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Population fragmentation in the small-scaled skink (*Oligosoma microlepis*): the consequences of human landscape transformation on a habitat specialist's distribution, morphology, and genetics.

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Abstract

The endemic small-scaled skink (*Oligosoma microlepis*) is restricted to the central North Island of New Zealand. Strong preference for exposed rock piles, a relatively rare habitat, has created a fragmented distribution and restricted subpopulation sizes. The Department of Conservation lists the species as in serious decline, with IUCN listing it as vulnerable. At the stronghold of this species in the southern part of its range, subpopulations exist on small rock piles separated by up to 11 km of pastureland. Scattered northern subpopulations are separated by more than 19 km. These distances may act as dispersal barriers, as might the lack of refugia between subpopulations on pastureland. Lack of migration between subpopulations could reduce genetic diversity and increase inbreeding. Reduced genetic diversity could decrease resistance to disease, parasitism and environmental change, whereas inbreeding may reduce fertility, lifespan and juvenile survival.

This thesis used a combination of survey data, morphological measurements and population genetics to investigate potential causes and consequences of population fragmentation on the small-scaled skink, with the aim of providing information to guide the long-term conservation of this species.

A survey of known small-scaled skink subpopulations was conducted to determine if the species is in decline. In addition, potential small-scaled skink habitat was searched, including previously surveyed sites. Evidence of decline was inconclusive, with three subpopulations appearing to be in decline and discovery of five new subpopulations.

Genetic (16S mitochondria and microsatellites) and morphological analysis was used to examine subpopulation differences in relation to species distribution, including investigating inbreeding within subpopulations. Relatedness between subpopulations was consistent with isolation by distance, indicating that small-scaled skink dispersal is limited by distance, but not significantly limited by pasture between subpopulations. Inbreeding was not detected within any subpopulation.

The main findings of this thesis were that evidence of species decline was inconclusive, with possible species expansion and undetected subpopulations. Dispersal was limited by dispersal distance but not by pasture and no subpopulation was subject to significant inbreeding. Current research indicates that pasture does not negatively affect the species, and may be beneficial in the formation and maintenance of habitat.

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General introduction

Figure 1.0: Typical basking behaviour of small-scaled skinks. Top photo: Andrew Blayney.

This thesis investigates potential causes and consequences of population fragmentation on the small-scaled skink (*Oligosoma microlepis*) with the aim of providing information to guide a long-term conservation strategy for this species.

Developing a conservation strategy for a species requires an understanding of the key factors shaping the species distribution and abundance. Yet to be established for small-scaled skinks is whether their current fragmented distribution is the result of preference for a naturally rare habitat or the result of species decline. Secondarily, the consequences of their fragmented distribution have not been investigated.

The cause of population fragmentation strongly affects the consequences; a population fragmented by anthropogenic means will be subject to different stressors than a naturally fragmented population. Caughley (1994) applied this concept in two paradigms; the small population paradigm that considers the effects of small population size (such as inbreeding and genetic drift), and the declining population paradigm that attempts to identify and mitigate the causes of small population size (such as predation and habitat loss). Determining whether the small-scaled skink is a naturally rare species or rare due to anthropogenic effects will resolve the optimal conservation protocol for this species.

The consequences of population fragmentation are many and varied; anthropogenic fragmentation has been linked to genetic drift, increase of genetic load, inbreeding depression, and greater susceptibility to environmental and demographic fluctuations (Willi, Van Kleunen, Dietrich, & Fischer, 2007). When combined with small population size these factors can result in an extinction vortex (Gilpin & Soule, 1983); a rapid decline to extinction (Tanaka, 2000). However, many species possess innate measures of avoiding or mitigating inbreeding, and if genetic issues are detected in a population they can be mitigated by transfer of individuals between populations (outbreeding) (Willi, Van Kleunen, et al., 2007).

1.1 The effects of habitat fragmentation on dispersal and gene flow

All matrices are not equal

The type of matrix between habitats matters; some matrices may prove less of a barrier to dispersal than others depending on matrix heterogeneity (Revilla, Wiegand, Palomares, Ferreras, & Delibes, 2004) and the similarity of the matrix to the species habitat (Ricketts, 2001). If dispersal across a matrix is limited it may result in population fragmentation; whereby the population size becomes restricted (particularly in species with large territories), populations become genetically differentiated, gene flow is reduced and genetic diversity within populations' decreases (Willi, Van Buskirk, Schmid, & Fischer, 2007).

The consequences of reduced genetic diversity

Reduced diversity within small, isolated populations can have a number of consequences: genetic drift may result in the loss of rare alleles, which if sustained will lead to fixation of a single allele for each locus (homozygosity) (Frankham, Ballou, & Briscoe, 2010). Homozygosity corresponds to reduced adaptive potential and vulnerability to environmental stochasticity (Amos & Balmford, 2001; Willi, Van Buskirk, & Hoffmann, 2006). Homozygous individuals are also susceptible to genetic load, an accumulation of deleterious alleles that may be fatal (Frankham, et al., 2010). In small populations genetic drift may override natural selection, resulting in loss of adaptive variation (Willi, Van Buskirk, & Fischer, 2005).

Small populations have higher proportions of related individuals (i.e. a higher likelihood that any chosen mate will be related), thus increasing the likelihood of inbreeding (Keller & Waller, 2002). Sustained rates of inbreeding can cause inbreeding depression, defined as a reduction in mean reproductive fitness (Wright, 1977). Inbreeding depression has been observed in a number of captive species; in birds it can reduce clutch size, hatch rate, and fledgling survival (Briskie & Mackintosh, 2004). For other species inbreeding can result in decreased sperm mobility, increased juvenile mortality, reduced lifespan, and increased vulnerability to disease, parasites, and environmental change (Cassinello, 2005; Keller & Waller, 2002). The occurrence and severity of inbreeding depression in wild populations have been questioned (Keller &

Waller, 2002), but some research suggests wild inbreeding depression is prevalent and more costly than in captive populations (Crnokrak & Roff, 1999).

Innate measures of avoiding inbreeding and inbreeding depression

There is some evidence to counter the risks of reduced diversity, namely inbreeding avoidance and purging of genetic load. Inbreeding avoidance can be achieved by a variety of mechanisms including sex-biased dispersal, kin recognition and delayed maturation (Pusey & Wolf, 1996). Inbreeding avoidance has been documented in a number of species (Pusey, 1987), including skinks (Stow & Sunnucks, 2004). Unfortunately habitat fragmentation can prevent sex-biased dispersal, thus allowing inbreeding to occur, as has been observed in grand skink populations separated by pastureland (Berry, Tocher, Gleeson, & Sarre, 2005).

While purging removes deleterious alleles it does not prevent homozygosity, so the adaptability of a population is still in jeopardy (Charlesworth & Charlesworth, 1999). Purging in small populations selectively removes the most detrimental alleles, thus slightly detrimental alleles will be subject to drift and selection (Willi, et al., 2005). Furthermore, if two purged populations are reunited subsequent breeding can result in outbreeding depression whereby individuals adapted for different habitats produce offspring adapted for neither due to the breakup of co-adapted genes (Pusey & Wolf, 1996).

In summary, isolation of small populations is often associated with loss of genetic diversity. Loss of genetic diversity can result in loss of fitness in terms of reproductive capacity and adaptive potential. However, inbreeding avoidance can prevent inbreeding depression and loss of genetic diversity to an extent.

1.2 The history of habitat transformation in New Zealand

Developing a conservation strategy requires knowledge about species distribution and abundance, including evidence of decline and potential causes for decline. Significant climatic events in the history of a species may have influenced the pattern of distribution observed today by either aiding or restricting population growth or expansion. There are a multitude of potential threats to a species but often the most

significant threats come from anthropogenic interference through habitat loss, habitat modification, and introduction of plants and animals to a naive ecosystem. The following section describes major habitat alterations that have occurred in the recent history of New Zealand, focusing especially on events, threats and locations specific to the small-scaled skink.

Pre human habitat transformations

The Pleistocene was an age of global climate fluctuations that took place from ~2.56 ma (million years ago) to 10 ka (thousand years ago) (Gibbs, 2006). During this time large tracts of the South Island were periodically covered by ice sheets and glaciers and a lowered sea level created a land bridge between the North and South Islands (Carter, 2005).

The last glacial maximum (LGM, 23-13 ka) was a period of maximum cooling in the central and lower North Island (Pillans, et al., 1993). During this period sub-alpine flora dominated the central North Island including herbs, grasses and trees associated with high altitude in modern times (Pillans, et al., 1993; Trewick & Bland, 2011; Wilmshurst & McGlone, 1996). Bog cores taken from Three Kings bog indicate that vegetation within Inland Patea district was minimal or absent during the Pleistocene, with tall forest trees emerging about 6 ka (McGlone, 1989b). The dominance of grassland habitats may have facilitated larger populations of grassland-dependant species, as was seen in the northern hemisphere during the same period (Guthrie, 1968; Yurtsev, 2001).

Many New Zealand taxa were significantly affected by the Pleistocene, which is evident in their genetic affinities (Avise, 1998; Neiman & Lively, 2004; Shepherd, Perrie, & Brownsey, 2007). Some lizard species show shallow divergence during the Pleistocene (D. Chapple, Daugherty, & Ritchie, 2008; K.M. Hare, Daugherty, & Chapple, 2008), while others maintained genetic partitioning that had been established by earlier geological events (Greaves, Chapple, Daugherty, Gleeson, & Ritchie, 2007, 2008; Trewick, 2001).

Anthropogenic habitat transformation

Habitat loss and modification

Habitat loss frequently occurs in conjunction with habitat fragmentation, the combination of which has been established as a major threat to wildlife and one of the most common causes of species rarity (Fahrig, 2003). Prior to human arrival, forest covered an estimated 82% of New Zealand; by 2002 this had dropped to 24%, representing a loss of 14 million ha of forest (Ewers, et al., 2006). Much of New Zealand's forests were cleared by deliberate burning, either by Maori or European settlers (McGlone, 1983, 1989a, 2001). Maori-lit fires are estimated to have destroyed 50% of forest cover, often replacing the forest with bracken (*Pteridium esculentum*) and native grasses (Poaceae) (McGlone, 2001).

Habitat loss is an ongoing threat to New Zealand wildlife; deforestation (Ewers, et al., 2006), modification of dunelands (Jamieson, 2010) and wetlands (Hunt, 2007) continues unabated.

Introduction of mammals

Polynesian colonisers introduced Kiore (*Rattus exulans*) to New Zealand approximately 1 ka (McGlone, 1989a). The combination of Maori hunting and Kiore predation was responsible for extinction of numerous native birds, in addition to other wildlife (Tennyson & Martinson, 2006). The impact of Kiore on reptiles is well documented; Tuatara (*Sphenodon sp*.) populations are non-viable on islands where Kiore are present due to high juvenile predation (Cree, Daugherty, & Hay, 1995; Crook, 1973; Newman, 1988; Tyrrell, Cree, & Towns, 2000). Duvaucel's geckos (*Hoplodactylus duvaucelli*) can persist sympatrically with Kiore, but are present at much lower densities and have lowered recruitment rates compared to rat-free populations (Hoare, Pledger, Nelson, & Daugherty, 2007).

European colonisation introduced a variety of mammals including two species of rat (*R. rattus* and *R. norvegicus*), grazing animals and predators. The extinction of several endemic birds, two frog species, one bat and one skink species have been directly linked to invasion of rats (Towns, Atkinson, & Daugherty, 2006). Rats were a major cause of range contractions of native reptiles, with several once widespread lizard species now restricted to mammal free offshore islands (Towns & Daugherty, 1994).

Species introduced by European settlers and known to prey on lizards include hedgehogs, rats, cats, stoats and ferrets (Middlemiss, 1995; Norbury, 2001; Spitzen–van der Sluijs, Spitzen, Houston, & Stumpel, 2009).

Herbivorous species introduced by Europeans have been both direct and indirect drivers of environmental change. Large tracts of native forest and wetland were burnt, drained and converted into pastureland for grazing livestock (Ewers, et al., 2006). Invasive herbivores continue to cause significant damage to remaining forest habitats (Husheer, Coomes, & Robertson, 2003; Stewart, Wardle, & Burrows, 1987). Rabbits are known to support high numbers of cats and ferrets in tussock grasslands; when rabbit populations are reduced, the predators switch to consuming higher numbers of native skinks (Norbury, 2001).

Small-scaled skinks continue to exist on pastureland despite (or perhaps because of) the abundance of mammals and the drastic anthropogenic habitat modification. It is plausible that grazing and exotic pasture in general are beneficial to the species (Teal, 2006), but the risk of predation remains unknown.

1.3 The small-scaled skink

Species description

The small-scaled skink is a scincid lizard endemic to New Zealand. It was first discovered in 1971 but not formally identified until 1990 (Patterson & Daugherty, 1990). The small-scaled skink is distinguished from other similar New Zealand species by a high scale row count of 38-44 as well as by a tear-drop shaped marking below the eye shared with few other species (Fig. 1.1) (Patterson & Daugherty, 1990). The maximum snout-vent length of this species was recorded as 73 mm (Gill & Whitaker, 1996) with the intact tail length often exceeding body length (Appendix 2). Information such as the annual clutch output (offspring per female per year) and lifespan have not been documented in small-scaled skinks. However, the lifespan is likely to be similar to other New Zealand species, which can live up to 16 years in the wild and over 30 years in captivity (Norbury, Reardon, & McKinlay, 2006).

Figure 1.1: A small-scaled skink showing the tear-drop pattern and relatively small scales that are distinguishing features for this species.

Habitat preferences

Small-scaled skinks are diurnal, saxicolous and have a strong propensity to bask (Whitaker, 1991), restricting the species to rock piles and outcrops that occur in discrete patches (Teal, 2006). The majority of small-scaled skink sites are on heavily grazed pastureland with only three known skink sites found on relatively unmodified land; this may not be an indicator of skink preference but of survey effort. Two surveys (Hutchinson, 1992; Whitaker, 1997) have attempted to survey outside of pastureland; one in the Kaimanawa forest park, the other in Kaweka forest park, both of which are large areas of difficult terrain (Department of Conservation, 2005; Tongariro-Taupo Conservation Board, 2007). Both of these surveys were conducted over a very short period that prevented a thorough search of these ranges. Pastureland requires considerably less effort to survey due to potential habitats being easily spotted from a distance and often accessible by vehicle.

The erosion caused by stock tracks, roads and streams appears to have formed most small-scaled skink habitats (personal observation). Teal (2006) found small-scaled skinks preferred sites nearby stock tracks or roads, likely due to the high rate of disturbance. The possible beneficial effects of a high disturbance regime include; refreshing habitat (i.e. overlaying fresh layers of rock on top of existing habitat), expanding habitat, creating new habitats and reduced vegetation height within and around habitats through grazing (preventing basking areas from shading by vegetation).

However, there may also be negative consequences of high disturbance. Livestock disturbance can cause small rock slides within small-scaled skink habitats (personal observation), with the potential to harm skinks. Heavy grazing of pasture between skink habitats may decrease the number of refugia between sites, reducing the likelihood of successful dispersal between habitats (Berry, et al., 2005). Long-term reduced dispersal could result in genetic differentiation of neighbouring skink subpopulations and potentially increase inbreeding (Berry, et al., 2005). Lowered dispersal ability would reduce the species ability to colonise new habitat as it becomes available, and prevent them finding new habitat if their current one becomes unsuitable.

Berry (2004) conducted research on the dispersal abilities of grand skinks (*Oligosoma grande*), which live in rock outcrops within matrices of either exotic pasture or native tussock-scrubland, not unlike the small-scaled skink. These studies found grand skinks had lower rates of successful dispersal over pasture compared to tussock grassland, which correlated to higher genetic structuring and lower genetic diversity within populations within the exotic pasture matrix (Berry, et al., 2005).

Distribution

The small-scaled skink is restricted to the central North Island of New Zealand (Fig. 1.2). In some texts (such as Whitaker 1997) each small-scaled skink site is considered to be a separate population; this seems unlikely from a genetics perspective, given that the shortest distance between two populations is 20 metres. For the purposes of this project, each skink site will be considered a subpopulation, with the entire species

considered to be one population. The highest density of subpopulations occurs in Inland Patea district, approximately 25 km northeast of Taihape. Inland Patea district consists of several sheep and beef stations, with heavily grazed pasture between skink sites. Scattered subpopulations occur northwards on the borders of Kaimanawa, Kaweka and Te Urewera forest parks. The northern sites are divided by a range of land types including native forest, exotic forest, pasture and scrubland.

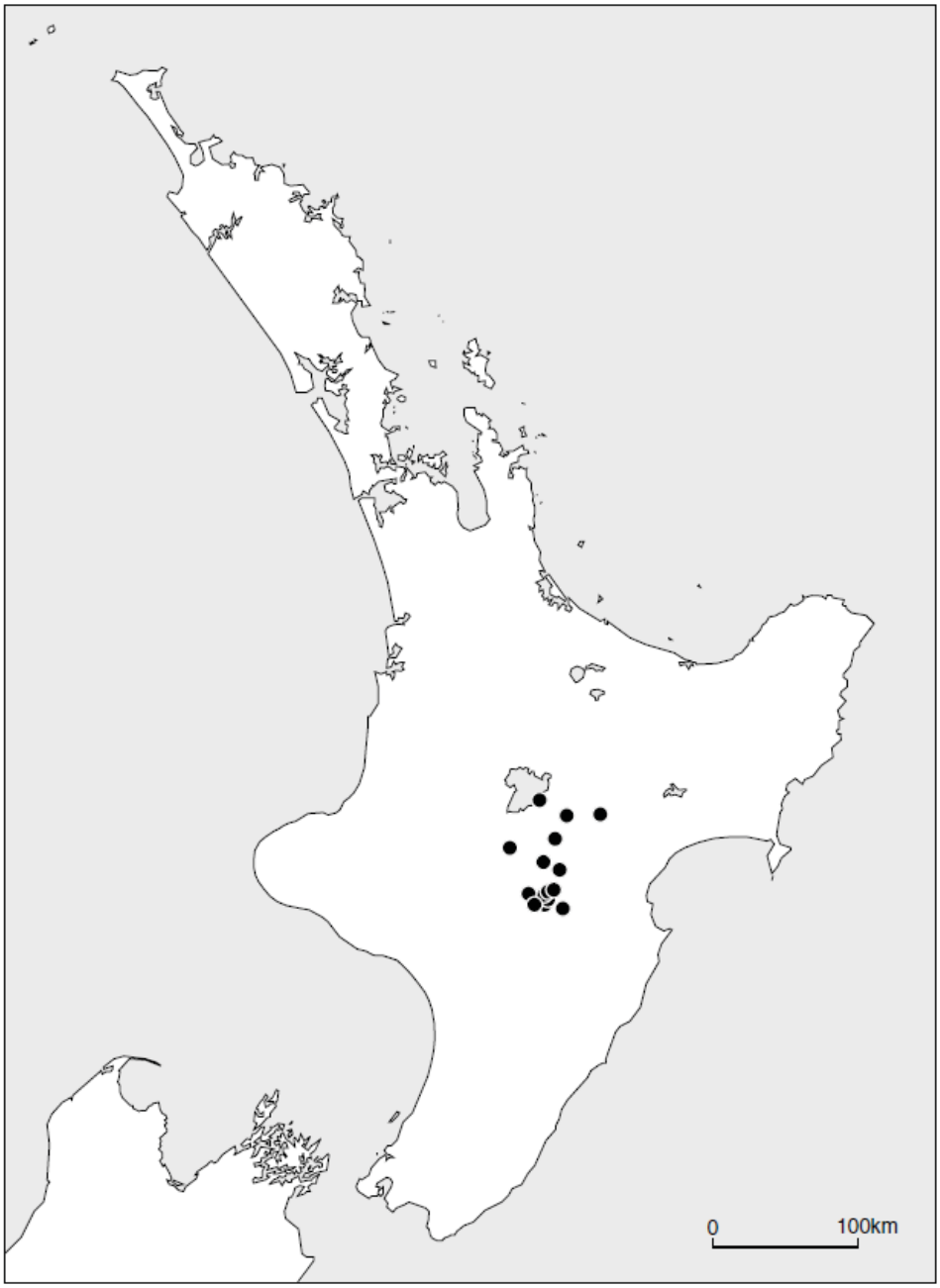


Figure 1.2: The distribution of small-scaled skinks. Image obtained from the North Island *Oligosoma* spp. recovery plan (Towns, Neilson, & Whitaker, 2002).

The small-scaled skink distribution is notably different than other North Island lizard distributions. Excluding island endemics, North Island skink distributions tend to be widespread with several coastal populations (Fig. 1.3). By contrast, small-scaled skinks have a very restricted central distribution and no subpopulations near a coastline.

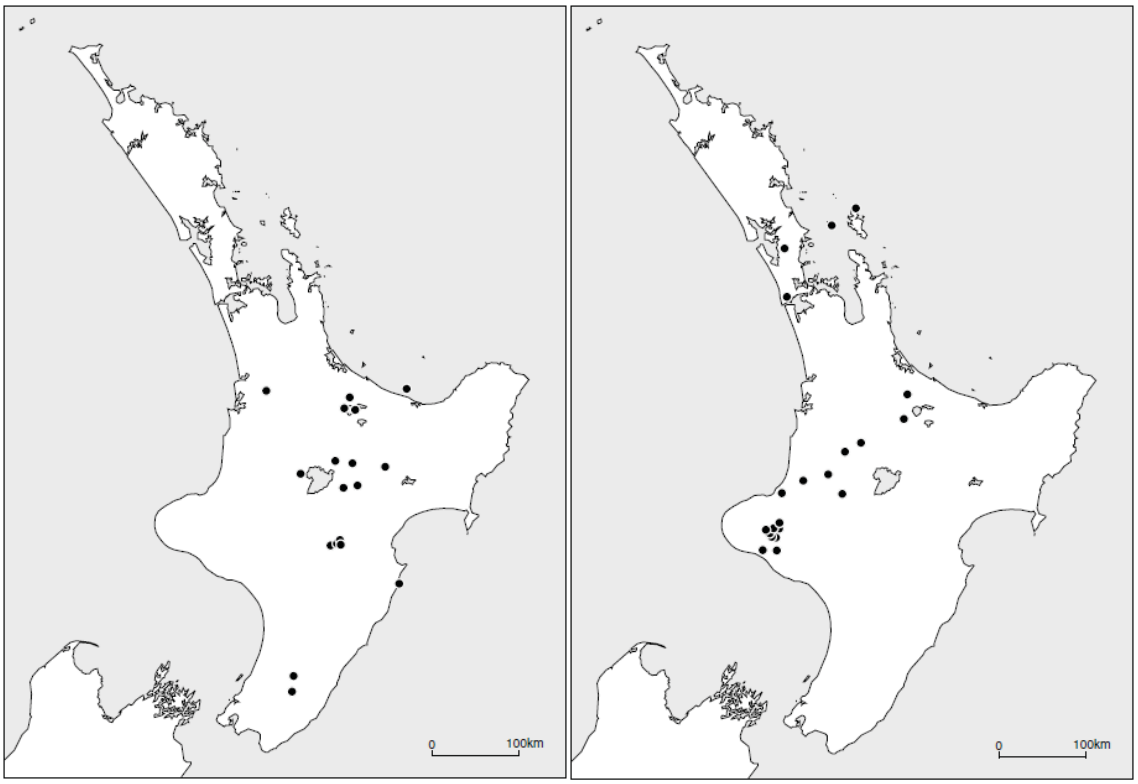


Figure 1.3: The distribution of the speckled skink (*O. infrapunctatum*) (left) and the striped skink (*O. striatum*) (right). Images obtained from the North Island *Oligosoma* spp. recovery plan (Towns, et al., 2002).

