), an Endangered New Zealand Rail

Abstract

The mating system of island takahe (*Porphyrio mantelli*) was determined using multilocus DNA profiling of 37 chicks from 11 presumptive families. No evidence of extra-pair paternity was found for the 25 chicks to which paternity could be confidently assigned, supporting the assumption that takahe are genetically monogamous. The paternity of 12 chicks could be not resolved, due to low levels of minisatellite DNA variation. For two of these chicks, paternity was assigned to the presumed father on the basis of territory isolation. Island takahe exhibit low levels of minisatellite DNA variation in comparison with other New Zealand bird species. Further research is necessary before the implications of low genetic variation in island populations of takahe can be placed in perspective.

Introduction

The takahe (*Porphyrio mantelli*) is an endangered flightless rail endemic to New Zealand. Thought to be extinct earlier this century, the takahe was "rediscovered" in the Murchison Mountains, Te Anau, in 1948, in what has been hailed as one of the most spectacular ornithological discoveries of this century. Considered to be a first priority species ("Category A") by the Department of Conservation (Molloy, 1994), takahe now number approximately two hundred.

While no longer in imminent danger of extinction, management of takahe by the Department of Conservation continues to be intensive. Key management components include regular monitoring, deer culling, predator control, egg manipulation, captive rearing, and translocation. Long-term goals include the establishment of at least two self-sustaining populations of 500 birds each, and several small (<30 individuals) island populations (Crouchley, 1994).
The captive rearing unit (Burwood Bush, Te Anau) was established in 1985 in response to the population decline noted during the 1960s and 1970s. In 1981, the population reached a low of approximately 120 birds, representing a 40% decline in numbers over the previous decade (Mills et al., 1984). Juvenile mortality (survival to 1 year of age) in the wild is high (up to 70%), but can be decreased to 10% - 20% by raising birds in captivity for the first year of their life (Eason, 1992). Captive-reared birds have been used in an attempt to found a second wild population, support the existing population, and provide founders for predator-free islands.

Four island populations of takahe were established during the mid-80s to early 90s, using small numbers of founders. Since then, a few captive-reared birds have been added to these populations, and some birds transferred between islands. Island populations have slowly expanded from 24 founding individuals to a current total of ~56 takahe (19 breeding pairs). Generally, transfers are motivated by the availability of unpaired mates, although there have been other reasons, such as aggression, poor fertility, the prevention of matings between closely related individuals, and safety (e.g. mice eradication on Mana Island in 1989). Concern over disease risks and other issues have led to the formulation of a policy regarding one-way transfers between certain locations. A summary of takahe management and allowable movement can be found in appendix one.

**Genetic issues related to management of takahe: translocation, genetic variation, and mating system**

Translocation involves the capture and selection of a number of founding individuals, which subsequently reproduce and establish a new population. Translocations may be undertaken for numerous reasons, such as habitat degradation, the arrival of predators, or the need for an “insurance policy” – an additional population(s) that provides a back up, should the original population expire. Translocations form an integral part of New Zealand’s conservation strategy; by 1990 over 200 separate translocation events had taken place (Atkinson, 1990).
In theory, island populations may be more vulnerable to extinction due to disrupted gene flow (Blackwell and Doerr, 1995) and increased likelihood of inbreeding (mating between relatives) (Frankham, 1998). In addition, there may be founder effects, whereby the founding individuals represent only a portion of the genetic diversity present in the original population. Collectively, these processes can result in a loss of genetic variability, which in turn is thought to confer reduced adaptive potential for a given population in variable environments (Lacy, 1997; Fleischer et al., 1994; Simberloff, 1988; Lande and Barrowclough, 1987; Gilpin and Soule, 1986; Frankel and Soule, 1981).

From a management perspective, there are therefore two central goals in the genetic management of endangered populations. The first of these goals is to maintain the genetic variation of the population in order to preserve its evolutionary potential. The second goal is the prevention of excessive inbreeding in order to minimise potential negative effects of inbreeding depression (Wang, 1997).

Crucial to both goals is the need to elucidate the genetic mating system of the species under consideration and consider the associated consequences for management. Knowledge of the genetic mating system of a species allows pedigrees to be constructed, from which levels of inbreeding may be estimated. In many captive populations of endangered species, pedigree records are used to manipulate matings in order to minimise or prevent inbreeding.

The type of mating system shown by a species may also influence the speed at which genetic variation is lost within a population. In some species (e.g. lek-breeders, or those which form packs or harems), relatively few males may sire the majority of offspring. The resulting offspring will collectively be less genetically variant than offspring sired by different fathers in a monogamous mating system. This leads to a faster rate of allele fixation (the process whereby all individuals in a population end up with the same allele at a given locus).

There is currently much debate on how to manage populations for maximal genetic diversity, and exactly what constitutes an “acceptable” level of genetic variation for population persistence over the long term (Caughley and Gunn,
1996). Genetic considerations may be futile when other factors contributing to the decline of a population, such as habitat destruction, are not being addressed (Haig, 1998). However, for species like the takahe, which persist as multiple populations in protected habitats, translocation provides a potential means of minimizing further losses in genetic variation.

What is the mating system of takahe?
Takahe are assumed to be genetically monogamous. This assumption is based on observations of long-term pair bonding and defence of large, all-purpose territories. However, not all island takahe are strictly pair-bonded; there is a two-female, male trio on Mana Island, and a two-male, female trio on Tiritiri Matangi Island. Island takahe pedigrees, constructed by the Department of Conservation, are used to calculate relatedness coefficients between pairs and inbreeding coefficients for individuals, both of which are used to monitor inbreeding.

The advent and widespread use of molecular means of determining mating systems have shed serious doubt on the reliability of pedigrees based on observational data. "True" or genetic monogamy may be the exception rather than the rule for birds: although 80-90% of bird species are socially monogamous (Lack, 1968; Moller, 1986), genetic monogamy is rare (Gilbert et al., 1998). The incidence of extra-pair paternity varies considerably between species, and in some cases within populations of the same species (reviewed by Petrie and Kempenaers, 1998, and Westneat and Sherman, 1997; Birkhead and Moller, 1992; Westneat et al., 1990).

Mating events of island takahe are rarely witnessed (Ryan, 1996). The potential for extra-pair fertilisations does exist, as territories of several breeding pairs on some islands are in close proximity. If island takahe do engage in extra-pair fertilisations, the parentage of the resulting offspring will be erroneously represented by pedigree data. Inaccurate pedigrees may inhibit the detection of any potentially adverse effects from inbreeding on reproductive fitness. Secondly, extra-pair paternity among island takahe will lead to offspring that are collectively less genetically variant (since they are sired by fewer males). It is therefore important to determine whether or not social monogamy equates to
genetic monogamy for takahe, using DNA profiling (also known as DNA fingerprinting).

**DNA profiling**

DNA profiling is a molecular technique capable of producing individual-specific “fingerprints” – unique bar-code-like patterns of electrophoretically separated bands, or minisatellite DNA sequences (Jeffreys et al., 1985a; Amos, et al., 1992). Minisatellite DNA consists of short (10-100 base pair) repeats in tandem arrays of up to several hundred copies (Burke et al., 1996). Initially discovered in humans, minisatellites have since been found in a wide range of organisms, and appear to be widely distributed throughout the genome.

Minisatellites may be used as individual-specific markers by virtue of their polymorphic nature. The length of minisatellite arrays is extremely variable between individuals, reflecting the rapid rate at which they lose or gain repeat units (Amos et al., 1992). Minisatellites are detected using restriction digestion and Southern blotting of agarose gels, forming what are known as restriction-fragment-length-polymorphism (RFLP) patterns (Figure 1). Since many minisatellite loci share a relatively conserved core region, they may be detected simultaneously by hybridisation to a matching probe sequence. This process is known as multi-locus fingerprinting.

DNA profiling may be used to assign parentage and detect the genetic similarity between individuals. Genetic similarity is calculated from the proportion of bands that are shared between two individuals in a restriction-fragment-length-polymorphism (RFLP) pattern, and correlates with their relatedness (Wetton et al., 1987; Lynch 1988; Kuhnlein et al., 1989). When averaged across a large number of pair-wise comparisons, the resulting index (Bandsharing or Similarity Index) provides a measure of genetic variation for that population or species.

**Objectives of this study**

The primary aim of this study was to test the assumption that island takahe are genetically monogamous, using DNA profiling. Should this assumption prove
Figure 1  Schematic representation of DNA profiling. DNA is extracted from nucleated red blood cells (using phenol/chloroform extractions) and digested with restriction enzyme to yield fragments of various sizes. These fragments are electrophoretically separated in an agarose gel and transferred to a nylon membrane by Southern blotting. Membranes are subsequently hybridised with a radioactively-labelled probe and exposed to X-ray film. This film is then developed to produce the DNA fingerprint.
to be invalid, more accurate pedigrees can be constructed using molecular paternity data.

The second aim of this study was to compare levels of genetic variation in takahe to those observed in other New Zealand bird species, as reflected by minisatellite DNA. Ultimately, knowledge of mating system and genetic variation should aid management of island takahe.
Methods

Pilot study

DNA from available tissue samples (n = 15) of takahe from the Murchison Mountains was used in a pilot DNA fingerprinting study. Such studies are recommended before embarking on extensive studies on species which have not been studied in this way. The aim of the pilot study was to identify restriction enzyme/probe combinations that exhibit reasonable levels of genetic variation (Burke et al., 1989).

The use of different DNA probe and restriction enzyme combinations result in profiles which typically differ in the number of detectable fragments, their distribution across the molecular weight range, and the index of similarity between unrelated individuals (Hanotte et al., 1992; Holmes, 1994). Ideal profiles are characterised by low background (i.e. non-specific binding of probe) and a moderate number of clearly resolved fragments spread evenly across a molecular weight range (Holmes, 1994). While a large number or bands may reduce the probability of false inclusion (the probability that parentage is assigned incorrectly to a non-parent), the accuracy with which profiles are scored may be compromised.

DNA digested with either HaeIII, AluI, or Hinfl, was used to generate profiles with probes PV47-2 (Longmire et al., 1990), human probes 33.15 and 33.6 (Jeffries et al., 1985b), YNH24 (van Ede et al., 1990), and 3'HVR (Goodbourne et al., 1983; Fowler et al., 1989), according to the methods outlined below.

Collection of samples and DNA extraction

Forty island takahe were captured in April 1997 using a combination of hand nets and large mist nets set at ground level (Appendix Two). Up to 5 ml of blood was collected from each bird from the inter-tarsal vein, using a 26-gauge sterile butterfly needle fitted to a 5 ml disposable seringe. Samples were immediately transferred to liquid nitrogen and stored at -80°C until analysis. Twelve samples collected on previous occasions were also made available. In total, 37 chicks from eleven presumptive families were analysed for paternity. Takahe pedigrees for
each island were constructed from Department of Conservation records, and can be found in Appendix Three.

Whole blood (15 µl) was mixed with 400 µl SET buffer (0.1 M NaCl, 1 mM EDTA, 0.1 M Tris-HCl pH 8.0), 10 µl of Proteinase K (20 mg/ml), 10 µl 20% SDS, and incubated overnight at 55°C with gentle rotation. DNA was extracted once with phenol, twice with phenol/chloroform/isoamyl alcohol (25:24:1), and once with chloroform/isoamyl alcohol (1:1). Precipitations (using 3 M NaOAc pH 5.2) and resuspensions were performed according to Sambrook et al., (1989).

