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Genetic diversity and gene flow in a rare New Zealand skink despite fragmented habitat in a volcanic landscape

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Anthropogenic habitat fragmentation often restricts gene flow and results in small populations that are at risk of inbreeding. However, some endangered species naturally occupy patchy habitat where local population extinction and recolonization are normal. We investigated population fragmentation in the range-restricted New Zealand small-scaled skink (*Oligosoma microlepis*), documenting changes in habitat occupancy and analyzing mitochondrial, microsatellite, and morphological variation sampled across the geographical range of the species (approximately 100 km^2). Small-scaled skinks have a strong preference for rocky outcrops that exist in a mosaic of other habitat types. A metapopulation structure was indicated by both local extinction and colonization of new sites. We found relatively high mtDNA nucleotide site diversity within this narrow range ($\pi = 0.004$; 16S), evidence of inter-patch gene flow, and no statistical support for inbreeding. Gene flow was limited by geographical distance, although the existence of pasture between habitat patches apparently has not prevented skink dispersal. Generalized linear models indicated an association between body size and location suggesting a local environmental influence on phenotype. Prior to human-induced habitat modification, native forest probably separated preferred sites and, less than 2000 years ago, volcanic activity devastated much of the area currently occupied by *O. microlepis*. This skink appears able to re-establish populations if other human-linked factors such as agricultural intensification and introduced predators are limited. Although in contrast to expectations for a scarce and localized species living in a highly modified landscape, this lizard may have previously adapted to a dynamic, mosaic environment mediated by volcanism. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **119**, 37–51.

KEYWORDS: conservation genetics – metapopulation – mtDNA haplotype – *Oligosoma* – patchy habitat – reptiles – size variation.

INTRODUCTION

There are many consequences of population fragmentation and the anthropogenic fragmentation of natural populations has been linked to decreased genetic variation, inbreeding depression, and increased susceptibility to environmental and demographic fluctuations (Frankham, 1998, 2005; Willi *et al.*, 2007). Arising from small population size, these factors can result in what has been described as an extinction vortex, where low density provides a positive feedback to further reduce population size (Gilpin & Soulé, 1986). However, naturally fragmented populations of sexually reproducing species may possess adaptations to avoid inbreeding, such as sex-biased dispersal, kin recognition, and delayed maturation (Pusey & Wolf, 1996), and gene flow among fragmented populations is likely to depend on geographical distance and matrix heterogeneity (Ricketts, 2001; Revilla *et al.*, 2004). Species in metapopulations are expected to have active and dynamic patterns of local extinction and colonization among habitat patches (Hanski, 1998). For putatively fragmented populations, analysis of genetic structure can reveal the degree of isolation (Hedrick, 2005) and the rate of successful migration between populations (Slatkin, 1987).

The small-scaled skink *Oligosoma microlepis* (Patterson & Daugherty, 1990) is IUCN red-listed as vulnerable with a 'high risk of extinction in the

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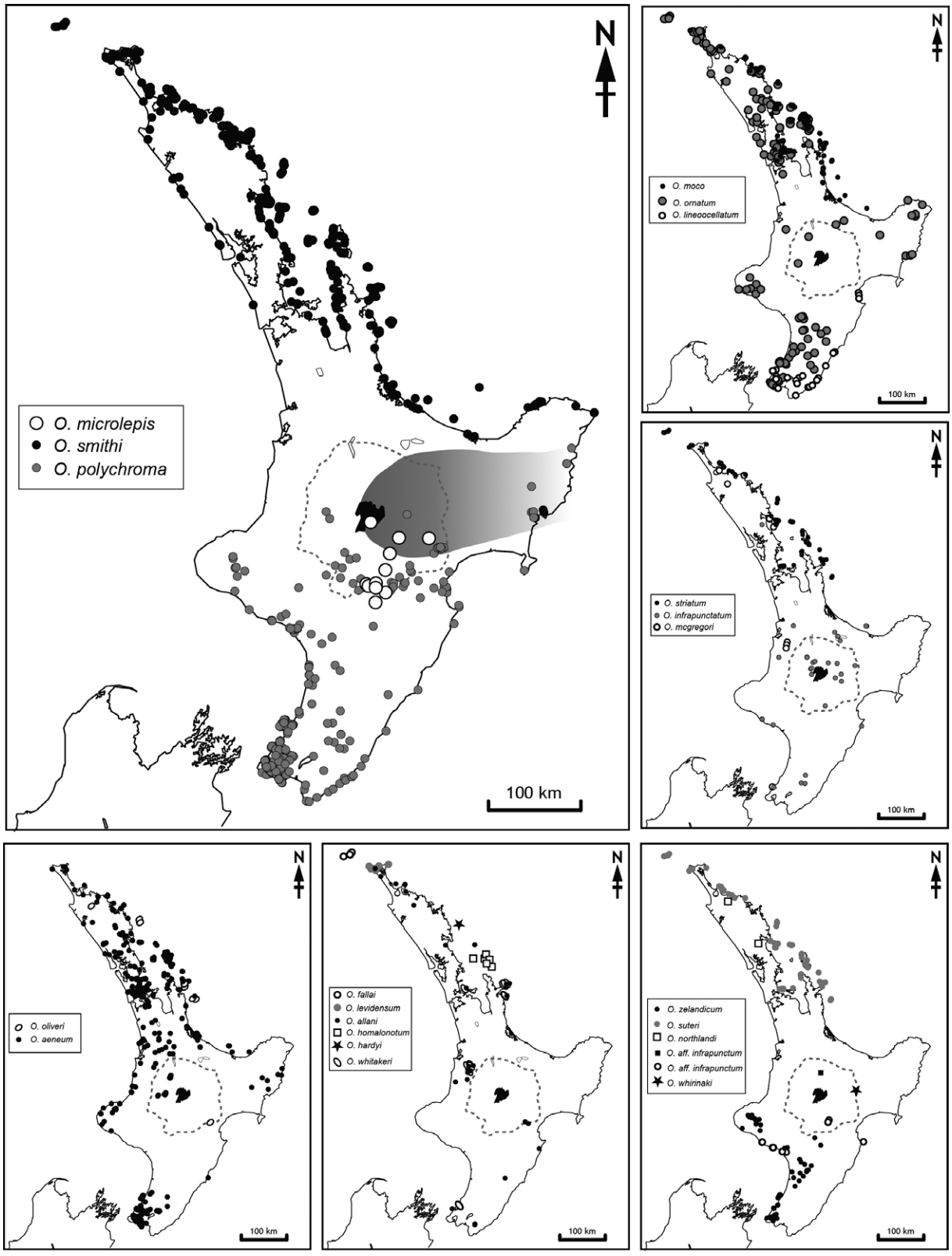
Figure 1. Recorded locations of native skinks in North Island, New Zealand, including the focal species *Oligosoma microlepis* the small-scaled skink. Location data were drawn from the New Zealand Herpetofauna database (http://www.doc.govt.nz/nzherpatlas). Lake Taupo, near the centre of North Island, is indicated by a black fill. Areas affected by ash fall and pyroclastic-flow from the Taupo eruption (approximately 1800 years bp) are indicated by graded grey fill and dashed line, respectively (Wilson, 1993).

