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

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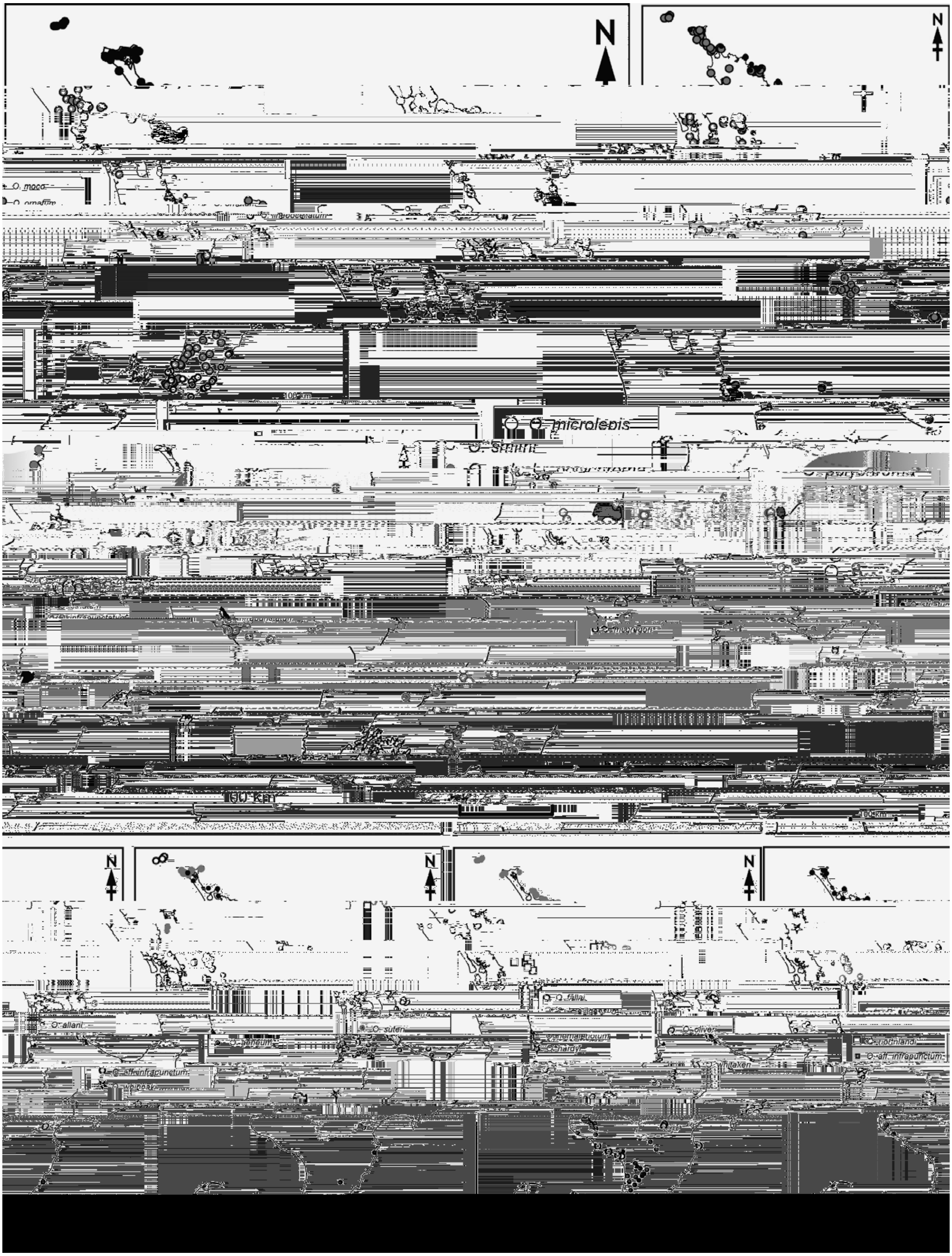
Received 6 January 2016; revised 23 February 2016; accepted for publication 24 February 2016

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

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 ($< 100\ \mathrm{km}^2$) 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


N
O. moco
O. ornatum
100 km
O. microlepis
O. smithi
O. infrapunctatum
O. allani
O. aenum
O. aff. infrapunctum
O. suteri
O. fallai
O. hardyi
O. oliveri
O. whitakeri
O. aff. infrapunctum
100 km

