



## Mating system and genetic variation in the endangered New Zealand takahe

Marieke Lettink<sup>1</sup>, Ian G. Jamieson<sup>1\*</sup>, Craig D. Millar<sup>2,3</sup> & David M. Lambert<sup>2</sup>

<sup>1</sup>Department of Zoology, University of Otago, PO Box 56 Dunedin, New Zealand; <sup>2</sup>Institute of Molecular Bio-Sciences, Massey University, Private Bag 11-222, Palmerston North, New Zealand; <sup>3</sup>Present address: School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand (\*Author for correspondence: E-mail: ian.jamieson@stonebow.otago.ac.nz; Fax: 64-3-479-7584)

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### Abstract

The takahe (*Porphyrio hochstetteri*) is a highly endangered flightless rail that is endemic to New Zealand. Only one remnant population of takahe (~120 adults) is left in the wild in Fiordland, which has been the source for introductions to four predator-free islands. The objective of the present study was to determine the mating system and amount of genetic variation in takahe using multilocus DNA profiling, in order to assist in the management of the island populations. There was no evidence of extra-pair paternity for the 27 (73%,  $n = 37$ ) offspring to which paternity could be resolved. The paternity of the remaining 10 offspring could not be resolved due to low levels of minisatellite DNA variation, but in none was the resident male excluded. Overall, the DNA results along with behavioral and life history information indicate that extra-pair fertilizations should be rare or absent, and takahe join a small but growing list of long-lived species of birds that have been shown to exhibit genetic monogamy. In addition, the levels of minisatellite DNA variation detected in takahe are low relative to those reported for most other known outbred avian populations, and are consistent with the evidence of the takahe's persistence as a small, isolated population in Fiordland over at least the last 100 years. The low genetic variation is discussed in relation to possible evidence of environment depended inbreeding depression in translocated island populations of takahe.

### Introduction

Management of endangered species to preserve their gene pools requires knowledge of demographic and life history characteristics, an understanding of genetic relationships among individuals in a population, and measurements of genetic diversity in the current population (Haig et al. 1990). Two areas where molecular genetics has played a significant role in endangered species management are confirming a species' mating system and estimating the degree of genetic variation within and among populations. Knowledge of a species' mating system, along with other life history parameters, is valuable when calculating the effective population size and its effects on the rate of inbreeding and loss of genetic variation (Nunney and Elam 1994;

Frankham 1995). At a more specific level, validating pedigrees based on observational data with molecular methods has become increasingly frequent in avian species because of the widespread occurrence of extra-pair copulations (Petrie and Kempenaers 1998). Some researchers have even indicated that "true" or genetic monogamy may be the exception rather than the rule in birds (Birkhead and Møller 1992).

Even if the pedigrees maintained for captive or free-ranging populations were accurate, they are not necessarily a good basis for calculating genetic diversity or the degree of inbreeding in a population because the pedigrees might only cover a few generations. This is particularly the case if a population has previously gone through a genetic bottleneck (Bensch et al. 1994).

In this study, we use multilocus minisatellite data to determine the mating system and estimate the level of genetic variation in the highly endangered takahe (*Porphyrio hochstetteri*) (formerly *mantelli*; Trewick 1996). The takahe is a large (~3 kg) flightless rail endemic to New Zealand. Thought to be extinct earlier this century, the takahe was "rediscovered" in 1948 in alpine habitat in the isolated Murchison Mountains, Fiordland. Presently, there are approximately 120 adult takahe in Fiordland (Maxwell 2001).

A total of 24 birds (mostly yearlings) was translocated during the mid-80s to early 90s from the Fiordland population to four offshore islands from which introduced mammals had been removed or eradicated (Crouchley 1994; Bunin and Jamieson 1995). Since then, some birds have been transferred between islands, primarily because of the unavailability of mates and to prevent matings between closely related individuals. Island populations slowly increased to a total of 59 birds of one year of age and older (Jamieson and Ryan 2001). The slow growth rate has been partly due to island breeders having high rates of egg infertility and significantly lower hatching and fledging rates than pairs breeding in Fiordland (Bunin et al. 1997; Jamieson and Ryan 2000).

