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Assessing genetic diversity for conservation management: a case study of a threatened reptile

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Abstract

The consequences of inbreeding in small isolated populations are well documented, yet populations are often managed in isolation to avoid irreversibly mixing genetic lineages and to maintain the historic integrity of each population. Three remaining populations of Whitaker's skink (*Cyclodina whitakeri*) in New Zealand, remnants of a once wider distribution, illustrate the conflict between this genetic goal (separate management of populations) with the more tangible and immediate threats of small population size and inbreeding. Middle and Castle Islands harbour populations of *C. whitakeri* and have been separated from each other and from the mainland for ~10 000 years. The single mainland population at Pukerua Bay is extremely small, declining and deemed a high priority for management. We sequenced a 550 bp region of mitochondrial DNA (mtDNA,ND2) and genotyped animals from all three populations at 13 microsatellite loci. The population of *C. whitakeri* at Pukerua Bay showed marked differences from the island populations at both mtDNA (unique, fixed haplotype) and microsatellite loci (F_{ST}~0.20), and private alleles were detected at a high frequency (24% of all alleles). However, we attribute this pattern to an historic genetic gradient coupled with rapid genetic drift. Further, animals in captivity show genetic signatures of both Pukerua Bay and island populations, despite the goal to maintain a pure Pukerua Bay stock. The mixed genetic stock in captivity provides an opportunity for the addition of skinks from Middle Island to evaluate the risks of further population hybridization, including the disruption of potential local adaptation, while mitigating the risks of inbreeding.

Introduction

Balancing the immediate demographic threats to a population with long-term goals for genetic management can be a difficult and daunting task. The factors that cause population declines (e.g. disease or introduced predators) are often severe and pose a more tangible and immediate threat of extinction than the effects of inbreeding (Thorne & Williams, 1988; La Marca *et al*., 2005; Tocher & Norbury, 2005). Indeed, genetic factors such as inbreeding depression will have relatively little impact while populations are rapidly declining due to high predation rates or disease (Jamieson, 2007). Yet during population recovery, inbreeding depression may have a large impact on population growth rates and extinction risk (Frankham, 2005; O'Grady *et al*., 2006).

Inbreeding is inevitable in small closed populations (Allendorf & Luikart, 2007); thus management of threatened species in isolation may impose unnecessary inbreeding depression. Recovery programs in New Zealand have been criticized for neglecting the potential problems of inbreeding (Jamieson, Wallis & Briskie, 2006), particularly when genetic exchange among populations could minimize these effects (Allendorf, 2001). Interpopulation hybridization can result in an increase in fitness (Hedrick, 1995; Westemeier *et al*., 1998; Madsen *et al*., 1999). Alternately, hybridization may reduce fitness in first, second or later generation hybrids (Tallmon, Luikart & Waples, 2004). Empirical evidence for inbreeding depression outweighs that of outbreeding depression (Saccheri *et al*., 1998; Crnokrak & Roff, 1999), but the available information shows that the effects of outbreeding are severe (Marr, Keller & Arcese, 2002; Edmands, 2007 for review). In the case of threatened species, where decisions about management are both difficult and central to species survival, it becomes particularly apparent that information on the genetic differences among populations is important for adequate management.

New Zealand's lizard fauna may be the most diverse of any temperate archipelago (Daugherty *et al*., 1990), but human colonization led to radical changes in the abundance and distribution of many species. The primary cause of decline has been introduced mammalian predators,

especially rats (*Rattus* spp., Towns & Daugherty, 1994). At least 40% of New Zealand's herpetofauna is now restricted mainly or entirely to predator-free offshore islands (Daugherty, Patterson & Hitchmough, 1994). The ground-dwelling, relatively large-bodied, nocturnal skinks in the genus *Cyclodina* (Scincidae) are particularly susceptible to the effects of introduced mammals. Seven of the 10 species of *Cyclodina* have been effectively eliminated from mainland New Zealand by introduced predators (Daugherty *et al*., 1994; Towns & Daugherty, 1994; Chapple, Daugherty & Ritchie, 2008).