medium-term future due to severe population fragmentation, declining area of occupancy, declining habitat quality and reduced number of locations' (IUCN, 2014). The New Zealand conservation status of *O. microlepis* is given as Threatened–Nationally Vulnerable (Range Restricted, Sparse). The Nationally Vulnerable status is triggered by the species having ≤ 15 subpopulations and ≤ 500 mature individuals in the largest subpopulation (Hitchmough *et al.*, 2013).

The narrow spatial distribution (< 100 km^2) of *Oligosoma microlepis* intersects wider ranging skink species that live in more diverse habitats (Fig. 1). For example, Northern grass skinks (*Oligosoma polychroma*) occur widely in North Island from coastal to subalpine habitats, and are sympatric with *O. microlepis*, and speckled skinks (*Oligosoma infrapunctatum*) share habitat with *O. microlepis* in the Rangitikei catchment (Towns, Neilson & Whitaker, 2002). Several skink species in North Island, New Zealand, have their range margins near the active Taupo Volcanic Zone in central North Island (Fig. 1). The former presence of coastline in this area during the Pliocene has been suggested as the origin for modern species limits (McCann, 1955; Hare, Daugherty & Chapple, 2008), although most of southern North Island and its topography is mid-Pleistocene in age (Trewick & Bland, 2012). More recently, volcanoes developed in the area, and Lake Taupo itself was formed approximately 27 000 years ago after the massive Oruanui eruption of more than 1000 km^3 of material (Trewick & Bland, 2012). The most recent violent eruption at Taupo occurred approximately 1800 years ago. Volcanic eruptions have periodically deposited dense layers of ash and pumice over a wide area; during the Taupo eruption, pyroclastic flows filled valleys and overtopped mountain ranges within 80 km of the eruption site, cooling to form a deep layer of ignimbrite (Fig. 1). Almost all habitats within this zone would have been obliterated or severely damaged by fluctuations in temperature and noxious gases. Thus, the current distribution of *O. microlepis* must represent range expansion occurring less than 2000 years ago, from areas not buried in ash during past eruptions. The range of *O. microlepis* might have formerly extended eastwards and possibly north and south on the axial mountain ranges (Towns *et al.*, 2002).

Oligosoma microlepis is diurnal, saxicolous and has a strong propensity for open basking on rock piles and outcrops (Whitaker, 1991) that typically occur in small (< 3 m^2), discrete patches (Teal, 2006). Most of the rock patches now used by *O. microlepis* are on heavily grazed pastureland; only three *O. microlepis* populations are known on relatively unmodified land. Many occupied sites were formed by human activity such as roading, stock tracks, and deforestation (M. Nelson-Tunley, pers. observ.). All but three are on pastureland that was forested prior to human disturbance. Of the pastureland sites, four are immediately adjacent to active roads and two are the result of small-scale quarrying. Other sites on pastureland resulted from erosion of rocky outcrops by streams and/or rainfall, exacerbated by deforestation. Only one *O. microlepis* site appears to have been relatively unaffected by human activity: the cliff-faces of Motutaiko Island in Lake Taupo. Every other *O. microlepis* location has been subject to deforestation for the production of pastureland, milling of timber, and accidental fire. Although heavy grazing may decrease the availability of refuges between sites and potentially limit dispersal between primary habitat patches as inferred for grand skinks (*Oligosoma grande*; Berry *et al.*, 2005), moderate grazing could reduce vegetation height and so increase basking habitat. Prior to human colonization (approximately 700 years ago), habitat patches occupied by *O. microlepis* were likely to have been interspersed among shading forest and scrubland, and so were naturally fragmented in sparse sites.

Strong habitat preferences and limited distribution in *O. microlepis* suggest that habitat fragmentation may have resulted in small populations, low levels of genetic diversity, and elevated inbreeding. However, recent range expansion is expected to leave a signature of reduced genetic diversity in leading edge populations (northern) and little genetic structure. In the present study, we investigate population fragmentation of *O. microlepis* sampling from approximately 80% of its range and $> 80\%$ of all known populations. We examine the distribution of mitochondrial haplotype and microsatellite diversity, estimate inbreeding rates, and document morphological variation.


N
O. microlepis
O. smithi
O. polychroma
100 km
O. moco
O. ornatum
O. lineoocellatum
O. striatum
O. infrapunctatum
O. mcgregori
O. oliveri
O. aeneum
O. fallai
O. levidensum
O. allani
O. homalonotum
O. hardyi
O. whitakeri
O. zelandicum
O. suteri
O. northlandi
O. aff. infrapunctum
O. aff. infrapunctum
O. whirinaki

MATERIAL AND METHODS

Sample collection

Oligosoma microlepis are scarce and restricted to an area < 100 km^2 in central North Island, New Zealand, with the highest density of occupied sites in the area between the Ruahine and Kaimanawa ranges (Fig. 2A). This district (sometimes referred to as Inland Patea) is predominantly farmland with heavily grazed exotic pastureland between known *O. microlepis* sites. Scattered habitat sites occur northwards on the borders of conservation areas in the Kaimanawa and Kaweka ranges; these are separated by native forest, exotic forest, pastureland, and scrubland.

Patchy habitat for *O. microlepis* within a matrix of less suitable landscape generates habitat foci for these skinks and we treat patches as putative populations in a metapopulation framework. We documented the occupancy of known habitat patches and evidence of local extinction by surveying almost all sites where this species had previously been recorded ($N = 17$). Four known *O. microlepis* sites on private land or under traditional protection/tapu were, however, inaccessible (Whitaker, 1991, 1997). New populations were sought by identifying potential rock outcrop habitat from aerial imagery and conducting visual searches using binoculars from the roadside or farm track, as well as from landowner knowledge of rock piles or skink sightings. We used visual searches for active skinks and for skink scat on basking rocks that is characteristic of *Oligosoma* sp. occupancy (Whitaker, 1991, 1997).