Except for the rare occasions when they defend a territory as a polyandrous or polygynous trio, takahe usually breed as pairs. Pedigree data on island takahe maintained by the Department of Conservation assume that takahe breeding pairs are genetically monogamous. This assumption is based on observations of long-term pair bonding, extensive bi-parental care and defense of large, all-purpose territories. However, mating events of island takahe are rarely witnessed (Ryan 1997) and there is potential for extra-pair fertilizations, as territories of several breeding pairs on some islands are in close proximity. If island takahe do engage in extra-pair fertilizations, the parentage of the resulting offspring will be erroneously represented by pedigree data. Inaccurate pedigrees may preclude the detection of any adverse effects of inbreeding on reproductive fitness.

Therefore one immediate objective of the present study was to determine whether social monogamy equates to genetic monogamy for takahe, using DNA profiling, and to relate these results with those of recent paternity studies of other long-lived avian species. DNA profiling can also be used to calculate the degree of band-sharing between individuals within a group or a population, thereby providing a measure of genetic variation in takahe. The low genetic variation

detected in takahe is discussed in relation to possible evidence of environmental depended inbreeding depression.

## Methods

### *Pilot study*

DNA from blood samples that were previously collected from takahe in the Fiordland population ( $n = 17$ ) was used in a pilot DNA profiling study. The aim of the pilot study was to identify restriction enzyme/probe combinations that exhibit appropriate minisatellite DNA profiles and levels of genetic variation. DNA digested with either *Hae*III, *Alu*I, or *Hinf*I, was used to generate profiles with probes pV47-2 (Longmire et al. 1990), human probes 33.15 and 33.6 (Jeffreys et al. 1985), YNH24 (van Ede et al. 1990), and 3'HVR (Fowler et al. 1988), according to the methods outlined below. Probes pV47-2 and 33.6 in combination with restriction enzyme *Hae*III were subsequently selected for DNA profile analysis, based on (comparatively) low band-sharing values and profile clarity (Lettink 1999).

### *Collection of samples and DNA methods*

Pedigrees constructed from Department of Conservation records were used to identify presumptive families ( $n = 11$ ) for which both putative parents and one or more offspring (maximum = 8) could be sampled. Forty island takahe ranging from five months to 15 years of age were captured in April 1998 using a combination of hand nets and large mist nets set at ground level. Up to 5 ml of blood (some of which was used for work on disease screening) were collected from each bird from the inter-tarsal vein. Samples were immediately transferred to liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until analysis. Previously collected samples from 12 island birds together with a random sample of 17 birds from Fiordland were also available.

Adult takahe normally lay two eggs per clutch and raise a maximum of one chick to independence per season; thereafter annual survival rates are high (70–90%) and adult birds can be long lived ( $> 15$  years) (Bunin et al. 1997; I. Jamieson, unpublished data). Since this study sampled multiple generations, some takahe were represented both as offspring, and as parents of the subsequent generation. Of the 52 birds that were sampled, 37 were offspring; 7 were approximately five-month old juveniles still on their parents'

territory, 10 were non-territorial 1–2 year olds, and the remaining 20 were breeding adults. Five of the presumptive families were complete (i.e. consisting of all offspring raised by a breeding pair over the duration of their pair-bond), while six families were incomplete due to the death of one or more offspring prior to the sampling period. Attempts were made to sample all adult males that could have been potential fathers, but some of these had also died. The numbers of breeding pairs on each of the four islands at the time of the study were: five (Tiritiri Matangi Island), six (Mana Island), six (Maud Island), and two (Kapiti Island).

Whole blood (15  $\mu$ l) was mixed with 400  $\mu$ l SET buffer (0.1 M NaCl, 1 mM EDTA, 0.1 M Tris-HCl pH 8.0) to which SDS and proteinase K were added at final concentrations of 0.5% and 0.5 mg/ml respectively, 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 (24 : 1). Ethanol precipitations (using 3 M NaOAc pH 5.2) were performed according to Sambrook et al. (1989). Genomic DNA samples of approximately 20  $\mu$ g 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  $\mu$ g/ml). A further 10 units of restriction enzyme was added the following day, and incubation continued for at least 1 h. All digested samples were visualized in 0.8% agarose minigels and DNA concentrations determined using a Hoefer TK0-100 DNA fluorometer.

DNA electrophoresis, transfer, and Southern blot hybridization were performed according to Ardern et al. (1997), with the following modifications: 5  $\mu$ g *Hae*III digested DNA was loaded per gel lane and allowed to run for approximately 72 h at 55 V. After hybridization, membranes hybridized with pV47-2 were washed twice for 30 min with  $5 \times$  SSC, 0.1% SDS at 55 °C, and membranes hybridized with 33.6 were washed twice with  $1 \times$  SSC, 0.1% SDS at 61 °C.

