Low genetic diversity and small population size of Takahe *Porphyrio hochstetteri* on European arrival in New Zealand

CATHERINE E. GRUEBER & IAN G. JAMIESON*

Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand

The rediscovery of the Takahe *Porphyrio hochstetteri* in 1948 in the remote mountains of Fiordland, New Zealand, has been described as one of the greatest moments in ornithological history. The subsequent management of the population has become a model for avian recovery programmes, yet questions still remain regarding the population size at the time of, and prior to, its rediscovery. We used 20 microsatellite markers to genotype samples of the three surviving museum specimens (1849–1898) collected prior to the initial declaration of extinction to estimate levels of genetic diversity and effective population size. These estimates were compared with equivalent estimates from DNA samples of three specimens preserved at the time of rediscovery (1949) and with 20 contemporary samples. Using rarefaction simulations to account for the limited sample sizes, the results suggest that only slightly more genetic diversity (allelic diversity and numbers of polymorphic loci) existed in the earliest Takahe sampled and that levels of genetic diversity at the time of rediscovery were very similar to those today. Effective population size estimates showed a similar pattern. Contemporary samples from a widespread congener to Takahe, the Pukeko *Porphyrio porphyrio*, showed consistently higher levels of genetic diversity and greater effective population size, even after equivalent rarefaction to the same small sample sizes available for Takahe. It is likely that the population size of Takahe in Fiordland at the time of European arrivals in the 1800s was similar to its current size. These results provide molecular support for the hypothesis that Takahe were common throughout most coastal and eastern parts of the South Island of New Zealand before being hunted to extinction in these regions by early Maori, and persisted as a relatively small and isolated population in Fiordland where they may never have been very common. This is in marked contrast to other New Zealand endemic birds found in Fiordland, such as the Kakapo *Strigops habroptilus* and several forest passerines, which remained relatively numerous until the time of European arrival before undergoing rapid declines thereafter.

Keywords: effective population size, microsatellites, mitochondrial DNA, museum specimens, *Porphyrio*, threatened species.

When the Takahe *Porphyrio* (formerly *Notornis*) *hochstetteri*, the largest flightless rail in the world, was rediscovered in a remote alpine region of New Zealand in 1948, the news drew worldwide media attention, provoking this headline in the *Illustrated London News*: 'A sight never before seen by white men: the discovery of a colony of the extinct Notornis or Takahe' (Ballance 2001). Described as one of the 'greatest ornithological moments in history' (Fitzpatrick 2001), the story of the Takahe, its rediscovery and subsequent management, is central to changes to New Zealand's wildlife conservation consciousness, both institutionally and in a broader societal context (Wallace 2002), and serves as a model for avian recovery programmes around the

*Corresponding author.
Email: ian.jamieson@stonebow.otago.ac.nz

world. Although Takahe are known to have gone through a severe population bottleneck and currently have low levels of genetic diversity (Jamieson *et al.* 2006, Jamieson 2009), the size of the historical Takahe population prior to and at the time of its rediscovery remains unknown. To gain a better understanding of historical population patterns of Takahe prior to their more recent declines and recovery (Maxwell 2001, Hegg 2007), it is important to first consider the circumstances leading to the species' earlier presumed extinction.

Subfossil evidence suggests that prior to human arrival (in approximately 1200 AD, Higham *et al.* 1999), Takahe were widespread throughout the South and North Islands of New Zealand (Reid 1974, Trewick & Worthy 2001), although morphological and molecular evidence indicate that the now-extinct North Island Takahe *Porphyrio mantelli* was a separate species (Trewick 1996, 1997). With the arrival of early Polynesian settlers, Takahe populations declined, probably due to hunting pressure (Trewick & Worthy 2001). The Takahe was first scientifically described from skeletal remains in 1848 by Richard Owen, but few observations of live Takahe were recorded from the wild (Williams 1960). Indeed, the birds were so rare that European naturalists, otherwise avid collectors of biological specimens, preserved only four Takahe (as compared with the Huia *Heteralocha acutirostris*, for example, which was also rare at this time but of which several thousand specimens were collected; Morris & Smith 1988). All four Takahe specimens from this time, the most recent of which was collected in 1898 near Lake Te Anau, originated from the Fiordland region of the South Island (Fig. 1; Williams 1960, Ballance 2001). Subsequently, no further accepted sightings were reported, and the species was thought to be extinct in 1930 (Williams 1960, Reid 1974, Lee & Jamieson 2001).