Whitaker's skink (*Cyclodina whitakeri*) is an excellent example of the complexity of applying genetic data to the management of fragmented populations. Though once distributed across the North Island (Worthy, 1987), *C. whitakeri* now naturally occurs on two small islands and one mainland site. It is listed as 'vulnerable' (D1 + D2) by the World Conservation Union (2007), and its national threat status was heightened in 2007 by the New Zealand Department of Conservation (DOC) because of population declines in the previous 3 years (Hitchmough, Bull & Cromarty, 2007).

The three remaining natural populations of *C. whitakeri* total <20 ha of habitat (Towns, 1992*b*) and are separated by up to 500 km (Fig. 1). Two island populations are found on predator-free Middle and Castle Islands, and a single mainland population is located at Pukerua Bay. The largest population (estimated at 1300–12 000 individuals; Southey, 1985) on Middle Island (13 ha) is considered stable. Castle Island (3 ha) is privately owned and thus not managed by DOC. The skink population on Castle Island is not given a high level of security (Towns, 1992*b*, 1999), but is presumed stable because mammalian predators are not present. Surveys have not been conducted in the last 15 years because of limited access to the island. The mainland population of *C. whitakeri* at Pukerua Bay is confined to <1 ha of habitat and persists only in deep boulder banks (Towns, 1992*b*; Towns & Elliott, 1996). This population declined drastically during a 23-year monitoring period between 1984 and 2006, presumably due to continued predation by introduced mammals (Hoare *et al*., 2007).

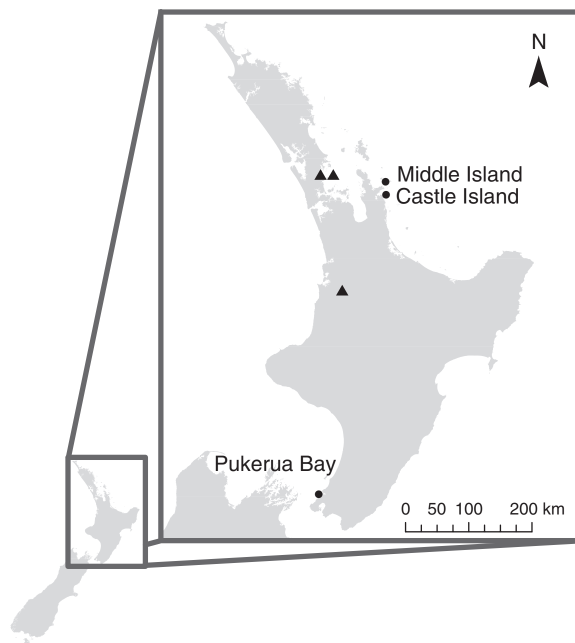

Figure 1 Map of *Cyclodina whitakeri* distribution (New Zealand). Closed circles indicate extant populations; triangles indicate locations of subfossil remains (from Worthy, 1987).

The genetic relationships among populations of *C. whitakeri* are not well understood. Middle and Castle Islands have been separated from each other and the mainland for ~10 000 years (Hayward, 1986; Towns, 1994). Lizards are generally good colonizers of oceanic islands (i.e. dispersing over saltwater, Thomas & Whitaker, 1996; Censky, Hodge & Dudley, 1998), but *C. whitakeri* has one of the highest rates of cutaneous water loss among lizards and is physiologically intolerant to dehydrating conditions (Cree, 1994). Contemporary gene flow (by dispersal) between populations is unlikely, but the three populations show no differentiation at allozyme loci (Towns & Daugherty, 1994). Shallow divergence in mitochondrial DNA (mtDNA) suggests that gene flow across the range of *C. whitakeri* persisted until the late Pleistocene (Chapple *et al*., 2008), and this estimate is consistent with the separation of the island populations from the mainland.

