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Genetic consequences of a century of protection: serial founder events and survival of the little spotted kiwi (*Apteryx owenii*)

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We present the outcome of a century of post-bottleneck isolation of a long-lived species, the little spotted kiwi (*Apteryx owenii*, LSK) and demonstrate that profound genetic consequences can result from protecting few individuals in isolation. LSK were saved from extinction by translocation of five birds from South Island, New Zealand to Kapiti Island 100 years ago. The Kapiti population now numbers some 1200 birds and provides founders for new populations. We used 15 microsatellite loci to compare genetic variation among Kapiti LSK and the populations of Red Mercury, Tiritiri Matangi and Long Islands that were founded with birds from Kapiti. Two LSK native to D'Urville Island were also placed on Long Island. We found extremely low genetic variation and signatures of acute and recent genetic bottleneck effects in all four populations, indicating that LSK have survived multiple genetic bottlenecks. The Long Island population appears to have arisen from a single mating pair from Kapiti, suggesting there is no genetic contribution from D'Urville birds among extant LSK. The N_e/N_C ratio of Kapiti Island LSK (0.03) is exceptionally low for terrestrial vertebrates and suggests that genetic diversity might still be eroding in this population, despite its large census size.

1. Introduction

Conservation management is often crisis management. In a worst-case scenario, only the last individuals of rare species are protected, either in the wild remnants of their native habitat or in captivity [1,2]. It is hoped that intense protection will allow all individuals to survive and reproduce, and that offspring can be returned to their native range via translocation (the intentional movement of individuals, populations and species across landscapes to enhance or maintain biodiversity [3]). This strategy has saved entire species from extinction by meeting the first critical step of allowing endangered populations to recover demographically (e.g. cheetah, *Acinonyx jubatus* [4]; Chatham Island black robin, *Petroica traversi* [5,6]; Mexican wolf, *Canis lupus baileyi* [7]; takahe, *Porphyrio hochstetteri* [8]). A number of risks, however, are inherent in the translocation and isolation of limited numbers of individuals to represent species. For example, local adaptations may be lost if populations are removed from their native habitats [3], and reproduction and rearing of young may be impaired if important social complexes are disturbed (e.g. Allee effects [9,10]).

Perhaps the greatest long-term risk associated with intense protection and isolation of a population or species is the potential for profound loss of genetic diversity [11]. Genetic variation allows populations to respond to selection and survive disturbance, and so is critical to long-term persistence [12,13]. Translocation of few individuals can result in substantial loss of genetic variation owing to founder effects (genetic bottlenecks associated with the founding of

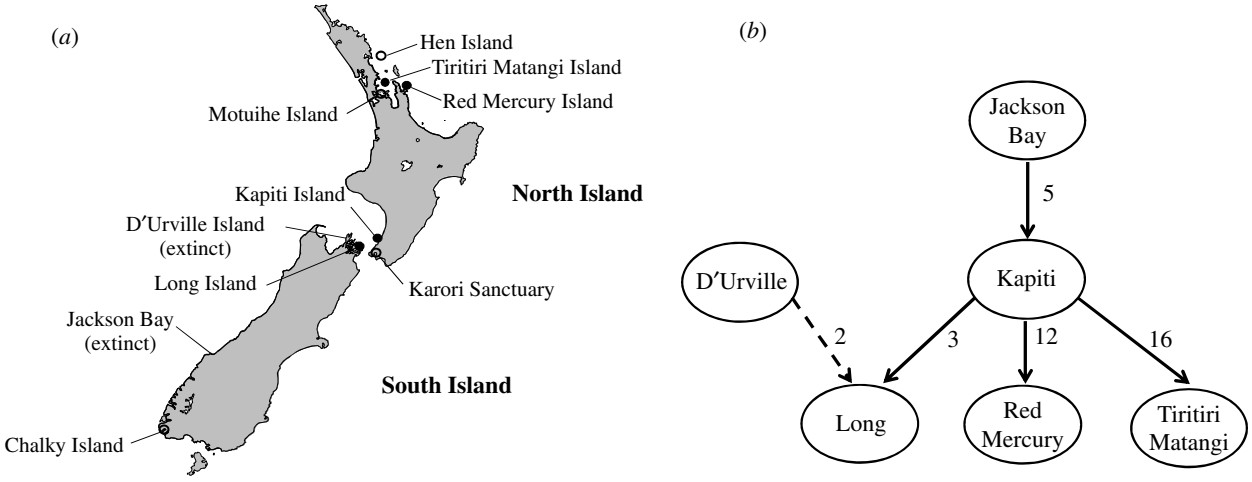

Figure 1. (*a*) Current distribution of extant LSK populations and (*b*) translocation history of those included in this study. LSK were once found throughout New Zealand but now comprise eight closed sanctuary populations (circles) that were founded via translocation of birds. Four extant populations (filled circles) are included in this study.

new populations) which may then be exacerbated by high variance in reproductive success among founders post-translocation [14,15]. In addition, small population size can lead to elevated inbreeding within protected populations [7], further reducing genetic diversity. Long-term isolation of protected populations, either owing to a lack of wild conspecifics, or intentionally as part of a management strategy to avoid outbreeding depression [16], leads to continued erosion of genetic variation over time [17]. Effects may be intensified in species with large body size (which limits numbers of individuals that can be protected), limited dispersal or mobility (which enhances isolation), long generation interval, low reproductive rate, and high parental investment (which hinder population growth) and monogamous mating (which exacerbates the effects of inbreeding). These traits are not uncommon among endangered species, but limit experimental study of long-term genetic consequences of isolation. Here, we present the results of a century-long natural experiment in post-bottleneck isolation of one such species, the little spotted kiwi (*Apteryx owenii*, LSK).

Kiwi (Family Apterygidae) are flightless ratite birds endemic to New Zealand. They are treasured icons of New Zealand culture and represent an ancient and distinctive evolutionary lineage occupying an unusual ecological niche. Five kiwi species are currently recognized, and all have experienced extreme reductions in abundance and geographical distribution owing to habitat destruction and the introduction of mammalian predators to New Zealand [18]. Management has focused on predator control and translocation of kiwi to predator-free sanctuaries, but the genetic consequences of such management are unclear.

LSK are the second rarest kiwi species, with approximately 1600 individuals remaining [18]. They were once found throughout mainland New Zealand but declined rapidly in the 1800s, and were extinct in the North Island by 1900 owing to introduced predators and an enormous trade in their skins for export to Europe for muffs and museum collections (figure 1*a*; [19,20]). In the South Island, LSK remained common until the early 1900s but then declined rapidly owing to predation from stoats, cats and dogs, and were virtually extinct by the 1980s [18]. Two populations are known to have survived beyond 1980: one on D'Urville Island and another on Kapiti Island (figure 1*a*; [18,21,22]). The D'Urville Island population appears to have been a natural remnant population that has since gone extinct [20,23]. Historical records suggest that the Kapiti Island population originated in October 1912 with the introduction of five birds from Jackson Bay of the South Island (figure 1*a*,*b*) and that no LSK have been translocated to Kapiti since ([24,25]; H. Robertson 2012, unpublished data; but see [23]). While all other LSK populations dwindled to extinction, the Kapiti population increased, and now represents the largest extant population comprising approximately 1200 birds [18].

