

Distinguishing historical fragmentation from a recent population decline – shrinking or pre-shrunk skink from New Zealand?

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Abstract

Species that are rare when first described present a practical management problem because it may be unclear whether the taxon is in the final stages of an anthropogenic decline, or is naturally uncommon, and each scenario dictates a distinct approach to management. We analysed mitochondrial and microsatellite DNA data with population genetic and phylogeographic tools to distinguish between these possibilities in a rare lizard from southern New Zealand. Grand skinks, *Oligosoma grande*, are large rock-dwelling lizards that have a fragmented distribution consisting of a western and eastern cluster of populations separated by ca. 120 km. This distribution could result from human disturbance, pre-human climatic and vegetation changes, or both. All populations were highly genetically structured (overall F_{ST} 0.171, R_{ST} 0.235), indicating that populations were demographically independent and skinks are unlikely to expand their range without human intervention. In addition, the current fragmented distribution is likely to have both historical and recent anthropogenic elements. Two eastern populations showed evidence of being historically large (high θ mtDNA genetic diversity), although they are now small, supporting anecdotal data that grand skinks have declined in historical times. However, eastern and western populations were reciprocally monophyletic for mtDNA lineages, suggesting long independent evolutionary histories that predate the arrival of humans in New Zealand. Eastern and western populations fulfil many criteria to be considered as evolutionarily significant units, but such a classification must be balanced against addressing more immediate threats to the species' survival, such as introduced predators.

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1. Introduction

Humans and their commensals have caused the endangerment and extinction of countless species. Although the declines of charismatic or commercially important species and the agents responsible are often well documented (e.g. the northern elephant seal, *Mir-*

ounga angustirostris, Stewart et al., 1994), less obvious species may be rare before they are even described (e.g. the white-bellied frog, *Geocrinia vitellina*, Roberts et al., 1990). Such cases present a practical problem for wildlife managers because it will be unclear whether the taxon is in the final stages of a major decline caused by human activities, or is naturally uncommon, and each scenario dictates a distinct approach to management. For example, if the species has suffered a recent decline, a key to successful conservation will be to identify the causes so that their impacts can be reduced or eliminated. In contrast, if a species is naturally rare,

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seeking proximate biological causes for rarity such as predation, competition or habitat degradation will be fruitless. Distinguishing declining from naturally rare species is a critical step in the conservation management of species (Caughley, 1994).

1.1. Inferring declines

Recent range contractions can be inferred for a few species from subfossil deposits, which may also allow the agents of decline to be identified. For example, the Tuatara, *Sphenodon punctatus*, is now restricted to offshore islands around New Zealand (Townes et al., 2001), yet subfossil bones indicate that it was widespread on the mainland until 1000 years ago (Worthy, 1997). Their disappearance from mainland New Zealand coincides with the arrival of Polynesian settlers and the Pacific rat *Rattus exulans*. Further experimental work has also verified that *R. exulans* has a detrimental effect on Tuatara populations (Cree et al., 1995). Similar patterns exist for many taxa in New Zealand (Whitaker, 1978; Worthy, 1987), the Pacific islands (Pregill, 1989), and Madagascar (Dewar, 1984).

More commonly, fossil records are not available, and the demographic history of an animal species must be inferred by other means. Increasingly, this is by analysis of molecular data (e.g. Avise, 1992; Grant and Bowen, 1998; Goldstein et al., 1999). One widely employed approach to diagnosing population declines is by comparison of the observed and equilibrium heterozygosities calculated from codominant DNA markers (e.g. Cornuet and Luikart, 1996; Luikart et al., 1998; Luikart and Cornuet, 1998). However, this method is only useful where bottlenecks have been fairly drastic ($N_e \leq 20$ individuals), and have occurred recently ($2-4N_e$ generations; Luikart et al., 1998). Consequently, bottleneck tests are not well suited to identifying ongoing population reduction where a species still numbers in the hundreds or thousands. Yet reversing declines before they get to a critical bottleneck stage is vital to successful conservation (Caughley, 1994).

Range disjunctions may also indicate that a once widespread species has declined, but it could equally arise from ancient vicariance driven by geological or climatic events (Firestone et al., 1999). One approach to distinguishing these processes is phylogeographical analyses of mitochondrial DNA (mtDNA) sequences (e.g. Carpenter et al., 2001; Williams, 2002). Mitochondrial DNA is well suited to studying demographic processes because its small effective population size ($1/4N_e$) makes it highly responsive to genetic drift. In addition, because mtDNA typically is not subject to recombination, the dynamics of the lineage sorting process and how population demography affects it are well understood (e.g. Avise et al., 1984; Neigel and Avise, 1986; Rogers and Harpending, 1992). In situations where a disjunct range

is encountered, a useful qualitative measure of time since isolation is the predicted progression through time from lineage polyphyly via paraphyly to reciprocal monophyly caused by the stochastic sorting of mtDNA lineages (Neigel and Avise, 1986). In some circumstances, the inference can be extended to a more quantitative measure of time since isolation by use of a molecular clock to date lineage divergence (e.g. Treweek and Wallis, 2001; Waters et al., 2001). Mitochondrial DNA data can also provide information about historical effective population sizes because the time to mtDNA lineage coalescence depends on female effective population size (N_{ef}), rate of population growth, and mutation rate (Avise et al., 1984, 1988; Rogers and Harpending, 1992; Kuhner et al., 1998; Crandall et al., 1999). Comparisons of historical effective population size with estimates of current census size enable recent population declines or expansions to be identified (e.g. Ball and Avise, 1992; Roman and Palumbi, 2003).

