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Comparative landscape genetics of two widespread, endemic species, the common and McCann's skink in Canterbury and Otago, New Zealand

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Johnathon Ridden

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of B.sc (Hons).

Comparative landscape genetics of two widespread, endemic species, the common and McCann's skink in Canterbury and Otago, New Zealand

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Understanding how genetic variation within a particular species is spatially structured is important for knowing how populations are connected and how landscape configuration affects population connectivity. Landscape genetics provides an ideal toolbox to determine patterns and processes structuring populations. These techniques were applied to two species of New Zealand skink, the common skink *Oligosame nigraplantare polychorma* and McCann's skink *Oligosoma maccanni*, to investigate how these populations are structured in Canterbury and Otago, New Zealand. Specific objectives for this study were (1) to determine the genetic structure of both species, (2) to determine the influence of landscape features on genetic structure, (3) to determine how geography and genetic structure influence patterns of morphological variation and (4) to use this information to recommend conservation management plans for these species. Microsatellite genotyping was used to determine genetic structuring for both species. Distance matrices were created for genetics, land use, Euclidean distance and morphology. Population genetic structure was calculated using GenAlEx. All realtionships between distance matrices were analysed using Mantel and partial Mantel tests. The results showed signicant genetic structure in both species. Landscape and geographic distances had a significant relationship with genetic distance for the common skink, but not for McCann's ski nk. Morphology was not correlated with genetic distance in either species, but there was some correlation between geography and morphology. Based on this, the study has highlighted that populations of congeneric species, that are sympatric and ecologically similar, are not necessarily influenced by the same landscape features. This has implications for conservation, indicating that species-specific conservavtion strategies should be applied.

Keywords: comparative landscape genetics, *Oligosoma maccanni*, *Oligosoma nigraplantare polychroma*, population genetics, microsatellites, endemic species, Mantel test, partial Mantel test, land use change, connectivity, isolation by distance, morphological variation

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Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1 Landscape genetics

To understand how genetic variation within a particular species is spatially structured, it is important to know how populations are connected, and how landscape configuration influences population connectivity. Landscape genetics is the field of research that aims to quantify explicitly the effects of landscape composition, configuration and matrix quality on gene flow and spatial variation (Manel et al. 2003). Since the inception of this field by Manel et al. (2003), there has been a move towards integrating landscape ecology, spatial statistics and population genetics. By incorporating real world features, such as landscape configuration and barriers affecting connectivity of populations, landscape genetics provides better understanding and knowledge of a population's ecology (Holderegger & Wagner 2006; Storfer et al. 2006). Many different taxa have been studied under this framework, including amphibians (Spear et al. 2005; Murphy et al. 2010), mammals (Schwartz et al. 2003; Coulon et al. 2004), birds (Petren et al. 2005; Barr et al. 2008), lizards (Smith et al. 2009; Blair et al. 2013), invertebrates (Holzhauer et al. 2006; Sander et al. 2006) and plants (Hirao & Kudo 2004; McRae & Beier 2007).

Genetic structuring is an important component of landscape genetics. Molecular data and analyses are used to infer connectivity between populations via gene flow, for example using microsatellite data (Pritchard et al. 2000; Balloux & Lugon-Moulin 2002). Connectivity is important as it relates to predicting how the flow of genetic material influences population structure (Manel et al. 2003). Gene flow can be related to how populations interact within the landscape, via connectivity or isolation. Other methods for inferring connectivity between populations, such as tracking and observation studies, are difficult and expensive to achieve for many taxa, promoting the use of genetic methods (Kool et al. 2013). With recent rapid advances in molecular technology, computational power, and rapidly decreasing costs of DNA sequencing and genotyping, genetic analysis promises to provide greater resolution of information on population ecology.

The other component of landscape genetics is the effect of landscape on population connectivity, and how it relates to the genetic structuring. Storfer et al. (2010) reviewed the importance of landscape features and variables for landscape genetics, identifying the importance of linking the fields of landscape ecology and population genetics. Generally, landscape genetic studies have focussed on the effects of landscape features such as topographic relief e.g. (mountain, valleys and

elevation gradients) (Spear et al. 2005; Giordano et al. 2007), and fragmented habitat and changing land use types (Keyghobadi et al. 2005; Sacks et al. 2005). Specific landscape features have more relevance and influence on population structure, depending on the study species. Freshwater ecosystems are influenced by factors such as drainage patterns, and direction and speed of water flow, as shown for example in zooplankton (Michels et al. 2001). However for mammals, habitat fragmentation can influence population genetic structure; for example, population structure in European roe deer was found to be associated with woodland corridors, with no relationship of genetic structuring to disturbed habitat patches (Coulon et al. 2004). This highlights that different landscape features affect population genetic structure, depending on the taxa or species of interest.

Landscape genetics can answer many different questions relating to population structure and connectivity of species in their natural environments. These questions relate to quantifying the effect of potential barriers to dispersal, such as rivers and mountains, on population structure and connectivity (Storfer et al. 2010). With rapid land use change due to anthropogenic factors, such as deforestation and modification of landscapes, many populations of species become isolated, so quantifying the effect of land use change on population structure is an ideal application of landscape genetics (Sork et al. 1999). Depending on the taxon and its biology, different landscape genetic approaches can be used to understand how landscape features affect population structure.

1.2 Landscape genetics methods

The application of landscape genetics relies on two sources of information, genetic data and landscape data. Genetic data used in landscape genetic studies usually takes the form of highly variable polymorphic genetic markers, such as Amplified Fragment Length Polymorphisms (AFLPs) or microsatellites (Selkoe & Toonen 2006; Storfer et al. 2010). Storfer et al. (2010) did a meta-analysis of landscape genetics, which looked at the molecular markers used in 655 studies. They found that microsatellites were the most common marker used, in 70% of papers studying animals and 32% of papers studying plants (Storfer et al. 2010). Microsatellites provide several benefits over other markers such as a decrease in cost and time necessary to carry out research using them, which allows researchers insight into fine-scale ecological questions, and a large amount of population genetic studies using them, which means they are well understood (Selkoe & Toonen 2006; Storfer et al. 2010). For landscape geneticists interested in the effects of recent land use change, microsatellites can provide information on the contemporary effects of landscape change on populations (Selkoe & Toonen 2006). Genetic distance matrices can be computed using microsatellite data, allowing comparisons with other measures of population structure and connectivity such as physical distance and landscape features.

There are many different methods for understanding population connectivity in the environment. Pairwise distance matrix correlations are a common method used to relate physical connectivity to other measures of connectivity, such as genetic relatedness (Manel et al. 2003; Storfer et al. 2006; Lowe & Allendorf 2010). The most commonly used methods for analysing population structure are isolation by distance, least cost path distance and isolation by resistance (Balkenhol et al. 2009; Guillot et al. 2009; Spear et al. 2010). Isolation by distance examines the effect of straight line Euclidean distance on genetic structuring of populations, assuming that specimens farther away from each other are likely to be less genetically similar (Manel et al. 2003; Storfer et al. 2006; Wang et al. 2009). Least cost path analysis assumes that the landscape configuration influences connectivity between populations, so populations are connected based on the optimal route through the habitat configuration separating them (Spear et al. 2005; Storfer et al. 2006; Wang et al. 2009). Isolation by resistance is an extension of least cost path analysis that recognises that there may be multiple pathways connecting populations (McRae 2006; McRae et al. 2008; Spear et al. 2010). For least cost path analysis and isolation by resistance, resistance surfaces are created using software such as ArcGIS (Michels et al. 2001) and Circuitscape (Etherington 2011), and these surfaces classify different land use types on the basis of their suitability for dispersal, based on the ecology of the study species.

Landscape genetics provides an ideal toolbox to determine the patterns and processes structuring populations. Many studies have documented deep evolutionary population genetic structuring (evolutionary relationships over a period of millions of years) relating to biogeographical features in the South Island of New Zealand for different taxa (Trewick & Wallis 2001), such as cicada (Buckley et al. 2001), beetles (Marske et al. 2011), and weta (Trewick 2001). A benefit of landscape genetics is that it can uncover relatively contemporary patterns and processes and their impact on populations (Pavlacky Jr et al. 2009; Storfer et al. 2010), and show that contemporary landscape change can have rapid effects on genetic structure, e.g. over a period of 50 years (Landguth et al. 2010). Not all systems show a short time lag between landscape change and genetic structure, e.g. in the bush cricket *Metrioptera roeseli* (Holzhauer et al. 2006), in which genetic structuring is not related to the contemporary landscape, which was modified over 50-100 years ago (Holzhauer et al. 2006). However, most landscape genetics studies show landscape configuration influencing genetic structure of populations.

Contemporary landscape factors that may influence recent gene flow and population structure can be identified using landscape genetics (Landguth et al. 2010). Zellmer and Knowles (2009) showed how contemporary landscape features affect population structure, by comparing the effect of land cover from three time periods (pre- and post-European settlement and current land use), on the

population genetics of the wood frog *Rana sylvatica* in Michigan USA. They found that after controlling for the landscape structure of each time period, contemporary patterns of genetic differentiation were reflected by recent landscape features (Zellmer & Knowles 2009). Goldberg and Waits (2010) showed that two amphibian species from the Palouse bioregion of northern Idaho, the Columbian spotted frog *Rana luteiventris* and long-toed salamanders, *Ambystoma macrodactylum,* had different population genetic structures based on the landscape. A moisture gradient and wetter land use types explained the genetic structure of *A. macrodatylum*, with agricultural and shrub/clearcut habitat explaining the genetic structure of *R. luteiventris* (Goldberg & Waits 2010).

There are many examples in the literature of the use of landscape ecology and population genetics to examine population connectivity and structure, focussing on the importance of landscape features. Murphy et al. (2010) showed that *R. luteiventris,* had strong genetic structure. Ridgelines were found to be a barrier to gene flow, meaning that populations separated by ridgelines were genetically isolated, with basins facilitating gene flow, and populations separated by basins being genetically similar (Murphy et al. 2010). Spear et al. (2005) presented evidence that the tiger salamander *Ambystoma tigrinum melanostictum* had a relatively genetically homogenous population structure in Yellowstone National Park USA, based on eight microsatellite loci. The low degree of population genetic structure observed was attributed to the effect of main land use types, such as rivers and open shrub habitat, which facilitated gene flow (Spear et al. 2005). These relationships are examples of the application of landscape genetics, which can be used to determine the influence of landscape on wildlife population connectivity (Manel et al. 2003; Storfer et al. 2006; Pavlacky Jr et al. 2009).

1.3 New Zealand lizards

New Zealand has two genera of skinks in the family Scincidae, *Oligosoma* and *Cyclodina*, with the latter genus only found in the North Island (Chapple et al. 2009). The taxonomy of New Zealand's skink fauna has been revised on several occasions (Patterson & Daugherty 1995; Chapple et al. 2009). Patterson and Daugherty (1995) reclassified the New Zealand skink fauna from *Leiolopisma,* which is an Australian skink genus, to *Oligosoma*, based on unique morphology, including overall size, measured as snout vent length (SVL), colours and patterns, such as stripes, and genetics, based on allozyme data. *Oligosoma* are characterised by shallow pointed heads, long limbs and toes, and oval body shape in cross section. Chapple et al. (2009) readdressed the taxonomic assignment of the New Zealand Scincidae using mitochondrial and nuclear DNA sequence data, which resulted in taxonomic re- classification for several described species.