The rapid expansion of the Kapiti population has raised suspicions that LSK were present on the island prior to the 1912 transfer. However, there are no known sightings of LSK on Kapiti prior to 1929 despite there being a significant indigenous Maori settlement, whaling station and multiple farms on the island. In addition, the Inspector of Scenic Reserves, E. Phillip Turner, noted the absence of kiwi on Kapiti in March 1912 and suggested they be transferred there 'before they become extinct' [26]. Finally, it is entirely possible that the Kapiti population could have grown to its current size from just five founders. Mean annual population growth rate in LSK populations 3–14 years post founding is 7.7 per cent (range 4–11%; [21]; H. Robertson 2012, unpublished data). Assuming exponential growth at this rate, the Kapiti population would have reached 1200 birds by the mid-1980s.

The last-known surviving D'Urville Island LSK were captured and moved to the predator-free sanctuary of Long Island in 1982 (one female) and 1987 (figure 1*a*,*b*; one male). Two males from Kapiti were initially placed on Long Island with the D'Urville female, but one was replaced with a Kapiti female in 1989 [21,23]. Thus, a maximum of five birds representing two source populations could have contributed to the founding of the Long Island population (figure 1*b*; two D'Urville, three Kapiti), four of which potentially remain, including one male and one female from D'Urville Island. Six additional populations have been founded since 1983, each with 12–40 LSK from Kapiti (figure 1*a*,*b*; [21]; R. Colbourne 2012, unpublished data). As a result, seven of eight extant populations have arisen from a single source population, which itself may have been founded from only five LSK. The Long Island population is the only extant population with the potential to harbour genetic diversity from another source, D'Urville Island.

Adult LSK form socially monogamous pair bonds that can persist for decades, and mating pairs typically produce one to two chicks per year [18,22]. It is difficult to determine the generation interval (mean age of parents) for kiwi, because their maximum age and latest age of reproduction are unknown. Estimated generation interval of North Island brown kiwi is 15 years, assuming first reproduction occurs at 4 years of age, a maximum lifespan of 100 years and lower survival at greater ages (E. Weiser & I. Jamieson 2012, unpublished data). LSK reproduce annually, can begin reproducing at two years of age, have an estimated mean life expectancy of 45 years (95%CI 27–83 years; [27]), and are able to produce young at least into their 30s (R. Colbourne & H. Taylor 2012, unpublished data). Thus, LSK are capable of high rates of reproduction and 20–25 years is a reasonable estimate of their generation interval, with all populations having been recently founded (perhaps four to five generations for Kapiti Island and a single generation for all others).

An understanding of the current amount of genetic variation in LSK and its distribution among populations is urgently needed [18]. LSK have experienced recent and acute genetic bottlenecks and subsequent isolation, and it is imperative that management maintains the genetic variation remaining. In this study, we assess the amount and distribution of genetic variation among four extant populations of LSK and ask: (i) how much genetic variation remains in the source population of Kapiti Island? (ii) how much genetic variation was lost with the founding of new populations? and (iii) does the Long Island population contain genes contributed by the D'Urville Island founders?

2. Material and methods

(a) Sample collection and microsatellite genotyping

Six to eight pin feathers (developing feathers containing blood) were plucked from 167 LSK and blood drawn from 14 LSK for a total of 14–99 birds sampled from each of four populations (table 1 and figure 1*a*; Kapiti, Tiritiri Matangi, Red Mercury and Long Islands). Feathers were also collected from the two D'Urville LSK translocated to Long Island. Samples were assigned to the island on which the bird hatched, regardless of where the bird may have been moved to subsequently. The Kapiti sample included 14 of the 16 founders placed on Tiritiri Matangi Island and the two Kapiti founders still residing on Long Island. Handling and sampling of kiwi was carried out according to the Kiwi Best Practice Manual [28], under New Zealand Department of Conservation authority, with approval of the Victoria University of Wellington Animal Ethics Committee, and with guidance from the indigenous Maori communities of the sampling areas.

Genomic DNA was extracted from feathers with a Qiagen DNEasy extraction kit (Qiagen Inc) and from blood using a standard proteinase K phenol–chloroform protocol [29] followed by ethanol precipitation. Individuals were amplified at 15 microsatellite loci via polymerase chain reaction (PCR) in an Eppendorf mastercycler and visualized with universal M13 fluorescent labelling [30]. Loci, PCR conditions and amplicon-sizing procedures were as described in Ramstad *et al*. [31], with the exception of *Apt 59* which was amplified as in Shepherd & Lambert [32]. Reactions containing all constituents but template DNA were included in each PCR to detect sample contamination. Genotypes obtained from pin feather and blood samples were identical within 14 birds amplified at five loci, thus only feather samples were subsequently genotyped. Loci amplified successfully in 93 per

Table 1. Population size, founding history and genetic diversity of four populations at 15 microsatellite loci. (N_C, current estimated census population size (H. Robertson & R. Colbourne 2012, unpublished data); N_F, number of founders; *n*, number of birds genotyped; N_e, effective population size based on gametic disequilibrium and AR, allelic richness standardized to a sample size of 14 diploid individuals. Heterozygosity (H_E) is that expected at Hardy–Weinberg proportions with loss H_E expressed relative to the source population of Kapiti Island. Number of founders (N_F) includes all birds that could have potentially produced young on a given island. Effective founders is the estimated number of founders to explain the observed loss in H_E by a single generation bottleneck using the expected rate of loss of heterozygosity of $1/(2N + 1)$. $^{***}p < 0.0001$.)

population	N_C	N_F	year founded	*n*	N_e (95% CI)	mean H_E	loss H_E (%)	effective founders	mean F_{IS}	mean AR	no. fixed loci
Kapiti (source)	1200	5	1912	99	38.6 (11.2–130.9)	0.391	—	—	−0.035	2.4	0
Long	45	5	1982–9	14	—	0.315	20	2.1	−0.344***	1.8	5
Red Mercury	60	12	1983	27	12.3 (6.1–23.1)	0.356	9	5.0	0.027	2.2	2
Tiritiri Matangi	65	16	1993–5	27	3.6 (2.4–7.4)	0.376	4	12.6	−0.039	2.1	1

cent (*Aptowe 23*) to 100 per cent (*Aptowe 3*, *7* and *31*, *Apt 59*) of individuals assayed with a mean successful amplification rate of 98.6 per cent over all loci. Repeat amplification of 252 genotypes (10% of the final dataset) revealed four genotyping errors from three loci and a mean genotyping error rate of 1.6 per cent. Microsatellite genotypes were deposited in the Dryad repository (http://dx.doi.org/10.5061/dryad.nm341; [33]).