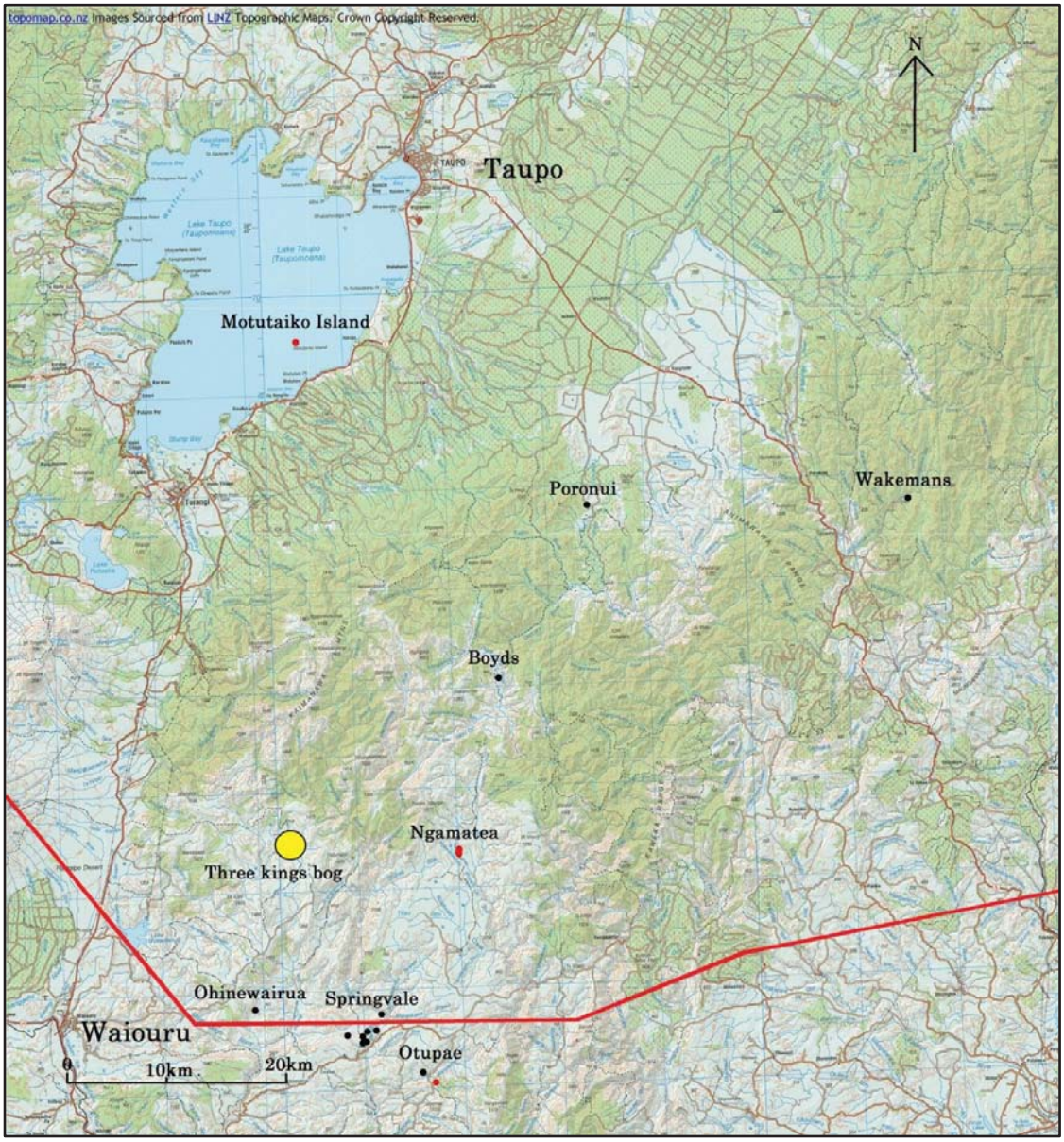


Figure 1.4: Topographical map (LINZ) showing the known distribution of small-scaled skinks. Sites marked with black dots were surveyed for this research whereas sites marked with red (Ngamatea station, Motutaiko Island and one Otupae site) were not. Also included on this map are the southern extent of the 186 AD Taupo eruption deposit (red line) and the location of Three Kings bog (McGlone, 1989b; Wilmshurst & McGlone, 1996).

The role of Taupo volcanism in the small-scaled skink distribution

The Taupo volcanic zone has a volatile history, dominated by a series of eruptions beginning 2 ma (Wilson, et al., 1995). The largest of these was the Oruanui eruption 26.5 ka; it ejected approximately 530 km^3 of magma, including 320 km^3 of pyroclastic

density currents (PDC) (Wilson, Blake, Charlier, & Sutton, 2006). The PDC mostly consisted of ignimbrite that covered ~12,000 km^2 and occurred up to 90 km from the source, with maximum depths of greater than 200 m (Wilson, 2004). Leeward sides of mountains received less deposition so pockets of habitat may have persisted (V. Neall, personal communication). This event caused massive restructuring of the caldera lake and significantly altered the flow of the Waikato River (Wilson, 2004). Vegetation recovery was slow due to the magnitude of impact as well as the cool climate (Pillans, et al., 1993).

The most recent significant Taupo eruption occurred 1.8 ka, c. 186 AD (Manville, Newton, & White, 2005; Manville, et al., 2009). An estimated 30 km^3 was deposited over 20,000 km^2 by a single PDC event at temperatures of 150-500°C and a velocity exceeding 100 m/s (Manville, et al., 2009). The flow reached 80±10 km from the source, travelling furthest towards east-south-east (Wilson, 1985). The pyroclastic flow overtopped the axial mountain range within 60 km of the vent, causing significant habitat destruction within the Kaimanawa and Kaweka ranges (Wilson, 1985). Charcoal deposits found at the base of this layer are remains of pre-eruption vegetation. Beyond 65 km the ignimbrite was largely confined to valleys and was less destructive (Wilson, 1985).

At least two small-scaled skink subpopulations are in habitats formed by the 186 AD eruption; Poronui and Boyds (Figs. 1.4 & 1.5). The impact of the eruption on other small-scaled skink sites is unknown, but a 26 cm layer of ignimbrite and charcoal consistent with the eruption was found in three kings bog, Inland Patea district, ca. 70 km from the vent (Fig. 1.4) (McGlone, 1989b). The Ngamatea small-scaled skink subpopulations are at similar latitude to Three Kings bog and so were likely affected to the same extent; Ohinewairua and Springvale were possibly affected to a lesser extent.

Figure 1.5: Photograph of the 186 AD Taupo eruption ignimbrite layer in the upper Ngaruroro Valley ca. 2 km downstream (south) of the Boyds small-scaled skink subpopulation, which resides on habitat formed from riverine erosion of this layer.

Conservation status

The small-scaled skink is listed on the IUCN red list as vulnerable with the species predicted to face a high risk of extinction in the wild in the mid-term future due to severe population fragmentation, declining area of occupancy, declining habitat quality and reduced number of locations (Australasian Reptile & Amphibian Specialist Group 1996). The Department of Conservation lists the small-scaled skink as in serious decline (Towns, et al., 2002).

The accuracy of these threat statuses are questionable; firstly both the IUCN and Department of Conservation threat ratings were produced more than ten years ago so recent research of their habitat preferences (Teal, 2006) and population densities (Gebauer, 2009b) have not been taken into account. Secondly, the decline of this species has yet to be quantitatively established; there is little evidence of subpopulations declining or becoming extinct. Finally, it is unknown whether this

species occurs in small, isolated subpopulations due to anthropogenic modification of the landscape or because it's preferred habitat is naturally uncommon. These different hypotheses lead to different management approaches; for naturally small populations the primary concern is to mitigate the consequences of rareness (such as genetic drift), while for species in decline management focuses on preventing or reversing further decline (often using pest control or other forms of habitat restoration) (Caughley, 1994).

1.4 Thesis aims and structure

Aims

The information above leads to five aims relevant to the conservation of the small-scaled skink. I first list these in order of likely relevance, and then explain how I will test/address these aims in the subsequent chapters of my thesis.

Aim 1

I aim to investigate evidence for decline of small-scaled skinks within their known distribution, in addition to surveying nearby potential habitats for undetected subpopulations. Small-scaled skinks may not be in serious decline as described by the Department of Conservation's threat status, but rather they may be naturally rare due to their specific habitat requirements.

Aim 2

I aim to examine the genetic diversity and morphological variation observed between subpopulations in order to determine if it is consistent with isolation by distance (i.e. strong positive correlation between genetic distance and geographic distance). Isolation by distance might have resulted in high genetic diversity between subpopulations and low genetic diversity within subpopulations, with evidence of genetic drift and inbreeding in particularly isolated subpopulations. Significant differences of morphological characters between subpopulations will indicate a dispersal barrier (which may be distance).

A secondary factor of this aim is to determine whether the pasture matrix present in Inland Patea district restricts dispersal to a greater extent than Euclidean isolation by

distance due to lack of refugia between habitats that would otherwise facilitate dispersal.

Aim 3

I aim to estimate rates of inbreeding within subpopulations and assess whether these could result in inbreeding depression. It is possible the most isolated subpopulations have been subject to inbreeding, which could increase the vulnerability of these subpopulations to disease and environmental variation.

Aim 4

Evidence of historical population size changes will be examined using estimates of genetic diversity and structure. The Taupo eruption likely caused local extinction of small-scaled skinks within the ignimbrite zone, and all subpopulations now within this area are probably the result of post eruption colonisation. There may be evidence of recent bottlenecks within these subpopulations and/or close relatedness with subpopulations outside the ignimbrite zone, indicating recent dispersal. In addition, the small-scaled skink population may have been abundant during the Pleistocene when prevalent grassland and frequent erosion meant exposed rocky habitats were widespread.

Aim 5

The final aim is to assess the conservation value of the captive small-scaled skink subpopulation, based on the genetic diversity within the subpopulation and the source of the founders.

Thesis structure

Chapter 2 describes a survey of the small-scaled skink distribution, conducted as part of the sampling process for later chapters. This chapter attempts to determine if any subpopulations have become extinct or are in obvious decline, in which case management of the small-scaled skink should be considered from Caughley's (1994) declining population perspective rather than his small population perspective.

Chapter 3 investigates morphological variation between and within subpopulations of small-scaled skink, with two main objectives; to determine if any subpopulations show

evidence of developmental stress (either environmental or genetic) and to use morphological differences between subpopulations to infer dispersal barriers. Genetic drift may be revealed by some subpopulations showing significant deviation in characters compared to the rest of the population. If there are few or no significant differences in morphology between subpopulations it could indicate moderate levels of gene flow between all subpopulations.

Chapter 4 details the process of genetic analysis of the small-scaled skink population, which address aims 2-5. A combination of maternally inherited mitochondrial DNA (mtDNA) and highly variable microsatellite loci was used, as mtDNA can provide information about historical divergences while the microsatellite data reveals current diversity levels and allows estimates of inbreeding.

Chapter 5 relates each of the previous chapters to the relevant aims to create a cohesive pattern that can be used to provide management recommendations for conservation of the small-scaled skink.

Chapter two

Site survey

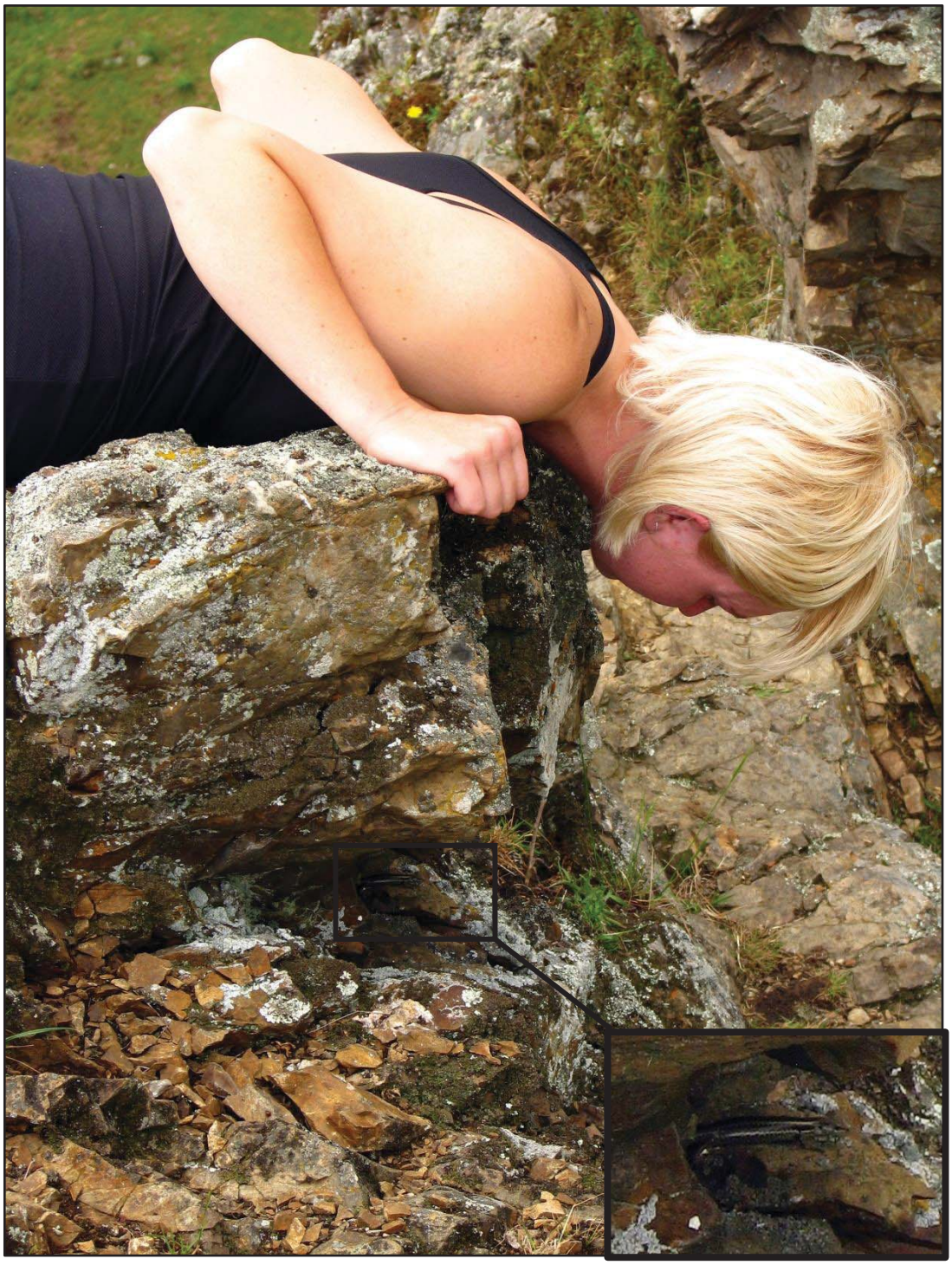


Figure 2.0: Finding the skink is tricky; catching it is an even greater challenge. Photo: Andrew Blayney

2.1 Introduction

Under Caughley's (1994) paradigms of conservation biology, a species with a small population size may be naturally so, or it may be rare due to decline in abundance or distribution. Determining whether a species is naturally rare or rare as a result of decline is imperative. For a naturally rare population the focus will be on the effects of limited size (such as inbreeding and genetic drift), whereas for a species in decline the focus will be on determining and reversing the causes of decline (which could include predation or habitat loss) (Caughley, 1994). A species that has a naturally fragmented distribution may have adaptations to cope with inbreeding (Chapter 1), but landscape modification may circumvent the species innate response. For example, sex biased dispersal appeared to be a method of inbreeding avoidance in the grand skink, but was ineffective in sites where pastureland reduced dispersal (Berry, et al., 2005).

The small-scaled skink is currently classified by the IUCN as vulnerable (Australasian Reptile & Amphibian Specialist Group, 1996) and by the Department of Conservation as in serious decline (Towns, et al., 2002). These classifications treat the small-scaled skink under the declining species paradigm with little evidence to suggest population decline over time. Recent surveys of small-scaled skink subpopulations have not been comprehensive; several subpopulations have not been surveyed since their initial discovery in 1991 or 1997 (Whitaker), nor has any survey determined the limits of this species' distribution. Since species records began, only one known subpopulation has become extinct (due to flooding) and there is no evidence for subpopulations declining over time, as mark-recapture estimates have only been conducted once (Gebauer, 2009b). In short, the decline of this species has yet to be quantitatively established. Furthermore, the IUCN threat status was assessed prior to the discovery of several subpopulations, thus it is out of date.

Small-scaled skinks were first described on Motutaiko Island, Lake Taupo in 1971 as a sub-species of common skink (*Oligosoma nigriplantare*) (Patterson & Daugherty, 1990). Several subpopulations were subsequently found within Inland Patea District in 1978 (Fig. 1.4). Although acknowledged as having an unusually high scale row count, they were not officially described as a distinct species until 1990 (Patterson & Daugherty).

Since 1990 there have been numerous surveys, most of which were conducted within Inland Patea district (Flannagan, Blackwell, & Ravine, 2001; Gebauer, 2009b; Hutchinson, 1992; Teal, 2006; Whitaker, 1991). In 1997 three more subpopulations were discovered to the north of Inland Patea district (Whitaker 1997), but these were not surveyed again until 2009 (Nelson- Tunley, 2009). These surveys have established Inland Patea district as the stronghold of the species, with outlying subpopulations at the northern extent of the species distribution (Fig. 1.4). In comparison to other mainland North Island *Oligosoma* species, the small-scaled skink has a very restricted distribution and is one of the few North Island species that has an entirely inland distribution (Towns, et al., 2002). Their restricted distribution may be partially due to limited survey effort for this species outside of Inland Patea district; suitable habitat that has not been surveyed exists in the nearby Kaimanawa, Kaweka, Ruahine, Te Urewera and Ahimanawa ranges.

A secondary aspect of their restricted distribution is their specific habitat requirements. Small-scaled skinks are saxicolous heliotherms, thus they require an exposed rocky habitat (Whitaker, 1991). This habitat is naturally rare, and sometimes patches can be isolated from one another by considerable distances, which could act to reduce migration and colonization. Major determining factors for *O. microlepis* presence/absence indicate the species prefers habitats with maximum exposure, allowing greater temperatures (Teal, 2006). Likewise, small-scaled skinks are more likely to be detected in warm temperatures, meaning that surveys are most effective during warm, sunny weather in the summer months (Teal, 2006).

The aim of this chapter is to evaluate evidence for decline of the small-scaled skink population. This will be investigated by; a) determining presence/absence of small-scaled skinks at previously surveyed sites, b) searching for small-scaled skinks within suitable habitats nearby known subpopulations, c) examining habitat changes in previously surveyed subpopulations. Previously surveyed subpopulations were searched for small-scaled skinks during optimal detection times (summer, warm weather). Small-scaled skink detection is highest when rock temperature is above 20°C (warm enough for basking and activity), and might also be higher during mornings (skinks typically bask in the morning) and in December (when skinks are foraging more

to compensate for winter dormancy) (Teal, 2006). Absence of detection may indicate subpopulation decline or extinction, which could be due to habitat changes (hence the third aim). If small-scaled skinks are detected at a previously unrecorded site, either this site has not been surveyed before (in which case it provides support for further survey effort) or the site has previously been surveyed, at which time no small-scaled skinks were detected. If the latter, small-scaled skinks were either not present at the time of the last survey (and have since colonized it, implicating population expansion), were present but poor weather resulted in low detection probability, or were present but at very low densities thus reducing detection probability (suggesting subpopulation increase).

2.2 Methods

Site descriptions

The 16 known small-scaled skink sites searched were at Springvale Station, Ohinewairua Station and Otupae Station in Inland Patea district, Ngaruroro river catchment in Kaweka forest park, Poronui Station in Taupo district, and Pohokura Station and Tataraakina Trust on the southern edge of Te Urewera National Park (Fig. 1.4 and Table 2.1). Two areas where *O. microlepis* had previously been recorded, but could not be accessed for this study, were Ngamatea Station on the southern edge of Kaimanawa forest park and Motutaiko Island, Lake Taupo (Fig. 1.4 and Table 2.1).

Potential small-scaled skink habitat was identified by a combination of examining aerial photographs (Google Earth) for rock outcrops, visual searches using binoculars from the roadside or farm track and asking landowners where/if they had seen skinks or rock outcrops or rock piles.

Basic information about each site was recorded; GPS co-ordinates, aspect, altitude, rock type and dominant vegetation (Tables 2.2 and 2.3). Other lizard species detected were also recorded.

Skink detection and identification

Small-scaled skink detection methods were similar to previous surveys, with a combination of visual searches for active skinks and for skink scat on top of good basking rocks (a characteristic of small-scaled skink occupancy) (Whitaker, 1991, 1997). Initially a site would be surveyed from a distance (about 20 m) using binoculars so as not to disturb active skinks. After 5 minutes the observers approached the site and watched for active skinks for at least 30 minutes or until skinks were spotted. If no skinks were detected at a site, the site was re-visited again at least twice more, using the same observation protocol. When the weather was inappropriate for basking (low air and rock temperature, strong winds, and/or rain), we set funnel traps and searched for skinks sheltering under rocks. Funnel traps were set on the hope that weather conditions may improve while observers were not present, allowing skinks to become active and perhaps be caught.

Identification of small-scaled skinks was possible due to their scale pattern. *O. microlepis* has a tear-drop pattern below the eye, a feature not shared with the sympatric common (*O. nigriplantare polychroma*) and speckled skinks (*O. infrapunctatum*). Furthermore, small-scaled skinks can be distinguished from all other skinks by their relatively high scale count (i.e. small scales) of 38-44 scale rows as opposed to 26-34 rows (Patterson & Daugherty, 1990). When skinks were seen at a distance scale counts were not possible; however, identification could still be conducted with some confidence given the differences in scale colouration and markings between the three sympatric species.

Skinks caught during the site survey were genetically and morphologically sampled as outlined in later chapters. The skinks were marked with a xylene-free ink pen to prevent resampling.

2.3 Results

Survey

Small-scaled skinks were found at all but two of the sites where they had been previously recorded; they were not found at Otupae site's track and gully (described by

Teal, 2006). Small-scaled skinks were not detected at Springvale Bridge, but they have not been observed here for multiple surveys due to flooding causing a significant change in the former skink habitat (Gebauer, 2009b). The site remains highly sedimented, and is unlikely to be recolonised by skinks in the near future.

Five previously undetected small-scaled skink subpopulations were discovered, which are recorded as Ohinewairua Station 2, Wakemans 2, Springvale 6, Springvale 7 and Black Hill. Whitaker (1991) surveyed Springvale 6 and Black Hill in 1991 without detecting small-scaled skinks; however, speckled skinks were seen at Springvale 6. Whitaker reported the weather during these surveys as fine, warm weather suitable for basking skinks (1991). Basic site statistics are provided in Tables 2.2 & 2.3, see appendix for detailed site descriptions.

Table 2.1: All known small-scaled skink sites, showing which sites were surveyed by whom including the most recent survey. Y= surveyed, *O. microlepis* present, N= surveyed, no *O. microlepis* observed, blank= not surveyed. Modified from Gebauer (2009a).

Site	Whitaker 1991	Hutchinson 1992	Whitaker 1997	Flannagan et al. 2001	Teal 2006	Gebauer 2008	Nelson -Tunley 2009	Nelson-Tunley (this research)
Motutaiko Island							Skink tracks	
Ngamatea Station 1	Y							
Ngamatea Station 2				Y				
Ohinewairua Station 1	Y			Y				Y
Ohinewairua Station 2								Y
Boyds			Y				Y	Y
Poronui			Y				Y	Y

Wakemans 1			Y			Y
Wakemans 2						Y
Otupae 1		Y		Y	Y	
Otupae gully/ 2				Y	Y	N
Otupae ridge/ 3				Y	N	Y
Otupae track				Y		N
Mt Aorangi					Y	
River flat	Y			N	Y	N
Springvale bridge	Y				N Habitat changed	N	N
Springvale quarry	Y			Y	Y	Y	Y
Springvale huts	Y			Y	Y	Y	Y
Springvale 2	Y					Y	Y
Springvale 3	Skink scat					Y	Y
Springvale 4	Skink scat					Y	Y
Springvale 5	Y					Y	Y
Springvale 6	N						Y
Springvale 7							Y
Black Hill	N						Y

Habitat data

Several skink habitats were on south or south-west facing slopes but the majority had a northern or western component, as was previously identified by Teal (2006). Altitude ranged from 563-961 m altitude, with a mean of 690 m altitude (Table 2.2). The rock type at all but two sites was greywacke, either in the form of an outcrop or

rock pile (Table 2.3). Rock outcrops and rock piles were often present at the same site, but both substrates were recorded only if lizards were found on both. The dominant vegetation type was not different than recorded in previous surveys, but increased vegetation cover was noted at Poronui (Fig. 2.1) and Otupae track (surveyed by Teal in 2006) based on previous site photos. At Poronui cessation of grazing resulted in increased height of grass and manuka (*Leptospermum scoparium*) near the skink habitat, encroachment of grass onto the habitat. At Otupae track it appears that native shrubs (including *Myrsine divaricata*, manuka, and *Muehlenbeckia* spp.) have increased in size and density and now shade much of the potential habitat.

Figure 2.1: Poronui small-scaled skink site in December 2009 (top) and January 2011 (bottom). Note the increased grass growth on and below the slope as well as the increased growth of manuka (*Leptospermum scoparium*) on the edges of the exposed rock pile.

Table 2.2: Aspect and altitude of surveyed sites, including sites where small-scaled skinks were not detected but had previously been recorded. Coordinates presented in NZ Geodetic Datum 2000.