**DNA digestion, electrophoresis and transfer**

Genomic DNA samples of approximately 20 µg were digested overnight at 37°C with restriction enzyme *Hae*III (10 units) in the presence of 4 mM spermidine trihydrochloride and bovine serum albumin (100 µg/ml). A further 10 units of restriction enzyme was added the following day, and incubation continued for at least one hour. All digested samples were visualised in 0.8% agarose minigels to confirm complete digestion and check relative concentrations. The concentration of restricted DNA was determined using a Hoefer TK0-100 DNA fluorometer.

Approximately 5 µg of restricted genomic DNA was loaded per gel lane. DNA fragments were resolved in a 0.8% agarose gel (200mm wide x 300mm long) in TBE running buffer (134 mM Tris, 74.9 mM boric acid, 2.55 mM EDTA pH 8.8) for 72 hours at approximately 55 volts. Molecular weight markers were loaded in outside gel lanes. After electrophoresis, the gel was soaked in depurination solution (0.25 M HCl) for 15 minutes, denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 45 minutes, followed by neutralising solution (1.5 M NaCl, 0.5 M Tris HCl pH 7.2, 1 mM EDTA) twice for 15 minutes. DNA restriction fragments were transferred onto nylon membrane (Amersham Hybond-N) by Southern blotting using 6 x SSC ((1 x SSC: 0.15 M NaCl, 0.015 M sodium citrate) overnight. After blotting, membranes were washed briefly in 6 x SSC, air dried, and baked at 80°C for approximately 2 hours.
DNA hybridisation

The probes pV47-2 (Longmire et al., 1991) and 33.6 (Jeffreys et al., 1985b) were labelled with radioactive $^{32}$P dCTP by random priming (RadPrime DNA labelling system). Membranes were pre-hybridised in 0.5 M disodium hydrogen orthophosphate pH 7.2, containing 0.5 M EDTA and 7% SDS, at either 55°C (pV47-2) or 61°C (33.6) for 2 hours before the addition of the probe. After addition of the probe, hybridisation was continued for approximately 18 hours. Membranes hybridised with pV47-2 were washed twice for 30 minutes with 5 x SSC, 0.1% SDS at 55°C (pV47-2), and membranes hybridised with 33.6 were washed twice with 1 x SSC, 0.1% SDS at 61°C.

Membranes were subsequently exposed to X-ray film (Fuji, RX) for 24 hours at 80°C, followed by a second expose for 3-7 days depending on the intensity of the first autoradiograph. After hybridisation with each probe, membranes were stripped with 0.4 M NaOH at 45°C for 30 minutes, followed by two neutralisation washes in 0.1 x SSC, 0.1% SDS, 0.2 M Tris-HCl pH 7.5 for 15 minutes, then air dried and stored at room temperature.

Fingerprint analysis

For each family, DNA samples from the mother, chick(s), and potential fathers were analyzed in adjacent lanes on the same agarose gel. Where possible, all families from the same island were analyzed on the same gel. For each profile, a presence-absence matrix was constructed for all fragments in the 6-23Kb region. A restriction fragment was scored as shared by two individuals their centers differed in electrophoretic mobility by less than 0.5 mm. Bandsharing coefficients were calculated for all pair-wise comparisons using the formula $D=2n_{AB}/(n_A+n_B)$, where $n_A$ and $n_B$ are the total numbers of bands scored in individuals A and B respectively, and $n_{AB}$ is the number of shared bands (Wetton et al., 1987). Heterozygosity values were calculated using the Macintosh-based computer program ThumbPrint (Marshall, 1992).

Genetic variation was assessed by computation of $D$, the band sharing index. $D$ was calculated separately for first order relatives (sibling-sibling pairs, mother-
offspring pairs, and father-offspring pairs), unrelated individuals, founders, pairs of breeding birds, and populations on each island. It should be noted that the latter does not provide a meaningful comparison of genetic variation between island populations, as some individuals are represented more than once as a consequence of having been subject to multiple translocation events between islands (maximum number of translocations = 3). Since island populations of takahē were formed from, and continue to be modified by translocations, there is effectively no “natural” population structure. Similarly, the band sharing index calculated for all islands is biased due to the inclusion of birds represented more than once. “True” estimates of within-and between-population variability could therefore not be made.

In order to assign paternity, profiles were analyzed for band matching, unattributable fragments, and band sharing between offspring and potential fathers (Ardern et al., 1997; Lambert et al., 1994; Decker et al., 1993; Graves et al., 1993; Westneat et al., 1990). Each of these methods has limitations, which can be largely overcome by using an integrated approach (Lambert, pers. com). In cases where maternity is known, any bands in the offspring’s profile that are not present in the mother must have been inherited from the father, with the only exception being bands that have arisen by mutation (Jeffreys, 1991). Thus chicks for which all bands not shared by the mother could be found in only one of the potential fathers could be confidently assigned paternity.

Apart from those relatively few bands that are expected to arise by mutation, unattributable bands will be present in a chick when the presumed father is not the biological father. In order to distinguish between these two possibilities, a comparison of band sharing between chicks and fathers, and chicks and non-fathers was made (where fathers are designated by virtue of having no unattributable bands). Ideally, such a comparison yields a bimodal distribution, which should permit the classification of potential males with one (or more) unattributable bands as being either true fathers (with unattributable alleles arising from mutation), or non-fathers.
Results

Pilot study
Probes pV47-2 and 33.6 in combination with restriction enzyme HaeIII were selected for DNA fingerprinting of island takahe, based on (comparatively) low band sharing values and profile clarity (Table 1).

Table 1  Comparison of probe/restriction enzyme combinations for Fiordland takahe profiles obtained using multilocus DNA fingerprinting (D = band sharing index, H = heterozygosity, * = profile could not be scored due to an excessive number of bands and/or high background).

<table>
<thead>
<tr>
<th>Probe</th>
<th>Restriction Enzyme</th>
<th>Number of bands scored (6-23 Kb)</th>
<th>Number of pair-wise comparisons</th>
<th>D</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>pV47-2</td>
<td>HaeIII</td>
<td>27</td>
<td>15 (136)</td>
<td>0.43</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>AluI</td>
<td>26</td>
<td>15</td>
<td>0.63</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>HinfI</td>
<td>38</td>
<td>15</td>
<td>0.75</td>
<td>0.29</td>
</tr>
<tr>
<td>33.6</td>
<td>HaeIII</td>
<td>45</td>
<td>15</td>
<td>0.67</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>AluI</td>
<td>47</td>
<td>15</td>
<td>0.74</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>HinfI</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>N/A</td>
</tr>
<tr>
<td>33.15</td>
<td>HaeIII</td>
<td>30</td>
<td>15</td>
<td>0.72</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>AluI</td>
<td>31</td>
<td>15</td>
<td>0.72</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>HinfI</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>N/A</td>
</tr>
<tr>
<td>YNH24</td>
<td>HaeIII</td>
<td>28</td>
<td>15</td>
<td>0.59</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>AluI</td>
<td>37</td>
<td>15</td>
<td>0.73</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>HinfI</td>
<td>29</td>
<td>15</td>
<td>0.74</td>
<td>0.35</td>
</tr>
<tr>
<td>3'HVR</td>
<td>HaeIII</td>
<td>33</td>
<td>15</td>
<td>0.66</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>AluI</td>
<td>39</td>
<td>15</td>
<td>0.83</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>HinfI</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Fingerprint Analysis
An example of the minisatellite DNA profiles produced by hybridisation of HaeIII-digested takahe DNA with probe pV47-2 is shown in Figure 2. The average number of scorable fragments (between 6.0 to 23.0 kb) per individual was typically greater for probe 33.6 (15.9 ± 2.1) than pV47.2 (12.15 ± 2.18). When combining data for both probes, an average of 14.0 ± 2.8 bands per individual was obtained.
Figure 2 DNA profile of two presumptive families of takahe on Tiritiri Matangi Island. The first family contains one female, seven chicks, and four potential fathers (1, 2, 3, 5), while the second family consists of one female, one chick, and five potential fathers (1-5). Open-ended arrows indicate bands diagnostic of paternity, which for family 1 can be assigned to male 1. Note that chick 4 subsequently became potential father 4, and chick 1 the mother of the second family. The paternity of chick 8 could not be assigned due to a lack of diagnostic bands, a consequence of the relatedness between individuals of the two families. The outside lanes contain molecular weight markers.
Band sharing ($D$) values were consistently higher for probe 33.6 (Table 2). Therefore, separate values for each probe were calculated in addition to a combined value. Standard deviations are included in all estimates and reveal a greater range for pV47-2 than 33.6. As band sharing values obtained with 33.6 were high and spread across a small range, data from this probe were of limited use.

Band sharing was lowest for pair-wise comparisons of founding individuals, followed by unrelated birds and breeding pairs (Table 2). The band sharing values obtained for the populations on Tiritiri Matangi and Mana islands were almost identical, but higher than those obtained for the Maud and Kapiti Island populations.

**Paternity Analysis**

The paternity of 21 (57%) chicks could be assigned based on unattributable bands using probe pV47-2 alone, 14 of which were confirmed by probe 33.6. The use of probe 33.6 allowed for two additional paternities to be resolved. Collectively, the paternity of 23 (62%) chicks was assigned, and in all cases found to be consistent with the resident male. Failure to assign paternity to the remaining 14 (38%) chicks was due to an inability to exclude one or more potential males other than the resident male as a result of high levels of band sharing. For the three chicks from the two-female, one-male trio on Mana Island, neither maternity or paternity could be resolved.

For the 14 chicks for which paternity could not be resolved, band sharing data and cuckoldry opportunities (based on the proximity of neighboring territories) were considered. In general, assigning paternity based on band sharing alone was not reliable, as non-fathers (i.e. males which were positively excluded) often had levels of band sharing with chicks that approached, or even slightly exceeded, that of chicks and fathers (Figure 3). Having said this, in most cases band sharing data was consistent with paternities assigned using the unattributable bands method.