#### *Minisatellite DNA profile analyses*

It was assumed that the resident female was the mother of the offspring, as frequent monitoring of nests did not reveal any cases of eggs being added to nests (i.e. intraspecific brood parasitism) during the incubation period (I. Jamieson, unpublished data). For each family, DNA samples from the mother, offspring, 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 6–23 kb region; internal lane molecular weight markers were not used, however significant distortions were not detected in any gels. A restriction fragment was scored as shared by two individuals if the centers of bands differed in electrophoretic mobility by less than 0.5 mm.

In order to assign paternity, profiles were initially analyzed for band matching, unattributable fragments, and comparisons of the proportion of bands that were shared between chicks and fathers, and chicks and non-fathers (where fathers are designated by virtue of having no unattributable bands). In the latter case, such comparisons ideally yield a bimodal distribution, which should permit the precise determination of potential males with one (or more) unattributable bands as being either true fathers (with unattributable alleles arising from mutation), or non-fathers. However, in general, assigning paternity based on band-sharing was not reliable as non-fathers (i.e. males that were positively excluded due to unattributable bands) often had levels of band-sharing with chicks that approached, or even slightly exceeded, that of chicks and fathers (Lettink 1999). Therefore we used unattributable bands to exclude individual males as fathers; if the attending male was the only one that could not be excluded, we assigned the paternity to him.

#### *Genetic variation*

Following Gilbert et al. (1991), we calculated the average percent difference (APD) in band sharing for pair-wise comparisons of all individuals in a group or population as a measure of genetic variation. There is a difficulty in the estimation of similarity levels arising from a lack of independence when multiple pair-wise comparisons are made among the same group of individuals (Danforth and FreemanGallant 1996). However, we calculated APD values for the sole purpose of comparing these to values reported for other species of birds. Hence any increased variance on estimates of APD will not affect comparisons of the estimate itself. APD values were generated for a random sample of Fiordland birds and for a sample of non-relatives and first-order relatives (sibling-sibling pairs, mother-offspring pairs, and father-offspring pairs) of takahe living on islands. Because of the frequent transfers of birds between islands (17–29% of breeding adults on

any one island have come from another island, with some individuals having been transferred 2–3 times; I. Jamieson, unpublished data), APD values were not calculated for individual islands.

## Results

### *Paternity analysis*

The average number of scoreable fragments (between 6.0 to 23.0 kb) per individual was slightly greater for probe 33.6 ( $15.9 \pm 2.1$  SD) than pV47-2 ( $12.15 \pm 2.18$ ), and the degree of overlap between the two probes was low ( $0.10 \pm 0.03$  ( $n = 15$ )). However, paternity for the majority of offspring were resolved using probe pV47-2 alone; probe 33.6 was used to resolve paternity for two additional offspring only (see below), as the degree of band-sharing was high and spread across a small range. An example of the minisatellite DNA profiles using probe pV47-2 is shown in Figure 1.

In all cases ( $n = 37$  offspring from 11 families), the resident males' profiles had no unattributable bands and therefore could not be excluded. Using both probes, the paternity of 27 offspring (73%) from 9 families was assigned to the resident male. For the remaining 10 offspring (27%) spread over five families, paternity could not be resolved due to high levels of band-sharing with other nearby males (Table 1). Of the five families with unassigned offspring, two (consisting of one and three chicks each) had no offspring that could be assigned. For the other three families, 1/3, 3/6 and 6/7 of the offspring were assigned to the resident male.

The probability that there were no extra-pair offspring if the true rate of EPFs was 15%, a rate not uncommon for many birds (Petrie and Kempeneers 1998), is significantly different from chance for both a sample of 37 offspring (Binomial test,  $P = 0.0024$ ), and a sample of 27 offspring ( $P = 0.012$ ). A binomial power test (cf Fleischer et al. 1997) indicated that we could have detected a significant difference as low as 8% EPFs with our sample of 37 offspring and 10.5% with a sample of 27 offspring.

