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How do reproductive skew and founder group size affect genetic diversity in reintroduced populations?

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Abstract

Reduced genetic diversity can result in short-term decreases in fitness and reduced adaptive potential, which may lead to an increased extinction risk. Therefore, maintaining genetic variation is important for the short- and long-term success of reintroduced populations. Here, we evaluate how founder group size and variance in male reproductive success influence the long-term maintenance of genetic diversity after reintroduction. We used microsatellite data to quantify the loss of heterozygosity and allelic diversity in the founder groups from three reintroductions of tuatara (*Sphenodon*), the sole living representatives of the reptilian order Rhynchocephalia. We then estimated the maintenance of genetic diversity over 400 years (~10 generations) using population viability analyses. Reproduction of tuatara is highly skewed, with as few as 30% of males mating across years. Predicted losses of heterozygosity over 10 generations were low (1–14%), and populations founded with more animals retained a greater proportion of the heterozygosity and allelic diversity of their source populations and founder groups. Greater male reproductive skew led to greater predicted losses of genetic diversity over 10 generations, but only accelerated the loss of genetic diversity at small population size (<250 animals). A reduction in reproductive skew at low density may facilitate the maintenance of genetic diversity in small reintroduced populations. If reproductive skew is high and density-independent, larger founder groups could be released to achieve genetic goals for management.

Keywords: heterozygosity, inbreeding, polygyny, *Sphenodon*, translocation, tuatara

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Introduction

Maintaining genetic diversity is a common goal for species management, yet routine management actions such as reintroductions may impose genetic bottlenecks. After a bottleneck, reduced genetic diversity can result in short-term decreases in fitness (inbreeding depression; Jamieson *et al*. 2007), increased extinction risk (Saccheri *et al*. 1998), and reduced evolutionary potential (Frankham 1999, 2005). Effective management of genetic diversity in reintroduced populations is therefore important for both short- and long-term success (Fitzsimmons *et al*. 1997; Armstrong & Seddon 2008), but founder group sizes are often small (Griffith *et al*. 1989), and reintroduced populations often have lower genetic diversity than their sources at neutral and functional loci (Williams *et al*. 2000; Miller & Lambert 2004). Predicting how management actions affect genetic diversity in reintroduced populations is therefore a priority both for species recovery (e.g. Towns 1999) and in the broader field of reintroduction biology (Armstrong & Seddon 2008).

Several factors will differentially affect the maintenance of genetic diversity in reintroduced populations. First, while reintroductions, particularly those with small founder groups, are likely to cause a genetic bottleneck and promote losses of genetic diversity, losses of diversity could be minimised when populations expand rapidly after reintroduction (Allendorf &

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Luikart 2007). Second, unequal reproduction (i.e. founder representation in the offspring) may lead to a small effective population size, but a high degree of generation overlap may minimise this effect (Nunney 1993). Lastly, density dependence in the mating and social systems (Kokko & Rankin 2006) may accelerate or slow the loss of genetic diversity.

In many species, mating success is unequal among individuals (Emlen & Oring 1977), and this variance can lead to decreases in effective population size (Anthony & Blumstein 2000). Indeed, highly polygynous mating systems, including harem and dominance polygyny, can lead to very low effective population sizes when generations are nonoverlapping (Nunney 1993; Parker & White 1997), but as the degree of generation overlap increases, the impact of varying reproductive success is harder to quantify. As the generation interval increases, the reduction in the effective population size should be minimised (Nunney 1993), yet at small population sizes, even a slight reduction in the effective population size could result in rapid genetic drift. The effect of variation in male reproductive success is unclear when population size is increasing (e.g. after reintroduction). If a few males dominate reproduction in a reintroduced population, inbreeding may increase rapidly and exacerbate the effects of a genetic bottleneck.

Mating systems and the determinants of male reproductive success are often density-dependent (Kokko & Rankin 2006). When males interact locally, for example, dominant males may thwart mating attempts of nearby subordinate males. When male–male competition increases with density, theory predicts that a smaller proportion of males will successfully mate at higher density (Kokko & Rankin 2006). However, this relationship does not always hold. Large male seed bugs (*Neacorphyus bicrucis*) defend territories against smaller males in order to acquire mates. At high density, these large males are less likely to monopolize territories, and smaller males are more likely to mate (McLain 1992). At very high densities, the energetic costs of site defence outweigh the reproductive benefits (Brown 1969; Emlen & Oring 1977), and males may abandon territoriality altogether (Maher & Lott 2000). Thus, variation in male reproductive success may change at differing population densities.

We used population viability analyses to assess how unequal reproductive success and founder group sizes influence the long-term maintenance of genetic diversity in reintroduced populations of tuatara. Tuatara (*Sphenodon*) are threatened reptiles endemic to New Zealand, and are the sole representatives of the ancient reptilian order Rhynchocephalia (Cree & Butler 1993). Reintroductions are commonly used for tuatara conservation, but male reproduction is highly skewed (only 25–30% of males mate, Moore *et al.* in press-a). We use the term reproductive skew to specify the percentage of males that do not mate during their lifetime (i.e. those excluded from mating by dominant males, Kokko & Rankin 2006).

The mating system of tuatara

Tuatara are medium-sized (~200 mm snout-vent length) and extremely long-lived (possibly 100 years, Dawbin 1982; Cree 1994). They reach sexual maturity around 14 years of age (Cree *et al.* 1992). Although males can breed every year, females reproduce asynchronously once every 4 years (Cree *et al.* 1992). Tuatara are territorial, and mate guarding results primarily in monogamy within a season, and polygyny and polyandry across seasons (Moore *et al.* in press-a). Reproduction is dominated by large males, as they monopolize areas where females are most dense and prevent smaller males from mating by interfering with courtships (Moore *et al.* in press-b). Large body size is the primary determinant of male reproductive success, but mate choice is also influenced by disassortative mating based on major histocompatability complex (MHC) genotype (Miller *et al.* 2009). Tuatara body size is most probably influenced by resource availability rather than genetic factors such as heterozygosity or MHC genotype (Miller *et al.* 2009), and there is no evidence for alternative male reproductive strategies (Moore *et al.* in press-a). Discrepancies in male reproductive success mean that male reproductive skew is high, with as few as 30% of males obtaining mates across years (i.e. 70% reproductive skew, Moore *et al.* in press-a). The level of reproductive skew may vary slightly with density, as the average body size of males that successfully mate is smaller in lower density habitats (Moore *et al.* in press-a). Further, in a captive population of eight tuatara, three of the four males successfully sired offspring over 15 years, but the largest male sired 80% of all offspring (Moore *et al.* 2008).