Sampling took place during the austral summer between December 2010 and February 2011. Skinks were caught using funnel traps and hand catching. Handling followed New Zealand Department of Conservation procedures and was conducted under Massey University Animal Ethics Committee protocol number 10/95. Each skink was photographed, measured, tail-clipped, and released at the point of capture. Tail tips were stored in 98% ethanol. Because this is an endangered and protected species, we also obtained data and tissue samples from eight captive bred *O. microlepis* for comparison.

DNA extraction

Genomic DNA was isolated using overnight incubation at 37 °C of approximately 5 mm finely diced tail tip tissue in 300 μL of TNES buffer (50 mM Tris, pH 7.5, 400 mM NaCl, 20 mM ethylenediaminetetraacetic acid and 0.5% SDS) with 2 μg of proteinase K. Proteins were precipitated by adding 85 μL of 5 M NaCl followed by vigorous shaking, and then pelleted by centrifuging at 16 060 ***g*** for 5 min. DNA was precipitated from the remaining supernatant with an equal volume of cold absolute ethanol, then pelleted, washed, and dried, before being dissolved in 50 μL of water.

mtDNA sequencing and analysis

A fragment of the mtDNA 16S gene was amplified using primers 16Sc (Reeder, 1995) and H3056 (Palumbi, 1996) in accordance with the methods of Fitness, Hitchmough & Morgan-Richards (2011). Polymerase chain reactions (PCR) were performed in 20-μL reactions containing 200 μM dNTPs, 2 mM MgCl, 1pM of each primer, 0.2 U of RedHot DNA polymerase (ABgene, Epsom), and 1–5 ng of template DNA. Thermocycling consisted of denaturation at 94 °C for 60 s then 35 cycles of 94 °C for 20 s, 50 °C for 15 s, and 72 °C for 90 s. The resulting 760 bp DNA fragment was subjected to capillary sequencing with an ABI3730 (Applied Biosystems). Sequence data were edited and assigned to haplotypes using SEQUENCHER, version 4.7 (Gene Codes Corporation).

The relationship between sample size and haplotype/allele richness was examined for wild *O. microlepis* population samples. Nucleotide diversity and pairwise ϕ_{ST} were calculated in ARLEQUIN (Excoffier, Laval & Schneider, 2005). Pairwise ϕ_{ST} estimates greater than zero indicate significant population differentiation and therefore all pairwise ϕ_{ST} values were tested to determine whether they differed significantly from zero based on frequency and sequence divergence and controlling for sample size (Excoffier *et al.*, 2005). The isolation-by-distance model (IBD) predicts that neutral genetic divergence of populations will be proportional to the geographical distance between them (Wright, 1943). Genetic diversity within a species will deviate from IBD following recent expansion that results in little genetic variation among populations, when geographical distance is insufficient to inhibit dispersal, or when factors other than spatial distance inhibit dispersal. Evidence of IBD was sought using a Mantel test of the correlation of logged pairwise geographical distances and pairwise ϕ_{ST} (or F_{ST} for microsatellite genotypes) with 10 000 randomizations using the IBDWS (Jensen, Bohonak & Kelley, 2005). A median-joining haplotype network (Bandelt, Foster & Röhl, 1999) was constructed using POPART (Leigh & Bryant, 2015).

Microsatellite genotyping and analysis

Four polymorphic microsatellite loci (Oligr6, Oligr14, Oligr19, Oligr20) were amplified using primers designed for New Zealand skinks with optimized

PCR conditions (Berry, Gleeson & Sarre, 2003). Six additional microsatellite loci previously amplified in a congeneric species (Berry *et al.*, 2003) were tested and were either monomorphic or did not amplify in *O. microlepis*. Microsatellite PCR primers had fluorescence labels and products were genotyped using the GeneScan-500 LIZ (Thermo Fisher) size standard on an ABI3730 (Applied Biosystems). Microsatellite allele lengths were identified using GENEMAPPER, version 4 (Applied Biosystems, 2004), followed by detection of null alleles, stuttering, large allele dropout, and homozygosity ratios using MICRO-CHECKER, version 2.2.3 (van Oosterhout *et al.*, 2004). Evidence of pairwise linkage disequilibrium among all loci was examined using GENPOP, version 4.2 (Raymond & Rousset, 1995; Rousset, 2008), with Markov chain parameters of 10 000 dememorization, 100 batches, and 10 000 iterations per batch. Evidence of linkage disequilibrium used probability tests with Bonferroni correction for multiple tests ($N = 6$; $P < 0.008$). Microsatellite data were then analyzed using Bayesian clustering and Markov chain Monte Carlo simulation implemented in STRUCTURE, version 2.3.4 (Pritchard, Stephens & Donnelly, 2000). STRUCTURE analyses were run using an admixture model and correlated allele frequencies with a burn-in of 100 000 replicates discarded followed by 1 000 000 generations with the number of groups (K) set from 1 to 10. STRUCTURE analyses, in conjunction with data transformation recommended by Evanno, Regnaut & Goudet (2005), were used to infer the number of genetic populations sampled (optimal K). The microsatellite genotypes were processed with FSTAT (Goudet, 2002) to estimate pairwise F_{ST} and population F_{IS}. All pairwise F_{ST} estimates were tested to determine whether population samples were genetically differentiated (> 0).

Morphology and fluctuating asymmetry

Each *O. microlepis* was sexed and its tail condition (intact or lost) noted. A digital balance accurate to 0.1 g was used to weigh each skink. Standard snout–vent length (SVL) and tail width at the base of the tail were measured using Vernier callipers accurate to 0.02 mm. Inbreeding can lead to developmental abnormalities that are detected as fluctuating asymmetry of individuals. Right and left hind limbs were measured and head photographs taken so that bilateral size and the scale symmetry of each individual could be scored. Scales were considered asymmetrical if the number of supraorbital scales on the left and right side of the head differed (Sarre, 1996).

Bivariate plots, analysis of variance (ANOVA), and regression and cluster analyses were undertaken in R (R Project for Statistical Computing). We used the model-based clustering package MCLUST version 4 package (Fraley *et al.*, 2012) implemented in the R environment to estimate data clusters. MCLUST uses maximum-likelihood estimation of multivariate mixture models without priors to assign individuals to clusters (Fraley & Raftery, 2002). The optimality of alternative models is compared using the Bayesian information criterion, with each individual being assigned an estimated uncertainty. Because each population sample contained adults and juveniles, we analyzed morphological variation using an ANOVA type model fitted using a linear model (lm) function in R. This allowed us to detect differences between quantitative predictors and qualitative factors as explanatory variables, and to construct the appropriate design matrix and model. We used skink weight as the response variable to determine whether collection location had any explanatory power in shape variation. We included, as factors, SVL, tail width, tail condition, sex, and population, as well as all interactions of these factors. When no significant interactions were detected, we re-ran the analyses without interactions. Analyses were repeated with and without the sample of captive *O. microlepis*.