In 1948, Dr Geoffrey Orbell and three other expedition members discovered a population of Takahe in the remote Murchison Mountains of Fiordland (Fig. 1). The remoteness of the Murchison Mountains may have been key to the species' persistence (Trewick & Worthy 2001): Maori were virtually absent from the alpine area of Fiordland, which was labelled 'unexplored' on European maps until the mid 1900s (Ballance 2001). Upon rediscovery of Takahe, conservation action was initiated by the New Zealand Wildlife Service (now the Department of Conservation, DOC; Lee & Jamieson 2001) and the Murchison Mountains were designated a 'Special Area', initially under a policy of minimum intervention. Over the next 50 years, more focused conservation actions were undertaken and Takahe management was established as an exemplar of pest control, captive rearing, translocation and conservation management in general in New Zealand (Lee 2001, Lee & Jamieson 2001). Nowadays, Takahe are classed as Endangered (IUCN 2009) and persist in the remnant population in the Murchison Mountains (*c.* 650 km^2), as well as on a number of offshore islands to which they have been translocated as part of conservation management since the early 1980s (Fig. 1, see also Jamieson & Ryan 2001, Grueber & Jamieson 2008). Among ongoing threats to the species, such as introduced mammalian predators, current management of the Takahe population also takes into consideration the importance of genetic management of the species (Wickes *et al.* 2009).

Recently, the use of museum specimens for elucidating historical demographic parameters has increased (Wandeler *et al.* 2007). In the current study, museum samples of Takahe are used to estimate historical effective population sizes, potentially providing an indication of the true rate of historical decline. Perhaps due to the remoteness of the Murchison Mountains habitat, the Takahe population size around the time of rediscovery remains unclear. Based on surveys and territory size estimates, Takahe numbers were estimated at approximately 200 pairs in the mid- to late 1960s (Reid 1969, Reid & Stack 1974). Although thought to be an overestimate (as it is unlikely that all territories are occupied every year, Mills & Lavers 1974), it seems remarkable that more recent surveys indicate fewer than half this many, with a total number of adults around 150 (Crouchley 1994, Clout & Craig 1995, Wickes *et al.* 2009). Several authors in the 1960s and 1970s suggested that the Takahe population was declining (Reid 1969, Mills & Lavers 1974, Mills 1975) but it is not clear whether these discrepancies were indicators of a substantial population crash, or whether the early population size was simply overestimated.

This study uses samples taken from three of the 19th century museum specimens (the fourth, which was collected on the eastern shore of Lake Te Anau, is thought to have been destroyed when the Dresden Museum in Germany was bombed

during World War II; Ballance 2001) to evaluate trends in Takahe population size over the last *c.*150 years. Although the sample size is extremely small, molecular techniques may offer some insight into the effective population size and genetic diversity of the Takahe population at around the time of European settlement (Wandeler *et al.* 2007, Pruett & Winker 2008). We compare genetic diversity of the earliest samples with that of three additional museum specimens collected at the time the species was rediscovered in 1948, and with diversity of contemporary samples from Fiordland birds.

As a reference for comparing temporal patterns of genetic diversity in Takahe, we also assess levels of genetic diversity in a contemporary population of the Takahe's closest extant relative, the volant New Zealand Pukeko *Porphyrio porphyrio melanotus* (Trewick & Worthy 2001). The subspecies present in New Zealand is one of 13 distributed around the Southern Hemisphere and is most closely related to birds in Australia, after natural colonization from there within the past 1000 years (Trewick 1996, 1997, Trewick & Worthy 2001). Although the Pukeko population is likely also to have experienced a population bottleneck as a result of this founder event, a large portion of the genetic diversity of the founders can be maintained if the population expands rapidly (Lacy 1987), as appears to be the case for Pukeko (Trewick & Worthy 2001).

METHODS

Samples

The small sample size of historical specimens gathered for this study is symptomatic of the rarity of documented encounters with Takahe in 19th century New Zealand. Three specimens were available from the 19th century prior to the species being considered extinct (the 'early' samples) and three were collected close to the time of rediscovery in the mid-19th century (the 'rediscovery' samples) (Table 1). Samples were small (< 1 cm) toe-pad fragments (Mundy *et al.* 1997) taken from these six museum specimens, held in collections in New Zealand and the UK (Table 1) and were obtained following guidelines described in Taylor *et al.* (2007). In addition, we included samples of 20 contemporary Takahe originating from the Murchison Mountains remnant population. These were collected for a previous study (Lettink *et al.* 2002) and stored as whole blood at −80 °C until use. The analysis also included a sample of 20 contemporary South Island Pukeko *Porphyrio porphyrio*, collected from a single site on the Taieri Plain (near the city of Dunedin) for a previous study (Jamieson *et al.* 1994) and stored at 4 °C in lysis buffer (Seutin *et al.* 1991) until use.

