Integrating species distribution models, genetics and morphology to infer species dynamics of New Zealand *Phaulacridium* grasshoppers

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Abstract

Species comparison studies have used a number of different methods that can contribute to our understanding of processes that influence the differences and similarities observed between species. This thesis describes the geographic distribution, spatial genetics, and morphology of two New Zealand *Phaulacridium* grasshoppers, the widespread *P. marginale* and the restricted *P. otagoense*. The primary focus was on *Phaulacridium* populations from the region of the southern South Island where the two species ranges overlap, for the purpose of examining the evolutionary and ecological interactions of the species.

The geographic distribution of the two species was analysed using the recorded and potential modern distribution of *Phaulacridium* grasshoppers. Models of environmental envelopes for each species demonstrated that the potential distribution of *P. marginale* covered the majority of New Zealand. In contrast, the potential distribution of *P. otagoense* is restricted to patches of land primarily in the southern South Island where this species is known to occur.

The phylogeographic structure of *Phaulacridium* species was analysed using dense population samples. Two main mtDNA COI sequence groups were found, one was shallow but geographically widespread, while the other was more diverse but geographically restricted. Within the southern South Island region both mitochondrial lineages co-occur within a single location. Demographic history analysis suggested that the widespread range of *P. marginale* is the result of recent population, and the restricted *P. otagoense* was recently represented in large populations.

The morphological variation of *Phaulacridium* grasshoppers was explored using traditional and geometric techniques. Two distinct morphotypes were apparent, the larger morph was geographically widespread and the smaller morph was restricted to the southern South Island. Both morphotypes co-occur in locations within the southern South Island region. Furthermore, several individuals could not be classified into a discreet morphotype, suggesting that these individuals had a mixture of morphological features, as expected of a hybrid.

Comparing the morphological and genetic data from the current study demonstrates the first reported case of introgression between *P. marginale* and *P. otagoense*. It is evident that *Phaulacridium* F_1 hybrids exist in the wild, however it is unknown whether these F_1 hybrids are fertile and also if F_2 hybrids (backcrossed from parental species or F_1 hybrids) are viable and fertile.

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Contents

List of Figures

List of Tables

Chapter 1

General introduction

Chapter 1: General introduction

New Zealand is a continental temperate island in the Southern Hemisphere that has suffered a range of geological catastrophic events, which has influenced the distribution and diversity of plants and animals. New Zealand underwent repeated cycles of glaciation during the Pleistocene that covered up to 30% of the South Island with ice (Wallis and Trewick 2009). However, large areas of the country went unscathed and became refugia for a range of New Zealand fauna and flora. This is seen in several regions, such as Nelson-Marlborough and Northland, where many species have been shown to exist along with high genetic diversity (Marshall *et al.* 2009; Buckley *et al.* 2010). Reforestation began as the glaciers retracted as the result of climate warming, and recently the majority of the country was covered by forest (Trewick and Morgan-Richards 2009). This allowed the expansion of forest invertebrates south (e.g. tree weta, Bulgarella *et al.* 2014). Widespread deforestation after the arrival of humans (~1260 AD) resulted in the conversion of native forest to open vegetation (McWethy *et al.* 2014).

New Zealand is home to a range of Orthoptera, such as cave weta (Raphidophoridae), true weta (Anostostomatidae), crickets, and grasshoppers. Grasshoppers are a particularly interesting Orthoptera group due to their huge genomes and phenotypic variation (Bensasson *et al.* 2001). There are five genera of flightless New Zealand short-horned grasshoppers (Orthoptera: Acrididae), of which four are endemic (Bigelow 1967). The majority of the fifteen New Zealand species are located in the South Island in the subalpine native grasslands above the tree line (Bigelow 1967). Prior to the arrival of humans, but post-glacial most of New Zealand was covered in dense forest (McGlone 1985; McGlone *et al.* 1995, 2001). Therefore, grass (grasshopper habitat) was mostly confined to the mountain ranges of the South Island (Dowle *et al.* 2014). Species that occurred at lower altitudes would have been limited to areas of grassland patches associated with disturbed environments around rivers and frost flats (Goldberg *et al.* 2015). Forest destruction in the wake of human arrival may have allowed the expansion of lowland grasshoppers into previously unoccupied areas.

The grasshopper genus *Phaulacridium* Brunner v. Wattenwyl, 1893 comprises of two species in Australia [*P. vittatum* (Sjöstedt, 1920) and *P. crassum* Key, 1992], one on Lord Howe Island (*P. howeanum* Key, 1992) and two in New Zealand [*P. marginale* (Walker, 1870) and *P. otagoense* (Westerman and Ritchie, 1984] (Key 1992). The geographic distribution, morphology, and genetics of two *Phaulacridium* species have been examined (Westerman and Ritchie 1984; Key 1992; Goldberg *et al.* 2015). This study focuses on the two New Zealand *Phaulacridium* species that primarily inhabit lowland habitat up to 1200m above sea-level (Westerman and Ritchie 1984). The larger species, *P. marginale*, occurs in mesic habits throughout mainland New Zealand and many off-shore islands (Westerman and Ritchie 1984). In contrast, *P. otagoense* is restricted to more arid areas in Central Otago and Central Canterbury, South Island (Westerman and Ritchie 1984).

1.1 Thesis objectives and plan

The overall aim for the thesis was to describe and contrast the geographic distribution, spatial genetics, and morphology of two New Zealand *Phaulacridium* species, the widespread *P. marginale* and the restricted *P. otagoense*. The primary focus was on *Phaulacridium* populations from the region of the southern South Island where the two species ranges overlap, for the purpose of investigating the possibility of introgression between the two species.

Chapter 2: Geographic distribution of New Zealand *Phaulacridium* grasshoppers

Species distribution can be influenced by a number of different abiotic and biotic factors. The importance of these factors can be inferred through species distribution models which can indicate the potential past, present and future distribution of a species' range. Chapter 2 describes and contrasts the geographic distribution of the two grasshopper species by analysing the recorded and potential modern distribution of New Zealand *Phaulacridium*. And using abiotic variables, the environmental envelopes of *P. marginale* and *P. otagoense* were observed. Additionally, the preferred temperature, a factor known to affect species

distribution and physiological behaviours, was tested in the lab using a temperature gradient.

Chapter 3: Spatial genetics of New Zealand *Phaulacridium* grasshoppers

Widespread versus restricted congeneric species generally show different trends of genetic variation. However, historical factors, such as range shifts, may have an overriding effect on the extent and distribution of genetic variation observed in species. In Chapter 3 the phylogeographic structure of *Phaulacridium* species was analysed using population samples. Additionally, the demographic history of each species was also examined.

Chapter 4: Morphological variation of New Zealand *Phaulacridium* grasshoppers

Traditionally the taxonomic classification of organisms was based on the description of their morphology. Advancing technology has allowed for morphological variation between species to be tested through geometric morphometric techniques, along with the more traditional morphometric methods. Chapter 3 explored the morphological variation of *Phaulacridium* grasshoppers using traditional and geometric methods.

Chapter 5: General discussion and future directions

Chapter 5 summarises the main findings of the above research chapters. This chapter specifically discusses the comparison of genetic and morphological data, particularly regarding evidence of introgression through the mismatch of morphology and genetic data. Lastly, this chapter identifies and discusses future research perspectives for understanding the potential introgression between New Zealand *Phaulacridium* species.

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Chapter 2

Geographic distribution of New Zealand *Phaulacridium* grasshoppers

Chapter 2: Geographic distribution of New Zealand *Phaulacridium* grasshoppers

Abstract

Species distribution can be influenced by a number of different abiotic and biotic factors. The importance of these factors can be observed through species distribution models which can indicate the potential past, present and future distribution of a species' range. The present study aimed to describe the geographic distribution of two New Zealand *Phaulacridium* species by analysing the recorded and potential modern distribution of the grasshoppers. The environmental envelopes of *P. marginale* and *P. otagoense* were observed using abiotic variables. The preferred temperature of *P. marginale*, a factor known to affect species distribution and physiological behaviours, was tested in the lab using a temperature gradient.

Models of environmental envelopes for each species demonstrated that the potential distribution of *P. marginale* covered the majority of New Zealand. In contrast, the potential distribution of *P. otagoense* is restricted to patches of land primarily in the central/southern South Island where this species is known to occur. The Cromwell and Alexandra areas in the South Island had a high probability of both species being present. Suitable habitat for *P. otagoense* is present in the Awatere Valley of northern South Island, however, this species has only been recorded as far north as the Mackenzie Basin.

Phaulacridium marginale exhibited a bimodal distribution of temperature preference. Some individual grasshoppers changed their preferred temperature between observations. It is possible that the observed bimodal distribution detected for this species is the result of the difference in optimal temperatures required for feeding and digestion.

2.1 Introduction

In recent years, predictive modelling of species distribution has become a valuable tool used to address various issues in evolution, ecology, conservation biology, biogeography, and climate change research (e.g. Araujo *et al.* 2006; Medley 2010; Bulgarella *et al.* 2014). Species distribution models (SDM) are empirical models relating observed occurrence localities to environmental/ecological predictor variables based on statistically or theoretically derived response surfaces (Guisan and Zimmermann 2000). Occurrence data exist in the form of georeferenced coordinates of latitude and longitude for confirmed species localities that typically derive from vouchered museum or herbarium specimens and from published reports (Anderson *et al.* 2003). The environmental/ecological variables used for the modelling normally examine relatively few of the possible ecological-niche dimensions (Anderson *et al.* 2003).

The subsequent model is then projected onto a map of the study region, allowing the species' potential geographic distribution to be shown (Peterson and Vieglais 2001). The models are generally based on the species' fundamental niche (Hutchinson 1957). Therefore, some parts designated by the model as regions of potential presence may be occupied by closely related species, or may signify suitable areas to which the study species has unsuccessfully dispersed to or in which the species has gone extinct (Anderson *et al.* 2003). This has evolutionary and ecological applications for comparing the potential and realised distributions of species.