RESULTS

Eighteen suitable rock patches in the known range of *O. microlepis* were investigated. *Oligosoma microlepis* had previously been recorded at thirteen of these habitats but, at three of them, we found no sign of this species (see Supporting information, Table S1). Five new *O. microlepis* populations were identified and two of these were sampled (Fig. 2A). The linear geographical distance between each site ranged from 400 m to 32 km, with the maximum span of occupied area being no more than 83 km. At Springvale, sites were separated by ≤ 4 km (Fig. 2A, inset). The nine southern populations were within 8 km of each other, whereas the northern three populations were up to 32 km apart. Sampling was limited by rarity of the target species, legal access, and ethical consideration. In total, samples were obtained from 12 populations with sample sizes ranging from 2 to 17 (see Supporting information, Table S1). Tail tissue samples were taken from 129 individuals and 127 individuals were measured.

mtDNA

Fifteen haplotypes were found within the 126 *O. microlepis* successfully sequenced for the 760 bp fragment of mtDNA 16S (Table 1; see also Supporting information, Table S2). Maximum nucleotide site diversity per population sample (π) was 0.004. Haplo-

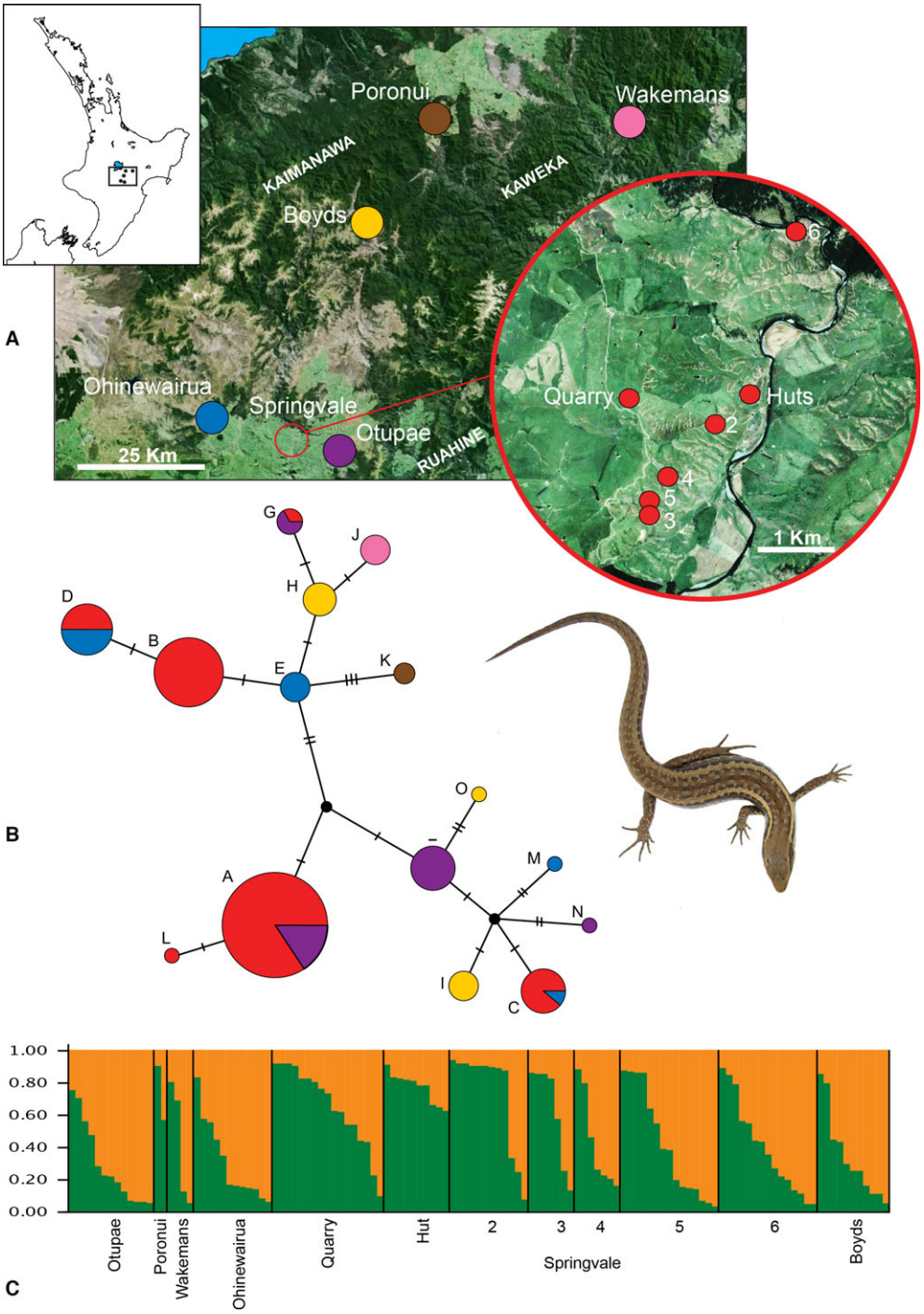


Figure 2. Genetic variation within the endangered New Zealand small-scaled skink *Oligosoma microlepis*. A, sampling locations in central North Island. Most populations are in the area known as Inland Patea between the Kaimanawa (north west) and Ruahine (south east) ranges. Pale green is pasture, dark green is forest, grey/brown is exposed soil, rock or dry scrub. B, minimum spanning network of 16S mtDNA haplotypes with image of adult *O. microlepis*. The size of circles indicates the number of individuals and segment colours indicate the locations as used on the map. C, Bayesian assignment analysis of data from four nuclear loci in STRUCTURE (Pritchard *et al.*, 2000) reveals little population structure ($K = 2$).