(b) Statistical analyses

Allelic richness (AR, the number of alleles corrected for sample size; [34]) per population and locus were calculated in FSTAT v. 2.9.3 [35]. Observed (H_O) and expected (H_E) heterozygosity were calculated and departures from Hardy–Weinberg proportions (HWP) assessed in GENEPOP v. 3.3 [36]. We tested for an excess of heterozygotes ($F_{IS} < 0$) according to Rousset & Raymond [37] for each population (over all loci) and for individual loci within populations after sequential Bonferroni correction for multiple comparisons [38]. Gametic disequilibrium (GD; sometimes referred to as linkage disequilibrium; [39]) was assessed for all pairwise locus comparisons within populations in GENEPOP after sequential Bonferroni correction for multiple comparisons between loci [38].

Recently bottlenecked populations often exhibit a mode shift in allelic frequency distribution owing to loss of rare alleles [40,41], and increased heterozygosity relative to that expected at mutation–drift equilibrium [42–44] owing to loss of alleles generally. We therefore assessed mode shifts in allelic frequencies within populations graphically [40] and heterozygosity excess (relative to a non-bottlenecked population in mutation–drift equilibrium having the same number of alleles) in BOTTLENECK v. 1.2.02 [42,45]. For the latter test, we assumed a two-phase mutation model (variance of 12.0, probability of 95%) appropriate for microsatellites [45,46] and assessed significance over all loci with a one-tailed Wilcoxon signed-rank test [42,43].

It is important to distinguish excess of heterozygotes from heterozygosity excess. The former compares the number of observed heterozygotes with that expected under HWP given the allele frequencies of the population [37], whereas the latter assesses mean H_E observed in a population relative to that expected of a non-bottlenecked population with the same number of alleles [42,43]. An excess of heterozygotes can be owing to differences in allele frequencies between sexes [47], non-random mating or hybridization, and genotypic proportions will return to HWP within a single generation of random mating [39]. Heterozygosity excess is an indication of a recent genetic bottleneck effect and can persist for a number of generations equal to four times the effective population size (N_e; [40,42]).

For populations where at least 20 birds were genotyped, inbreeding N_e was estimated in LDNe v. 1.31 [48,49]. N_e calculations assumed a monogamous mating system and excluded alleles present in a frequency of less than 0.05 to minimize bias owing to rare alleles [49]. Confidence intervals (95% CI) for N_e were calculated by jackknifing disequilibrium values among pairs of loci [48]. Long Island was excluded from this analysis, because low numbers of individuals ($n = 14$) and polymorphic loci ($n = 10$) provided highly imprecise N_e estimates [49]. Because GD-based estimates of N_e can be downwardly biased in species with overlapping generations, we also calculated N_e for the Kapiti Island population in ONESAMP, which uses an approximate Bayesian computation and eight summary genetic statistics to estimate N_e [50]. For the ONESAMP analysis, we assumed a true N_e between two and 500 individuals as priors. For recently translocated populations, number of effective founders was estimated by $1/(2N + 1)$, and reflects the amount of heterozygosity expected to be lost in a single generation with N founders [51].

The proportion of total genetic variation among birds owing to population subdivision was estimated as F_{ST} in FSTAT according to Weir & Cockerham [52], with significance assessed by comparison with a null distribution computed by bootstrapping over loci. Pairwise tests for heterogeneity of allelic frequencies between populations were conducted in GENEPOP. Significance was assessed for each pairwise population comparison by combining over all loci using Fisher's combined probability test [53], and for individual loci after sequential Bonferroni correction for multiple comparisons [38].

3. Results

(a) Genetic variation

Populations displayed between one and five alleles per locus and 27 (Long) to 38 (Kapiti) alleles in total across 15 loci. When all loci were combined, F_{IS} did not differ statistically from zero in the Kapiti, Red Mercury and Tiritiri Matangi populations, but was significantly less than zero in the Long Island population (table 1; $p < 0.0001$, $F_{IS} = -0.344$). A significant excess of heterozygotes (negative F_{IS}) was evident in a single locus in the Kapiti population (*Aptowe 3*; $p = 0.001$), and three loci in the Long population (*Aptowe 29*, *31*, *35*; $p = 0.003$) when loci were considered individually. GD was found between *Aptowe 1* and *29* in the Kapiti and Red Mercury populations, between *Aptowe 1* and *31* in the Kapiti and Tiritiri Matangi populations, and between *Aptowe 29* and *31* in the Kapiti population. No pairs of loci exhibited GD in more than two populations surveyed.

(b) Bottleneck signatures

Significant signs of recent and acute genetic bottleneck effects were found in all four populations. All populations had low H_E (0.315–0.391) and AR (1.8–2.4), and recently translocated populations exhibited reduced genetic variation relative to their source population on Kapiti Island (table 1). Loss of heterozygosity in recently translocated populations relative to Kapiti Island ranged from 4 to 20 per cent, and was consistent with a single generation bottleneck of 2–13 effective founders (table 1). Recently translocated populations also had a reduced total number of alleles (27–33 alleles), reduced AR, and fewer polymorphic loci than Kapiti Island (table 1; 38 alleles). The Long Island population had the lowest H_E and AR, and the highest number of fixed loci of all the populations surveyed (table 1).

Recently translocated populations displayed shifted modes in their allelic frequency distributions (figure 2). Tiritiri Matangi and Red Mercury LSK had lower proportions of rare alleles (0.094 and 0.156, respectively) than Kapiti LSK (0.184), and the Long Island population had no alleles present at a frequency less than 0.2 (figure 2). Kapiti birds used to found Tiritiri Matangi and Long Islands lacked four and 11 alleles, respectively, found at low frequency (Tiritiri Matangi < 0.10, Long < 0.27) in the Kapiti population. Kapiti LSK did not exhibit a shifted mode, but had a lower proportion of rare alleles than would be expected of neutral loci in a population at mutation : drift equilibrium [40,54]. All four populations displayed significant heterozygosity excess ($p < 0.025$), with the most extreme signal found among Long Island LSK ($p < 0.001$; figure 3).