Conservation of tuatara by reintroduction

Tuatara were once found throughout mainland New Zealand, but are now restricted to ~30 offshore islands that are free of introduced mammalian predators (Cree & Butler 1993). Between 1995 and 2008, tuatara were reintroduced to 12 sites. A total of nine source populations were used, but only a single source was used to found each reintroduction. Five reintroductions were sourced from two islands: Stephens Island (two reintroductions) and North Brother Island (three reintroductions). Stephens Island (150 ha, 40°40′S, 174°00′E) has

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the largest population of tuatara (30–50 000 individuals, Newman 1987) and high levels of genetic diversity at both neutral and functional loci (Miller *et al.* 2007; Hay & Lambert 2008). North Brother Island (4 ha, 41°07′S, 174°27′E) has a small population of tuatara (~350 adults; Newman 1878; Nelson *et al.* 2002b) and very low levels of genetic diversity at neutral and functional loci (Miller *et al.* 2008). Tuatara were translocated from North Brother Island as the population was then recognized as a distinct species (*Sphenodon guntheri*, Daugherty *et al.* 1990), but recent genetic work indicates that *Sphenodon* is best described as a single species (Hay *et al.* 2003, in press).

Initial survival of adults in the first year after reintroduction is high (at least 80%, and probably higher, McKenzie 2007), and tuatara show rapid increases in both body size and condition (Nelson *et al.* 2002a; McKenzie 2007). Successful reproduction has only been confirmed in three reintroduced populations as hatchling and juvenile tuatara are extremely difficult to detect, but may have occurred elsewhere. Because of the extreme longevity, long reproductive interval, and cryptic behaviour of tuatara, it is difficult to measure the population responses to management. Demographic and genetic models are important for predicting the effects of possible management actions and are likely to influence management decisions.

We used three well-monitored reintroduced populations of tuatara to measure losses of genetic diversity in the founder groups and to predict the maintenance of that diversity over 10 generations. In order to determine the effects of reproductive skew and founder group sizes on genetic diversity in reintroduced populations of tuatara, we used microsatellite data from source populations with high and low levels of genetic diversity to quantify the initial genetic bottleneck (i.e. the loss of alleles in the founder groups), and to predict the loss of heterozygosity and allelic diversity over 10 generations with different founder group sizes.

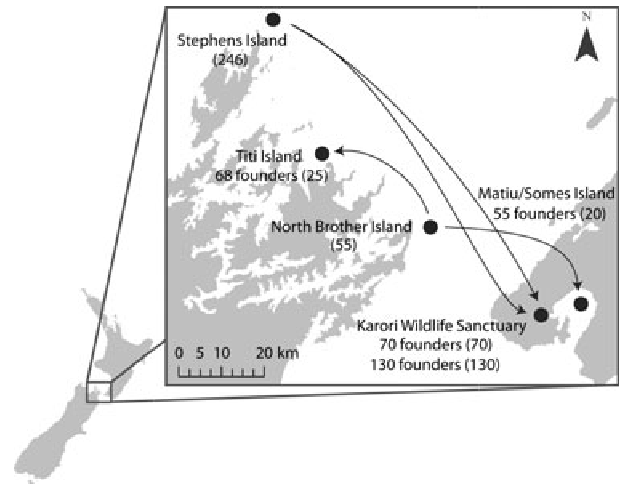

Fig. 1 Map of Cook Strait, New Zealand, showing the source populations (North Brother and Stephens Islands) and reintroduction sites (Titi Island, Matiu/Somes Island and Karori Wildlife Sanctuary) in this study. The number of founders released during each reintroduction is specified, and the number of founders sampled is given in parentheses.

Methods

Microsatellite genotyping

Tuatara were reintroduced from North Brother Island to Titi and Matiu/Somes Islands in 1995 and 1998 (with 68 and 55 founders, respectively, Fig. 1). In 2005, 70 tuatara were reintroduced from Stephens Island to Karori Wildlife Sanctuary, a fenced reserve on the New Zealand mainland cleared of mammalian predators; this population was supplemented with an additional 130 tuatara in 2007. We sampled DNA from tuatara in both the source and reintroduced populations (Fig. 1), by taking buccal swabs or blood samples. We sampled 246 tuatara from Stephens Island and 55 tuatara from North Brother Island. Animals reintroduced to Karori Wildlife Sanctuary were sampled at the time of translocation, and animals on Titi and Matiu/Somes Islands were sampled between December 2006 and March 2007 (Austral summer). Only a subset of tuatara in reintroduced populations are caught in any one season, due to their cryptic behaviour and the short duration of most survey trips (Nelson *et al.* 2002a). Therefore, sample sizes from Titi and Matiu/Somes Islands are smaller than the number of founders released (Fig. 1).

We extracted total genomic DNA using a standard proteinase K phenol–chloroform protocol (Sambrook *et al.* 1989) followed by ethanol precipitation or using a Qiagen DNEasy kit. We genotyped all animals from North Brother Island at six polymorphic microsatellite loci (*A12N, C11P, C12F, E11N, H5H* and *H4H*; Aitken *et al.* 2001; Hay & Lambert 2008). Animals from Stephens Island were genotyped at one additional locus (*C2F*). Polymerase chain reaction was carried out on an Eppendorf Mastercycler thermocycler as outlined in Moore *et al.* (2008), and products were run on an ABI3730 Genetic Analyzer (Applied Biosystems, Inc.). Allele sizes were scored manually using GeneMapper 3.7 (Applied Biosystems, Inc.).