Table 1. Genetic diversity of endangered New Zealand small-scaled skink (*Oligosoma microlepis*) population samples at the 16S mtDNA locus

Site		π	±	*N*	Haps	OT	PO	WK	OH	SQ	SH	S2	S3	S4	S5	S6
Otupae	OT	0.003	0.00199	12	3 (2)											
Poronui	PO	0	0	2	1 (1)	**0.628**										
Wakemans	WK	0	0	4	1 (1)	**0.682**	1									
Ohinewairua	OH	0.00296	0.00197	12	4 (2)	**0.439**	**0.447**	**0.528**								
Springvale quarry	SQ	0.00351	0.0022	17	5	**0.431**	**0.429**	**0.527**	**0.279**							
Springvale huts	SH	0.00359	0.00238	9	3	**0.427**	**0.436**	**0.506**	**0.289**	–0.043						
Springvale 2	S2	0.00392	0.00248	12	3	**0.47**	**0.491**	**0.565**	**0.243**	0.022	0.005					
Springvale 3	S3	0.00251	0.00185	7	2	**0.546**	0.62	**0.698**	**0.405**	0.152	0.127	**0.329**				
Springvale 4	S4	0.00316	0.0023	6	2	**0.499**	**0.538**	**0.642**	**0.351**	0.003	–0.046	0.136	–0.074			
Springvale 5	S5	0.00347	0.00219	15	5	**0.454**	**0.48**	**0.547**	**0.32**	–0.009	–0.053	–0.004	0.179	–0.026		
Springvale 6	S6	0.00309	0.002	15	3	**0.495**	**0.529**	**0.589**	**0.36**	0.033	–0.039	0.067	0.113	–0.087	–0.046	
Boyds	BO	0.00417	0.00266	10	3 (3)	**0.464**	**0.48**	**0.564**	**0.336**	**0.318**	**0.333**	**0.368**	**0.43**	**0.374**	0.137	**0.401**

Nucleotide diversity (π), sample size (*N*), and number of haplotypes (Haps) with private haplotypes presented in brackets. Pairwise ϕ_{ST} are shown in bold where the value was significantly > 0 at an indicated adjusted nominal level for multiple comparisons (0.00064).

types were similar, differing by a maximum of 6 bp (0.79%). The fully resolved haplotype network (Fig. 2B) revealed no major divisions within this diversity and many haplotypes were found in more than one population sample.

Ten haplotypes were restricted to single population samples, with two or three private haplotypes found in samples from Otupae, Ohinewairua, and Boyds (Fig. 2). Each of these populations was > 6 km from its nearest neighbour in our sample. The most common haplotype (A, 60%) was found only in Springvale populations (Fig. 2, Table 1). The three private haplotypes in the Boyds population sample (O, H, K) did not form a cluster in the network (Fig. 2B), suggesting that they may have originated in different places before being united at their current location. Thus, nucleotide diversity was highest in the Boyds population sample ($N = 11$, $\pi = 0.00417$). By contrast, samples from Poronui ($N = 2$), Wakemans ($N = 4$), and the captive specimens had no haplotype variation (Table 1). Observed nucleotide diversity was positively correlated with population sample size ($r^2 = 0.833$; $P < 0.001$), suggesting that additional diversity might exist. Sample size was also positively, although weakly, correlated with latitude (larger samples in the south; $r^2 = 0.324$; $P = 0.031$). An analysis of variance for the fit of a linear model including both sample size and latitude found a significant effect of the interaction between sample size and latitude ($P = 0.00189$) on observed nucleotide diversity.

Variation in haplotype frequency among population samples resulted in significant genetic structure within *O. microlepis*. Estimates of pairwise ϕ_{ST} were significantly greater than zero for the majority of comparisons (49/77) (Table 1). Among Springvale populations, only Springvale 2 and Springvale 3 showed a significant departure from 0 ($\phi_{ST} = 0.33$) (Table 1). mtDNA differentiation among population samples followed a model of IBD (linear $r^2 = 0.488$, $Z = -33.4727$, $r = 0.6983$, Mantel test: $P < 0.0001$) (Fig. 3A).

MICROSATELLITES

Four microsatellite loci showed length polymorphism within our *O. microlepis* sample. There was no evidence of stuttering or large-allele dropout, nor were values of expected and observed heterozygosity significantly different from random for any of the four loci, indicating that none of the population samples deviated from Hardy–Weinberg expectations. Evidence of a null allele was detected only within the Ohinewairua sample ($N = 12$) at Oligr6 but, because null alleles were not detected in any other population sample, the locus was retained in the analysis. Positive correlations were found between microsatellite

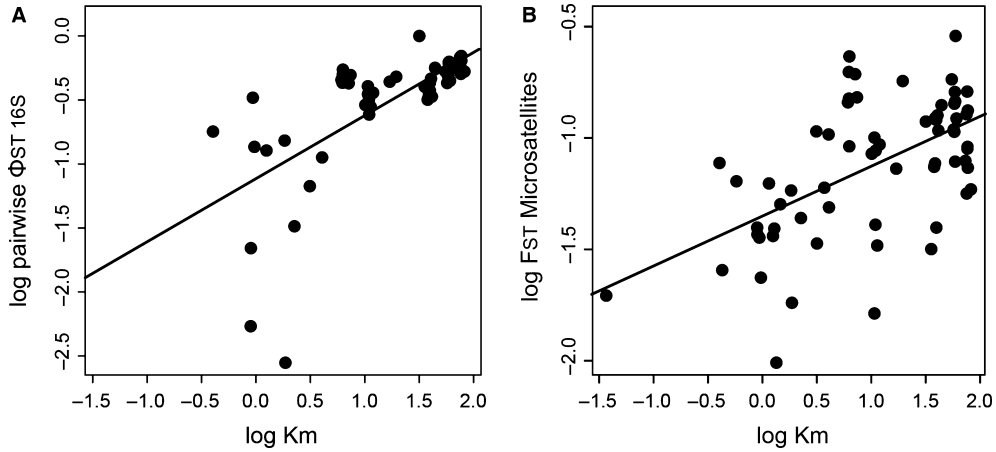


Figure 3. Positive correlation between geographical distance (log km) and genetic distance within the small-scaled skink (*Oligosoma microlepis*). A, log population pairwise ϕ_{ST} of 16S mitichondrial DNA (linear $r^2 = 0.488$, $Z = -33.4727$, $r = 0.6983$, Mantel test $P = 0.0001$). B, log population pairwise F_{ST} of microsatellite diversity (linear $r^2 = 0.320$, $Z = -67.0816$, $r = 0.5660$, Mantel test $P = 0.0041$).

allele diversity and sample size, for all loci and for overall diversity ($r^2 = 0.581$; $P = 0.004$). By contrast, microsatellite allele diversity did not correlate significantly with latitude, which was used as a proxy for site proximity under a range expansion model ($P = 0.0512$).

The total number of alleles per locus ranged from three (Oligr20) to 23 (Oligr6) (Table 2). Seven of the population samples had private alleles at one or more loci. The distribution of alleles at the four nuclear loci revealed geographical structure and resulted in significant pairwise differences ($F_{ST} > 0$ for 29/78 population comparisons) (Table 2). The spatial distribution of microsatellite differentiation followed a model of IBD (linear r^2 value = 0.320, $Z = -67.0816$, $r = 0.5660$, Mantel test $P = 0.0041$) (Fig. 3B). However, analysis of the microsatellite data using Bayesian assignment analyses (with STRUCTURE) did not reveal strong genetic division of putative populations, with $K = 2$ being optimal according to the Evanno method. Examination of assignment probabilities (Fig. 2C, bar plots) revealed that $K = 1$ could not be rejected because the estimated membership fraction of most individuals was close to 0.5.