Site	Reference	Coordinates	Aspect	Altitude (m)
Otupae track	Teal 2006	39 31 04.790 S 176 05 21.110 E	Variable	637
Otupae gully	Teal 2006	39 30 44.829 S 176 05 37.171 E	S	741
Otupae ridge	Teal 2006	39 30 56.506 S 176 05 25.296 E	SW	717
Poronui	Whitaker 1997	39 00 01.493 S 176 16 54.364 E	SW	676
Wakemans 1	Whitaker 1997	38 59 44.701 S 176 38 51.743 E	NW	563
Wakemans 2	New site	38 59 38.844 S 176 39 05.861 E	N	585
Ohinewairua 1	Whitaker 1991	39 27 43.318 S 175 54 21.026 E	W (outcrop), NW (scree)	824
Ohinewairua 2	New site	39 28 54.350 S 175 55 58.540 E	NW	600
Springvale quarry	Whitaker 1991	39 28 52.268 S 176 01 14.459 E	NE	813
Springvale huts	Whitaker 1991	39 28 43.028 S 176 02 09.365 E	NE	568
Springvale 2	Whitaker 1991	39 29 06.510 S 176 01 47.090 E	E	700
Springvale 3	Whitaker 1991	39 29 31.870 S 176 01 25.530 E	E	631
Springvale 4	Whitaker 1991	39 29 33.060 S 176 01 25.460 E	NE	639
Springvale 5	Whitaker 1991	39 29 24.190 S 176 01 39.180 E	E	613
Springvale 6	New site	39 27 33.740 S 176 02 39.970 E	W	586
Springvale 7	New site	39 27 07.910 S 176 01 23.240 E	NW	868
Black hill	New site	39 29 44.298 S 176 01 21.269 E	S	696
Boyds	Whitaker 1997	39 09 24.700 S 176 10 49.480 E	W	961

Table 2.3: Rock type, vegetation type and other lizard species recorded at all sites.

Site	Rock type	Dominant Vegetation	Other lizards detected	*O. microlepis* seen
Otupae track	greywacke outcrop	exotic grass, divaricates	*W. maculata*	N
Otupae gully	greywacke pile	exotic grass	*W. maculata, O. n. polychroma*	N
Otupae ridge	greywacke outcrop	exotic grass, divaricates	*W. maculata*	Y
Poronui	pumice pile	manuka regen, exotic grass		Y
Wakemans 1	greywacke pile	manuka regen, exotic grass	*W. maculata*	Y
Wakemans 2	greywacke pile	manuka regen, exotic grass		Y
Ohinewairua 1	greywacke pile and outcrop	exotic grass, divaricates	*W. maculata*	Y
Ohinewairua 2	greywacke pile and outcrop	exotic grass	*W. maculata*	Y
Springvale quarry	greywacke pile	exotic grass		Y
Springvale huts	greywacke pile	exotic grass, divaricates	*W. maculata*	Y
Springvale 2	greywacke pile	exotic grass	*W. maculata*	Y
Springvale 3	greywacke pile	exotic grass, lichen	*W. maculata*	Y
Springvale 4	greywacke pile	exotic grass, divaricates	*W. maculata*	Y
Springvale 5	greywacke pile	exotic grass		Y
Springvale 6	greywacke pile and boulder field	exotic grass, divaricates	*W. maculata, O. infrapunctatum*	Y
Springvale 7	greywacke pile	native scrub		Y
Black hill	greywacke pile	exotic grass		Y
Boyds	pumice pile	native grass, native scrub		Y

2.4 Discussion

Survey

The discovery of new subpopulations of small-scaled skinks increased the number of known subpopulations of this species from 19 to 24 (excluding Springvale Bridge). These new locations of *O. microlepis* populations were found with a minimum of search effort well within the known range for this species. This highlights the need for a thorough survey for lizards within the central North Island, as there are possibly more populations of *O. microlepis* to be discovered.

The detection of small-scaled skinks at previously surveyed sites could be indicative of colonization of these sites since the last survey, or it could mean *O. microlepis* were present but not detected during the last survey. Whitaker (1991) reported the weather and time of year of the last survey as appropriate for high detection (Teal, 2006), so it could be a matter of survey time span or low skink density. Of these two options, low skink density seems the most likely; we determined skink presence at these sites within 10 minutes of observation because both were of moderate to high density, whereas at a lower density site (like Wakemans 1 & 2) skink presence may take several hours to confirm. Furthermore, Whitaker detected speckled skinks at Springvale 6 which behave much more cryptically than small-scaled skinks (the later bask during fine weather whereas the former stay hidden). Supporting evidence for this idea is that some sites where skinks were not detected after being found previously (namely Otupae gully and River flat) appeared to have very few skinks recorded when they are detected (Table 2.1). Thus it is my opinion that either small-scaled skinks were either not present at Springvale 6 and Black hill during the last survey or that they were present but at very low densities.

It is important at this point to stress that lack of detection does not necessarily mean extinction; if several surveys fail to detect skinks then a conclusion may be reached. In good conditions a 15 minute small-scaled skink survey has a detection probability of 0.54 for rock piles and 0.46 for rock outcrops (Teal, 2006), so it fair to assume two surveys will increase the likelihood of finding skinks. There is fair cause to believe the Springvale bridge skink subpopulation is extinct; several surveys since 1991 have failed to detect skinks, and previous surveyors have recorded the site as significantly altered (Teal, 2006). Repeated surveys are the only way to confirm presence/absence at Otupae track and gully where small-scaled skinks were not detected this time. Otupae ridge provides an excellent example of a subpopulation that was not detected once (Gebauer, 2009b), then observed two years later. Obviously weather plays an important role in detection probability (Teal, 2006), and so later surveys should continue to search during optimal conditions.

Worthy of mention is the possible role of increased vegetation in small-scaled skink declines. Vegetation cover had increased noticeably at the Otupae track site within a 5

year period; the increased vegetation may have made the habitat unsuitable for small-scaled skinks, as they have been found to avoid shaded sites (Teal, 2006). A similar case may be underway at the Poronui habitat; vegetation noticeably increased within a year of the previous survey, and small-scaled skinks appeared to have declined. The increased vegetation noted at Poronui was due to reduced grazing as a response by the managers to the detection of small-scaled skinks. We advised the managers to graze the site more frequently to reduce grass growth.

The Aorangi stream (Inland Patea district) and Mokomokonui stream (southern edge of Te Urewera national park) both have suitable, unsurveyed habitat adjacent to known *O. microlepis* populations. The discovery of small-scaled skinks at Springvale 6 and Black hill demonstrates that regular resurveys of potential skink habitat would be a valuable practice; Ngamatea Station has not been surveyed since 1991 and Motutaiko Island has not had small-scaled skink presence conclusively confirmed. Furthermore, reports of unidentified skinks within the vicinity of Wakemans (D. Puna, personal communication) as well as within the Kaimanawa ranges (J. Scrimgeour, personal communication) should be investigated, as these may represent unconfirmed small-scaled skink subpopulations.

Small-scaled skinks may also be in locations further abroad that share the sub-alpine/greywacke habitat, such as on New Zealand Defence Force land east of the Desert Road, and at moderate altitudes in the Kaimanawa, Kaweka, Ruahine, Te Urewera, Whirinaki, and Ahimanawa ranges. These remote locations could be initially aerially surveyed to identify potential small-scaled skink habitat (using Teal's (2006) habitat preference data), followed by a ground-based visual survey of appropriate sites.

Habitat data

Other than the previously described increased vegetation, no major habitat changes were noted and all new sites had similar habitats to those already recorded.

Summary

This survey further confirms that small-scaled skinks have a fragmented distribution due to specific preferences for a naturally fragmented habitat; regardless of whether a subpopulation is found within pastureland or native forest, it is still isolated from other subpopulations by a matrix of unsuitable (i.e. shaded) habitat. The Wakemans sites clearly show this trend; there were two subpopulations in a relatively pristine environment that were bisected by forest/scrub habitat that does not provide the required basking conditions for small-scaled skinks. Further potential skink habitats within the vicinity were also isolated from one-another by dense forest.

Although this survey alone is not sufficient data to conclusively determine the appropriateness of the Department of Conservation threat status for *O. microlepis*, the discovery of five new subpopulations with little effort may indicate that the current known distribution may not represent the species' true distribution. The discovery of small-scaled skinks at previously surveyed sites suggests that *O. microlepis* may have increased in distribution and/or abundance in some areas, instead of declining as stated in the *Oligosoma* recovery plan (Towns, et al., 2002). If nothing else, this survey shows the need to review the current threat status of this species by; conducting regular surveys of existing subpopulations as well as potential habitats to investigate ongoing presence/absence (and thus potentially colonization and extinction), conducting long-term mark-recapture studies of several subpopulations to determine whether these subpopulations are stable, declining or increasing, and conducting surveys of the wider central North Island to search for unrecorded subpopulations.

Since this species can be considered to be naturally fragmented with no obvious decline in distribution or abundance, it should be considered under the small population paradigm, rather than the declining population paradigm (Caughley, 1994). Therefore the remainder of this thesis will consider the effects of small population size, rather than the potential causes.

Chapter three

Morphological variation

Figure 3.0: Photographs of the heads of several small-scaled skinks, showing the variation in facial markings (particularly the tear-drop pattern) that have previously been used for individual identification (Gebauer, 2009b; Nelson- Tunley, 2009). Photos: Andrew Blayney.

3.1 Introduction

Analysis of morphological variation among populations of animals is regularly applied to a wide range of questions including defining species (e.g. Will & Rubinoff, 2004), identifying different subpopulations (e.g. Fitness, Hitchmough, & Morgan-Richards, 2011), examining hybrid zones (e.g. Morgan-Richards & Townsend, 1995), and examining the effects of population fragmentation (e.g. Sarre, 1996). When investigating morphological variation among populations it is often sufficient to search for significant differences between averaged measures of characters such as body length, limb length or body mass. Such variation provides the basis for testing effects of population subdivision and environment.

Morphological variation among populations can also provide indicators of population "health", with the frequency of abnormalities providing evidence of congenital syndromes and location specific environmental effects. Fluctuating asymmetry, as measured by examining random departures from evenness in bilateral characteristics (Van Valen, 1962), has been used as an indicator of environmental or genetic stress during development, which can include exposure to toxins, poor maternal nutrition, deleterious mutations, and high homozygosity (Clarke, 1995). Fluctuating asymmetry measures can be more sensitive than direct comparison of other morphological characteristics (Sarre, 1996). Unfortunately some characters may not show fluctuating asymmetry even when stress is confirmed, such as head scale asymmetry of *Oligosoma suteri* that were experimentally subjected to developmental stress (Longson, Hare, & Daugherty, 2007). However, head scales have proved an effective measure of fluctuating asymmetry in two Australian reptile species, with hind limb length also being effective in one of these studies (Sarre, 1996; Sarre & Dearn, 1991).

Other types of morphological information can provide valuable insights into population fragmentation, thus I include here data on toe loss, tail loss and parasitism. Numerous researchers have shown a relationship between inbreeding (that is usually linked to small subpopulation size and isolation) and parasite susceptibility (Cassinello, Gomendio, & Roldan, 2001; Charpentier, Williams, & Drea, 2008; Van Oosterhout, et al., 2007). These mites have been linked to pregnancy failure in captive McCann's skinks (*O. maccanni*) (K. M. Hare, Hare, & Cree, 2010), as well as anecdotally linked

with reduced reproductive output in Otago skinks (Norbury, et al., 2006). Ecoparasitic mites found on New Zealand skinks include *Odontacarus lygosomae,* a chigger mite often located in the armpits, and *Ophionyssus scincorum*, a species that burrows underneath tail scales (Connolly & Cree, 2008; K. M. Hare, et al., 2010; Reardon & Norbury, 2004).

Caudal autotomy (self facilitated tail loss) is a common anti-predation adaption in squamates (Bateman & Fleming, 2009), although it can also occur during intraspecific competition (Pianka & Vitt, 2003). A third possible cause of tail loss is instability of the rocky habitats these skinks use; in highly disturbed environments a lizard's tail may become pinned between two surfaces whereby caudal autotomy will release the trapped lizard (Pianka & Vitt, 2003). Toe loss and scarring of the head and body may also result from interaction with predators, con-specifics or unstable habitat.

This chapter continues to investigate the causes and effects of population fragmentation on the small-scaled skink (*O. microlepis*) by examining morphological variation between subpopulations. A secondary part of this chapter is to interpret incidental findings of variation between subpopulations, namely rates of parasitism and indicators of predation.

The aims of this chapter are to: a) determine whether there are morphological differences between subpopulations of small-scaled skink and examine whether this variation is consistent with the species spatial distribution. In addition, assess morphological characters to investigate the occurrence of fluctuating asymmetry. Significant morphological differences at the subpopulation level could indicate a high level of population structuring (and perhaps low dispersal); while significant differences between groups of subpopulations could indicate that these represent different populations. b) Examine morphological differences between wild and captive small-scaled skinks. The captive subpopulation may show differences associated with small subpopulation size and/or husbandry practices. It is expected that captive small-scaled skinks will be heavier and longer (snout-vent length, SVL) than wild skinks due to easy access to food and little energy expenditure. c) Examine rates of fluctuating asymmetry among subpopulations. If fluctuating asymmetry is detected it could be the

result of either inbreeding or environmental effects, so the genetics of affected skinks will be examined in later chapters. d) Compare rates of ectoparasitism among subpopulations, and if significant differences exist, assess whether this correlates with patterns of fluctuating asymmetry, direct measures of inbreeding (Chapter 4) or other variables. e) Examine rates of toe and tail loss in small-scaled skinks and consider possible causes for variation. f) Assess sex ratio in the populations studied.

3.2 Methods

Catching and sampling procedure

Wild small-scaled skinks were sampled from 12 subpopulations throughout their distribution (Chapter 2), in addition to sampling 10 captive skinks. Collapsible funnel traps were the primary method of skink capture, supplemented with hand and noose catching methods. The funnel traps were composed of shade cloth and wire and measured 18 cm square by 75 cm long. Trap density varied with the size of the skink habitat, with up to 18 traps used on a ~100 m^2 site. The traps were baited with fruit, usually banana, as it had previously been an effective lure (Nelson- Tunley, 2009). Skink handling methods were carried out according to the Department of Conservation's standard operating procedure, and were approved by the Massey University Animal Ethics Committee.

Measurements taken for morphological analysis were snout-vent length (SVL, mm), tail width at the base of the tail (mm) using vernier callipers accurate to 0.02 mm and weight using a digital balance accurate to 0.1 g. Fluctuating asymmetry measures were the presence of asymmetrical head scales assessed from photographs of the head of each skink and difference between the length of hind limb length (to 0.1 mm) was measured using vernier callipers. Evidence of tail loss (missing or regrown segment), and toe loss were recorded. The presence of ectoparasites (mites) was recorded along with their position and abundance, the sex of each skink examined by presence/absence of hemipenes (Appendix 2) and the presence of other sympatric lizard species at each site were recorded (Table 2.3). Morphological data for both

adults and juveniles was examined; skinks less than 45mm SVL were classed as juveniles, while anything larger was considered adult.

In addition, ten skinks from the captive subpopulation housed by Dennis Keall were sampled. The captive small-scaled skink subpopulation was founded by two or three individuals (one female) in 1993. One male escaped within a year and the subpopulation has since grown to between ten and twenty individuals. Genetics results indicate this subpopulation was founded by individuals collected from Springvale (Chapter 4).

Data analysis

Raw morphological data (SVL, weight and tail width; Appendix 2) were grouped in four different ways; by subpopulation (all subpopulations compared to one another), by Springvale sites versus non-Springvale sites, by southern sites versus northern sites, and by wild skinks versus captive skinks. Captive skinks were included only in captive versus wild analysis due to their unknown origins at the time of analysis. The Springvale grouping represents a cluster of sites within 2 km of one another and separated by pasture, whereas the non-Springvale grouping represents much more isolated subpopulations (up to 31 km from the nearest neighbouring subpopulation). The southern versus northern grouping separates subpopulations up to 10 km apart from populations at least 19 km apart (Fig. 3.1).

The data were analysed using permutation analysis of variance (PERMANOVA) to explore similarities and differences between the groups described above using Primer 6.1 (Clarke & Gorley, 2006). Similarity percentage (SIMPER) analysis (also in Primer 6.1) was used to assess which variables (SVL, weight or tail width) were primarily responsible for the differences observed. Analysis of variance (ANOVA) (Microsoft Excel 2007) was conducted post-hoc on the main contributing factor to determine whether the observed difference was significant.

The same analysis was also applied using tail loss (Y/N) and toe loss (Y/N) in place of site groupings to examine whether SVL, tail width and weight influenced observed rates of toe and tail loss; this analysis will indicate whether toe or tail loss is more common in older skinks. In addition, a two-way ANOVA was conducted on tail and toe

loss to investigate distribution-based variation using the subpopulation groupings described above.

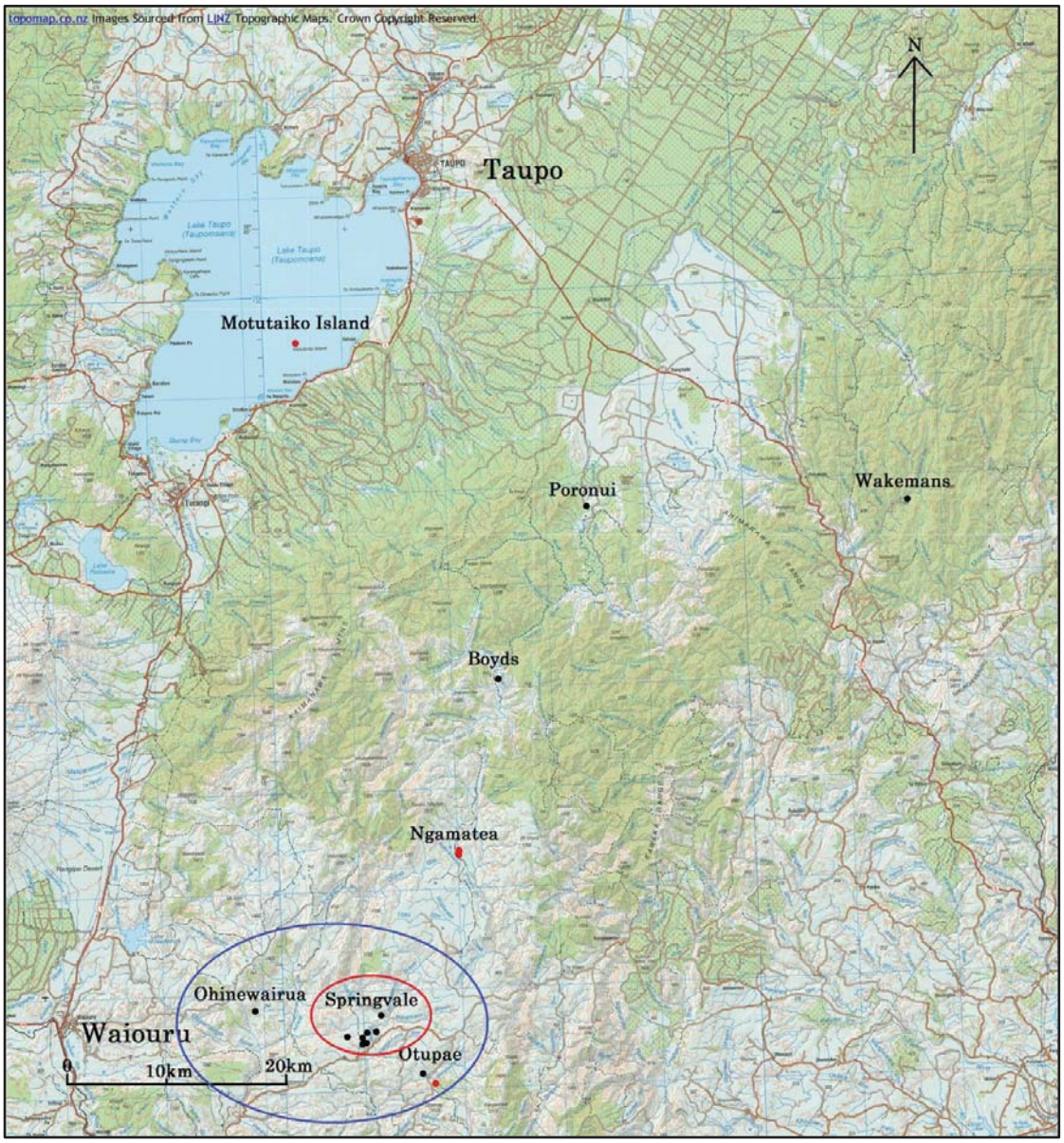


Figure 3.1: Locations at which small-scaled skinks were studied, showing the designated groupings used for analysis. The base topographical map is from LINZ. The red ellipse represents the Springvale grouping, with the non-Springvale sites being those outside this. The blue ellipse indicates the southern grouping, with the northern sites being those outside. Sites marked in red (Ngamatea, Motutaiko Island and one Otupae site) have previously been identified as skink subpopulations, but were not available for sampling.

3.3 Results

Morphological measurements were obtained from 127 wild small-scaled skinks, representing 12 subpopulations, in addition to ten captive skink samples. 86 skinks were within the Springvale group (41 within the non-Springvale group) and 109 skinks were within the southern group (18 the within northern group).

Morphological variation

There was a significant overall difference between measurements of small-scaled skinks for the Springvale versus non-Springvale group (*pseudo*-$F_{1,123}$= 7.07, P= 0.005) as well as the northern versus southern group (*pseudo*-$F_{1,123}$= 7.14, P= 0.008). However, there was no significant differences among ungrouped subpopulations (*pseudo*-$F_{11,113}$= 1.05, P= 0.413). Simper analysis revealed that SVL was the major contributing factor for the difference between Springvale and non-Springvale subpopulations (contribution of dissimilarity= 76.97%) as well as the main difference between northern and southern subpopulations (contribution of dissimilarity= 77.12%). Post hoc Single factor ANOVA revealed these contributions to be significant (Springvale versus non-Springvale P= 0.026, northern versus southern P= 0.022). No overlap existed between the SVL of Springvale and non-Springvale subpopulations and between northern and southern subpopulations (Fig. 3.2).

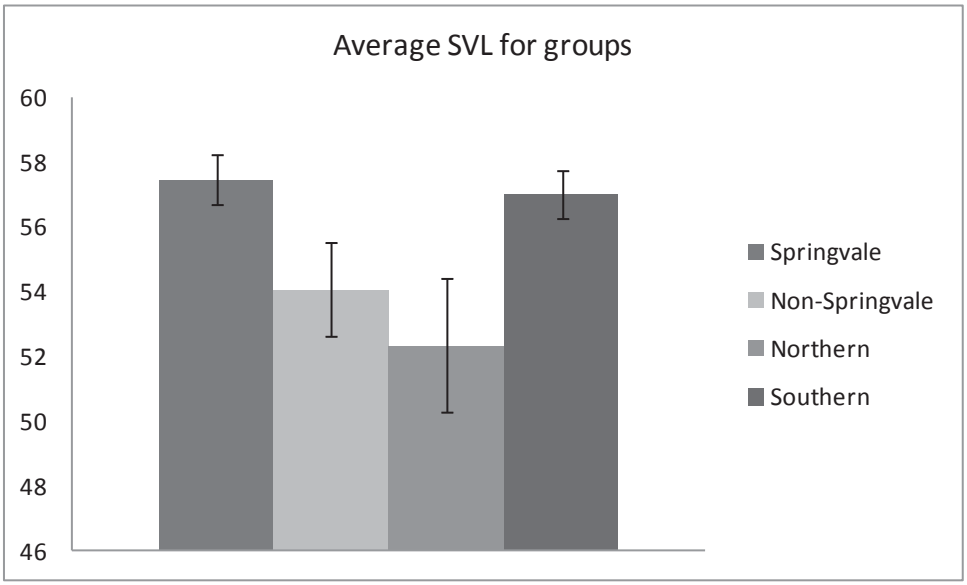


Figure 3.2: Average SVL of the assigned groups (Springvale versus non-Springvale and southern versus northern) including standard errors. There is no overlap of standard error between Springvale and non-Springvale subpopulations, or for southern and northern subpopulations.