1.2. The case of the grand skink

In this study, we examine a situation where a rare species has a disjunct range that could be explained by either recent anthropogenic landscape changes or more ancient processes associated with pre-Holocene and pre-human climate change. The grand skink, *Oligosoma grande*, is a large rock-dwelling lizard endemic to montane grasslands in southern New Zealand. The holotype specimen was described in 1845 (Hardy, 1977), but few records were made during the following century until systematic distribution surveys were undertaken in the 1980s (Whitaker and Loh, 1995). Fewer than 5000 individuals exist, and the species is considered vulnerable to extinction by IUCN criteria (Hilton-Taylor, 2000; Whitaker and Houston, 2002). Grand skinks have a curious disjunct distribution (Fig. 1). Most populations occur patchily in a limited region of eastern Otago, but several small populations are also known from western Otago over 120 km away. Aside from a single eastern population at Macraes Flat that consists of several thousand individuals (Patterson, 1992), repeated surveys have revealed that most populations are extremely small and probably consist of tens to hundreds of individuals (G. Loh, NZ Dept. Conservation, pers. comm.).

The current vulnerable status and approach to management of this species is based on the presumption that it was formerly widespread and has suffered a major range decline since human settlement in New Zealand, retracting to its current distribution, in particular, since the introduction of rabbits *Oryctolagus cunicularis*, 200 years ago (Whitaker and Houston, 2002). With little historical documentation, evidence for the decline is largely anecdotal, and mainly based on three points: (1) the occurrence of apparently suitable habitat in the region between the current populations; (2) anecdotal accounts

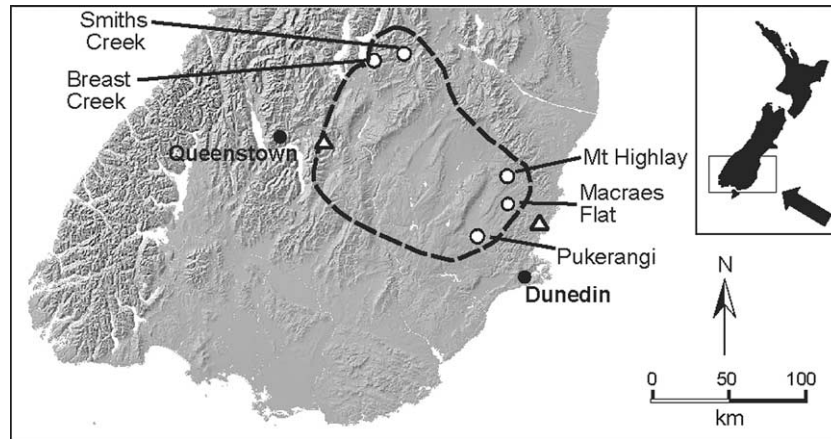


Fig. 1. The distribution of grand skinks, *Oligosoma grande*, in southern New Zealand. Open circles are sampled populations, and the open triangles are the approximate locations of historical samples. The dashed line indicates the estimated (pre-human) former range (adapted from Whitaker and Houston, 2002) and includes most of the district of Otago.

of “large lizards” in central Otago; and (3) two specimens collected outside the current range in 1907 and 1910 (Fig. 1; Hardy, 1977; Whitaker and Loh, 1995).

An alternative yet unexplored hypothesis is that grand skink populations experienced vicariance associated with pre-Holocene climatic changes, and that the isolated populations represent ancient refugia, predating human arrival in New Zealand. The arguments for a more ancient cause of the distribution are: (1) in the last 2–5 million years southern New Zealand experienced tectonic mountain building, greatly fluctuating temperatures, extensive glacial activity, and pronounced vegetational changes (Kershaw, 1988; Pillans et al., 1992; Cooper and Millener, 1993; McGlone et al., 1995) that may have affected the distribution of skinks; (2) preliminary evidence for morphological differences between individuals from the western and eastern parts of the range, which may be indicative of a long period of separation (Whitaker and Loh, 1995); (3) the presence of a series of mountainous ridges and wide valleys bisecting the region between the eastern and western populations that could limit dispersal, particularly in combination with climatic changes (Fig. 1); and (4) several taxa from southern New Zealand exhibit pronounced phylogeographic structure that have been attributed to the dynamic geological history of the region (e.g. geckos, Hitchmough, 1997; invertebrates, Trewick et al., 2000; Trewick and Wallis, 2001; Chinn and Gemmell, 2004; galaxiid fish, Waters et al., 2001). Furthermore, genetic studies of New Zealand lizards have revealed a large number of cryptic species, prompting extensive taxonomic revision (Daugherty et al., 1990; Hitchmough, 1997; Patterson, 1997).

We used mtDNA control region sequences and nuclear microsatellite DNA loci to evaluate two related questions: (1) do the western and eastern grand skink populations represent ancient refugia that are a legacy

of Pleistocene glacial cycles; and (2) is there evidence for recent population declines?

2. Methods

2.1. Sampling

Sixty-five grand skinks were sampled from most known populations throughout the species’ range between November 2000 and March 2002 (Fig. 1). Despite extensive searching, few samples ($n = 2$) could be obtained from two populations (Mt Highlay and Smiths Creek) where skinks were at extremely low densities (~ 0.67 skinks sighted day⁻¹; O. Berry unpubl.). Skinks were captured with nylon fishing line nooses. Toe-clips or tail tips (< 2 mg tissue) were taken and stored in liquid nitrogen or 95% ethanol and skinks were released at their point of capture. DNA was isolated by a salting-out procedure (Sunnucks and Hales, 1996).