Data analysis

Deviations from Hardy–Weinberg equilibrium (HWE) were tested in GENEPOP4.0 (Rousset 2008). Tests of significance were combined over all loci using Fisher's

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combined probability test, and significance was assumed at $P < 0.05$. The expected number of alleles, $E(n')$, represented in founder groups of N individuals from North Brother and Stephens Islands was calculated using the formula:

$$E(n') = n - \sum_{j=1}^{n} (1 - p_j)^{2N},$$

where n is the starting number of alleles and p_j is the frequency of the jth allele (Allendorf 1986). We then compared the number of alleles detected in each of the reintroduced populations to that expected with the number of founders. All founders of the reintroduction to Karori Wildlife Sanctuary were sampled, so the number of alleles detected is the true number of alleles in the founder group. Whilst only a subset of founders of the reintroductions to Titi and Matiu/Somes Islands were sampled, they also represent the true number of alleles in the founder group, as 100% of the source population diversity was detected (see Results). The expected proportion of the original heterozygosity remaining in a founder group of N individuals (Allendorf 1986) was calculated using the formula:

$$1 - \frac{1}{2N}.$$

We used VORTEX v 9.92 (Lacy 1993) to model the expected loss of heterozygosity (h) and allelic diversity (A) over 10 generations in reintroduced populations of tuatara from Stephens and North Brother Islands (for demographic input parameters, see Table 1). The mean generation interval in our simulations, calculated in VORTEX from a stable age distribution, was 39.5 using our input parameters. Therefore, we ran each simulation for 400 years (~10 generations). VORTEX assigns two unique alleles to each founder; in each iteration, these hypothetical alleles are 'dropped' through the simulated population from parents to offspring according to Mendelian inheritance. At the end of each iteration, the proportion of h that remains (or $1 - f$, the inbreeding coefficient) in the population is calculated using this infinite allele model. Thus, with all other demographic parameters being equal, different h-values result from differences in either the number of founders or carrying capacity. We used microsatellite data from Titi Island and Karori Wildlife Sanctuary to calculate allele retention when reintroductions are founded from low- (North Brother Island) and high-diversity (Stephens Island) source populations. Microsatellite diversity in tuatara reflects the amount of diversity seen at MHC loci (Miller *et al.* 2008), which are the most variable known vertebrate

Table 1 Input parameters used in VORTEX for tuatara

	High-diversity source	Low-diversity source	Reference
Maximum age of reproduction	100	100	Dawbin (1982); Castanet *et al.* (1988)
Age at maturity	14	14	Cree *et al.* (1992)
% Adult females breeding annually	25	25*	Cree *et al.* (1992)
Annual clutch size	9.1 ± 0.4	9.1 ± 0.4†	Nelson *et al.* (2004)
Annual recruitment‡§	5.07%	5.07%	Castanet *et al.* (1988)
Annual adult mortality§	2%	2%	Nelson (1998)
Maximum number of mates/male/year	2	2	Moore *et al.* (in press-a)
Carrying capacity	10 000	1500	Cree and Butler (1993)¶
Genotype frequencies of founders	Karori Wildlife Sanctuary	Titi Island	This study

Life-history characteristics were the same in all models, because known differences in many life-history characteristics between the high- (Stephens Island) and low-diversity (North Brother Island) source populations are most likely due to resource limitation on North Brother Island (Hoare *et al.* 2006), and thus may not be relevant after reintroduction. Carrying capacity and genotype frequencies of the founder groups were different for reintroduced populations from high- and low-diversity source populations.
*The average gravidity rate of mature females is lower in the low-diversity source population (~11%, North Brother Island; Mitchell *et al.* in review), but is likely due to resource limitation (Hoare *et al.* 2006). After reintroduction this rate should increase as female body condition improves (Nelson *et al.* 2002a).
†Mean clutch size is smaller in the low-diversity source (six eggs; North Brother Island), because of the small size of females (Mitchell *et al.* in review). Within 5 years of reintroduction, females increase in body size and condition (Nelson *et al.* 2002a), and mean clutch size would therefore increase.
‡The percentage of eggs laid that survive into adulthood.
§Annual adult and juvenile mortality was assumed to be equal for males and females.
¶Using an estimate of 100 tuatara/ha over half of the available habitat.

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genes. We ran 1000 iterations for each scenario to provide estimates of the loss of h and A given the stochastic natures of inheritance and population growth.

Prior to running models of different founder group sizes and levels of reproductive skew, we tested the influence of inbreeding depression on the loss of h after 400 years in our models. We used groups of 30 adults with each of three levels of reproductive skew (70%, 50% and 0%) with no inbreeding depression and with inbreeding depression (3.14 lethal equivalents/individual, the median for mammalian species, Ralls *et al.* 1988; 50% due to recessive lethals). Inbreeding depression had little effect on the amount of h retained after 400 years, even at an unrealistically low carrying capacity (5000 individuals) for the population in Karori Wildlife Sanctuary (<0.1% less h retained relative to models with no inbreeding depression). Therefore, we ignored inbreeding depression in further models.

To determine how well h and A are maintained for 10 generations with different founder groups, we modelled four different founder group sizes for translocations from Stephens and North Brother Islands: 200 adults, 70 adults, 30 adults and 30 juveniles. Two hundred adults represent the maximum number of wild founders used for reintroduction; 70 adults represent the first reintroduction to Karori Wildlife Sanctuary and a similar founder group to Titi and Matiu/Somes Islands. As mainland fenced reserves are becoming more common in New Zealand, the number of tuatara reintroductions is expected to increase, and it is likely that founder groups will be comprised of ~30 adults for most of these reintroductions (P. Gaze, personal communication, New Zealand Department of Conservation). Thirty juveniles is a similar founder group to some reintroductions of captive-bred/reared populations. Ages of adult founders were modelled based on a stable age distribution. The age of all juvenile founders was set to 5 years (the age at which captive-reared tuatara are generally released).