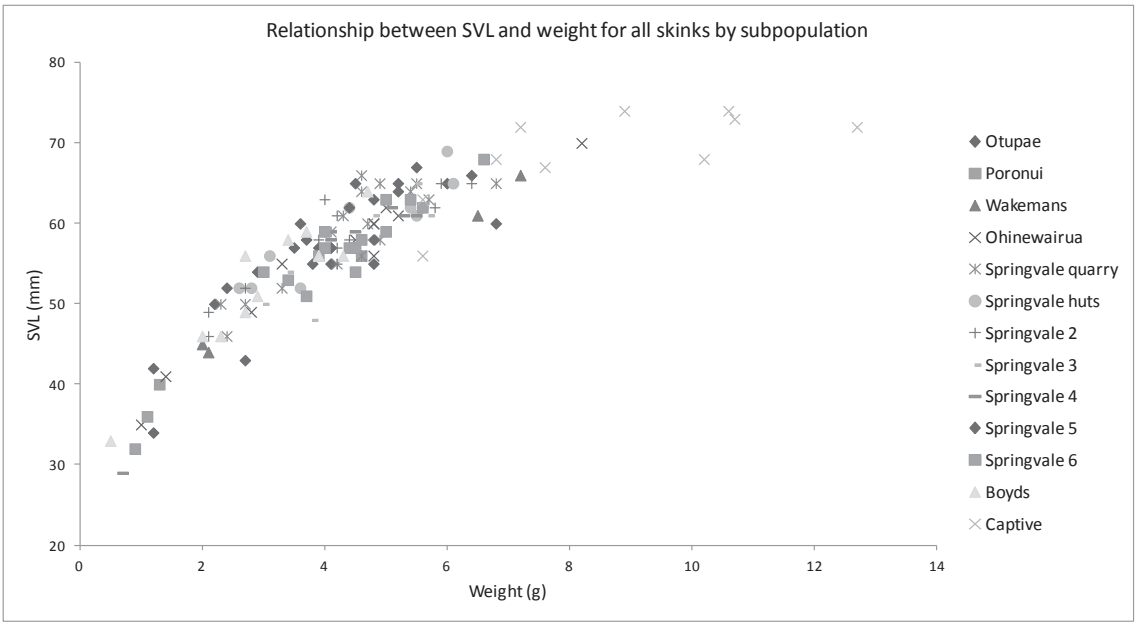


Figure 3.3: Relationship between SVL and weight for all skinks, grouped by subpopulation.

When SVL was plotted against weight a clear positive power relationship was discovered, with R^2= 0.8935 and $Y=36.935x^{0.3081}$. Captive small-scaled skinks dominated the upper range of SVL and weight, and a cohort of skinks under 45mm and

2g was observed in the lower end of the range (Fig. 3.3). When this image was enlarged, there appeared to be two less distinct cohorts dividing skinks around 3.5 g and 55 mm and around 4.7 g and 60 mm.

Captive small-scaled skinks were significantly different from their wild counterparts (*pseudo*-$F_{1,133}$= 22.68, P= 0.001), once again with SVL as the major contributing factor (contribution of dissimilarity= 67.34%). ANOVA showed that SVL was significantly different between wild and captive skinks (P= 5.12×10^{-6}). Captive skinks were much larger than wild skinks over all characters (SVL, weight and tail width), with no overlap of standard errors (Table 3.1). Single factor ANOVAs of weight and tail width confirmed that these variables were significantly higher in captive individuals (weight P= 6.04×10^{-15}, tail width P= 4.46×10^{-10}). On average captive small-scaled skinks were over twice the weight and 22% longer (SVL) than their wild counterparts.

Table 3.1: Average snout-vent length, weight and tail width (mm) of wild and captive small-scaled skinks, including standard errors.

	Wild	Captive	P- value
SVL	56.4 ±0.7	68.7 ±1.8	5.12×10^{-6}
Weight	4.1 ±0.1	8.6 ±0.8	6.04×10^{-15}
Tail width	5.6 ±0.7	7.4 ±0.2	4.46×10^{-10}

Fluctuating asymmetry

Left and right hind limb lengths did not differ (within 0.1 mm) in any of the skinks examined, so this could not be used as a measure of fluctuating asymmetry. There was however, some variation in the patterns and symmetry of head scalation.

Several different patterns of head scalation were observed in small-scaled skinks (Fig. 3.4). The majority of sampled skinks had what we considered normal scale morphology (Fig. 3.4a). When quantifying bilateral asymmetry, I excluded skinks with head scarring that may have caused the asymmetry (Fig. 3.4b). 9.5% of sampled skinks had ridged scales (Fig. 3.4c), which could not be used as fluctuating asymmetry measures due to scale symmetry being retained. Eight skinks (6.9% of total skinks) sampled had bilateral asymmetry without obvious scarring (Fig. 3.4d), possibly indicating fluctuating

asymmetry in a small proportion of the sampled population. Several skinks with this form of bilateral asymmetry were observed at the Boyds and Otupae subpopulations, with three other subpopulations (within Springvale) each having one skink showing this condition.

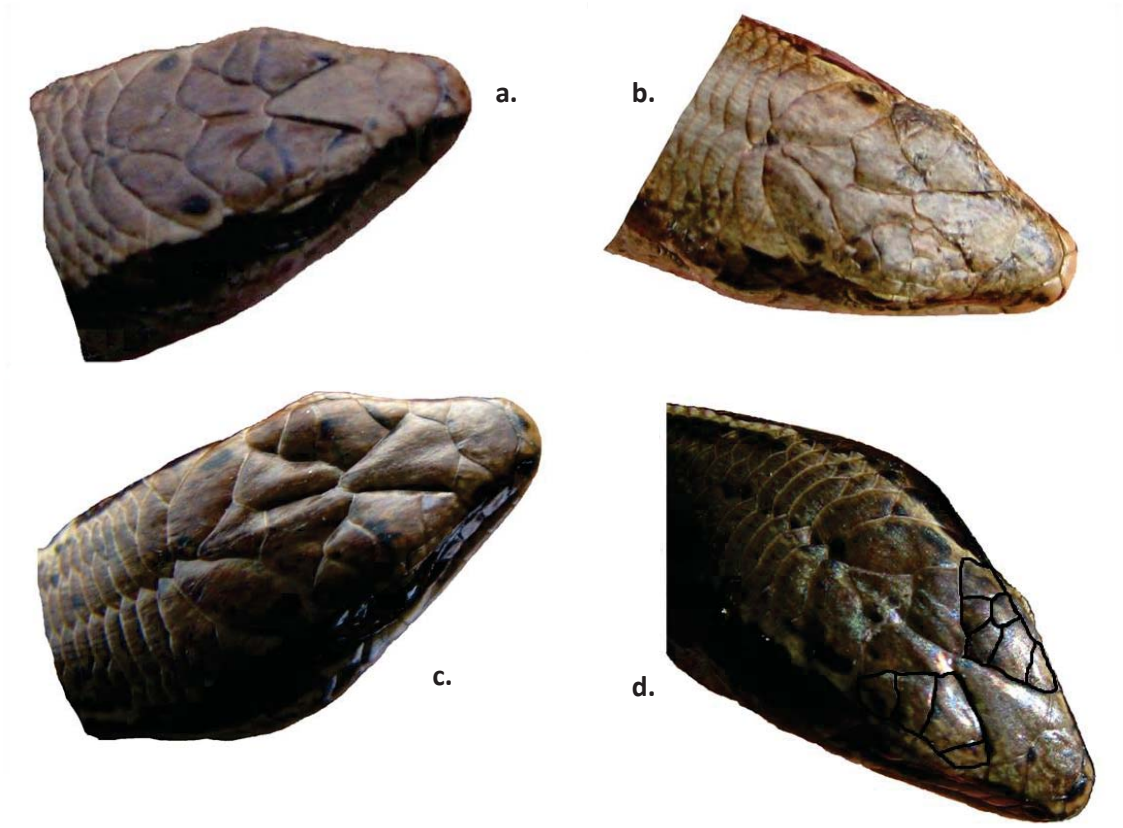


Figure 3.4: Head scale variation among small-scaled skinks. a; normal symmetrical scale morphology, b; asymmetrical scale morphology caused by scaring, c; heavily ridged but symmetrical morphology, d; asymmetrical scale morphology of unknown cause. Supraorbital scales have been outlined in Fig. 3.4d to exemplify the difference between the skinks' left (abnormal) and right (normal) scalation. Photos: Andrew Blayney.

Toe and tail loss

Tail loss was evident in all subpopulations, and was more common than toe loss. No toe loss was recorded at three of the wild subpopulations, although small sample sizes may have influenced this result. Regression analysis indicated that there was not a

significant relationship between tail and toe loss (R^2 =0.2159, P= 0.1499), suggesting that they may have different causes.

PERMANOVA analysis of toe and tail loss revealed a significant difference between morphological measurements of skinks that had lost toe(s) and/or tails and those that had not (tail loss: *pseudo*-$F_{1,123}$= 10.56, P= 0.001; toe loss: *pseudo*-$F_{1,123}$= 5.41, P= 0.018). For both toe and tail loss the main contributing factor was SVL (tail loss contribution of dissimilarity = 77.01%; toe loss contribution of dissimilarity= 76.55%). Skinks that had lost tail or toe(s) (or both) were significantly longer SVL than skinks that had not lost either tail or toe(s) (tail loss P= 0.001, toe loss P= 0.012; Table 3.2).

Table 3.2: Average SVL (mm, including standard error) of small-scaled skinks that have or have not lost tail and toe(s). The P values indicate that skinks that have lost tail or toe(s) are significantly larger than skinks that have not lost tail or toe(s).

	Loss	No loss	P value	N
Tail loss	58.24±0.76	53.62±1.27	0.001	127
Toe loss	60.55±1.27	55.69±0.79	0.012	126

A two way ANOVA of tail and toe loss revealed a significant difference in toe loss between Springvale versus non-Springvale sites (P= 0.02), with toe loss in a higher proportion of Springvale skinks. Neither of the other site groupings (northern versus southern, captive versus wild and individual subpopulations) was significantly different for toe loss, and no groupings were significantly different for tail loss.

Parasitism

Ectoparasites (prostigmatid mites) were found on skinks at only four sites (Ohinewairua, Springvale huts, Springvale 5 and Springvale 6). At Ohinewairua (n=11) and Springvale huts (n=10) only one skink was found with mites (9% and 10% respectively), whereas mites were found on 41% (n=17) of skinks at Springvale 6 and 50% (n=16) of skinks at Springvale 5. The number of parasites per skink ranged from zero to eight.

All parasites recorded were off-white or red coloured mites found in the underarm of the front limbs (Fig. 3.5). The hind limbs, ears and eyes were also checked for mites. Some mites were collected for future identification.

Figure 3.5: Four mites found on an *O. microlepis* skink. Photo: Andrew Blayney

Post-hoc ANOVA analysis revealed significant differences in parasite prevalence depending on the number of lizard species present (P= 0.0005), with highest parasite counts at sites with three species and lowest parasite counts at sites with only *O. microlepis* (Fig. 3.6). See Table 2.3 for details of the lizard species present at each location.

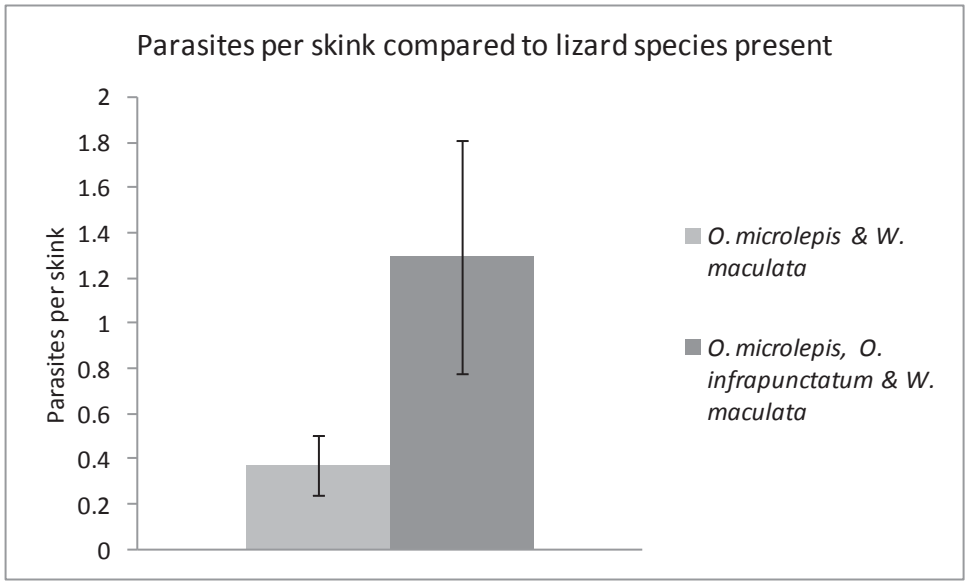


Figure 3.6: Parasites per (small-scaled) skink compared to lizard species present at site, with error bars indicating standard error. No parasites were detected at sites with only small-scaled skinks (n=18). N=92 for sites with small-scaled skink and *W. maculata* and n=17 for sites with all three species.

Sex ratio

Sex skew was observed at several sites with the most severe being at Springvale 6 (5 males and 10 females sampled) and Boyds (7 males and 4 females sampled), however binomial tests revealed that neither of these were significant (Springvale 6 P= 0.09, Boyds P= 0.16). Overall 60 males, 58 females and 9 undefined (very young) were sampled showing there was no species-wide sex skew. There were no significant differences detected between sexes (no difference in SVL, weight, tail width, or presence of toe loss, tail loss or parasites), nor were sex ratios significantly different between assigned groups (Springvale versus non-Springvale and northern versus southern).

3.4 Discussion

Morphological variation

Significant differences were observed in length (SVL) of the Springvale versus non-Springvale and northern versus southern subpopulations; longer skinks were found at

Springvale and southern localities. This shows that there are small, yet significant differences between small-scaled skinks associated with their geographical distribution, with larger skinks within the area considered to be the species stronghold (Chapter 2). There were no significant morphological differences detected between ungrouped subpopulations.

The possible causes of size differences among the locations are many, including age structure, prey availability, seasonality, or genotype. Northern and non-Springvale sites could have a greater proportion of young skinks, which would cause the average SVL to be smaller. However, numerous juveniles were detected at northern and non-Springvale sites, and the number of juvenile skinks caught (here considered to be skinks smaller than 45mm SVL) was similar throughout all sites. An age-related factor that could explain the observed variation is a poor reproductive season several years ago which has lead to an under-representation of a cohort in Springvale and southern subpopulations. Small-scaled skinks appear to have a juvenile cohort representing the most recent generation and indistinct cohorts for the rest of the population, with an overall power function relationship between SVL and weight (Fig. 3.3). Absence or reduced size of a cohort from several generations ago would result in less subadult sized skinks within a subpopulation, leading to a larger average SVL without a noticeable absence of juvenile skinks.

Prey availability and seasonality (i.e. winter length) can affect growth rate, thus either of these may have caused small adult size of northern skinks. However, the difference between Springvale and non-Springvale skinks is unlikely to be due to seasonality or prey availability because some of the non-Springvale subpopulations are within the same latitude and habitat matrix as the Springvale subpopulations (Fig. 3.1).

There may be genetic differences between Springvale and non-Springvale skinks or between northern and southern skinks, such as different levels of diversity within or between subpopulations or genetic adaption to the different locations (which may be evident in a division in their phylogeny). These genetic differences could cause the observed morphological differences and so will be discussed in later chapters (Chapter 4 and 5).

Fluctuating asymmetry

The lack of fluctuation in femur length does not mean that fluctuating asymmetry does not occur in this species, as it is possible for some features to express it while others do not (Clarke, 1998). This may happen when strong selection exists for the character to be symmetrical, which is likely for femur length as asymmetry could impair survival (Clarke, 1995).

Four main types of head scale morphology were identified, one of which may be an indicator of fluctuating asymmetry. Since fluctuating asymmetry can be caused either by genetic or environmental stress during development, determining the cause of asymmetry is not possible with the current information. The following chapter (Chapter 4) investigates the genetic diversity of small-scaled skinks; if any subpopulations containing abnormally scaled skinks are inbred this could confirm head scale asymmetry as an indicator of genetic stress induced fluctuating asymmetry.

Small-scaled skinks were observed to vary in other features that may have been suitable fluctuating asymmetry measures (shape of patterns on either side of body and length between eye and rostrum); however these features were not noticed until after commencement of the sampling process and so could not be included.

Captive versus wild variation

There was a significant difference in length (SVL) between wild and captive small-scaled skinks, with captive skinks being significantly longer than wild ones. In addition, captive skinks were significantly heavier with fatter tails than wild skinks. The average captive skink was more than twice the weight of the average wild skink. Obesity in lizards has been linked to reduced reproductive rate (Connolly & Cree, 2008), which is an important consideration for a species listed as declining in the wild (Towns, et al., 2002). It has been noted that the captive small-scaled skink population produced very few young each season (Keall, D., personal communication); this may have caused a slight over-estimation of the difference between wild and captive skinks since the wild samples included juveniles and sub-adults. As the captive population ages its reproductive capacity may decline, as has been observed in captive birds and mammals (Ricklefs, Scheuerlein, & Cohen, 2003). At present the captive population has

unlimited access to food and a considerably smaller habitat size than in the wild; altering this regime may reduce the weight of the captive population.

Figure 3.7: Direct visual comparison between the heaviest captive skink (left) and the heaviest wild skink (right). Note the comparatively thick tail and upper body of the captive skink. Both skinks were female, and the wild skink appeared heavily pregnant.

Tail and toe loss

Instances of tail and toe loss were significantly higher in larger skinks. This can be most readily explained as a correlation between age and tail/toe loss; the older a skink is, the more likely it is to encounter a situation that results in tail or toe loss. Although causes of tail and toe loss were not investigated directly, I propose three likely causes for losses; predation, competition and habitat instability. As previously mentioned, tail loss has been observed as a predator response as well as during interspecific competition. No predation was observed during small-scaled skink sampling, but numerous instances of intraspecific interactions took place, none of which resulted in tail loss. During sampling I noted that small-scaled skink habitats are often unstable, with loose rocks able to move against one another; a rock slide may result in amputation or autotomy for skinks pinned by moving rocks. The fact that captive skinks that are not subject to predation, and do not have unstable habitat, nevertheless experience tail loss suggests that intraspecific aggression is a likely cause of captive tail loss. Toe loss (which is absent in captive skinks) was significantly higher in Springvale subpopulations than non-Springvale subpopulations, possibly indicating higher predation, higher habitat instability, higher competition, or a combination of the three, in Springvale subpopulations. However, a simpler explanation is that both

the greater amount of toe loss and larger SVL of Springvale skinks have the same cause; Springvale skinks may be older on average than non-Springvale skinks.ws

Parasitism

It is likely that the mites detected on small-scaled skinks and speckled skinks during this study were Odontacarus spp., as they match the description of mites found on other native species. No tail scale mites were detected, although this could be due their detection difficulty rather than an absence of this type of mite. Rates of parasitism were significantly higher in sites with three lizard species, and absent in sites with only *O. microlepis*. However, there was only one site where three lizard species were present, which was the only small-scaled skink location that had *O. infrapunctatum* recorded. In order to more thoroughly test the relationship between parasite prevalence and lizard species present it would be necessary to examine other sympatric species. A variety of potential hosts may be beneficial for mite populations as it provides greater habitat heterogeneity through host switching. However, one study of a New Zealand lizard assemblage found no mite sharing between geckos and skinks (Reardon & Norbury, 2004), which could mean the higher mite densities observed here were not due to host switching among sympatric lizards. Alternatively, higher host densities are known to correlate with higher parasite densities (Arneberg, Skorping, Grenfell, & Read, 1998), so it is possible that sites with greater lizard diversity may also have greater densities of each lizard species, thus facilitating greater parasite densities. It is theoretically conceivable that a habitat can be optimal for several species with different habitat requirements, and if the species occupy different niches competition may be low enough for each species to reach high densities.

Susceptibility to parasites and disease is thought to be higher within inbred populations (Cassinello, et al., 2001; Charpentier, et al., 2008; Van Oosterhout, et al., 2007), so parasite occurrence will be compared to genetic diversity in later chapters to determine whether inbred subpopulations have higher parasitism. No link was found between parasite presence and scale asymmetry in the present study.

Sex ratio

The cause of the sex skews observed at Springvale 6 and Boyds is unknown, and could be due to random variation within small sample sets. Factors determining sex in New Zealand lizards are poorly understood, although maternal basking regime during pregnancy does not influence sex in laboratory conditions (K. M. Hare & Cree, 2010). Sex skew is a particular concern for the Boyds subpopulation due to its geographic isolation from other subpopulations; demographic stochasticity can lead to extinction. Juvenile skinks were observed at Boyds, so the subpopulation was reproducing even if female numbers were low.

Conclusions

Small-scaled skinks showed some morphological differences based on geographic distribution, but not to the extent that subpopulations were different from one another. Head scale asymmetry was observed, but cannot be conclusively determined to be fluctuating asymmetry without evidence of genetic or environmental stress. Captive skinks were considerably longer and heavier than wild skinks, to the extent that reducing their weight is advisable. Tail and toe loss occurred more often within larger (and presumably older) skinks, and toe loss was more common within Springvale subpopulations. Three possible causes for tail and toe loss were discussed, but further data collection is required to resolve this. Parasitism appears to be more prevalent in small-scaled skink subpopulations which are sympatric with *W. maculata* and *O. infrapunctatum*. As with tail and toe loss, further data collection may provide resolution.

Chapter four

Population genetics

Figure 4.0: A small-scaled skink poised to strike a fly.

4.1 Introduction

Genetics overview

Genetic information can be used in the study of biogeography (K.M. Hare, et al., 2008; Liggins, Chapple, Daugherty, & Ritchie, 2008), taxonomy (D. G. Chapple, et al., 2011), hybridisation (Morgan-Richards, et al., 2009), dispersal (Stow, Sunnucks, Briscoe, & Gardner, 2001) and inbreeding (Miller, Chapple, Towns, Ritchie, & Nelson, 2009). For rare or endangered species, inferences from genetic analysis can be directly applied to their conservation (Allendorf & Luikart, 2007). In small or fragmented populations, analysis of genetic differences between populations can determine whether the effects of isolation (genetic drift and inbreeding) are being reduced by gene flow (Hedrick, 2005).

The isolation by distance hypothesis (Wright, 1943) states that neutral genetic divergence of two populations should be proportional to the geographic (or Euclidean) distance between them (Slatkin, 1993). Species deviate from isolation by distance through one of two causes; either distance does not inhibit dispersal or there are factors greater than distance inhibiting dispersal. The former would indicate a species which has little difficulty dispersing large distances, but for the latter it indicates a strong dispersal barrier such as a greatly unsuitable matrix between habitat patches (Ricketts, 2001).

Population genetics can additionally be used to infer the biogeographical history of a species. High genetic structure throughout the species, with unique alleles in isolated populations, is indicative of fragmentation and/or isolation by distance, while low genetic diversity can indicate either high gene flow between populations or a recent population bottleneck (Excoffier, Foll, & Petit, 2009; Templeton, 2006). Population bottlenecks reduce genetic diversity compared to stable large populations where standing diversity is proportional to effective population size (Charlesworth, 2009). Knowledge of a species' biogeographical history can aid conservation by understanding the processes that formed a species distribution; from this information we can better predict the effects of different management practises (Templeton, 2006).

The small-scaled skink

The small scaled skink (*Oligosoma microlepis*) has a narrow distribution, fragmented population and is of conservation concern (Chapter 1). A small captive subpopulation (10-20 individuals) of this species exists that was founded by two or three individuals (one female) from an unknown location (D. Keall, personal communication, 4/5/2011). The skinks were caught illegally and later transferred to a reputable reptile breeder, resulting in no formal captive management plan for this species. The IUCN states that the primary objectives of a captive population should be to support conservation of a threatened taxon by protecting its genetic diversity and habitat (IUCN, 2002), thus a captive population should be a fair representative of wild genetic diversity. The usual purpose of a captive population is to produce individuals for eventual reintroduction into the wild (Tenhumberg, Tyre, Shea, & Possingham, 2004; Theodorou & Couvet, 2004; Williams & Hoffman, 2009). Until recently, this was not identified as a goal for small-scaled skinks or indeed for many of the New Zealand lizard species currently in captivity, which are instead kept for advocacy and education (New Zealand Hepetological Society, 2012). Small-scaled skinks are currently being considered for a captive breeding for release program (D. van Winkle, personal communication, 6/7/2012), which means establishing the origins of the founders and determining their genetic diversity relative to the wild population has become much more important.

Two types of genetic data have been generated for this study. First, mitochondrial DNA (mtDNA) sequences were used because mitochondria are maternally inherited and not subject to recombination so that haplotype relatedness can be readily traced (Allendorf & Luikart, 2007). The use of mtDNA in many studies of New Zealand lizards means the data can be readily compared to both common and rare species (e.g. Chapple (2011), Fitness et al. (2011) and Greaves (2008)). Second, length polymorphisms of nuclear microsatellite loci were used. Microsatellites are highly variable short repeating segments of DNA (Allendorf & Luikart, 2007). The length of repeating strand is used to identify different alleles, but unlike mtDNA relatedness cannot be traced as the same allele can be derived from different lineages through reversible stepwise mutation (Hedrick, 2005). Microsatellites have a high mutation rate which means even small populations show high polymorphism, thus they are

excellent for examining genetic diversity at the subpopulation level (Allendorf & Luikart, 2007).