DNA was extracted from the museum samples in duplicate. We used the Qiagen DNeasy kit (Qiagen GmbH, Hilden, Germany) following the methods
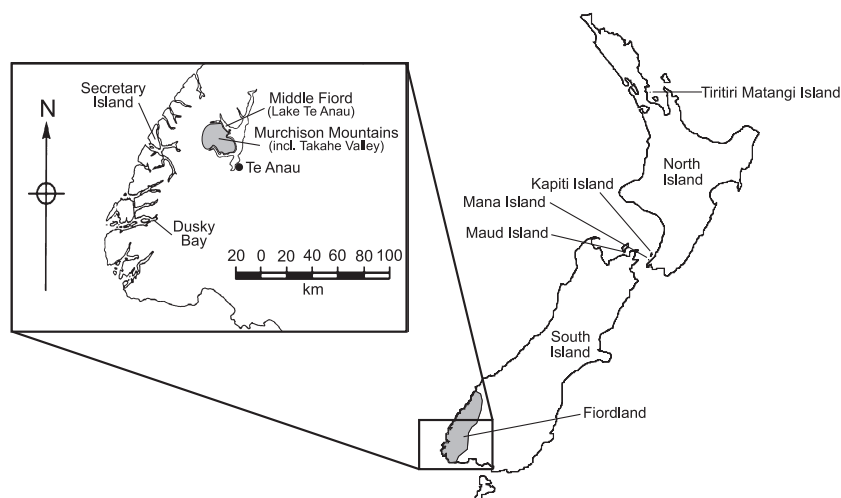

Figure 1. Important Takahe sites. The main figure indicates the four offshore islands on which Takahe populations have been established recently. The inset shows part of the Fiordland region, wherein the shaded region indicates the Murchison Mountains, the primary range of contemporary remnant population. The collection sites of the museum specimens are indicated.

Table 1. Collection sites and dates of the Takahe museum specimens used in this analysis.

Sample ID	Collection site[a]	Collection date	Institution	Preservation note	Yield in first, second extractions (ng/μL)	Number of loci successfully genotyped
'Early' samples						
NH	Dusky Bay	1849	Natural History Museum, UK		6.5, 2.8	13
TP1	Secretary Island	1851	Te Papa Museum of New Zealand	Legs and feet were painted[b]	14.1, 9.7	6
OM	Middle Fiord, Lake Te Anau[c]	1898	Otago Museum, Dunedin, NZ	Reportedly the best preserved of the early specimens[d]	9.3, 0.9	20
'Rediscovery' samples						
TP3	Takahe Valley	1949	Te Papa Museum of New Zealand	Specimen was dried out[b]	14.5, 3.6	20
TP2	Takahe Valley	1958	Te Papa Museum of New Zealand	Only the skin was preserved[b]	1.3, 1.0	6
TP4	Takahe Valley	1958	Te Papa Museum of New Zealand		2.0, 0.1	3

[a]See Figure 1.
[b]Te Papa Museum of New Zealand curator, pers. comm.
[c]Adjacent to the Murchison Mountains.
[d]Ballance 2001.

outlined in Taylor *et al.* (2007), wherein DNA from a third to half of each tissue sample was extracted, alongside a negative control, which was checked for contamination. Contamination is a frequent problem in the molecular analysis of historical specimens (Pääbo *et al.* 2004) and so extraction and amplification followed the specifications and guidelines of Cooper and Poinar (2000) and Taylor *et al.* (2007). These included: carrying out molecular work at a dedicated lab facility (with dedicated equipment such as pipettes, racks, etc.) at the University of Otago, where no amplified avian product had ever been present; performing DNA extraction and PCR setups under a hood that was irradiated with UV light after each laboratory session; performing negative controls with every PCR; and amplifying approximately half of the genotypes at least twice. We used a Nano Drop ND1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) to establish DNA extraction yields.

DNA was extracted from recent Takahe and Pukeko samples using a standard 5% Chelex method with 40 μg proteinase K (Roche Ltd, Basel, Switzerland; Walsh *et al.* 1991). Working stock DNA samples were stored at 4 °C, with the remainder frozen at −20 °C.