### *Genetic variation*

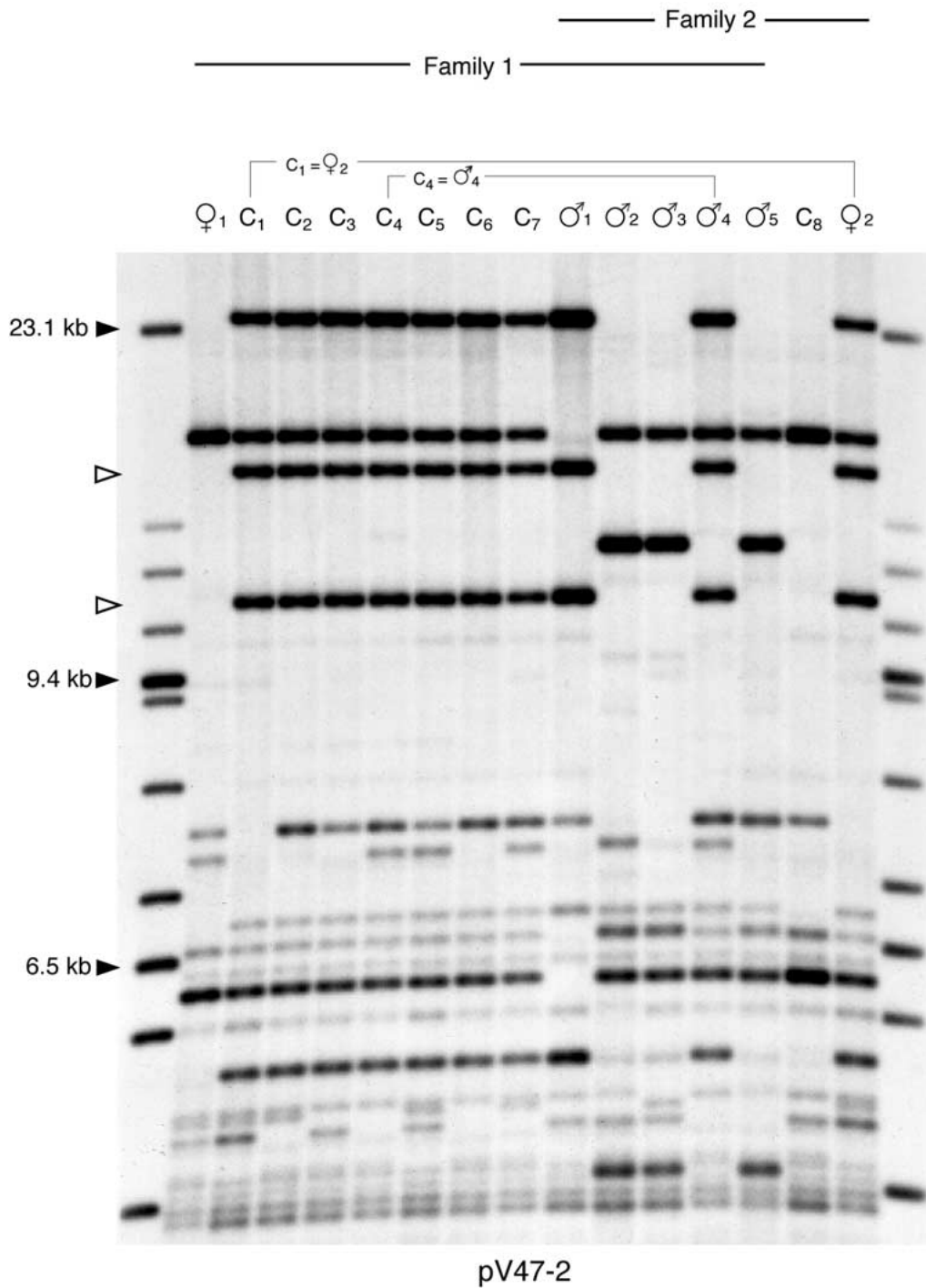
APD measures were consistently lower for probe 33.6 than probe pV47-2, thus values generated from both probes are presented in Table 2. A random sample

of takahe from Fiordland and a sample of unrelated takahe on islands showed similar amounts of genetic variation, and these APD values were considerably higher than first-order relatives when compared between each probe, as would be expected (Table 2). Nevertheless, the APD values for the unrelated takahe are low relative to those calculated in other species (see Discussion). The low APD values for takahe are partly a consequence of the presence of a number of apparently homozygous bands in takahe multilocus profiles. For example, in Figure 1, bands indicated by an open arrow are inherited by all chicks. In both cases indicated, these fragments are present in all seven chicks. The probability of this occurring by chance in the case of a band that segregates as a Mendelian allele is 0.0078.