For probe pV47-2/HaeIII profiles, mean levels of band sharing between father and chicks, and non-fathers and chicks were $0.731 \pm 0.074$ and $0.529 \pm 0.110$, **
Table 2  Band sharing values for pair-wise comparisons of founders, breeding pairs, first order relatives, unrelated birds, and island populations of takahe for probes pV47-2 (Longmire et al., 1990) and 33.6 (Jeffreys et al., 1985b)(n = number of pair-wise comparisons, S.D. = standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>PV47-2</th>
<th>S.D.</th>
<th>n</th>
<th>33.6</th>
<th>S.D.</th>
<th>n</th>
<th>combined</th>
<th>S.D.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Founders</td>
<td>0.413</td>
<td>0.179</td>
<td>11</td>
<td>0.732</td>
<td>0.069</td>
<td>11</td>
<td>0.572</td>
<td>0.210</td>
<td>22</td>
</tr>
<tr>
<td>Breeding pairs</td>
<td>0.561</td>
<td>0.202</td>
<td>10</td>
<td>0.735</td>
<td>0.033</td>
<td>10</td>
<td>0.648</td>
<td>0.167</td>
<td>20</td>
</tr>
<tr>
<td>First order relatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Sib-sib pairs</td>
<td>0.821</td>
<td>0.113</td>
<td>72</td>
<td>0.854</td>
<td>0.077</td>
<td>72</td>
<td>0.837</td>
<td>0.098</td>
<td>144</td>
</tr>
<tr>
<td>b) Mother-offspring pairs</td>
<td>0.704</td>
<td>0.113</td>
<td>34</td>
<td>0.838</td>
<td>0.053</td>
<td>34</td>
<td>0.771</td>
<td>0.111</td>
<td>68</td>
</tr>
<tr>
<td>c) Father-offspring pairs</td>
<td>0.731</td>
<td>0.074</td>
<td>21</td>
<td>0.869</td>
<td>0.063</td>
<td>14</td>
<td>0.791</td>
<td>0.097</td>
<td>35</td>
</tr>
<tr>
<td>Unrelated birds</td>
<td>0.545</td>
<td>0.143</td>
<td>148</td>
<td>0.774</td>
<td>0.073</td>
<td>148</td>
<td>0.660</td>
<td>0.161</td>
<td>296</td>
</tr>
<tr>
<td>Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) All islands</td>
<td>0.646</td>
<td>0.034</td>
<td>382</td>
<td>0.808</td>
<td>0.012</td>
<td>382</td>
<td>0.727</td>
<td>0.024</td>
<td>764</td>
</tr>
<tr>
<td>b) Tiritiri Matangi</td>
<td>0.685</td>
<td>0.161</td>
<td>123</td>
<td>0.772</td>
<td>0.071</td>
<td>123</td>
<td>0.729</td>
<td>0.064</td>
<td>247</td>
</tr>
<tr>
<td>c) Mana</td>
<td>0.686</td>
<td>0.113</td>
<td>115</td>
<td>0.806</td>
<td>0.074</td>
<td>115</td>
<td>0.746</td>
<td>0.028</td>
<td>230</td>
</tr>
<tr>
<td>d) Maud</td>
<td>0.553</td>
<td>0.191</td>
<td>66</td>
<td>0.791</td>
<td>0.089</td>
<td>66</td>
<td>0.672</td>
<td>0.072</td>
<td>132</td>
</tr>
<tr>
<td>e) Kapiti</td>
<td>0.660</td>
<td>0.178</td>
<td>78</td>
<td>0.863</td>
<td>0.061</td>
<td>78</td>
<td>0.765</td>
<td>0.083</td>
<td>156</td>
</tr>
</tbody>
</table>
Levels of band sharing between chick/father dyads (black), and chick/non-father dyads (grey), using combined data for probes pV47-2 and 33.6 respectively. For probe 33.6/HaeIII profiles, the corresponding values were 0.869 ± 0.063 (chick/fathers dyads), and 0.763 ± 0.063 (chick/non father dyads). For two chicks, paternity was assigned based on high band sharing with the resident male (0.815, 0.870) and low band sharing with the only other non-resident male (0.308, 0.273), using values obtained with probe pV47-2. Furthermore, the two males also did not share adjacent territories. The paternities of two additional chicks were assigned solely according to territory isolation.

Therefore, combining the above criteria, I was able to assign paternity of 27 chicks (73%) to the resident male, and unable to resolve 10 others (27%).
Discussion

The results obtained in this study support the notion that island takahe are genetically monogamous. However, given the small sample size, these results cannot be taken as conclusive, nor would it be correct to assume that mating patterns will remain constant as island populations of takahe increase. At best, it can be concluded that if extra-pair fertilisations do occur, they are not likely to be frequent events. Unfortunately, the paternity of over a quarter of all chicks could not be resolved using DNA profiling with probes pV47-2 and 33.6, due to the low levels of minisatellite variation recorded. Towards the end of the study, an additional probe, \( \text{per} \), was tested and revealed promising levels of minisatellite variation, but due to a lack of time and resources this option could not be investigated further. For the time being, it would seem reasonable to assume that island takahe pedigrees constructed from observational records reflect the true relationships of breeders.

DNA profiling revealed that island takahe exhibit low levels of genetic variation, as reflected by minisatellite DNA. In order to place this result in perspective, it is worthwhile considering a recent review by Papangelou et al., (1998). Papangelou and co-workers reviewed DNA fingerprint variation in 70 avian species to obtain baseline band sharing data for outbreeding populations and small and/or inbred populations. Populations were classified as inbred and/or the product of strong genetic drift only when a second publication provided clear evidence of such, independent of its DNA fingerprinting results. Estimates for band sharing among first order relatives and unrelated individuals were obtained for both types of populations, yielding frequency distributions.

These frequency distributions provide a baseline against which band sharing in other bird species can be evaluated for the potential effects of inbreeding and genetic drift. Papangelou et al., (1998) concluded that, in general, obtaining mean band sharing values of 0.50 - 0.65 (or greater) for unrelated individuals from avian populations is indicative of a "genetically depauperate" population. In contrast, for outbreeding populations, mean band sharing values are significantly different (0.2 - 0.3). Band sharing among unrelated individuals from inbred
populations is similar to that reported for first order relatives from outbreeding populations.

When comparing band sharing values obtained for island takahe to those generated by Papangelou et al., (1998), it is evident that band sharing values for unrelated takahe fall within the small/inbred population category (Table 3). Island takahe are therefore genetically depauparate according to the classification generalisations proposed by Papangelou et al., (1998). Genetically depauparate populations may result from a number of processes acting either in combination or single-handedly. These include inbreeding, recent bottlenecks or founder effects, and influence of genetic drift due to small population sizes.

Given what is known about takahe, it is not surprising to find low DNA minisatellite variation. Subfossil evidence indicates that takahe were once widespread throughout New Zealand. Although debate over the exact habitat preferences of takahe is considerable (reviewed by Bunin and Jamieson, 1995), there is general agreement that numbers declined dramatically after the arrival of humans, and that takahe had become quite rare by the time of European colonisation (Bunin and Jamieson, 1995). Takahe are thought to have persisted in Fiordland because of the area’s remoteness from activities associated with human colonisation, such as deforestation, hunting and the introduction of mammalian predators (Bunin and Jamieson, 1995). Although this population has been protected since its discovery, the decline in numbers has continued to the present day.

The low levels of DNA minisatellite variation observed in island takahe profiles are potentially indicative of translocation-induced bottlenecks and/or persistence in a single small population over time. The ability to discriminate between these two possibilities will necessitate a comparison of genetic variation between island and Fiordland takahe. Such comparisons are ideally performed with some knowledge of the relationships between individuals. Commonly reported measures are band sharing between first-order relatives and unrelated individuals. Since monitoring of Fiordland takahe has been based on territories rather than individuals, such a comparison is not possible at present.
Table 3: Comparison of band sharing values for unrelated and first order relatives of island takae (Porphyrio mantelli) to those reported by Papangelou et al., (1998) for outbreeding populations and inbred/small populations of birds ($D =$ mean band sharing index).

<table>
<thead>
<tr>
<th></th>
<th>D unrelated</th>
<th>S.D.</th>
<th>D 1st order relatives</th>
<th>S.D.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Takahe P. mantelli</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PV47-2</td>
<td>0.545</td>
<td>0.143</td>
<td>0.704 - 0.812</td>
<td>-</td>
<td>Current study</td>
</tr>
<tr>
<td>33.6</td>
<td>0.774</td>
<td>0.073</td>
<td>0.838 - 0.869</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>combined</td>
<td>0.660</td>
<td>0.161</td>
<td>0.771 - 0.837</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Outbreeding</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>populations</td>
<td>0.243 *</td>
<td>0.092</td>
<td>0.603 *</td>
<td>0.062</td>
<td>Papangelou et al., 1998</td>
</tr>
<tr>
<td><strong>Small/inbred</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>populations</td>
<td>0.506 *</td>
<td>0.162</td>
<td>-</td>
<td>-</td>
<td>Papangelou et al., (1998)</td>
</tr>
</tbody>
</table>

*band sharing values were converted from mean average proportional difference (APD) scores to mean band sharing indexes ($D$) using the equation $APD = 1-D$ (Papangelou et al., 1998; Wetton et al., 1987).
An alternative approach consists of comparing DNA minisatellite variation from a random population sample of Fiordland takahē to band sharing values reported for unrelated island takahē in this study. A similar approach was used to derive the most likely origin of low levels of genetic variation in the Chatham Island black robin *Petroica traversi*, with one notable difference: similarly bottlenecked populations of the closely related New Zealand robins *P. australis* were used as control populations (Ardern and Lambert, 1997). Results from this study suggest that the extremely low levels of genetic variation observed in black robins are a consequence of the species’ persistence as a small single population over the last ~100 years, rather than the extreme bottleneck experienced in during the late 1970s, at which time just five individuals remained.

Island takahē and black robins are not the only New Zealand bird species for which low genetic variation has been reported. Others include the kakapo, South Island robin populations on Motuara island, Auckland Island teal, island populations of pukeko (Table 4), and Campbell Island teal (Dave Lambert, pers. com.). In the international literature, there are many more populations or species of birds for which low genetic variation has been reported (reviewed by Papangelou *et al.*, 1998). The million dollar question for such species or populations is “what are the consequences, if any, of low genetic variation?”

**Genetic variation: how much is enough?**

At present, there is little consensus over how much genetic variation a given population or species requires in order to ensure survival over the long term. Attempts to derive a theoretical, universally desirable level of genetic variation are essentially meaningless, as the relative importance of genetic factors and demographic ones varies between species according to the combined effects of a number of variables. These variables include past and present population size/structure, mating system (including inbreeding history), and current levels of genetic variation.

According to Hartley (1994), a risk analysis should be performed for New Zealand species for which genetic factors are thought to pose a risk to survival. This analysis should consist of the following components, in order of importance:
Table 4  Number of bands and bandsharing indices for pairwise comparisons of presumptive unrelated individuals belonging to a number of New Zealand avian species. All samples were digested with HaeIII restriction enzyme (reproduced with permission from Lambert and Millar, 1995).