Microsatellite genotyping

Samples of both Takahe and Pukeko were genotyped using microsatellite markers developed for Takahe. There is therefore some potential to introduce a bias with respect to relative diversity within Pukeko, as cross-species microsatellite amplifications can result in underestimation of genetic diversity in the non-target species (e.g. Akst *et al.* 2002, Grueber 2005). We minimized this bias by using a combination of loci that were initially found to be polymorphic and monomorphic in contemporary Takahe. We therefore genotyped 20 microsatellite loci that had been developed specifically for Takahe, including five that were polymorphic in contemporary samples (Pho11, Pho28, Pho44, Pho46, Pho47; Grueber *et al.* 2008) and 15 additional loci that were initially found to be monomorphic when screened during development using a sample of 25 contemporary Takahe (nine males and 16 females; see Supporting Information, Table S1). PCR and genotyping protocols followed those of Grueber *et al.* (2008).

Mitochondrial sequencing

Because all of the 'early' museum specimens were collected from sites outside the current range of the modern Takahe population (Table 1, Fig. 1), we assessed whether these birds were likely to have come from a single regional population (rather than discrete, genetically distinct subpopulations). To this end, we sequenced part of the first hypervariable region of the mitochondrial control region for all museum specimens and for two of

the extracted contemporary Fiordland birds. Mitochondrial DNA amplification targeted two overlapping fragments using unpublished primers that were designed using the Takahe mitochondrial sequence deposited in GenBank (Morgan-Richards *et al.* 2008): TakdloopF (5′–3′: CCTATTTACAT TAAAATTCATGCAC) paired with Takdloop2 (AAGGGGTTTGTGTATTTAGTCCG) and Takdloop1 (AAACCTAAATTAATGGAACTCGG) paired with Takdloop4 (TACCTGGTTCTCTG AGACCGG), following the method of Boessenkool *et al.* (2009). PCR products were sequenced in both directions, resulting in a total of 250 bp used for further analysis with SEQUENCHER (Schneider 1998).

Statistical analysis

The disproportionately small sample sizes of museum specimens, relative to the sample sizes of contemporary Takahe and Pukeko populations, may bias conventional summary statistics such as observed heterozygosity (H_O) and alleles per locus (N_A) (Leberg 2002, Pruett & Winker 2008). We thus controlled for the disproportional sample sizes across groups by taking a rarefaction approach for comparing allelic diversity (Petit *et al.* 1998, Leberg 2002, Kalinowski 2004, Pruett & Winker 2008) using a resampling macro in VISUAL BASIC (implemented in MSEXCEL 2003). This approach also allowed us to calculate confidence intervals for our genetic diversity estimates. For each locus, we identified the size of the group ('early', 'rediscovery', recent Fiordland, recent Pukeko) with the smallest number of genotypes. For some loci this was only one individual – two alleles – due to poor amplification of some museum specimens (see Results). We randomly sampled the equivalent number of alleles (with replacement) from within the pool of genotyped individuals for each group and calculated the number of unique alleles per locus (N_A), which was then averaged across all 20 loci. We also evaluated the number of polymorphic loci. After repeating this process 10 000 times, we compared the median values for N_A and polymorphism rate across groups, and generated 95% quartiles of the distributions as a measure of the error of the estimate.

To determine whether observed differences in the medians between Takahe samples from the three time points differ from those expected by chance, we defined a null hypothesis that:

$$GD_{early} = GD_{rediscovery} = GD_{recent\ Fiordland}\quad (H0)$$

where *GD* is the genetic diversity estimate (either N_A or number of polymorphic loci). Pukeko genotypes were excluded from this part of the analysis. We took a similar resampling approach to model this null hypothesis, but with the assumption that all observed Takahe genotypes came from a single population of constant size. It was necessary to assume that the observed allele frequencies among sampled Takahe were representative of population allele frequencies so that we could randomly sample alleles from the pool of all available alleles (regardless of source population) without sampling effort bias. We assigned the sampled alleles (with replacement) to each of the three Takahe populations, again rarefied to the smallest sample size for each locus as above. Repeating across loci allowed us to calculate mean N_A and overall polymorphism rate, and repeating this 10 000 times provided a distribution of values for each of these two metrics, as predicted under the null hypothesis.