Pukerua Bay is a 'Key Place' in the Wellington Conservancy's Conservation Management Strategy (Anon, 1996); management of this reserve is targeted at the protection of *C. whitakeri*. Additionally, management of this population and translocation of animals from the mainland to predator-free island sites are high priorities for the species (Miskelly, 1999; Towns, 1999). However, the current capture rate of *C. whitakeri* at Pukerua Bay (0.03 per 100 trap nights) is insufficient to produce a propagule for translocation; only three animals have been captured since 2001. Construction of a predator-proof fence is the most effective way to protect mainland populations from introduced predators, and would provide the best chance of capturing *C. whitakeri* (Hoare *et al*., 2007). However, this measure has several challenges, including high start-up costs. If the population at Pukerua Bay is genetically distinct, it may influence whether this management action is deemed a priority.

Skinks from Pukerua Bay are held in captivity with the intention of using captive-bred animals as founders of a new population on a predator-free island. All 12 F1 offspring in the program have been produced by a single pair: a female from Pukerua Bay and a male (of presumed Pukerua Bay origin) that have been part of the breeding program for ~10 years. The male was taken into the captive breeding program after a private breeder abandoned his collection, and confirmation of its origin was not obtained. A second male, taken into captivity from Pukerua Bay by DOC in 2005, has sired offspring from F1 females.

Here we evaluate both mitochondrial and microsatellite diversity for the three populations of *C. whitakeri* to inform conservation management. We aim to determine

conservation units under traditional definitions, and assess the validity of those units given the challenges faced in managing *C. whitakeri* and the limitations encountered when sampling is constrained by current population status. Lastly, we aim to resolve the origin of the captive male (of presumed Pukerua Bay origin) to make recommendations for the future management of the captive population.

Materials and methods

Sample collection and DNA extraction

We sampled 51 animals: 44 from Middle Island (preserved specimens and field collected), all available samples from Castle Island (one preserved specimen) and Pukerua Bay (three preserved specimens and two animals in captivity), and the captive male of presumed Pukerua Bay origin. Tail or toe samples (~3 mm) from each animal were stored in 70% ethanol. Total genomic DNA extraction was performed using a standard proteinase K phenol–chloroform protocol (Sambrook, Fritsch & Maniatis, 1989) followed by ethanol precipitation. DNA was quantified using a NanoDrop® (Thermoscientific, Wilmington, DE, USA) ND-1000 Spectrophotometer at 260 nm.

mtDNA sequencing

We sequenced a ~600 bp portion of the mitochondrial gene ND2, a region commonly selected for work at comparable taxonomic levels in squamate reptiles (Keogh, Scott & Hayes, 2005; Hare, Daugherty & Chapple, 2008) and previously used to investigate the phylogeography of the genus *Cyclodina* (Chapple *et al.*, 2008). We attempted to sequence all 51 samples, but 10 samples from Middle Island failed to amplify. The primers used to amplify and sequence ND2 were L4437 (Macey *et al.*, 1997) and ND2r102 (Sadlier *et al.*, 2004). PCR and sequencing were conducted as outlined in Greaves *et al.* (2007). Sequence data were edited using ContigExpress v9.1.0 (Invitrogen, Carlsbad, CA, USA) and aligned using the default parameters in Clustal X (Thompson *et al.*, 1997). The edited alignment comprised the 550 bp sequence reported by Chapple *et al.* (2008).