<table>
<thead>
<tr>
<th>Species</th>
<th>Number sampled (combinations)</th>
<th>Minisatellite probe used</th>
<th>Mean number of bands scored</th>
<th>Mean bandsharing Index (D)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auckland Island teals (<em>Anas aucklandica aucklandica</em>)</td>
<td>16 (120)</td>
<td>33.15</td>
<td>11</td>
<td>0.66</td>
<td>Lambert, 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3'HVR</td>
<td>7</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>pV47-2</td>
<td>15</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Brown skua (<em>Catharacta longbergi</em>)</td>
<td>12</td>
<td>33.15</td>
<td>14</td>
<td>0.33</td>
<td>Millar et al., 1994a</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>33.6</td>
<td>10</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>South Polar skua (<em>Catharacta maccormicki</em>)</td>
<td>33</td>
<td>33.15</td>
<td>27</td>
<td>0.20</td>
<td>Millar et al., 1997</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>33.6</td>
<td>27</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>pV47-2</td>
<td>26</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Black Robins (<em>Petroica traversi</em>)</td>
<td>15</td>
<td>33.15</td>
<td>7</td>
<td>0.87</td>
<td>Holmes, 1994</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>33.6</td>
<td>9</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>pV47-2</td>
<td>3</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>South Island robins (<em>Petroica australis australis</em>) - Motuara Island</td>
<td>17</td>
<td>33.15</td>
<td>13</td>
<td>0.53</td>
<td>Holmes, 1994</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>33.6</td>
<td>10</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>pV47-2</td>
<td>8</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>North Island robins (<em>Petroica australis longipes</em>)</td>
<td>15 (27)</td>
<td>33.15</td>
<td>25</td>
<td>0.21</td>
<td>Holmes, 1994</td>
</tr>
<tr>
<td></td>
<td>15 (27)</td>
<td>33.6</td>
<td>10</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 (27)</td>
<td>pV47-2</td>
<td>35</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Blue ducks (<em>Hymenolaimus malacorhynchos</em>) between populations</td>
<td>- (55)</td>
<td>33.15</td>
<td></td>
<td>0.17-0.24</td>
<td>Triggs et al. 1992</td>
</tr>
<tr>
<td>within populations</td>
<td>- (63)</td>
<td>33.15</td>
<td></td>
<td>0.36-0.51</td>
<td></td>
</tr>
<tr>
<td>Pukeko (<em>Porphyrio porphyrio melanotus</em>)</td>
<td>17</td>
<td>pV47-2</td>
<td>18</td>
<td>0.6</td>
<td>Lambert et al. 1994</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>3'HVR</td>
<td>16</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>per</td>
<td>5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Adélie penguins (<em>Pygoscelis adeliae</em>) Northern Cape Bird colony, Ross Island, Antarctica</td>
<td>23 (179)</td>
<td>33.15</td>
<td>-</td>
<td>0.16</td>
<td>Monehan, 1994</td>
</tr>
<tr>
<td></td>
<td>23 (179)</td>
<td>33.6</td>
<td>-</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

Band sharing between pairs of individuals is calculated as: $D=2n_{AB}/(n_A + n_B)$ where $n_A$ and $n_B$ are the numbers of bands scored in individuals A and B respectively, and $n_{AB}$ is the number of shared bands (Wetton et al. 1987).
1) an evaluation of the risks associated with environmental stochasticity, 2) an identification of the factors that cause the decline of the population or species, with subsequent action to prevent further decline due to external factors, and 3) an evaluation of the risk posed by genetic factors.

Caughley and Gunn (1996) distilled the genetic variation dilemma down to three questions. Firstly, is the population of interest characterised by reduced variation? Secondly, if this is the case, is there any cause for concern? And lastly, can the population be managed in a way that minimises or mitigates the perceived impact of low genetic diversity? As stated previously, it may be futile to be concerned with maintaining adequate levels of genetic diversity when the short-term future of a population is under threat from non-genetic factors (Haig, 1998; Hartley, 1994).

The second question may be the hardest to answer, due to the difficulty in pinpointing a decline in “fitness” in a population over time. Fitness has many components, and their expression is confounded through environmental factors and stochastic fluctuations. Some inbred New Zealand bird species, such as kakapo and takahe, have low reproductive success. However, we simply do not know whether this is a result of environmental factors, or a consequence of low genetic variation manifesting itself in inbreeding depression. Although researchers have been quick to conclude that inbreeding depression is either absent from a population, or not an issue for various reasons, these claims are often made without detailed assessment of fitness traits (Wallis, 1994).

There is also some controversy concerning the appropriateness of using selectively neutral molecular markers for the assessment of genetic variation. Since minisatellite DNA is typically non-coding, variation levels may not necessarily be of functional significance (Ardern and Lambert, 1997; Wallis, 1994). As our knowledge of functional genomics increases, we may be able to assess genetic variation at appropriate loci in the future. Candidate loci are those involved in disease resistance (MHC, or major histocompatability complex loci; Hughes, 1991) and reproduction. Although progress is being made with regard to the former, these techniques have yet to be applied to molecular studies of New Zealand birds (Wallis, 1994).
In summary, we cannot conclude that low genetic variation *per se*, as reflected by minisatellite DNA, is a cause of concern for island populations of takahe. Further research is needed to determine whether or not genetic variation is declining in takahe (by comparing levels of genetic variation in Fiordland and island populations), and secondly, by investigating appropriate fitness traits (see Chapter Two).
Literature cited


Appendix One

Takahe management and allowable takahe movement (reproduced from Crouchley, 1994).
Appendix Two

Details of takahe included in the paternity analysis (* = takahe samples collected on previous occasions).

<table>
<thead>
<tr>
<th>Bird</th>
<th>Band no.</th>
<th>Sex</th>
<th>Age</th>
<th>Sire</th>
<th>Dam</th>
<th>Island</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adot</td>
<td>31878</td>
<td>F</td>
<td>1</td>
<td>Stormy</td>
<td>JJ</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Ahikiaea</td>
<td></td>
<td>F</td>
<td>1</td>
<td>Greg</td>
<td>Pounamu</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Albert*</td>
<td>31873</td>
<td>M</td>
<td>13</td>
<td>B33M</td>
<td>B33F</td>
<td>Maud</td>
</tr>
<tr>
<td>Alec*</td>
<td></td>
<td>M</td>
<td></td>
<td>D13M</td>
<td>D13F</td>
<td>Mana</td>
</tr>
<tr>
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<td></td>
<td>F</td>
<td>1</td>
<td>Lucky</td>
<td>Toni/Tilly</td>
<td>Mana</td>
</tr>
<tr>
<td>Aroha</td>
<td>34990</td>
<td>F</td>
<td>6</td>
<td>Stormy</td>
<td>JJ</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Beaker</td>
<td>32917</td>
<td>F</td>
<td>3</td>
<td>Taku</td>
<td>Squeak</td>
<td>Kapiti</td>
</tr>
<tr>
<td>Benzi</td>
<td>32914</td>
<td>F</td>
<td>4</td>
<td>Emie</td>
<td>Terri</td>
<td>Mana</td>
</tr>
<tr>
<td>Bunchy</td>
<td>32929</td>
<td>F</td>
<td>2</td>
<td>Selwyn</td>
<td>Victoria</td>
<td>Mana</td>
</tr>
<tr>
<td>Eric</td>
<td>34945</td>
<td>M</td>
<td>4</td>
<td>Albert</td>
<td>Maud</td>
<td>Maud</td>
</tr>
<tr>
<td>Ernie*</td>
<td>36063</td>
<td>M</td>
<td>10</td>
<td>Mr Black</td>
<td>Mrs Black</td>
<td>Mana</td>
</tr>
<tr>
<td>Glencoe</td>
<td>32500</td>
<td>M</td>
<td>3</td>
<td>Stormy</td>
<td>JJ</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Greg</td>
<td>34973</td>
<td>M</td>
<td>6</td>
<td>C35M</td>
<td>C35F</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Henry/jetta</td>
<td>n/a</td>
<td></td>
<td></td>
<td></td>
<td>Emie</td>
<td>Mana</td>
</tr>
<tr>
<td>Hilda</td>
<td></td>
<td>F</td>
<td>2</td>
<td>Albert</td>
<td>Maud</td>
<td>Maud</td>
</tr>
<tr>
<td>Iti</td>
<td>34281</td>
<td>F</td>
<td>9</td>
<td>Taku</td>
<td>Squeak</td>
<td>Kapiti</td>
</tr>
<tr>
<td>JJ</td>
<td>36066</td>
<td>F</td>
<td>8</td>
<td>Becka</td>
<td>Maud</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Kahurangi</td>
<td>32526</td>
<td>M</td>
<td>3</td>
<td>Emie</td>
<td>Terri</td>
<td>Mana</td>
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<td>4</td>
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<td>Tiritiri</td>
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<td>4</td>
<td>Stormy</td>
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<td>Tiritiri</td>
</tr>
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<td>Kris</td>
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<td>F</td>
<td>4</td>
<td>Emie</td>
<td>Terri</td>
<td>Mana</td>
</tr>
<tr>
<td>Kristin</td>
<td>32499</td>
<td>n/a</td>
<td>3</td>
<td>Greg</td>
<td>Pounamu</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Lucky</td>
<td>32911</td>
<td>M</td>
<td>5</td>
<td>Emie</td>
<td>Iti</td>
<td>Mana</td>
</tr>
<tr>
<td>Manawanui</td>
<td></td>
<td>F</td>
<td>2</td>
<td>Stormy</td>
<td>JJ</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Manuiti</td>
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</tr>
<tr>
<td>Maud*</td>
<td>31863</td>
<td>F</td>
<td>14</td>
<td>E43M</td>
<td>E34F</td>
<td>Maud</td>
</tr>
<tr>
<td>Mowhai</td>
<td>32930</td>
<td>m</td>
<td>2</td>
<td>Lucky</td>
<td>Toni/Tilly</td>
<td>Mana</td>
</tr>
<tr>
<td>Mr Black*</td>
<td>31883</td>
<td>M</td>
<td>15</td>
<td>D34M</td>
<td>D34F</td>
<td>Maud</td>
</tr>
<tr>
<td>Mr Blue*</td>
<td>31865</td>
<td>M</td>
<td></td>
<td>C27M</td>
<td>C27F</td>
<td>Tiritiri</td>
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<tr>
<td>Mrs Black*</td>
<td>31885</td>
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<td>15</td>
<td>D12M</td>
<td>R28412</td>
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<tr>
<td>Mrs White*</td>
<td>31886</td>
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<td></td>
<td>F19M</td>
<td>F19F</td>
<td>Maud</td>
</tr>
<tr>
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<td></td>
<td>F</td>
<td>2</td>
<td>Kaitiaki</td>
<td>Aroha</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Pounamu</td>
<td>34991</td>
<td>F</td>
<td>6</td>
<td>Green</td>
<td>Yellow</td>
<td>Tiritiri</td>
</tr>
<tr>
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<td>32293</td>
<td>F</td>
<td>3</td>
<td>Lucky</td>
<td>Toni/Tilly</td>
<td>Mana</td>
</tr>
<tr>
<td>Rangi</td>
<td>32919</td>
<td>M</td>
<td>2</td>
<td>Taku</td>
<td>Squeak</td>
<td>Kapiti</td>
</tr>
<tr>
<td>Robin*</td>
<td>34290</td>
<td>M</td>
<td>6</td>
<td>Taku</td>
<td>Squeak</td>
<td>Maud</td>
</tr>
<tr>
<td>Selwyn*</td>
<td>34942</td>
<td>M</td>
<td>5</td>
<td>Mr Black</td>
<td>Mrs Black</td>
<td>Mana</td>
</tr>
<tr>
<td>Shy*</td>
<td>34328</td>
<td>F</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sneatch</td>
<td></td>
<td>M</td>
<td></td>
<td>Mr Black</td>
<td>Mrs Black</td>
<td>Maud</td>
</tr>
<tr>
<td>Squeak</td>
<td>31879</td>
<td>F</td>
<td>12</td>
<td>Taku</td>
<td>Squeak</td>
<td>Kapiti</td>
</tr>
<tr>
<td>Stormy*</td>
<td>34329</td>
<td>M</td>
<td>9</td>
<td>Pipi</td>
<td>Mrs White</td>
<td>Tiritiri</td>
</tr>
<tr>
<td>Tahi</td>
<td></td>
<td>M</td>
<td></td>
<td></td>
<td>Albert</td>
<td>Maud</td>
</tr>
<tr>
<td>Taku</td>
<td>34259</td>
<td>M</td>
<td>14</td>
<td>T77001</td>
<td>Red Left</td>
<td>Kapiti</td>
</tr>
<tr>
<td>Teebee</td>
<td>32924</td>
<td>F</td>
<td>3</td>
<td>Emie</td>
<td>Terri</td>
<td>Mana</td>
</tr>
<tr>
<td>Terri</td>
<td>34289</td>
<td>F</td>
<td>9</td>
<td>Ralph</td>
<td>Betty</td>
<td>Mana</td>
</tr>
<tr>
<td>Tilly</td>
<td>36068</td>
<td>F</td>
<td>6</td>
<td>Albert</td>
<td>MrsWhite</td>
<td>Mana</td>
</tr>
<tr>
<td>Titahi</td>
<td>32927</td>
<td>F</td>
<td>2</td>
<td>Emie</td>
<td>Terri</td>
<td>Mana</td>
</tr>
<tr>
<td>Toni*</td>
<td>32322</td>
<td>F</td>
<td>7</td>
<td>Taku</td>
<td>Squeak</td>
<td>Mana</td>
</tr>
<tr>
<td>unnamed</td>
<td></td>
<td>F</td>
<td>1</td>
<td>Taku</td>
<td>Squeak</td>
<td>Kapiti</td>
</tr>
<tr>
<td>Victoria*</td>
<td>34285</td>
<td>F</td>
<td>7</td>
<td>Emie</td>
<td>Iti</td>
<td>Mana</td>
</tr>
<tr>
<td>Whakame</td>
<td>32496</td>
<td>M</td>
<td>4</td>
<td>Greg</td>
<td>Pounamu</td>
<td>Tiritiri</td>
</tr>
</tbody>
</table>
Appendix Three
Island takahe pedigrees constructed from records held by the Department of Conservation, Te Anau