Aims

Here I investigate the causes and consequences of population fragmentation on the small-scaled skink using genetic evidence, with the aim of providing information to guide long-term conservation strategies for this species. a) I will determine whether isolation by distance explains a significant amount of observed genetic variation. b) To determine the risk of inbreeding depression I will examine estimates of inbreeding and deviations from expected genotype frequencies under models of random mating (Hardy-Weinberg). c) Evidence of historical population size changes will be examined using estimates of genetic diversity and structure. d) I aim to determine the origins of the captive subpopulation of small-scaled skinks by comparing mitochondrial haplotypes of captive animals to those found in wild subpopulations. Based on this I will consider the relevance of this subpopulation to conservation of the species.

Isolation by distance would be evident in a close correlation of geographic distance and genetic distance between subpopulations. Genetic distance is measurable by pairwise F_{ST} (using microsatellite loci) and pairwise ϕ_{ST} (using mtDNA), which is then correlated to pairwise geographic distance. The lack of refugia provided by the pasture matrix in Inland Patea district may restrict dispersal between skink habitats despite dispersal distances being relatively short. If this is the case, subpopulations will be more genetically dissimilar than expected under Euclidian distance. In addition, the presence of private alleles or haplotypes (mutations found within only one subpopulation) can be used as evidence of restricted gene flow. Significant F_{IS} values indicate non-random mating, which is strong evidence of inbreeding within a subpopulation.

The Taupo eruption would have caused local extinction of small-scaled skinks within the ignimbrite zone, and all subpopulations now within this area are likely the result of post-eruption colonisation. There may be evidence of recent bottlenecks within these subpopulations and/or close relatedness with subpopulations outside the ignimbrite zone, indicating recent dispersal. The small-scaled skink population may have been

abundant during the Pleistocene when prevalent grassland and frequent erosion meant exposed rocky habitats were widespread. This hypothesis would be supported by numerous haplotypes showing a complex pattern of relatedness, indicative of consistently large population size and no loss of diversity through population bottlenecks.

4.2 Materials & Methods

DNA extraction, amplification, sequencing and genotyping

129 tail tissue samples were collected from 12 sites representing most of the known small-scaled skink distribution (see Chapter 1). In addition, 10 tail tissue samples were obtained from the captive subpopulation.

Total genomic DNA was extracted using the salting out technique (Sunnucks & Hales, 1996) and was followed by polymerase chain reaction (PCR) conducted using four different primers (Table 4.1). The PCR protocol for the amplification and sequencing of the mitochondrial sequence (16S) was that of Fitness (2010). Primers and PCR protocol for the microsatellite markers are given in Berry et al. (2003).

Table 4.1: Oligonucleotide primers used in this study of variation at five genetic loci in the small scaled skink. Primer sequences for 16S were obtained from Reeder (1995) (16Sc) and Palumbi (1996) (H3056). Primers for the microsatellite markers were from Berry et al. (2003).

Locus	Location	Forward Primer	Sequence	Reverse Primer	Sequence	Product Length (BP)
16S	Mitochondria	16Sc	GT[A/C]GGCCTA AAAGCAGCCAC	H3056	CTCCGGTCTGAACT CAGATCACGTAGG	760
Oligr6	Nuclear (microsatellite)	Oligr6f	TTTGGTGCCTT ATTGCCTTTG	Oligr6r	GGTCTTTGGG TCTATGCTTTG	151-224
Oligr14	Nuclear (microsatellite)	Oligr14f	TCTGGTTAACA GAGATTCCAC	Oligr14r	AGACAGTGGTG AAGTTTGAAG	258-296
Oligr19	Nuclear (microsatellite)	Oligr19f	CTGTCTGCTGC TAATGGAGAG	Oligr19r	AAACACCCCT CTCGTTGTAC	155-172
Oligr20	Nuclear (microsatellite)	Oligr20f	TTGCTGCTTCT ATCCCTTCTC	Oligr20r	TGGTGTGCCTT GTCAATAGTC	272-285

Sequence data for 16S was edited and assigned to haplotypes using Sequencher (Gene codes Corporation) in conjunction with Geneious (Drummond A.J., et al., 2010) to produce a haplotype matrix. Abundance and diversity of haplotypes at each subpopulation was overlaid onto the small-scaled skink distribution. The relationship between sample size and haplotype/allele richness was examined for wild skinks. Nucleotide diversity was calculated in Arlequin (Excoffier, Laval, & Schneider, 2005).

Isolation by distance web service (Jensen, Bohonak, & Kelley, 2005) was used to produce pairwise ϕ_{ST} values (i.e. genetic distance) and to conduct Mantel tests of log (ϕ_{ST}) versus log (distance) with 10,000 randomisations. Construction of the minimum spanning network used principles of parsimony without aid of computer software.

Microsatellite lengths were determined using Genemapper (Applied Biosystems, 2004), followed by detection of null alleles, stuttering, large allele drop out, and homozygosity ratios using Micro-Checker ver.2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). Data were then processed by Structure (Pritchard, Wen, & Falush, 2007) which in conjunction with data transformation recommended by Evanno et al. (2005) determined optimal K, the number of populations sampled. The microsatellite data were processed by Fstat (Goudet, 2002) to estimate pairwise F_{ST} and population F_{IS}. Isolation by distance web service (Jensen, et al., 2005) was used to conduct a mantel test for a significant correlation between genetic distance (log F_{ST}) and geographic distance (log km). Allele abundance and diversity at each subpopulation were overlaid onto the small-scaled skink distribution.

4.3 Results

mtDNA

From the 136 skinks sequenced for 16S (760bp) 15 haplotypes were observed (Tables 4.2 & 4.3). These haplotypes differed by a maximum of 6 BP (0.79%). The fully resolved haplotype network (Fig. 4.1) revealed no major divisions within this diversity. Ten haplotypes were restricted to single subpopulation samples and the most common haplotype (A) was observed in seven wild subpopulations (Fig. 4.2) and within the captive population, with a maximum distance of 82.4 km (Table 4.4) between subpopulations. Haplotype diversity was dependent on sample size (positive correlation $R^2 = 0.776$). Fortunately, nine of the thirteen subpopulations sampled have ten or more samples. The subpopulation samples from Boyds, Otupae, and Ohinewairua each had two or three private haplotypes and these subpopulations are at least 6 km from the nearest recorded neighbouring subpopulation.

Table 4.2: 16S haplotypes of small-scaled skinks, showing the nucleotide substitutions which differ from the most common haplotype (A).

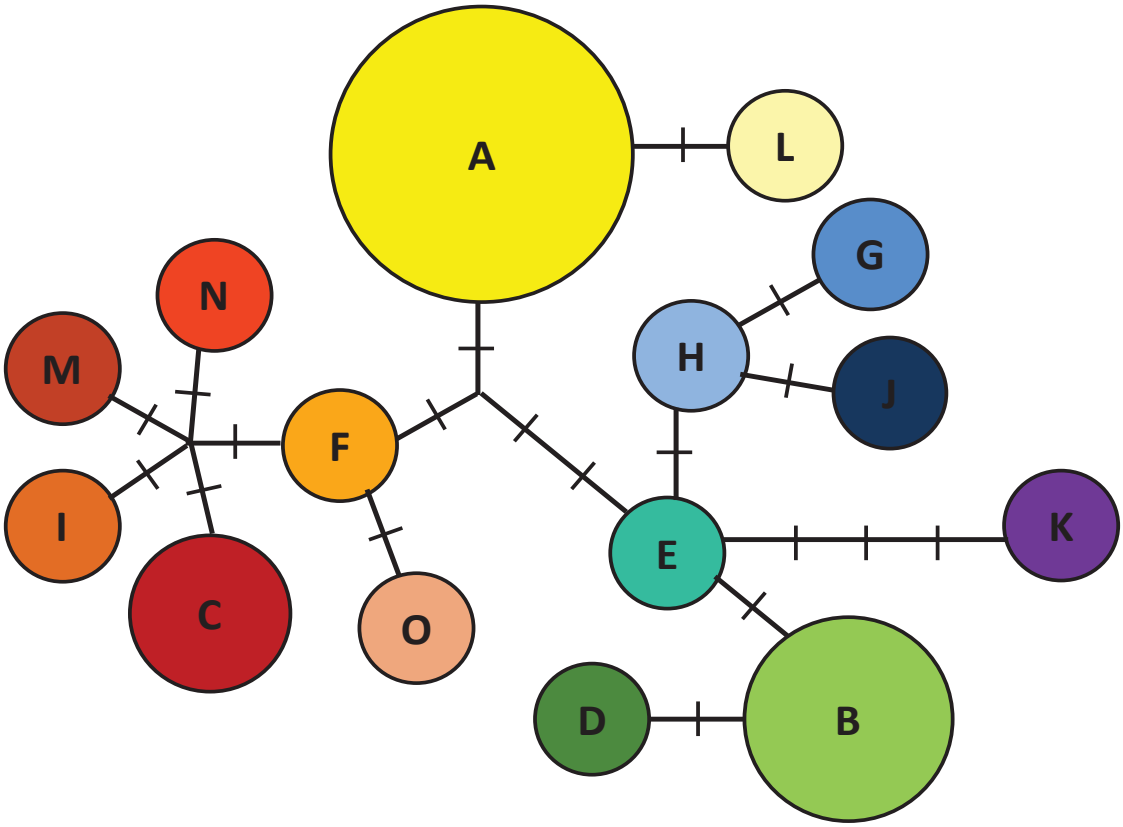
Haplotype A	C	C	T	G	C	C	T	G	A	T	G	G	A	A	C	G	G	T	T
Haplotype B	.	T	.	.	T	.	.	.	G	.	.	.	.	G	.	.	.	.	.
Haplotype C	.	.	.	.	T	.	.	.	.	.	A	.	.	.	T	.	.	.	C
Haplotype D	.	T	.	.	T	.	.	.	G	.	.	.	G	G	.	.	.	.	.
Haplotype E	.	T	.	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.
Haplotype F	.	.	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.
Haplotype G	.	T	.	.	T	.	C	A	G	.	.	.	.	.	.	.	.	.	.
Haplotype H	.	T	.	.	T	.	.	A	G	.	.	.	.	.	.	.	.	.	.
Haplotype I	.	.	.	.	T	.	.	.	.	.	A	.	.	C	T	.	.	.	.
Haplotype J	.	T	.	A	T	.	.	A	G	.	.	.	.	.	.	.	.	.	.
Haplotype K	.	T	.	.	T	.	.	.	G	A	.	.	.	.	.	.	A	C	.
Haplotype L	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Haplotype M	.	.	C	.	T	T	.	.	.	.	A	.	.	.	T	.	.	.	.
Haplotype N	.	.	.	.	T	.	.	.	.	.	A	A	.	.	T	A	.	.	.
Haplotype O	.	.	C	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.

Table 4.3: The number of alleles (microsatellite loci) or haplotypes (mtDNA) identified at each subpopulation of small-scaled skinks. The number of haplotypes/alleles unique to a subpopulation is presented in parentheses. n = total number of skinks sampled from each subpopulation.

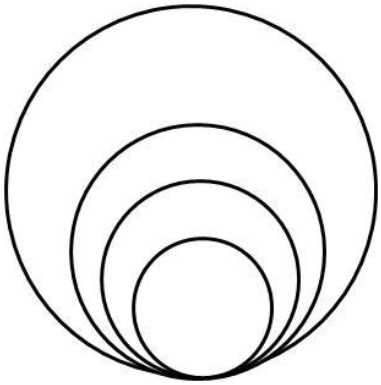
	Microsatellites				mtDNA	
Site	Oligr20	Oligr19	Oligr14	Oligr6	16S	n
Otupae (OT)	2	3	2	7	3(2)	13
Poronui (PO)	1	3	1	2	1(1)	2
Wakemans (WK)	2	2	2	4	1(1)	4
Ohinewairua (OH)	3	4(1)	3	8(1)	4(2)	12
Springvale quarry (SQ)	3	4	4(1)	15(2)	5(1)	17
Springvale huts (SH)	3	3	3(1)	7	3	10
Springvale 2 (S2)	3	4(1)	3	6	3	12
Springvale 3 (S3)	3	3	3	5	2	7
Springvale 4 (S4)	3	5(1)	2	8	2	7
Springvale 5 (S5)	3	3	3	6	5	16
Springvale 6 (S6)	2	5	3	13(1)	3	15
Boyds (BO)	2	3	2	8	3(3)	11
Captive (DK)	1	2	3	2	1	10
Total	3	8	6	23	15	136

Table 4.4: Pairwise geographical distance (km) between subpopulations of *Oligosoma microlepis*.

	PO	WK	OH	SQ	SH	S2	S3	S4	S5	S6	BO
OT	59.5	75.1	17.0	6.2	7.1	6.2	6.3	6.3	6.1	7.4	40.6
PO		31.7	60.7	57.2	57.9	58.0	59.0	59.0	59.0	55.0	19.5
WK			82.4	75.3	76.4	76.1	77.1	77.1	76.7	73.2	44.2
OH				11.3	10.1	11.0	10.7	10.7	10.9	11.9	41.3
SQ					1.3	0.9	1.8	1.9	1.5	2.3	37.8
SH						0.9	1.3	1.3	1.2	3.2	38.5
S2							0.9	1.0	0.6	3.1	39.6
S3								0	0.4	4.1	39.6
S4									0.4	4.1	39.6
S5										3.7	39.3
S6											35.6



Key:



Largest circle = 41-50 samples

Second largest circle = 21-30 samples

Second smallest circle = 11-20 samples

Smallest circle= 1-10 samples

Figure 4.1: Minimum spanning network of mtDNA haplotypes based on 760 BP of 16S from small-scaled skinks. The key provides observed abundance of each haplotype. The colours used within this network were used to present haplotypes frequencies within each subpopulation (Fig. 4.2). Marks between haplotypes represent single nucleotide substitutions.

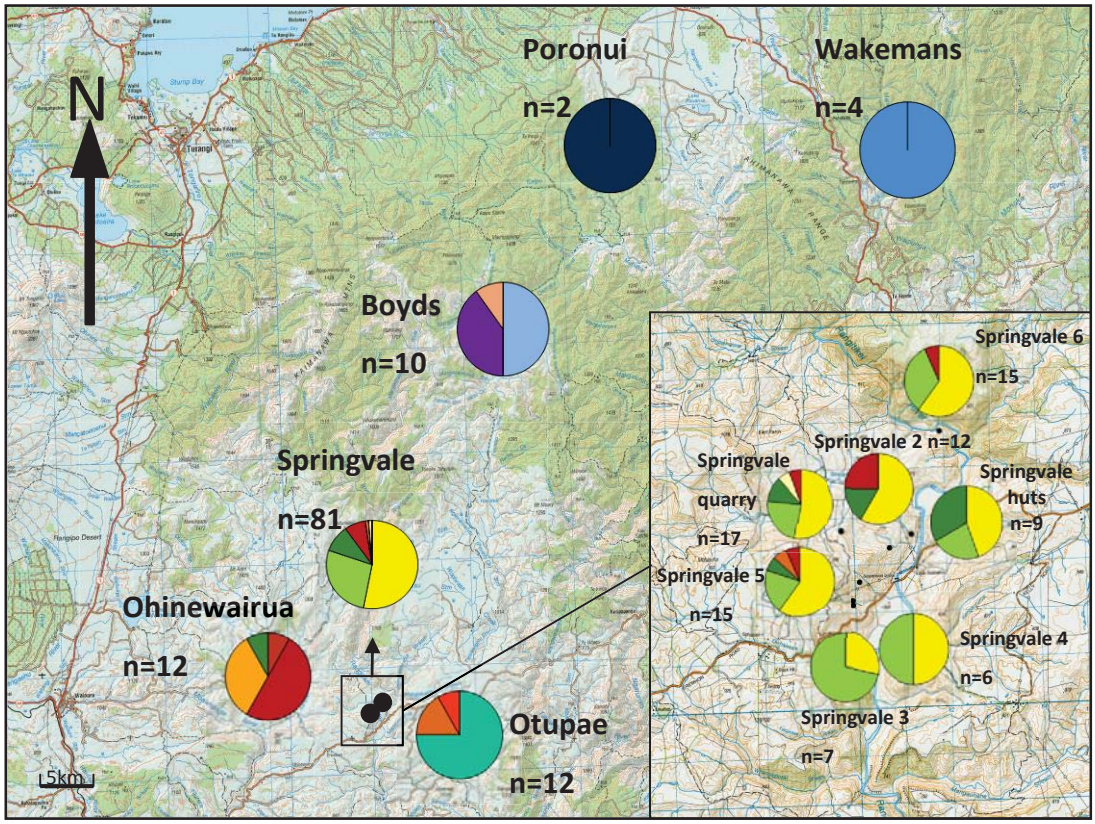


Figure 4.2: Distribution and frequency of 16S haplotypes amongst small-scaled skink subpopulations (see Fig. 4.1 for relationships among haplotypes). N= number of individuals sequenced at each site. Topographical map from LINZ.

The most common haplotype (A) is restricted to the Springvale subpopulations (Fig. 4.2), comprising of more than 60% of the total skink dataset. Haplotypes B and L are also restricted to the Springvale region. The related haplotypes coloured in orange and red (F, I, C, N, M) are found only within the Inland Patea district (with the exception of haplotype O), while the blue coloured haplotypes (G, J, H) are only within northern subpopulations. The three private haplotypes of Boyds subpopulation sample (O, H, K) are not closely related, suggesting that they may have originated in different places before being united at their current location. The captive subpopulation was not displayed in Figure 4.2 due to its origins being unknown, but the single haplotype found within the captive subpopulation was haplotype A, found only within the seven Springvale subpopulations.

Table 4.5: 16S nucleotide diversity of subpopulation of *O. microlepis*.

Subpopulation	π	±
Otupae	0.00300	0.00199
Poronui	0	0
Wakemans	0	0
Ohinewairua	0.00296	0.00197
Springvale quarry	0.00351	0.00220
Springvale huts	0.00359	0.00238
Springvale 2	0.00392	0.00248
Springvale 3	0.00251	0.00185
Springvale 4	0.00316	0.00230
Springvale 5	0.00347	0.00219
Springvale 6	0.00309	0.00200
Boyds	0.00417	0.00266
Captive	0	0

Nucleotide diversity was highest at the Boyds subpopulation (π= 0.00417 ±0.00266) and lowest at the captive, Poronui and Wakemans subpopulations (π=0) (Fig. 4.5). The remaining subpopulations varied between π= 0.00251 and 0.00392, with a large margin of overlap within the standard errors.

Table 4.6: Pairwise ϕ_{ST} computed using mitochondrial haplotype frequencies. Bold type indicates significantly different pairs based on an indicated adjusted nominal level of 0.000641. See Table 4.3 for subpopulation abbreviations.

Site	OT	PO	WK	OH	SQ	SH	S2	S3	S4	S5	S6	BO
PO	**0.628**											
WK	**0.682**	1										
OH	**0.439**	**0.447**	**0.528**									
SQ	**0.431**	**0.429**	**0.527**	**0.279**								
SH	**0.427**	**0.436**	**0.506**	**0.289**	-0.043							
S2	**0.470**	**0.491**	**0.565**	**0.243**	0.022	0.005						
S3	**0.546**	0.620	**0.698**	**0.405**	0.152	0.127	**0.329**					
S4	**0.499**	**0.538**	**0.642**	**0.351**	0.003	-0.046	0.136	-0.074				
S5	**0.454**	**0.480**	**0.547**	**0.320**	-0.009	-0.053	-0.004	0.179	-0.026			
S6	**0.495**	**0.529**	**0.589**	**0.360**	0.033	-0.039	0.067	0.113	-0.087	-0.046		
BO	**0.464**	**0.480**	**0.564**	**0.336**	**0.318**	**0.333**	**0.368**	**0.430**	**0.374**	0.137	**0.401**	
DK	**0.742**	**1**	**1**	**0.607**	**0.329**	0.181	0.196	**0.686**	0.461	0.137	0.214	**0.649**

The variation in haplotype frequency among subpopulations results in significant genetic structure within *O. microlepis.* Estimates of pairwise ϕ_{ST} were significantly greater than 0 for the majority of sample comparisons (49/77; Table 4.6). In contrast within Springvale the only significant subpopulation sample differences were between Springvale 2 and Springvale 3 (ϕ_{ST} = 0.33; Table 4.6) due to the similarity of haplotype frequencies within the Springvale subpopulations. The captive subpopulation (DK) was not significantly different from five of the Springvale samples (Springvale Huts, Springvale 2, 4, 5, 6) suggesting one or more of these subpopulations is the likely source of the captive subpopulation.

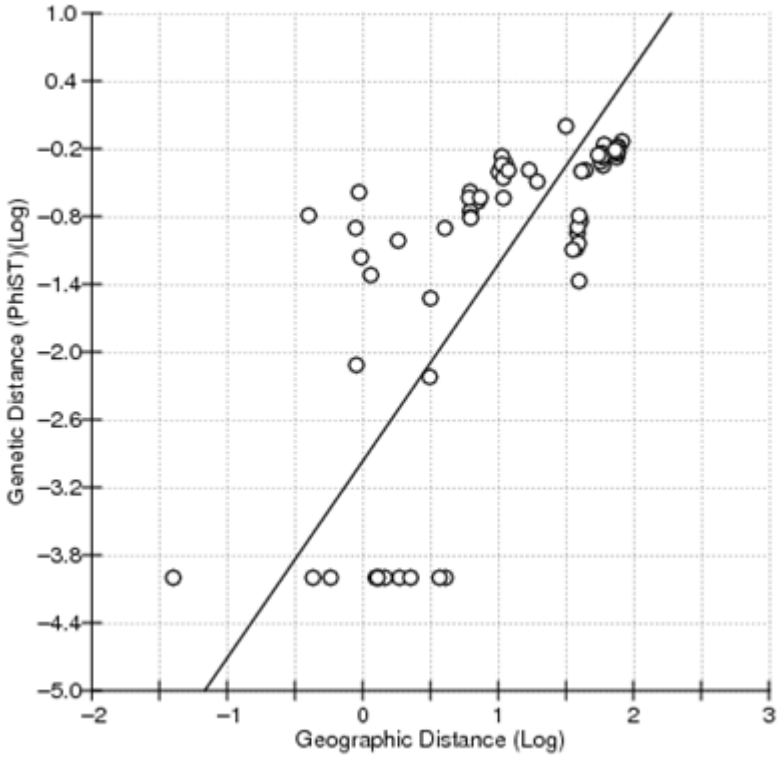


Figure 4.3: Positive correlation between geographical distance (log Km) and genetic distance (log ϕ_{ST}) in the small scaled skink (mantel test P = 0.0001).

MtDNA differentiation followed a model of isolation-by-distance (linear R^2 value= 0.488, Z = -33.4727 and r = 0.6983 mantel test: *P* = 0.0001; Fig. 4.3).

Microsatellites

Four microsatellite loci showed length polymorphism within our sample of small scaled skink. There was no evidence of stuttering or large allele drop out, nor were any of the differences between expected and observed homozygosity significant for any of these four loci, indicating that none of the subpopulation samples deviated from Hardy-Weinberg expectations. Evidence of null alleles was detected only at Ohinewairua (n= 12) within Oligr6, but as null alleles were detect at no other site the locus was retained in the analysis. Poronui or Wakemans samples were not analysed due to small sample sizes (n = 2 and 4). Positive correlations were found between microsatellite allele diversity and sample size, and were strongest for Oligr6 (R^2 = 0.77) and Oligr14 (R^2= 0.69).

Total number of alleles per locus ranged from three (Oligr20) to 23 (Oligr6). Seven of the 13 population samples had private alleles at one or more loci. The distribution of alleles at the four nuclear loci revealed geographic structure (Figs. 4.5-4.8) and resulted in significant pairwise differences (F_{ST} > 0 for 29/78 population comparisons; Table 4.7). However, analysis of the microsatellite data using Structure did not reveal strong genetic divisions of subpopulations. Although K=2 was optimal with a delta K value of 4.38 (Fig. 4.4), this analysis did not indicate the species is divided into clusters of differentiated subpopulations. It is possible that K=1 would be more accurate due to there being no clear distinction between different subpopulation samples at K=2 (Fig. 4.4).