To estimate the effective population sizes, N_e, of all populations, we again controlled for variation in sampling effort by taking a rarefaction resampling approach to calculate expected heterozygosity, H_E. This was calculated for each population at each locus, on comparable sample sizes, using the formula:

$$H_E = 1 - \sum_{i=1}^{N_A} p_i^2 \qquad (1)$$

where p_i is the frequency of the *i*th of the total number of unique alleles (N_A) at a locus, as based on the Hardy–Weinberg principle (Allendorf & Luikart 2007). This value was then used to calculate effective population size, N_e, defined as the closed, idealized population size that would maintain the observed level of diversity at mutation-drift equilibrium (Hedrick 2000; Leberg 2005). Following the method of Akst *et al.* (2002), this procedure was performed using the stepwise mutation model for microsatellite evolution (Lehmann *et al.* 1998):

$$N_e = \left[\left(\frac{1}{1 - H_E} \right)^2 - 1 \right] \cdot \frac{1}{8\mu} \qquad (2)$$

where H_E is derived from Equation 1 and μ is the mutation rate for microsatellites. It can be seen from Equation 2 that N_e is sensitive to the mutation rate selected and we therefore followed the recommendations of others (Bagley *et al.* 1999,

Akst *et al.* 2002) and used the conservative estimate of 1×10^{-4} (Dallas 1992; Weber & Wong 1993). We used this method in preference to maximum-likelihood estimators (e.g. Miller & Waits 2003), such as those implemented in NEESTIMATOR (Peel *et al.* 2004) because the small sample sizes of the historical sample periods prevented likelihood functions from converging. Approximate Bayesian computation of N_e (in ONESAMP; Tallmon *et al.* 2008) also failed due to insufficient sample size in the historical sample periods. To calibrate our mutation rate-based method, however, we calculated N_e for the Pukeko sample, as well as a larger sample (total $n = 40$) of contemporary Takahe using ONESAMP (Tallmon *et al.* 2008). N_e estimates were calculated using genotype data from all loci where the number of possible genotypes was > 1, and averaged across polymorphic loci to estimate N_e for each population.

RESULTS

Among Takahe samples from all time periods, only one mitochondrial haplotype was observed, identical to the published haplotype on GenBank (Morgan-Richards *et al.* 2008). This result is consistent with a single regional Takahe lineage, indicating no evidence of significant phylogeographic structure across the Fiordland region.

Despite repeated amplification attempts, microsatellite genotyping of museum specimens had varying success (Table 1), such that only small numbers of genotypes (< 10) were available for half of the samples, precluding conventional population genetic statistics.

Overall observed levels of polymorphism were similar among the three time periods: five loci were polymorphic among the early samples, three in the rediscovery birds and five in the recent Fiordland population (Fig. 2). Many alleles were shared across Takahe sampling periods (see Supporting Information). Fourteen of the 20 loci genotyped (70%) were polymorphic in the contemporary Pukeko population. Under the assumption that over this *c.* 150-year period, alleles were likely to have been either lost or maintained (but not gained), the detection of polymorphism among the few Takahe sampled here, at four loci that are now monomorphic, indicates that microsatellite diversity was probably higher among the historical sampled populations than among contemporary birds.
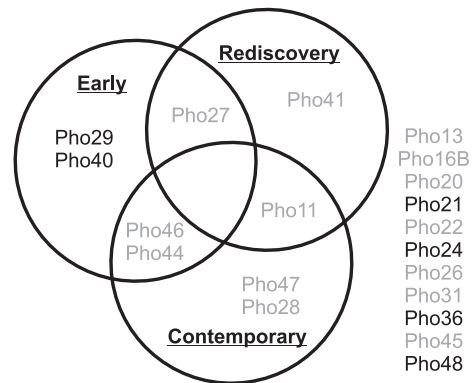

Figure 2. Circles represent sampling of the Fiordland population of Takahe at three time points: 'early' (mid-1800s, $n = 3$), 'rediscovery' (mid-1900s, $n = 3$) and 'contemporary' (1999, $n = 20$). Locus names within circles are polymorphic for that specific time period; loci excluded from all circles were monomorphic among all Takahe sampled. Locus names in grey were polymorphic in contemporary Pukeko (1994, $n = 20$).

After using a simulation approach to control for unequal sampling effort among populations, we observed a trend for the early samples to show slightly higher mean N_A (Fig. 3a) per locus, and slightly more polymorphic loci (Fig. 3b), than the 'rediscovery' and recent samples. The errors associated with all of these estimates are high, however, and because the observed values fall within the 95% confidence interval of the null distribution of the hypothesis, we cannot exclude the possibility that samples from Takahe at all three time points show equal genetic diversity (Fig. 3a). Although Pukeko sample sizes were similarly restricted by rarefaction, the population showed higher mean N_A per locus and more polymorphic loci than all Takahe populations; but again, sample error was high (Fig. 3a,b).