Tiritiri Matangi Island Takahe Pedigree

<table>
<thead>
<tr>
<th>Breeding Season</th>
<th>91/92</th>
<th>92/93</th>
<th>93/94</th>
<th>94/95</th>
<th>95/96</th>
<th>96/97</th>
<th>97/98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key:
- Female
- Male
- Sample collected/provided utility
- Died
- Sex unknown
Mana Island Takahe Pedigree

Mana Island
Founded 1988

Breeding Season 88/89
89/90
90/91
91/92
92/93
93/94
94/95
95/96
96/97
97/98

Key

- female
- male
- sex unknown
- sample collected previously
- died

From Maud
Selwyn
Bunchy

From Kapiti
Tyler
Xana

From Maud
T2

From Maud
From Kapiti
Tatu

From Kapiti
Toro

From Maud
Mowha

From Maud
Amazon

From Maud
Tilly

From Kapiti
Lucky

From Kapiti
Tenise

From Kapiti
Benz

From Kapiti
Tahnee

From Kapiti
Kahurangi

33
Kapiti Island Takahe Pedigree

Kapiti Island Founded 89/90

89/90
- Terri
  - To Mana

90/91
- Ili
  - Bubble

91/92
- Tiri
  - To Mana

92/93
- To Maud
  - Mana
  - To Mana

93/94
- From Burwood
  - Papa
    - Mate for Rangi

94/95
- Kaitiaki
  - To Tiri

95/96
- Beaker
  - Rangi
  - fostered to Ili & Lyall

96/97
- Taku
  - Squeak

97/98
- From Maud
  - Emie
Mating With Your Relatives: Does Inbreeding Affect the Reproductive Success of Island Takahe (*Porphyrio mantelli*)?

Abstract

Pedigree records were used to evaluate the reproductive (hatching and fledging) success of pairs of island takahe over a seven year period. Hatching and fledging success were modelled separately using binomial general linear models. Predictors examined in these models included variation among years, pairs, and individuals, whether or not individuals were inbred, and pairs related. Pairs in which the female was inbred had significantly lower hatching success. Fledging success was also lower for pairs with inbred females, but the effect was not significant, possibly due to small sample size. There was no significant difference in reproductive success between related and unrelated pairs. These results have implications for management of takahe breeding on islands.

Introduction

Inbreeding (mating between relatives) is a phenomenon of fundamental concern to conservation biologists for several reasons. In many animal populations, inbreeding has adverse affects on fitness-related traits, including viability, growth rate, disease resistance, physiological efficiency, mating ability, and fecundity (Lacy, 1997; Caughley and Gunn, 1996). Collectively, such effects are known as inbreeding depression. Lowered fecundity and survival of inbred individuals can decrease a populations’ growth rate, with an associated increase in the likelihood of extinction. In addition, populations may lose their capacity to persist in changing environments (Lacy, 1997).

Inbreeding depression has been documented in a wide range of plant and animal populations (reviewed by Thornthill, 1993), and is often an unavoidable consequence of small population size (Keller, 1998; Lande, 1988; Soule, 1986). Most studies on the effect of inbreeding have been based on captive populations (reviewed by Ralls *et al.*, 1988). Results of these studies have been used to estimate the extent to which wild populations are affected by inbreeding. Since captive populations are not subjected to many of the
causes of mortality that their wild counterparts experience, such as variations in environmental conditions, predation, and disease epidemics, the validity of such extrapolations is debatable (Jimenez et al., 1994). Clearly, more studies on the impacts of inbreeding in natural populations are necessary (Ardern and Lambert, 1997; Lacy, 1997).

Unfortunately, the effects of inbreeding in natural populations are difficult to document, as this necessitates long-term studies of individually marked animals (Keller, 1998). Natural vertebrate populations for which inbreeding depression has been documented include several species of passerine birds (Keller, 1998; Kempenaers et al., 1996; Bensch et al., 1994; van Noorwijk and Scharloo, 1981; Greenwood et al., 1978; Bulmer, 1973), two baboon species (Alberts and Altmann, 1995; Packer, 1979), golden lion tamarin (Dietz and Baker, 1993), and the common shrew (Stockley et al., 1993). Traits commonly affected include measures of reproductive success (primarily hatching success in birds) and survival to various developmental stages (weaning/fledging, maturity).

Other approaches to the study of inbreeding in natural populations include the experimental manipulation of mating systems and the choice of a non-vertebrate model organism with high fecundity and/or a short life span. Recently, Saccheri et al., (1998), provided the first direct evidence that inbreeding can lead to extinction, using a wild butterfly (Melitaea cinxia) metapopulation. In their analysis, inbreeding was found to account for 26% of the variation in extinction rate among different populations.

However, the picture is by no means clear. First of all, there are a number of studies for which inbreeding depression was not found for any of the traits examined (Grant and Grant, 1995; Hoogland, 1992; Gibbs and Grant, 1989; Bulger and Hamilton, 1988). Secondly, it has been argued that in some cases inbreeding may be beneficial to future population viability by increasing the rate at which deleterious recessive mutations are removed from the gene pool (Lacy, 1997). This "purging" process may be controlled by manipulation of mating regimes in captive populations (Fu et al., 1998; Wang, 1997a; Wang, 1997b; Backus et al., 1995; Spielman and Frankham, 1992). This notion is largely theoretical, and has been criticised as being risky, of limited use, and lacking in practical application (Ballou, 1997). Thirdly, there is mounting evidence that the degree to which inbreeding depression becomes manifest in a population is in part, environmentally dependent (Keller et al., 1994; Pray et al., 1984). Lastly, there may be species-specific
differences in the response to inbreeding (Ralls et al., 1988; Wayne et al., 1991). Birds are particularly interesting in this regard, as they collectively utilise a variety of mating systems and are relatively well studied with respect to inbreeding.

**Inbreeding depression in avian species**

Studies of wild populations of birds show that the most common trait affected by inbreeding depression is hatching success (Table 1). Effects on survival have also been reported, but are more difficult to interpret, as not all studies examine these, and the way in which survival is categorised varies between studies. Not all species examined demonstrate a reduction in the fitness of the examined traits in response to inbreeding. This could reflect historical differences in size and structure (Keller et al., 1994). In addition, it appears that some species are able to avoid inbreeding (Wheelwright and Mauck, 1998), while others are able to compensate for any negative effects by virtue of high fecundity relative to survival (Keller and Arcese, 1998). Means whereby inbreeding avoidance may be achieved include mate choice, dispersal, and the suppression of reproduction in offspring in the presence of related adults.

Among New Zealand birds, inbreeding has been reported for a number of species, most notably pukeko (Porphyrio porphyrio; Craig and Jamieson, 1988), blue duck (Hymenolaimus malacorhynchos; Triggs et al., 1992), yellow-eyed penguin (Megadyptes antipodes; Richdale, 1957), and the Chathams Island black robin (Petroica traversi; Ardern and Lambert, 1997). New Zealand birds have a history of inbreeding as a consequence of persisting as small, relict populations. The effects of inbreeding may therefore be less extreme due to populations having being purged of deleterious alleles over time (Craig, 1991). None of the studies on New Zealand birds have reported a significant degree of inbreeding depression, however, the appropriate comparisons between inbred and outbred populations, or related and unrelated birds were not carried out.

**Reproductive success of Fiordland and island takahe**

Takahe were presumed to be extinct until 1948, at which time a remnant population of ~250 birds was discovered in the Murchison Mountains, Fiordland. During the 1970s, monitoring revealed that the population was declining, prompting the implementation of a number of management strategies, including egg manipulation, control of introduced competitors (deer), stoat control, the establishment of a captive rearing unit,
<table>
<thead>
<tr>
<th>Species</th>
<th>Traits affected by inbreeding depression</th>
<th>Incidence of inbreeding</th>
<th>Number of breeding attempts</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue tit <em>Parus caeruleus</em></td>
<td>hatching success</td>
<td>rare (&lt;1%)</td>
<td>469</td>
<td>Kempenears et al., 1996</td>
</tr>
<tr>
<td>Great reed warbler</td>
<td>hatching success</td>
<td>rare</td>
<td>104</td>
<td>Bensch et al., 1994</td>
</tr>
<tr>
<td><em>Acrocephalus arundinaceus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Great tit <em>Parus major</em></td>
<td>recruitment</td>
<td>rare (1.8%)</td>
<td>397</td>
<td>Bulmer, 1973</td>
</tr>
<tr>
<td></td>
<td>hatching and fledging success</td>
<td>rare (&lt;2%)</td>
<td>885</td>
<td>Greenwood et al., 1978</td>
</tr>
<tr>
<td></td>
<td>hatching success with compensatory recruitment</td>
<td>variable between sites (5.47%)</td>
<td>&gt;1000</td>
<td>Van Noordwijk and Scharloo, 1981</td>
</tr>
<tr>
<td>Mexican jay <em>Aphelocoma ultramarina</em></td>
<td>brood size (possibly due to hatching failure)</td>
<td>rare (4.8%)</td>
<td>657</td>
<td>Brown and Brown, 1998</td>
</tr>
<tr>
<td></td>
<td>juvenile survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Song sparrow</td>
<td>juvenile survival</td>
<td>not available</td>
<td>1574</td>
<td>Keller et al., 1994</td>
</tr>
<tr>
<td>Moorhen <em>Gallinula chloropus</em></td>
<td>juvenile survival</td>
<td>rare for pairs (1.8%)</td>
<td>162</td>
<td>McRae, 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>majority of polygynous groups</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>
and translocations of small numbers of captive-reared birds to predator-free islands (Crouchley, 1994). At present, the remnant Fiordland population numbers approximately 120 birds (Jamieson and Ryan, 1999). Numbers appear to be limited by harsh alpine climatic conditions and introduced mammalian predators (Bunin and Jamieson, 1995; Maxwell and Jamieson, 1997; Jamieson and Ryan, 1999). Takahe are classified as a "Category A" (highest priority for conservation) species by the New Zealand Department of Conservation (Molloy, 1994).