Oligosoma are generally characterised by several distinct features of their ecology. They are diurnal and prefer open habitats to bask in the sun, and are more active during warmer seasons of the year, such as summer and spring (Patterson & Daugherty 1995; Chapple et al. 2009). Skink diets usually consist primarily of invertebrates, as well as berries from shrubs like *Coprosma* spp., while some species are described as having a generalist diet (Freeman 1997; Hickson et al. 2000). One interesting feature of New Zealand skinks is that they are viviparous, meaning they give birth to live young, which is thought to be due to the cold climate (Cree 1994) as viviparous females can thermoregulate and develop young under optimal conditions *in utero* (Guillette 1993; Cree 1994), whereas the eggs of oviparous females would experience lower temperatures in the nest that could slow or even prevent embryonic development (Guillette 1993).

Fragmentation and land use change has been attributed to reduced gene flow in populations of many species (Storfer 2010). Many taxa in New Zealand have suffered population declines and isolation throughout their geographic ranges, primarily due to habitat loss and modification since human settlement (Towns & Elliott 1996). New Zealand skinks occur in a broad range of habitats and are a useful group for studying the effects of ecological change (Hickson et al. 2000). A large majority of pre-human settlement habitat has been changed into many different land uses, the main one being agriculture. Habitat preference of skinks in New Zealand is for indigenous habitat types such as tussock grassland, shrubland and stony/gravel areas (Patterson & Daugherty 1995; Walker et al. 2014). Therefore, it is interesting to see how this land use change has influenced population structure of different New Zealand lizard species.

1.4 Biology and ecology of the McCann's and common skinks

This study focusses on two species the common skink *Oligosoma nigraplantare polychroma* (Patterson & Daugherty 1990), and McCann's skink *Oligosoma maccanni* (Patterson & Daugherty 1990). Both of these species are found throughout most of the South Island of New Zealand (Liggins et al. 2008a; O'Neill et al. 2008), with common skink also found in the lower North Island (Liggins et al. 2008a). These species have been selected for this study for several reasons. First, they are sympatric, co-occurring taxa that are closely related to each other. No landscape genetics study has ever compared the landscape genetics patterns of two co-occurring, closely related species that can be sampled together. Second, they are widely distributed in Canterbury and Otago, which have experienced significant land use change, habitat destruction and fragmentation (Patterson & Daugherty 1990). Both species have been relatively well studied, so information on their taxonomic relationships and ecology is available. They also exhibit interesting morphology, with each species showing variation in Otago and Canterbury (Freeman 1997).

McCann's skink is widespread in Canterbury and Otago, with a history of taxonomic reclassification. Patterson and Daugherty (1990) revised the then *Leiolopisma nigriplantare maccanni* species complex into five distinct species (*inconspicuum, maccanni, microlepis, notosaurus*) and subspecies *L. nigriplantare polychroma* (the common skink). Phylogeographic research has shown that Pliocene and Pleistocene tectonic and mountain building processes have shaped McCann's skink populations, with multiple geographically and genetically distinct clades found throughout Canterbury and Otago (O'Neill et al. 2008). McCann's skink has been documented with habitat preferences for open, dry areas such as shrublands, rocks and rocky outcrops in tussock grasslands (Patterson & Daugherty 1990; O'Neill et al. 2008; Walker et al. 2014). Freeman (1997) found McCann's skinks use of habitat on Kaitorete spit to be correlated with dune vegetation, such as herbs/shrublands, and grasses like pingao. Habitat use in by McCann's skink in Central Otago may contrast with that in Canterbury, with McCann's skink preferring dry arid environments and vegetation with low soil development, such as herbs and shrubs, rocks and rocky outcrops in Otago, compared with using grass marram or grasses like pingao on dunelands in coastal Canterbury (Freeman 1997). McCann's skink has been noted to have a preference for stone/gravel habitats, so this land use type may facilitate connectivity (Freeman 1997; O'Neill et al. 2008).

The common skink is the most widespread species of skink in New Zealand, ranging from Southland to the lower North Island, just North of Wellington (Patterson 1992). It has been suggested that the common skink could consist of multiple different species, due to large amounts of morphological variation (Patterson & Daugherty 1990). However, recent genetic work has shown that this species is in fact only one species that has five geographically and genetically distinct clades (Liggins et al. 2008a). These clades are thought to have arisen due to Pleistocene glacial processes (Liggins et al. 2008a). This species contains a sub-specific epithet to denote its similarity to its closest relative, the Chatham Island skink *Oligosoma nigraplantare nigraplantare*, which can be distinguished by morphological (Daugherty et al. 1990) and genetic divergence (Liggins et al. 2008b). The habitat preferences of common skinks vary slightly from that of McCann's skinks. They have a preference for shrubs and grasses, such as tussock (Patterson 1992), with specimens studied at Kaitorete spit in Canterbury being found in shrubland and grassland with substrates that have high moisture retention (Freeman 1997). This habitat preference was consistent with a study of skinks in the Rock and Pillar ranges of Central Otago, which found that common skinks prefer grassland or tussock vegetation (Patterson 1992).

One interesting feature of both species is that they appear to have geographically opposite morphological patterns when the morphology of Otago populations is compared to those in Canterbury (Freeman 1997). McCann's are striped in Canterbury, but speckled in Otago, whereas

common skink is speckled in Canterbury and striped in Otago (Freeman 1997). Determining the processes or reasons for this morphological change requires taking a landscape genetics approach.

These species provide an ideal system to carry out a comparative landscape genetics study of closely related taxa. Storfer et al. (2010) found in their meta-analysis of landscape genetics that 90% of papers focussed on single species, while 7% focussed on two or more species, which were not congeneric. By understanding how landscape features influence the population structure and connectivity of these species in Canterbury and Otago, the impact of land use modification on common widespread species can be investigated. Most skink species in New Zealand have suffered population declines and isolation throughout their geographic ranges, primarily due to habitat loss and modification since human settlement (Towns & Elliott 1996). Understanding the effect of current landscape configuration and barriers such as rivers on McCann's and common skink populations is important for understanding how their populations may be relatively isolated or connected, which has important ecological and conservation implications.

1.5 Aims and Objectives

In the landscape genetics literature, most studies focus on how landscape features influence genetic structuring of populations of single species (Manel et al. 2003; Driscoll et al. 2012). This study aims to compare two congeneric species, McCann's and common skink, and examine the similarities and differences in patterns of their population structure and the processes that are inferred to drive and cause these patterns. There are few examples of comparative landscape genetic studies like this, and to my knowledge, none that compare congeneric sympatric species. A specific list of objectives is given below.

Objectives

1) Determine the genetic structure of McCann's skink and common skink populations in Canterbury and Otago.

   Prediction: There will be significant genetic structure for both species. They will not be in Hardy-Weinberg equilibrium, indicating that they are not panmictic across the sampled range (Spear et al. 2005).

2) Determine whether genetic structure of each species correlates with particular landscape features.

Prediction: Similar barriers will be shared by these congeneric, sympatric species, so landscape factors will have similar effects on genetic structure in each case (Petren et al. 2005).

3) Determine the correlation between morphological patterns and genetic or landscape structure for both species.

   Prediction: Morphological patterns will be related to either geographic or genetic patterns, or both (Francuski et al. 2013).

4) Identify areas of restricted gene flow within each species and make recommendations for conservation management of these species.

The overall aim is to discover whether these two closely related species, exhibit similar population structure at the landscape level, and determine the features and processes producing these patterns. This will combine landscape configuration, morphology, geographic distance and genetic distance and will provide an insight into the generalisability of the results of comparative landscape genetics studies.

Chapter 2

Methods

2.1 Study area and specimen collection

Common and McCann's skinks were sampled from locations all over Canterbury and Otago during the summer of 2010/2011. The study area is defined by the regional boundaries of Canterbury and Otago. There are several major rivers that dissect both regions including the Waitaki, Rakaia, Waimakiriri, Clutha and Mataura. The Waitaki River defines the boundary between Canterbury and Otago. A total of 92 skinks were sampled, 48 McCann's skinks and 44 common skinks, from 24 different sites (Figure 2.1.1). Measurements of snout vent length (SVL), vent–tail length (VTL), age, sex, and weight were taken, and each skink was identified to species, with a confidence of identification recorded as a percentage. The GPS coordinates, altitude and location names were recorded for each site. If multiple samples were collected from one site, one GPS waypoint was recorded for that site and a single general habitat description was recorded. A small portion of the skink's tail tip was cut off and stored in 100% ethanol for DNA analysis. Ventral, dorsal and lateral photographs of each individual were taken. Sampling was done under a high impact DoC permit.

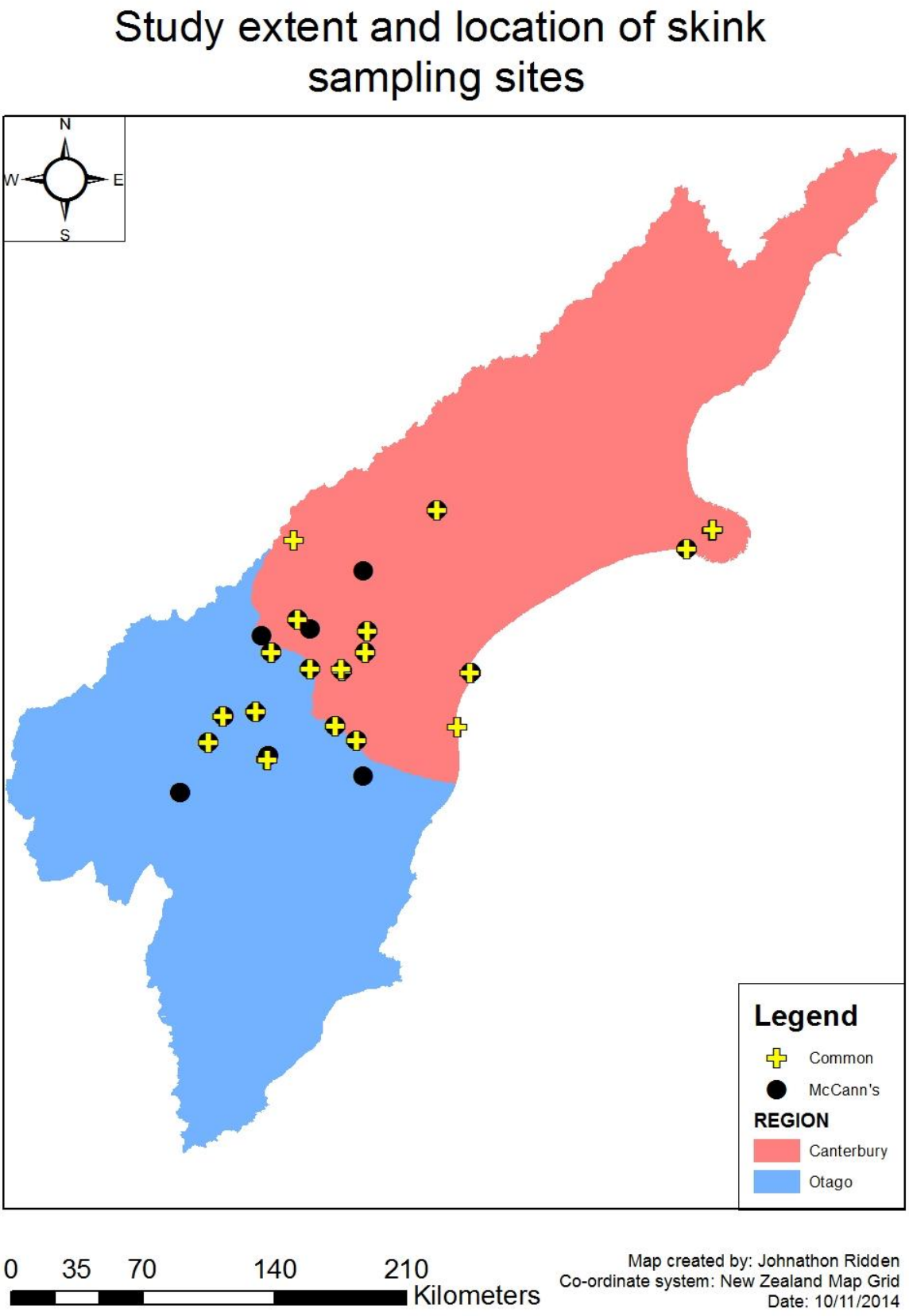

Figure 2.1.1: Map of study extent and 24 sampling location in Canterbury and Otago. McCann's skinks sampling locations are black circles, with common skink sampling locations are represented by yellow crosses.