## Discussion

The paternity of approximately three quarters of all takahe offspring (73%,  $n = 37$ ) could be resolved using multilocus DNA profiling, despite the low levels of minisatellite DNA variation recorded in takahe generally (see below). In all of these cases ( $n = 27$ ), paternity was assigned to the resident male, and in none of the unresolved cases ( $n = 10$ ) was the resident male excluded. The power to detect relatively low rates of extra-pair fertilizations (EPFs) with the sample sizes we had was high. Therefore, if extra-pair fertilizations do occur in takahe, they are not likely to be frequent events, even though the density of breeding pairs on three of the four translocated island populations was high relative to the source population in Fiordland. The results obtained in this study are consistent with the assumption that island takahe are genetically monogamous (Jamieson and Ryan 2000), and that pedigrees constructed from observational records are likely to reflect the true relationships of breeders.

The need to carry out DNA profiling on socially monogamous birds arose because early genetic studies of mating systems detected high rates of EPFs (see Birkhead and Moller 1992). However, high rates of EPFs are a more prevalent feature of socially monogamous passerines than non-passerines (Fleischer 1996). By contrast, extremely low rates of EPFs appear to be the rule in many long-lived, non-passerine species such as seabirds, which have long-term pair bonds, lay few eggs, and have offspring that require extensive bi-parental care (Fleischer 1996; Piper et al. 1997; Gilbert et al. 1998, Mauck et al. 1999; Quill-



*Figure 1.* DNA profile of two presumptive families of takaha on Tiritiri Matangi Island. Family 1 contains one female, seven chicks (C<sub>1</sub>–C<sub>7</sub>), and four potential fathers (1–4), while Family 2 consists of one female, one chick (C<sub>8</sub>), and five potential fathers (1–5). Note that C<sub>4</sub> subsequently became one of the putative fathers, and C<sub>1</sub> became the mother of Family 2. Open arrows indicate bands diagnostic of paternity, which for Family 1 can be assigned to the first male. The paternity of C<sub>8</sub> 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, as shown by the dark arrows.

Table 1. Summary of paternity analyses based on unattributable bands in multilocus DNA profiles of island takahe

Percentage of offspring (N = 37)	Level of resolution achieved
100	Resident male not excluded
73	Paternity assigned to resident male
3	Resident male and two sons not excluded
3	Both resident males <sup>a</sup> , and brother and father of one resident male not excluded
5	Resident male and one other unrelated male <sup>b</sup> not excluded
8	Resident male and two other unrelated males <sup>b</sup> not excluded
8	No males excluded due to a lack of exclusive paternal bands

<sup>a</sup>Represents offspring from a two male, one female trio on Tiritiri Matangi Island.

<sup>b</sup>“Unrelated” is defined as a lack of common ancestors over the depth of the available pedigree (in most cases pedigrees include at least three generations).

Table 2. Genetic variation in a random sample of Fiordland takahe and in two samples of island takahe (non-relatives and first-order relatives), calculated as the average percent difference (APD) in band sharing for pairwise combinations of all individuals in the above designated groups. APD measures were consistently lower for probe 33.6 than probe pV47-2, thus values generated from both probes are presented

Takahe population	Probe	No. of individuals/ no. of pairwise comparisons	APD ± SD
Fiordland	33.6	6/15	32.6 ± 7.2
	pV47-2	17/136	41.0 ± 13.0
Islands			
	Non-relatives		
	33.6	18/153	22.6 ± 7.3
	pV47-2	18/153	45.5 ± 14.3
First-order relatives			
	Sib-sib pairs		
	33.6	13/78	14.6 ± 7.7
	pV47-2	13/78	17.9 ± 11.3
Mother-offspring pairs			
	33.6	9/36	16.2 ± 5.3
	pV47-2	9/36	29.6 ± 11.3
Father-offspring pairs			
	33.6	6/15	13.1 ± 6.3
	pV47-2	7/21	26.9 ± 7.4

feldt et al. 2001). Genetic monogamy may also be common in species such as loons (*Gavia immer*) and takahe where vigorous defense of breeding territories by both males and females is needed to prevent territory takeovers (Piper et al. 1997; Ryan 1997). Loon and takahe pairs also show very low rates of copulation (Piper et al. 1997; Ryan 1997), indicating that sperm competition may not be prevalent in these species (Birkhead and Moller 1992). Thus behavioural and life history data also support our conclusion that extra-pair paternity in takahe is rare or absent, and takahe could be added to a growing list of birds for which EPFs have been shown to be low or non-existent.