The first translocations of takahe to islands took place during the mid 80s, at which time nine juveniles were transferred to Maud Island in the Marlborough Sounds. Between 1987 and 1993, an additional 15 birds were released on Mana, Kapiti, and Tiritiri Matangi Islands. Island populations are slowly increasing, with a current total of ~56 adult birds spanning four generations. Takahe are long lived and have low reproductive rates. Individuals typically start breeding from the age of two years, and produce clutches of 1-3 eggs, most often two. In a single season, a pair of takahe can produce up to two replacement clutches, but rarely raise more than one chick.

Although island takahe produce more eggs per pair than Fiordland takahe, a lower proportion of these hatch, and fewer juveniles per egg are produced (Table 2; Jamieson and Ryan, 1999; Bunin et al., 1997). These differences are not immediately obvious, as survival rates of independent juveniles and adults on islands exceed those of Fiordland equivalents, due to the absence of predators and a milder climate (Jamieson and Ryan, 1999). Mean annual adult survivorship of island takahe has been estimated to be almost 95%, while that of Fiordland birds ranges from 73% to 97% depending on year and location (Bunin et al., 1997).

A variety of hypotheses to explain the comparatively low hatching success of takahe on island have been proposed (Bunin et al., 1997; Jamieson and Ryan, 1999). Briefly, these include nutritional deficits, the use of "poor quality" founders, effects of transfer on individual birds, inbreeding, and abnormal egg shell morphology and development arising from differential rates of water loss caused by altitude differences (sea level vs 600+ meters).

Support for several of these hypothesis (nutritional deficits, transfer effects, egg shell
Table 2  Comparison of reproductive success (mean ± s.d) of island and Fiordland takahe (including captive-reared eggs) analysed by combining all breeding pairs across years (pair-years) or by averaging individual pairs across years (pairs). Adapted with permission from Bunin et al., (1997).

<table>
<thead>
<tr>
<th></th>
<th>Island</th>
<th>S.D.</th>
<th>Fiordland</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>By pair-years</strong></td>
<td></td>
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</tr>
<tr>
<td>eggs per year</td>
<td>3.5</td>
<td>1.5</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>chicks per egg</td>
<td>0.30</td>
<td>0.31</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>juveniles per egg</td>
<td>0.18</td>
<td>0.19</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>By pairs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eggs per year</td>
<td>3.4</td>
<td>1.5</td>
<td>1.9</td>
<td>0.5</td>
</tr>
<tr>
<td>chicks per egg</td>
<td>0.26</td>
<td>0.29</td>
<td>0.61</td>
<td>0.37</td>
</tr>
<tr>
<td>juveniles per egg</td>
<td>0.15</td>
<td>0.19</td>
<td>0.37</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*all differences were statistically significant when compared using ANOVA (p<0.05).

morphology) has not been found (reviewed by Jamieson and Ryan, 1999). Similarly, a detailed analysis of reproductive success of island takahe (Jamieson and Ryan, 1999) failed to reveal a relationship between whether or not paired birds were related, and egg infertility (proportion of infertile eggs laid) and productivity (number of juveniles produced). High egg infertility appears to be characteristic of all island takahe, whether breeders were related or not, and did not significantly vary between years or islands. The only significant finding to emerge from this analysis was that fertility rates improved with re-nesting and the laying of a second clutch within a season.

Overall, the reproductive success of island takahe did not improve over the period of the study (1991-1997). The high inbreeding coefficient and consistently high infertility rates have led Jamieson and Ryan (1999) to conclude that island takahe are suffering from environmentally induced inbreeding depression as a consequence of translocating individuals from an inbred population to a markedly different habitat (i.e. from alpine tussock habitat to temperate lowland islands with pasture grasses).

However, the above study focused only on the potential relationship between inbreeding and egg fertility. This may have led Jamieson and Ryan (1999) to underestimate the effects of inbreeding on fitness, since other studies have shown that
later stages in the avian reproductive cycle, especially hatching success, may be affected by inbreeding (Table 1). There is therefore a need to re-evaluate takahe productivity data.

Objectives of this study
The aim of this study is to determine if, and to what extent, takahe breeding on islands are affected by inbreeding depression. Hatching success (proportion of eggs that hatch) and fledging success (proportion of hatched eggs that produce juveniles) will be compared between:

• pairs of related and unrelated takahe
• pairs containing inbred females and pairs that do not
• pairs containing inbred males and pairs that do not
• pairs with high and low genetic similarity, using data obtained from multilocus DNA fingerprinting (Chapter One).

If a relationship between inbreeding and reproductive success is evident, management options formulated to mitigate the problem will be identified and discussed.
Methods

Reproductive Success

Reproductive success was calculated from data collected by the Department of Conservation. Each year, nests are located and inspected several times, during which eggs are candled, and infertile (i.e. non-developing) or addled eggs removed. Nests are also checked at the end of the incubation period to record how many eggs hatched. Chicks are considered fledged if they survive 4 or more weeks. Nesting birds are identified by unique combinations of coloured leg bands. Juveniles are banded at approximately four to six months of age, at which time they receive an aluminium band in addition to up to three coloured bands.

Although takahe have bred on islands since 1986, only data for the time period 1991-1997 was included in the analysis, as records prior to 1991 were incomplete. Two measures of reproductive success were examined: the proportion of eggs that hatched ("hatching success"), and the proportion of hatched eggs that produced juveniles ("fledging success"). Data from multiple clutches was combined into an annual average (i.e. = 1 pair-year/breeding attempt).

Inbreeding estimates

The level of inbreeding in a population is typically measured by calculation of $F$, the coefficient of inbreeding (Wright, 1977). Briefly, $F$ represents the probability that two alleles at a locus in an individual are identical by descent (IBD). $F$ may be calculated for individual breeders or for any offspring produced by a particular pair. The two measures are related in that the inbreeding coefficient for a pair is identical to the inbreeding coefficient of any resulting offspring (Keller, 1998).

Inbreeding coefficients for island takahe were calculated from pedigree data, using the computer program "SPARKS". Pedigrees were constructed by Department of Conservation staff from observations of established territories and pair bonding, and are likely to be correct given the lack of extra pair paternity revealed by DNA fingerprinting (Chapter One). For most takahe born on islands, pedigrees extend to at least grandparents. Pedigree information for founders is less extensive, extending to parents or territories in Fiordland only. Pedigree depth is an important consideration when
investigating inbreeding effects, as “shallow” or incomplete pedigrees can result in an underestimation of inbreeding (Keller, 1998). Researchers often guard against this potential bias by performing inbreeding analyses with only those pairs for which all grandparents are known, or alternatively, perform the analysis with and without pairs with shallow pedigrees.

Pairs were considered to be inbred if they were related, i.e. share a common detectable ancestor, and individuals were considered inbred if their parents were related. Pairs that did not share a common detectable ancestor (“unrelated” pairs) were given an inbreeding coefficient of zero. Therefore, offspring resulting from unrelated pairs also inherited an inbreeding coefficient of zero. To aid clarity, pairs that are inbred will be referred to as “related”, and inbred individuals as “inbred”.

**Statistical Analysis**

Hatching and fledging success were modelled separately using General Linear Models (GLM, binomial family) in S-Plus. Binomial GLMs are commonly applied to logistic regression problems, such as this one, where the aim is to predict the probability of an event occurring as a function of a set of predictors (MathSoft, Inc. 1995). This procedure was deemed appropriate since hatching and fledging success (response variables) are variables with distributions that do not conform to normality. Each pair-year represent one trial (= one replicate) made up of a number of “successes” and “failures” (i.e. proportions), indicating whether or not an event has occurred (eggs either hatched or failed to hatch, and hatched eggs either produced juveniles or did not).

Based on knowledge of takahē reproductive ecology and literature on inbreeding depression in birds, the following factors (“predictors”) were identified as having potential effects on takahē reproductive success: year, pair, male, female, breeding experience (first year or >first year), whether pairs were related (“inbred pair”), and whether individuals in each pair were inbred (“inbred female”, and “inbred male”). Since pairs were either related or unrelated, and individuals were inbred or not inbred, data was coded as “yes” or “no” for these factors.

Since some of the predictor variables were related (and therefore non-independent), it was not statistically valid to combine all factors into a single model. To illustrate this
problem, consider the relationship between "female" and "inbred female". All the variance in the response associated with "inbred female" is effectively a subset of the variance due to "female". Such correlations between predictors affect the order in which they are removed from a model during stepwise (backward stepping) procedures. In this case, the predictor of interest ("inbred female") will be masked by "female", leading to elimination from the model. The same is true for "inbred pair" and "inbred male". In order to avoid this problem, the approach outlined in Figure 1 was taken.

During step one, the variables that were not of direct interest were explored in order to determine their relative influence on hatching and fledging success. It was anticipated that "pair" and "year" would not be effective predictors, based on previous analyses of takahe productivity data, in which only breeding experience was significant (Jamieson and Ryan, 1999). If "pair", "female" or "male" remained in the model during model selection (Step Two), the corresponding coefficients were examined for inbreeding effects, i.e. do unrelated pairs/non-inbred individuals have greater coefficients than related pairs/inbred individuals? If this was found to be the case, it was assumed that the exclusion of "pair", "female" and "male" was valid for subsequent modelling with inbreeding predictors (figure 1, Step Four).

**Reproductive success and genetic similarity**

Measures of "genetic similarity" between pairs (i.e. band sharing values from multilocus DNA profiles obtained in chapter one) were examined in relation to inbreeding coefficients and average reproductive success for 13 takahe pairs. This was done for two reasons: 1) to test for the positive (theoretical) relationship between band sharing and relatedness (inbreeding coefficients), and 2) to determine whether or not genetic similarity can be used as an indicator of reproductive success, since Bensch et al., (1994) found that hatching success declined in pairs of genetically similar great reed warblers. A relationship between high band sharing and reproductive failure was also documented for endangered Puerto Rican parrots (Brock and White, 1992).

Genetic similarity was not included as a predictor variable in the GLM models generated for hatching and fledging success, as it is theoretically correlated with
Figure 1  Statistical procedure used to model hatching (H) and fledging (F) success of island takahē

Step One
Model H independent of inbreeding predictors to determine the relative importance of “year”, “pair”, “female”, “male”, and breeding experience (“exp”), i.e.,

\[ H = \text{year} + \text{pair} + \text{female} + \text{male} + \text{exp} \]

Step Two
Perform stepping procedure to generate a series of models for H and select “best” model (‘model I”), according to AIC score.