Microsatellite genotyping

All individuals were genotyped at 13 microsatellite loci (Berry, Gleeson & Sarre, 2003) adapted with 5′ M-13 tags (TGTAAAACGACGGCCAGT; Schuelke, 2000). PCR was performed in 15 μL reactions containing: PCR buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.4 $\mu g\,mL^{-1}$ bovine serum albumin, 0.2 mM each dNTP, 0.375 U Taq DNA polymerase (Invitrogen) and ~35 ng genomic DNA. Locus-specific forward primer concentrations were between 0.25 and 0.53 μM; fluorescently labelled M-13 universal primers (Schuelke, 2000) were at equal concentrations with reverse primers. PCR was carried out on an Eppendorf Mastercycler (North Ryde, NSW, Australia) thermocycler. The PCR profile consisted of one cycle of 94 °C for 3 min, 30 cycles of (94 °C for 30 s, 57 °C for 45 s and 72 °C for 45 s), eight cycles of (94 °C for 30 s, 53 °C for 45 s and 72 °C for 30 s) and a final extension at 72 °C for 15 min. PCR products were pooled for genotyping on an ABI3730 Genetic Analyzer (Applied Biosystems Inc., Scoresby, VIC, Australia). Allele sizes were scored manually by KAM using GeneMapper software v.3.7 (Applied Biosystems Inc.).

Data analysis

The captive male of presumed Pukerua Bay origin was removed from all analyses except for the population assignment test. The Castle Island individual was removed for all statistical microsatellite analyses because only one sample was available.

Haplotype diversity (*h*) and nucleotide diversity (π) were calculated in DnaSP 4.10.9 (Rozas *et al.*, 2003), and uncorrected mtDNA sequence divergence were calculated in MEGA3.1 (Kumar, Tamura & Nei, 2004). A haplotype network was created in TCS 1.21 (Clement, Posada & Crandall, 2000).

We calculated the frequency of null alleles in FreeNA (Chapuis & Estoup, 2007), and found that two loci (*Oligr3* and *Oligr13*) had a high frequency of null alleles in the Middle Island population (0.19 and 0.08, respectively). Two loci (*Oligr2* and *Oligr17*) had a high frequency of null alleles in Pukerua Bay (0.29 and 0.15, respectively). Null alleles affect estimates of population differentiation by reducing intra-population genetic diversity (Paetkau & Strobeck, 1995), and F_{ST} is generally overestimated in the presence of null alleles (Chapuis & Estoup, 2007). We therefore excluded *Oligr3* and *Oligr13* from analyses of population differentiation and assignment tests. We did not exclude *Oligr2* or *Oligr17* from any analyses, because null alleles were not detected in the Middle Island sample, and we could not rule out that detection of null alleles may have been related to the sample size of the Pukerua Bay population.

Departures from Hardy–Weinberg proportions (Guo & Thompson, 1992) and F_{ST} (Weir & Cockerham, 1984) were calculated in Genepop 4.0 (Rousset, 2008). Tests of significance were combined over all loci using Fisher's combined probability test. We also conducted an AMOVA using GenAlEx 6.1 (Peakall & Smouse, 2006). Using FSTAT 2.9 (Goudet, 1995), we calculated allelic richness (number of alleles corrected for sample size) at all 13 loci in the Middle Island population based on a sample of five individuals (i.e. the sample size from Pukerua Bay).

Assignment/exclusion tests were conducted in GeneClass2 (Piry *et al.*, 2004) which assigns individuals to a population and computes the probability that the individual's multilocus genotype would be encountered in each reference population. Probabilities were calculated using the Monte-Carlo resampling method of Paetkau *et al.* (2004) to reflect the sampling variance associated with the dataset. An assignment test was run on all individuals from our reference populations (Middle Island and Pukerua Bay) to assess the power of our dataset. A second test was run to assign the

captive male of presumed Pukerua Bay origin to one of the two reference populations.

Results

mtDNA variation

We obtained 41 mtDNA sequences and observed 11 ND2 haplotypes in total (GenBank accession numbers: EU852568–EU852578). All Pukerua Bay animals shared a single haplotype that was not found in either Middle Island or Castle Island samples. The Castle Island animal shared a haplotype with Middle Island. Overall haplotype diversity was high (0.89 ± 0.003; Table 1), as was diversity within Middle Island (0.88 ± 0.005). The relationships among haplotypes are shown in Fig. 2. The captive male of presumed Pukerua Bay origin shared a haplotype with both Middle and Castle Islands.