Under rarefaction for disproportional sample sizes, Equations 1 and 2 were used to evaluate the effective population sizes (N_e) of all groups (Fig. 4). The heterozygosity-based N_e of the widespread contemporary Pukeko was higher than that of all Takahe populations, as expected (Fig. 4). Using ONESAMPLE (Tallmon *et al.* 2008), the N_e (median and 95% credible interval) for the contemporary populations of Takahe and Pukeko were 21.2 (12.8, 44.6; $n = 5$ loci; 40 individuals) and 13.9 (11.4, 18.3; $n = 13$ loci with sufficient data; 20 individuals), respectively. The observed values are slightly lower than the results based on mutation rate, indicating that the latter may be overestimated, especially for
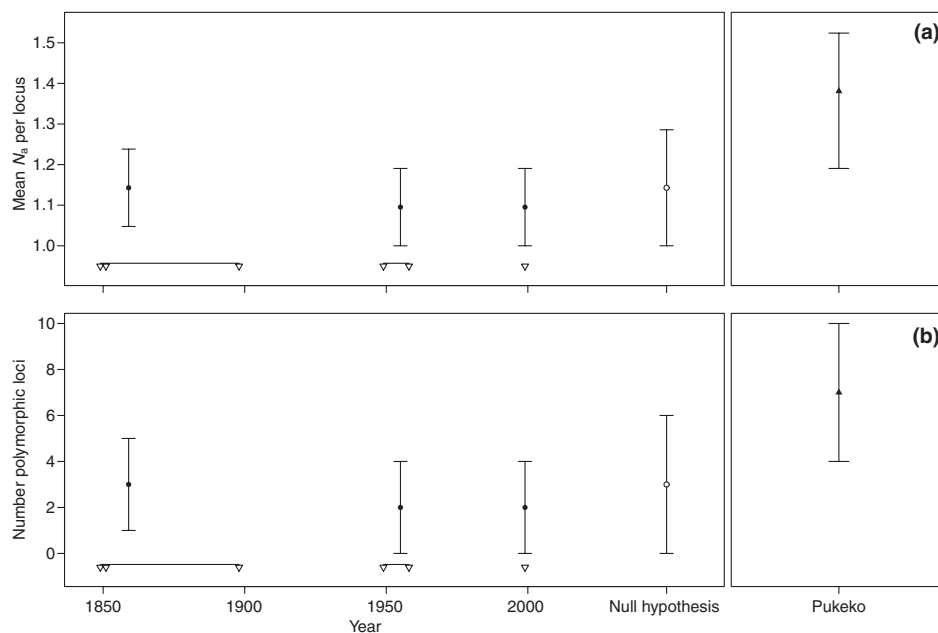
Figure 3. Mean allelic diversity (N_A) per locus (a) and number of polymorphic loci (b) among up to 20 microsatellites for Takahe from three time points (filled circles) compared with the null hypothesis (open circle) that all populations share equal genetic diversity (based on 10 000 randomizations). White arrows indicate the year samples were taken; see Methods for sample sizes. Shown for comparison are the genetic diversity values for Pukeko (triangles). All points represent the median of 10 000 resampling iterations to control for the disproportional sample sizes; error bars indicate the 95% quartiles (see Methods).
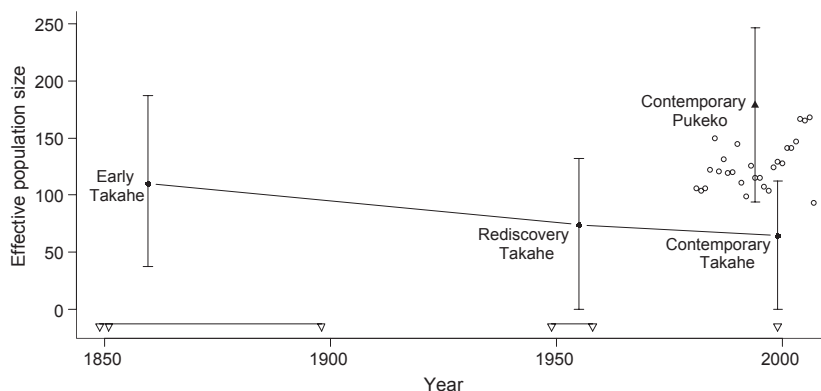

Figure 4. Effective population size (N_e) based on the unbiased estimated heterozygosity of up to 20 microsatellite loci of Takahe populations at three sampled time points (filled circles), and a contemporary Pukeko population (filled triangle). Filled points represent the median of 10 000 resampling iterations to control for disproportional sample sizes; error bars indicate the 95% quartiles. Open circles indicate recent census estimates of Takahe. White arrows indicate the year samples were taken; see Methods for sample sizes.