2.2. Isolation of mtDNA control region sequences

The hypervariable region I (HVRI) of the mtDNA control region has been widely used to recover intraspecific genealogies because of its relatively rapid rate of sequence evolution (e.g. Wenink et al., 1993). However, control region sequences have only been described from a single (and distantly related) skink species (*Eumeces egregius*; Kumazawa and Nishida, 1999). To identify suitable regions for primer placement in HVRI we characterised the control region in *O. grande* by long-range PCR anchored to flanking genes, followed by inwards sequencing with primer walking. Initially we sequenced a 306 nt fragment of the mitochondrial cytochrome *b* gene of four skinks from the Macraes Flat population using universal primers (L14841 and H15149; Kocher

pairwise F_{ST} values between populations from haplotype frequencies.

Prior to phylogenetic analysis we used the computer program MODELTEST 3.06 (Posada and Crandall, 1998) to determine the appropriate model of sequence evolution for the data. We used unweighted maximum parsimony (MP), and maximum likelihood (ML) analysis to infer phylogenetic relationships among haplotypes using the program PAUP* 4.01 (Swofford, 1998). For the ML analysis we conducted an initial heuristic search using the tree bisection and reconnection (TBR) option with 10 random additions of taxa, then reoptimised the model parameters on this tree before running a final heuristic search. A heuristic search was also conducted for the maximum parsimony criteria using TBR with 10 random additions of taxa. Support for the branching pattern in the ML analysis was established using 100 nonparametric bootstrap replicates, and the MP analysis with 1000 replicates. PAUP* was also used to calculate a pairwise distance matrix between all haplotypes using the HKY85 + Γ model of substitution. We tested for clock-like sequence evolution using a likelihood difference test (Huelsenbeck and Crandall, 1997).

2.4. Effective population size estimates

We used a maximum likelihood approach implemented in the computer program FLUCTUATE 1.4 (Kuhner et al., 1998) to estimate the genetic diversity parameter θ in the Macraes Flat and Pukerangi populations, for which estimates of census size were available. This method makes use of the information about a population's history contained in genealogical data. The method allows for genealogical uncertainty by sampling over a range of genealogies weighted by their likelihoods to estimate θ . θ is a product of the female inbreeding effective population size ($2N_{ef}$) and the substitution rate per generation (μ), and thus if substitution rate is known, θ can be used to estimate the effective population size (see Vilá et al., 1999; Roman and Palumbi, 2003). Substitution rates for the mtDNA control region have not been characterised in lizards. We used a relatively fast estimate of 2.85×10^{-8} substitutions/site/year taken from cytochrome *b* sequence of a lacertid lizard ("fast clock", Paulo et al., 2001). This rate is the most rapidly evolving molecular clock that has been published for a lizard, and therefore represents a conservative lower bound for the rate of evolution in *O. grande*. In addition, because it is generally accepted that the mtDNA control region evolves 3–5 times as rapidly as protein coding genes such as cytochrome *b* (Aquadro and Greenberg, 1983; Avise, 2000; but see Ruokonen and Kvist, 2002), we also used 1.0×10^{-7} , to cover the likely range of substitution rates in skinks. Generation time was estimated from the average age of female skinks giving birth, which was 6.4 years (Berry unpubl.

data). FLUCTUATE assumes no selection, recombination or migration and we also assumed a constant population size. Our microsatellite data indicated that gene flow between populations was negligible. However, for population Macraes Flat there was evidence of historical gene flow, which would inflate θ estimates (see results). Therefore we calculated two values of θ – one that included lineages that might have originated from outside Macraes Flat, and another without these lineages. We used estimates for T_i/T_v ratio and base frequencies as estimated from a run of MODELTEST as starting values for the analysis.

2.5. Hypothesis testing

We used parametric bootstrapping (Hillis et al., 1996) to test the null hypothesis that eastern and western populations were reciprocally monophyletic for mtDNA lineages. A model tree was selected using a maximum parsimony search with the dataset constrained to conform to the reciprocal monophyly hypothesis. We used the difference between the parsimony score of the best constrained MP tree and the best unconstrained MP tree as the test value. We then generated 100 simulated sequence datasets using maximum likelihood estimates of model parameters and branch lengths from the best constrained MP tree using the computer program SEQ-GEN (Rambaut and Grassley, 1997), and conducted two heuristic searches on each dataset. The first was to find the best tree overall, and the second to find the best tree compatible with the constraint. The differences in parsimony scores for each dataset were used to construct a null distribution of these differences. The significance of the test was assessed by direct comparison of the test value for the actual data with the null distribution (Hillis et al., 1996; Goldman et al., 2000).

2.6. Microsatellite analyses

All skinks were genotyped with 12 microsatellite loci designed for *O. grande* (Berry et al., 2003). Skinks from the western populations did not amplify at two loci (Oligr11 and Oligr15) and these loci were removed from the analysis. We calculated mean and pairwise F_{ST} (Weir and Cockerham, 1984) between populations with FSTAT 2.9.3 (Goudet, 1995), and also calculated values of weighted R_{ST} (Rousset, 1996), which accounts for stepwise mutation in microsatellite DNA. In addition, we calculated the shared allele distance between all pairs of individuals (D_{as}), and constructed a neighbour-joining (NJ) tree using the program POPULATIONS 1.2.28 (Langella, 2000). We also constructed a NJ tree based on the average D_{as} between populations. Support for nodes was established by bootstrapping over loci and over individuals.

3. Results

We characterised a 3804 nucleotide sequence of the grand skink mtDNA genome. This consisted of the majority of the cytochrome *b* gene, tRNA-phenylalanine, tRNA-threonine, the control region, tRNA-proline, and 383 nt of the 12s ribosomal RNA gene.