(c) Genetic divergence

The Kapiti population exhibited strong genetic divergence from the Long ($F_{ST} = 0.109$) and Tiritiri Matangi ($F_{ST} = 0.019$;

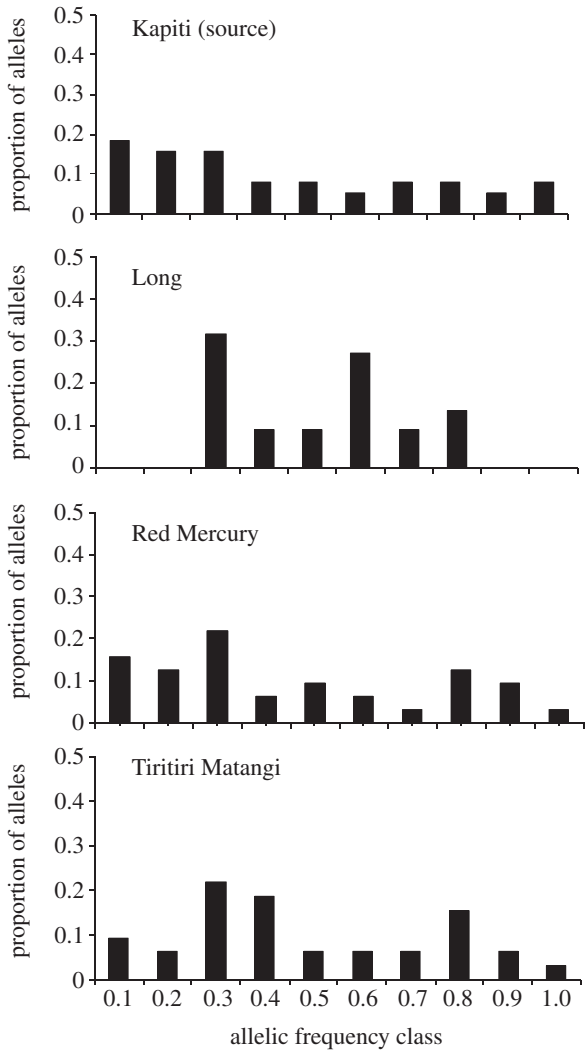

Figure 2. Distribution of allelic frequencies within four populations at 15 microsatellite loci.

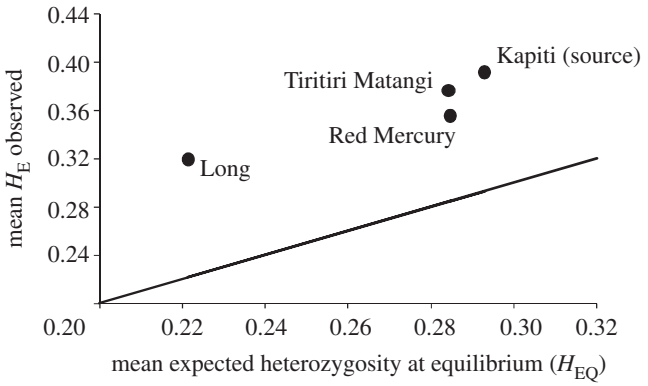

Figure 3. Relationship between mean expected heterozygosity observed (H_E) and expected at mutation : drift equilibrium (H_{EQ}) at 15 microsatellite loci for four LSK populations. Line represents equality between H_E and H_{EQ} under a two-phase model of mutation.

Table 2. Genetic differentiation among four populations at 15 microsatellite loci. (Pairwise F_{ST} is given above the diagonal, with significance based on bootstrapping over loci. Number of loci with significant differences in allelic frequencies ($p \leq 0.05$ after sequential Bonferroni correction) are given below the diagonal; all comparisons were significant when combined over all loci (Fisher's combined probability). *$p < 0.05$; **$p < 0.01$.)

	Kapiti (source)	Long	Red Mercury	Tiritiri Matangi
Kapiti	—	0.109**	0.010	0.019**
Long	6	—	0.165**	0.171**
Red Mercury	0	4	—	0.023*
Tiritiri Matangi	1	7	2	—

$p < 0.01$) populations, but no divergence from the Red Mercury population (table 2; $F_{ST} = 0.010$, statistically equal to zero). Allelic frequencies differed significantly over all loci between the Kapiti Island and all recently translocated populations ($p < 0.02$), with the Kapiti population differing significantly from the Long population at six loci and the Tiritiri Matangi population at a single locus, but not differing from the Red Mercury population at any pairwise locus comparison (table 2).

All recently translocated populations showed strong genetic divergence from one another. Pairwise F_{ST} between recently translocated populations was significantly greater than zero (table 2; range 0.023–0.171, $p < 0.05$), with the Long Island population exhibiting the greatest F_{ST} values and strong divergence from the Red Mercury and Tiritiri Matangi populations (table 2). Significant differences in allelic frequencies were evident in all pairwise population comparisons when loci were combined ($p < 0.0001$), reflecting differences in allelic frequencies at two to seven loci per comparison (table 2).

(d) Effective population size

Kapiti Island N_e estimated by LDNe (38.6, 11.2–130.9 95% CI) did not differ statistically from the estimate obtained in ONESAMP (39.1, 30.1–71.8 95% CI). Thus, we further discuss only the inbreeding N_e estimates from LDNe, which ranged from 3.6 to 38.6 birds and was much lower than census size estimates (N_C) in all populations (table 1). The number of founders is less than N_e on Kapiti, equals N_e on Red Mercury, and is significantly greater than N_e on Tiritiri Matangi Island (table 1). Effective to census population size ratio (N_e/N_C) was less than 0.10 for the Kapiti (0.03, 0.01–0.11 95% CI) and Tiritiri Matangi (0.06, 0.04–0.11 95% CI) populations, and 0.21 (0.10–0.39 95% CI) for the Red Mercury population.

(e) Long Island founder event

The two D'Urville birds translocated to Long Island are genetically distinct from both the Kapiti and Long Island populations. At the 15 loci genotyped, there are 12 alleles present in the D'Urville birds that are absent in both the Kapiti founders and LSK hatched on Long Island. For example, the Kapiti founders and the Long Island hatched birds are fixed for the same allele at *Aptowe 2*, whereas the two D'Urville birds are fixed for a different allele. Similarly, the Kapiti founders and D'Urville birds have different alleles at *Aptowe 8*, *23* and *35*, and the Long Island birds exhibit only those found in the Kapiti founders.

Nearly all of the Long Island LSK are probably the first-generation progeny of a single mating pair. For example, at

Table 3. Genotypes and allelic frequencies observed (expected) in 14 Long Island LSK. (Letters designate alleles, and expected values assume Mendelian segregation of the genotypes of the two Kapiti Island founders remaining on Long Island. $F_{IS} <$ zero; $^{**}p < 0.01$; $^{***}p < 0.0001$.)