2.2 DNA extraction

Genomic DNA was isolated from the skink tail tip samples in the Molecular Ecology Laboratory at Lincoln University using the QIAGEN DNeasy tissue and blood kit, following the manufacturer's instructions but with an overnight lysis step. All DNA extracts were stored at -20°C.

2.3 PCR amplification

DNA extracts were amplified using a selection of primers to see if DNA extraction was successful. However, the primers used did not work successfully for both species. Since the only successful amplifications were with MlepF1 and HCO for McCann's skink, none of these primers could be used to check for successful DNA extraction on both species (Table 2.3.1). Microsatellite primers that had been created for a closely related species, the Grand skink (*Oligosoma grande*) (Berry et al. 2003) were therefore used to test for DNA extraction success instead. One microsatellite locus, Oligr8, which had successfully been used to amplify DNA from 20 other New Zealand skink species (Berry et al 2003), was used to confirm the success of DNA extraction for both species, due to low success of CO1 and ITS gene regions (Table 2.3.1).

Table 2.3.1: Amplification success rates for nuclear rDNA and mtDNA genes tested on skink DNA extracts. Success is represented by a fraction under each species column.

Forward primer	Reverse primer	Gene region	McCann's	Common	Annealing temperature
HCO	HCO	CO1	0/6	0/6	54°C
LCO	HCO	CO1	0/6	0/6	45°C
CAS18sF1	CAS28sB1d	ITS	0/6	0/6	54°C
CAS18sF2	CAS5p8s1d	ITS1	0/6	0/6	54°C
CAS18sF1	CAS28sB1d	ITS	0/6	0/6	45°C
MLepF1	HCO	CO1	6/6	0/6	54°C
LCO	LepR1	CO1	0/6	0/6	54°C

All 15 primers developed by Berry et al (2003), were then tested to see which loci would work on the common and McCann species. All loci were tested using 6 DNA extracts, 3 of common skinks and 3 of McCann's skinks (Table 2.3.2).

Table 2.3.2: Amplification success rates for 15 microsatellites tested on skink DNA extracts. The same 2 or 3 extracts were used for each locus

Forward primer	Reverse primer	Annealing temperature (°C)	Success rate McCann's	Success rate Common
Oligr1F	Oligr1R	61	2/2	2/3
Oligr2F	Oligr2R	57	2/3	2/3
Oligr3F	Oligr3R	59	3/3	1/3
Oligr4F	Oligr4R	57	2/3	3/3
Oligr6F	Oligr6R	57	2/3	3/3
Oligr7F	Oligr7R	57	3/3	1/3
Oligr8F	Oligr8R	59	3/3	3/3
Oligr10F	Oligr10R	57	3/3	3/3
Oligr11F	Oligr11R	59	3/3	3/3
Oligr13F	Oligr13R	58	2/2	2/3
Oligr14F	Oligr14R	55	2/2	3/3
Oligr15F	Oligr15R	59	0/2	0/3
Oligr17F	Oligr17R	55	2/2	3/3
Oligr19F	Oligr19R	55	3/3	3/3
Oligr20F	Oligr20R	58	2/2	3/3

PCR was carried out for these 6 samples using a standard thermo-cycling protocol. A master mix was prepared using the required materials for each test locus. Each master mix contained a pair of primers, specific to each locus. PCR amplification was performed in a 25µl reaction volume using 12.5 µl of GoTaq® Green Master Mix (Promega, Madison, WI, USA), 1 µl of each primer, 1µl of 10 mg/ml purified bovine serum albumin (BSA) 100X (New England BioLabs, Ipswich, MA, USA) (to reduce the impact of PCR inhibitors), and 8.5 µl sterile autoclaved water. 22 µl of master mix and 3 µl of each DNA template were added to separate PCR tubes, to make up the 25 µl reaction volumes. If a high quantity of primer dimer was observed on a gel, then the amount of primer added to the master mix was halved for subsequent PCRs

All PCR samples were then placed in a PCR thermocycling machine with the following cycle: initial denaturation at 94°C for 2 minutes: 35 cycles of 94°C for 30 seconds, $T_{annealing}$ (3 degrees less than the average annealing temperature of both primers, as recommended by the manufacturer Invitrogen) for 45 seconds, and 72°C for 2 minutes for extension, with a final extension at 72°C for 7 minutes. The PCR product was then run on a 1.5% agarose gel to test whether PCR amplification was

successful. The length of the PCR product was estimated by comparison with a standard DNA ladder. This was compared to the length reported by Berry et al. (2003) for each locus to confirm that the PCR product was of the expected length.

2.4 Genotyping

Based on the microsatellite testing described above, a panel of 7 microsatellite loci were selected for analysis and genotyping. These 7 loci were put into two groups, one with four primers and one with three (Table 2.4.1). The group of four loci had the 5' end of the forward primers labelled with a fluorescent dye (6-FAM, VIC, NED or PET, Applied Biosystems). This was to distinguish the loci based on colour when genotyped. The group of three were labelled with three different fluorochrome tags. Every DNA extract was run as a singleplex PCR, with each labelled primer, using the optimal annealing temperature (Table 2.3.2). Then PCR products for each group were pooled in a single PCR tube for each sample, with relative quantities of PCR product depending on intensity of bands in the gel and the intensity of the dye, as the blue and green tagged product were more intense than yellow and red. Different volumes of PCR products were added so that the heights of the chromatogram peaks would be similar (Table 2.4.1).

Table 2.4.1: Combination of microsatellite loci used for poolplexing of PCR product for each sample. The dye attached the forward primer in each locus primer pair is shown, with the colour it represents on the chromatogram. The volume of singleplex PCR product added for each group is given in μl.

Group	Locus	Dye	Colour	Volume added to pool
1	Oligr8	6FAM	Blue	1ul
	Oligr10	PET	Red	2ul
	Oligr14	VIC	Green	1ul
	Oligr17	NED	Yellow	3ul
2	Oligr1	6FAM	Blue	2ul
	Oligr6	NED	Yellow	1ul
	Oligr19	VIC	Green	0.3ul

1μl of pooled PCR product for each sample was genotyped using the sequencer AbiPrism3750. 10-12μl of HiDi formamide was used to re-suspend the samples, and a LIZ1200 size standard was used to analyse the PCR product allele sizes. The genotype output files (chromatograms) were analysed in Genemarker v2.6.3 (Figure 2.4.1). The peaks were manually scored by the author and recorded in two excel spreadsheets, one for each species using the GenAlEx format. Each sample was scored for two alleles at each locus. If there was one peak, then the genotype was scored as homozygous; if there were two peaks, the genotype was scored as heterozygous. Alleles with 1bp difference at any

locus were scored as the same allele, as software error can occur leading to incorrect over-scoring of alleles. Any sample that did not have successful amplification at an individual locus was scored as zero.

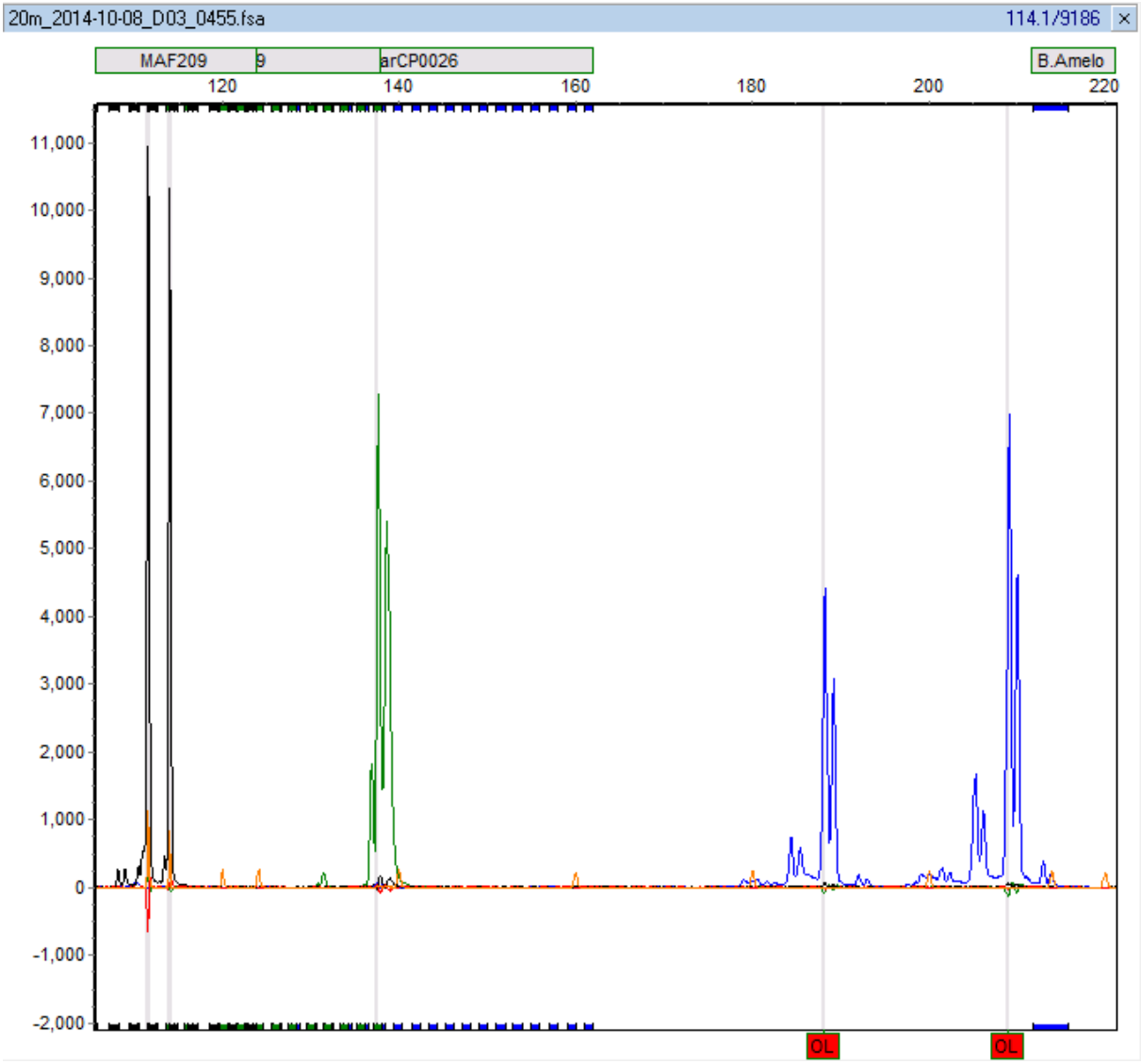

Figure 2.4.1: Chromatogram of pooled PCR product for sample 20. The peaks represent the NED tagged PCR product (black), VIC tagged PCR product (green) and 6FAM tagged PCR product (blue). Each peak was scored from its tip on the right most peak, based on the size given in the top right hand corner (e.g. 114.1) by rounding it to the nearest whole number.