Temperature is one of the main environmental factors affecting organisms because it influences all biological rates and functions. Ectothermic animals are particularly sensitive to the physiological effects of temperature variation, and have to exploit solar radiation by adjusting their behaviour to maintain an optimal body temperature. Animals typically move to their preferred ambient temperature when a choice of a temperature gradient is available (Forsman 2000; Samietz *et al.* 2005; Harris *et al*. 2012, 2013). The ability to regulate body temperature behaviourally to attain a preferred temperature range has fundamental consequences for features such as the rate of development, reproduction,

locomotion, habitat selection, predator avoidance, and the rate of feeding and digestion (Heinrich 1993; Blandford and Thomas 2000; Forsman 2000; Forsman *et al.* 2002; Harris *et al.* 2013). A majority of Orthoptera species, in particular many members of the Acrididae, have proven to be active behavioural thermoregulators; individuals move at a microhabitat scale to optimize conditions (Springate and Thomas 2005; Harris *et al.* 2012). Experimental thermal gradients have been used to test the preferred temperature of *Phaulacridium vittatum* (Harris *et al.* 2013) among other Acrididae (Chapman 1965; Forsman 2000; Samietz *et al.* 2005; O'Neill and Rolston 2007).

The grasshopper genus *Phaulacridium* occurs throughout Australasia, with two species endemic to New Zealand. *Phaulacridium marginale* (Walker 1870) is found widely through New Zealand, including Stewart Island and various other off-shore islands up to 1200 above sea-level (Westerman and Ritchie 1984; Key 1992). The species prefers open grassland habitat with low erosion rates, mostly produced by anthropogenic clearing of forests (Westerman and Ritchie 1984; Key 1992). In contrast, *P. otagoense* (Westerman and Ritchie 1984) is restricted to the Mackenzie Basin and Central Otago regions of the South Island (Westerman and Ritchie 1984; Key 1992). This species occurs predominantly along the Ahuriri and Waitaki river courses, in the Lindis Valley, the Clutha Valley to Alexandra, and in the Manuherikia Basin (Westerman and Ritchie 1984; Key 1992). *Phaulacridium otagoense* prefer microclimatically more arid, degraded habitats generated by grazing and erosion of the hillsides (Westerman and Ritchie 1984; Key 1992). Populations of the two *Phaulacridium* species only intermix where the two distinct habitat types are adjacent, such as damp gullies and stream courses that pass through exposed north- and west-facing slopes as observed in the Lindis Valley (Westerman and Ritchie 1984; Key 1992).

Here I examine in detail the geographic distribution of New Zealand *Phaulacridium* species by analysing the known distribution and potential modern distribution of these grasshoppers. Current distributions suggest that the environmental conditions underlying the predicted distributions of *P. marginale* and *P. otagoense* will be different. In addition, I use laboratory experiments to assess temperature

preferences of *Phaulacridium* grasshoppers collected from different New Zealand locations.

2.2 Methods

Recorded distribution

The distributions of *P. marginale* and *P. otagoense* were mapped using all available locality records that were deemed reliable (i.e. accompanied by location data). Specimen locations came from numerous sources including published work, Crown Pastoral Tenure Reviews, and Massey University Phoenix Group's collection. Only mainland New Zealand locations were used for mapping the known distribution although *P. marginale* does occur on the Chatham Islands (Goldberg *et al.* 2015). All available specimen records yielded 245 localities, 219 of those for *P. marginale* (see Appendix 1.1) and 26 for *P. otagoense* (see Appendix 1.2). Locality records of *Phaulacridium* grasshoppers were mapped onto a New Zealand hypsometric raster (100m) (Koordinates n.d.) using Quantum GIS (QGIS) software v2.6.0 (QGIS Development Team 2015) under the coordinate reference system WGS 84 EPSG: 4326.

Potential distribution

The potential inferred distributions of *P. marginale* and *P. otagoense* were analysed using climate data from the Land Environments of New Zealand (LENZ) database (Leathwick *et al.* 2002) obtained from Land Resource Information Systems Portal (LRIS Portal) (LRIS Portal n.d.). Preliminary analyses indicated that five climate layers were most influential for New Zealand *Phaulacridium* grasshoppers, and these were incorporated into the modelling: annual water deficit (mm); mean minimum temperature of the coldest month (July, representing winter minimum temperature, $^{\circ}$C); October vapour pressure deficit (when persistent westerly winds result in strong geographical variation in vapour deficits across New Zealand, kPa); mean annual temperature ($^{\circ}$C); and mean annual solar radiation (kJ/day/m^2) (Leathwick *et al.* 2002, 2003). These climate layers are derived from mathematical surfaces (thin-plate splines) that use information from observed meteorological

data (climate, location and elevation, from 1950 to 1980) (Leathwick *et al.* 2002, 2003).

Species distribution models were generated with Maxent v3.3.3k (Phillips *et al.* 2006; Phillips and Dudík 2008), which uses the maximum entropy method for modelling presence-only species distribution data, by distinguishing presence from random. The model was based on 217 georeferenced localities for *P. marginale* and 26 for *P. otagoense*. The default convergence threshold was used in Maxent, maximum iterations were increased to 5000 to allow the algorithm to converge naturally, and regularization values and functions of environmental variables were selected automatically by the program based on sample size. The modern distributions of each species were modelled 15 times, using 90% of the localities to train the model and 10% to test the model. Jackknifing was performed to measure the importance of the climate variables. Model performance was evaluated using the area under the receiver operating characteristic curve (AUC), which reflects the model's ability to distinguish between presence records and random background points (Hanley and McNeil 1982; Phillips *et al.* 2006). The AUC varies between 0.5 (localities equally likely to be designated presence or absence) to 1 (perfect assignment of presence and absence), although AUC is below 1 in practice (Phillips *et al.* 2006). Values above 0.7 indicate adequate determination (Swets 1988). Model predictions were visualised in QGIS v2.6.0 (QGIS Development Team 2015).

Preferred temperature experiment

A preliminary study into the preferred temperature of adult *P. marginale* grasshoppers was undertaken. *Phaulacridium otagoense* grasshoppers were not used due to a limited sample size. Live *P. marginale* grasshoppers were collected by net or hand from six locations around New Zealand during two time periods (South Island 14th–16th February, 2015; North Island 8th–14th March, 2015) (Table 2.1). All grasshoppers were held in the laboratory under identical conditions for a minimum of seven days prior to the experiment. The populations were housed at room temperature in plastic containers with mesh netting to allow air flow and an overhead heat lamp that was turned on for a period of four hours each day. Each

day a combination of plantain (*Plantago major*) leaves, exotic grasses and clover leaves was provided along with water.

Table 2.1: Source locations of adult *Phaulacridium marginale* grasshoppers used for the preferred temperature experiment.

Population Location	Sample Size	Sex	
		Male	Female
Palmerston North	10	5	5
Marshall Road, Alexandra	1	1	0
Cardrona Ski Field	5	0	5
Lake Pukaki Dam	1	1	0
Hokio Beach, Levin	8	3	5
Pohangina Valley	14	4	10
Total	**39**	**14**	**25**

The experiment apparatus was designed to generate a temperature gradient along an aluminium plate 100cm long by 20cm wide (Figure 2.1) modified from similar approaches (Dyck 1969; Forsman 2000; Harris *et al.* 2013). The plate was partitioned into four separate 5cm wide longitudinal runways using Perspex strips. A Perspex box, 60cm long by 20cm wide and 10cm high with a movable Perspex lid was placed onto the gradient to prevent loss of grasshoppers. A centre hole in the lid, allowed animals to be introduced into the runways and two elongated slots either side of the centre hole (25cm long by 1cm wide) for air circulation; mesh was placed on top of the lid to prevent escape. The temperature gradient was established in the aluminium plate by placing one end on a thermostatically controlled electronic hot plate and the other on a refrigeration plate. The temperature ranged from 10˚C to 50˚C in the section of the plate accessible by grasshoppers during the experiment, and remained stable throughout. The temperature gradient was set up in a 15˚C room with fluorescent tubes in the ceiling to provide a constant overhead light source, and a handheld IR thermometer (Digitech QM7221) used to monitor conditions.

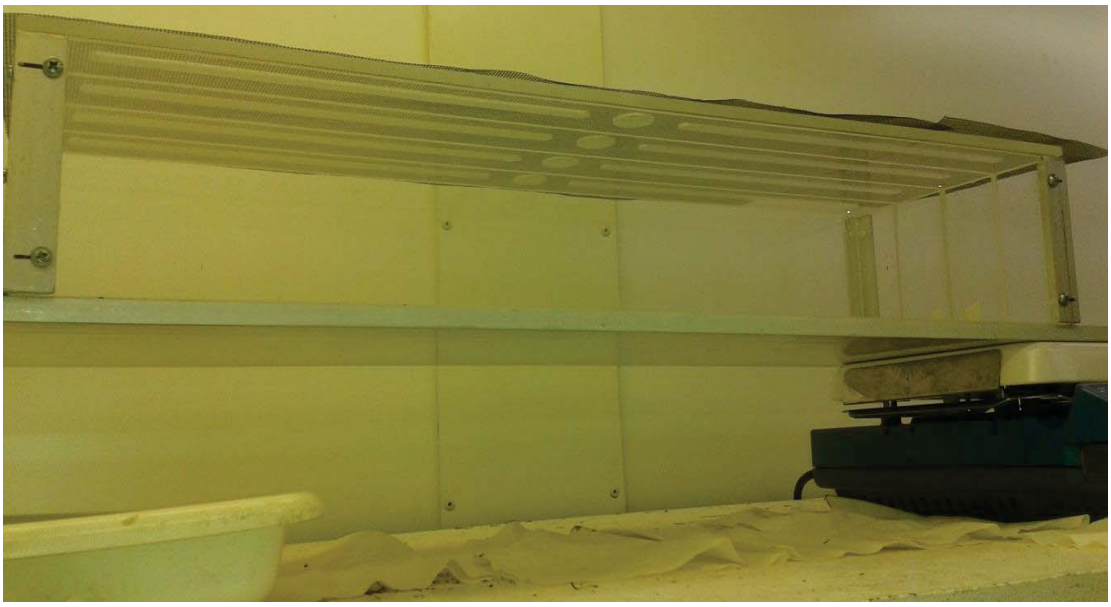

Figure 2.1: Gradient apparatus used for preferred temperature experiment on *Phaulacridium marginale* individuals.

An hour before each experimental run, grasshoppers were transferred into small plastic containers to allow for easy transfer to the centre of the gradient without handling. Runs were performed for 30 minutes, with the surface temperature and behaviour of the grasshoppers being measured at 15 minute intervals. Surface temperature was measured at the point at which the grasshopper came to rest, using an infrared thermometer (Digitech QM7221). Individuals were able to move freely within the gradient. For each run a mixture of individuals of different sexes and colour morphs were included, to account for confounding effects of experimental run or gradient. At the end of a run, an individual that was on the perspex wall or showed lack of activity by not jumping to escape a probe was excluded. The grasshoppers were then returned to the plastic vivarium with fresh plantain leaves and given a code; this was to allow for a total of three experiment trials to be run over a period of days to determine if there was individual variation in the preferred temperature of the grasshoppers. After the three experiment trials were finished grasshoppers were frozen at -20°C.