Analysis of microsatellite data revealed no evidence for deviation from random mating within populations. F_{IS} estimates were never greater than zero, indicating no detectable evidence of inbreeding within *O. microlepis* population samples (see Supporting information, Table S3).

Body size variation

There was a positive relationship between individual length (SVL) and weight ($r^2 = 0.75$, residual SE = 0.9732, d.f. = 135, weight = −6.834 + 0.197 SVL) as expected; however, a linear regression did not appear to describe the size distribution observed (Fig. 4). Cluster analysis using the R Project package MCLUST identified three size groups in the data. In addition to the main group, one corresponded to putative juvenile skinks, and the other comprised mostly captive skinks (8/10). The cohort of skinks < 45 mm and < 2 g (clustered in our model) were considered juvenile and linear regression excluding these and the captive individuals resulted in a better fit to the remaining data with lower residuals ($r^2 = 0.74$, residual SE = 0.634, d.f. = 116, weight = −6.537 + 0.185 SVL). The proportion of juveniles within any population sample ranged between 0% and 18% (for $N > 4$).

Accommodating the variable contribution to samples of different skink age classes, we used a linear model with weight as the response variable. Skink weights were influenced by both body length and tail width (see Supporting information, Table S4). Sex was also a significant factor (females were heavier than males, $P = 0.0218$), although only when captive individuals were excluded from the analysis. Captive individuals were among the heaviest *O. microlepis* in our sample (Fig. 4) and their inclusion in the analyses produced a significant effect of tail condition on weight (tail loss reduced weight; $P = 0.0020$). However, in the absence of the captive samples, population location also had a significant effect on weight even when length (SVL) and tail condition were accounted for ($P = 0.0068$).

There was no detectable asymmetry in hind limb lengths and only eight of the *O. microlepis* examined had a degree of bilateral asymmetry in head scale

Table 2. Genetic differentiation among population samples of the endangered New Zealand small-scaled skink (*Oligosoma microlepis*)

Site	Oligr20	Oligr19	Oligr14	Oligr6	N	OT	PO	WK	OH	SQ	SH	S2	S3	S4	S5	S6
Otupae	2	3	2	7	13											
Poronui	1	3	1	2	2	0.287										
Wakemans	2	2	2	4	4	0.128	0.119									
Ohinewairua	3	4 (1)	3	8 (1)	12	0.073	0.122	0.059								
Springvale Quarry	3	4	4 (1)	15 (2)	17	0.193	0.107	0.09	0.085							
Springvale Huts	3	3	3 (1)	7	10	0.15	0.109	0.056	0.033	0.01						
Springvale 2	3	4 (1)	3	6	12	0.198	0.142	0.162	0.088	0.04	0.037					
Springvale 3	3	3	3	5	7	0.232	0.078	0.133	0.1	0.036	0.058	0.036				
Springvale 4	3	5 (1)	2	8	7	0.092	0.146	0.074	0.016	0.039	0.018	0.024	0.02			
Springvale 5	3	3	3	6	16	0.145	0.161	0.091	0.041	0.063	0.05	0.064	0.077	0.026		
Springvale 6	2	5	3	13 (1)	15	0.152	0.183	0.079	0.093	0.034	0.044	0.107	0.104	0.049	0.06	
Boyds	2	3	2	8	11	0.126	0.18	0.14	0.108	0.77	0.074	0.123	0.121	0.04	0.121	0.032

Number of microsatellite alleles per site, with private alleles presented in brackets. Population pairwise F_{ST} are in shown bold where estimates are significantly > 0 at indicative adjusted nominal level (5%) for multiple comparisons (0.00064). For abbreviations, see Table 1.

numbers (6.9% of sample) (see Supporting information, Fig. S1).

DISCUSSION

Oligosoma microlepis is part of an endemic New Zealand radiation of more than fifty skink species that evolved from a colonizing lineage since the mid-Miocene (Chapple, Ritchie & Daugherty, 2009). Approximately half of the extant *Oligosoma* species appear to be the product of diversification within North Island (Fig. 1) and, among these, *O. microlepis* is unusual in being restricted to a small inland area (Towns *et al.*, 2002). Many North Island skinks are coastal specialists, including the closest relative of *O. microlepis*, the shore skink *O. smithi* (Fig. 1), with which it is estimated to have shared a common ancestor in the late Pliocene (Hare *et al.*, 2008; Chapple *et al.*, 2009). By their nature, coastal environments comprise sustained and extensive exposed rocky habitat ideal for the concealment and basking of skinks. By contrast, rocky habitat is sparse and patchy inland, below the alpine zone.

Habitat mosaics

Studies of endangered species have found human modification of the environment to be a likely cause of population fragmentation and local extinction (Ehrlich & Ehrlich, 1970; Soulé, 1983; Ewers & Diham, 2007). Range size has been shown to correlate strongly with extinction risk, both globally (Böhm *et al.*, 2016) and in New Zealand lizards (Tingley, Hitchmough & Chapple, 2013), and the negative implications of small range size could be exacerbated in species such as *O. microlepis* that use highly fragmented habitat. Although fragmentation is considered a key threat among New Zealand skinks (Whitaker, 1996; Berry *et al.*, 2005), many reptiles are naturally confined to small habitat patches interspersed among unsuitable landscape matrix (Hanski, 1994). Demographic responses to habitat fragmentation vary, even in the same landscape (Drisccoll, 2004). Species that inhabit rocky outcrops are predisposed to existence in habitat mosaics, although conditions between preferred patches are expected to have a strong influence on metapopulation dynamics. For example, patch extinctions are countered by colonization of vacant patches in grand (*O. grande*) and Otago (*Oligosoma otagense*) skinks inhabiting a native grassland matrix in South Island, New Zealand, although the same species experience more extinctions than recolonizations in exotic pasture (Houton & Linkhorn, 2002; Gebauer *et al.*, 2013). Our detailed field searching indicated