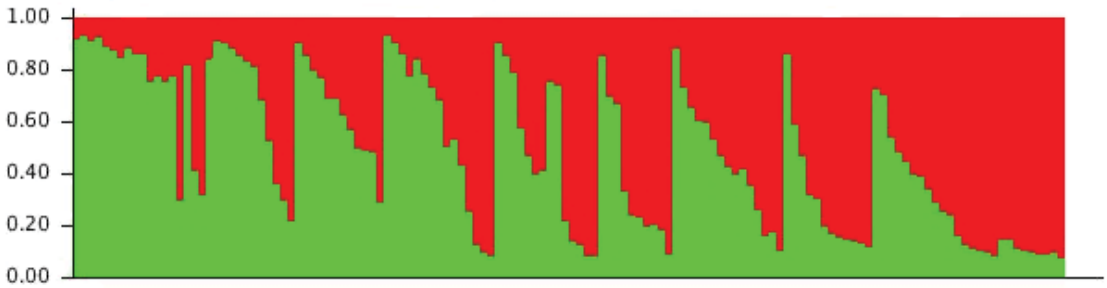


Figure 4.4: Diagram of the optimal (K=2) population structure of small-scaled skinks using four polymorphic nuclear loci, which indicated there were two populations of small-scaled skink within the samples. Each bar represents the structure within an individual skink, with skinks from the same subpopulation grouped together and ordered by their proportion of green from left to right.

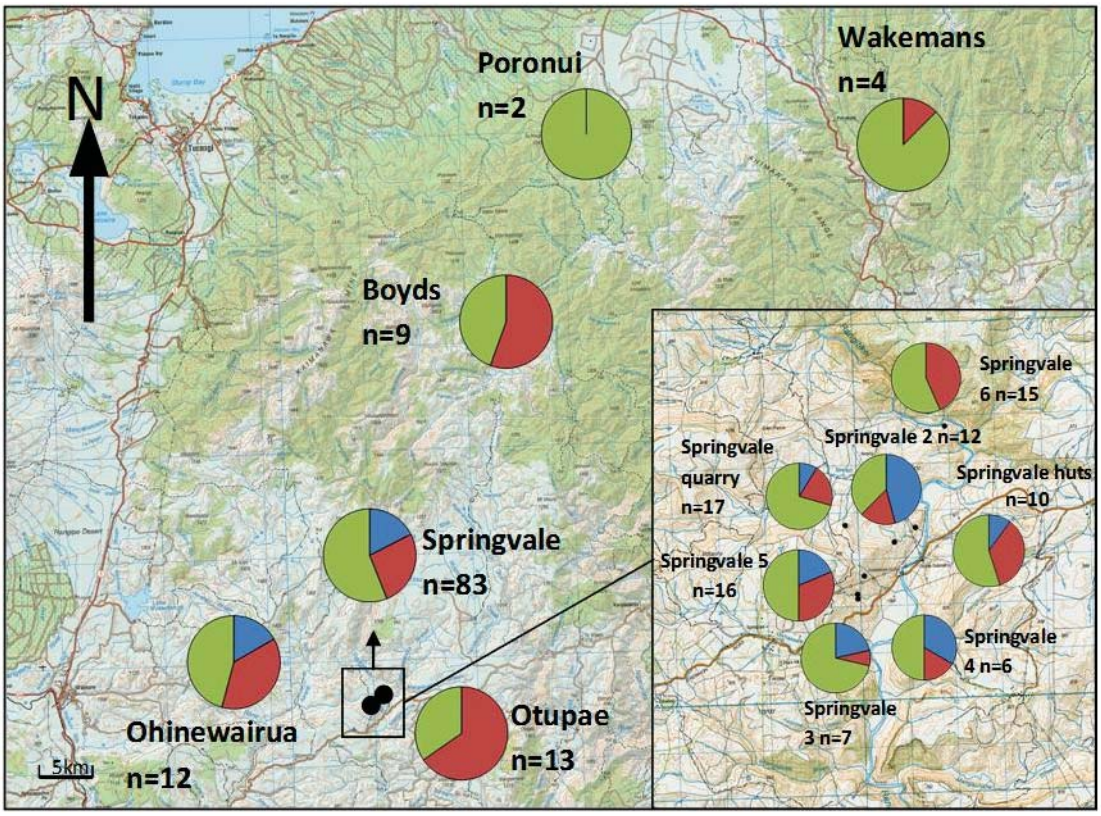


Figure 4.5: Distribution and frequency of alleles at the microsatellite locus Oligr20 among small-scaled skink subpopulations. n= number individuals genotyped. Seven Springvale samples presented grouped and separate (insert), topographical map from LINZ.

Two of the three alleles at Oligr20 were common to all sites except Poronui (n = 2), with one allele restricted to Springvale and Ohinewairua subpopulations (Fig. 4.5).

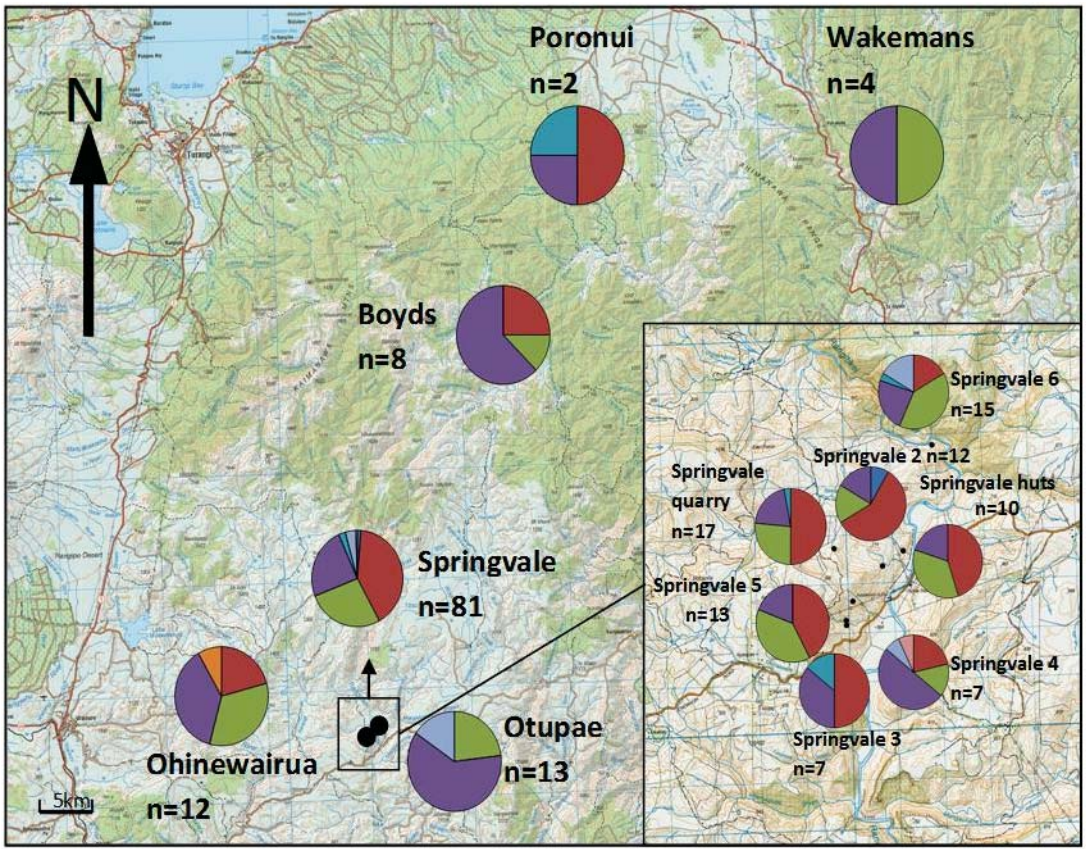


Figure 4.6: Distribution and frequency of alleles at the microsatellite locus Oligr19 among small-scaled skink subpopulations. n= number individuals genotyped. Seven Springvale samples presented grouped and separate (insert), topographical map from LINZ.

Three Oligr19 alleles were widespread and 4 were restricted (Fig. 4.6). Private alleles were present at both Ohinewairua and Springvale 4, in addition to an allele shared between Otupae, Springvale 4 and Springvale 6, and an allele shared between Poronui, Springvale quarry, Springvale 4 and Springvale 6 (Fig. 4.6).

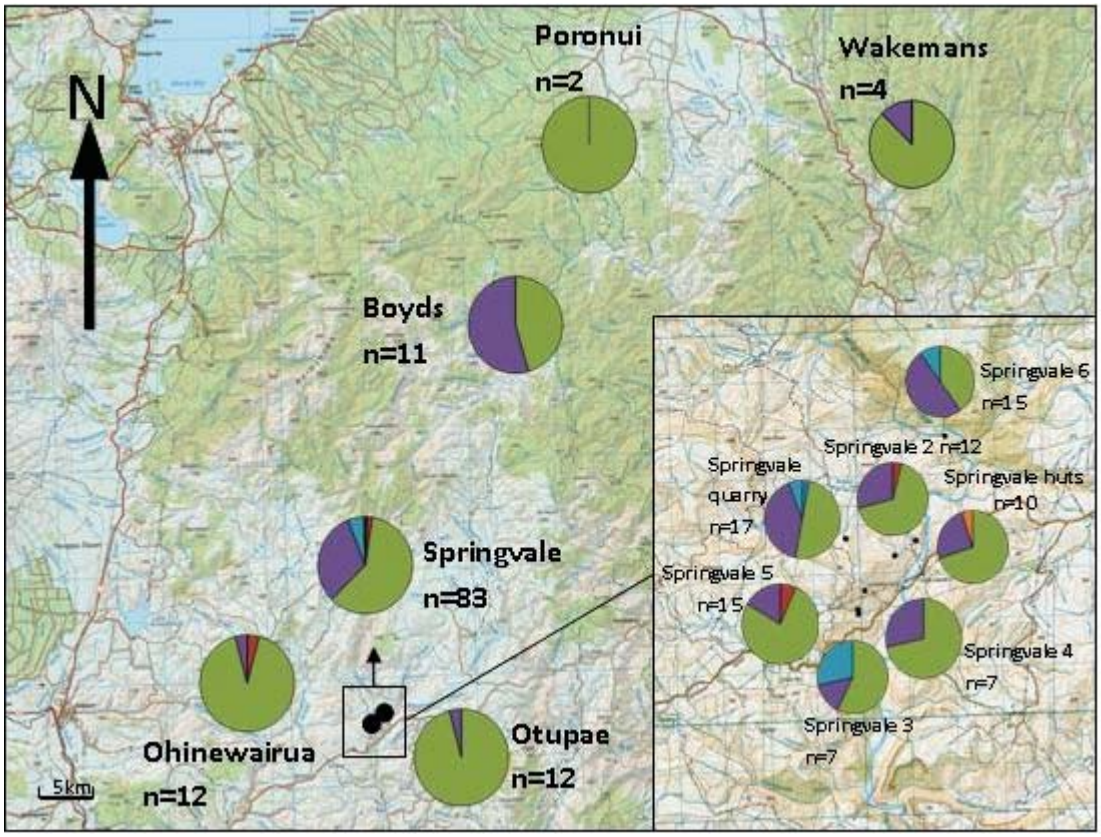


Figure 4.7: Distribution and frequency of alleles at the microsatellite locus Oligr14 among small-scaled skink subpopulations. n= number individuals genotyped. Seven Springvale samples presented grouped and separate (insert), topographical map from LINZ.

One abundant Oligr14 allele was throughout the small-scaled skink distribution, along with one other widespread allele (Fig. 4.7). One allele is shared between Ohinewairua, Springvale 5 and Springvale 2. Another allele is shared between Springvale quarry, Springvale 3 and Springvale 6, in addition to private alleles at Springvale huts and Springvale quarry (Fig. 4.7).

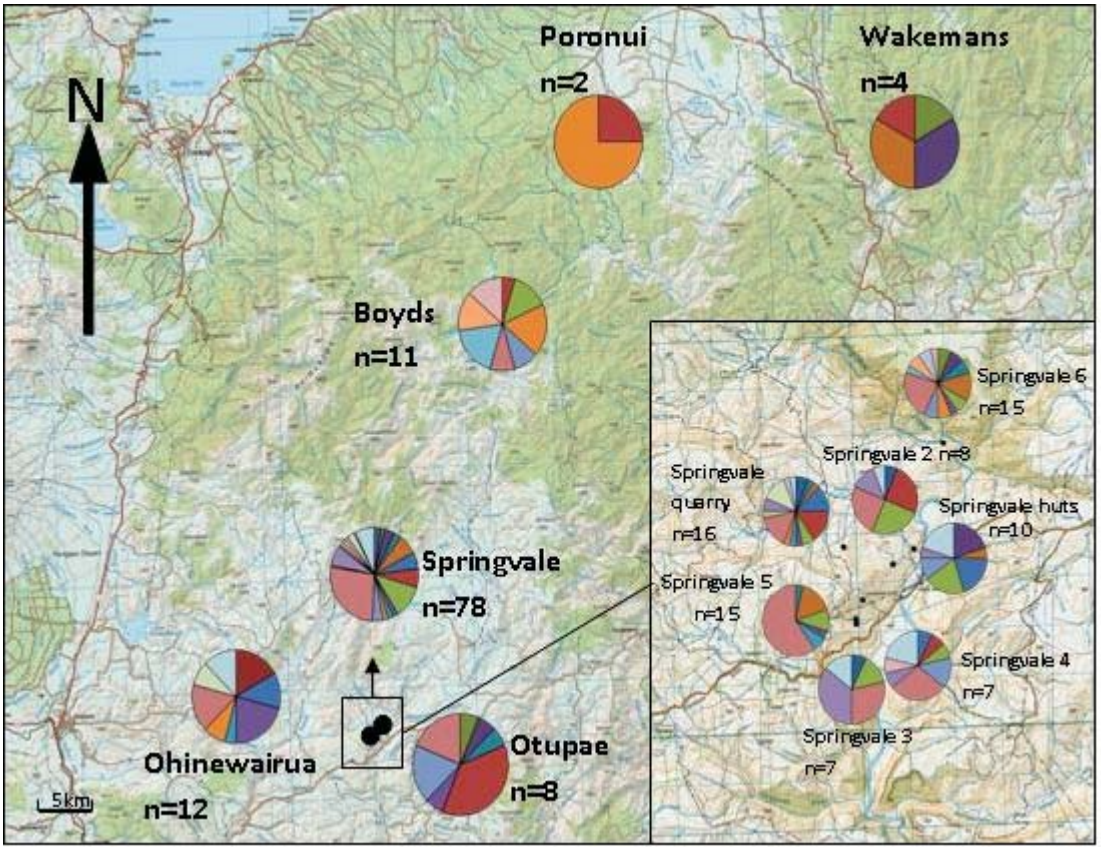


Figure 4.8: Distribution and frequency of alleles at the microsatellite locus Oligr6 among small-scaled skink subpopulations. n= number individuals genotyped. Seven Springvale samples presented grouped and separate (insert), topographical map from LINZ.

There was an abundance of alleles for Oligr6, especially within Springvale quarry and Springvale 6. Boyds had seven of its eight alleles in common with Springvale 6 (Fig. 4.8).

Table 4.7: Estimates of population pairwise F_{ST} values based on four microsatellite loci from 13 small-scaled skink subpopulations. Bold type indicates significantly >0 based on the indicative adjusted nominal level (5%) for multiple comparisons (0.00064). See Table 4.3 for subpopulation abbreviations.

Site	OT	PO	WK	OH	SQ	SH	S2	S3	S4	S5	S6	BO
PO	0.287											
WK	0.128	0.119										
OH	**0.073**	0.122	0.059									
SQ	**0.193**	0.107	0.090	0.085								
SH	**0.150**	0.109	0.056	**0.033**	0.010							
S2	**0.198**	0.142	0.162	**0.088**	0.040	0.037						
S3	**0.232**	0.078	0.133	**0.100**	0.036	0.058	0.036					
S4	0.092	0.146	0.074	0.016	0.039	0.018	0.024	0.020				
S5	**0.145**	0.161	0.091	0.041	0.063	**0.050**	0.064	**0.077**	0.026			
S6	**0.152**	0.183	0.079	**0.093**	0.034	**0.044**	**0.107**	**0.104**	0.049	0.060		
BO	0.126	0.180	0.140	0.108	**0.77**	**0.074**	0.123	0.121	0.040	**0.121**	0.032	
DK	**0.413**	0.327	0.288	**0.226**	**0.156**	**0.149**	**0.206**	**0.214**	**0.262**	**0.219**	**0.228**	**0.322**

The samples from Poronui and Wakemans are not significantly different from other subpopulations as these small sample sizes (n= 2, 4) have no private alleles. In contrast, the larger sample from Otupae has no private alleles but frequency differences make it differ significantly from eight other population samples. The captive subpopulation differs significantly from all except Poronui and Wakemans, thus failing to confirm the inference from mtDNA data about the origins of the captive subpopulation. Interestingly, Boyds, in the middle of the sampled skink distribution differs only from two Springvale subpopulations.

Table 4.8: F_{IS} calculated per loci and per site. All results did not significantly vary from zero based on an indicated adjusted nominal level of 0.00096.

	OT	PO	WK	OH	SQ	SH	S2	S3	S4	S5	S6	BO	DK
Oligr 20	-0.151	NA	0	-0.302	-0.012	0.339	-0.302	0.415	-0.071	0.237	0.084	-0.297	NA
Oligr 19	-0.178	0.5	0.143	-0.031	-0.079	0.107	0.341	0.579	-0.2	0.222	-0.063	0.576	-0.778
Oligr 14	0	NA	0	-0.023	-0.404	0.153	-0.023	0.077	-0.333	0.5	0	0.661	0.652
Oligr 6	0.255	0	0.667	0.45	0.12	-0.038	0.271	-0.273	-0.135	0.249	0.059	-0.01	-0.455
All	0.027	0.333	0.36	0.078	-0.069	0.121	0.093	0.167	-0.168	0.281	0.018	0.209	-0.304

There were no significant F_{IS} values found for any subpopulation within the loci tested (Table 4.8), so there is no evidence for deviation from random mating.

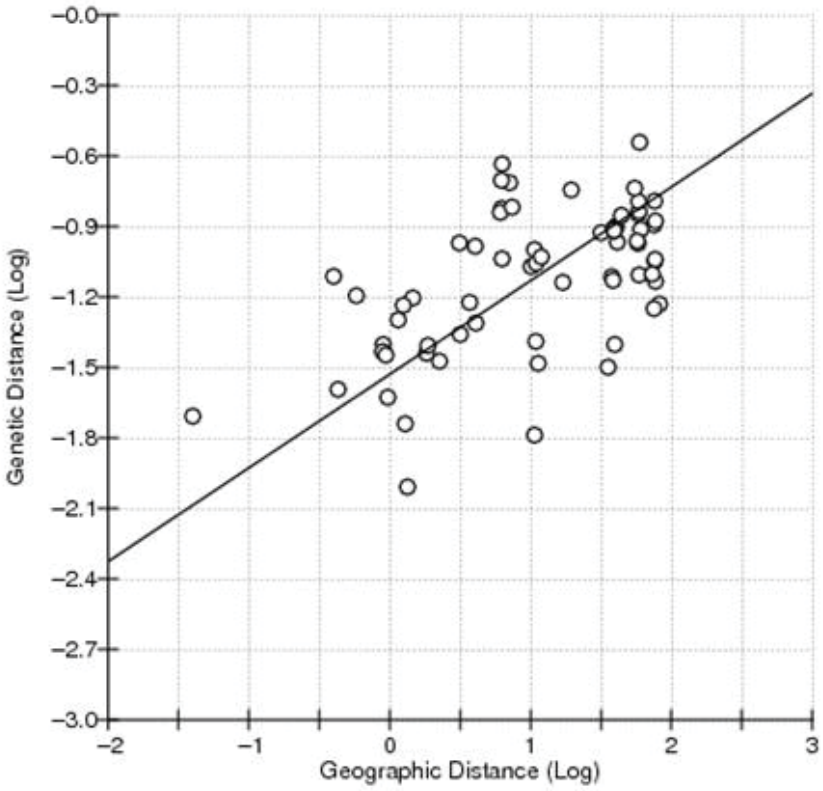


Figure 4.9: Mantel test for matrix correlation between log (genetic distance (F_{ST})) and log (distance) using combined microsatellite loci.

Microsatellite differentiation followed a model of isolation-by-distance (linear R^2 value = 0.320, Z = -67.0816 and r = 0.5660; mantel test P= 0.0041) (Fig. 4.9).

4.4 Discussion

Isolation by distance

Significant correlation between geographic distance and genetic distance was found in both the mitochondrial and nuclear data, consistent with the isolation by distance hypothesis (Figs. 4.3 & 4.9). For example, several examples of rare alleles shared between two or more nearby sites were detected (see Figs. 4.2, 4.5, 4.6, 4.7 & 4.8), which suggests that when a new allele occurs in a subpopulation it is more likely to migrate to a nearby subpopulation than a more distant subpopulation (Slatkin, 1985). It is not particularly surprising that a small-bodied ectotherm has dispersal limited by distance between suitable habitats of up to 82km, although it is remarkable that the apparently barren pasture matrix does not act as a significant barrier. Berry (2004)

found that the New Zealand grand skink (*Oligosoma grande*) frequently dispersed within a habitat patch (up to 350m), but very seldom dispersed more than 800m. If these distances were applied to small-scaled skinks frequent dispersal would only occur between Springvale 3 and Springvale 4 and rare dispersal events would occur between Springvale 2, Springvale 3, Springvale 4 and Springvale 5. Judging by the sharing of alleles and haplotypes between all Springvale subpopulations and to a lesser extent the Inland Patea district, gene flow appears to be higher in small-scaled skinks than in grand skinks.

Between Springvale subpopulations there were no potential habitats that were not already occupied by small-scaled skinks, so the distances between subpopulations in Springvale reflected the true minimum distance a skink would have to travel before reaching another suitable habitat. However, within the wider small-scaled skink distribution, a countless number of potential small-scaled skink habitats exist, many of which have not been surveyed. There are almost certainly subpopulations closer to Boyds, Wakemans and Poronui that have not been detected, which could facilitate dispersal between known subpopulations by acting as stepping stones (Kimura & Weiss, 1964). Thus the geographic and genetic distances used here could only be used to measure the isolation between known subpopulations and the subpopulations that appear isolated by this analysis may be less isolated in reality.

Gene flow across pasture

Inland Patea subpopulations are generally not genetically distinct from one another, suggesting that gene flow connects these subpopulations preventing differentiation. At neutral loci, one individual per generation is enough to prevent differentiation (Slatkin 1987). Therefore the pasture matrix separating these subpopulations does not appear to present a barrier to dispersal across the distances examined here. Even a distance of up to 10km (between Ohinewairua and the closest Springvale subpopulation) appears to allow some gene flow.

The ramifications of this result are valuable for the management of this species. The stronghold of this species is within Inland Patea district, an area which is almost entirely pastureland. The amount of gene flow observed within Inland Patea district,

coupled with high within subpopulation diversity, shows that current farm management practises do not appear to be detrimental to the skinks. Indeed, current management may benefit small-scaled skinks as they have been shown to prefer habitats close to farm or stock tracks (Teal, 2006). Grazing inhibits growth of vegetation, allowing greater solar radiation on rock surfaces which is of great benefit to a saxicolous heliotherm such as the small-scaled skink. Furthermore, erosion from farming (including trampling by stock, quarrying for gravel and general erosion) that results in exposure of greywacke bedrock produces potential habitat for small-scaled skinks. This species may be an exception to the rule within New Zealand fauna; the small-scaled skink is a rare example of an endemic species apparently not disadvantaged by farming and may even benefit from it.

Ideally grazing should be continued at current stocking rates, with potential benefit for the species being obtained by pest control (primarily rodents and cats) or creation of artificial stepping stone habitats between existing subpopulations.

Potential inbreeding

Inbreeding is not currently occurring at a detectable level in small-scaled skinks. This was shown by the F_{IS} values indicating none of the subpopulations of small-scaled skink sampled deviated significantly from Hardy-Weinberg equilibrium ratios. However, inbreeding has certainly been taking place within the captive population as this was founded by a single female and one or two males and has since grown to 10-20 individuals with very low genetic diversity. Low nucleotide diversity was detected in Wakemans, Poronui and captive subpopulations; in the former two this was likely due to small sample size, but for the captive subpopulation it was strongly indicative of small effective subpopulation size due to a small total subpopulation size several generations ago (i.e. founder effect) (Charlesworth, 2009). The nucleotide diversity of all other subpopulations had overlapping standard errors, thus were not markedly different from one another.

The lack of detectable inbreeding in wild subpopulations is good news for the subpopulations that have been identified as isolated under the isolation by distance hypothesis; despite the lack of gene flow between these subpopulations and other

known subpopulations they do not appear to be inbreeding as may be expected in a small, isolated subpopulation. Two possibilities arise from this; either subpopulations are not as small as anticipated, or subpopulations are not as isolated as expected. Typically one migrant per generation is considered sufficient to maintain heterozygosity while allowing genetic divergence among subpopulations, although this has been shown to be an oversimplification of some natural populations (Mills & Allendorf, 1996). One or both of these possibilities could apply to several sites; the Boyds site has a much larger habitat size than typical of small-scaled skink habitats and there are possibly undetected subpopulations nearby with which gene flow could occur. Wakemans has a very small habitat size, but there are numerous potential small-scaled skink habitats in the vicinity. Unfortunately, the Poronui site is small and shrinking due to overgrowth of vegetation and surveys within the area have failed to detect neighbouring subpopulations (Chapter 2; Nelson- Tunley, 2009).