3.1. Sequence heteroplasmy in the mtDNA control region

The control region contained two heteroplasmic regions. The first, at the 5' end of HVRI, consisted of 50 bp repeat units. In two clones sequenced from a single skink one had a single motif and the other had four identical repeats. In addition, in two skinks sequenced for this region, two different repeat motifs were identified. The repeats differed by an A ↔ T and a C ↔ T substitution. Heteroplasmy was also observed in a poly-A string near the central conserved domain. In six clones sequenced from a single individual A₁₀, A₁₁, A₁₂ and A₁₃ haplotypes were recorded.

3.2. Characteristics of the mtDNA data

530 bp of sequence could be resolved from all individuals and we detected 47 polymorphic sites (Table 2). Thirty-six sites were parsimony informative, and 11 were singletons. The most likely model of nucleotide substitution as identified by MODELTEST was HKY85 + Γ. The estimated nucleotide frequencies in the dataset according to this model were A: 0.298, C: 0.093, G: 0.259, T: 0.350, the gamma shape parameter was 0.1737, and the transition to transversion ratio was 3.5689:1. The sequences have been deposited in GenBank Accession Nos. AY700064–AY700080.

3.3. MtDNA phylogenetic analyses

No populations shared haplotypes (Table 2). Both eastern populations Pukerangi and Macraes Flat had high haplotypic and nucleotide diversity relative to the

western population at Breast Creek (Table 3). The Macraes Flat population contained three groups of highly divergent haplotypes, with corrected HKY85 distances between haplotypes ranging between 0.2% and 6.4% (average 3.1% ± 0.4 SE). Trees produced by maximum likelihood and maximum parsimony criteria had very similar topologies, resolving the same four well supported clades (Fig. 2), but neither analysis could resolve whether eastern and western populations were reciprocally monophyletic. However, the most parsimonious tree constrained to the reciprocal monophyly hypothesis only required an additional three steps more than the observed tree, and parametric bootstrapping analyses indicated that the null hypothesis of reciprocal monophyly of eastern and western populations could not be rejected ($p = 0.53$; Fig. 3). The likelihood difference test showed no significant difference between the log-likelihood of phylogenetic trees with and without a molecular clock enforced ($2\Delta\log\text{-likelihood} = 23.54$, critical $\chi^2_{15} = 25.00$, $p > 0.05$).

3.4. θ and Historical effective population sizes

Values of the genetic diversity estimator θ for populations Macraes Flat and Pukerangi were high (Table 4), and translated into estimates of effective female population size that were greater than census sizes in most cases, regardless of the assumed substitution rate or whether putative historical migrant haplotypes were included.

3.5. Microsatellite DNA data

All populations exhibited high levels of microsatellite DNA variation (Table 3), although the eastern populations Mt Highlay and Pukerangi were genetically more variable than the only well-sampled western population at Breast Creek based on all measures of diversity. Paired *t* tests based on allelic richness, which accounts for differences in sample size (Petit et al., 1998), showed that both Macraes Flat and Pukerangi were significantly

Table 3
Descriptive statistics for microsatellite and mtDNA data

Population	<i>n</i>	Microsatellite				MtDNA				
		H_{exp}	H_{obs}	AR	<i>A</i>	hap	<i>h</i>	<i>II</i>	Dist ^a ± SE	
Eastern	MF	42	0.82 ± 0.04	0.78 ± 0.11	3.14 ± 0.14	11.5 ± 1.0	9	0.82 ± 0.04	0.02541	3.1 ± 0.4
	PK	7	0.90 ± 0.03	0.64 ± 0.18	3.37 ± 0.12	7.7 ± 0.7	4	0.80 ± 0.17	0.00693	0.8 ± 0.1
	MH	2	0.60 ± 0.09	0.60 ± 0.32	2.30 ± 0.21	2.3 ± 0.2	1	0	–	0
Western	BC	11	0.70 ± 0.04	0.50 ± 0.16	2.62 ± 0.13	5.2 ± 0.4	2	0.22 ± 0.17	0.00043	0.2 ± 0.2
	SC	2	0.78 ± 0.08	0.75 ± 0.26	2.90 ± 0.28	2.9 ± 0.3	1	0	–	0
Total	64	0.78 ± 0.02	0.65 ± 0.03	3.4 ± 0.09	20.8 ± 1.9	17	0.37 ± 0.03			3.3 ± 0.2

AR, allelic richness (based on a maximum of 2 diploid individuals); H_{exp} , gene diversity; H_{obs} , observed heterozygosity; *A*, average number of alleles; hap, number of haplotypes recorded; *h*, haplotype diversity; *II*, nucleotide diversity.

^a Dist = average HKY85 + Γ distance between haplotypes within population.