Pukeko. However, it is clear from genetic diversity estimates that Pukeko have much higher diversity (Fig. 3, Table S2). Insufficient data were available for the historical samples to calculate N_e using this Bayesian method.

The ratio of $N_e : N_c$ (census population size) in the contemporary Takahe population was 0.451, based on the population census size of 129 in 1999, when the contemporary DNA samples were collected (Fig. 4). Under the assumption that this ratio has remained constant across the recent history of the Takahe population, we estimated that the probable census sizes of the early and rediscovery populations were 232 and 135 individuals,

respectively, although these values are likely to be prone to error due to the wide confidence intervals around the N_e estimation. It is worth noting that the Takahe population in the Murchison Mountains had increased recently partly due to a captive rearing and release programme (Hegg 2007), with the census size reaching a high of 168 birds in 2006, before it crashed in 2007 to 93 due to a population irruption of Stoats *Mustela erminea* (Greaves *et al.* 2008, Wickes *et al.* 2009; Fig. 4).

DISCUSSION

Subfossil evidence indicates that Takahe were once widespread throughout the South Island (Trewick & Worthy 2001), but the rarity of encounters by European settlers indicates that the population had undergone a dramatic decline before the mid-19th century (Lee & Jamieson 2001). The low overall genetic diversity in Takahe, even from the earliest samples, supports this view. Visual inspection of the data suggests that there was more genetic diversity in the 'early' and 'rediscovery' Takahe populations than at present, as indicated by uniquely polymorphic loci in these groups (Fig. 2), despite the small sample sizes. Taking a rarefaction approach to comparing genetic diversity, we observed similar levels of allelic diversity (N_A) (Fig. 3a) and polymorphism (Fig. 3b) across the three time periods and that the effective population size may have declined slightly, although the high errors around these estimates cannot rule out the possibility that the population has remained small but relatively constant (Fig. 4). Bayesian approximation estimates of N_e were slightly lower than mutation-rate values, indicating that the latter may be overestimated. Whichever method is used, the results indicate that the effective population size of Fiordland Takahe was historically very small (i.e. < 200).

Estimates of genetic diversity, especially allelic diversity, are sensitive to sampling effort (Leberg 2002, Pruett & Winker 2008) and it is therefore possible that the genetic diversity of the early samples is underestimated relative to the contemporary samples, where larger numbers were available. However, analysis of Pukeko samples, which showed high microsatellite diversity (Fig. 2, Supporting Information Table S2), was similarly restricted through rarefaction to the smallest Takahe sample sizes and thus provides a useful scale for evaluating relative genetic diversity loss.

After rarefaction, the widespread Pukeko displayed higher genetic diversity (Fig. 3a) than even the earliest Takahe samples. This suggests that the current method of analysis has accurately represented the measured levels of genetic diversity in early Takahe samples, as compared with contemporary birds. Overall, however, the small number of genotypes available for many loci (Table 1) leads to high error around all genetic diversity estimates, as indicated by the very wide 95% quartiles for all populations and all metrics, precluding detection of statistically significant differences.

Our genetic data were used to estimate the effective population sizes of Takahe over three time periods. Due to the unavoidably small historical sample sizes, our approach is likely to be subject to considerable uncertainty. Uncertainty here is likely to be due to a number of assumptions including the microsatellite mutation rate (which we chose following the conservative recommendations of Bagley *et al.* 1999 and Akst *et al.* 2002), processes that influence N_e and N_e estimation, such as fluctuations in census size (N_c) (Charlesworth 2009), as well as sampling considerations (Antao *et al.* 2010). However, by applying the same statistical treatment to each population, we were able to assess whether effective population size estimates followed a similar pattern to that observed in the genetic diversity data. In natural populations, N_e is usually less than N_c due to deviations from ideal population demography, such as unequal sex ratio, non-random mating, variance in reproductive success and population size fluctuations, among others (Wright 1977, Franckowiak *et al.* 2009). For Takahe, we found that the calculated ratio of $N_e : N_c$ (0.451) is similar to the mean value of 0.459 estimated from demographic data for 17 bird populations (range: 0.09–0.80, Frankham 1995). The extrapolated point estimate of the population size at the time of rediscovery (135) is much smaller than the estimates of at least 400 adults (*c.* 200 pairs) presented in the early 1970s (Reid 1969, Mills & Lavers 1974, Reid & Stack 1974). These earlier estimates are currently thought to be an overestimate (Lee 2001), and the molecular findings support this conclusion. Indeed, the population size estimate for the rediscovery period is close to the maximum recent census size of adult Fiordland Takahe of 168 (2006 census, Greaves *et al.* 2008, Wickes *et al.* 2009; Fig. 4) and only slightly smaller than the estimate for the population in the 1800s (232). This indicates that