To thoroughly assess the effects of male reproductive skew, we ran 10 models of a reintroduction of each founder group from both low- (North Brother Island) and high-diversity (Stephens Island) source populations. In each model, we specified a different level of male reproductive skew. We ran eight density-independent models (0–70% reproductive skew, at 10% increments) and two density-dependent models. These values represent realistic levels of reproductive skew in many species, including tuatara. Reproductive skew (R_N) at a given population size (N) was directly proportional to population density in the latter models, where

$$R_N = R_L + [(R_H - R_L) * (N/K)],$$

and R_H is the reproductive skew at high density, R_L is the reproductive skew at low density, and K is the carrying capacity. We used a linear function, as the exact changes in reproductive skew at differing density are unknown. In the first of these models, we used 20% reproductive skew at low density and 70% at high density (i.e. $R_L = 20$ and $R_H = 70$). In the second density-dependent model, we used 0% reproductive skew at low density and 50% at high density (i.e. $R_L = 0$ and $R_H = 50$). We based these values on data from captivity and Stephens Island (respectively), where reproductive skew is 25% at low density (Moore *et al.* 2008) and 70% at high densities (Moore *et al.* in press-a). To make comparisons with the density-independent models, we used 20% skew at low density rather than 25%. As data from captivity and Stephens Island were collected over 15 and 3 years, respectively, and may overestimate reproductive skew over the lifespan of a tuatara, we used the more conservative estimates in the second density-dependent model.

Results

Expected heterozygosity per locus ranged from 0.071 to 0.497 (mean = 0.406) on North Brother Island and from 0.730 to 0.927 (mean 0.782) on Stephens Island. Following Bonferroni correction, only one locus (*H5V*) deviated significantly from HWE in the North Brother and Stephens Island populations, but it was included in models of allelic diversity. Mean allelic diversity was 14.4 on Stephens Island and 2.3 on North Brother Island.

The proportion of alleles retained in founder groups of each of the three reintroduced populations sampled ranged from 84.2% to 100%, and was within 2% of the predicted values (Fig. 2). Groups of 30 founders from Stephens Island were predicted to retain only 70.9% of the allelic diversity of the source, but 30 founders from North Brother Island were expected to retain 99.2% of the allelic diversity on North Brother. Founder groups of 30–200 individuals were predicted to represent between 98.3 and 99.8% of the original heterozygosity.

Models showed that after 10 generations, populations with larger founder groups retained a greater proportion of the h and A of both the source population and the founder groups themselves (Fig. 3). For example, populations founded with 200 adults from the high-diversity source population (Stephens Island) with 50% reproductive skew would retain 98.8% h and 83.2% A of the source (corresponding to a loss of 1.0% h and 11.2% A from the founder group), but a population founded with only 30 adults would retain 92.4% h and 52.4% A of the source population (a loss of 5.9% h and 18.5% A from the founders). Populations founded with 30 juveniles would retain less h and A over 10 generations than those founded with 30 adults. Predicted

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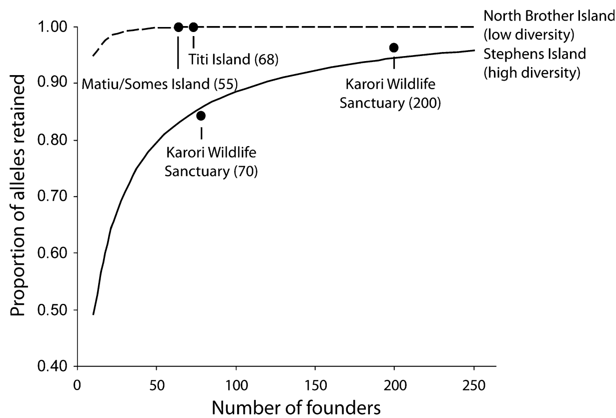

Fig. 2 Comparison between the predicted loss of alleles with different founder group sizes, based on allele frequencies in high-diversity (Stephens Island; solid line) and low-diversity (North Brother Island; broken line) populations. The actual proportion of alleles retained in the reintroduced populations of tuatara is indicated with circles. The number of tuatara reintroduced is indicated in parentheses.

losses of allelic diversity from populations with low-diversity sources were small (6.5%) under even the most extreme conditions (30 juvenile founders; 70% reproductive skew; Fig. 3D). On the other hand, populations with high-diversity sources lost at least 13.2% of alleles even under scenarios that promote retention of allelic diversity (200 adult founders; 0% reproductive skew; Fig. 3C). Probabilities of extinction were 0% for all populations founded with 30, 70 or 200 adults. The probability of extinction was 0.4–4.8% for populations founded with 30 juveniles.

Greater male reproductive skew led to significant predicted losses of h and A after 10 generations (Fig. 3). The loss of h was between 0.2% and 2.9% greater with each 10% increase in reproductive skew (i.e. with 10% more males excluded from mating), and was minimal after population size exceeded ~250 animals. The predicted effect of reproductive skew on h was slightly more severe when carrying capacity was smaller (Fig. 3B). Reproductive skew had a greater effect on the loss of alleles in the high-diversity populations (Fig. 3C). The loss of A was 0.7–3.7% with each 10% increase in reproductive skew in the high-diversity populations, but 0.1–1.4% in the low-diversity populations. Populations from a high-diversity source population are predicted to retain between 43.1% and 86.8% of the source A, depending on the number of founders and the degree of reproductive skew (Fig. 3C), while populations