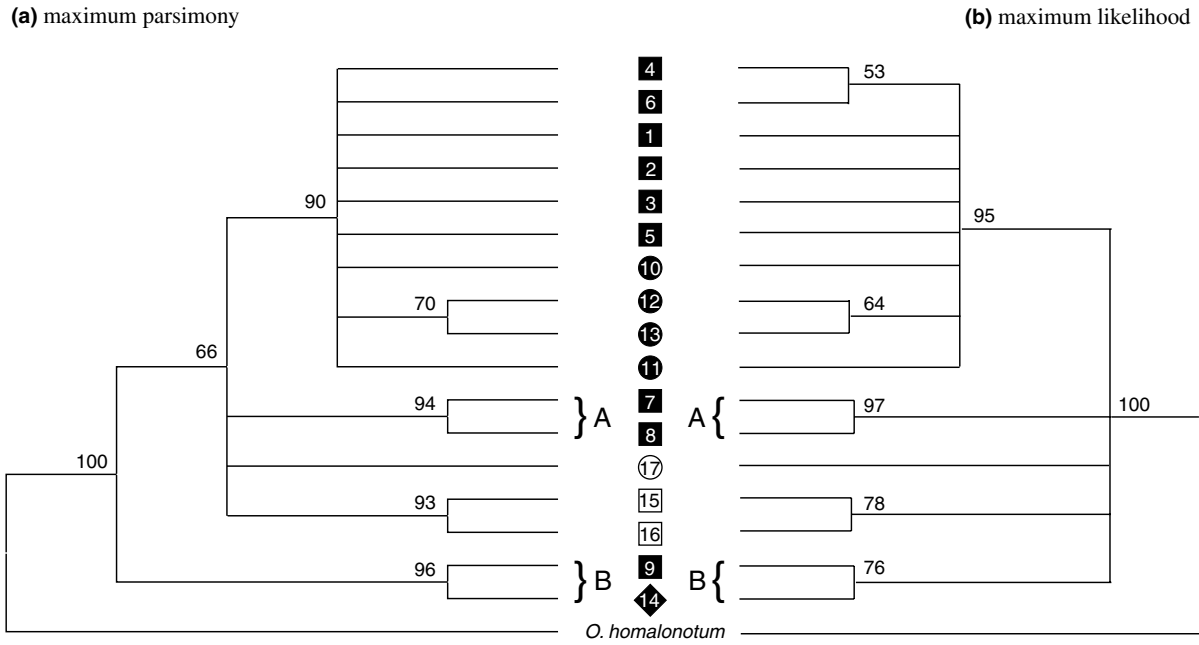


Fig. 2. Phylogenetic trees based on control region mtDNA sequence from *Oligosoma grande*. (a) Maximum parsimony analysis showing strict consensus of 3750 most parsimonious trees with 198 steps. (b) Maximum likelihood analysis under a HKY85 + Γ model of sequence evolution (majority rule bootstrap consensus). Numbers above nodes are non-parametric bootstrap values. Symbols identify populations: black square Macraes Flat, black circle Pukerangi, black diamond Mt Highlay, open square Breast Creek, open circle Smiths Creek. The lineages labelled A and B are referred to in the text, and the numbers inside the symbols refer to haplotypes in Table 2.

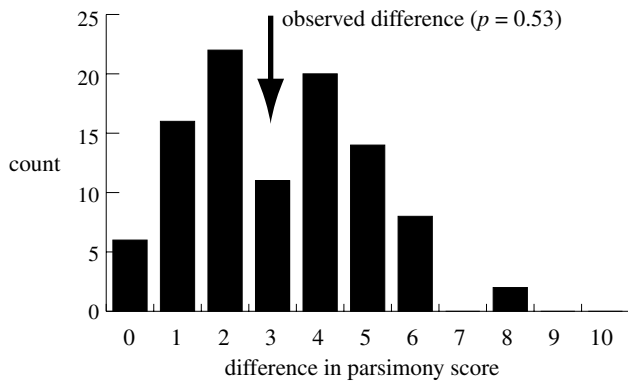


Fig. 3. Results of parametric bootstrapping to test the null hypothesis that eastern and western grand skink populations were reciprocally monophyletic for mtDNA lineages. Arrow indicates the difference in parsimony scores from the observed data.

more variable than Breast Creek (Macraes Flat vs. Breast Creek $t_{9, 0.05} = 4.83$, $p < 0.001$; Pukerangi vs. Breast Creek $t_{9, 0.05} = 2.26$, $p < 0.001$). Sample sizes for the Mt Highlay and Smiths Creek populations were too small for useful comparisons to be made.

The overall level of genetic subdivision among populations as measured by F_{ST} was 0.171 (± 0.022 SE), and by weighted R_{ST} was 0.235 (± 0.082 SE). All populations were highly genetically divergent, although the small samples from Smiths Creek and Mt Highlay mean that the results for these populations are provisional (Table

5). In addition, clustering analysis showed that all individuals grouped according to their population of origin (Fig. 4). Clustering analysis of populations showed that the two western populations (Breast Creek and Smiths Creek) and two of the eastern populations (Macraes Flat and Pukerangi) grouped separately from one another with relatively high bootstrap support, but the position of the Mt Highlay population relative to these clades was not well resolved (Fig. 5).

4. Discussion

Are grand skink populations natural relics or recent fragments? Distinguishing which of these scenarios is more likely will have practical and potentially financial importance for the management strategy adopted. For example, captive breeding and translocation are being considered as possible management tools (Whitaker and Houston, 2002). If the current populations represent lineages that persisted in ancient refugia, their evolutionary distinctiveness may demand that animals from different populations are not interbred or that existing historical relationships among populations are taken into account if translocations were made or captive colonies assembled (Dunham and Minckley, 1998; Moritz, 1999). Alternatively, recently formed and small isolates that are vulnerable to stochastic demographic or genetic processes may be supplemented by translocations from

Table 4

Estimates of female effective population size (N_{ef}) at Macraes Flat and Pukerangi populations derived from estimates of θ

Population/clades included	Substitution rate/gen (μ)	Genetic diversity (θ)	Effective female population size (N_{ef})	95% confidence intervals of N_{ef}	Census size
Macraes Flat					1800 ^a
Partial haplotypes	1.84×10^{-7} 6.44×10^{-7}	0.0034	9170 2613	3170–13,351 903–3805	
Macraes Flat					
All haplotypes	1.84×10^{-7} 6.44×10^{-7}	0.0196	53,304 15,192	34,624–82,134 9868–23,408	
Pukerangi					100s ^b
	1.84×10^{-7} 6.44×10^{-7}	0.0079	21,584 6,151	7,143–76,146 2,036–21,702	

Partial haplotypes refers to calculations based on haplotypes H-Og1-6 only, and all haplotypes refers to calculations based on all haplotypes recorded from Macraes Flat (see text for explanation).