The preferred temperature of *P. marginale* grasshoppers across the three experimental trials at each 15 minute time period was visualized by bar graphs using the program R v3.1.0 (R Core Team 2014). Male and female preferred temperatures were cexamined to see if the sex of a grasshopper influenced temperature preference. The largest difference in preferred temperature across

the three experimental trials was calculated for each individual to determine if an individual had a similar preferred temperature across the three trials.

2.3 Results

Recorded distribution

The known modern occurrences of *P. marginale* and *P. otagoense* were mapped with a total of 245 data points (Figure 2.2). *Phaulacridium marginale* were recorded throughout both major islands of New Zealand as well as on Stewart Island and many surrounding off shore islands (Figure 2.2). There were few recorded sightings of *P. marginale* in the Northland, Auckland, and Taranaki regions of the North Island, and the West Coast and Southland regions of the South Island. These low sightings could be the result of low sampling or represent areas with true abundance of *P. marginale*. In contrast, the distribution of *P. otagoense* was restricted to the central/southern South Island (Figure 2.2). Mackenzie Basin, Lindis Pass/Valley, and Alexandra/Cromwell areas were the three areas where *P. otagoense* was recorded.

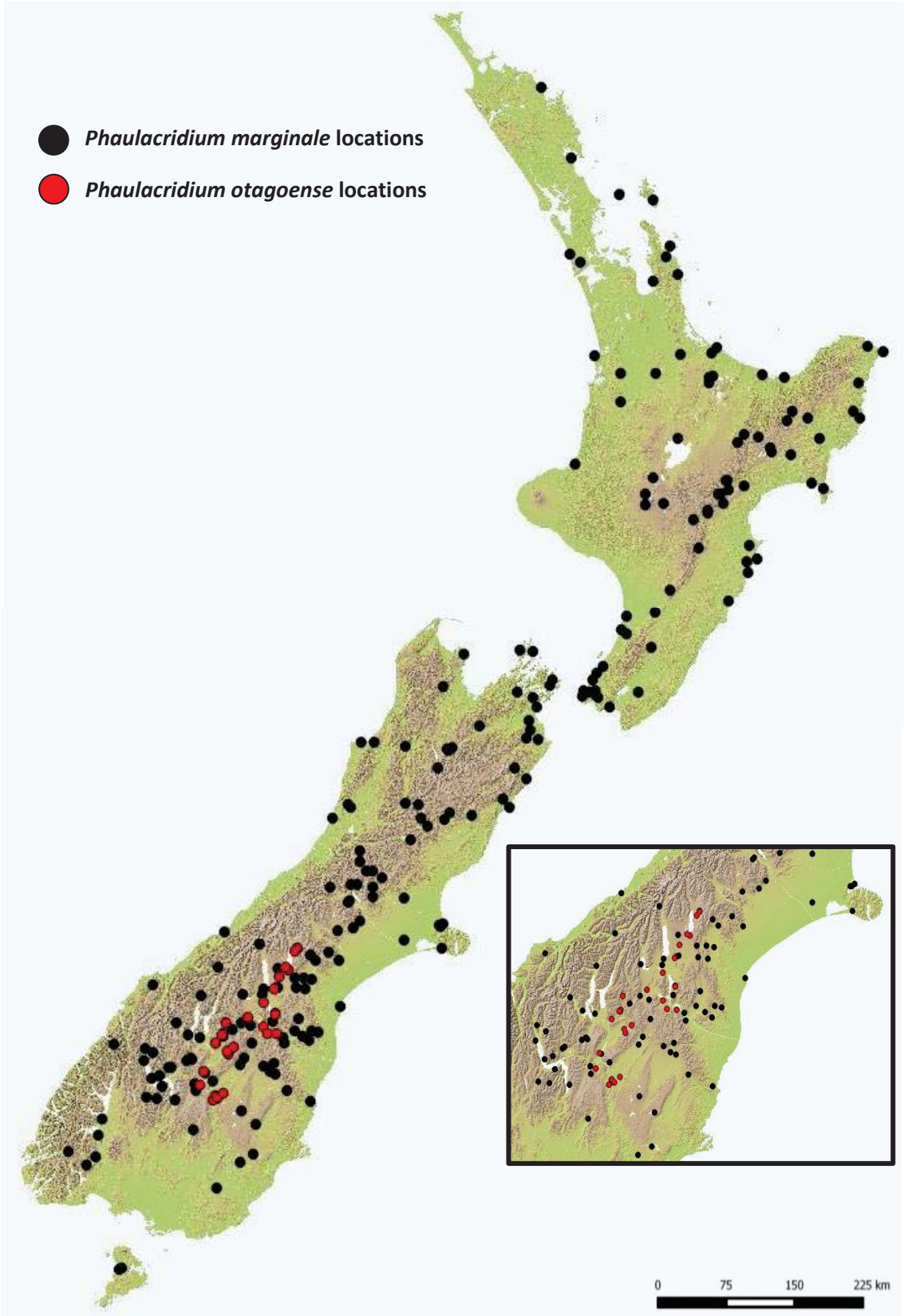

Figure 2.2: Known mainland New Zealand locations of grasshoppers from the genus *Phaulacridium*, *P. marginale* (black circles) and *P. otagoense* (red circles), with a close up of the central/southern South Island where the two species co-occur.

Potential distribution

The potential distribution for *P. marginale*, modelled using Maxent, encompassed the majority of New Zealand (Figure 2.3). The test AUC (the area under the receiver operating characteristic curve for the test data) for the SDM, averaged across all 15 runs, was moderately low (0.626, standard deviation = 0.059, range = 0.518-0.738). Analysis of the climatic variable contribution showed that for the distribution of *P. marginale*, October vapour pressure deficit was the variable of highest importance (Table 2.2). Mean minimum temperature of the coldest month and mean annual temperature were the second and third most important predicators, with the annual water deficit the least (Table 2.2). October vapour pressure deficit was the variable with the highest training gain when used in isolation, according to jackknife tests of variable importance, suggesting that this variable contained the most useful information by itself. Furthermore, this variable also decreased the gain the most when it was omitted; therefore the variable appears to have the most information that isn't present in the other variables.

The potential distribution for *P. marginale* includes the majority of New Zealand, with low probability of presence in areas of high elevation and parts of the Bay of Plenty and Waikato regions of North Island, the West Coast, Fiordland, and Southland regions of the South Island (Figure 2.3). Areas of relative high probability of *P. marginale* occurrence included the base of the Mahia Peninsula, East Cape, Fairlie area and along the eastern coastline of southern North Island and the northern South Island. The highest probability of *P. marginale* presence was in the Alexandra and Cromwell areas of Central Otago (Figure 2.3).

Table 2.2: Relative contributions and ranks of the five climatic variables to the Maxent models for New Zealand *Phaulacridium* species.

	Percentage Contribution		**Climatic Rank**	
	P. marginale	*P. otagoense*	*P. marginale*	*P. otagoense*
Annual solar radiation	13.3	4.2	4	3
Annual water deficit	3.6	56.7	5	1
Mean annual temperature	16.5	0.7	3	4
Mean minimum temperature of the coldest month	21.0	38.1	2	2
October vapour pressure deficit	45.6	0.3	1	5

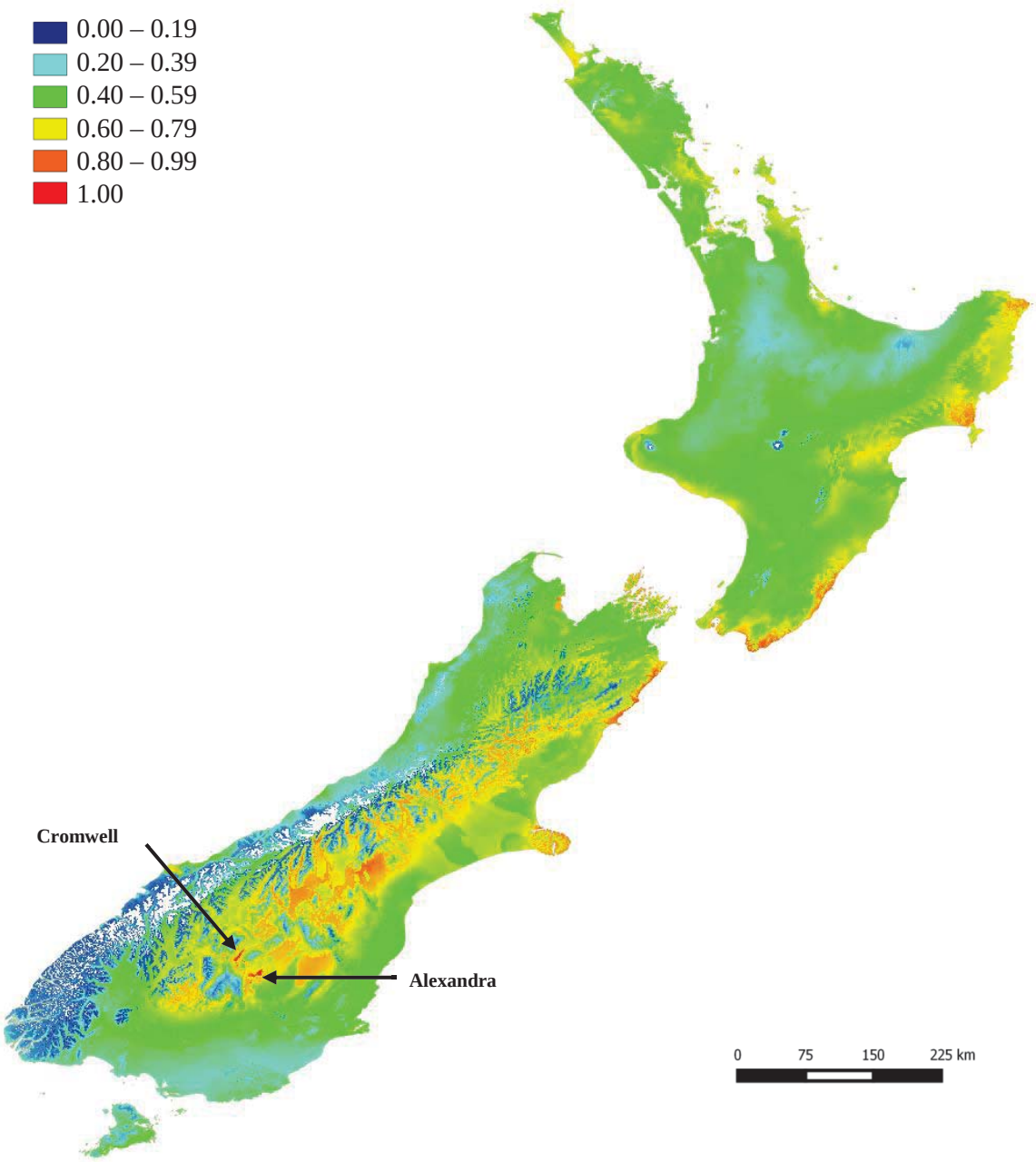

Figure 2.3: Species distribution model projections for the current distribution of the New Zealand grasshopper, *Phaulacridium marginale*. Environmental variables for the Maxent model are: annual water deficit; mean minimum temperature of the coldest month; October vapour pressure deficit; mean annual temperature; and mean annual solar radiation. Warmer colours indicate land inferred to be within the niche and colder colours represent land outside of the niche. An area where no environmental variable data is available is white. The areas of highest probability of *P. marginale* occurrence are shown.