To account for scoring error, 30 samples were randomly re-scored (15 for each species) to check the accuracy of the initial scoring (Table 2.4.2). All scoring was re-checked and errors identified were resolved. The spreadsheet was saved in GenAlEx and GenePop formats for subsequent analysis.

Table 2.4.2: Random re-scoring of alleles for 15 samples to test for consistency and accuracy of scoring.

	% alleles scored the same as original scoring						
Species	Oligr8	Oligr10	Oligr14	Oligr17	Oligr1	Oligr6	Oligr19
McCann	86.6	86.6	86.6	95.4	93.3	100	86.6
Common	90	93.3	93.3	100	92.3	100	80

A genetic distance matrix for each species was created from the genotype data in GenAlEx (Peakall & Smouse 2012). Populations were assigned within each species based on *a priori* criteria. The genetic matrix was calculated using the Distance>Genetic option. The default parameters were used including 'Codom-genotypic' as the input data format, 'Output Total Distance Only' and Sample for pairwise calculations. The matrix was selected 'As Tri Matrix'. The 'Interpolate Missing' box was checked, for samples that did not have alleles scored. The distance matrix output was saved in a new spreadsheet, which was then saved as a text file for subsequent analysis.

2.5 Euclidean distance matrix

Euclidean distance matrices were calculated to test for Isolation by distance. Two matrices were produced for each species, using GPS points for the sampling location of every skink. The matrices contained the physical pairwise distance between every sample point. Both were calculated using R (R Core Team 2012).

2.6 Geographic resistance surface

Creation of resistance surfaces started with collecting several GIS data layers into an ArcGIS geodatabase (ESRI 2011), including the Landcare Research land cover database v4.0 layer, a New Zealand regional boundaries layer and the collected skink data. The regional boundaries layer is a polygon feature class, which was clipped to include only the Canterbury and Otago regions. The land cover layer, which is a polygon feature class, was also clipped to this same extent. The skink data was stored as a point feature class, with sample locations found within the Canterbury and Otago regions. All layers were projected in New Zealand Map Grid 1984.

The land cover feature class was used to classify skink habitat preference, based on its suitability as for skink movement. There were 32 different land cover types, which were converted to raster format for the analysis using the 'Polygon to Raster' tool, with a 100 x 100 cell size resolution. The output surface produced was reclassified so that every habitat type was scored either 1 (low resistance) or 2 (high resistance). Resistance scores for habitat types were selected based on the

habitat preference of both the common and McCann's skink, as recorded in the literature (Patterson 1992; Freeman 1997), and the type of habitat the skink data were collected from (Table 2.6.1).

Table 2.6.1: Classification of resistance scores for each land use type for resistance surface with two resistance types. The low values correspond to low resistance surfaces, i.e. higher likelihood of passage through that habitat, the high values correspond to high resistance surfaces, i.e. lower likelihood of passage through habitat. Land use categories are based on land cover data layer v4.0

Resistance scores	Land use type
1	Low producing grassland, manuka and/or kanuka, sub-alpine shrubland, tall tussock grassland, fernland, sand or gravel, gravel or rock, matagouri or grey Scrub, depleted grassland, flaxland, alpine Grass/herbfield
2	High producing exotic grassland, exotic forest, herbaceous freshwater vegetation, gorse and/or broom, indigenous forest, orchard, vineyard or other perennial crop, river, urban parkland/open space, built-up area (settlement), surface mine or dump, lake or pond, short-rotation cropland, estuarine open water, deciduous hardwoods, broadleaved indigenous hardwoods, herbaceous saline vegetation, forest – harvested, transport infrastructure, landslide, permanent snow and ice

The 'Reclassify' tool was used to reclassify the different habitat types based on the above criteria. For the Circuitscape analysis, the resistance surface needs to be in ASCII format, so the conversion tool 'Raster to ASCII' was used to produce the ASCII file. The other file input for Circuitscape is the focal node locations. This refers to the locations where the skinks were sampled. The GPS coordinates are required to compute the pairwise geographic distance between each point, based on the habitat between them. For the analysis, the focal node file requires a node ID (sample number), and the northing and easting GPS co-ordinates, each in separate columns.

Geographic distance matrices were created using Circuitscape software (Shah & McRae 2008; McRae & Shah 2009). The input data type selected was 'Raster', with the modelling mode selected as 'Pairwise: iterate across all pairs in focal node file'. For the 'Input resistance data' the ASCII raster resistance file was entered. The focal node file was entered as specified above, with one analysis run using the common skink focal node file, and the other using the McCann's focal node file. The output of the analysis was saved in an .OUT format as a square matrix of pairwise distances, with the output files named based on the species matrix being calculated.

2.7 Morphological distance matrix

Photographs were taken of the dorsal and underside of every skink collected. These skinks were then classified as having one of three distinct pattern types: striped, checkered, or a combination of striped and checkered. Each sample had the pattern type recorded in a spreadsheet. Maps were produced for both species showing where the different patterns occur using ArcGIS (Figure 2.7.1 and Figure 2.7.2). This data was then used to create a distance matrix in R (R Core Team 2012) using the package *labdsv* (Roberts 2007), splitting them based on species.

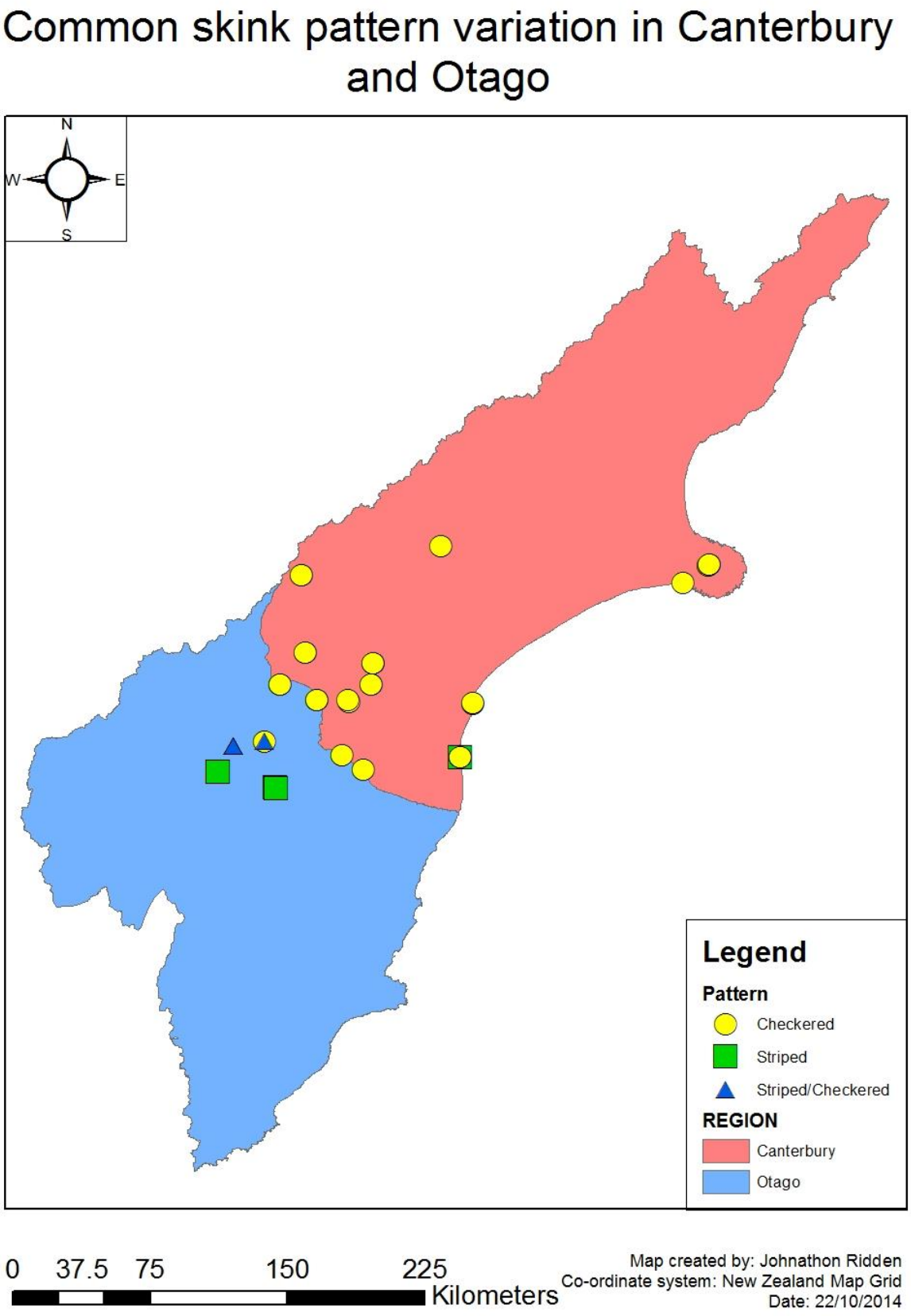

Figure 2.7.1: Map showing the morphological pattern of specimens from each sampling location for common skink.

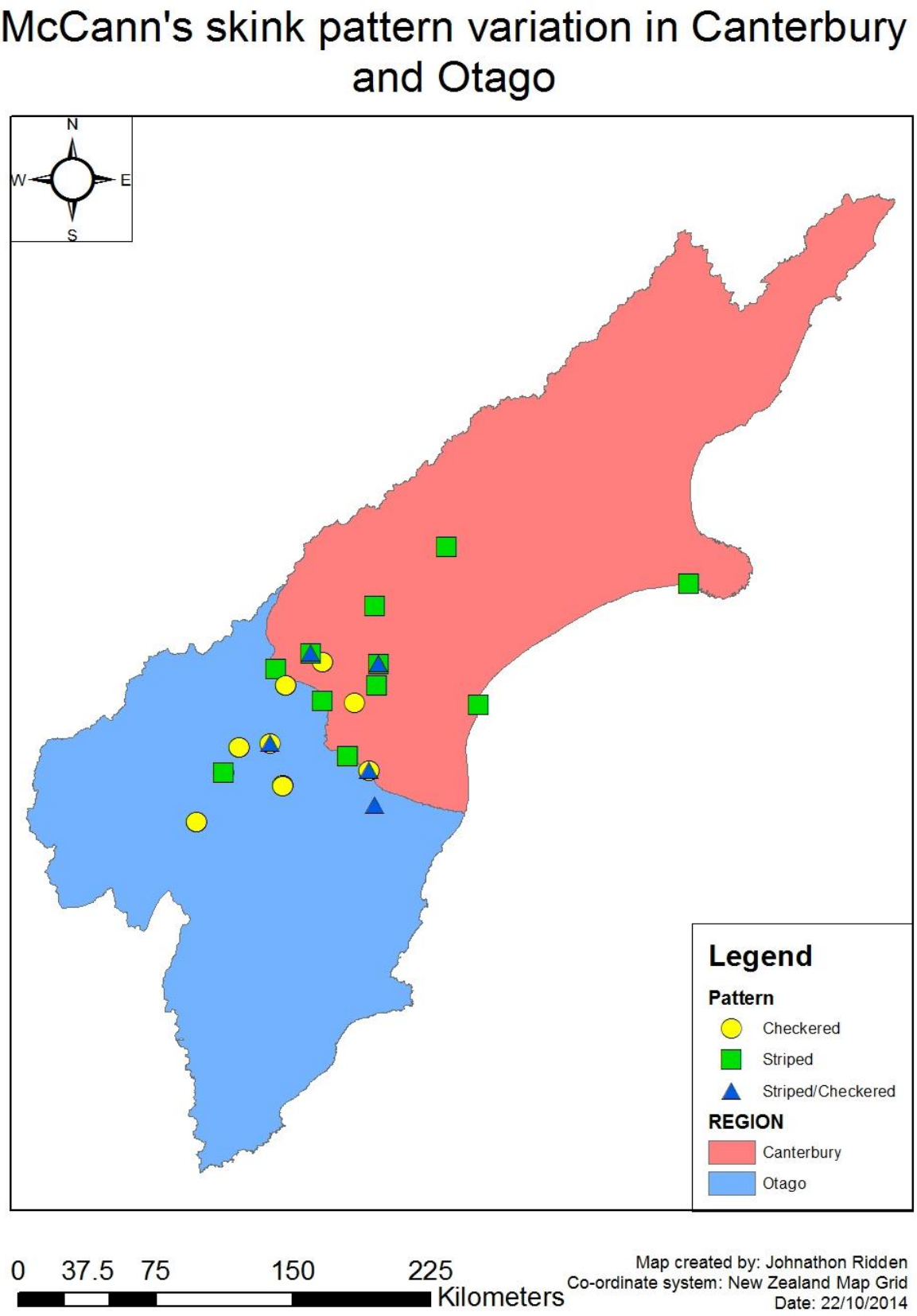

Figure 2.7.2: Map showing the morphological pattern of specimens from each sampling location for McCann's skink.