Step Three
Construct a new model (“model II”), combining model I with inbreeding predictors, i.e.,

\[ H = \text{predictors in model I} + \text{inbred pair} + \text{inbred female} + \text{inbred male} \]

Step Four
Perform stepping procedure to generate a series of models for H and select “best” model (‘model II”), according to AIC score.

Step Five
Use model II to predict H

Step Six
Repeat steps one to five for F

inbreeding, and band sharing data was only available for a 13 out of 37 takahē pairs. Band sharing values obtained with probe pV47-2 were used (chapter one).

Comparisons were made in two ways. The first method involved simple correlations between average hatching and fledging success, inbreeding coefficients, and band sharing values for each pair. The second method consisted of dividing all pair-years for which band sharing data was available into “low genetic similarity” (band sharing < 0.6) or “high genetic similarity” (band sharing > 0.6), and comparing relative measures of
hatching and fledging success. For the purpose of these analyses, the four individual island populations were treated as a single, large population.
Results

Reproductive trends and frequency of inbreeding
In the time period 1991-1997, a total of 101 breeding attempts (pair-years) from 37 pairs (combinations of 26 males and 25 females) were observed. In total, 316 eggs were laid, of which 105 hatched, and 55 produced juveniles. Most individuals (82.3%) were represented in more than one pair-year, with either one (57%), two (39%), three (2%), or four (2%) different partners. Almost half the males (46%) and females (44%) have never been in pairings which produced a juvenile (Figure 2).

![Graph showing number of male and female takahe involved in successful pair-years](image)

Figure 2  Number of male and female takahe involved in successful pair-years (n = 43), i.e. pair-years which resulted in the production of (at least) one juvenile

Almost a third (30.7%) of breeding attempts were made by related pairs (n = 13), with the remainder (69.3%) being made up by pairs which were not related (n = 24). Seven individual birds (5 females and 2 males) were inbred themselves, representing approximately 14% of the total number of birds included in the analysis. It should be noted that this is an underestimate of the current number of inbred birds on islands, as the analysis does not include "non-breeders" - birds which had not reached breeding
age by the 1997/1998 breeding season (23 takahe, of which approximately half (3 males and 9 females) are inbred).

The degree of kinship for related pairs ranged from full-sibs to half-1st cousins twice removed (Table 3). Pedigrees tended to be complex, with some ancestors represented a number of times over the depth of the pedigree. The average inbreeding coefficient for the 37 takahe pairs in the analysis was 0.0418. For individuals, the average F coefficient was 0.0233. This number will increase as non-breeders are recruited into the breeding population, and as a function of pedigree depth (Table 4).

Table 3 Summary of relationships between inbred island takahe pairs

<table>
<thead>
<tr>
<th>Pair (male + female)</th>
<th>Relationship of male to female</th>
<th>Inbreeding coefficient</th>
<th>Pedigree relatedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whetu + Aroha</td>
<td>full-brother</td>
<td>0.3437</td>
<td>0.5</td>
</tr>
<tr>
<td>Tussock + Rima</td>
<td>father</td>
<td>0.3125</td>
<td>0.5</td>
</tr>
<tr>
<td>Selwyn + Victoria</td>
<td>full-uncle</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>T2 + Rima</td>
<td>half-uncle</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Vick and Hinewai</td>
<td>full-nephew</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Stormy + JJ</td>
<td>full 1st cousin</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Tuarua + Redleft</td>
<td>full-grandson</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Lucky + Rima</td>
<td>half-nephew</td>
<td>0.0781</td>
<td>0.125</td>
</tr>
<tr>
<td>Bubble + Terri</td>
<td>full-1st cousin</td>
<td>0.0625</td>
<td>0.125</td>
</tr>
<tr>
<td>Ernie + Terri</td>
<td>half-1st cousin</td>
<td>0.0312</td>
<td>0.0625</td>
</tr>
<tr>
<td>Chester + Heidi</td>
<td>half-1st cousin once removed</td>
<td>0.0312</td>
<td>0.125</td>
</tr>
<tr>
<td>T2 + Puffin</td>
<td>half-1st cousin twice removed</td>
<td>0.0312</td>
<td>0.0625</td>
</tr>
<tr>
<td>Thumper + JJ</td>
<td>half-1st cousin once removed</td>
<td>0.0312</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Note: Inbreeding coefficients do not always match pedigree relatedness values. Inbreeding coefficients more accurately reflect relatedness between pairs, as all common ancestors over the depth of the pedigree are taken into account.

Hatching and fledging success

The proportion of hatched eggs averaged across all pair-years was 0.326 ± 0.031 (S.E., n = 101). For eggs that hatched, the mean proportion that produced a fledgling was 0.363 ± 0.055 (n = 64 pair-years). Mean reproductive success for related and unrelated pair-years was similar (Figure 3 A,B; statistical analysis to follow). Both hatching and
Figure 3  Comparisons of:
A  hatching success for related and unrelated pair-years
B  fledging success for related and unrelated pair-years
C  hatching success for pair-years with inbred females and non-inbred females
D  fledging success for pair-years with inbred females and non-inbred females
E  hatching success for pair-years with inbred males and non-inbred males
F  fledging success for pair-years with inbred males and non-inbred males
Note  error bars represent standard errors
Table 4  Average inbreeding coefficients (F) for takahe breeding on islands.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>All birds included in analysis</td>
<td>51</td>
<td>0.0233</td>
</tr>
<tr>
<td>All birds included in analysis with known grandparents</td>
<td>33</td>
<td>0.0360</td>
</tr>
<tr>
<td>All birds in analysis plus non-breeders *</td>
<td>74</td>
<td>0.0351</td>
</tr>
<tr>
<td>All birds in analysis with known grandparents plus non-breeders *</td>
<td>56</td>
<td>0.0463</td>
</tr>
</tbody>
</table>

* non-breeders were individuals which had not reached reproductive maturity by the 1997/1998 breeding season

Fledging success were lower for pair-years with inbred females than for pair-years with non-inbred females (Figure 3 C,D). This trend was not evident for pair-years with inbred males (Figure 3 E,F).

Pair-years from inbred females (n = 13) were classified further according to whether or not those females paired up with males they were related to. This was done to see whether there was further reduction in hatching success arising from females being related to their mates in addition to being inbred. Although hatching success was reduced further for pair-years where this was the case (0.094 ± 0.023, compared with 0.125 ± 0.035 for pair-years with inbred females which were unrelated to their mates), the sample sizes were too small to permit a meaningful inference to be drawn (n = 6 and n = 7, respectively).

Hatching success was most accurately predicted by whether or not the female in a pair was inbred (Table 5). During the first modelling step (Model I), “year”, “exp”, “pair”, and “male” were identified as being the best predictors. A close examination of male coefficients revealed large variation. This partly reflects individual variation in hatching success, but was exacerbated by a lack of replication at the individual level (i.e. cases where males only bred once). This was typical of female and pair coefficients also. Predicted mean hatching success for pair-years with inbred females (0.143 ± 0.058) was significantly lower than predicted mean fledging success for pair-years for non-inbred females (0.361 ± 0.031; t = -2.68).
Table 5  Summary of GLM binomial models generated for hatching success of island takahe

<table>
<thead>
<tr>
<th>Hatching success</th>
<th>Model</th>
<th>AIC score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model I</td>
<td>year+exp+male</td>
<td>166.60</td>
</tr>
<tr>
<td></td>
<td>year+exp+male+pair+female</td>
<td>154.90</td>
</tr>
<tr>
<td></td>
<td>year+exp+male+pair</td>
<td>147.04</td>
</tr>
<tr>
<td>Model II</td>
<td>inbred female+ inbred pair+exp+inbred male+year</td>
<td>161.23</td>
</tr>
<tr>
<td></td>
<td>inbred female+ inbred pair+exp+inbred male</td>
<td>152.07</td>
</tr>
<tr>
<td></td>
<td>inbred female+ inbred pair+exp</td>
<td>151.82</td>
</tr>
<tr>
<td></td>
<td>inbred female+ inbred pair</td>
<td>150.61</td>
</tr>
<tr>
<td></td>
<td>inbred female</td>
<td>149.66</td>
</tr>
</tbody>
</table>

Fledging success was also most accurately predicted by whether or not the female was inbred, and breeding experience alone was the best predictor of fledging success for Model I (Table 6). Predicted mean fledging success for pair-years with inbred females (0.167 ± 0.148) was lower than predicted mean fledging success pair-years for non-inbred females (0.545 ± 0.0489), however this difference was not significant (t = -1.62). The non-significance of this result is possibly a consequence of the small number of pair-years with inbred females, and therefore, the large corresponding standard error.

Table 6  Summary of GLM binomial models generated for fledging success of island takahe

<table>
<thead>
<tr>
<th>Fledging success</th>
<th>Model</th>
<th>AIC score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model I</td>
<td>exp+male+year+pair+female</td>
<td>177.53</td>
</tr>
<tr>
<td></td>
<td>exp+male+year+pair</td>
<td>172.17</td>
</tr>
<tr>
<td></td>
<td>exp+male+year</td>
<td>171.42</td>
</tr>
<tr>
<td></td>
<td>exp+male</td>
<td>161.27</td>
</tr>
<tr>
<td></td>
<td>exp</td>
<td>159.82</td>
</tr>
<tr>
<td>Model II</td>
<td>inbred female+inbred pair+exp+inbred male</td>
<td>152.07</td>
</tr>
<tr>
<td></td>
<td>inbred female+inbred pair+exp</td>
<td>151.61</td>
</tr>
<tr>
<td></td>
<td>inbred female+inbred pair</td>
<td>150.61</td>
</tr>
<tr>
<td></td>
<td>inbred female</td>
<td>149.66</td>
</tr>
</tbody>
</table>
Reproductive success and genetic similarity

There were 13 pairs for which band sharing data was available. Band sharing correlated poorly with inbreeding coefficients ($r = 0.182$) and average hatching success ($r = 0.226$). Average fledging success declined with increased band sharing ($r = -0.484$). When band sharing was considered in relation to pair-years ($n = 49$), no significant or consistent patterns were evident. Pair-years from related pairs made up similar proportions of the “low” (6/26) and “high” (9/23) categories of genetic similarity (figure 4 A-F). Overall, there was no evidence that genetically similar pairs have lower reproductive success than pairs that are not genetically similar, nor was were the two measures of relatedness (pedigree and band sharing values) correlated.
Figure 4  Comparisons of:
A  hatching success for pair-years with low and high genetic similarity
B  fledging success for pair-years with low and high genetic similarity
C  hatching success for related and unrelated pair-years with high genetic similarity
D  fledging success for related and unrelated pair-years with high genetic similarity
E  hatching success for related and unrelated pair-years with low genetic similarity
F  fledging success for related and unrelated pair-years with low genetic similarity

Low genetic similarity refers to band sharing values of <0.6, and high genetic similarity corresponds to band sharing values that are > 0.6. Band sharing values were obtained by DNA fingerprinting with probe pV47.2.