	genotype						allelic frequency		
locus	*aa*	*ab*	*bb*	*ac*	*bc*	*cc*	*a*	*b*	F_{IS}
Aptowe 2, 7, 8, 24, 34	14	0	0	—	—	—	1.0	—	—
	(14)	(0)	(0)	—	—	—	(1.0)	—	—
Aptowe 3	3	8	3	—	—	—	0.500	—	−0.106
Aptowe 15	2	8	4	—	—	—	0.429	—	−0.130
Aptowe 23	6	7	1	—	—	—	0.679	—	−0.110
	(3.5)	(7)	(3.5)	—	—	—	(0.500)	—	—
Aptowe 31	1	13	0	—	—	—	0.464	—	−0.857**
	(0)	(14)	(0)	—	—	—	(0.500)	—	—
Aptowe 1	8	6	0	—	—	—	0.786	—	−0.238
Aptowe 28	7	7	0	—	—	—	0.750	—	−0.300
	(7)	(7)	(0)	—	—	—	(0.750)	—	—
Aptowe 39	0	9	5	—	—	—	0.321	—	−0.444
Apt 59	0	6	8	—	—	—	0.214	—	−0.238
	(0)	(7)	(7)	—	—	—	(0.250)	—	—
Aptowe 29	0	0	0	7	6	1	0.250	0.214	−0.509**
	(0)	(0)	(0)	(7)	(7)	(0)	(0.250)	(0.250)	—
Aptowe 35	0	6	1	0	7	0	0.214	0.536	−0.509**
	(0)	(7)	(0)	(0)	(7)	(0)	(0.250)	(0.500)	—
mean									−0.344***

four loci (*Aptowe 1*, *28*, *39* and *Apt 59*), three of the four alleles present in the Kapiti founders are identical, but no Long Island hatched birds are homozygous for the rare allele, which would indicate a second or later-generation offspring (table 3). F_{IS} values are negative across all 10 polymorphic loci in the Long Island population, yielding a 34 per cent mean excess of heterozygotes (table 3). F_{IS} values were significantly less than zero at *Aptowe 29*, *31* and *35*, which displayed exceptionally high proportions of heterozygotes (0.93) and suggests that Long Island LSK are progeny of two individuals that are homozygous for different alleles at these loci. In addition, the allelic frequencies among Long Island LSK approximate 1.0, 0.75, 0.5 and 0.25, which are the only allelic frequencies possible in a full-sibling family (table 3). Three birds are homozygous at one of *Aptowe 29*, *31* or *35* (table 3) and so cannot be first-generation offspring of the Kapiti founders to Long Island. These birds probably represent second-generation offspring or have resulted from first-generation offspring backcrossing with one of the founders.

4. Discussion

(a) Founder effects

All four populations surveyed exhibit signs of recent and acute genetic bottleneck effects, including low H_E and AR, loss of rare alleles, GD and heterozygosity excess. In recently translocated populations, genetic diversity was reduced relative to the source population of Kapiti Island and modes of allelic frequencies were shifted. These are signatures expected of genetic bottleneck effects that occurred less than five generations (approx. 100 years) ago and involved fewer than 20 founders [40,42,55], making our data consistent with historical LSK translocation records.

Recently translocated LSK populations have experienced an additional genetic bottleneck associated with their founding. Loss of heterozygosity relative to Kapiti suggests a single generation bottleneck of two to 13 effective founders, which in all cases is lower than the actual number of founders transferred to the island. Low genetic diversity is coupled with strong genetic divergence, such that recently translocated populations do not reflect the allelic frequencies of their source population on Kapiti. Genetic differences among populations are due to genetic drift and do not represent genetic divergence resulting from selection or local adaptation. Reduced genetic diversity and strong genetic divergence of recently translocated populations relative to Kapiti are due to a small number of founders and failure of some founders to produce offspring post-translocation. For example, founders to Tiritiri Matangi lacked four rare alleles found among Kapiti LSK, and two of the founders died within a year of release, contributing little, if any, to recruitment [56].

(b) N_e/N_C and continuing genetic erosion

The large census size of the Kapiti Island population resulted in a mean N_e/N_C ratio of 0.03, which is exceptionally low for terrestrial vertebrates [57,58]. Empirical estimates among wild populations provide median N_e/N_C values of 0.10–

0.15, with large variation among populations and species [57,58]. Mean N_e/N_C was 0.45 among 52 terrestrial vertebrate species [57], with several N_e/N_C estimates less than 0.10 (leopard frog, *Rana pipiens*, 0.01; great toad, *Bufo marinus*, 0.016–0.088; white-winged wood duck, *Cairina scutulata*, 0.052–0.094; bison, *Bison bison*, 0.069–0.084; red-spotted newt, *Notophthalmus viridescens*, 0.073; acorn woodpecker, *Melanerpes formicivorus*, 0.09). The N_e/N_C for Kapiti Island LSK clearly is at the lower end of all these scales.

The accuracy of our Kapiti Island N_e estimate is unclear as it may be downwardly biased owing to residual GD from the Kapiti Island founding event, overlapping generations, or population subdivision. Residual GD may be of minor effect given its expected high rate of post-bottleneck decay [55,59]. However, it is unclear how quickly GD from the Kapiti founder effect might decay and similarly the magnitude of its effect on our N_e estimate. The long generation interval and overlapping generations of LSK would allow birds from several different generations to pair and produce young, potentially resulting in mixture disequilibrium that could downwardly bias N_e estimates [49,58]. Finally, LSK were sampled primarily from the northern half of Kapiti Island. Thus, our N_e estimate may reflect only the northern part of the island and not the Kapiti population as a whole [59]. Assuming half of the 1200 LSK on Kapiti inhabit the northern half of the island, our N_e/N_C estimate would be 0.06 and still well below that expected of terrestrial vertebrate populations [57].

Inbreeding N_e is a measure of the rate of loss of heterozygosity [58], and our estimate suggests that Kapiti Island LSK are continuing to lose heterozygosity at a rate between 0.4 per cent and 4 per cent per generation. The low N_e/N_C ratio suggests that the Kapiti population is more sensitive to genetic stochasticity than its census size would indicate, and therefore at greater conservation risk than currently recognized. The low N_e of the Tiritiri Matangi and Red Mercury Island populations and the apparently small numbers of breeding LSK on Long Island suggest these recently translocated populations are also continuing to lose genetic variation over time owing to drift. These populations will thus be at even greater risk than the Kapiti Island population as they have lower N_e, lower N_C and were derived from Kapiti.

(c) Loss of D'Urville Island genetic diversity

The effort to save what was thought to be the last-surviving LSK from the last remnant population appears to have failed. Although a D'Urville male and female were put on Long Island with Kapiti LSK approximately 30 years ago, none of the Long Island birds sampled had microsatellite alleles that are private to the D'Urville birds. The genotypic frequencies in the Long Island birds are consistent with nearly entirely (11 of 14 genotyped) first-generation offspring of two founders from Kapiti Island. Three birds are exceptions to this pattern but clearly not of D'Urville descent. The most parsimonious explanation of our data is that the Long Island population arose from only two Kapiti birds of the original five founders placed on the island and that these three birds represent their second- or later-generation offspring. A 20 per cent loss of heterozygosity in Long Island LSK relative to the Kapiti population supports this conclusion as it equals the loss expected from a single generation bottleneck of two individuals. Low N_e has also resulted in the large and negative F_{IS} observed among Long Island LSK.