Table 1 Variation in mitochondrial DNA across the three populations of *Cyclodina whitakeri*

	Sample size	Number of haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)
Middle Island	34	10	0.88 ± 0.005	0.79%
Pukerua Bay	5	1	0.0	0.0
Castle Island	1	1	–	–
Total	40	11	0.89 ± 0.003	0.87%

Overall haplotype diversity was high, but was mostly influenced by intra-population diversity in Middle Island.

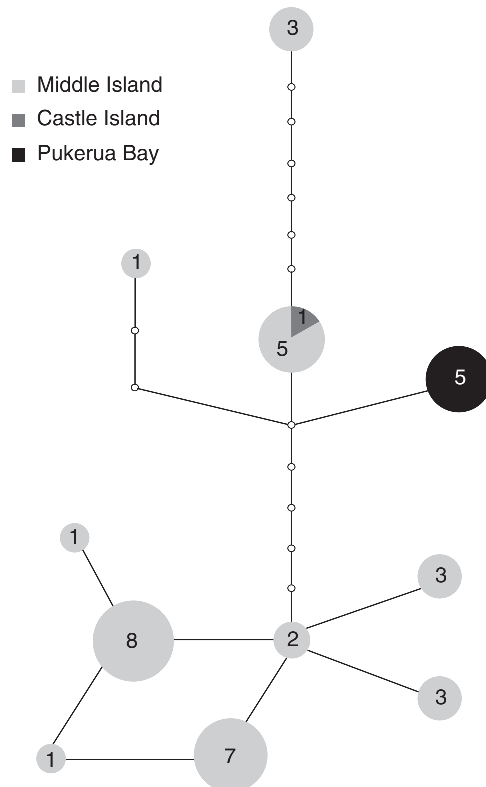

Figure 2 Network showing the relationship among haplotypes of *Cyclodina whitakeri*. Each circle represents one haplotype, and sizes indicate the number of samples (inset) with each haplotype. Each line represents one base pair change between sequences.

Geographic structuring among populations was minor (mean sequence divergence across all samples = $0.87\% \pm 0.23$). Sequence divergence between Pukerua Bay and the island populations (Middle Island: $1.14\% \pm 0.36$; Castle Island: $0.36\% \pm 0.25$) was not greater than between the island populations (Middle to Castle Island = $1.05\% \pm 0.33$) or the mean sequence divergence within Middle Island ($0.80\% \pm 0.20$).

Microsatellite variation within populations

Twelve of 13 microsatellite loci surveyed were polymorphic (2–18 alleles each; Table 2). After removal of *Oligr3* and *Oligr13* (loci with high frequencies of null alleles), both Middle Island and Pukerua Bay populations were in Hardy–Weinberg equilibrium ($P = 0.17$ and 0.08, respectively). Only one locus (*Oligr2*) in Pukerua Bay deviated significantly from Hardy–Weinberg proportions ($P = 0.02$ after sequential Bonferroni correction).

Genetic differentiation between Middle Island and Pukerua Bay was high ($F_{ST} = 0.19$) across 11 loci. The estimate of differentiation between populations was higher using an AMOVA framework, which considers relationships between alleles ($\varphi_{PT} = 0.27$, $P < 0.001$). The population at Pukerua Bay had fewer alleles per locus than the population on Middle Island, even after correction for discrepancies in sample sizes (Table 2).

Private alleles were only labelled in Pukerua Bay and Castle Island populations (Table 2), as sample sizes were unequal between populations and the detection of alleles is highly dependent on sample size. Five loci had a total of eight private alleles in Pukerua Bay, representing 24.2% of the total alleles found in Pukerua Bay samples across all 13 loci (Table 2). All Pukerua Bay animals had between one and four private alleles. The single sample from Castle Island had private alleles at four loci, representing 19% of the total alleles found across 13 loci (Table 2).