Post-eruption colonisation

A species that has small subpopulations and a meta-population structure (where extinction and re-colonisation is common) is expected to have low levels of genetic diversity. In a species recovering from a bottleneck the expected haplotype network would be one with a single common haplotype and several rare haplotypes branching from it with few mutations (Excoffier, et al., 2009). The mtDNA haplotype network for the small scaled skink is not dominated by a common ancestral sequence. Unique haplotypes were found at Boyds, Poronui and Wakemans subpopulations, which suggest that these subpopulations were probably not the result of dispersal from Inland Patea district during the last 2,000 years, but must have resulted from recolonisation from other sources. The presence of several haplotypes in the Boyds subpopulation suggests the Taupo eruption was followed by recolonisation that did not result in a severe bottleneck and/or gene flow with undetected or now-extinct subpopulations resulted in mixing of genetic diversity from more than one location.

The Poronui and Boyds habitats are formed from erosion of the ignimbrite layer, deposition of which would have caused the extinction of any in-situ skinks (Chapter 1). The skink subpopulations currently within these sites probably colonised these areas post-eruption, having dispersed from outside of the eruption zone or from refugia

within the eruption zone. Given the genetic differences between haplotypes O, H and K it seems quite likely that the Boyds subpopulation was founded by individuals from several different subpopulations which are either now extinct or have not been sampled (discovered). The Boyds subpopulation of small-scaled skinks is adjacent to both the Kaimanawa and Kaweka ranges, neither of which has been extensively surveyed and both with the potential to hold many more skink subpopulations (Chapter 2).

Historical abundance

The high mitochondrial diversity observed within and between subpopulations of small-scaled skink rules out the possibility of recent population (or subpopulation) bottlenecks and indicates this species has been subject to isolation by distance for a considerable length of time. The observed pattern of relatedness appears to have developed over a long time period without significant population declines. I previously hypothesised that small-scaled skinks would have benefited by the lowered tree line during the Pleistocene as it would have caused a higher abundance of grassland and greater exposure and erosion of bedrock, creating small-scaled skink habitat (Chapter 1). The initial evidence presented here tentatively supports this hypothesis as the high diversity suggests that this species was historically abundant, but no dating methods were undertaken to estimate the age of mitochondrial divergences. However, comparison with studies of other New Zealand skink species within the genus *Oligosoma* does suggest the mtDNA diversity is fairly low. For example, the fragmented *O. otagense* has haplotypes that differ by as much as 4.9% (ND2; Chapple, Birkett, Miller, Daugherty, & Gleeson (2012)), and more widespread species have 2 -8 % divergence between haplotypes (Greaves, et al., 2007, 2008). Here, 0.79% in 16S (which is likely to be more slowly evolving that ND2 (Hills, Trewick, & Morgan-Richards, 2011)) is observed over just 82.4 km.

The pattern of genetic structure also indicates that anthropogenic modification within the small-scaled skink distribution has probably not resulted in a significant bottleneck; otherwise much lower haplotype diversity would have been revealed. This is a very important finding for conservation of this species, as most subpopulations are found on private land and current management practises do not appear to cause diversity

loss and/or inhibit gene flow. It could be postulated that the Inland Patea district habitat and matrix (greywacke rock piles and outcrops amongst exotic pasture) approximate the habitat and matrix available during the Pleistocene (similar habitat, but amongst native grassland or scrubland). It is possible that a native grassland/scrubland matrix would facilitate dispersal more readily than exotic pasture, but this is inconsequential given the knowledge that pasture does not appear to significantly inhibit gene flow.

Origin and conservation value of the captive population

The only 16S haplotype found within the captive subpopulation was haplotype A, confirming that this subpopulation was founded by a female collected from one of the Springvale subpopulations. Although pairwise ϕ_{ST} excludes Springvale quarry and Springvale 3, this is based on haplotype frequencies and the single female could have come from any of the subpopulations with haplotype A. However, the year of collection rules out Springvale 6 since that subpopulation was discovered during the course of this research (Chapter 2; Appendix 1). Thus Springvale huts, 2, 4 and 5 are likely sources. F_{ST} derived from nuclear loci was not informative as it showed the captive subpopulation as significantly different from all other subpopulations. The captive subpopulation usually had the most common microsatellite alleles, but in Oligr14 there was an allele exclusive to Springvale and in Oligr6 there was an allele found within Otupae, Ohinewairua, Springvale 5, Springvale 6, and Springvale quarry. Although these alleles may occur in low frequency in other subpopulations it does mean that the most likely origin of the captive skinks is Springvale 5. The sample of captive skinks never had more than three alleles per locus and thus there is no evidence that the founding size was any greater than two individuals.

The low genetic diversity found within the captive subpopulation could be improved by addition of wild individuals from the subpopulations mentioned above; using individuals from the same source as the original captive founders would result in a captive subpopulation that adequately represents the genetic diversity of those wild subpopulations. It is unlikely that the captive subpopulation will ever be sustained at sizes that would allow representation of the entire wild population, but representation of a subset of the population is achievable and should be a goal. However, before this

can be conducted it is imperative to create a captive management plan for this species that examines the fundamental purpose of the captive subpopulation of small-scaled skinks. Fortunately this species is currently being considered for a breeding for release program (D. van Winkle, personal communication, 6/7/2012), so a captive management plan may be formalised within the near future.

Genetic diversity, scale asymmetry and parasitism

Scale asymmetry and parasitism rates were higher in some subpopulations than others; it was thought that this may relate to the homozygosity of the individual, or the genetic diversity of the subpopulation (Chapter 3). However, subpopulations affected by parasites were no less genetically diverse than non-parasitized subpopulations. Likewise, subpopulations containing skinks with asymmetrical scales (Fig. 3.2) had similar genetic diversity to all other subpopulations, and the asymmetrical individuals were no more homozygous on the loci tested than other skinks. Therefore, there was no link between parasitism rates or scale asymmetry to genetic diversity. This means that scale asymmetry may not be a good indicator of genetic stress in this species, but could still be an indicator of environmental stress.

Summary

Isolation by distance has a strong affect on small-scaled skinks. Anthropogenic modification of Inland Patea district (i.e. conversion to pasture) had not noticeably impaired small-scaled skink dispersal, and may have benefited the species by increasing the number of available habitats via erosion and exposure of bedrock. No evidence of inbreeding in wild subpopulations was found, and most of the wild subpopulations had high genetic diversity. The high genetic diversity observed further indicates that this species has not undergone recent population bottlenecks and was historically abundant. The diversity observed within the Boyds subpopulation strongly suggests it was founded subsequent to the 186AD Taupo eruption and was founded by two or more subpopulations that have either become extinct or have not been discovered. The captive subpopulation was founded by 2-3 individuals collected from Springvale. The captive subpopulation has very low genetic diversity but does not yet appear to be affected by inbreeding depression. The conservation value of the captive

population could be improved by the addition of several wild individuals collected from the Springvale area.

Chapter five

Summary and recommendations

Figure 5.0: A small-scaled skink basking on the upper edge of a ten metre cliff. Skinks were also observed climbing the cliff and within the Muehlenbeckia sp. pictured.

5.1 Summary

Conservation biology can be considered as being divided between two concepts; the declining population paradigm and the small population paradigm. The declining population paradigm investigates the causes of small population size and attempts to counter them, while the small population paradigm investigates the consequences of small population size and attempts to counter them (Caughley, 1994). Although these concepts are complimentary, distinguishing between them is important. For example, for a species in rapid decline it is more important to instigate habitat restoration or pest control (causes of decline) than to investigate reduced gene flow (a consequence of decline). Likewise, in a naturally rare species techniques to increase population size may be unnecessary and/or ineffective, while improving gene flow may increase population viability. As previously described, (Chapter 1) naturally fragmented populations may have adaptations to counter the effects of fragmentation, but these adaptations may not be successful if the matrix between habitats changes (such as in grand skinks (Berry, et al., 2005)).

The present research addressed potential causes and consequences of population fragmentation in the small-scaled skink, with the aim of guiding conservation strategies for this species. The cause of fragmentation may have been either natural preference for rare habitat or human habitat modification. If the small-scaled skink population was fragmented due to human habitat modification, the declining population paradigm would be applied with the aim of reversing the fragmentation. In contrast, a naturally fragmented species should be considered under the small population paradigm, whereby the goal becomes to deal with the consequences of fragmentation rather than attempting to alter a natural distribution. The consequences of population fragmentation are largely the result of genetic isolation of subpopulations, which would result in lower genetic diversity within subpopulations due to genetic drift via inbreeding, as well as increased differences between subpopulations due to lack of migration (Willi, Van Buskirk, et al., 2007). Low genetic diversity within subpopulations can result in an accumulation of deleterious alleles and increased susceptibility to disease, parasitism and environmental change, thus increasing the overall risk of subpopulation extinction (Willi, et al., 2006). A further

issue is present if the matrix between usable habitats is particularly uninhabitable for the species, in which case it may act as a dispersal barrier (Ricketts, 2001). The dispersal barrier may prevent migration between subpopulations as well as colonisation of suitable vacant habitat patches, thus preventing species expansion.

The small-scaled skink is currently listed as in serious decline due to population fragmentation and small population size (Australasian Reptile & Amphibian Specialist Group 1996; Towns, et al., 2002). It is a saxicolous heliotherm with a strong preference for exposed, rocky habitats that typically occur in small patches (Teal, 2006). The cluster of subpopulations considered to be the species stronghold (Inland Patea district) consists of small patches of habitat separated by up to 11 km of highly modified pastureland. In the northern part of the range there are 3 known subpopulations separated from one another by more than 19 km of pasture, scrub and forest. The pasture matrix might have acted as a dispersal barrier leading to little or no migration between subpopulations, with the sometimes great distance between subpopulations also inhibiting dispersal. If migration between subpopulations has ceased, the long-term viability of small-scaled skink subpopulations in question and species decline due to inbreeding may be imminent.

Evidence for decline

The first part of this research dealt with examining evidence for population decline in the small-scaled skink; despite the species threat status decline has not been documented. If evidence of decline was found, it would be imperative to determine and counter the causes of decline. Conversely, if there is little evidence for decline then conservation of this species should focus on the effects of small population size rather than the causes. This was examined by conducting an almost comprehensive survey of the known small-scaled skink subpopulations, some of which had not been visited since their discovery, to determine presence/absence of small-scaled skinks. In addition, nearby potential small-scaled skink habitat patches were surveyed, including some sites that had previously been surveyed without small-scaled skink detection. Small-scaled skinks appeared to have declined at three locations and at two small-scaled skinks were not detected at after several hours of searching. At two of these sites the habitat had been noticeably altered by increased vegetation cover, which

would have reduced basking area. Five previously undescribed subpopulations were discovered, two of which were in sites that had been previously surveyed. These results indicated that subpopulation decline has occurred in some instances due to habitat change, perhaps from decreased grazing of the skink habitat by livestock. In most cases subpopulation decline could be reversed by increased grazing of habitats or by weed control. The finding of new subpopulations indicates that the true distribution of small-scaled skinks remains unknown, with a high possibility of undetected subpopulations. Additionally, the detection of small-scaled skinks in sites previously surveyed without detection indicates that these subpopulations have either colonised since the last survey (population expansion), or have increased in density (population growth). This evidence contradicts the threat status of serious decline; although the species may be declining at some sites, it appears to be stable in the majority of subpopulations and may even be expanding into new sites. It seems very likely that the small-scaled skink's fragmented distribution is the result of habitat specificity rather than primarily human induced decline. Therefore this species should not be considered under the declining population paradigm, but instead under the small population paradigm. Thus the remainder of the thesis dealt with investigating the consequences of small population size rather than the causes.

Causes of population structuring

Differences between subpopulations were investigated using several different methods. Morphological differences (SVL, weight and tail width) were compared between subpopulations and between groups of subpopulations based on distribution. One pair of groups separated Springvale subpopulations (all within 2 km of one another) from non-Springvale subpopulations (more than 6 km from one another), while the other pair of groups separated southern subpopulations (within 11 km from one another) from northern subpopulations (more than 19 km from one another). In addition to these measures, population genetic structuring was examined using a combination of mitochondrial (16S) and microsatellite (Oligr20, Oligr19, Oligr14 and Oligr6) loci. The genetic analysis compared genetic difference (F_{ST} and ϕ_{ST}) to geographic distance between subpopulations to test for isolation by distance (correlation between geographic distance and genetic difference). If isolation by

distance was not evident in small-scaled skinks, it could indicate that pasture within the species stronghold was acting as a dispersal barrier. Evidence of gene flow could also be detected by the sharing of mitochondrial haplotypes between subpopulations.

Morphological differences were detected in the subpopulation groupings; with significantly different SVL between groups (Springvale and southern skinks were longer than non-Springvale and northern skinks). Genetic analysis confirmed that isolation by distance was occurring within the small-scaled skink population. Mitochondrial haplotype sharing was found within Inland Patea district, indicating recent or current gene flow across the pasture matrix. These results showed that there are differences between subpopulations based on their distribution, with geographically close subpopulations sharing genetic similarities. Furthermore, skink size appears to correlate to isolation, with more isolated subpopulations containing smaller skinks. The apparent gene flow between Inland Patea district subpopulations suggested that pasture does not act as an absolute dispersal barrier to small-scaled skinks, thus this species is not being detrimentally affected by the modified landscape within which most of the population exists. Indeed, it is quite likely that the small-scaled skink is benefited by some aspects of landscape modification, due to erosion and grazing acting to create and maintain exposed rocky habitat.

Inbreeding

Inbreeding within small-scaled skink subpopulations was investigated by two methods. Fluctuating asymmetry, a measure of developmental instability, was investigated by examining head scale asymmetry and hind limb length within subpopulations. Occurrence of fluctuating asymmetry could be indicative of either environmental or genetic stress (i.e. inbreeding) affecting the animal during development. In addition, genetic diversity within small-scaled skink subpopulations was examined using significant F_{IS} as an indicator of inbreeding.

No subpopulations had significant F_{IS} values, indicating that there was no significant inbreeding happening within small-scaled skink subpopulations. There were no occurrences of hind limb length asymmetry although this has been recorded in other lizard species, but there were eight skinks with asymmetrical head scalation. These

asymmetrical skinks did not occur in subpopulations with low genetic diversity, nor were these individuals more genetically homozygous than other wild skinks. Thus, there is no evidence to suggest that head scale asymmetry was an indicator of genetic stress (inbreeding); it may have been caused by environmental stress during development (e.g. poor maternal nutrition, sub-optimal incubation temperatures etc). Overall, there was no evidence for significant inbreeding within small-scaled skink subpopulations thus the threat of the species developing inbreeding depression is minimal so long as recent rates of gene flow are maintained.

Historical population size

In order to understand the effect of human habitat modification on a species it is important to compare the abundance of a species before and after human influence. This can be difficult to achieve without records of historical abundance, but modern-day genetic diversity and structure can act as a substitute. Mitochondrial (16S) diversity and structure was examined across the small-scaled skink distribution, particularly for evidence of recent colonisation and population bottlenecks. Key periods of environmental change that may have affected small-scaled skink abundance were the human colonisation of New Zealand and the 186AD Taupo eruption. Environmental changes resulting from Pleistocene glaciations have probably been overwritten by these more recent events. Each of these events may have resulted in more skink habitat, but the Taupo eruption and formation of pastureland may have temporarily reduced the small-scaled skink population through the widespread destruction that took place.

Fifteen mitochondrial haplotypes were identified within the small-scaled skink population, ten of which were found only within one subpopulation. A high degree of structuring with shallow divisions between haplotypes (maximum of 6 BP difference) was revealed. The Boyds subpopulation, which exists within the area most severely affected by the Taupo eruption, contained three private haplotypes that were not closely related to one another. The high genetic diversity combined with the complex spatial structuring observed suggest that the small-scaled skink population had not undergone a significant population bottleneck recently. Possibly the Taupo eruption and human colonisation did not severely reduce the abundance of small-scaled skinks.

The amount of diversity observed indicates that isolation by distance has been operating on this species for a considerable length of time, thus at some time there was more small-scaled skink habitat, and it did not necessarily increase connectivity between subpopulations. The Boyds habitat was formed by erosion of the ignimbrite (eruption deposition) layer (Fig. 1.5), thus must have been colonised since the eruption. The haplotype diversity observed at the Boyds subpopulation was indicative of colonisation by small-scaled skinks from several sources which are either extinct or undescribed. This once again indicates that the currently unidentified distribution probably does not include all subpopulations, so more comprehensive surveys are needed.

Conservation value of captive subpopulation

The captive small-scaled skink subpopulation was founded by two or three individuals of unknown origins and now consists of between 10 and 20 skinks. The origins and genetic diversity within the captive subpopulation is of fundamental value; without knowing these, the subpopulation cannot be translocated. Morphological (SVL, weight and tail width) differences between captive and wild small-scaled skinks were compared in addition to investigating the genetics of the captive subpopulation to determine its origins (using 16S mitochondrial haplotypes) and diversity (16S and microsatellite loci).

Only one mitochondrial haplotype was found within the captive small-scaled skinks, showing that the record of one founding female is probably correct (D. Keall, pers. comm.). This haplotype was common to all Springvale subpopulations and found in no other wild subpopulations, thus the captive founders must have been collected from one of the Springvale subpopulations. Genetic diversity was very low within captive skinks due to several generations of inbreeding; however F_{IS} did not detect it at significant levels, so inbreeding depression is unlikely to be imminent. Captive skinks had significantly higher SVL, weight and tail width than wild skinks, likely due to relaxed environmental stresses (e.g. predation, food scarcity etc). The average captive skink weight was more than twice the average wild skink weight, strongly suggesting the captive small-scaled skinks are obese. Now that the origins of the captive subpopulation are known, the captive skinks could act as contingency for those

subpopulations in case of decline in the wild. While inbreeding has not yet affected the captive small-scaled skinks, it is likely to in the future unless fresh genetic stock is obtained through the addition of wild caught skinks. I suggest collecting additional skinks from Springvale subpopulations, so that the captive subpopulation continues to represent these subpopulations.

5.2 Recommendations

- Negotiate with landowners/Iwi of Ngamatea station and Motutaiko Island to survey for both described and undescribed small-scaled skink subpopulations within their land. Conduct follow up surveys of potential small-scaled skink habitat nearby existing subpopulations, most notably along Aorangi and Mokomokonui Streams.
- Identify potential small-scaled skink habitats utilising aerial photography and Teal's (2006) habitat preference and site occupancy research, followed up with a survey to determine *O. microlepis* presence. Defence Force land (east of Ohinewairua station), Kaimanawa forest park, Kaweka forest park, Te Urewera forest park, Ahimanawa ranges and northern Ruahine ranges are all within the small-scaled skink distribution and contain potential habitat.
- Conduct regular resurveys of core *O. microlepis* sites to examine long term occupancy. Establish a long-term population estimate and viability study at several core sites.
- Evaluate the option of creating artificial habitat at sites where small-scaled skink subpopulations appear to be declining in addition to discussing increased grazing in sites with increased vegetation cover. Artificial habitat can be created by forming rock piles of a similar size, depth and rock size to skink-inhabited sites.
- Advise landowners to continue grazing *O. microlepis* habitat as it prevents vegetation from overgrowing and making the site unsuitable. If landowners are enthusiastic about facilitating small-scaled skinks, advise on pest control programs and creation of artificial habitat. The extent that predators affect

small-scaled skinks is unknown, but pest control would likely benefit a range of species.

- Consider investigating the dispersal ability of small-scaled skinks (or other New Zealand lizards) by creating artificial habitats at set distances from high density sub-populations and regularly survey the artificial habitat to determine the time taken to colonise.
- Determine a purpose for the captive small-scaled skink subpopulation, whether it be breeding for release, contingency, research or advocacy, then develop a captive management plan to execute this purpose. Whatever the case, the genetic diversity of the captive subpopulation should be increased by the addition of wild-caught skinks. I recommend that supplementary skinks should be collected from Springvale as this is the origin of the founders of the captive subpopulation, so further additions from the same region would result in a fair representation of the genetic diversity of skinks from this region. Declining subpopulations appear to be affected by reduced habitat quality, in which case the addition of captive reared skinks would not be beneficial to the subpopulation. “Healthy” subpopulations are already very densely populated and so don’t require additional skinks. In my opinion, the best translocation option for captive small-scaled skinks is to introduce them to currently unoccupied habitats (natural or artificial) within their known distribution (and within Springvale if translocating the skinks currently within captivity), which would improve connectivity between subpopulations.

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Appendices

Appendix 1

New site descriptions

Black Hill

The Black hill site consisted of a single large greywacke rock pile (c.a. 50m x100m) (Fig. 6.1) with a smaller rock pile (c.a. 50m^2) beside it. The survey was conducted from the roadside using binoculars as permission had not been sought to access Black hill property. Three small-scaled skinks were seen foraging along the upper edge of the large rock pile during a 15 minute visual search. Given the large size of this habitat patch, there is potential for this site to support a large skink population.

Figure 6.1: The larger rock pile slope at Black hill.

Springvale 6

The 1991 survey of Springvale 6 revealed speckled skink presence, but small-scaled skinks were not detected. During our survey both speckled skinks and small-scaled skinks were detected at this site, with small-scaled skinks quite abundant. A total of 19 small-scaled skinks were caught at Springvale 6 during a three day period, excluding several recaptures (skinks were marked on their first capture).

The site consists of a prominent greywacke outcrop on the margin of the true right of the Rangitikei River (Fig 6.2). The large outcrop has created a series of rock piles of varying dimensions. Smaller greywacke outcrops along the ridge have created a boulder field on the steeper slopes and rock piles at the base of the hill. Small-scaled skinks were observed basking and foraging in both the rock piles and boulder field.

Figure 6.2: Springvale 6, showing the prominent greywacke outcrop on the left of the image.

Springvale 7

Springvale 7 was approximately two kilometres north-west of Springvale 6, bordered on the northern side by the Rangitikei River, with Ngamatea Station opposite. It was an area of dense but patchy native scrub with small patches of bare clay or greywacke rock pile interspersed (Fig. 6.3). Two skinks were seen in approximately 0.5 person/hours search time, one of which was caught and identified as *O. microlepis*.

Figure 6.3: Springvale 7 viewed facing north.

Wakemans 2

Wakemans 2 was located approximately 300m east-nor-east of Wakemans 1, on the same track on the true left of Mokomokonui River. The rock piles consisted of very loose greywacke rock on a steep angle, preventing permanent vegetation from taking hold (Fig. 6.4). On either side of the rock pile was regenerating manuka (*Leptospermum scoparium*). Two small-scaled skinks were caught at Wakemans 2 over a period of four half-days. This catch rate is very low when compared to catches at sites in the Inland Patea district, perhaps due to a much smaller population size. However, we noted Wakemans 1 & 2 skinks were easily disturbed and would not return to basking after

the initial disturbance, unlike skinks observed on pastureland. We believe this is due to skinks on pastureland becoming habituated to disturbance by stock, ultimately making them easier to detect and catch.

Figure 6.4: One of the rock pile slopes at Wakemans 2, complete with funnel traps set. A photo of the entire site was not possible due to the steep and forested terrain.

Discussion with the Pohokura Station manager and a Tataraakina Trust representative revealed that unidentified skinks have been seen in other locations within several kilometres of Wakemans clearing. Numerous suitable rock piles were observed along Mokomokonui River and along the road to Wakemans hut. It is quite likely more populations will be found there given time and search effort.

Ohinewairua 2

Ohinewairua 2 was situated on an active greywacke quarry on the true left of the Aorangi stream, approximately 100 metres south of the shearing sheds. There was evidence of lizards (presumably W*oodworthia maculata*) inhabiting the outcrop being directly quarried, but the main area of lizard activity appeared to be in the rock pile near the stream (Fig. 6.5), which appears to have been formed by quarrying activities. There were patches of rocks suitable for skinks between the outcrop and major rock pile. The major rock pile may be at risk of flooding during high rainfall. Many skink scats were seen on the rock pile and two small-scaled skinks were seen during 0.5 person/hours searching. No further effort was made to catch skinks or estimate abundance due to time constraints.

Several other rock pile slopes were seen upstream and downstream of the quarry, which appeared to be suitable for small-scaled skinks. Further examination of the Aorangi stream using Google Earth© revealed potential skink habitat along much of its length.

Figure 6.5: Ohinewairua 2. The quarrying activity was occurring on the top edge of the slope pictured, which has created the rock pile at the bottom of the image, where the small-scaled skinks were observed.