The lack of D'Urville offspring on Long Island was unexpected, and causes of the probable reproductive failure of the D'Urville founders are unclear. The D'Urville founders could have died some time ago (the female was last detected in 1988, the male in 1995; R. Colbourne 2000, unpublished data) or been infertile or past reproductive age when translocated. It is also possible that there are D'Urville offspring on Long Island that have not yet been detected. Our data represent 14 of approximately 40 LSK estimated to have hatched on Long Island [60], and further surveys are warranted.

Sampling founders is critical to understanding the genetic effects of translocation. Feather samples from the D'Urville birds placed on Long Island allowed us to determine that these birds were genetically divergent from those on Kapiti and did not parent any of the Long Island LSK genotyped. It is remarkable and extremely fortunate that managers thought to collect feathers from the D'Urville birds prior to the advent of PCR [61] and the widespread use of microsatellites and non-invasive sampling. At present, it should be standard management practice to collect tissue samples from all translocated individuals, kiwi or otherwise, to determine their subsequent reproductive success.

(d) Conservation implications

LSK have the lowest genetic diversity of all the kiwi species [31], an observation that cannot be explained solely by the Kapiti Island founder effect, as the heterozygosity reduction in LSK relative to other kiwi species (mean 41%, range 34–46%; [31]) far exceeds that expected of a bottleneck of five birds (10%). A prolonged period at low population size also cannot explain this extreme loss of heterozygosity as LSK have only been on Kapiti Island for approximately five generations and have recovered quickly demographically in that time. The low overall genetic diversity in LSK also reflects historical losses that pre-date the Kapiti Island bottleneck [62]. In particular, extirpation of LSK from mainland New Zealand resulted in a significant loss of genetic diversity, which was substantially higher among historical ($n = 9$ birds collected 1888–1987, AR = 4.3) than extant LSK ($n = 99$ Kapiti Island birds, AR = 2.3; K. Ramstad 2012, unpublished data).

The Kapiti Island population is currently the best hope for the long-term survival of LSK in the wild—it has the largest population, the greatest genetic diversity and is the source of all other LSK populations. However, the Kapiti Island population displays clear evidence of having experienced a strong genetic bottleneck, and a maximum of five alleles per locus suggests the number of founders could be as few as three birds.

How can we reconcile the extremely low genetic diversity in LSK with their strong population expansion? Demographic dynamics relative to loss of genetic diversity are complex and difficult to predict. Many populations have grown in size following extreme losses of genetic variation [6,63,64]. This is because population growth rates may be reduced following a bottleneck, but remain positive. Thus, a small population exhibiting reduced genetic diversity and suffering substantial inbreeding depression may still be growing in size [6,63], and growing populations with large census sizes might still be suffering reduced fitness owing to low genetic diversity and inbreeding [64]. Both of these scenarios describe

populations that are vulnerable to changing environments and stochastic events.

There has been considerable debate regarding the appropriate population size thresholds for conservation [65–67]. As a guiding principle, an isolated population should have a minimum N_e of 50 to avoid inbreeding depression and ensure short-term persistence and a minimum N_e of 500 to maintain long-term adaptive potential [67,68]. The estimated N_e of Kapiti Island LSK (38.6, 11.2–130.9 95% CI) places this population near and below these two threshold sizes. Kapiti Island is thought to be at carrying capacity with 400–500 territory holding pairs of LSK (H. Robertson & R. Colbourne 2012, unpublished data). Most pairs attempt to breed annually, and common encounters with juveniles and subadults suggest successful recruitment. However, our N_e estimate of approximately 40 birds indicates that fewer birds are effectively contributing to recruitment than census data suggest. This could be owing to strong variance in reproductive success among breeding pairs or significant bias in our N_e estimate [57]. Confirming the validity of the surprisingly low N_e/N_C estimate for Kapiti Island LSK will be crucial for their future management.

LSK are the only kiwi species not considered threatened by the International Union for Conservation of Nature (near threatened) and the New Zealand Department of Conservation (DOC) (at risk/recovering; [69,70]). This is because all LSK populations are protected in predator-free sanctuaries, have increased in size to near carrying capacity, and appear to lack ill effects from inbreeding or low genetic variation [18,21]. Nevertheless, LSK are conservation-dependent. All populations with the exception of Kapiti are too small to be demographically viable or act as reservoirs of genetic variation, and predator-free status of LSK sanctuaries requires active management. The loss of genetic variation resulting from the Kapiti founding event is a potential threat to the long-term persistence of LSK because reduced fitness may only become apparent under future environmental stress [71,72, but see 6,73,74]. For example, LSK have extremely low variation at major histocompatibility complex genes [75], suggesting they may be limited in their ability to respond effectively to disease challenges. Our results support the DOC's current plan to translocate and subsequently monitor additional Kapiti birds to the smaller, recently founded LSK populations. This will make LSK a metapopulation occupying different sites that are connected through migration, and offer some protection against the threats of introduced predators and genetic erosion.

Intense protection has ensured a century of survival of the LSK despite their extinction on mainland New Zealand. It has also resulted in extreme loss of genetic variation and isolation of LSK populations. In the case of the D'Urville Island LSK, protection of the last remaining individuals could not guard against reproductive failure and extinction. The survival of LSK thus far provides hope for the survival of similarly isolated and bottlenecked species. Certainly low genetic diversity does not doom a species to imminent extinction [73,74]. It can, however, result in poor fitness that may become apparent only under stressful environmental conditions [71,72], precisely when at-risk species are most vulnerable. Thus, genetic risks to population persistence may be greater than apparent, even at large census population sizes and for growing populations.

Handling and sampling of kiwi was carried out according to the Kiwi Best Practice Manual [28], under New Zealand Department of Conservation authority, with approval of the Victoria University of Wellington Animal Ethics Committee, and with guidance from the indigenous Maori communities of the sampling areas.