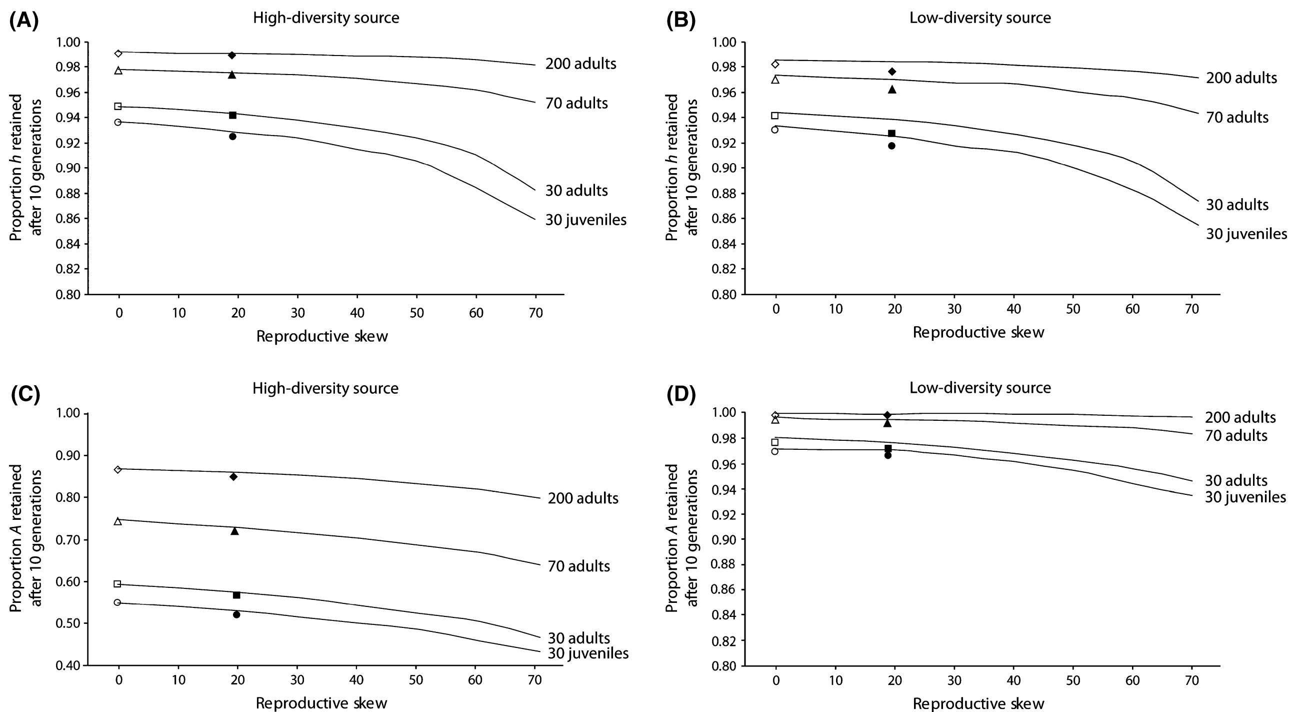

Fig. 3 Predicted proportion of source heterozygosity (h) and allelic diversity (A; note differences in scale) retained 10 generations after reintroduction of founder groups with different levels of reproductive skew (presented as the percentage of males that do not mate). Solid lines indicate the retained diversity predicted from density-independent models; symbols indicate the retained diversity predicted from density-dependent models (●: 30 juvenile founders, ■: 30 adult founders, ▲: 70 adult founders, ◆: 200 adult founders). Open symbols indicate the retained diversity predicted when 0% of males are excluded from mating at low density, and 50% are excluded at high density. Closed symbols indicate the retained diversity predicted when 20% of males are excluded at low density, and 70% are excluded at high density.

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reintroduced from a low-diversity source population are predicted to retain 93.4–100% of the source A.

The predicted losses of h and A in density-dependent models were similar to, but slightly greater than losses predicted by the percentage of males mating at low density (Fig. 3). For example, with 20% reproductive skew at low density and 70% at high density, populations retained similar levels of h and A after 10 generations as predicted by density-independent models with 20–30% reproductive skew.

Discussion

Reintroduced populations of tuatara should retain a relatively high proportion of the heterozygosity (86–99%) and allelic diversity (43–100%) of their source populations after 10 generations. Founder groups released into Karori Wildlife Sanctuary, Titi Island, and Matiu/Somes Island have retained 84–100% allelic diversity of their source populations. The amount of genetic diversity in the source populations affects the retention of diversity in the founders, with populations reintroduced from high-diversity sources (Stephens Island) losing more diversity than those from low-diversity sources (North Brother Island). As expected, populations founded with more animals were predicted to retain a greater proportion of heterozygosity and allelic diversity of both the source populations and the founder groups after 10 generations than smaller founder groups. Greater male reproductive skew led to greater predicted losses of heterozygosity and allelic diversity over 10 generations, but the effect of reproductive skew was minimal after the populations expanded to >250 animals.

Losses of genetic diversity

It is generally agreed that the more heterozygosity retained, the better (Franklin & Frankham 1998; Lynch & Lande 1998), but cited goals for genetic management of threatened species are typically ~90–95% heterozygosity retained over 100–200 years (Soulé *et al.* 1986; Lacy 1987; Allendorf & Ryman 2002). As tuatara are extremely long-lived (possibly 100 years, Dawbin 1982; Cree 1994) with a long generation interval, targets for genetic management in reintroduced populations over 100 years have little meaning. However, we applied these targets (90–95% heterozygosity) to tuatara over 10 generations.

The rate of population expansion has a large effect on how well genetic diversity is maintained, and factors that increase population growth rates may help to maintain genetic diversity (Allendorf & Luikart 2007). After reintroduction, several factors may result in greater population growth rates. Release from competition results in higher body condition and increases in body size (Nelson *et al.* 2002a; McKenzie 2007). Female tuatara in better body condition may reproduce more frequently, and those with larger body size produce more eggs per clutch (Newman *et al.* 1994). Females in captivity, where resources are not limited, reproduce on average every 2 years (Moore *et al.* 2008). Additionally, at low density, the top predator of juveniles (adult tuatara) will be sparse, and juvenile survival may be higher. Under these conditions, the loss of heterozygosity and allelic diversity may be lower than predicted by our models.

Genetic drift in small populations generally outweighs selection at functional loci (e.g. MHC; Miller & Lambert 2004; Campos *et al.* 2006; but see Aguilar *et al.* 2004). In tuatara, the loss of genetic variation at MHC loci after a bottleneck is comparable to that lost at neutral loci (Miller *et al.* 2008). Although models of neutral genetic variation may have limited ability to predict the effects of a bottleneck on quantitative (polygenic) variation (Reed & Frankham 2001), they can be used to evaluate how well adaptive variation (at least at MHC loci) may be maintained after reintroduction. The loss of genetic variation at these fitness-related genes may reduce the ability of the populations to respond to novel disease threats and increase the risk of an epidemic causing a population crash.