Appendix 2

Table 6.1: Small-scaled skink raw morphological data. Site refers to the subpopulation the skink was caught at, while I.D. is an individual identification number. Weight is in grams. SVL, tail length, tail regrowth and tail width are in mm.

Site	I.D.	Weight	SVL	Tail length	Tail regrowth	Tail loss	Toe loss	Tail width	Parasites	Sex
Otupae	OT01	3.9	57	55	5	Y	Y	6.1	0	M
	OT02	2.9	54	40	0	Y	N	5.6	0	M
	OT03	2.2	50	49	0	Y	N	6.1	0	Un
	OT04	6.8	60	76	0	N	N	6.1	0	F
	OT05	1.2	42	50	0	N	N	4.2	0	F
	OT06	4.1	55	76	3	Y	N	6.1	0	M
	OT09	5.6	62	46	40	Y	N	6.6	0	F
	OT10	5.5	67	65	30	Y	N	7.0	0	M
	OT12	6	65	51	41	Y	N	6.9	0	F
	OT13	1.2	34	51	0	N	N	3.7	0	M
	OT14	4.8	55	57	10	Y	N	7.3	0	M
	OT15	6.4	66	90	0	N	N	5.8	0	F
Poronui	P07	4.6	56	19	8	Y	N	5.9	0	M
	P08	1.3	40	42	2	Y	N	3.9	0	Un
Wakem-ans	WK16	7.2	66	78	0	N	N	6.1	0	F
	WK17	2.1	44	47	31	Y	N	4.7	0	F
	WK18	6.5	61	62	41	Y	N	5.1	0	F
	WK19	2	45	56	24	Y	N	4.7	0	M
Ohine-wairua	OH20	2.8	49	70	39	Y	N	5.1	0	M
	OH21	4.8	60	71	10	Y	N	6.1	0	M
	OH22	4.5	58	55	40	Y	N	5.6	0	F
	OH23	4.8	56	60	40	Y	N	6.5	0	F
	OH24	1	35	50	0	N	N	3.6	0	Un
	OH25	1.4	41	38	12	Y	Y	4.1	0	Un
	OH26	4.8	60	42	30	Y	N	5.6	0	M
	OH27	5	62	67	0	N	N	6.0	0	M
	OH28	8.2	70	86	0	N	N	6.7	0	F
	OH29	3.3	55	65	15	Y	N	4.8	0	F
	OH31	5.2	61	78	0	N	N	5.6	5	M
Spring-vale quarry	SQ32	4.9	65	62	42	Y	N	6.1	0	M
	SQ54	4.2	55	80	0	N	N	6.3	0	M
	SQ55	2.3	50	69	0	N	N	4.9	0	M
	SQ56	4.9	58	73	0	N	N	5.7	0	M

	SQ57	4.3	61	70	5	Y	N	6.0	0	F
	SQ58	4.6	66	70	20	Y	N	5.8	0	F
	SQ59	6.8	65	61	48	Y	N	6.3	0	F
	SQ60	5.7	63	58	40	Y	Y	6.3	0	F
	SQ61	2.4	46	60	26	Y	N	4.2	0	F
	SQ62	2.7	50	65	0	N	N	4.2	0	F
	SQ63	4.6	64	46	42	Y	N	5.9	0	F
	SQ64	5.4	64	63	0	N	N	5.8	0	M
	SQ65	4.6	56	74	0	N	N	5.6	0	M
	SQ66	5.5	65	72	20	Y	N	6.3	0	M
	SQ67	4.7	60	73	0	N	N	5.9	0	M
	SQ68	3.3	52	65	0	N	N	4.5	0	F
	SQ69	4.1	59	59	34	Y	Y	5.1	0	F
Spring-vale huts	SH33	5.6	62	83	0.8	Y	N	6.1	0	F
	SH34	3.1	56	65	0	N	N	4.3	0	F
	SH35	4.4	62	55	47	Y	Y	5.2	0	M
	SH36	6	69	65	50	Y	Y	7.5	0	M
	SH37	2.8	52	74	0	N	N	4.7	0	M
	SH47	3.6	52	76	0	N	N	5.7	0	M
	SH48	6.1	65	79	26	Y	N	6.3	3	M
	SH49	5.5	61	87	6	Y	N	6.2	0	F
	SH50	5.4	62	82	0	N	Y	6.2	0	F
	SH101	2.6	52	55	46	Y	N	4.7	0	M
Spring-vale 2	S238	4.2	55	82	20	Y	N	5.6	0	M
	S239	3.9	58	42	0	Y	N	5.3	0	M
	S240	5.8	62	54	42	Y	N	5.1	0	F
	S241	4	63	71	9	Y	N	5.9	0	M
	S242	2.1	46	65	0	N	N	4.4	0	Un
	S243	6.4	65	57	43	Y	Y	5.6	0	F
	S244	2.7	52	75	0	N	N	5.1	0	F
	S245	4.2	57	52	22	Y	N	6.2	0	F
	S246	4.4	58	61	31	Y	N	6.1	0	M
	S251	5.9	65	78	12	Y	N	6.8	0	F
	S252	2.1	49	60	0	N	N	4.2	0	M
	S253	4.2	61	77	0	N	N	5.8	0	F
Spring-vale 3	S372	4.6	58	82	0	N	Y	6.1	0	M
	S373	4.8	61	79	0	N	Y	6.1	0	M
	S374	3.8	48	68	11	Y	N	4.8	0	F
	S375	5.5	65	62	48	Y	N	6.5	0	M
	S376	3	50	74	0	N	N	4.9	0	F
	S377	3.4	54	78	0	N	N	5.4	0	M
	S378	5.7	61	70	12	Y	N	6.5	0	F
Spring-vale 4	S480	4.5	59	82	3	Y	Y	5.8	0	M

	S481	5.1	62	82	12	Y	Y	4.9	0	M
	S482	4.1	58	84	0	N	N	5.6	0	F
	S483	5.3	61	88	0	N	N	6.1	0	M
	S484	4.1	59	79	0	N	N	5.6	0	M
	S485	5.5	61	75	0	N	Y	6.2	0	F
	S486	0.7	29	35	0	N	N		0	Un
Spring-vale 5	S5102	4.4	62	47	24	Y	Y	6.5	0	F
	S5103	5.2	65	77	0	N	Y	6.4	0	M
	S5104	4	59	70	0	N	Y	5.5	2	M
	S5105	2.7	43	72	0	N	N	4.5	0	F
	S5106	4.5	65	68	19	Y	N	5.7	0	F
	S5107	4.5	57	77	0	N	N	5.5	5	M
	S5108	3.8	55	67	17	Y	N	5.1	4	F
	S5109	4.1	57	67	5	Y	N	5.7	2	F
	S5110	5.2	64	61	35	Y	N	5.7	1	M
	S5111	3.7	58	60	27	Y	N	5.4	3	M
	S5112	3.5	57	60	41	Y	N	5.7	0	M
	S5113	4.8	58	62	40	Y	N	5.9	1	F
	S5114	4.1	57	71	4	Y	N	5.8	0	F
	S5115	2.4	52	50	0	N	N	4.3	0	F
	S5116	4.8	63	65	40	Y	Y	5.7	0	M
	S5117	3.6	60	41	0	Y	N	5.2	8	M
Spring-vale 6	S670	4.4	57	64	36	Y	N	5.1	1	M
	S671	5.6	62	72	15	Y	N	5.7	2	F
	S679	4.5	54	74	0	N	N	5.7	0	F
	S687	0.9	32	42	0	N	N	3.1	0	Un
	S688	5	59	84	24	Y	N	6.1	0	M
	S689	3.4	53	66	9	Y	N	5.4	0	F
	S690	4	59	60	32	Y	N	6.2	5	F
	S691	6.6	68	78	25	Y	Y	7.1	7	F
	S692	1.1	36	37	0	Y	N		0	Un
	S693	3	54	65	13	Y	N	4.8	4	M
	S694	5.4	63	84	11	Y	N	6.1	0	F
	S695	4.5	57	65	25	Y	Y	5.9	0	F
	S696	3.9	56	70	0	N	N	4.9	2	M
	S697	4.6	58	69	0	N	Y	5.9	0	M
	S698	5	63	71	4	Y	N	5.4	0	F
	S699	3.7	51	74	0	N	N	5.7	1	F
	S6100	4	57	80	0	N	N	5.5	0	F
Captive	DK118	12.7	72	85	0	N	N	8.2	0	F
	DK119	10.7	73	79	0	N	N	8.0	0	F
	DK120	5.6	56	66	18	N	N	6.5	0	M
	DK121	10.2	68	84	0	N	N	7.3	0	F
	DK122	5.6	63	70	19	N	N	6.3	0	F
	DK123	10.6	74	81	22	N	N	7.1	0	M

	DK124	7.6	67	79	0	N	N	7.4	0	F
	DK125	6.8	68	91	0	N	N	7.1	0	M
	DK126	7.2	72	89	9	N	N	7.2	0	M
	DK127	8.9	74	79	14	N	N	8.5	0	F
Boyds	B133	0.5	33	44	0	N	N	0.3	0	Un
	B134	2.7	56	72	0	N	N	0.4	0	F
	B135	4.7	64	69	18	Y	N	0.6	0	F
	B136	2.3	46	66	0	N	N	0.5	0	M
	B137	3.7	59	44	0	Y	N	0.6	0	M
	B138	3.9	56	75	0	N	N	0.7	0	F
	B139	2.9	51	63	0	Y	N	0.5	0	M
	B140	2	46	76	0	N	N	0.5	0	M
	B141	3.4	58	71	14	Y	N	0.6	0	M
	B142	3.9	56	38	2	Y	N	0.5	0	M
	B143	2.7	49	72	0	N	N	0.6	0	M
	B144	4.3	56	75	0	N	N	0.6	0	F

Appendix 3

Table 6.2: Small-scaled skink sequence lengths for microsatellite loci and 16S mitochondrial haplotype.

Site	ID	Oligr20		Oligr19		Oligr14		Oligr6		16S
Otupae	OT01	278	278	164	164	274	274	-	-	E
	OT02	278	285	162	172	275	275	174	174	E
	OT03	278	285	162	164	275	275	-	-	E
	OT04	278	278	162	164	275	275	161	194	E
	OT05	278	285	162	164	275	290	174	194	E
	OT06	278	278	162	164	275	275	181	194	E
	OT09	278	285	164	164	275	275	190	190	E
	OT10	278	285	164	172	274	274	-	-	I
	OT11	278	285	164	164	275	275	173	173	N
	OT12	278	278	162	164	274	274	-	-	E
	OT13	278	285	164	164	275	275	166	190	E
	OT14	278	278	164	164	-	-	-	-	-
	OT15	285	285	164	172	274	274	165	173	I
Poronui	P07	285	285	164	166	275	275	186	186	J
	P08	285	285	160	160	275	275	174	186	J
Wakemans	WK16	278	285	162	164	274	274	161	174	G
	WK17	285	285	164	164	275	290	169	169	G
	WK18	285	285	162	162	275	275	-	-	G
	WK19	285	285	162	164	274	274	165	165	G
Ohinewairua	OH20	273	278	160	164	275	275	181	184	C
	OH21	278	285	162	164	275	275	156	193	C
	OH22	272	278	162	164	275	275	185	194	C
	OH23	278	285	160	164	263	275	172	172	F
	OH24	273	278	164	164	275	275	182	194	F
	OH25	285	285	160	164	275	275	182	182	F
	OH26	273	285	162	164	275	275	180	180	M
	OH27	278	285	162	162	275	275	221	221	F
	OH28	278	285	164	170	274	290	172	185	D
	OH29	278	285	162	162	274	274	156	156	C

	OH30	278	285	160	170	275	275	181	181	C
	OH31	285	285	160	162	274	274	156	194	C
Springvale quarry	SQ32	278	285	160	164	290	259	198	198	B
	SQ54	278	285	162	166	275	290	170	221	A
	SQ55	285	285	162	162	275	275	173	194	B
	SQ56	273	285	160	162	290	290	174	174	L
	SQ57	285	285	160	160	275	290	152	194	A
	SQ58	273	285	160	162	275	290	152	178	A
	SQ59	285	285	160	160	275	290	172	194	B
	SQ60	285	285	160	162	275	296	170	172	D
	SQ61	278	285	162	164	274	290	184	221	A
	SQ62	278	285	160	164	275	290	-	-	A
	SQ63	278	285	160	164	275	290	168	172	A
	SQ64	285	285	160	164	275	296	172	196	D
	SQ65	285	285	160	164	275	275	193	221	A
	SQ66	278	278	160	160	275	290	173	182	B
	SQ67	285	285	160	162	275	290	170	225	A
	SQ68	285	285	162	164	275	290	173	178	A
	SQ69	273	285	160	160	275	290	194	221	C
Springvale huts	SH33	278	278	160	160	275	288	172	178	-
	SH34	278	285	162	164	275	290	182	182	A
	SH35	285	285	160	162	275	290	169	189	D
	SH36	285	285	160	160	275	275	178	189	B
	SH37	278	285	160	162	275	275	169	173	B
	SH47	273	285	160	160	274	290	172	178	A
	SH48	285	285	162	164	275	275	169	172	D
	SH49	278	278	160	164	275	275	178	182	A
	SH50	273	278	162	162	290	290	169	198	D
	SH101	285	285	162	164	275	275	172	182	A
Springvale 2	S238	273	285	160	160	275	275	-	-	D
	S239	273	278	164	164	275	290	-	-	D
	S240	273	285	160	160	275	290	-	-	A

	S241	273	285	160	160	275	275	182	194	A
	S242	273	273	159	159	274	274	198	198	A
	S243	278	285	160	164	275	275	174	178	C
	S244	273	285	162	164	274	290	-	-	A
	S245	273	273	160	162	275	290	174	174	C
	S246	278	285	160	162	290	290	178	178	C
	S251	278	285	160	160	275	290	178	194	A
	S252	273	285	160	162	275	275	152	194	A
	S253	273	285	160	160	275	275	174	194	A
Springvale 3	S372	285	285	160	166	275	290	178	194	A
	S373	285	285	160	160	275	296	194	198	B
	S374	278	285	160	160	275	290	152	198	B
	S375	285	285	164	166	296	296	194	198	B
	S376	285	285	160	160	275	296	194	198	B
	S377	273	273	164	164	274	274	178	182	A
	S378	273	285	164	164	274	274	182	198	B
Springvale 4	S480	278	285	160	164	275	290	182	190	B
	S481	285	285	160	164	275	290	178	182	A
	S482	273	285	164	164	274	274	182	194	A
	S483	273	285	162	164	275	275	172	194	B
	S484	278	285	160	164	275	275	193	199	A
	S485	273	273	155	162	275	290	173	217	B
	S487	278	285	164	172	275	290	190	194	A
Springvale 5	S5102	285	285	162	164	274	274	194	194	A
	S5103	273	285	162	164	275	275	178	194	I
	S5104	-	-	-	-	-	-	-	-	A
	S5105	277	277	162	162	275	275	193	193	-
	S5106	272	272	0	0	274	274	194	194	B
	S5107	285	285	162	162	275	275	194	194	A
	S5108	273	278	160	164	290	290	180	194	A
	S5109	278	285	160	160	274	274	169	194	D
	S5110	278	285	160	160	275	290	194	194	B
	S5111	285	285	160	164	274	274	169	184	B
	S5112	284	284	160	162	275	275	169	169	A

	S5113	285	285	162	162	275	290	169	193	A
	S5114	277	284	164	164	274	290	184	194	A
	S5115	278	285	160	162	275	275	194	194	C
	S5116	277	277	-	-	274	274	177	177	A
	S5117	278	285	160	164	274	274	168	194	-
Springvale 6	S670	278	285	162	162	290	290	169	217	B
	S671	278	278	160	164	275	290	206	209	A
	S679	278	285	162	162	290	290	168	194	C
	S688	285	285	162	162	275	296	168	178	A
	S689	278	285	160	162	275	275	161	194	B
	S690	278	285	160	162	275	296	169	178	A
	S691	278	278	160	172	274	274	161	181	B
	S693	285	285	164	172	275	290	189	194	A
	S694	278	285	162	172	275	290	185	194	A
	S695	278	285	164	172	275	290	190	194	A
	S696	285	285	164	166	290	296	185	194	B
	S697	285	285	162	164	275	290	206	209	A
	S698	278	278	164	172	290	290	165	165	A
	S699	278	285	162	164	275	290	194	201	A
	S6100	285	285	160	162	290	290	169	169	B
Captive	DK118	-	-	-	-	-	-	-	-	A
	DK119	285	285	160	160	290	290	178	180	A
	DK120	284	284	160	162	275	275	178	178	A
	DK121	285	285	160	162	275	275	178	178	-
	DK122	285	285	160	162	274	274	178	181	A
	DK123	285	285	160	162	275	275	178	178	A
	DK124	285	285	160	162	275	275	178	180	A
	DK125	285	285	160	162	274	274	178	180	A
	DK126	285	285	160	162	275	296	178	181	-
	DK127	285	285	160	162	275	275	178	181	A
Boyds	B133	278	285	164	164	274	274	194	205	-
	B134	278	285	160	160	290	290	200	217	H
	B135	278	285	164	164	275	290	186	186	H
	B136	285	285	164	164	275	290	201	205	K

	B137	278	285	162	164	275	275	190	207	K
	B138	-	-	164	164	290	290	186	193	H
	B139	278	285	160	160	290	290	201	218	H
	B140	278	285	-	-	290	290	174	178	-
	B141	278	278	162	164	275	275	178	190	K
	B143	-	-	-	-	275	275	178	186	H
	B144	278	278	-	-	290	290	201	218	K

Appendix 4

Table 6.3: Raw sequence data for O. microlepis haplotypes

Haplotype A	CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT CGGCATCTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCGCTATGTCTGGCT TAATAGCGTTAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTGTTTATTCGA GCTTTTGCACTCGAAGATCAGTTTCACTGGTCAATTATAAGAGACAGGTCCATC CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTGTTTCACTGGGCAGGCAGG ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG GTTTGACAGTCTGAGGTATGTTGTTGTCCCTGTTTTGGTCTGTTAATATCCAGT AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGT TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT GGCT
Haplotype B	CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT CGGCATTTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCGCTATGTTTGGCT TAATAGCGTTAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTGTTTATTCGA GCTTTTGCACTCGAAGATCAGTTTCACTGGTCGATTATAAGAGACAGGTCCATC CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTGTTTCACTGGGCAGGCAGG ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG GTTTGACAGTCTGAGGTGTGTTGTTGTCCCTGTTTTGGTCTGTTAATATCCAGT AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGT TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT GGCT

Haplotype C
CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
CGGCATCTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA
AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCGCTATGTTTGGCT
TAATAGCGTTAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTGTTTATTCGA
GCTTTTGCACTCGAAGATCAGTTTCACTGGTCAATTATAAGAGACAGGTCCATC
CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTATTTCACTGGGCAGGCAGG
ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG
GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG
GTTTGACAGTCTGAGGTATGTTGTTGTTCCTGTTTTGGTCTGTTAATATCCAGT
AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG
TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGC
TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT
GGCT

Haplotype D
CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
CGGCATTTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA
AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCGCTATGTTTGGCT
TAATAGCGTTAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTGTTTATTCGA
GCTTTTGCACTCGAAGATCAGTTTCACTGGTCGATTATAAGAGACAGGTCCATC
CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTGTTTCACTGGGCAGGCAGG
ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG
GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG
GTTTGACAGTCTGGGGTGTGTTGTTGTCCCTGTTTTGGTCTGTTAATATCCAGT
AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG
TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGT
TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT
GGCT

Haplotype
CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA

E GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
CGGCATTTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA
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TAATAGCGTTAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTGTTTATTCGA
GCTTTTGCACTCGAAGATCAGTTTCACTGGTCGATTATAAGAGACAGGTCCATC
CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTGTTTCACTGGGCAGGCAGG
ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG
GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG
GTTTGACAGTCTGAGGTATGTTGTTGTCCCTGTTTTGGTCTGTTAATATCCAGT
AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG
TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGT
TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT
GGCT

Haplotype F CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
CGGCATCTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA
AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCGCTATGTTTGGCT
TAATAGCGTTAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTGTTTATTCGA
GCTTTTGCACTCGAAGATCAGTTTCACTGGTCAATTATAAGAGACAGGTCCATC
CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTATTTCACTGGGCAGGCAGG
ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG
GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG
GTTTGACAGTCTGAGGTATGTTGTTGTCCCTGTTTTGGTCTGTTAATATCCAGT
AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG
TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGT
TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT
GGCT

Haplotype G CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
CGGCATTTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA

AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCGCTATGTTTGGCT
TAATAGCGTCAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTATTTATTCGA
GCTTTTGCACTCGAAGATCAGTTTCACTGGTCGATTATAAGAGACAGGTCCATC
CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTGTTTCACTGGGCAGGCAGG
ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG
GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG
GTTTGACAGTCTGAGGTATGTTGTTGTCCCTGTTTTGGTCTGTTAATATCCAGT
AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG
TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGT
TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT
GGCT

Haplotype H

CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
CGGCATTTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA
AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCGCTATGTTTGGCT
TAATAGCGTTAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTATTTATTCGA
GCTTTTGCACTCGAAGATCAGTTTCACTGGTCGATTATAAGAGACAGGTCCATC
CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTGTTTCACTGGGCAGGCAGG
ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG
GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG
GTTTGACAGTCTGAGGTATGTTGTTGTCCCTGTTTTGGTCTGTTAATATCCAGT
AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG
TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGT
TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT
GGCT

Haplotype I

CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
CGGCATCTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA
AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCGCTATGTTTGGCT
TAATAGCGTTAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTGTTTATTCGA

GCTTTTGCACTCGAAGATCAGTTTCACTGGTCAATTATAAGAGACAGGTCCATC
CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTATTTCACTGGGCAGGCAGG
ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG
GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG
GTTTGACAGTCTGAGGTCTGTTGTTGTTCCTGTTTTGGTCTGTTAATATCCAGTA
GGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAGT
TTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGTT
TGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGTG
GCT

Haplotype J CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
CGGCATTTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA
AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCACTATGTTTGGCT
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GCTTTTGCACTCGAAGATCAGTTTCACTGGTCGATTATAAGAGACAGGTCCATC
CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTGTTTCACTGGGCAGGCAGG
ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG
GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG
GTTTGACAGTCTGAGGTATGTTGTTGTCCCTGTTTTGGTCTGTTAATATCCAGT
AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG
TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGT
TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT
GGCT

Haplotype K CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
CGGCATTTCTTTGACTTGTTGGTCTAAGTTAGAGTGGTTGGCTCTGTGCTCGGA
AGTTTTATTTTATTCCGAAGTCGCCCCAACTTAAAACTTAGCGCTATGTTTGGCT
TAATAGCGTTAGTTTTAAGCTCCACAGGGTCTTCTCGTCTTATGTGTTTATTCGA
GCTTTTGCACTCGAAGATCAGTTTCACTGGTCGATTATAAGAGACAGGTCCATC
CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC

TTTGCACGGTTAGGATACCGCGGCCGTTAAAAGTGTTTCACTGGGCAGGCAGG
ACCTTTAATACTTGTTTAGCTAAAGGCTATGTTTTTGGTAAACAGTTGGGATGG
GGTTTGCTGAGTTCCTTATGTAATTTTTAACCTTCCTTGTGGCACTCCGGTGTTG
GTTTGACAGTCTGAGGTATGTTGTTGTCCCTGTTTTGGTCTGTTAATATCCAGT
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TTTTAGCATTAGTGCTGCTATTGATATAGCGTGGCTCGGTAATTTACTAGGGGT
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GGCT

Haplotype L

CATGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
GATAGCGCTGTTATCCCTGGGGTAACTTGGTTCGTTGTTCAGTAGGACTGGGT
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CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
TTTGCACGGTTAGGATACCGCGGCCGTTTAAAGTGTTTCACTGGGCAGGCAGG
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AGGTTTTCTATTTCTGGGGCACGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAG
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TTGGTTGGGTTAGTGTTATTTATGATTTTTGTGCTGTGACGCTTTATTTATTGGT
GGCT

Haplotype M

CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
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CGGCATCTCTTTGACTTGTTGGTCTAAGTTAGAGTGGCTGGCTCTGTGCTCGGA
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CTCATTAGGCCGTTCATACTAGTCTTTATTTAAAAGACAAGTGATTACGCTACC
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GGCT

Haplotype N

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GCT

Haplotype O

CCTGATCCAACATCGAGGTCGTAAACCTTCTTGTCGATATGAACTCTTGAAGAA
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CGGCATCT-
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GGCACTCCGGTGTTGGTTTGACAGTCTGAGGTAT-

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CGCGGGTGTGTAGAGAAGTTTTCTTGTTACTAGTTTTAGCATTAGTGCTGCTAT

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