Species distribution models for *P. otagoense* were more restricted than *P. marginale* with limited areas only in the South Island (Figure 2.4). The average test AUC for the replicate runs was 0.981 (standard deviation = 0.009, range = 0.980-0.982). In contrast to *P. marginale*, the climatic variable of most importance to the distribution of *P. otagoense* was the annual water deficit (Table 2.2). Mean minimum temperature of the coldest month was the second most important predicator with the October vapour pressure deficit being the least (Table 2.2). Jackknife tests of variable importance that annual water deficit was the variable with the highest gain when used in isolation and also the most gain decreased when it was omitted.

The potential distribution for *P. otagoense* included only small patches of the South Island (Figure 2.4). The highest probability of the presence of *P. otagoense* was around the Alexandra area and from Cromwell to Tarras. Lake Aviemore, Lake Benmore, and Lake Tekapo of the Mackenzie Basin also have a relatively high presence probability. Furthermore, patches of the Mackenzie Basin and the Ranfurly area had a high potential of *P. otagoense* being present. The potential distribution of *P. otagoense*, based on the Maxent model, suggested that the Awatere Valley in the northern South Island had a high probability of *P. otagoense* occurrence, although this species has only been recorded as far north as the Mackenzie Basin (Westerman and Ritchie 1984; Key 1992).

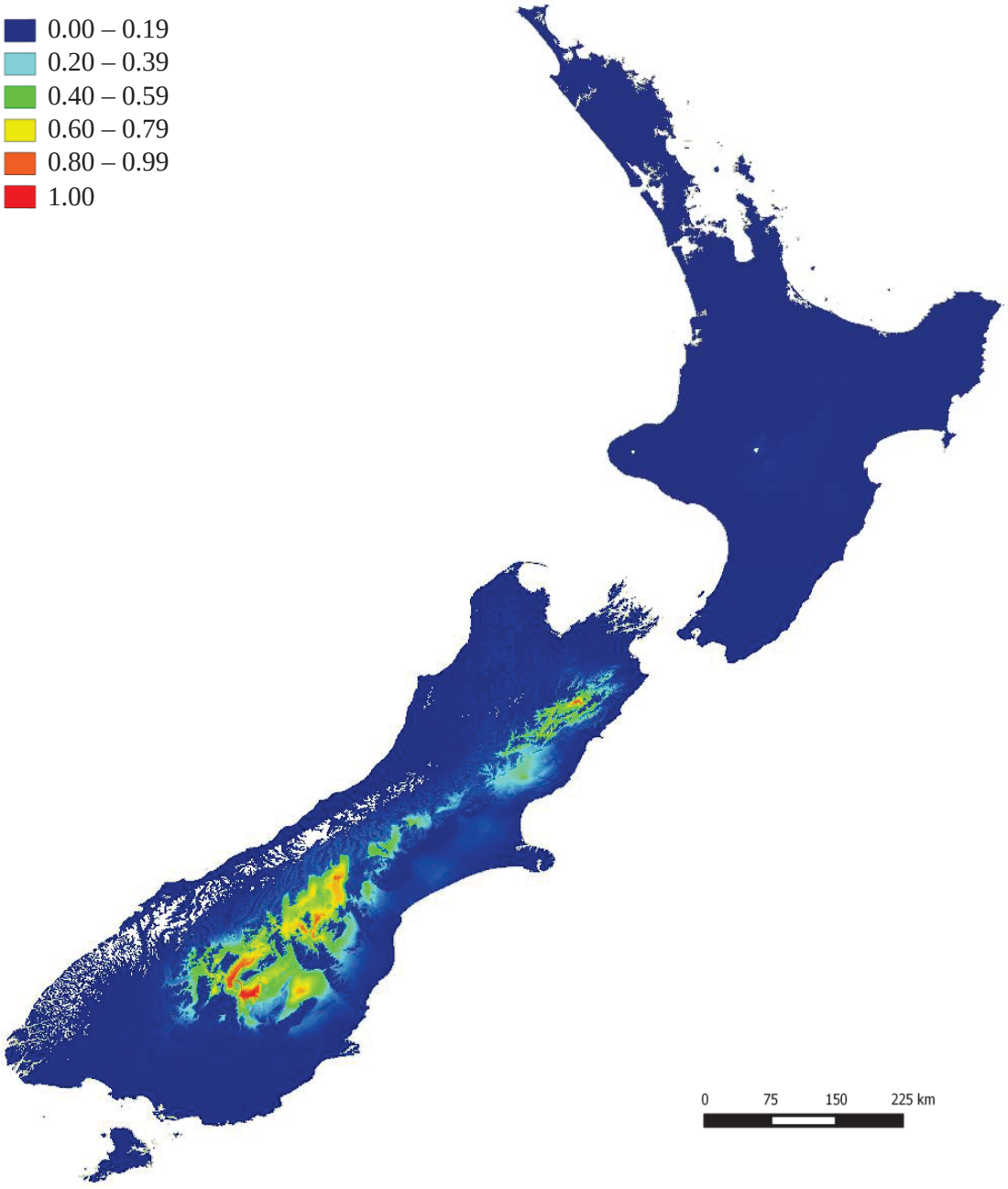

Figure 2.4: Species distribution model projections for the current distribution of the New Zealand grasshopper, *Phaulacridium otagoense*. Environmental variables for the Maxent model are: annual water deficit; mean minimum temperature of the coldest month; October vapour pressure deficit; mean annual temperature; and mean annual solar radiation. Warmer colours indicate land inferred to be within the niche and colder colours represent land outside of the niche. An area where no environmental variable data is available is white.

Preferred temperature experiment

Phaulacridium marginale grasshoppers (n=39) had a wide range of preferred temperatures across the three trials, ranging from 11.3°C to 49.5°C. The distribution of preferred temperatures for *P. marginale* grasshoppers was bimodal in all three experimental trials (Figure 2.5). One peak fell in the range 15.0-25.0°C and the other in the region of 40.0-42.5°C. The lower peak preferred temperature range was 17.5–25.0°C in males (n=14) and 15.0–25.0°C in females (n=25). The high preferred temperature range was 40.0–42.5°C for both male and female *P. marginale* grasshoppers.

The temperatures preferred by a grasshopper at the 15 minutes and 30 minutes intervals were similar. The majority of individuals (77.8%) had a 0-3°C difference between the time intervals across the three trials, with only a few individuals (11.1%) having more than a 10°C difference between the two measurements. Grasshopper behaviour ranged from the abdomen being held flat on the plate with the antennae either up or touching the plate, to the abdomen being slightly raised from the plate with the antennae held up.

The majority of grasshopper individuals showed little difference in the preferred temperature across the three trials (Figure 2.6). However, there were several *P. marginale* individuals that settled at different temperatures during the three experimental trials (Figure 2.6). This would suggest that grasshopper individuals were switching between the two preferred temperature peaks across the period of experimental trials.

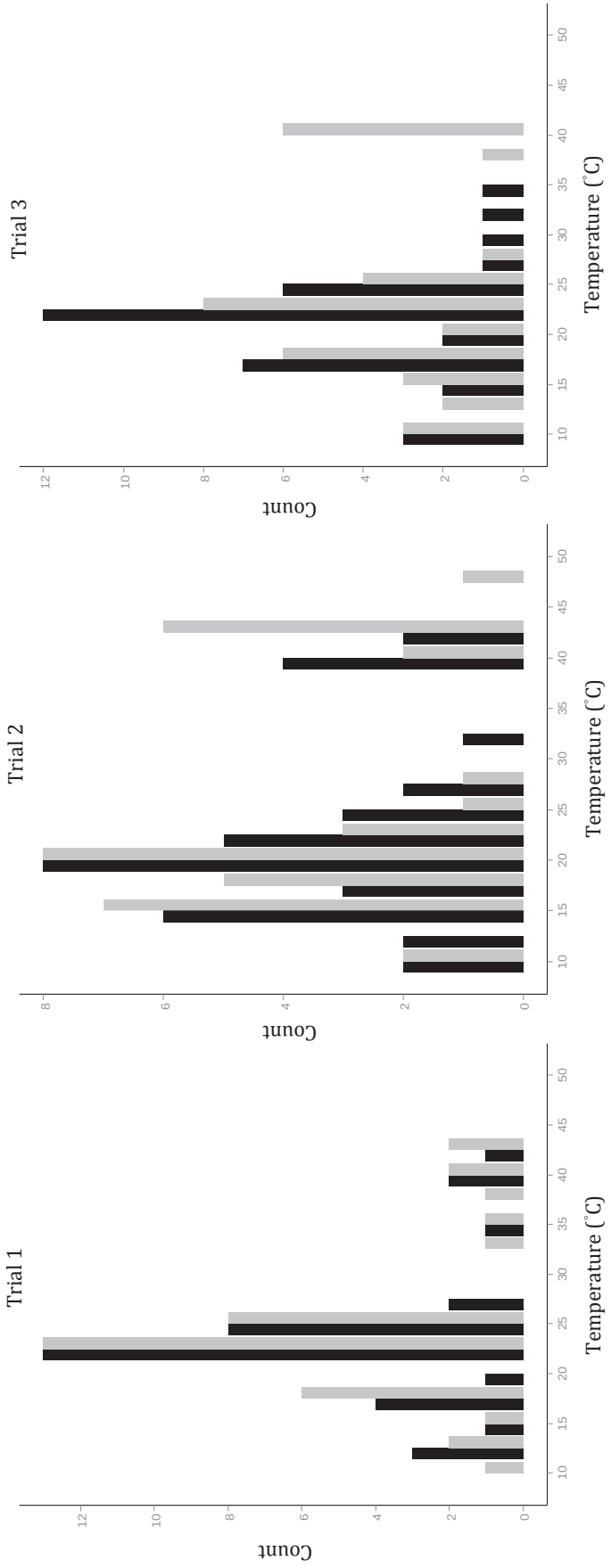

Figure 2.5: Preferred temperature of the New Zealand grasshopper *Phaulacridium marginale*, across a temperature gradient at 15 minutes (black) and 30 minutes (grey) for the three experiment trials.