Choice of source population

The assumption of no background inbreeding (i.e. all founders being unrelated) may be unrealistic, particularly for populations reintroduced from North Brother Island, which has a history of population bottlenecks and small population size (Newman 1878; Nelson *et al.* 2002b). We ignored inbreeding depression in our models, as it had relatively little impact on the amount of heterozygosity retained after 10 generations, but inbreeding (particularly when a small number of founders are reintroduced) could affect individual fitness and may reduce population growth rates (Briskie & Mackintosh 2004; Taylor *et al.* 2005). If deleterious alleles have been purged on North Brother Island because of its history of small population size and bottlenecks, then populations reintroduced from North Brother Island would be less affected by the increase in inbreeding than a population reintroduced from Stephens Island. However, purging is unlikely to reduce the negative effects of inbreeding (Ballou 1997; Frankham 2001), particularly when deleterious alleles have small rather than lethal effects (Hedrick 1994). Thus, it is likely that populations reintroduced from North Brother Island would be equally vulnerable to the potential effects of inbreeding.

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Populations reintroduced from North Brother Island would retain a larger proportion of the allelic diversity of the source than populations reintroduced from Stephens Island. This does not indicate that reintroduced populations from Stephens Island would have less adaptive potential. In fact, even when 50% of the original allelic diversity from Stephens Island is lost during reintroduction, it is higher than that on North Brother Island. Why, then, should North Brother Island (or any other population with low genetic diversity) be used as a source population? Tuatara were reintroduced from North Brother Island as part of an objective for species recovery to maintain genetic diversity across the existing range of tuatara. North Brother Island is the only naturally occurring population of a recently recognized species of tuatara (*S. guntheri*), but current genetic data indicates that *Sphenodon* is better described as a single species (Hay *et al.* 2003, in press). Genetic distinction of the North Brother Island population may have resulted from an historic bottleneck (Newman 1878) and subsequent genetic drift. In the absence of a species distinction, we recommend that high-diversity populations be selected as source populations over low-diversity populations.

Our results suggest that reintroductions from North Brother Island should lose little allelic diversity, but will have low diversity at both neutral and functional loci. Small populations with low diversity are at an increased risk of extinction (Saccheri *et al.* 1998), and hybridising populations, subspecies, or even species can reduce the effects of inbreeding depression and increase population growth rates (e.g. Madsen *et al.* 1999; Pimm *et al.* 2006). However, 'genetic rescue' of populations is complex and may lead to outbreeding depression; it is difficult to predict whether hybridisation will be beneficial to a given population (Tallmon *et al.* 2004). Reintroductions to islands provide an opportunity to assess the effects of population hybridisation for tuatara: individuals from a genetically distinct population could be added to one of the populations reintroduced from North Brother Island. Population growth rates and fitness could be monitored simultaneously in the reintroduced populations on Titi and Matiu/Somes Islands to assess whether there is evidence for inbreeding depression and whether hybridisation alleviates the effects.

Founder group size and composition

The number of founders released during reintroductions may be small to reduce impacts on fragile source populations (e.g. Towns & Ferreira 2001) or because of the inherent risk of reintroduction. Forty-six per cent of reintroductions of birds and mammals carried out between 1978 and 1986 had fewer than 30 founders (Griffith *et al.* 1989). Although the median number of founders increased to ~50 founders by 1993 (Wolf *et al.* 1996), founder groups are often still much smaller than 50, particularly for threatened species (e.g. Fitzsimmons *et al.* 1997; Towns & Ferreira 2001). It has been suggested that ~100 founders would be needed to maximise the probability of success (Griffith *et al.* 1989), but species with high population growth rates may have a negligible probability of extinction through demographic stochasticity after reintroduction of as few as four animals (e.g. saddlebacks, *Philesturnus caruncucatus*; Taylor *et al.* 2005). Similarly, our models showed a negligible probability of extinction with at least 30 adult founders.

Captive-reared juveniles are often used as founders for reintroduced populations (Griffith *et al.* 1989), and it is often possible to release more captive-reared juveniles than wild founders without damaging the source populations. These juveniles are often related clutch-mates, as the harvest an equivalent number of wild juveniles may be unfeasible for many cryptic species because juveniles are difficult to locate. Although captive-reared juveniles remain in captivity during the period of highest mortality (e.g. until age 5 for tuatara), survival of juveniles is lower than adults. Our results show that the release of juvenile founders would result in greater losses of genetic diversity than release of the same number of adults, because juvenile mortality effectively results in fewer founders. Additionally, reintroductions of juveniles have slightly higher probabilities of extinction than reintroductions of the same number of founders (Nelson 1998; this study). Therefore, adult founders should be used over an equivalent number of juvenile founders. Juveniles could be released if more founders are released to compensate for greater juvenile mortality and increased founder relatedness.

Larger founder groups will help to maximise genetic diversity, reduce inbreeding, and maintain genetic diversity across generations. Our models clearly indicate that with any source population or any level of reproductive skew, reintroduced populations founded with more individuals will have smaller losses of genetic diversity over 10 generations. However, populations founded with only 30 adult tuatara should meet genetic targets for management when reproductive skew is low (see next section). For species with relatively high reproductive output with low levels of reproductive skew, at least 30 founders should be released to achieve genetic goals.

Reproductive skew

High variance in male reproductive success, where few males obtain almost all of the matings in a population,

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results in low effective population size (Nunney 1993; Parker & White 1997), yet the effect of reproductive skew is difficult to quantify in species with overlapping generations. Our models showed that reproductive skew had relatively little impact on the loss of genetic diversity while population size was large. However, when population size is reduced (e.g. after reintroduction), reproductive skew has a significant impact on how well genetic diversity is maintained, despite a large generation interval. Thirty founders will therefore not be adequate for reintroductions of species with highly polygynous mating systems, where reproductive skew is high.