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References

1. Olecha W, Perzanowskib K. 2002 A genetic background for reintroduction program of the European bison (*Bison bonasus*) in the Carpathians. *Biol. Conserv.* **108**, 221–228. (doi:10.1016/S0006-3207(02)00108-8)
2. Jiang Z, Yu C, Feng Z, Zhang L, Xia J, Ding Y, Lindsay N. 2000 Reintroduction and recovery of Père David's deer in China. *Wildl. Soc. Bull.* **28**, 681–687.
3. Weeks AR *et al*. 2011 Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evol. Appl.* **4**, 709–725. (doi:10.1111/j.1752-4571.2011.00192.x)
4. O'Brien SJ *et al*. 1985 Genetic basis for species vulnerability in the cheetah. *Science* **227**, 1428–1434. (doi:10.1126/science.2983425)
5. Towns DR, Ballantine WJ. 1993 Conservation and restoration of New Zealand island ecosystems. *Trends Ecol. Evol.* **8**, 452–457. (doi:10.1016/0169-5347(93)90009-E)
6. Ardern SL, Lambert DM. 1997 Is the black robin in genetic peril? *Mol. Ecol.* **6**, 21–28. (doi:10.1046/j.1365-294X.1997.00147.x)
7. Fredrickson RJ, Siminski P, Woolf M, Hedrick PW. 2007 Genetic rescue and inbreeding depression in Mexican wolves. *Proc. R. Soc. B* **274**, 2365–2371. (doi:10.1098/rspb.2007.0785)
8. Grueber CE, Laws RJ, Nakagawa S, Jamieson IG. 2010 Inbreeding depression accumulation across life-history stages of the endangered takahe. *Conserv. Biol.* **24**, 1617–1625. (doi:10.1111/j.1523-1739.2010.01549.x)
9. Gusset M, Slotow R, Stomers MJ. 2006 Divided we fail: the impact of social integration for the re-introduction of endangered African wild dogs (*Lycaon pictus*). *J. Zool.* **270**, 205–511. (doi:10.1111/j.1469-7998.2006.00168.x)
10. Brashares JS, Werner JR, Sinclair ARE. 2010 Social 'meltdown' in the demise of an island endemic: Allee effects and the Vancouver Island marmot. *J. Anim. Ecol.* **79**, 965–973. (doi:10.1111/j.1365-2656.2010.01711.x)
11. Jamieson IG, Lacy RC. 2012 Managing genetic issues in reintroduction biology. In *Reintroduction biology: integrating science and management* (eds JG Owen, DP Armstrong, KA Parker, PJ Seddon), pp. 441–475. New York, NY: Wiley-Blackwell.
12. Reed DH, Frankham R. 2003 Correlation between fitness and genetic diversity. *Conserv. Biol.* **17**, 230–237. (doi:10.1046/j.1523-1739.2003.01236.x)
13. Spielman D, Brook BW, Frankham R. 2004 Most species are not driven to extinction before genetic factors impact them. *Proc. Natl Acad. Sci. USA* **101**, 15 261–15 264. (doi:10.1073/pnas.0403809101)

14. Jamieson IG. 2011 Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. *Conserv. Biol.* **25**, 115–123. (doi:10.1111/j.1523-1739.2010.01574.x)
15. Moore JA, Nelson NJ, Keall SN, Daugherty CH. 2008 Implications of social dominance and multiple paternity for the genetic diversity of a captive-bred reptile population (tuatara). *Conserv. Genet.* **9**, 1243–1251. (doi:10.1007/s10592-007-9452-6)
16. Edmands S. 2007 Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol.* **16**, 463–475. (doi:10.1111/j.1365-294X.2006.03148.x)
17. Wright S. 1951 The genetical structure of populations. *Ann. Eugen.* **15**, 323–354.
18. Holzapfel SA, Robertson HA, McLennan JA, Sporle W, Hackwell K, Impey M. 2008 Kiwi (*Apteryx* spp.) recovery plan 2008–2018. In *Threatened species recovery plan,* 60. Wellington, New Zealand: Department of Conservation.
19. Worthy TH, Holdaway RN. 2002 *The lost world of the moa: prehistoric life of New Zealand*. Bloomington: Indiana University Press.
20. Heather BD, Robertson HA. 2005 *The field guide to the birds of New Zealand*. Auckland: Viking.
21. Colbourne RM, Robertson HA. 1997 Successful translocations of little spotted kiwi (*Apteryx owenii*) between offshore islands of New Zealand. *Notornis* **44**, 253–258.
22. Jolly J. 1989 A field study of the breeding biology of the little spotted kiwi (*Apteryx owenii*) with emphasis on the causes of nest failures. *J. R. Soc. NZ* **19**, 433–447.
23. Jolly JN, Daugherty CH. 2002 Comparison of little spotted kiwi (*Apteryx owenii*) from Kapiti and D'Urville Islands. In *Science and research internal report*, pp. 57–64, 191. Wellington, New Zealand: Department of Conservation.
24. Turner EP. 1913 Report by the Inspector of Scenic Reserves. In Annual report of the Department of Lands & Survey, Appendix B. New Zealand Journal of the House of Representatives.
25. Turner EP. 1929 *Correspondence to Johannes Anderson, Librarian, Alexander Turnbull Library*. Document 32/3/2. Wellington, New Zealand: State Forest Service.
26. Turner EP. 1912 Report by the Inspector of Scenic Reserves. In Annual report of the Department of Lands & Survey, Appendix B. New Zealand Journal of the House of Representatives.
27. Robertson HA, Colbourne RM. 2004 Survival of little spotted kiwi (*Apteryx owenii*) on Kapiti Island. *Notornis* **51**, 161–163.
28. Robertson H, Colbourne R, Castro I, Miller C, Cresswell M. 2003 *Kiwi* (Apteryx *spp.*) *best practice manual*. Wellington, New Zealand: Department of Conservation.
29. Sambrook J, Fritsch EF, Maniatis T. 1989 *Molecular cloning: a laboratory manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
30. Schuelke M. 2000 An economic method for the fluorescent labeling of PCR fragments. *Nat. Biotechnol.* **18**, 233–234. (doi:10.1038/72708)
31. Ramstad KM, Pfunder M, Robertson HA, Colbourne RM, Allendorf FW, Daugherty CH. 2010 Fourteen microsatellite loci cross-amplify in all five kiwi species (*Apteryx* spp.) and reveal extremely low genetic variation in little spotted kiwi (*A. owenii*). *Conserv. Genet. Res.* **2**, 333–336. (doi:10.1007/s12686-010-9233-2)
32. Shepherd LD, Lambert DM. 2006 Nuclear microsatellite DNA markers for New Zealand kiwi (*Apteryx* spp.). *Mol. Ecol.* **6**, 227–229. (doi:10.1111/j.1471-8286.2005.01201.x)
33. Ramstad KM, Colbourne RM, Robertson HA, Allendorf FW, Daugherty CH. 2013 Data from: genetic consequences of a century of protection: serial founder events and survival of the little spotted kiwi (*Apteryx owenii*). *Dryad Digital Repository* (doi:10.5061/dryad.nm341)
34. El Mousadik A, Petit RJ. 1996 High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinos* (L.) Skeels] endemic to Morocco. *Theor. Appl. Genet.* **92**, 832–839. (doi:10.1007/BF00221895)
35. Goudet J. 1995 FSTAT (version 1.2): a computer program to calculate *F*-statistics. *J. Hered.* **86**, 485–486.
36. Raymond M, Rousset F. 1995 GENEPOP version 1.2: population genetics software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.
37. Rousset F, Raymond M. 1995 Testing heterozygote excess and deficiency. *Genetics* **140**, 1413–1419.
38. Holm S. 1979 A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**, 65–70.
39. Allendorf FW, Luikart G. 2007 *Conservation and the genetics of populations*. Malden, MA: Blackwell Publishing.
40. Luikart G, Allendorf FW, Cornuet JM, Sherwin WB. 1998 Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J. Hered.* **89**, 238–247. (doi:10.1093/jhered/89.3.238)
41. Allendorf FW. 1986 Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biol.* **5**, 181–190. (doi:10.1002/zoo.1430050212)
42. Cornuet JM, Luikart G. 1996 Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001–2014.
43. Luikart G, Cornuet JM. 1998 Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv. Biol.* **12**, 228–233. (doi:10.1046/j.1523-1739.1998.96388.x)
44. Maruyama T, Fuerst PA. 1995 Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics* **111**, 675–689.
45. Piry S, Luikart G, Cornuet JM. 1999 Bottleneck: a computer program for detecting recent reductions in effective size using allele frequency data. *J. Hered.* **90**, 502–503. (doi:10.1093/jhered/90.4.502)
46. Shriver MD, Jin L, Chakraborty R, Boerwinkle E. 1993 VNTR allele frequency distributions under the stepwise mutation model: a computer simulation approach. *Genetics* **134**, 983–993.
47. Robertson A. 1965 The interpretation of genotypic ratios in domestic animal populations. *Anim. Prod.* **7**, 319–324. (doi:10.1017/S0003356100025770)
48. Waples RS, Do C. 2008 LDNE: a program for estimating effective population size data on linkage disequilibrium. *Mol. Ecol. Res.* **8**, 753–756. (doi:10.1111/j.1755-0998.2007.02061.x)
49. Waples RS, Do C. 2010 Linkage disequilibrium estimates of contemporary *Ne* using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evol. Appl.* **3**, 244–262. (doi:10.1111/j.1752-4571.2009.00104.x)
50. Tallmon DA, Koyuk A, Luikart G, Beaumont MA. 2007 ONESAMP: a program to estimate effective population size using approximate Bayesian computation. *Mol. Ecol. Res.* **8**, 299–301. (doi:10.1111/j.1471-8286.2007.01997.x)
51. Wright S. 1931 Evolution in Mendelian populations. *Genetics* **16**, 97–159.
52. Weir BS, Cockerham CC. 1984 Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370. (doi:10.2307/2408641)
53. Sokal RR, Rohlf FJ. 1981 *Biometry: the principals and practice of statistics in biological research*, 2nd edn. San Fransisco, CA: WH Freeman and Company.
54. Nei M, Chakraborty R, Fuerst PA. 1976 Infinite allele model with varying mutation rate. *Proc. Natl Acad. Sci. USA* **73**, 4164–4168. (doi:10.1073/pnas.73.11.4164)
55. Waples RS. 2005 Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? *Mol. Ecol.* **14**, 3335–3352.
56. Girardet S. 2000 *Tools for protecting endangered species: eradication, translocation, triangulation*. Auckland, New Zealand: University of Auckland.
57. Frankham R. 1995 Effective population size/adult population size ratios in wildlife: a review. *Genet. Res.* **66**, 95–107. (doi:10.1017/S0016672300034455)
58. Palstra FP, Ruzzante DE. 2008 Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Mol. Ecol.* **17**, 3428–3447. (doi:10.1111/j.1365-294X.2008.03842.x)
59. Waples RS. 2006 A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conserv. Genet.* **7**, 167–184. (doi:10.1007/s10592-005-9100-y)
60. Robertson H, Colbourne R. 2008 Status of little spotted kiwi (*Apteryx owenii*) on Long Island, Marlborough Sounds, March–April 2008. DOCDM-317704. Wellington, New Zealand: Department of Conservation.
61. Mullis KB, Faloona FA. 1987 Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Meth. Enzymol.* **155**, 335. (doi:10.1016/0076-6879(87)55023-6)
62. Shepherd LD, Worthy TH, Tennyson AJD, Scofield RP, Ramstad KM, Lambert DM. 2012 Ancient DNA analyses reveal contrasting phylogeographic patterns amongst kiwi (*Apteryx* spp.) and a recently