2.8 Data analysis

Samples were assigned to populations based on an *a priori* criteria. Firstly, each species was spilt into two populations corresponding to specimens from Canterbury and Otago, either side of the Waitaki River, which is presumed to be a significant barrier to dispersal. The results of a preliminary analysis (see Results below) suggested that the Otago population of the common skink, and the Canterbury population of McCann's skink were not in Hardy-Weinberg equilibrium, with significant homozygote excess at several loci. These populations were therefore subdivided i nto four and three population respectively on geographical ground (see Figs. 2.8.1 and 2.8.2).

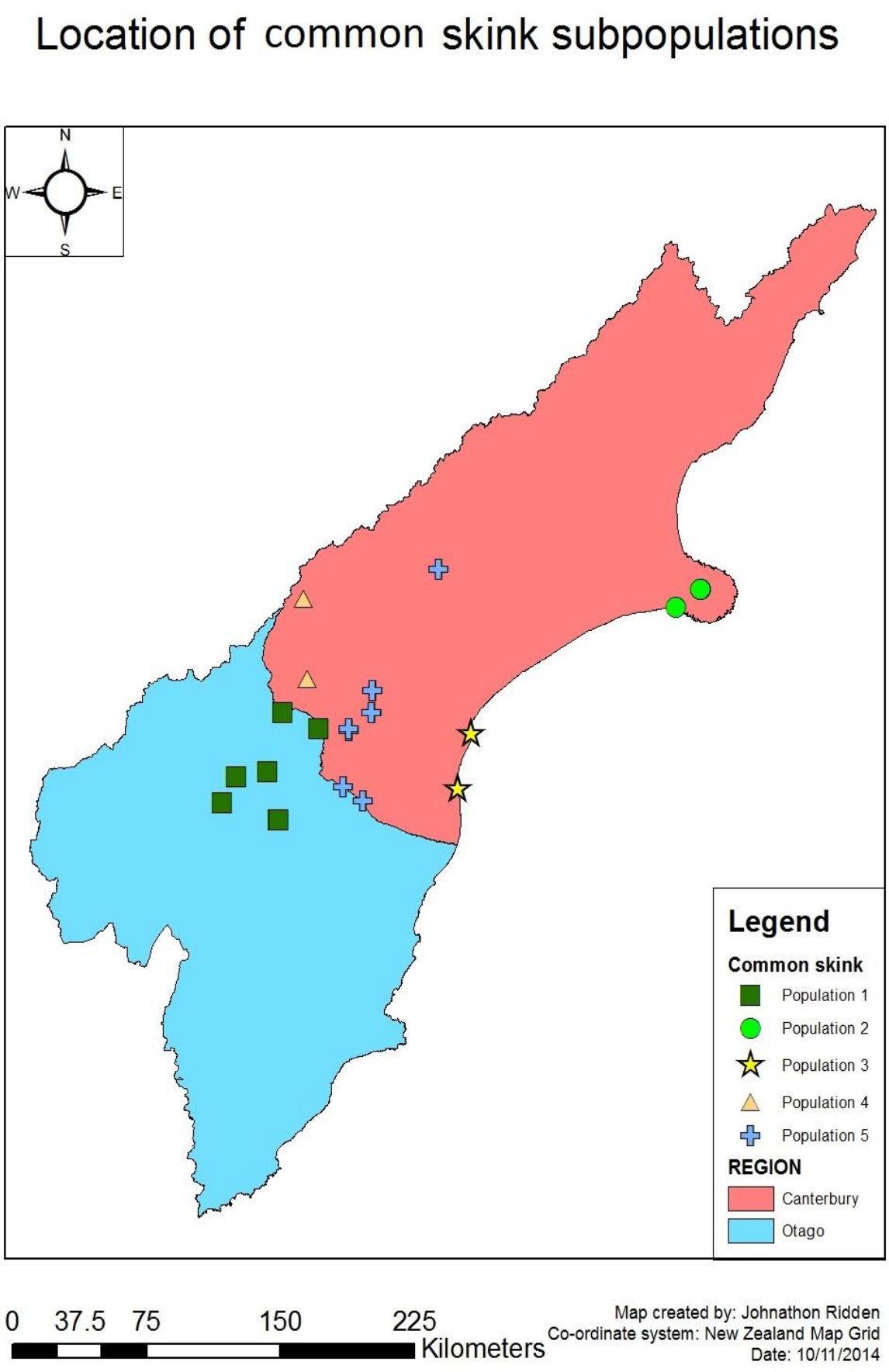

Figure 2.8.1: Map showing populations of common skinks assigned based on *a priori* geographical criteria.

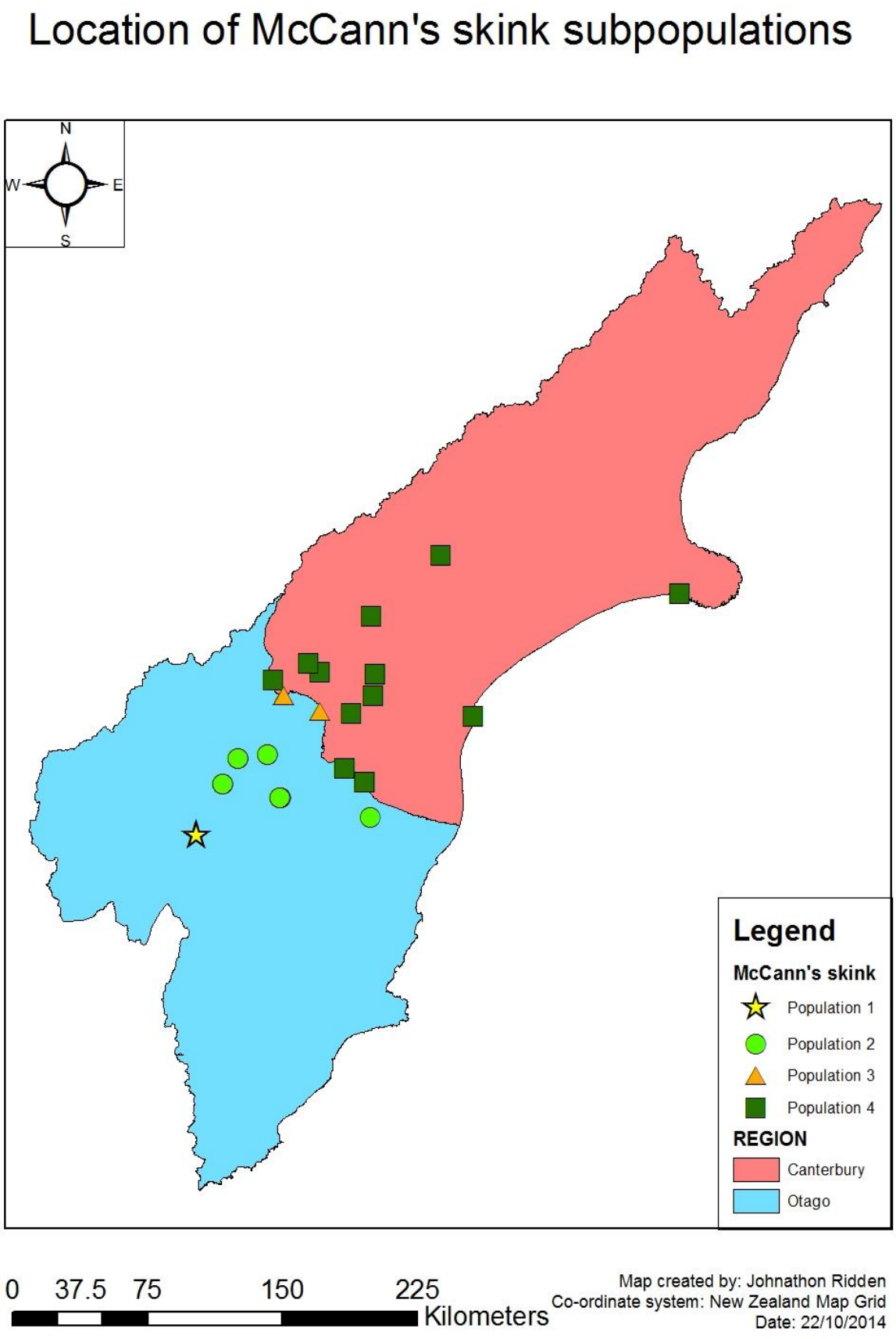

Figure 2.8.2: Map showing populations of McCann's skinks assigned based on *a priori* geographical criteria.

Data were checked for null alleles using Microchecker v2.2.3 (Van Oosterhout et al. 2004). For each locus the number of alleles and the observed and expected heterozygosities were calculated, and Hardy Weinberg tests were performed using GenAlEx v6 (Peakall & Smouse 2012). P-values were corrected for multiple tests in R (R Core Team 2012) using the false discovery rate approach of Benjamini and Hochberg (1995).

Mantel test and partial Mantel test were used to compare the distance matrices in R (R Core Team 2012) using the *permute* and *vegan* packages (Oksanen et al. 2007). Distance matrices produced for the Mantel and partial Mantel tests were: genetic distance, morphological distance, Euclidean distance, and resistance surface matrix. For the Mantel test, each matrix described above was paired with each other matrix to test for correlations (Table 2.8.1). For the partial Mantel tests, the same paired combinations were used as the Mantel test, and the effect of a third distance matrix was controlled for (Table 2.8.2).