^a Patterson, 1992.

^b Graeme Loh, NZ Department of Conservation pers. comm.

Table 5

Genetic differentiation between grand skink populations

	Macraes Flat	Pukerangi	Mt Highlay	Breast Creek	Smiths Creek
Macraes Flat	█	0.42	0.20	0.12	0.22
Pukerangi	0.09/0.18	█	0.29	0.27	0.33
Mt Highlay	0.20/0.32	0.13/0.40	█	0.35	0.16
Breast Creek	0.21/0.38	0.17/0.53	0.22/0.81	█	0.43
Smiths Creek	0.16/0.32	0.08/0.40	0.29/1.00	0.16/0.81	█

Proportion of shared microsatellite alleles above diagonal and F_{ST} below diagonal (microsatellite/mtDNA).

less vulnerable populations without risk of disturbing adaptive gene complexes (e.g. the Florida panther, *Felis concolor coryi*; Hedrick, 1995).

4.1. Eastern populations – genetically diverse and a recent decline

Despite their small size (ca. 100s–1000s of individuals), the eastern populations Macraes Flat and Pukerangi were characterised by high haplotypic, nucleotide and θ diversity. In part this is due to the presence of deeply divergent lineages in the Macraes Flat population, which most likely result from historical gene flow (see below). However, even when these lineages are excluded from analysis, both the Macraes Flat and Pukerangi populations are currently too small to have retained the levels of mtDNA genetic diversity recorded (Table 4). Importantly, this suggests that the current population sizes are a fraction of historical effective population size (N_{ef}). Furthermore, because adult sex ratios are approximately 1:1 (Whitaker, 1996), and effective population sizes are on average only 10% of adult census sizes (Frankham, 1995a), the historical census sizes would have been even greater than the N_{ef} estimates in Table 4. While the estimates of long-term effective female population size make several simplifying assumptions and rely on a single locus, the magnitude of the difference be-

tween observed and expected population sizes based on coalescent theory are large enough that the qualitative result appears robust. It is also congruent with anecdotal evidence for a widespread decline of grand skinks reported by Whitaker and Loh (1995). Studies that have employed similar approaches to understand demographic histories of populations have usually shown the opposite pattern to that observed here – effective female population sizes much smaller than observed census sizes (e.g. Avise et al., 1988; Ball and Avise, 1992; but see Roman and Palumbi, 2003), but the species examined (e.g. American eel, redwing blackbird) were numerous, geographically widespread and not known to be in decline.

The finding that at least two grand skink populations have declined recently is not unexpected, given that almost half of New Zealand's lizard species (26 of 62) occur as small populations or are restricted to offshore islands (Towns et al., 2001). Nevertheless, before this study, no direct evidence had been gathered that supported the theory put forward by Whitaker and Loh (1995) that grand skinks have declined markedly since human settlement in New Zealand. Our results reinforce the need to address the causes of grand skink endangerment, which include predation by feral animals, destruction of habitat and changes to landscape structure (Whitaker and Loh, 1995; Whitaker, 1996; Berry et al.,