All Middle Island and Pukerua Bay animals were correctly assigned to their population of origin ($P < 0.04$). The captive male of presumed Pukerua Bay origin was assigned to Middle Island and Pukerua Bay was excluded as a possible origin ($P < 0.001$).

Discussion

Patterns of genetic diversity at both mtDNA and microsatellites indicate that the island and Pukerua Bay populations of *C. whitakeri* are strongly differentiated. When threatened species are reduced to very few natural populations, we must consider the risks of managing discrete populations separately against the alternative of managing them together, particularly when one population is disproportionately vulnerable to extinction. Only the Middle

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Table 2 Expected heterozygosity (H_E), number of alleles, allelic richness (number of alleles corrected for sample size) and number of private alleles in populations of *Cyclodina whitakeri*

	Middle Island ($n=44$)			Pukerua Bay ($n=5$)			Castle Island ($n=1$)		
Locus	H_E	Number of alleles	Allelic richness	H_E	Number of private alleles	Number of alleles	H_E	Number of alleles	Number of private alleles
Oligr2	0.78	10	4.51	0.66[a]	4	1	1.00	2	1
Oligr3	0.80[a]	10	4.74	0.18	2	0	0.00	1	0
Oligr4	0.90	12	5.89	0.00	1	0	1.00	2	1
Oligr6	0.88	15	5.68	0.74	5	3	1.00	2	1
Oligr7	0.90	16	5.91	0.78	5	1	1.00	2	1
Oligr8	0.70	8	3.90	0.48	2	0	1.00	2	0
Oligr10	0.86	13	5.44	0.66	3	1	1.00	2	0
Oligr13	0.90[a]	17	5.95	0.70	4	2	1.00	2	0
Oligr14	0.11	3	1.41	0.00	1	0	0.00	1	0
Oligr15	0.39	6	2.65	0.00	1	0	0.00	1	0
Oligr17	0.07	2	1.47	0.47	2	0	1.00	2	0
Oligr19	0.00	1	1.00	0.00	1	0	0.00	1	0
Oligr20	0.02	2	1.26	0.32	2	0	0.00	1	0
All loci	0.51[b]	115	49.8	0.37[b]	33	8	0.64[b]	21	4

Both Middle Island and Pukerua Bay populations were in Hardy–Weinberg equilibrium across all combined loci (after removal of loci with a high frequency of null alleles, *Oligr3* and *Oligr13*). Because of disparity in sample sizes, private alleles were only identified in Pukerua Bay and Castle Island populations. Allelic richness in Middle Island was calculated relative to Pukerua Bay.

[a]Locus out of Hardy–Weinberg equilibrium ($P<0.05$).

[b]Excluding *Oligr3* and *Oligr13*.

Island population of *C. whitakeri* is stable (Towns, 1999), and the Pukerua Bay population is highly vulnerable.

Populations are often labelled as evolutionary significant units (ESUs) when they are reciprocally monophyletic at mtDNA and show significant divergences at nuclear loci (Moritz, 1994). This definition is used most frequently as it is simple to implement (De Guia & Saitoh, 2007), but small bottlenecked populations are more likely to be identified as ESUs when only neutral markers are considered (Allendorf & Luikart, 2007). The island and Pukerua Bay populations qualify as separate ESUs under this definition, but recent population declines limit its applicability. More robust ESU definitions use data on non-molecular traits, including adaptive differences and ecological exchangeability (Waples, 1991; Crandall *et al.*, 2000). Other authors suggest that both the specific management objectives for and the anthropogenic risks faced by the species must be considered (Taylor & Dizon, 1999), and that no single definition will work in all cases (Fraser & Bernatchez, 2001).