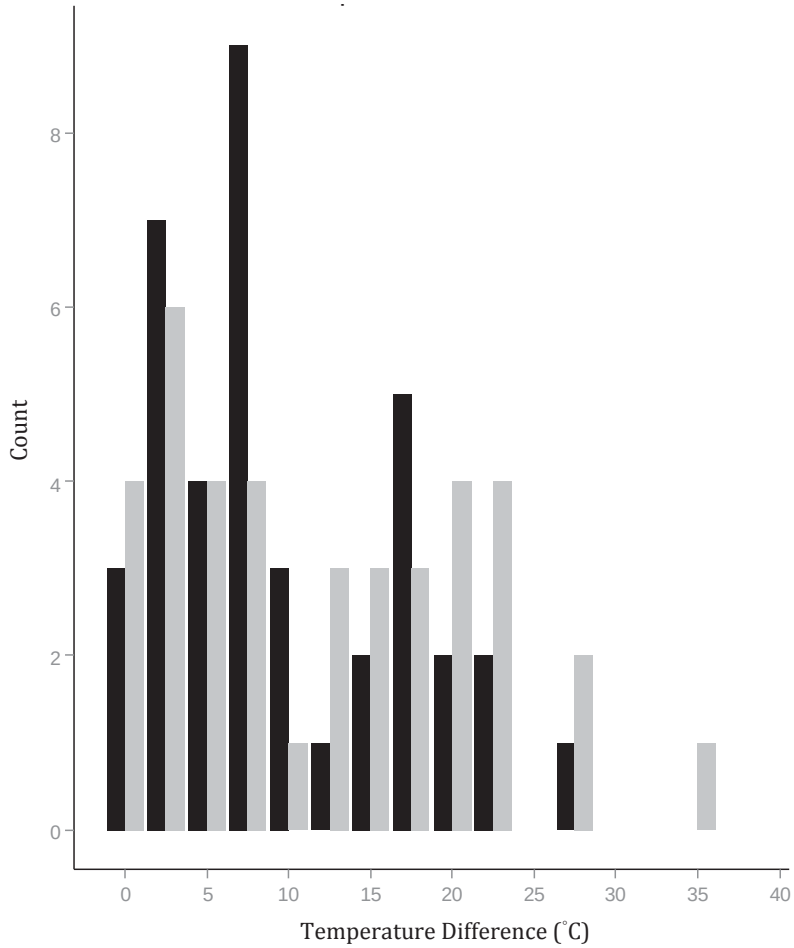

Figure 2.6: Largest temperature difference for the preferred temperature displayed by individual *Phaulacridium marginale* grasshoppers across the three experimental trials at 15 minutes (black) and 30 minutes (grey).

2.4 Discussion

Species distribution models for *P. marginale* demonstrated that its potential distribution covers the majority of New Zealand, which is consistent with the observed occurrences of this species. The SDM for *P. marginale* had a relatively low AUC (0.626), however, this was consistent with the species widespread distribution. As the probability of presence increases steadily with predicator values for widespread species, an accurate model will have low AUC values (Lobo *et al.* 2008). Therefore, the AUC provides information about the distribution of generalist and specialist species, but not about how good the performance of the model (Lobo *et al.* 2008).

In contrast, the potential distribution of *P. otagoense* was limited primarily to patches of land in the central/southern South Island, which is similar to the species known distribution. The modelled distribution of *P. otagoense* also showed that this species had a high potential to occur in the Awatere Valley of northern South Island. The Awatere Valley is one of the driest and sunniest areas of New Zealand (Williams 1989; Marra and Leschen 2004) with conditions similar to the central/southern areas of the South Island where this species is known to occur (Westerman and Ritchie 1984; Key 1992). *Phaulacridium otagoense* may have existed in this northern area sometime in the past but has since gone extinct through competition or habitat modification.

The most influential climate variable affecting the potential distribution of *P. marginale* was the October vapour pressure deficit, although this variable had the lowest influence on the distribution of *P. otagoense*. In contrast, annual water deficit contributed most to the potential distribution of *P. otagoense*, and the least to *P. marginale*. The climatic variable influencing the potential distribution of *P. otagoense* (annual water deficit) is consistent with the species known preference for microclimatically more arid habitats (Westerman and Ritchie 1984; Key 1992).

In reality additional factors not considered in the current modelling mean that *Phaulacridium* grasshoppers rarely occupy all areas with suitable climatic environments. Vegetation type is an obvious feature influencing the distribution of *Phaulacridium* grasshoppers, with the extent and continuity of grassland being

potentially the most influential. Furthermore, it has been hypothesised that widespread deforestation and conversion of native forest to open grassland for agriculture allowed *P. marginale* to expand its range (Goldberg *et al.* 2015). Additionally, soil characteristics (e.g. substrate, particle size, drainage capabilities, and chemical limitations to plant growth) could also influence the distribution of *Phaulacridium*. The influences of vegetation types and soil characteristics are apparent in the known habitat preference of the two species, with *P. marginale* preferring open grassland habitat with low erosion (Figure 2.7 a), and *P. otagoense* favouring more degraded habitats generated by grazing and erosion of the hillsides (Figure 2.7 b; Westerman and Ritchie 1984; Key 1992). Biotic interactions could also influence *Phaulacridium* distributions, through competition with rabbits and invertebrates including other species of grasshoppers or because of predation by vertebrates. Species distributions models using species competition as a factor have been successfully performed using two species of New Zealand tree weta, *Hemideina crassidens* and *H. thoracia* (Bulgarella *et al.* 2014).

a)

b)

Figure 2.7: Examples of habitats used by *Phaulacridium* grasshoppers in New Zealand. a) *P. marginale* habitat at Hokio Beach, Levin, North Island and b) *P. otagoense* habitat at Graveyard Gully, Alexandra, South Island.

Phaulacridium marginale exhibited a bimodal distribution of temperature preference similar to that observed in Tasmanian *P. vittatum* (Harris *et al.* 2013). Furthermore, the preliminary study showed that an individual could move between the two temperature peaks. There are several possible explanations for the bimodal distribution of temperature preferences found here. Differences in the developmental stage and reproductive state can be discounted, as all grasshoppers were adults, and males as well as females exhibited the same pattern. It is possible that feeding behaviours and digestion of *P. marginale* require different temperatures for optimal performance.

The rate of food intake for the grasshopper *Poecilocerus pictus* was shown to increase with increasing temperature, up to 30°C where the food intake started to decline with increasing temperature (Singhal 1979). Another study found that *Melanoplus bivittatus* feeding behaviour was suppressed at 10°C, but the portion of grasshoppers feeding did not vary between 15°C to 35°C (Harrison and Fewell 1995). Digestive enzymes operate best within a narrow temperature range, which is influenced by the maximum temperature an organism will typically experience in the field (Whitman 1988). Temperatures above 30°C were noted in the field in the present study. Furthermore, a higher body temperature has been shown to strongly increase the net energy intake of *M. bivittatus* by increasing digestive tract throughput (Harrison and Fewell 1995). Further work is needed to determine if the bimodal distribution in preferred temperature is the result of feeding behaviour and digestion. Additionally, a similar analysis of *P. otagoense* individuals would determine if the same bimodal distribution occurs and whether there are difference in preferred temperature, as the temperatures of the known locations of *P. otagoense* are generally higher than *P. marginale* (Westerman and Ritchie 1984; Key 1992).

The present study demonstrates that local climate conditions influence the current distribution of New Zealand *Phaulacridium* species. The climate condition that has the greatest effect on the distribution of the widespread *P. marginale* is the October vapour pressure deficit, with the annual water deficit highly influencing the distribution of the restricted *P. otagoense*. Although climate is important, a species distribution is also influenced by a number of environmental and

ecological factors, therefore further work needs to be performed to determine how factors such as vegetation type, soil characteristics, and species competition influence the distribution of New Zealand *Phaulacridium* grasshoppers. Species distribution models show that both species have high potential of being present in the Alexandra and Cromwell areas of Central Otago. Consequently, there is the possibility for gene flow to occur, therefore the phylogeographic structure of these species needs to be analysed.

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Chapter 3

Spatial genetics of New Zealand *Phaulacridium* grasshoppers

Chapter 3: Spatial genetics of New Zealand *Phaulacridium* grasshoppers

Abstract

Widespread versus restricted congeneric species generally show different trends of genetic variation. However, historical factors, such as range shifts, may have an overriding effect on the extent and distribution of genetic variation observed in species. The current study aimed to investigate the phylogeographic structure of New Zealand *Phaulacridium* grasshoppers using dense population samples. Additionally, the demographic history of *P. marginale* and *P. otagoense* was also examined.

Two main mtDNA COI sequence groups were found; the shallow but geographically widespread Group 1 (Lineage I) was considered to represent *P. marginale*, while the more diverse but geographically restricted Group 2 (Lineages II, III, IV) is inferred to be *P. otagoense*. The results also demonstrated that within the southern South Island region, the mitochondrial lineages attributed to *P. marginale* and *P. otagoense* co-occur within a single location. Therefore, it is possible that introgression between the two *Phaulacridium* species could occur.

Demographic history analysis suggested that the widespread range of *P. marginale* is the result of recent population expansion because low genetic diversity is best explained by small population size. In contrast, *P. otagoense* has a restricted range today, but their high genetic diversity suggests that this species was recently represented in large populations.

3.1 Introduction

The geographic range of a species has been regarded as one of the most significant factors influencing genetic diversity and its distribution (Hamrick and Godt 1989). Generally it has been accepted that species with restricted range exhibit lower genetic diversity than species that are more widespread. At equilibrium population size is positively correlated with genetic diversity (Charlesworth 2009) and widespread ranges are often linked to large populations. Species with restricted range are often found in small, isolated populations that are more susceptible to losses of genetic variation due to genetic drift, inbreeding and strong directional selection (Karron 1991; Broadhurst and Coates 2002; Coates *et al.* 2003). Furthermore, founder events associated with recent speciation could reduce genetic variation (Karron 1991; Broadhurst and Coates 2002; Coates *et al.* 2003).