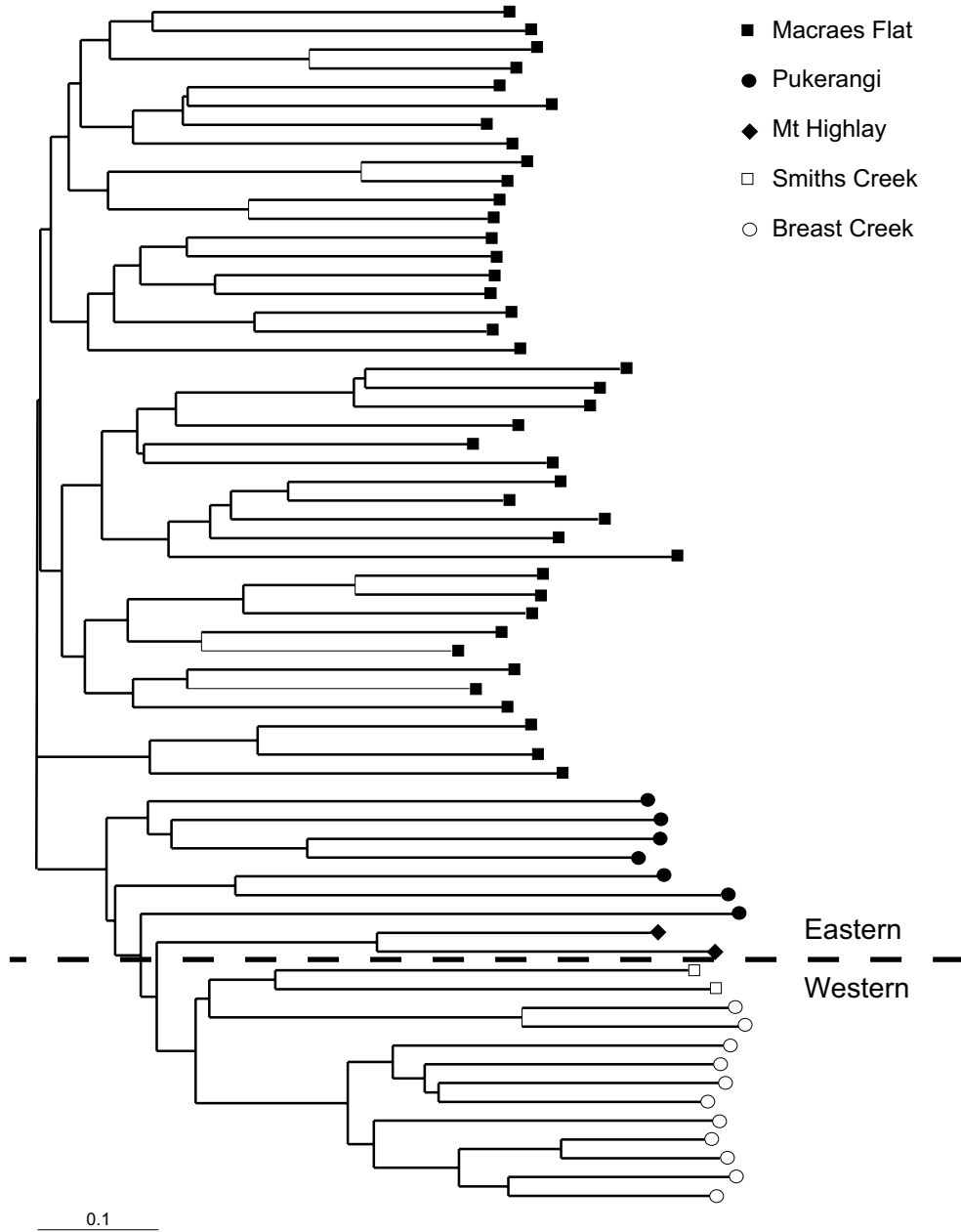


Fig. 4. Neighbour-joining tree based on the shared allele distance between individuals (D_{as}). Symbols refer to sampling populations and are the same as Fig. 2.

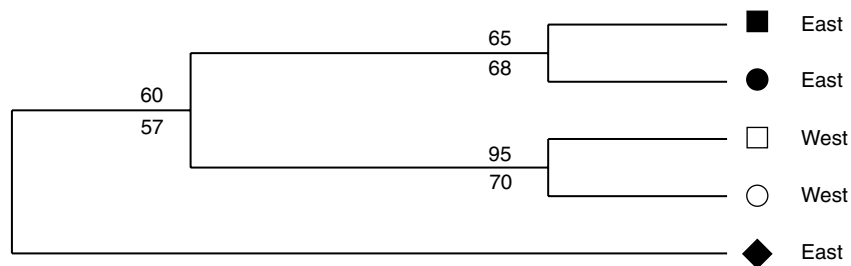


Fig. 5. Neighbour-joining tree based on the average shared allele distance between populations (D_{as}). Numbers at nodes refer to bootstrap support. Values above and below nodes were derived by bootstrapping over individuals and loci, respectively. Symbols indicate populations and are the same as Fig. 2.

in press). More generally, the results provide insight into the New Zealand biota prior to human settlement, where in the absence of terrestrial mammals, birds and reptiles may have existed at abundances that are now almost inconceivable. The results also illustrate how genetic evidence can be used to detect a decline even before it reaches a critical bottleneck stage, which is the most relevant time to take management action (Caughley, 1994). In this case tests based on departure from expected equilibrium heterozygosities in the microsatellite data (Cornuet and Luikart, 1996; Luikart et al., 1998) did not show any evidence for a population bottleneck in any grand skink populations (analyses not presented).

4.2. Population structure

Grand skink populations were highly genetically structured. By implication, inter-population dispersal must be negligible or zero. This is consistent with results from mark-recapture and fine-scale genetic studies, which showed grand skinks have limited dispersal capabilities on scales of several hundred metres (Houghton, 2000; Berry et al., in press). The significance of extremely limited dispersal is twofold. First, in combination with the small size of most populations, almost all face grave risk of extinction by stochastic processes (Lande, 1988), let alone the risks posed by abundant feral predators (Middlemiss, 1995). Second, their ability to recolonise vacant habitat will be very limited, and although suitable rocky habitat is abundant in southern New Zealand (Whitaker and Loh, 1995), they are unlikely to expand their range rapidly without human intervention. This isolation is likely to be exacerbated by the increasing modification of the native vegetation in the region (Berry et al., in press). Each population clearly represents a unit of management because their short-term viability is determined entirely by the internal rates of birth and death (Moritz, 1994a).

4.3. Eastern populations – deep lineages, ancient vicariance and ancient gene flow

Three highly distinct and well-supported mtDNA lineages were observed in skinks from Macraes Flat. This pattern is uncommon, and is usually interpreted as admixture of historically isolated populations (Grant and Bowen, 1998; Avise, 2000). There has been little characterisation of a molecular clock for lizards, but to provide a rough perspective on the time frame involved in the vicariance we use a relatively rapidly evolving molecular clock estimated from the cytochrome *b* gene of lacertid lizards (“fast clock”, Paulo et al., 2001), which evolves at 2.85% per million years. Based on this rate, the divergent mtDNA lineages at Macraes Flat, which differ from one another by a minimum

and maximum distance of 4.6% and 6.4% corrected sequence divergence respectively, coalesce between 1.6 and 2.24 million years ago. Significantly, this straddles the Pliocene-Pleistocene boundary (1.8 mya Fleming, 1979), which marks the end of relatively stable warm climates that had persisted since the Miocene, and the beginning of more extreme conditions associated with glacial cycles (Fleming, 1979; Kershaw, 1988; Pillans et al., 1992). Although the cytochrome *b* gene is generally thought to evolve more slowly than the control region (Aquadro and Greenberg, 1983), it does not always (Ruokonen and Kvist, 2002). The dates suggested here must therefore be viewed as provisional until further details of rates of molecular evolution in lizards become available. Nevertheless, even mutation rates an order of magnitude higher still place the divergence of these lineages prior to the Holocene.