When populations are extremely small and/or rare, it is difficult to obtain the required ecological information to evaluate more robust definitions of ESUs. However, local adaptation, particularly with respect to temperature in ectotherms, is possible when populations survive in atypical habitats. *Cyclodina whitakeri* typically live in seabird burrows (Southey, 1985), but occupy deep boulder banks at Pukerua Bay. Seabird burrows maintain stable temperatures (18–19 °C) and high humidity (>90%; Towns, 1992*a*), but are not found at Pukerua Bay as seabirds are also vulnerable to mammalian predators. Although use of boulder bank habitat most likely reflects a response to mammalian predators (Towns & Elliott, 1996; Hoare *et al.*, 2007), animals at Pukerua Bay may have adapted to thermal conditions. Temperature and humidity are major physiological constraints for *C. whitakeri*, and temperatures at Pukerua Bay are relatively low, as Pukerua Bay lies ~500 km south of the island populations. Temperature would be a major selective force for *C. whitakeri*, and animals in captivity have died during winter frosts typical in the Pukerua Bay region. It is possible that the population is locally adapted to conditions at Pukerua Bay, but without clear evidence of adaptive divergence, the island populations and Pukerua Bay should be treated as a single unit of conservation (Crandall *et al.*, 2000).

While the population of *C. whitakeri* at Pukerua Bay shows differences from the island populations at both mtDNA and microsatellite loci, this pattern is most likely a product of an historic genetic gradient coupled with strong genetic drift. Populations of *C. whitakeri* have declined since the introduction of mammalian predators. The three remnant populations are at opposite ends of the species historic range (Worthy, 1987); historic population structure due to isolation by distance could partially explain the relatively large proportion of private alleles (24.2% of all alleles found in Pukerua Bay; Table 2) and a unique mtDNA haplotype. With a population size almost certainly smaller than 100 individuals (extrapolated from Towns & Elliott, 1996; Hoare *et al.*, 2007), the population at Pukerua Bay is clearly in a demographic bottleneck. Genetic diversity is reduced at both mtDNA and microsatellites in the Pukerua Bay population, indicating that the genetic differences between Middle Island and Pukerua Bay skinks (F_{ST} = 0.19–0.27) could be due to rapid genetic drift. Differentiation is large and significant, but measures of F_{ST} do not necessarily reflect

variation in quantitative traits (Reed & Frankham, 2001, but see Merilä & Crnokrak, 2001). However, the effects of genetic drift may supersede adaptation in cases of severe bottlenecks (Miller, Miller & Daugherty, 2008).

The recent decline of *C. whitakeri* on the mainland has most likely produced the observed pattern of mitochondrial diversity. Patterns of mitochondrial diversity in two widespread *Cyclodina* species (*Cyclodina aenea* and *Cyclodina ornata*; Chapple *et al.*, 2008), whose distribution reflect the historic distribution of *C. whitakeri* (Worthy, 1987) support this assertion. These species inhabit similar habitat (lowland forest) and may provide insight into historic relationships among *C. whitakeri* populations. Populations of *C. aenea* from the lower North Island (including Pukerua Bay) and the Mercury Island group (the group containing Middle Island) belong to a single clade with sequence divergence equal to that seen across populations of *C. whitakeri*, yet populations of *C. aenea* within the Mercury Island group belong to two different clades (Chapple *et al.*, 2008). Populations of *C. ornata* from the lower North Island also group with a northern population (Chapple *et al.*, 2008). Further, the greatest source of mtDNA diversity was found north of the Mercury Islands in both *C. aenea* and *C. ornata*, indicating that perhaps the most divergent populations of *C. whitakeri* were once also found in that range.

Both mtDNA and microsatellite data confirm that the captive male of presumed Pukerua Bay origin likely belongs to one of the island populations. We cannot conclude from which of these populations he originated, as this male shared an mtDNA haplotype with both Middle and Castle Islands. Due to small sample size from Castle Island, it is currently not possible to use nuclear DNA to distinguish between Middle and Castle Islands as the origin of the captive male. However, the high frequency of private alleles in the single sample from Castle Island (19% of all alleles) indicates that further sampling is required to determine the extent of genetic differentiation of this population.