There has been growing interest in the comparison of widespread and restricted congeneric species in regards to their population genetics and conservation biology. Closely related species are likely to share many life history characteristics, that shape population genetic structure, therefore ruling out these characteristics will allow easier identification of the causes of observed differences in their genetic diversity (e.g. Karron *et al.* 1988; Hamrick and Godt 1996; Gitzendanner and Soltis 2000; Dodd and Helenurm 2002; Cole 2003). Such comparisons have highlighted that the trend of lower genetic variation in species with a restricted range is an overgeneralization (Gitzendanner and Soltis 2000). Although some range-restricted species exhibit low genetic variation (e.g. Broadhurst and Coates 2002; Castellanos-Morales *et al.* 2015), others maintain levels of diversity equal to (e.g. Edwards and Wyatt 1994; Takahashi *et al.* 2011), or exceeding those of widespread congeners (e.g. Lewis and Crawford 1995; Chen *et al.* 2007).

This suggests that historical factors may have a critical, and in some cases, overriding effect on the extent and distribution of genetic variation observed in species (Loveless and Hamrick 1988). One expression of this would be that the modern range of a species may not correspond with its past distribution (Stebbins and Major 1965; Kruckeberg and Rabinowitz 1985; Karron 1987). Range-restricted species might have higher genetic variation than expected because they

were once more widespread and have recently declined in range as a result of factors such as climate change, human activity, and competition from other species (e.g. Chen *et al.* 2007). Whereas, range and population expansion might be underway for widespread species that have low genetic diversity (e.g. Hewitt 2004). Most comparative studies using restricted and widespread congeneric species involve plants (see Karron 1987; Gitzendanner and Soltis 2000), whereas there are few studies considering animals (e.g. Black-Tailed Prairie Dogs *Cynomys ludovicianus* and *C. mexicanus*, Castellanos-Morales *et al.* 2015; coral reef fish, Bay and Caley 2011; Ridley sea turtles *Lepidochelys kempi* and *L. olivacea*, Bowen *et al.* 1998; grasshoppers *Sigaus australis* and *S. childi*, Dowle *et al.* 2014).

In New Zealand, *Phaulacridium* grasshoppers are an excellent opportunity to compare genetic variation and geographic structure in a pair of widespread and restricted congener species. Although the genus also occurs in Australia (Key 1992) two *Phaulacridium* species are endemic to New Zealand. These inhabit primarily lowland habitat up to 1200m above sea-level, in contrast to a separate endemic radiation of unrelated grasshoppers in the alpine zone (>1200m) (Bigelow 1967). The larger species, *P. marginale* (Walker 1870), occurs in mesic habitats throughout mainland New Zealand and many off-shore islands (Westerman and Ritchie 1984). In contrast, *P. otagoense* (Westerman and Ritchie 1984) is restricted to more arid areas in Central Otago and Central Canterbury, South Island (Westerman and Ritchie 1984). An assessment of mitochondrial DNA sequence variation in both species showed lower genetic diversity in the more widespread *P. marginale* (12 mitochondrial haplotypes in 65 individuals) compared to the more restricted *P. otagoense* (9 mitochondrial haplotypes in 12 individuals) (Goldberg *et al.* 2015). Furthermore, there was high geographic structure in *P. otagoense* populations, with separate clades for the Mackenzie Basin (Central Canterbury) and Alexandra (Central Otago) area (see Figure 3.1; Goldberg *et al.* 2015). These findings were interpreted as indicating recent population expansion of *P. marginale* and complementary range reduction of *P. otagoense* from a former larger distribution and population size during the Last Glacial Maximum (LGM) (Goldberg *et al.* 2015).

Here I examine in detail the phylogeographic structure of these species by analysing population samples. It is predicted that, at the species level, the restricted *P. otagoense* will show higher levels of genetic variation and geographic structure than the widespread *P. marginale*. Furthermore, I test the prediction of recent population expansion in *P. marginale*. The genetic structure and distribution of the two *Phaulacridium* species among population samples from the region of the southern South Island where the two species ranges overlap is examined, and I seek evidence of introgression between the two species.

3.2 Methods

Sampling

Grasshoppers were collected between the years 2012 to 2015 during the New Zealand summer season (January to March) when *Phaulacridium* grasshoppers are mature and active (Northcroft 1967). A total of 149 individuals were collected from roadside verges, public areas and open grassland from 18 locations by hand and with a sweep net (Table 3.1; Figure 3.1).

The North Island locations and northern South Island locations (White Bay, Greymouth, and Mt. Fitzwilliam) were selected on the expectation that these have homogeneous populations of *P. marginale* as determined by geographical location (Westerman and Ritchie 1984), and allowed for a comparison of population samples along a latitudinal gradient. Nine other locations were in southern South Island, the region where both *Phaulacridium* species have previously been reported. *Phaulacridium otagoense* has been identified at Lake Tekapo, Omarama, Lake Dunstan, and Alexandra sites through morphology and molecular data, with *P. marginale* recorded at Lake Pukaki (Westerman and Ritchie 1984; Goldberg *et al*. 2015). Lake Aviemore, Ahuriri River, and the Lindis Valley are recorded locations where the two species co-occur and are sites where introgression could potentially occur (Westerman and Ritchie 1984; Scott 1997) (Figure 3.1).

Table 3.1: Population sample size and location details of *Phaulacridium* grasshopper population samples in New Zealand used in the current study.

Population Location	Code	Latitude	Longitude	Elevation	Sample Size		
					Total	Males	Females
N.I., Auckland, Muriwai Beach	MB	-36.8241	174.4263	10m	10	5	5
N.I., Ahimanawa Range, Wharangi Road	WR	-39.0501	176.5780	490m	8	3	5
N.I., Hawkes Bay, Ngaruroro River	NR	-39.3880	176.3313	595m	6	0	6
N.I., Manawatu, Pohangina Valley	PV	-40.1834	175.8697	420m	7	3	4
N.I., Manawatu, Palmerston North	PN	-40.4145	175.6617	70m	8	5	3
N.I., Wellington, Titahi Bay	TB	-41.1076	174.8247	65m	10	4	6
S.I., Blenheim, Whites Bay	WB	-41.3859	174.0583	6m	7	3	4
S.I., West Coast, Greymouth	GM	-42.4981	171.1870	65m	10	5	5
S.I., Canterbury, Mt. Fitzwilliam	MF	-43.1738	171.5249	750m	10	5	5
S.I., Canterbury, Mackenzie Basin, Lake Tekapo	LT	-44.0022	170.4995	720m	6	1	5*
S.I., Canterbury, Mackenzie Basin, Lake Pukaki Dam	LP	-44.1908	170.1541	503m	9	4	5
S.I., Canterbury, Mackenzie Basin, Omarama	OM	-44.4653	169.8991	599m	8	4	4
S.I., Canterbury, Mackenzie Basin, Lake Aviemore	LA	-44.5873	170.1867	320m	10	5	5
S.I., Canterbury, Mackenzie Basin, Ahuriri River	AR	-44.4970	169.7585	875m	10	5	5
S.I., Otago, Lindis Valley	LV	-44.7482	169.5108	559m	9	2	7
S.I., Otago, Lake Dunstan	LD	-44.9677	169.2703	243m	4	1	3
S.I., Otago, Alexandra, Graveyard Gully	GG	-45.2610	169.3973	201m	9	4	5
S.I., Otago, Alexandra, Marshall Road	MA	-45.2477	169.3573	172m	8	4	4

* Includes one juvenile.

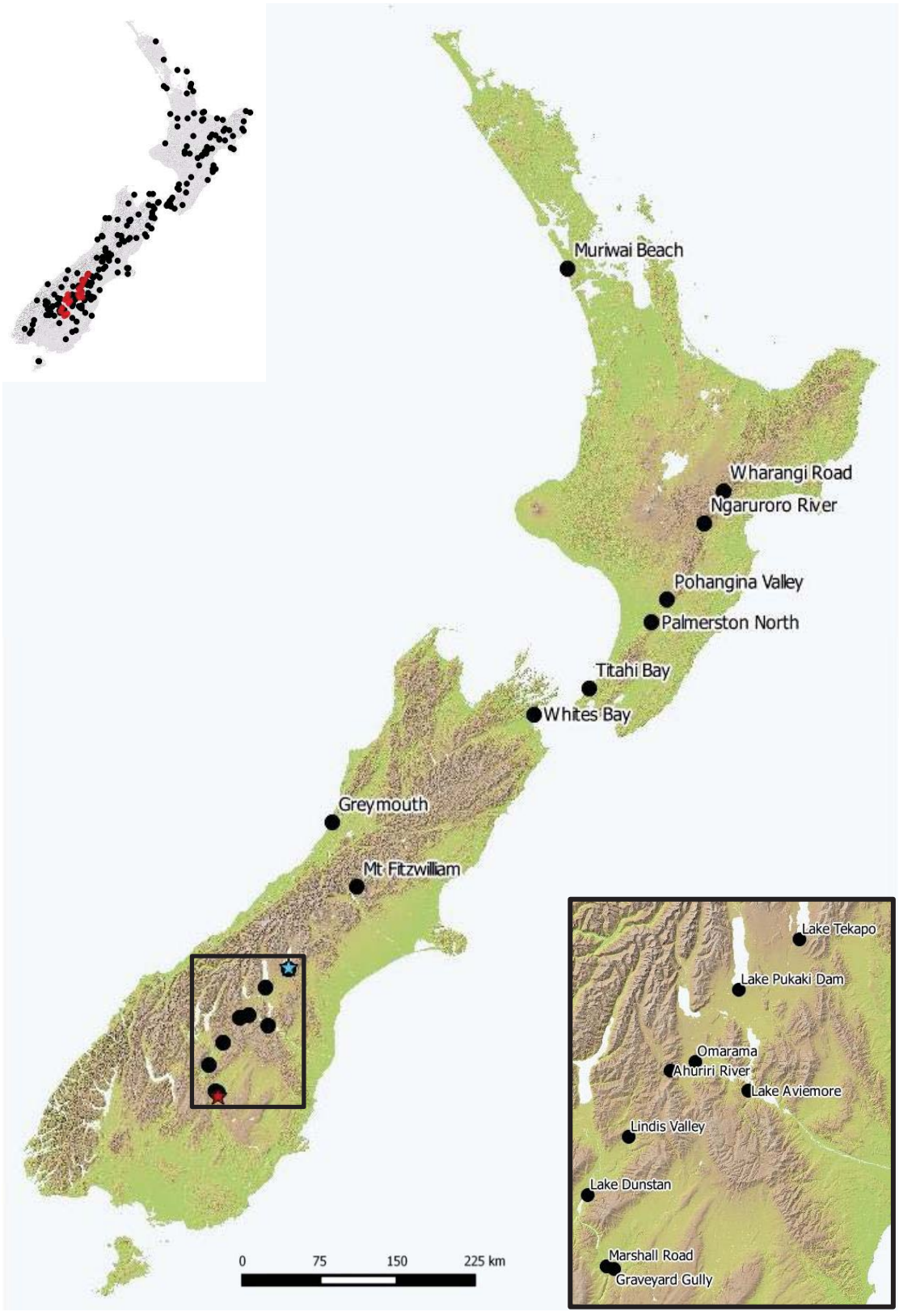

Figure 3.1: Sampling locations across New Zealand of 18 *Phaulacridium* grasshopper population samples used in the study, with a close up of the southern South Island locations. Insert New Zealand map shows known locations of *P. marginale* (black circles) and *P. otagoense* (red circles). Mackenzie Basin (blue star) and Alexandra (red star), were previously shown to support separate lineages of *P. otagoense* (Goldberg *et al.* 2015).