Table 2.8.1: Mantel tests were used to test the following correlations:

Mantel tests	
Matrix 1	Matrix 2
Genetic distance	Resistance distance
Genetic distance	Euclidean geographic distance
Genetic distance	Morphological distance
Morphological distance	Euclidean geographic distance
Morphological distance	Resistance distance
Resistance distance	Euclidean geographic distance

Table 2.8.2: Partial Mantel tests were used to test the following correlations

Partial Mantel tests		
Matrix 1	Matrix 2	Matrix 3
Resistance distance	Euclidean distance	Genetic distance
Resistance distance	Euclidean geographic distance	Morphological distance
Resistance distance	Morphological distance	Genetic distance
Resistance distance	Morphological distance	Euclidean geographic distance
Genetic distance	Euclidean geographic distance	Resistance distance
Genetic distance	Euclidean geographic distance	Morphological distance
Morphological distance	Euclidean geographic distance	Genetic distance
Morphological distance	Euclidean geographic distance	Resistance distance
Morphological distance	Genetic distance	Euclidean geographic distance
Morphological distance	Genetic distance	Resistance distance
Resistance distance	Genetic distance	Euclidean geographic distance
Resistance distance	Genetic distance	Morphological distance

Chapter 3

Results

3.1 Population structure of common skinks

When the common skink was divided into two populations a significant excess of homozygotes at several loci was observed in the Otago population (Table 3.1.1). There was no significant evidence for null alleles in common skinks, apart from locus Oligr8 with a low frequency of null alleles (Table 3.1.2); therefore the homozygote excess was interpreted as evidence for population structure.

Table 3.1.1: Population genetic structure of the common skink, based on Hardy Weinberg equilibrium for Pop1 as Canterbury, and Pop2 as Otago. Adj. p-value = p-value adjusted for multiple tests (see Methods). Sig. = significance (ns = not significant, * = 0.05 < adj. p-value <0.01, ** = 0.01 < adj. p-value <0.001, *** = adj. p-value <0.001).

Common	Locus	H_o	H_e	p-value	Adj. p-value	Sig.	Interpretation
Pop1	Oligr8	0.600	0.913	0.002	0.006	**	Homozygote excess
	Oligr10	0.875	0.881	0.180	0.320	ns	
	Oligr14	0.000	0.000				
	Oligr17	0.167	0.153	0.824	0.923	ns	
	Oligr19	0.000	0.000				
	Oligr1	0.875	0.939	0.680	0.828	ns	
	Oligr6	0.000	0.000				
Pop2	Oligr8	0.885	0.958	0.067	0.144	ns	
	Oligr10	0.893	0.952	0.336	0.495	ns	
	Oligr14	0.077	0.497	0.000	0.000	***	Homozygote excess
	Oligr17	0.316	0.723	0.013	0.033	*	Homozygote excess
	Oligr19	0.000	0.266	0.000	0.000	***	Homozygote excess
	Oligr1	0.958	0.947	0.435	0.609	ns	
	Oligr6	0.250	0.542	0.000	0.000	***	Homozygote excess

There was no significant evidence for null alleles in common skinks, apart from locus Oligr8 with a low frequency of null alleles (Table 3.1.2); therefore the homozygote excess was interpreted as evidence for population structure.

Table 3.1.2: Frequency of null alleles for common skink at seven loci. Statistically significance is indicated in **bold**.

common				**Null allele frequency**			
	Stutter	Dropout	Null alleles	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
Oligr8	No	No	**Yes**	0.1723	0.2070	0.1638	0.2610
Oligr10	No	No	No				
Oligr14	No	No	No				
Oligr17	No	No	No				
Oligr19	No	No	No				
Oligr1	No	No	No				
Oligr6	No	No	No				

When the Otago population of common skinks was subdivided into four separate populations there was little evidence for departure from Hardy Weinberg equilibrium (Table 3.1.3). Homozygote excess was observed at Oligr8 in population 1, Oligr19 in population 2, Oligr6 in population 3 and at Oligr 14 and Oligr 6 in population 5 but all of these results were only weakly statistically significant (p value <0.02-0.05).

Table 3.1.3: Population genetic structure of the common skink, based on Hardy Weinberg equilibrium. Adj. p-value = p-value adjusted for multiple tests (see Methods). Sig. = significance (ns = not significant, * = 0.05 < adj. p-value <0.01, ** = 0.01 < adj. p-value <0.001, *** = adj. p-value <0.001).

common	Locus	H_o	H_e	p-value	Adj. p-value	Sig.	Interpretation
Pop1	Oligr8	0.600	0.913	0.002	0.021	*	Homozygote excess
	Oligr10	0.875	0.881	0.180	0.634	ns	
	Oligr14	0.000	0.000				
	Oligr17	0.167	0.153	0.824	1.000	ns	
	Oligr19	0.000	0.000				
	Oligr1	0.875	0.939	0.680	0.847	ns	
	Oligr6	0.000	0.000				
Pop2	Oligr8	0.667	0.819	0.293	0.735	ns	
	Oligr10	1.000	0.917	0.477	0.930	ns	
	Oligr14	0.400	0.480	0.120	0.554	ns	
	Oligr17	0.400	0.320	0.576	0.930	ns	
	Oligr19	0.000	0.722	0.006	0.042	*	Homozygote excess
	Oligr1	0.800	0.860	0.363	0.847	ns	
	Oligr6	0.250	0.531	0.245	0.735	ns	
Pop3	Oligr8	1.000	0.861	0.564	0.930	ns	
	Oligr10	0.875	0.852	0.633	0.973	ns	
	Oligr14	0.000	0.000				
	Oligr17	0.200	0.620	0.107	0.554	ns	
	Oligr19	0.000	0.000				
	Oligr1	1.000	0.875	0.749	1.000	ns	
	Oligr6	0.000	0.219	0.005	0.039	*	Homozygote excess
Pop4	Oligr8	0.750	0.688	0.532	0.930	ns	
	Oligr10	0.750	0.719	0.530	0.930	ns	
	Oligr14	0.000	0.000				
	Oligr17	0.000	0.000				
	Oligr19	0.000	0.000				
	Oligr1	1.000	0.778	0.532	0.930	ns	
	Oligr6	0.333	0.278	0.729	1.000	ns	
Pop5	Oligr8	1.000	0.920	0.350	0.847	ns	
	Oligr10	0.900	0.940	0.333	0.847	ns	
	Oligr14	0.000	0.420	0.002	0.021	*	Homozygote excess
	Oligr17	0.429	0.633	0.123	0.553	ns	
	Oligr19	0.000	0.000				
	Oligr1	1.000	0.925	0.733	1.000	ns	
	Oligr6	0.444	0.580	0.002	0.021	*	Homozygote excess

The amount of within population diversity was not statistically significantly different between all five populations. This was calculated using two measures of genetic diversity, allelic diversity (N_a) and expected heterozygosity (H_e) (Table 3.1.4).

Table 3.1.4: Within population diversity of common skink within five populations. Pop1 is samples from Canterbury, with the populations highlighted in grey from Otago.

common	Pop1	Pop2	Pop3	Pop4	Pop5
	Canterbury	Otago			
N	16	6	8	4	10
Allelic diversity (Na)	8.286	5.714	5.143	2.714	9.000
Standard error	3.421	1.322	1.639	0.714	2.690
Expected heterozygosity (He)	0.412	0.664	0.489	0.352	0.631
Standard error	0.178	0.084	0.153	0.138	0.130

The allelic diversity was not significantly different among populations (paired two-tailed t tests; table 3.1.5).

Table 3.1.5: P-values (paired two-tailed t tests) showing the pairwise relationships between the allelic diversity (N_a) in each population of common skink. Significant p-values of <0.05 indicate differing amounts of within population variation between given populations. P-values have not been adjusted for multiple tests as they are all non-significant.

p-values	Pop1	Pop2	Pop3	Pop4
Pop2	0.658			
Pop3	0.538	0.801		
Pop4	0.436	0.124	0.340	
Pop5	0.884	0.385	0.268	0.177

The expected heterozygosity was also not significantly different among populations (paired two-tailed t tests; Table 3.1.6).

Table 3.1.6: P-values showing pairwise relationship between the expected heterozygosity (He) diversity found within each population of common skink. Significant p-values of <0.05 indicate differing amounts of within population variation between given populations.

p-values	Pop1	Pop2	Pop3	Pop4
Pop2	0.410			
Pop3	0.782	0.175		
Pop4	0.873	0.073	0.581	
Pop5	0.387	0.859	0.487	0.240

3.2 Population structure of McCann's skinks

When the McCann's skink was divided into two populations a significant excess of homozygotes at several loci was observed in the Canterbury population (Table 3.2.1).

Table 3.2.1 Population genetic structure of the common skink, based on Hardy Weinberg equilibrium for Pop1 as Canterbury and Pop2 as Otago. Adj. p-value = p-value adjusted for multiple tests (see Methods). Sig. = significance (ns = not significant, * = 0.05 < adj. p-value <0.01, ** = 0.01 < adj. p-value <0.001, *** = adj. p-value <0.001).

McCann's		H_o	H_e	p-value	Adj. p-value	Sig.	Interpretation
Pop1	Oligr8	1.000	0.946	0.820	0.923	ns	
	Oligr10	0.870	0.966	0.183	0.320	ns	
	Oligr14	0.000	0.635	0.000	0.000	***	Homozygote excess
	Oligr17	0.238	0.261	0.000	0.000	***	Homozygote excess
	Oligr19	0.348	0.771	0.000	0.000	***	Homozygote excess
	Oligr1	0.500	0.914	0.001	0.003	***	Homozygote excess
	Oligr6	0.313	0.664	0.000	0.000	***	Homozygote excess
Pop2	Oligr8	0.957	0.959	0.547	0.696	ns	
	Oligr10	0.875	0.967	0.266	0.414	ns	
	Oligr14	0.087	0.162	0.023	0.054	ns	
	Oligr17	0.421	0.382	0.492	0.656	ns	
	Oligr19	0.375	0.573	0.127	0.254	ns	
	Oligr1	0.917	0.959	0.233	0.384	ns	
	Oligr6	0.444	0.863	0.000	0.000	***	Null alleles

When this population was subdivided into three separate populations, most of this homozygote excess disappeared, however some remained. There was significant evidence of null alleles at loci Oligr14 and Oligr6 (Table 3.2.2). Homozygote excess observed at these loci could be attributed to these null alleles, which can confound estimates of population structure.

Table 3.2.2: Frequency of null alleles for McCann's skink at seven loci. Statistical significance is indicated in **bold**.

McCann's				**Null allele frequency**			
	Stutter	Dropout	Null alleles	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
Oligr8	No	No	No				
Oligr10	No	No	**Yes**	0.051	0.053	0.049	0.119
Oligr14	**Yes**	No	**Yes**	**0.442**	**1.000**	**0.388**	0.532
Oligr17	No	No	No				
Oligr19	No	No	**Yes**	0.262	0.378	0.239	0.306
Oligr1	No	No	**Yes**	0.227	0.293	0.216	0.594
Oligr6	No	No	**Yes**	0.249	0.360	0.211	**0.606**

Homozygote excess was observed at Oligr6, Oligr17, and Oligr14 in population 2 and Oligr14 in population 4 (Table 3.2.3). Of these, only Oligr4 in population 2 and Oligr6 in population 4 were strongly significant, and in these cases, homozygote excess is attributed to null alleles.

Table 3.2.3: Population genetic structure of the McCann's skink, based on Hardy Weinberg equilibrium. Adj. p-value = p-value adjusted for multiple tests (see Methods). Sig. = significance (ns = not significant, * = 0.05 < adj. p-value <0.01, ** = 0.01 < adj. p-value <0.001, *** = adj. p-value <0.001).