Takahe have persisted at a small population size for over 150 years, or a minimum of 21 generations (based on a mean generation length of 7.2 years; Grueber & Jamieson 2008), thus explaining its low genetic diversity (see also Trewick & Worthy 2001, Lettink *et al.* 2002, Grueber *et al.* 2008). However, these point estimates also hide the fact that Takahe numbers continued to decline up until the 1980s, at which point more effective management strategies were put in place (Maxwell 2001). More recent evidence indicates that since 1990 the slow recovery of the population through captive rearing and release of juvenile birds could have been offset by periodic irruptions of Stoats, which kill both juveniles and adults (Hegg 2007).

However, the finding that the estimated numbers of Takahe in Fiordland were relatively low prior to arrival of Europeans is perhaps more puzzling. In other parts of New Zealand, Takahe, like other large avian species such as Moa (the now-extinct family Dinornithidae, Tennyson 2010) and Yellow-eyed Penguin *Megadyptes antipodes* (Boessenkool *et al.* 2009), were probably a target food source for early Polynesian settlers in New Zealand. This hunting pressure, along with vegetation changes associated with deforestation and the burning practices of early Polynesians, is likely to have been a major contributor to the early decline and local extinction of lowland and coastal populations of Takahe (Trewick & Worthy 2001). In contrast, the Maori population around the Fiordland area was always small and transient, suggesting that hunting pressure on Fiordland Takahe may have been low. Furthermore, there are no sub-fossil remains of Takahe in Fiordland and generally very few deposits above the treeline across the South Island (Trewick & Worthy 2001). It is possible therefore that Takahe in Fiordland were naturally uncommon and had correspondingly low levels of genetic diversity. By way of comparison, the Kakapo was relatively common in Fiordland in the early 19th century (Powlesland *et al.* 2006) and one museum sample collected in Dusky Sound in 1884 had correspondingly high levels of microsatellite genetic variation, especially in comparison with insular Kakapo on Stewart Island (Robertson *et al.* 2000). Similarly, large reductions in genetic diversity among extant threatened passerines in Fiordland occurred during severe bottlenecks within the last 150 years (e.g. South Island Saddlebacks *Philesturnus carunculatus carunculatus*, South

Island Robins *Petroica australis* – Taylor *et al.* 2007, Mohua *Mohoua ochrocephala* – Tracy & Jamieson 2011), mainly due to the introduction of mammalian predators (Innes *et al.* 2010).

Low levels of genetic diversity can impact evolutionary potential and therefore long-term population persistence (Lacy 1987, Allendorf & Luikart 2007), the importance of which for Takahe was noted as early as 1969 (Reid 1969). Although Fiordland Takahe have persisted for a considerable period with low levels of genetic diversity, it is still important to prevent further losses, especially as Takahe are translocated and managed outside the Fiordland region. For example, an earlier pedigree analysis of Takahe introduced to small offshore island sanctuaries indicated that disproportional breeding among the founders led to a relatively high loss in founder genetic diversity over just a few generations (Grueber & Jamieson 2008). Grueber and Jamieson (2008) concluded that translocation of two Takahe per generation from the Fiordland population to the islands, as well as permitting the island population to expand, would maintain genetic diversity among these birds. The most practical strategy for maintaining genetic diversity for the Takahe population in the rugged Fiordland Mountains is the continued maximization of population growth rate through captive rearing of chicks and pest control (Hegg 2007).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Set of unpublished microsatellite loci developed using the methods outlined in Grueber *et al.* (2008).

Table S2. Allelic diversity and amplification success of 20 microsatellite loci across the Fiordland Takahe population sampled at three time points, and a contemporary Pukeko population.

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