Differences in fitness due to unequal mating success are the basis of sexual selection, and are common in natural populations (Emlen & Oring 1977). Thus, reproductive skew *per se* is not detrimental, but after reintroduction, it may have a large influence on whether the genetic goals for management are met. Density-dependent changes in reproductive skew, where more males mate at low density, may facilitate the maintenance of genetic diversity in the early stages of population growth after reintroduction. If reproductive skew is high and density-independent, larger founder groups could be released to achieve genetic goals for management. For example, populations of tuatara founded with 70 adults with 70% reproductive skew would retain similar amounts of heterozygosity and allelic diversity to a population founded with 30 adults with 0% reproductive skew. Although it is possible to intentionally bias founder sex-ratios to account for reproductive skew (Lenz *et al*. 2007), it will not always be possible to determine which males might be successful, particularly if more males mate at low density or there are multiple determinants of male reproductive success (e.g. body size and colouration, Stapley & Keogh 2006). For species with strong social structure where subordinate males may be entirely eliminated from mating, understanding how density influences social structure will be critical for understanding how genetic diversity is maintained and thus how reintroduction will impact population viability.

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References

Aguilar A, Roemer G, Debenham S *et al*. (2004) High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 3490–3494.

Aitken N, Hay JM, Sarre SD, Lambert DM, Daugherty CH (2001) Microsatellite DNA markers for tuatara (*Sphenodon* spp.). *Conservation Genetics*, **2**, 183–185.

Allendorf FW (1986) Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biology*, **5**, 181–190.

Allendorf FW, Luikart G (2007) *Conservation and the Genetics of Populations*. Blackwell, Oxford, UK.

Allendorf FW, Ryman N (2002) The role of genetics in population viability. In: *Population Viability Analysis* (eds Beissinger SR, McCullough DR), pp. 50–85. The University of Chicago Press, Chicago, IL, USA.

Anthony LL, Blumstein DT (2000) Integrating behaviour into wildlife conservation: the multiple ways that behaviour can reduce N_e. *Biological Conservation*, **95**, 303–315.

Armstrong DP, Seddon PJ (2008) Directions in reintroduction biology. *Trends in Ecology & Evolution*, **23**, 20–25.

Ballou JD (1997) Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. *Journal of Heredity*, **88**, 169–178.

Briskie JV, Mackintosh M (2004) Hatching failure increases with severity of population bottlenecks in birds. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 558–561.

Brown JL (1969) Territorial behavior and population regulation in birds. *Wilson Bulletin*, **81**, 293–329.

Campos JL, Posada D, Moran P (2006) Genetic variation at MHC, mitochondrial and microsatellite loci in isolated populations of brown trout (*Salmo trutta*). *Conservation Genetics*, **7**, 515–530.

Castanet J, Newman DG, Saintgirons H (1988) Skeletochronological data on the growth, age, and population structure of the tuatara, *Sphenodon punctatus*, on Stephens Island and Lady Alice Island, New Zealand. *Herpetologica*, **44**, 25–37.

Cree A (1994) Low annual reproductive output in female reptiles from New Zealand. *New Zealand Journal of Zoology*, **21**, 351–372.

Cree A, Butler D (1993) *Tuatara Recovery Plan (*Sphenodon *spp.)*. Department of Conservation, Wellington, New Zealand.

Cree A, Cockrem JF, Guillette LJ (1992) Reproductive cycles of male and female tuatara (*Sphenodon punctatus*) on Stephens Island, New Zealand. *Journal of Zoology*, **226**, 199–217.

Daugherty CH, Cree A, Hay JM, Thompson MB (1990) Neglected taxonomy and continuing extinctions of tuatara (*Sphenodon*). *Nature*, **347**, 177–179.

Dawbin WH (1982) The tuatara *Sphenodon punctatus*: aspects of life history, growth, and longevity. In: *New Zealand Herpetolog* (ed. Newman DG), pp. 237–250. New Zealand Wildlife Service, Wellington, New Zealand.

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Emlen ST, Oring LW (1977) Ecology, sexual selection, and evolution of mating systems. *Science*, **197**, 215–223.

Fitzsimmons NN, Buskirk SW, Smith MH (1997) Genetic changes in reintroduced Rocky Mountain bighorn sheep populations. *Journal of Wildlife Management*, **61**, 863–872.

Frankham R (1999) Do population size bottlenecks reduce evolutionary potential? *Animal Conservation*, **2**, 255–260.

Frankham R (2001) Inbreeding and extinction: effects of purging. *Conservation Genetics*, **2**, 279–285.

Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131–140.

Franklin IR, Frankham R (1998) How large must populations be to retain evolutionary potential? *Animal Conservation*, **1**, 69–73.

Griffith B, Scott JM, Carpenter JW, Reed C (1989) Translocation as a species conservation tool: status and strategy. *Science*, **245**, 477–480.

Hay JM, Lambert DM (2008) Microsatellite DNA loci identify individuals and provide no evidence for multiple paternity in wild tuatara (*Sphenodon*: Reptilia). *Conservation Genetics*, **9**, 1039–1043.

Hay JM, Daugherty CH, Cree A, Maxson LR (2003) Low genetic divergence obscures phylogeny among populations of Sphenodon, remnant of an ancient reptile lineage. *Molecular Phylogenetics and Evolution*, **29**, 1–19.

Hay JM, Sarre SD, Lambert DM, Allendorf FW, Daugherty CH (in press) Genetic diversity and taxonomy: a reassessment of species designation in tuatara (*Sphenodon*: Reptilia). *Conservation Genetics*. doi: 10.1007/s10592-009-9952-7.

Hedrick PW (1994) Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity*, **73**, 363–372.

Hoare JM, Pledger S, Keall SN *et al*. (2006) Conservation implications of a long-term decline in body condition of the Brothers Island tuatara (*Sphenodon guntheri*). *Animal Conservation*, **9**, 456–462.

Jamieson IG, Tracy LN, Fletcher D, Armstrong DP (2007) Moderate inbreeding depression in a reintroduced population of North Island robins. *Animal Conservation*, **10**, 95–102.

Kokko H, Rankin DJ (2006) Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **361**, 319–334.

Lacy RC (1987) Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology*, **1**, 143–158.

Lacy RC (1993) VORTEX – a computer simulation model for population viability analysis. *Wildlife Research*, **20**, 45–65.

Lenz TL, Jacob A, Wedekind C (2007) Manipulating sex ratio to increase population growth: the example of the Lesser Kestrel. *Animal Conservation*, **10**, 236–244.