McCann's	Locus	H_o	H_e	p-value	Adj. p-value	Sig.	Interpretation
Pop1	Oligr8	1.000	0.844	0.521	0.930	ns	
	Oligr10	1.000	0.875	0.464	0.930	ns	
	Oligr14	0.000	0.000				
	Oligr17	0.500	0.375	0.505	0.930	ns	
	Oligr19	0.750	0.688	0.677	0.996	ns	
	Oligr1	0.000	0.444	0.083	0.475	ns	
	Oligr6	0.250	0.219	0.775	1.000	ns	
Pop2	Oligr8	1.000	0.931	0.804	1.000	ns	
	Oligr10	0.857	0.949	0.168	0.633	ns	
	Oligr14	0.000	0.611	0.000	0.000	***	Null alleles
	Oligr17	0.231	0.278	0.004	0.036	*	Homozygote excess
	Oligr19	0.500	0.793	0.181	0.634	ns	
	Oligr1	0.375	0.813	0.175	0.634	ns	
	Oligr6	0.333	0.726	0.001	0.021	*	Homozygote excess
Pop3	Oligr8	1.000	0.840	0.628	0.973	ns	
	Oligr10	0.800	0.840	0.337	0.847	ns	
	Oligr14	0.000	0.000				
	Oligr17	0.000	0.000				
	Oligr19	0.000	0.000				
	Oligr1	1.000	0.880	0.516	0.930	ns	
	Oligr6	0.000	0.000				
Pop4	Oligr8	0.957	0.959	0.547	0.930	ns	
	Oligr10	0.875	0.967	0.266	0.761	ns	
	Oligr14	0.087	0.162	0.023	0.145	ns	
	Oligr17	0.421	0.382	0.492	0.930	ns	
	Oligr19	0.417	0.448	0.234	0.735	ns	
	Oligr1	0.917	0.959	0.233	0.735	ns	
	Oligr6	0.444	0.863	0.000	0.000	***	Null alleles

The amount of within population diversity was not statistically significantly different between all four populations (Table 3.2.4).

Table 3.2.4: Within population diversity of McCann's skink within four populations. Pop1, Pop2 and Pop3 are samples from Canterbury, with the populations highlighted in grey from Otago.

McCann's	Pop1	Pop2	Pop3	Pop4
	Canterbury			Otago
N	4	15	5	24
Allelic diversity (Na)	3.714	10.000	4.000	18.571
Standard error	1.040	2.920	1.543	5.698
Expected heterozygosity (He)	0.492	0.729	0.366	0.677
Standard error	0.124	0.087	0.172	0.128

The allelic diversity was not significantly different among populations (paired two-tailed t tests; Table 3.2.5).

Table 3.2.5 P-values (pairwise two-tailed t tests) showing the pairwise relationships between the allelic diversity (N_a) in each population of McCann's skink. Significant p-values of <0.05 indicate differing amounts of within population variation between given populations. P-values have not been adjusted for multiple tests as they are all non-significant.

p-values	Pop1	Pop2	Pop3
Pop1			
Pop2	0.293		
Pop3	0.889	0.265	
Pop4	0.304	0.267	0.261

The expected heterozygosity was also not significantly different among populations (paired two-tailed t tests; Table 3.2.6).

Table 3.2.6: P-values (pairwise two-tailed t tests) showing pairwise relationships between the expected heterozygosity (H_e) in each population of common skink. Significant p-values of <0.05 indicate differing amounts of within population variation between given populations. P-values have not been adjusted for multiple tests as they are all non-significant.

p-values	Pop1	Pop2	Pop3
Pop1			
Pop2	0.210		
Pop3	0.590	0.363	
Pop4	0.570	0.770	0.299

3.3 Mantel and partial Mantel tests

Results are shown in tables 3.3.1 – 3.3.4.

Table 3.3.1: Results of Mantel test correlations between six different distance matrix combinations for the common skink. Significant results are indicated in **bold**.

Matrix 1	Matrix 2	r statistic	Significance (p-value)
Genetic	Resistance	0.66	**<0.001**
Genetic	Euclidean	0.68	**<0.001**
Genetic	Morphological	-0.10	<0.840
Morphological	Euclidean	0.10	<0.154
Morphological	Resistance	-0.13	<0.926
Resistance	Euclidean	0.82	**<0.001**

Table 3.3.2: Results of partial Mantel tests showing correlation between 12 different distance matrix combinations for the common skink. In each combination matrix 1 is correlated with matrix 2, while controlling for matrix 3. Significant results are indicated in **bold**.

Matrix 1	**Matrix 2**	**Matrix 3**	**R statistic**	**Significance (p-value)**
Euclidean	Resistance	Genetic	0.67	**<0.001**
Euclidean	Resistance	Morphological	0.84	**<0.001**
Morphological	Resistance	Genetic	-0.09	<0.806
Morphological	Resistance	Euclidean	-0.38	<1.000
Genetic	Euclidean	Resistance	0.33	**<0.001**
Genetic	Euclidean	Morphological	0.70	**<0.001**
Morphological	Euclidean	Genetic	0.24	**<0.001**
Morphological	Euclidean	Resistance	0.37	**<0.001**
Morphological	Genetic	Euclidean	-0.24	<0.999
Morphological	Genetic	Resistance	-0.02	<0.597
Genetic	Resistance	Euclidean	0.25	**<0.009**
Genetic	Resistance	Morphological	0.66	**<0.001**

Table 3.3.3: Results of Mantel test correlations between 4 different distance matrix combinations for the McCann's skink.

Matrix 1	**Matrix 2**	**r statistic**	**Significance (p-value)**
Genetic	Resistance	0.07	<0.214
Genetic	Euclidean	0.13	<0.052
Genetic	Morphological	-0.01	<0.516
Morphological	Euclidean	0.19	**<0.002**
Morphological	Resistance	0.14	**<0.005**
Euclidean	Resistance	0.84	**<0.001**

Table 3.3.4 : Results of partial Mantel tests showing correlation between 12 different distance matrix combinations for the common McCann's skink. In each combination matrix 1 is correlated with matrix 2, while controlling for matrix 3. Significant results are indicated in **bold**.

Matrix 1	Matrix 2	Matrix 3	R statistic	Significance (p-value)
Euclidean	Resistance	Genetic	0.84	**<0.001**
Euclidean	Resistance	Morphological	0.83	**<0.001**
Morphological	Resistance	Genetic	0.14	**<0.009**
Morphological	Resistance	Euclidean	-0.04	<0.759
Genetic	Euclidean	Resistance	0.13	**<0.031**
Genetic	Euclidean	Morphological	0.14	<0.055
Morphological	Euclidean	Genetic	0.19	**<0.001**
Morphological	Euclidean	Resistance	0.14	**<0.001**
Morphological	Genetic	Euclidean	-0.03	<0.8
Morphological	Genetic	Resistance	-0.02	<0.614
Genetic	Resistance	Euclidean	-0.07	<0.793
Genetic	Resistance	Morphological	0.07	<0.199

Genetic distance was significantly correlated with Euclidean distance and resistance distance for the common skink (Table 3.3.1 and Table 3.3.2), but not for the McCann's skink, apart for a weak correlation between genetic distance and Euclidean distance, when controlling the effect of resistance distance (Table 3.3.3 and Table 3.3.4). Morphology was only significantly correlated with Euclidean distance, when controlled for the effect of resistance distance on morphology in the common skink (Table 3.3.1 and Table 3.3.2), where in McCann's skink, morphology was correlated with Euclidean distance and resistance distance. (Table 3.3.3 and Table 3.3.4)

Chapter 4

Discussion

4.1 Genetic structuring

There is evidence for significant genetic structure in both common and McCann's skink across the sampled range. For common skinks, when divided into five populations these appeared to be in Hardy Weinberg Equilibrium, indicating that each population was panmictic (Table 3.1.3). Four genetically distinct populations were located in Otago, indicating that population structure was more pronounced there compared to Canterbury, which contained a single population. No significant levels of null alleles were detected at any loci for the common skink (Table 3.1.2). Therefore, all homozygote excess is interpreted as evidence for genetic structure, which is likely due to isolation and lack of gene flow between these populations. The genetic variation observed within each population was similar, indicating that these populations contain equivalent levels of genetic diversity (Table 3.1.3). This is significant because if one population contained greater genetic diversity than the others, an inference of the ancestral range could be made, but no population exhibited significantly greater levels of diversity.

McCann's skink also exhibited significant population structuring, but this contrasts with the patterns observed for common skinks. When McCann's skinks were divided into four populations, they all appeared to be in Hardy-Weinberg equilibrium, which is interpreted as evidence for panmixis within these populations (Table 3.2.3). Three of these populations are located in Canterbury, indicating that population structure for this species is greater there than in Otago, which contained a single apparently panmictic population. However, there was significant evidence for null alleles at two loci in two populations (Table 3.2.2), which can cause homozygote excess, and can be confounded with population structure. As the homozygote excess was restricted to a few loci, and population structure is expected to affect all loci more or less equally (Table 3.2.3), it is assumed that the homozygote excess observed in some of these populations is due to null alleles rather than population structure. The structure observed is more than likely due to isolation and restricted gene flow between populations, as for the common skink. Similar amounts of genetic variation was observed within each population, indicating that all five populations contain equivalent levels of genetic diversity (Table 3.2.4). No population contained greater amounts of genetic diversity than the other, so the ancestral range could not be inferred from this analysis.

These results are consistent with the prediction that both species would show some degree of population structure. The results are consistent with other studies that have looked at the effects of fragmentation on population genetic structure (Coulon et al. 2004; Keyghobadi et al. 2005; Sacks et al. 2005). For both species, there must be barriers to dispersal between the populations. Barriers, like rivers or less preferential habitat, could significantly restrict gene flow between populations, and may be a potential factor influencing the population structure observed for the common and McCann's skink.

Sampling density has an influence on determining genetic structure in all species across Canterbury and Otago. As populations of both species did not appear to be in Hardy Weinberg equilibrium when they were subdivided into two populations across the range, the sampling density was sufficient for this study. However, there were some significant gaps in sampling, which mean the whole picture on genetic diversity is not shown. In Canterbury there was no sampling done on the Canterbury plains, which is a significant gap that would be beneficial to fill, as there appears to be genetic subdivision between samples from Banks Peninsula and Southern Canterbury (see Figs 2.8.1 and 2.8.2). Sampling on the Canterbury plains would be significant as this area has been subject to significant land use change from indigenous habitat to agriculture (Patterson & Daugherty 1990; Towns & Elliott 1996), so understanding how populations isolated in small parts of habitat on the plains are genetically structured, would contribute greatly to the results of this study. No samples were collected from any site North of Banks Peninsula, or from Southern Otago, which means this study only represents populations near the Canterbury/Otago regional boundary (see Figs 2.8.1 and 2.8.2). This means that there may be unsampled genetic diversity in Canterbury and Otago for both species that was not captured in this study.

4.2 Genetic structure in relation to landscape features

Different factors were identified that could influence population structure in these species. Common skink genetic diversity showed a pattern of isolation by distance (IBD) and was related to aspects of landscape configuration (Table 3.3.1, Table 3.3.2). However, for McCann's skinks there was no significant IBD signal, or relationship between population genetic structure and landscape configuration (Table 3.3.3, Table 3.3.4). As the relationship between landscape configuration and genetic structure differed for these two closely related congeneric species, with similar ecology, it is evident that the same landscape features, do not influence their population structure in the same way. However, when the effect of the resistance distance was controlled for, there was a weak IBD signal in McCann's skink (Table 3.2.4). With regards to objective 2, common skink genetic distance is significantly correlated with the landscape resistance surface, but the same is not the case for McCann's skink.