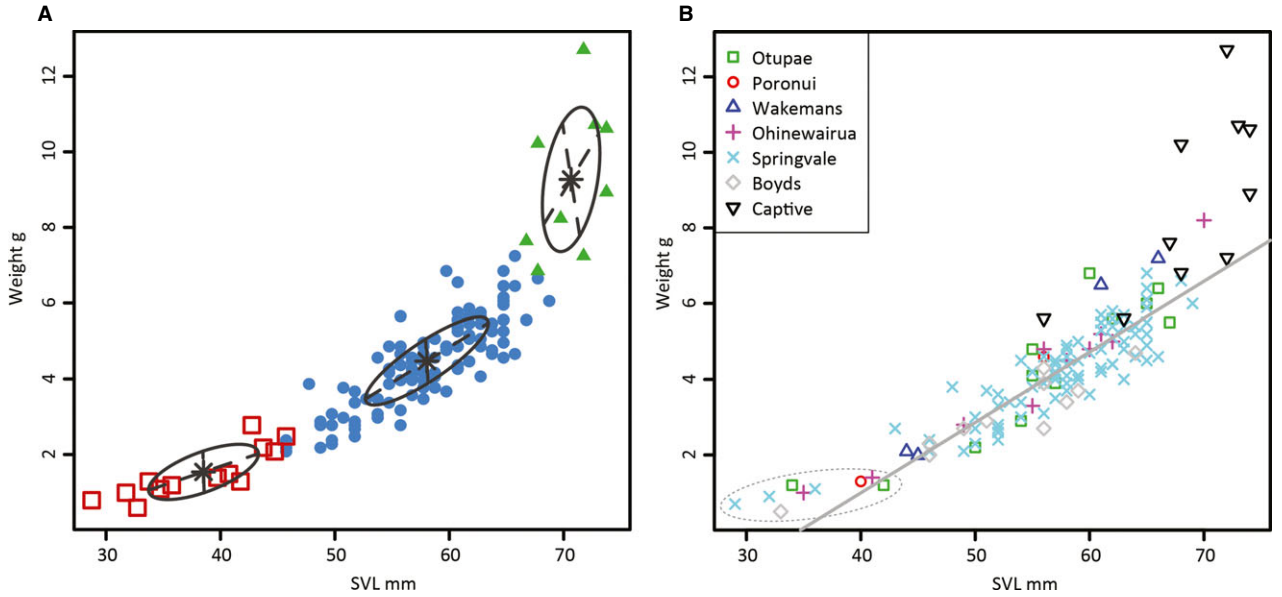


Figure 4. Relationship between snout–vent length (SVL) and weight for small-scaled skinks (*Oligosoma microlepis*). A, three groups detected with best fit VEV model (ellipsoidal, equal shape) and Gausian finite mixture model fitted by an expectation–maximization algorithm. Log likelihood −964.2494 d.f. = 25, Bayesian information criterion −2051.498 (MCLUST, version 4; R Project for Statistical Computing). B, linear regression fitted to weight and SVL excluding data from captive skinks and putative juveniles (d.f. = 117, $r^2 = 0.7525$). Populations as indicated by symbol shape/colour. Likely juveniles are indicated by the grey dashed elipse.

both extinction and recolonization of habitat patches by *O. microlepis* over as little as 5 years. *Oligosoma microlepis* were not detected at three habitat patches where they had previously been recorded, despite systematic searches for both skink sign and active animals in ideal climatic conditions. At one site, local extinction as a result of flooding in 2001 was likely, and the other two probably represent local extinctions since the last survey or exceedingly low densities (Teal, 2006). The species was, however, found at five new sites, two of which had previously been surveyed by experienced herpetologists without detection of *O. microlepis* (T. Whitaker, pers. comm.), supporting an inference of recent patch colonization (Whitaker, 1991). Taken together, a pattern of rapid local extinction and colonization looks likely for *O. microlepis* and the genetic outcome of such a metapopulation pattern will depend on the source of colonizers and the age of adjacent populations (Slatkin, 1977; Fields & Taylor, 2014).

Isolation and gene flow

Significant correlation between geographical distance and genetic distance was found in both the mitochondrial and nuclear data for *O. microlepis*, consistent with a model of IBD. For example, several rare alleles shared between two or more nearby populations were detected, which suggests that, when a new allele arises, it is more likely to be transferred to a nearby population than a more distant one (Slatkin, 1985). The signature of IBD suggests that populations have been relatively stable and connected by gene flow or subjected to a metapopulation process, in contrast to the expectations of range-expansion models (Excoffier, Foll & Petit, 2009). Because extant *O. microlepis* populations live in a region that would have been uninhabitable fewer than 2000 years ago, it appears that recolonization of habitat has been extensive. We found a positive correlation between genetic diversity and sample size, suggesting that our estimates of genetic diversity and gene flow might be minimized by sampling, which was restricted because of the conservation status of this species. Although detected with a comparatively slow-evolving mitochondrial gene region (16S) (Jiang *et al.*, 2007), genetic diversity in *O. microlepis* populations was relatively high (maximum $\pi = 0.004$) and similar to other New Zealand skinks for which data exist. *Oligosoma zelandicum* ($N = 17$) sampled across central New Zealand had a nucleotide diversity of 0.006 at combined ND2 and ND4 loci (O'Neill *et al.*, 2008), whereas a population of *O. otagense* ($N = 17$) had a diversity of 0.006 among Control Region sequences (Chapple *et al.*, 2012). Because nucleotide diversity is usually positively correlated with population size (Charlesworth, 2009), we can infer relatively large population sizes for *O. microlepis* despite its small spatial range today.

Inland Patea population samples were not genetically distinct from one another (Table 2), which suggests that gene flow (or metapopulation extinction and recolonization) prevents differentiation within the scale of detection (Slatkin, 1987; Mills & Allendorf, 1996). It appears that the improved pasture matrix separating the sampling sites has not created a major barrier to dispersal; sites within 10 km of each other (Ohinewairua and nearest Springvale) shared genotypes. Although these small-bodied ectotherms experience some restriction of gene flow at inter-patch distances in the region of 80 km, it is remarkable that the apparently barren pasture matrix appears to not impede gene flow entirely. In the Springvale area (Fig. 2A), there were no additional unoccupied habitat patches, and so the inter-patch distances (< 4 km) might reflect the true minimum that an individual would need to travel before reaching other small-scaled skinks. Elsewhere in the current range of *O. microlepis*, undiscovered sites (ghost populations) close to Boyds, Wakemans, and Poronui could be contributing to population cohesion (Slatkin, 2005).