Note  error bars represent standard errors
Discussion

Island takahe have high rates of infertility and poor hatching success compared with Fiordland takahe. Jamieson and Ryan (1999) argue that the high egg infertility rate observed for island takahe is a consequence of environmentally induced inbreeding depression. Results from this study show that hatching success is significantly lower for pairs of takahe containing inbred females than pairs with non-inbred females. Hatching success was not reduced for related pairs, or pairs with inbred males. This suggests that only females are affected by inbreeding depression, although the small sample sizes involved may render this conclusion premature. If this apparent female-specific effect of inbreeding is a real biological effect, there is cause for concern as a substantial proportion of females which have yet to reach reproductive maturity are inbred.

The situation that takahe are in is by no means unique. Many other native bird species persist as small remnant populations and/or inhabit islands with limited carrying capacities as a result of translocation events. In terms of inbreeding, there are a number of questions we could ask. First, does inbreeding occur, and if so, is there evidence that it is adversely affecting the population? Secondly, can the population be managed in such a way as to minimise or prevent inbreeding if we believe this to be a problem? And thirdly, what is the population size needed to ensure long-term survival, taking into account all operating factors?

To come back to the first question, we know that inbreeding occurs for a number of New Zealand bird populations. Craig (1991) argues this does not present a problem, as these populations have a history of bottlenecks and translocations, and are therefore likely to have become purged of their deleterious alleles in the process. However, this conclusion may be premature, as we do not know whether or not a given population would have done better in the absence of inbreeding. In order to make a meaningful comment on the effect of inbreeding on populations, we need to compare fitness estimates from inbred and outbred populations (Wallis, 1994; Jamieson and Ryan, 1999).

Unfortunately, we do not have the option to obtain such estimates for many endangered species. Even comparisons of inbreeding depression with common species are difficult: to obtain, given that the incidence of inbreeding is often low (Table 1). Two studies are
worth considering in some detail in light of results obtained for takahe; Keller’s (1998) research on song sparrows, and Sittman’s (1966) work on captive Japanese quail.

Keller (1998) found that inbred female song sparrows had lower lifetime reproductive success (LRS) and seasonal reproductive success (SRS) than non-inbred females. LRS was defined as the number of independent young produced over a lifetime, whereas SRS consisted of the following measures: number of nesting attempts, number of eggs laid, proportion of eggs hatched, number of independent juveniles produced, and proportion of hatched young that reached independence. This breakdown of SRS was designed to allow for the identification of the stage(s) of the nesting cycle most affected by inbreeding depression.

In terms of LRS, the daughters of full-sib pairs and first cousins had, on average, 52% and 87% the LRS of non-inbred females, respectively. Inbred females also had reduced SRS, affecting hatching success and the number of independent young produced. Other measures of SRS were unaffected. For daughters of full-sib pairs, the probability of an egg hatching was reduced by 36%. Inbreeding depression was evident for pairs of song sparrows with inbred males, but tended to affect only the earlier stages in the nesting cycle (number of nesting attempts, number of eggs laid). Hatching success was not reduced in pairs with inbred males, agreeing with the results from other studies (van Noordwijk and Scharloo, 1981; Kempenaers et al., 1996). However, the possibility that female song sparrows from these pairs were seeking extra-pair fertilisations, and therefore masking inbred male effects, could not be ruled out (Keller, 1998). Relatedness between pairs did not significantly reduce SRS in any of the measures that were examined.

The results from this study on island takahe are similar to that obtained by Keller (1998), i.e. hatching success is reduced for pairs with inbred females but not for related pairs. For takahe, this means that the effects of inbreeding depression will not become obvious until the daughters of related pairs have reached reproductive age, creating a time lag of 2 years. Since productivity is low and takahe are long-lived, changes in the inbreeding coefficient of the population as a whole will be slow. This is advantageous in that the theoretical worse case scenario (i.e. population decline/local extinction through cessation of reproduction caused by inbreeding depression) will be delayed in time, but
disadvantageous in that the effect of management interventions will be slow and difficult to evaluate.

Sittman (1966) showed that reproduction in captive Japanese quail (\textit{Coturnix cotunix japonica}) effectively ceases after three successive generations of full-sib matings. This study was initially motivated by the desire to develop viable inbred lines for genetic and biomedical research, using various mating strategies including successive full-sib matings. After three successive generations of full-sib matings, the probability of an egg hatching was reduced by 46%, but survival to reproductive maturity became practically nil. Once again, hatching success was the trait most affected by inbreeding depression, and declined by 7% for each 10% increment in inbreeding (\(F\)) of the offspring. Other traits examined were survival (from 0-5 weeks, and 5-16 weeks) and fertility.

These studies show that close inbreeding (\(F \geq 0.125\)) does affect reproductive fitness for the respective species, with the severity of the effect increasing as \(F\) increases. For the five inbred takahe females whose reproductive performance (13 pair-years) was considered in this analysis, four are the product of close inbreeding (\(F = 0.125\)). Although it can be argued that a larger sample size is necessary before these results can be accepted as providing conclusive evidence of inbreeding depression, a cautionary approach is probably is warranted and management options should be considered at this point in time. Inbreeding depression is unlikely to fully account for the overall low reproductive success of island takahe relative to Fiordland birds, as reproductive success is low for all birds, irrespective of whether or not they are inbred.

Keller \textit{et al.}, (1994) stress that the effects of environment and genetics may interact and should therefore be considered together rather than independently. As evidence, they found that inbred song sparrows were "selected against" during severe environmental stress (adverse winter conditions). Environmental factors are clearly important for takahe also, and have been held accountable for the high rates of egg infertility of island takahe relative to Fiordland takahe (Jamieson and Ryan, 1999). Unfortunately Jamieson and Ryan (1999) did not examine whether egg infertility was lower for pairs of island takahe as a consequence of individuals being inbred.
Tahake populations persist in two very different environments. Fiordland takahe experience harsh conditions and are likely to be inbred given their history of isolation and small size. When considering these two effects together, one might predict (in accordance with the song sparrow situation reported by Keller et al., 1994) that some degree of selection against inbred individuals could be occurring in the Fiordland population. This could potentially account for the comparatively higher reproductive fitness of Fiordland takahe, and represents an alternative notion to the idea that higher reproductive fitness of is a consequence of local adaptation.

These two possible scenarios have different implications for the interpretation of inbreeding depression in island takahe. If Fiordland takahe are locally adapted to their environment, then the lower reproductive success of island takahe could indeed be the result of translocation to a substantially different environment (i.e. environmentally-induced inbreeding depression). Alternatively, if Fiordland takahe are not locally adapted but regulated by selection against inbred individuals (=females), then the lower reproductive success of island takahe must be a result of inbreeding at the genetic level. According to this scenario, environmental conditions on islands should be favourable to reproduction and the survival of inbred individuals, and reproductive fitness will decline as generations become progressively more inbred.

In summary, this study found evidence that island takahe are affected by inbreeding depression. Pairs with inbred females had significantly lower hatching success than pairs with females which were not inbred. If one assumes that takahe are adapted to Fiordland conditions, then these results agree with those obtained by Jamieson and Ryan (1999) in that island takahe are adversely affected by inbreeding. There are differences however; this study implicates genetic factors, while the other stresses environmental causes. These differences could be the result of genetic and environmental factors affecting different stages of the reproductive cycle in different ways, but could also be the result of misinterpretation. It may be the case that Jamieson and Ryan (1999) failed to detect the effects of inbreeding on egg infertility at the genetic level because they did not consider whether or not individual female breeders were inbred. It is also possible that the results from this study are an artefact of small sample size.
I have proposed that the comparatively higher reproductive fitness of Fiordland takahe could also be the result of adverse environmental conditions selecting against inbred individuals. If this is the case, the results obtained by Jamieson and Ryan (1999) and this study are in conflict. Clearly, further research is necessary. Recommendations for research and management options are discussed in the following section.
Literature cited


Conclusions and Recommendations

1. **Island takahe appear to be genetically monogamous**
   Results from this study suggest that the assumption that social monogamy equates to genetic monogamy is valid for island takahe. Due to the small sample size (27 chicks to which paternity was assigned), the possibility that extra-pair fertilisations do occur at low frequency cannot be ruled out.

2. **Island takahe populations are genetically depauparate**
   Island takahe exhibit low levels of DNA minisatellite variation, which fall within the range reported for other New Zealand bird species. The low levels of genetic variation reported for island takahe are likely to reflect a history of persistence as a small, single population (Fiordland) and/or founder effects (translocation of small numbers of takahe to offshore islands).

3. **Inbreeding depression may be a factor contributing to the low reproductive success of takahe on islands compared to the Fiordland population**
   Pairs of island takahe with inbred females have significantly lower hatching success than pairs with non-inbred females. The same trend is evident for fledging success, but was not significant, possibly due to small sample sizes. Larger sample sizes are necessary before this result can be taken as conclusive.

Recommendations

1. **Translocation frequency should not be increased solely for the purposes of “maximising genetic diversity”**
   Low genetic variation alone does not constitute sufficient reason for changing current management practices for two reasons: 1) we do not know whether genetic variation is actually declining (i.e. lower for island takahe than Fiordland birds, and 2) takahe on islands are already effectively being managed as a meta-population, which is thought to
be a better strategy (in terms of maintaining genetic variation) than managing populations as isolated units.

2. A comparison of the levels of genetic variation for Fiordland vs island takahe should be performed
Since efforts are being made to collect samples from Fiordland takahe, and conditions have already been optimised, it would be relatively easy to profile a random sample of Fiordland birds. This analysis would allow for the detection of a decline in genetic variation due to founder effects (i.e. consequence of translocating small number of birds to islands).

3. A cost-benefit analysis should be performed to determine the merits of preventing matings between closely related pairs
Although the results from this study show that the effects of inbreeding appear to be female-specific, inbred females are the offspring of related pairs. A large proportion of females which have yet to reach reproductive maturity are inbred. These two factors taken together mean that, assuming that the findings of this study are real (i.e. not an artifact of small sample size), there will be a delay of one generation (>2 years) before adverse inbreeding effects are manifested.
Acknowledgments

A large number of people have shown their support during the course of this research project. First of all, I'd like to thank my supervisors, Dr Ian Jamieson and Prof Dave Lambert. Ian, thanks for the opportunity to work with takahe, and your endless supply of “just being there at the right time”. Your generally relaxed approach to academic life, approach to students, and sense of humour were greatly appreciated. Dave, I am extremely grateful to you for providing me a more expansive appreciation for the science behind molecular ecology (even if this consists of admitting that we don’t know all that much and shouldn’t pretend that we do), a great working environment, and also the opportunity to attend the Molecular Ecology Meeting at Tongariro. My three months at Massey were really enjoyable, and would not have been the same without the friends I have there. Nyree, Halema, Toni, Isabel, Elissa and Wayne – you’re all fantastic people who seriously enhance the “Palmerston North living experience”. The same can be said for the friends I made during my time in Dunedin – you know who you are. Thanks especially to the wildlife girls, Ken Miller, Petrina (for providing a real home atmosphere, friend included), and Peter Bayliss (for greatly improving my stats knowledge). Last of all, thank you Daniel for being the most awesome partner I could possibly want.