This result highlights the key point of this study, that populations of widespread congeneric species may not be structured by the same factors at the landscape level. There are several potential reasons for the patterns observed in these species. First, the landscape configuration surface may have been too simple. One possible reason for this is related to the fact that for both species the Euclidean distance matrix was significantly correlated with the resistance distance matrix, so that any influence of landscape on the genetic distance was minor. Second, the same resistance surface matrix was used for both species. Using the same resistance surface is a potential limitation as both species have varying habitat preferences, with McCann's preferring stone/gravel habitat, and common preferring grassland and shrubland (Freeman 1997). It may be worth creating landscape resistance surfaces based on the specific ecology of each species, to test the impact of specific landscape features.

Classification of good and bad habitat for the creation of the resistance surfaces was broad, based purely on where the samples were collected, and on literature pertaining to the ecology of both species (Patterson 1992; Freeman 1997). Quantifying the potential effect of landscape features on movement and connectivity of individuals between populations is an issue (Holderegger & Wagner 2008). This is related to potentially incorrect assumptions on which landscape features facilitate or restrict dispersal, which can influence applicability to real world systems. One way to overcome this would be to do field surveys at every land use type, and quantify to what extent these species of skink used the specific habitat type using count data, which could better inform the parameterization of the resistance surface. Another more realistic method would be to create multiple different landscape resistance surfaces, based on different landscape features, to see which are most correlated with genetic structure. These factors are related to contemporary landscape configuration, which may not necessarily relate to the genetic structure observed.

Historic landscape configuration was not used in this study. The genetic structure observed in McCann's skink may be attributed to previous landscape configurations. It would be worthwhile producing a pre-human land use resistance surface to compare to the genetic structure of both species. Using a pre human resistance surface could identify barriers to dispersal or corridors of habitat that may have facilitated gene flow between populations of McCann's skink in the past, with the genetic structure related to past, rather than current, landscape configuration, which is the case for the bush cricket *Metrioptera roeseli* (Holzhauer et al. 2006). Greater sampling may be required to fully tease out the relationship of the McCann's genetic data to the landscape. However, this is out the scope of this study, as the main aim was to compare the effect of landscape features on population connectivity in these congeneric species. Clearly, the same landscape factors do not influence genetic structuring the same way in both species.

4.3 Influence of genetic and landscape structure on morphological patterns

An interesting trait of both of these species, and one reason that they were good candidates for this study, is the morphological variation they exhibit. McCann's are striped in Canterbury, but speckled in Otago, whereas the common skink is speckled in Canterbury and striped in Otago (Freeman 1997). The relationship between morphology, genetics and landscape features was tested. There was no significant relationship between genetic distance and morphological distance. Genetic structuring observed for each species did not correlate with the change in morphology for either species. The lack of a significant relationship between genetics and morphology for either species is not surprising as both species have many different colour morphs documented throughout their range, which has been attributed to the influence of habitat (Patterson & Daugherty 1990; Freeman 1997). However, habitat use and land use type may be influenced by geography.

For the common skink, there was a significant relationship between Euclidean distance and morphology, when the effect of genetic distance and resistance distance was controlled for (Table 3.2.4). The McCann's skink also exhibited this relationship between Euclidean distance and morphological distance, with a significant relationship between resistance distance and morphology when controlling for genetic distance, and without controlling for other matrices (Table 3.2.3, Table 3.2.4). The reason for these relationships could be the change in habitat usage and composition with increasing Euclidean distance, by both species. This relationship could be attributed to habitat partitioning between the different regions, which has been documented with change in habitat use from Canterbury to Otago, as there is no relationship between these genetic data and morphology (Freeman 1997). Geographic patterns were correlated with the morphological patterns observed, with no relationship between genetic distance and morphology.

4.4 Implications of the research for skink conservation

One important reason for undertaking this research is to apply it to management of populations of both species. Such research can identify how connected or isolated populations of a species are, based on the landscape configuration (Schwartz et al. 2007). This study has identified (1) landscape level processes that affect the observed genetic structure and (2) population connectivity. These observations can be used to inform potential management options for these species. Because the resistance surface was correlated with genetic distance for the common skink, certain habitats could be conserved or restored to facilitate connectivity and gene flow between these populations. This research is also significant both these species are widespread, and not critically endangered (Hitchmough et al. 2010), which means that as the results between the two species differ, the results cannot be generalised for critically endangered skink species

Habitat such as grassland, shrublands, tussock and gravel/rock should be restored between the populations of the common skink. By restoring these habitat types as corridor restoration, it will facilitate movement of individuals and gene flow between populations. The main benefit of this is that gene flow can maintain local genetic variation by counteracting genetic drift as well as spreading potentially adaptive genes (Segelbacher et al. 2010). If populations become isolated they can lose genetic diversity, as they are usually much smaller, and are at greater risk of stochastic events decreasing local populations sizes to near extinction (Ellstrand & Elam 1993; Segelbacher et al. 2010). This study can only broadly suggest conservation implementation plans for the common skink, due to the very coarse aspect of the landscape land use type data. Ideally, quantifying the use of all land use types by the common skink and McCann's skink, to infer the most relevant landscape features influencing the isolation, would allow successful implementation of conservation management of species (Segelbacher et al. 2010). However, landscape genetics provides a way in which this information be gathered without the need for large amounts of intensive field work and money (Kool et al. 2013), by testing different hypothesises on which landscape features influence population structure the most.

There was no relationship between habitat configuration and genetic distance for the McCann's skinks. Based on this, no direct conservation implementation can be suggested. However, based on the genetic structure in Otago, a smaller scale landscape genetics study could be carried out there aimed at determining which habitat McCann's skink uses, and creating corridors for dispersal. Gravel and rocky habitats are more preferred by McCann's skink (Patterson & Daugherty 1990; Freeman 1997), so using farmland and fence lines, gravel or stone beds could be placed down. Further work needs to be done to determine how landscape connectivity influences population connectivity in both species.

4.5 Limitations

This study has several limitations. The sampling regime was one of the most limiting factors. For the genetic analysis, samples had to be pooled into distinct populations from different collection sites, which were sometimes located very far apart, between tens to hundreds of kilometres (Figure 2.8.1, Figure 2.8.2). These wide ranging populations that were pooled may not be very representative of the genetic structure, or physical populations for both species across the range of sampling. To strengthen any trends observed and reduce the chance of erroneous conclusions, sampling design should address issues of local and spatial autocorrelation influencing the results (Schwartz & McKelvey 2009). Sampling locations should have at least 5 specimens sampled for each species, with 10 or more samples being more representative of the population at each site (per comm. Marie Hale). An increased sampling size and randomised sampling strategy would also allow and more

robust statistical analysis to be completed, accounting for impacts of sampling on genetic data (Schwartz & McKelvey 2009). Samples were also collected from sites where they were expected to be found, which means sampling is not randomised (Segelbacher et al. 2010), however this is logistically more reasonable due to time and money constraints. Also, the high impact DoC permit only allowed 100 individuals to be collected, as animal welfare is an issue and over collection may have a negative effect on the sampling populations. The matrix analysis used in this study does not require multiple individuals from a site, so to improve the quantity of data, and strengthen trends observed, adding more microsatellite loci provides extra information, without extra sampling. However, there is no quantitative study showing the effect of tail tipping on fitness of skinks, so it is not known how negative the effect is of sampling populations.

Genotyping error is another source of error that can limit the results of the analysis (Bonin et al. 2004; Van Oosterhout et al. 2004). A positive control (Segelbacher et al. 2010) was not run with every sample, due to extra cost so variation could not be explicitly quantified, however there was no evidence of genotype error in the data, based on the genotype results given and the GenAlEx analysis. Small error rates in microsatellite genotype scoring from human error, can lead to a number of incorrect multilocus genotypes (Selkoe & Toonen 2006). This was mitigated by rechecking and re-scoring the microsatellite data, without looking at the initial scoring, which showed high levels of successful scoring (see Table 2.4.2). Any loci with large error rates, over 10% were re-scored. Some of the initial error was attributed to alleles being scored differently in each case e.g. a peak that was 166.5 could have been scored 166 or 167. However, all alleles were checked for consistency, and any peak observed within 1bp was scored the same, so peaks of similar size were scored them same, as it is uncommon to have alleles 1 bp apart.

The use and relevance of Mantel and partial Mantel tests, used in this study to compare distance matrices, has been the subject of some controversy in the population ecology and population genetics literature recently. Many authors have critiqued this method for having inflated type 1 error and low inferential power when samples are spatially structured (Raufaste & Rousset 2001; Balkenhol et al. 2009; Guillot et al. 2009). Based on this, p-values produced using partial Mantel tests may be subject to type I error, leading to false conclusions (Raufaste & Rousset 2001). Other authors have refuted these findings, claiming that the results of their tests do not support the criticism (Castellano & Balletto 2002). Mantel tests are justified for landscape genetics because studies that have used it produced meaningful results (Coulon et al. 2004). The analysis in this study has produced significant results that appear to make sense; however, future analysis should investigate hypothesis testing options, such as mixed effect models that can incorporate spatial and covariance structure of allele frequencies (Manel & Holderegger 2013).

4.6 Future work

An alternative method for landscape genetics could be an approach which looks at the genetic structure first and then overlaying it on landscape configuration. This could be done using Bayesian assignment tests from software like STRUCTURE to determine optimal clusters of genetic groups based on genotype data (Evanno et al. 2005; Segelbacher et al. 2010). Then different landscape features could be modelled to see which correlate the best with the genetic groups. Pritchard et al. (2000) showed that based on sampling scheme, this method can result in different clustering, which will influence the real world applicability when it comes to comparing landscape structure to genetic structure. The data presented here should also be analysed with STRUCTURE to compare against the GenAlEx output, but that is beyond the scope of this dissertation.

Further investigation is warranted to determine the cause of morphological variation in these species between Canterbury and Otago. It would also be worth reanalysing the pattern data against a range of different resistance surfaces, or focussing in on specific habitat use. It would be worth mapping the morphological patterning onto a phylogeny of New Zealand skinks dating back millions of years, to trace the deeper evolutionary history of this trait.

Quantifying the effect of landscape on population connectivity can be solved by sampling for skinks in all habitat types, collecting skink tail tips and habitat use data. This means would mean that the relative effect of every habitat is quantified with data, and skinks can be collected randomly from any habitat they are observed in. This would help to randomise the sampling scheme and strengthen the criteria used to create resistance surfaces based on current configuration.

There are several approaches that could further strengthen the trends observed in this study. Increasing sample size replication, and incorporating a random sampling design, that can capture the current habitat usage of both skink species, will improve the strength of the picture of genetic structuring in both species, however is not practical (see above). Creating more realistic landscape parameters to correlate with genetic data will provide greater real world application for conservation management. Creating more realistic landscape resistance surfaces and producing and testing many different models of landscape configuration, will better elicit explanations for how landscape features influence genetic structure in both common and McCann's skink. Finally, comparative landscape genetics should be applied to more closely related, sympatric taxa, to test whether the results of this study are specific to skinks, or generalizable for other taxa.

4.7 Conclusions

This study has highlighted the usefulness of comparative landscape genetics. If this study was carried out using only morphological data, the conclusions reached about population structure would have

been different. Most landscape genetics studies have focussed on single species population structure (Segelbacher et al. 2010; Storfer et al. 2010; Manel & Holderegger 2013), which is not informative for how generalizable the results are for other taxa. This study has shown that sympatric species with similar ecology can have different population structures and connectivity that is influenced by different features of the landscape, or undetermined features in the case of the McCann's skink. This study model should be applied in future work, to see if these results are consistent for other taxa, using more complex statistical methods such as Bayesian analysis.

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