extinct lineage of spotted kiwi. *PLoS ONE* **7**, e42384. (doi:10.1371/journal.pone.0042384)
63. Leberg PL. 1990 Influence of genetic variability on population growth: implications for conservation. *J. Fish Biol.* **37**(Suppl. A), 193–195. (doi:10.1111/j.1095-8649.1990.tb05036.x)
64. Williams SL. 2001 Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. *Ecol. Appl.* **11**, 1472–1488. (doi:10.1890/1051-0761(2001)011[1472:RGDIET]2.0.CO;2)
65. Frankham R, Brook BW, Bradshaw CJA, Traill LW, Spielman D. 2013 50/500 rule and minimum viable populations: response to Jamieson and Allendorf. *Trends Ecol. Evol.* (doi:10.1016/j.tree.2013.01.002)
66. Jamieson IG, Allendorf FW. 2012 How does the 50/500 rule apply to MVPs? *Trends Ecol. Evol.* **27**, 578–584. (doi:10.1016/j.tree.2012.07.001)
67. Jamieson IG, Allendorf FW. 2013 A school of red herring: reply to Frankham *et al*. *Trends Ecol. Evol.* (doi:10.1016/j.tree.2013.01.012)
68. Franklin IR. 1980 Evolutionary change in small populations. In *Conservation biology: an evolutionary-ecological perspective* (eds ME Soule, BA Wilcox), pp. 135–149. Sunderland, MA: Sinauer Associates.
69. IUCN. 2011 IUCN red list of threatened species, version 2011.2. www.iucnredlist.org. (accessed on 23 March 2012)
70. Miskelly CM, Dowding JE, Elliott GP, Hitchmough RA, Powlesland RG, Robertson HA, Sagar PM, Scofield RP, Taylor GA. 2008 Conservation status of New Zealand birds. *Notornis* **55**, 117–135.
71. Ambruster P, Reed DH. 2005 Inbreeding depression in benign and stressful environments. *Heredity* **95**, 235–242. (doi:10.1038/sj.hdy.6800721)
72. Keller LF, Grant PR, Grant BR, Petren K. 2002 Environmental conditions affect the magnitude of inbreeding depression in survival of Darwin's finches. *Evolution* **56**, 1229–1239.
73. Johnson JA, Tingay RE, Culver M, Hailer F, Clarke ML, Mindell DP. 2009 Long-term survival despite low genetic diversity in the critically endangered Madagascar fish-eagle. *Mol. Ecol.* **18**, 54–63. (doi:10.1111/j.1365-294X.2008.04012.x)
74. Milot E, Weimerskirch H, Duchesne P, Bernatchez L. 2007 Surviving with low genetic diversity: the case of albatrosses. *Proc. R Soc. B* **274**, 779–787. (doi:10.1098/rspb.2006.0221)
75. Miller HC, Bowker-Wright G, Kharkrang M, Ramstad KM. 2011 Characterisation of class II B MHC genes from a ratite bird, the little spotted kiwi (*Apteryx owenii*). *Immunogenetics* **63**, 223–233. (doi:10.1007/s00251-010-0503-7)