Lynch M, Lande R (1998) The critical effective size for a genetically secure population. *Animal Conservation*, **1**, 70–72.

Madsen T, Shine R, Olsson M, Wittzell H (1999) Restoration of an inbred adder population. *Nature*, **402**, 34–35.

Maher CR, Lott DF (2000) A review of ecological determinants of territoriality within vertebrate species. *American Midland Naturalist*, **143**, 1–29.

McKenzie KL (2007) *Returning tuatara (*Sphenodon punctatus*) to the New Zealand mainland*. MSc Thesis, Victoria University of Wellington, Wellington.

McLain DK (1992) Population density and the intensity of sexual selection on body length in spatially or temporally restricted natural populations of a seed bug. *Behavioral Ecology and Sociobiology*, **30**, 347–356.

Miller HC, Lambert DM (2004) Genetic drift outweighs balancing selection in shaping post-bottleneck major histocompatibility complex variation in New Zealand robins (Petroicidae). *Molecular Ecology*, **13**, 3709–3721.

Miller HC, Andrews-Cookson M, Daugherty CH (2007) Two patterns of variation among MHC class I loci in tuatara (*Sphenodon punctatus*). *Journal of Heredity*, **98**, 666–677.

Miller HC, Miller KA, Daugherty CH (2008) Reduced MHC variation in a threatened tuatara species. *Animal Conservation*, **11**, 206–214.

Miller HC, Moore JA, Nelson NJ, Daugherty CH (2009) Influence of MHC genotype on mating success in a free-ranging reptile population. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **276**, 1695–1704.

Mitchell NJ, Allendorf FW, Keall SN, Daugherty CH, Nelson NJ (2009) Demographic effects of temperature-dependent sex determination: will tuatara survive global warming? *Global Change Biology*. doi: 10.1111/j.1365-2486.2009.01964.x.

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). *Conservation Genetics*, **9**, 1243–1251.

Moore JA, Daugherty CH, Godfrey SS, Nelson NJ (in press-a) Social monogamy and multiple paternity in a wild population of a territorial reptile (tuatara). *Biological Journal of the Linnean Society*.

Moore JA, Daugherty CH, Nelson NJ (in press-b) Large male advantage: phenotypic and genetic correlates of territoriality in tuatara. *Journal of Herpetology*.

Nelson NJ (1998) *Conservation of the Brothers Island tuatara (*Sphenodon guntheri*)*. MConSc Thesis, Victoria University of Wellington, Wellington.

Nelson NJ, Keall SN, Brown D, Daugherty CH (2002a) Establishing a new wild population of tuatara (*Sphenodon guntheri*). *Conservation Biology*, **16**, 887–894.

Nelson NJ, Keall SN, Pledger S, Daugherty CH (2002b) Male-biased sex ratio in a small tuatara population. *Journal of Biogeography*, **29**, 633–640.

Nelson NJ, Thompson MB, Pledger S, Keall SN, Daugherty CH (2004) Induction of oviposition produces smaller eggs in tuatara (*Sphenodon punctatus*). *New Zealand Journal of Zoology*, **31**, 283–289.

Newman AK (1878) Notes on the physiology and anatomy of the tuatara (*Sphenodon guntheri*). *Transactions and Proceedings of the New Zealand Institute*, **10**, 222–239.

Newman DG (1987) Burrow use and population densities of tuatara (*Sphenodon punctatus*) and how they are influenced by fairy prions (*Pachyptila turtur*) on Stephens Island, New Zealand. *Herpetologica*, **43**, 336–344.

Newman DG, Watson PR, McFadden I (1994) Egg production by tuatara on Lady Alice and Stephens Island, New Zealand. *New Zealand Journal of Zoology*, **21**, 387–398.

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Nunney L (1993) The influence of mating system and overlapping generations on effective population size. *Evolution*, **47**, 1329–1341.

Parker PG, White TA (1997) Mating systems, effective population size, and conservation of natural populations. In: *Behavioral Approaches to Conservation in the Wil* (eds Clemmons JR, Buchholz R), pp. 243–261. Cambridge University Press, Cambridge, UK.

Pimm SL, Dollar L, Bass OL (2006) The genetic rescue of the Florida panther. *Animal Conservation*, **9**, 115–122.

Ralls K, Ballou JD, Templeton A (1988) Estimating lethal equivalents and the cost of inbreeding in mammals. *Conservation Biology*, **2**, 185–193.

Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution*, **55**, 1095–1103.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Saccheri I, Kuussaari M, Kankare M *et al.* (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Soulé M, Gilpin M, Conway W, Foose T (1986) The millennium ark: how long a voyage, how many staterooms, how many passengers? *Zoo Biology*, **5**, 101–113.

Stapley J, Keogh JS (2006) Experimental and molecular evidence that body size and ventral colour interact to influence male reproductive success in a lizard. *Ethology Ecology & Evolution*, **18**, 275–288.

Tallmon DA, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology & Evolution*, **19**, 489–496.

Taylor SS, Jamieson IG, Armstrong DP (2005) Successful island reintroductions of New Zealand robins and saddlebacks with small numbers of founders. *Animal Conservation*, **8**, 415–420.

Towns DR (1999) Cyclodina *spp. Recovery Plan 1999–2004*. Department of Conservation, Wellington, New Zealand.

Towns DR, Ferreira SM (2001) Conservation of New Zealand lizards (Lacertilia: Scincidae) by translocation of small populations. *Biological Conservation*, **98**, 211–222.

Williams RN, Rhodes OE, Serfass TL (2000) Assessment of genetic variance among source and reintroduced fisher populations. *Journal of Mammalogy*, **81**, 895–907.

Wolf CM, Griffith B, Reed C, Temple SA (1996) Avian and mammalian translocations: update and reanalysis of 1987 survey data. *Conservation Biology*, **10**, 1142–1154.

The authors' research group in the Allan Wilson Centre for Molecular Ecology and Evolution at Victoria University of Wellington has broad interests in conservation and evolutionary genetics, physiological and behavioural ecology, and management of New Zealand vertebrates, particularly reptiles. This study was part of Kim Miller's doctoral research that focused on genetic diversity in reintroduced populations.

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