DNA extraction, amplification and sequencing

A front leg from recently collected or alcohol preserved specimens was removed and cut into pieces and whole genomic DNA extracted using a salting-out method (Sunnucks and Hales 1996; Trewick and Morgan-Richards 2005). Polymerase chain reaction used the primers L2-N-3014 (5' TCCAATGCACTAATCTGCCATATTA 3'; Simon *et al.* 1994) and the newly designed hopp-1490 (5' TTTCAACAAACCATAAGGACATTGG 3'), modified from LCO1490 (Folmer *et al.* 1994), to target a 755bp fragment of the mitochondrial DNA gene cytochrome oxidase I (COI). PCR amplification was performed in 20 µL reactions containing 0.2 µL of MCLAB Taq (Molecular Cloning Laboratories), 2.0 µL of 10x MCLAB buffer (Molecular Cloning Laboratories), 2.0 µL of 2mM dNTPs, 1.4 µL of 25mM Mg^{2+}, 0.8 µL of each primer (1mM), and 2.0 µL of genomic DNA. Thermocycling conditions were 95°C for 90 seconds; 94°C for fifteen seconds, 52°C for fifteen seconds and 72°C for 90 seconds repeated 38 times. The amplified products were checked on 1% TAE agarose gels. The amplified COI fragments (L2-N-3014) were commercially sequenced by Macrogen Inc. (Korea). All sequences were edited, assembled, and aligned using Geneious 6.1.8 (Kearse *et al.* 2012).

Phylogenetic relationships of haplotypes

MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) was used to examine tree topology of the haplotypes found among the 149 individuals using one sequence of each haplotype. Sequences available from the Australian *Phaulacridium* species were included (Goldberg *et al*. 2015) along with an outgroup sequence from the New Zealand grasshopper *Sigaus australis* (Hutton 1898). The GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites was applied. Four MCMC chains with 10^6 generations sampled every 10^3 generations and a 10% burn-in was used for the Bayesian tree. Resulting posterior probabilities on the nodes were recorded.

Population genetic analysis

As a result of the information obtained from the haplotype tree topology, two groups of lineage clusters were identified and are believed to correspond to the

two *Phaulacridium* species. Haplotype networks of New Zealand *Phaulacridium* were constructed in PopART (Leigh and Bryant 2015) using the median joining algorithm (Bandelt *et al.* 1999) to assess the geographic structure of the two groups. DnaSP v5.0 (Rozas *et al.* 2003; Librado and Rozas 2009) was used to calculate haplotype diversity (*h*), nucleotide diversity (π, Nei 1987) and the average number of nucleotide differences (*k*) within *Phaulacridium* populations and clades.

Pairwise geographic distances between the 18 locations were computed using Quantum GIS (QGIS) software v2.6.0 (QGIS Development Team 2015) and relevant pairwise θ_{ST} values for the lineage clusters were calculated using 1,000 permutations in Arlequin v3.5 (Excoffier and Lischer 2010). Evidence of isolation-by-distance was looked for using a Mantel test of correlation of pairwise geographic distances and pairwise θ_{ST} for mtDNA using 1,000 permutations for the two lineage clusters using IBDWS v3.23 (Jensen *et al.* 2005). Separate Mantel tests were also performed for the North Island and South Island population samples of one of the lineage clusters.

Demographic history

The demographic histories of the lineages within the *Phaulacridium* grasshoppers were examined using three methods. Tajima's *D* (Tajima 1989) and Fu's F_s (Fu 1997) neutrality tests were performed using 1,000 coalescent simulations in DnaSP v5.0 (Rozas *et al.* 2003; Librado and Rozas 2009). A value near zero implies a constant population size; significant negative values imply population expansion or purifying selection; while positive values indicate population subdivision or a recent population bottleneck. Another neutrality test, Ramos-Onsins and Rozas's R_2 (Ramos-Onsins and Rozas 2002) was performed in DnaSP v5.0 (Rozas *et al.* 2003; Librado and Rozas 2009) using 1,000 coalescent simulations. This test and Fu's F_s are the best statistical tests for sensing population growth, with R_2 being better suited to small sample sizes compared to F_s which is more useful for larger population sizes (Ramos-Onsins and Rozas 2002). Significant R_2 values close to zero indicate that population expansion has occurred (Young *et al.* 2014).

Observed distribution of pairwise differences between sequences was calculated using DnaSP v5.0 (Rozas *et al.* 2003; Librado and Rozas 2009) and the expected distributions modelled under the demographic scenario of sudden population expansion (Rogers and Harpending 1992). Mismatch distributions from populations of constant size typically have a ragged profile and are often multimodal compared to smooth and unimodal mismatch distributions of populations that have been subject to recent demographic expansion (Slatkin and Hudson 1991; Rogers and Harpending 1992; Harpending 1994; Harpending *et al.* 1998). The sum of squares deviation (SSD; Schneider and Excoffier 1999), Harpending's raggedness index (Harpending *et al.* 1993; Harpending 1994), and their corresponding *P* values were obtained using Arlequin v3.5 (Excoffier and Lischer 2010) with 1,000 bootstrap resamples. These statistics are commonly used to test for the fit to a unimodal mismatch distribution, with a non-significant result indicating a history of population expansion.

The Rogers and Harpending (1992) model was used to calculate time since population expansion by estimating Tau (τ), θ_0, and θ_1 based on the mismatch distribution outputs from Arlequin. The parameter τ from the mismatch distribution was used to estimate the time since expansion (t) using the equation $t = \tau/2u$, where u is the mutation rate per sequence per generation (Rogers 1995; Schneider and Excoffier 1999). The general substitution rate for the insect mitochondrial genome recommended by Brower (1994) (2.3% divergence per million years between sequences) is widely used among Orthoptera and other insect species (i.e. Knowles 2000; Allegrucci *et al.* 2005; O'Loughlin *et al.* 2008; Swaegers *et al.* 2014). However, more recent studies have drawn attention to the variation between mitochondrial genes for substitution rates (Mueller 2006; Papadopoulou *et al.* 2010; Pons *et al.* 2010; Goldberg *et al.* 2014), with genes in the cytochrome family having a much higher rate of substitution (3-4% divergence per million years) compared to genes in the NAD family that evolve closer to the general rate of 2.3% divergence per million years. Furthermore, within Orthoptera rates of between 3% and 4% divergence per million years have been inferred for the COI gene (Shapiro *et al.* 2006; Allegrucci *et al.* 2011). The three mutation rates (u; per site per generation) used in the present analysis were as follows: $1.15 \times 10^{-8} \pm 0.14 \times 10^{-8}$ (rate of 2.3% divergence per Myr, Brower 1994); $1.33 \times 10^{-8} \pm 0.13 \times$

10^{-8} (global mtDNA rate of 2.6% divergence per Myr proposed by Papadopoulou *et al.* 2010); and $1.60 \times 10^{-8} \pm 0.19 \times 10^{-8}$ (faster rate of 3.2% divergence per Myr based on COI estimates, Shapiro *et al.* 2006; Papadopoulou *et al.* 2010; Allegrucci *et al.* 2011).

Thirdly, the Bayesian coalescent method (Bayesian skyline plot, BSP, Drummond *et al.* 2005) was implemented in BEAST v2.3.1 (Bouckaert *et al.* 2014) to visualise the dynamics of population size fluctuations over time. Bayesian MCMC simulations for the Group 1 sequences (see Results) were run for 110 million generations, sampling every 1000 generations, with the first 10% discarded as burn-in. Ten million generations were run for the Group 2 sequences consisting of the other three clades (see Results). Both sequences groups were modelled under the GTR + G model of nucleotide substitution using a relaxed uncorrelated lognormal molecular clock. Only one of the previously mentioned substitution rates was used for the analysis as only a comparison of BSP's between the two sequence groups was needed. The widely used arthropod mtDNA molecular clock rate of 2.3% pairwise sequence divergence per million years, equivalent to a rate of 0.0115 substitutions per site per million years was used for each group of sequences (Brower 1994). Results were examined using TRACER v1.5 (Rambaut and Drummond 2009) and checked to make sure the ESS values were more than 200; the same program was used to reconstruct the BSP's.

3.3 Results

Phylogenetic relationships of haplotypes

The aligned homologous COI sequences (755 bp) from 149 *Phaulacridium* individuals contained 56 unique mtDNA haplotypes. The 149 sequences analysed contained 106 variable sites (14%). Results from phylogenetic analysis of the haplotypes indicated the presence of four well-supported lineages (clades) among individuals from the genus *Phaulacridium* sampled in New Zealand (Figure 3.2). A shallow clade (Lineage I) comprised haplotypes sampled from the North Island and the South Island of New Zealand while three more diverse clades (Lineages II, III, and IV) comprised haplotypes sampled from southern/central South Island (Figure 3.2). Lineage I is equivalent to clade I (*P. marginale*) of Goldberg *et al.*

(2015), whereas Lineages III and IV correspond to the two clades assigned to *P. otagoense*. Lineage II has not previously been reported.