Evaluating the risks of intentional population hybridization is a complex task (Edmands, 2007), and genetic risks must be considered along with many others. The unique set of factors influencing management of a species will have an impact on whether intraspecific hybridization is a viable option for management (Westemeier *et al.*, 1998; Ebert *et al.*, 2002; Berry & Gleeson, 2005; Gleeson *et al.*, 2007). Inbreeding depression can be difficult to demonstrate in cryptic species. However, realistic levels of inbreeding depression influence extinction risks in natural populations, and inappropriate recovery strategies may be implemented if genetic factors are ignored (Frankham, 2005; O'Grady *et al.*, 2006). Isolated relict populations cannot be conserved indefinitely with small size and hybridization may be a viable option for recovery (Westemeier *et al.*, 1998). After introduction of genetic diversity from three populations to a small isolated population in Illinois, greater prairie chicken egg fertility increased by up to 25% (Westemeier *et al.*, 1998). Florida panthers (*Puma concolor coryi*) were hybridized with the subspecies from Texas (*P. stanleyana*) in 1995 with multiple benefits. Survival of hybrid kittens was three times higher than that of purebred kittens (Pimm, Dollar & Bass, 2006) and no hybrid animals suffered from cryptorchidism, a condition common in purebred Florida panthers that may reduce fertility (Mansfield & Land, 2002). Similarly, the release of adders *Vipera berus* from two non-isolated populations into a population suffering from inbreeding depression led to a rapid decrease in the proportion of stillborn young (Madsen, Stille & Shine, 1996) and an increase in recruitment (Madsen *et al.*, 1999).

When species are extremely rare, decisions about genetic management are often limited by small sample sizes. An adaptive management approach may be valuable in these situations. For *C. whitakeri*, genetic data indicate that management efforts should focus on implementing strategies to increase the size of the Pukerua Bay colony in captivity and on sampling individuals from Castle Island. Further, this approach should be used to evaluate the effects of hybridization in the captive colony. An adaptive management approach would require commitment to continued genetic monitoring, but would reduce uncertainty over time by accruing the data needed to improve future management.

Management implications

Current management is aimed at maintaining a pure Pukerua Bay population in captivity for translocation (Miskelly, 1999). However, the sire of all 12 F1 offspring in the program originated from an island population (this study). In light of our genetic data, there are four options for continued management of this population: (1) continuation of the program 'as is,' accepting that population hybridization has occurred; (2) restarting the captive program using only Pukerua Bay animals; (3) maintaining both pure and hybrid populations, while adding more animals from Middle Island (adaptive approach); (4) terminating the captive breeding program and transferring captive animals to a predator-free island. All of these options pose substantial risks of inbreeding depression if new animals are not introduced from the wild. However, an adaptive management approach (option 3) provides an opportunity to assess the risk of population hybridization by pairing animals of Pukerua Bay origin and comparing survival and fecundity of those offspring to that of hybrid offspring (about 30% juvenile mortality, D. Keall, pers. comm.) over several generations. If hybrid animals survive and reproduce as well as or better than pure offspring, animals from Middle Island could be intentionally introduced to the captive population to increase the founding population size. Hybrid animals could comprise a founder group for the proposed translocation to a predator-free site. While animals from Middle island could be released onto a predator-free island with captive animals (option 4), there would be little opportunity to evaluate survival and fecundity. However, the non-genetic risks, including the potential disruption of social interactions and introduction of parasites or disease, and the potential value of preserving historical integrity of a population (Schwartz, 1994; Edmands, 2007) need to be assessed by managers independently of genetic risks. As animals are extremely rare at Pukerua Bay, addition of skinks to the captive breeding program from an island population

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would be beneficial from both genetic and demographic perspectives.

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