MATERIAL AND METHODS

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PCR conditions (Berry, Gleeson & Sarre, 2003). Six

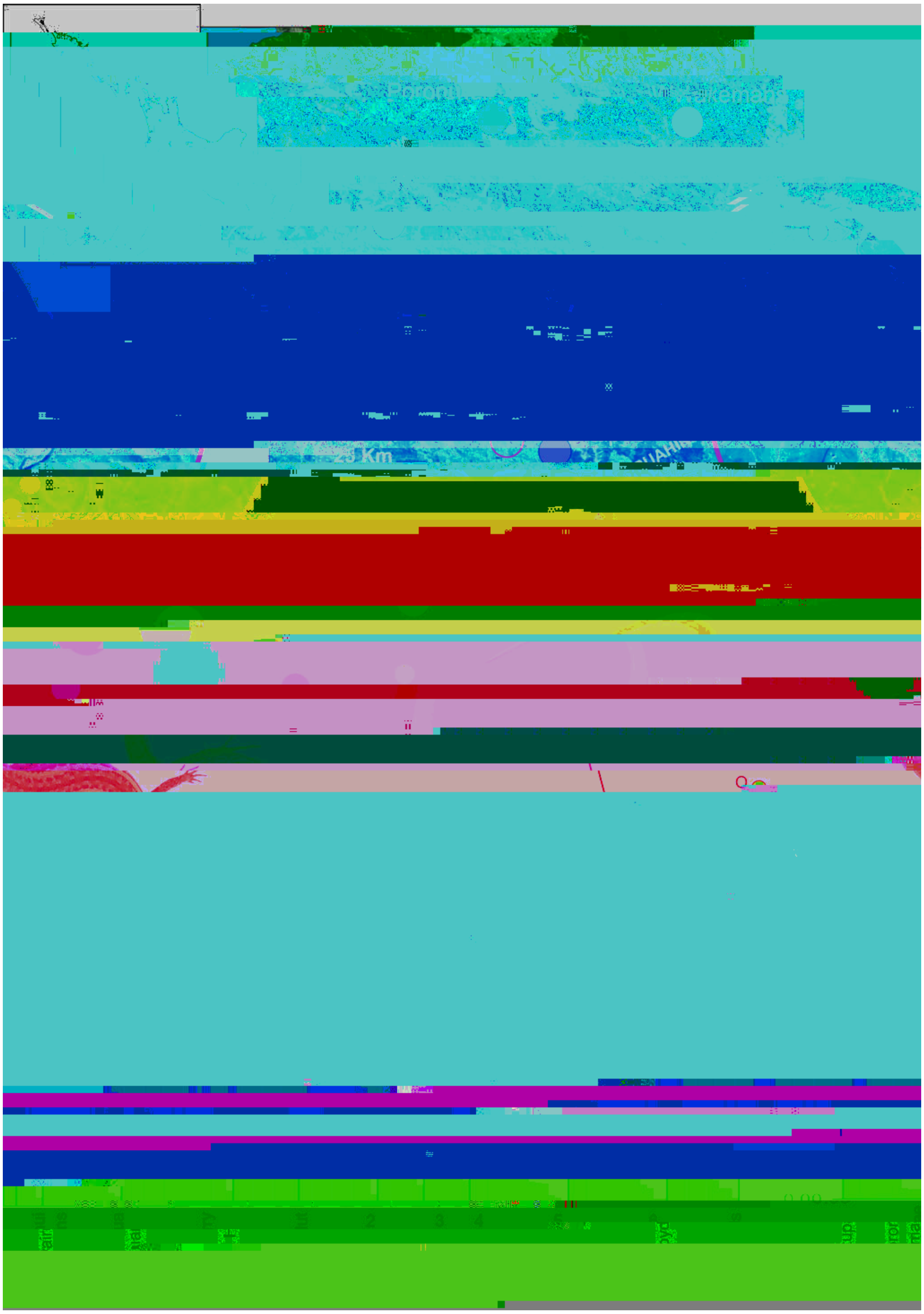

Figure 2. Genetic variation within the endangered New Zealand small-scaled skink Oligosoma microlepis. A, sampling locations in central North Island. Most populations are in the area known as Inland Patea between the Kaimanawa (north west) and Ruahine (south east) ranges. Pale green is pasture, dark 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).

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 populanumbers (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 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

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 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

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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.