Direct measures of migration are not available for *O. microlepis*, and inferences from population genetic analysis are likely to be influenced by temporal lag (Charlesworth, 2009). Although Polynesian settlers started clearing land in New Zealand using fire more than 500 years ago, and Europeans increased the rate of modification from the mid 1800s, their effects were not homogenous over New Zealand. In the region where *O. microlepis* is now found, the landscape remained dominated by native forest and scrub well into the 20th Century (Ward, 1956). This was because initial agricultural development was retarded by mineral deficiency (especially cobalt) of the volcanic soils, leading to so-called 'bush sickness' in livestock (McKinnon, 1997; Gordon, 2009). It was not until the 1950s when returning World War II servicemen settled the land and superphosphate fertilizers became available that a rapid increase in livestock numbers occurred in this region (McKinnon, 1997). Thus, anthropogenic changes in the habitat matrix may have yet to be fully realized in *O. microlepis* populations. As seen in other reptiles occupying modified environments (Levy *et al.*, 2013), the current genetic structure may not reveal current patterns of gene flow but, instead, reflect short-term lag in population structuring. Available estimates of *O. microlepis* population size obtained using mark–recapture suggest that all are small (from 9 ± 6 to 79 ± 8 individuals per habitat patch; Gebauer, 2009). Nevertheless, we did not detect evidence of inbreeding in *O. microlepis*, and fluctuating asymmetry in head scale counts was rare, suggesting that inbreeding depression is not currently a conservation concern for this species.

Although the total spatial range of *O. microlepis* is small and no major genetic subdivision was identified, we found some evidence of morphological variation among populations. The fact that captive individuals, which tend to be well fed, were the heaviest in our sample suggests that environment contributes significantly to size and shape in *O. microlepis* (Fig. 4). More significantly, location did have a significant effect on body condition even when SVL and tail loss was accounted for. For example, *O. microlepis* at Boyds were lightweight for their length, suggesting that there are small, yet significant ecological differences associated with geographical distribution. Size variation in New Zealand reptiles is sometimes considerable (Fitness *et al.*, 2011), although this can be associated with local variation in age structure, prey availability or genotype. Habitat patch quality is likely to be a determinant of phenotype and possibly also local extinction risk.

Conservation management and habitat patchiness

The stronghold of *O. microlepis* is an area now dominated by pastureland. The inferred degree of gene flow, coupled with high within-site diversity, suggests that current or recent farm management practices have not yet eroded genetic diversity. Grazing inhibits vegetation growth, allowing greater solar radiation on rock surfaces, and so could benefit saxicolous heliotherms such as *O. microlepis*. Trampling by stock, quarrying for gravel, and landslips arising from farming can expose the greywacke bedrock, producing potential skink habitat (Teal, 2006), although intensified agriculture and pastoral 'improvement' are also likely to degrade skink populations. Low fecundity, with only two or three live-born offspring per litter, one litter per year, and sexual maturity reached after 2 years or more (Patterson & Daugherty, 1990), means that predation by introduced mammal pests (stoats, cats, hedgehogs, and rodents; Wilson, Mulvey & Clark, 2007) is a key challenge to species' survival (Towns *et al.*, 2002).

The ecological effects of habitat fragmentation are well documented (Didham, 2010), although inferences made about the impact of anthropogenic habitat modification on natural populations are sensitive to the types of data used and local circumstances (Frankham, 1995; Williams, Nichols & Conroy, 2002; Driscoll & Hardy, 2005; Mona *et al.*, 2014). Inferences from population genetic data applied to lizards indicate diverse responses to changing circumstances (Richmond *et al.*, 2009; McCoy *et al.*, 2010; Tucker, McBrayer & Harrsion, 2014), although cause and effect are easily confounded. Variation in one or more life-history traits, such as population density,

recruitment, dispersal behaviour, and generation time, interact to give distinct population genetic signals (Heath *et al.*, 2012). The detection of population differences at very fine spatial scales can be achieved with sufficient loci and samples sizes, although their evolutionary and conservation significance usually remains uncertain (Moore *et al.*, 2008). In circumstances where population structure actually predates human habitat modification, the resulting coincidence of spatial structure is readily (but incorrectly) interpreted as anthropogenic (Stow & Briscoe, 2005). Conversely, a signal of high gene flow among natural populations might be retained in modern studies if environmental perturbations are very recent and animals sufficiently long-lived (Sumner *et al.*, 2004; Richmond *et al.*, 2009). In the most extreme cases, the impact of severe reduction in population size and cohesion can be masked by the survival of individuals from early in a bottlenecking event (e.g. white-tailed eagle: Halker *et al.*, 2006; greater one-horned rhinoceros: Dinerstein & McCracken, 1990). New Zealand skinks similar to *O. microlepis* can live for at least 10 years, although the life span of this species in the wild is not known.

We did not find evidence of low genetic diversity, impeded gene flow, inbreeding or its downstream effects on fluctuating asymmetry in *O. microlepis*, even though this species exists in a patchy habitat mosaic over a small range. This could be interpreted as evidence for human activity having had little impact on these skinks, or that there is a lag in the genetic response to population subdivision. However, although anthropogenic effects might date back only 100 years, the landscape occupied by *O. microlepis* likely presented a patchy environment for these skinks before the arrival of humans when forest was more extensive. Furthermore, most of the region was devastated by pyroclastic flow and ash within recent geological time (< 2000 years). Together with our observations of population demise and recovery, our data point to a mobile and dynamic ecology, consistent with the original metapopulation model of Levins (1969). Although specialized to rocky outcrops, *O. microlepis* must move between habitat hotspots, although the rate at which they do so is likely dependent on the ecology of intervening habitat.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Head scale variation among small-scaled skink *Oligosoma microlepis*. A, heavily ridged but symmetrical morphology. B, asymmetrical scale morphology caused by scaring. C, asymmetrical scale morphology of unknown cause. Supraorbital scales have been outlined in red to highlight the difference between the skinks' left (normal) and right (abnormal) scalation. Photographs by Andrew Blayney.

Table S1. Known wild small-scaled skinks *Oligosoma microlepis* sites as at March 2011, including confirmation of presence, sample size, and sample demography in the present study. Asterisks indicate new sites found during this study.

Table S2. Variation among 16S haplotypes of small-scaled skink *Oligosoma microlepis* Variable positions in a 760-bp fragment with nucleotide substitutions compared to the most common haplotype A. An alignment of full sequence haplotypes ia available at: http://evolves.massey.ac.nz/Data/Small-scaled_haplotypes.txt.

Table S3. No evidence of inbreeding was detected within New Zealand small-scaled skink samples (*Oligosoma microlepis*) at 13 sites based on four microsatellite loci. F_{IS} calculated per loci and per site. No estimates differed significantly from zero based on an indicated adjusted nominal level of 0.00096.

Table S4. Analysis of variance generalized linear model. Results from a reduced factorial model to explain variation in skink weight without interactions and without captive individuals. Response: weight.