Although there is no outbred population of takahe with which to compare, the APD values of unrelated takahe are low relative to those reported for most other known outbred avian populations. Papangelou et al. (1998) found that unrelated individuals from outbreeding populations have significantly higher APD (range 70–80%) than those from small and/or inbred populations (APD < 50%). Using these results as a guide, APD values for the random sample of takahe from Fiordland (32–41%) and for unrelated takahe on islands (23–45%) (see Table 2), fall within the range reported for small and/or inbred populations of birds (Papangelou et al. 1998), and are similar to those

in other inbred or bottlenecked population of New Zealand birds (Ardern and Lambert 1997).

Given what is known about takahe, it would not be surprising to find low genetic variation in this species. Takahe declined dramatically after the arrival of humans, and became very rare and restricted to the South Island by the time of European colonization in the 1800s (Beachamp and Worthy 1988). The last bird recorded outside the alpine region of Fiordland was in 1898 (Reid 1974). Takahe are thought to have persisted in mountainous Fiordland because of the area's remoteness from activities associated with human colonization, such as hunting and the introduction of mammalian predators (Bunin and Jamieson 1995). Although takahe have been protected since their rediscovery in 1948, the decline of the Fiordland population continued until the early 1980s when numbers stabilized at around 120 birds, corresponding to the reduction and control of introduced deer, a major food competitor (Maxwell 2001). Therefore the low APDs observed in DNA profiles of Fiordland takahe and those birds translocated to islands is consistent with the evidence of the takahe's persistence as a small, isolated population over at least the last 100 years. We have conservatively estimated the takahe population in Fiordland to have an inbreeding coefficient of 0.048 (Jamieson et al. unpublished ms), a value considered high for a natural population (Caughley 1994).

There is little consensus in the conservation genetics literature over the long-term effects of inbreeding or low genetic variation in a wild population (Caughley 1994). Most conservation biologists would agree that the impact of loss of habitat and introduction of alien predators in New Zealand far outweighs any potentially adverse genetic effects. For example, the growth of the takahe population in Fiordland appears to be primarily limited by introduced predators and competitors (Bunin and Jamieson 1995; Maxwell and Jamieson 1997). However, neither of these factors affect takahe on offshore islands where introduced predators/competitors have been eradicated. Yet ever since takahe were first introduced onto islands, their egg fertility, hatching and fledging rates have been significantly lower than birds in Fiordland (Bunin et al. 1997; Jamieson and Ryan 2000), despite both having similar estimates of genetic variation (Table 2). Yet, no detrimental environmental factors (e.g. toxins, dietary deficiencies), which might be peculiar to islands and could affect egg fertility or embryo development, have been identified (Jamieson and Ryan 1999, 2001; Jamieson and Easton 2001).

One possible explanation for the observed pattern of poor reproductive success is that takahe on islands are suffering from environment dependent inbreeding depression (Miller 1994; Bijlsma et al. 1999). Under this process, inbred populations are exposed to additional genetic load and consequently have reduced fitness when subjected to environmental conditions that differ from the prevailing conditions they experienced during previous purging of deleterious recessives (Bijlsma et al. 1999). The lowland islands to which Fiordland takahe have been translocated had been farmed in the past and now consist of a mixture of pasture grasses and regenerating native forest (Jamieson and Ryan 2001). Although translocated takahe had high adult survival relative to the Fiordland birds, and appeared healthy and nested successfully (Ryan and Jamieson 1998), they show all the signs of avian inbreeding depression (i.e. high rates of egg infertility, late embryo deaths, early juvenile deaths). This hypothesis is explored further in an analysis of relatedness, inbreeding and fitness estimates of island takahe (Jamieson et al. unpublished ms). By contrast, in the last remnant population of the Chatham Island black robin (*Petroica traversi*), which is another highly endangered New Zealand bird species, intense inbreeding and very low genetic variation were not associated with low reproductive rates (Ardern and Lambert 1997). Clearly, further research is still needed to determine the short and long term effects of inbreeding and reduced genetic variation, and their interactions with habitat/environmental conditions, in wild populations of endangered species.

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