Fossil evidence has shown that the Pleistocene glacial conditions profoundly affected the distribution of the New Zealand biota (Suggate et al., 1978; Fleming, 1979; McGlone, 1988), and our molecular data provide an independent demonstration of these impacts. Our results closely parallel data from the alpine weta, *Hemideina maori*, (Orthoptera) from the Rock and Pillar Range, which is immediately to the east of Macraes Flat (King et al., 2003). In that study, two deeply divergent mtDNA clades with corrected COII distance ca. 4.5% co-occur and are different colour morphs. This indicates that a common climatic process at the Pliocene-Pleistocene boundary may have caused both alpine invertebrates and sub-alpine skinks to retract to multiple refugia. Investigation of the phylogeographic structure of additional co-occurring but more common species may reveal further details on the nature of this significant biogeographic processes.

Our data also offer a perspective on the nature and timing of the expansion from these refugia. The presence of three divergent mtDNA lineages at Macraes Flat implies that at least two separate gene flow events occurred, and from different source populations. In addition, the two lineages (labelled A and B in Fig. 2) differ in the level of intra-lineage divergence between haplotypes and haplotypes in lineage A differ by 1.5% corrected sequence difference, whilst haplotypes in lineage B differ by only a single substitution (0.2%), implying that the geneflow occurred at different times. Since geneflow must have occurred prior to the divergence of haplotypes (cf. Bowen and Avise, 1990), the deep divergence in lineage A places it at ca. 525,000 years bp (significantly, a similar level of intra-lineage divergence was presented in King et al.’s (2003) study of weta). In contrast, the high similarity of lineage B haplotypes, which were recorded from both Macraes Flat and Mt Highlay, indicates that gene flow occurred from Mt Highlay to Macraes Flat much more recently, perhaps even during the current interglacial. Additional

sampling of the Mt Highlay population would provide greater precision on the timing of this process.

4.4. Population history: western populations – post-glacial founding

In contrast to the eastern populations, the western population Breast Creek had extremely low levels of haplotype and nucleotide diversity as well as lower microsatellite diversity. Such a pattern is typical of a historical population bottleneck or founding event (Grant and Bowen, 1998). Because of their close proximity to alpine regions and major glacial valleys such as Lake Hawea, which were periodically covered by extensive ice sheets and tundra during the Pleistocene (Fleming, 1979; Pillans et al., 1992) it is likely that the western populations were founded following the last glacial maximum 14,000 years ago, although because the mtDNA lineage is distinct from all others, the refugium was not shared by any of the eastern populations studied here. Further samples from the Smith Creek population would clarify its relationship to the Breast Creek population.

4.5. Pre-human distribution

Whitaker and Loh (1995) suggested that prior to European settlement in New Zealand approximately 200 years ago, grand skinks had a more or less continuous distribution throughout the central Otago region, and have since contracted to approximately 8% of their former range. Our data allow us to make two observations on the pre-human distribution of grand skinks. First, prior to the Holocene (ca. 10,000 ybp) grand skinks had a dynamic distribution that probably changed with the twenty or so Pleistocene glacial cycles that occurred (Suggate et al., 1978). Second, there is evidence that as recently as the Holocene, skink populations that now appear isolated (e.g. Macraes Flat and Mt Highlay) probably experienced gene flow – indicating a previously more continuous distribution. However, the high level of genetic structuring among the remaining populations makes it more likely that the distribution throughout Otago was naturally discontinuous, although the species could still have been widespread. An alternative interpretation that we cannot exclude is that the distribution was continuous, but a very strong isolation by distance effect existed because of the low dispersal capabilities of grand skinks.

4.6. The significance of evolutionary history to grand skink management

Do the western and eastern grand skink populations have long-independent evolutionary histories that pre-date human arrival in New Zealand? The answer is al-

most certainly yes. Both the mtDNA and microsatellite data indicate that they are strongly genetically differentiated, and they are indistinguishable from being reciprocally monophyletic for mtDNA lineages. In addition, preliminary evidence suggests that western and eastern skinks are subtly morphologically different (size and colour differences; Whitaker and Loh, 1995; O. Berry, pers. obs.). Overall, western and eastern populations probably fulfil most criteria to be considered evolutionarily significant units (Waples, 1991; Moritz, 1994b; Vogler and Desalle, 1994; Crandall et al., 2000; Fraser and Bernatchez, 2001). They are also more genetically divergent than almost all eight species of the *Galaxias vulgaris* species complex that are endemic to southern New Zealand rivers (Waters and Wallis, 2001). Yet is this sufficient for managers to give consideration to their evolutionary heritage? We believe that this case highlights the potential for conflict between short-term demographic versus long-term evolutionary goals in conservation.

Like all native New Zealand terrestrial vertebrates, grand skinks face the well-documented pressing threats of predation by invasive mammals as well as small population size (Towns, 1985; Towns et al., 2001; Whitaker and Houston, 2002). All but one of the populations studied here were small, and although precise population estimates are not known, all must face serious threat of extinction in the short term (Whitaker and Houston, 2002). A problem confronting managers is how to evaluate the relative importance of these known threats alongside less tangible long-term conservation goals such as ensuring future evolution (Crandall et al., 2000) and avoiding outbreeding depression (Frankham, 1995b). Both the short- and long-term goals are stated in policy that guides implementation of the New Zealand Conservation Act (1987) (Anonymous, 2003). It is likely that in practice, managers will have to be pragmatic in implementing the Conservation Act because ascribed evolutionary significance will have little conservation relevance for a species with more immediate threats. This situation parallels one in the management of New Zealand endemic frogs *Leiopelma* spp. (Holyoake et al., 2001), and probably many other taxa.

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