Contrasting geographic structuring was observed among the four mtDNA lineages. Individuals from Lineage I were found in all of the sampling locations except Lake Dunstan (Figure 3.1, see Appendix 2). In contrast, Lineages II, III and IV were geographically more restricted. Lineage III included only individuals from Alexandra, and Lineage IV population samples only from the Mackenzie Basin (Figure 3.3). Lineage II represented grasshoppers from Lindis Valley and Lake Dunstan (Otago) (Figure 3.3). Furthermore, Lineage II could be further divided into two sub clades representing these two locations (Figure 3.2). Lindis Valley and Lake Dunstan (Otago) were the only areas that contained a majority (77%) of individuals lacking Lineage I haplotypes. In contrast, only 26% - 24% of grasshoppers from the population samples from Mackenzie Basin and Alexandra were without Clade I haplotypes (Figure 3.3).

Two main sequences groups were formed on the basis of their geographic distribution, haplotype tree typology, and the correspondence to previously identified groups (Goldberg *et al.* 2015). Group 1 contains all Lineage I individuals, referred to as *P. marginale* grasshoppers by Goldberg *et al.* (2015). Individuals from Lineages II, III and IV, which with the exception of Lineage II, were referred to as *P. otagoense* by Goldberg *et al.* (2015), are here considered together as Group 2. These groups were used in genetic analyses with the initial hypothesis that they represent the two described species of New Zealand *Phaulacridium*.

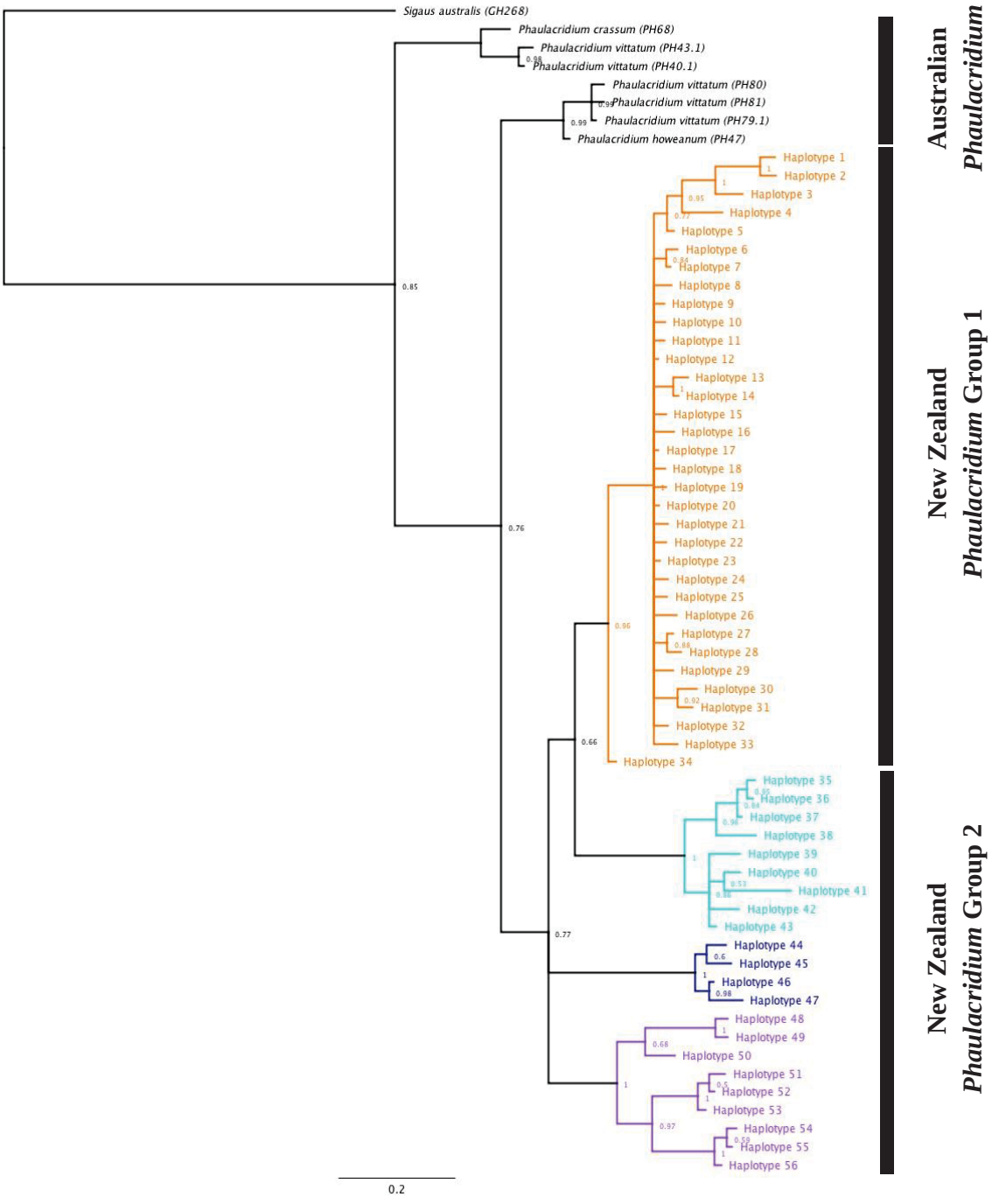

Figure 3.2: Phylogenetic tree of 56 mtDNA COI haplotypes represented in 149 New Zealand *Phaulacridium* individuals, generated in MrBayes with Bayesian posterior probabilities mapped to the branches. The tree was rooted with *Sigaus australis* from New Zealand, and individuals representing the Australian *Phaulacridium* species. Four New Zealand clades are indicated: Lineage I (orange), Lineage II (light blue), Lineage III (dark blue) and Lineage IV (purple), along with the two main sequence groups.

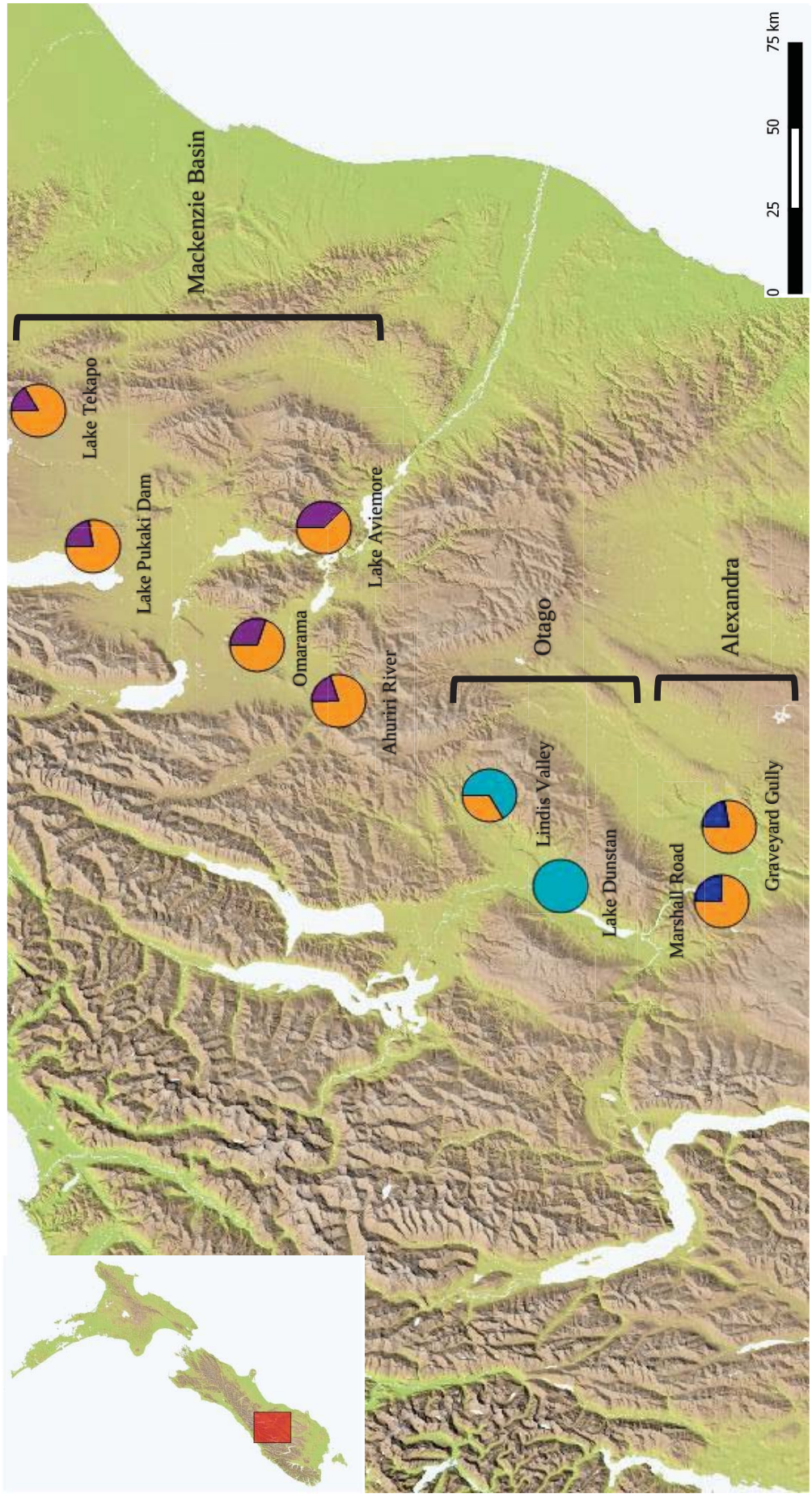

Figure 3.3: Representation of the proportion of four New Zealand *Phaulacridium* mtDNA COI lineages at southern South Island sites. Four lineages are identified by colour: Lineage I (orange), Lineage II (light blue), Lineage III (dark blue) and Lineage IV (purple).

Population genetic analysis

Median joining haplotype networks were generated for Group 1 and Group 2 sequences (Figure 3.4 a, b). Of the 124 *Phaulacridium* individuals in Lineage I, 34 unique COI haplotypes were discovered (Figure 3.4 a). Four haplotypes were observed at high frequency: Hap7 (n = 27, 22%), Hap12 (n = 21, 17%), Hap20 (n = 18, 15%), and Hap23 (n = 18, 15%), however the distribution of these main haplotypes around New Zealand were different. Hap7 and Hap20 were found only in the South Island, with Hap12 distributed throughout New Zealand, and the majority (94%) of Hap23 located in the North Island (Figure 3.4 a). Unique haplotypes were detected in all regions expect Marlborough and Otago (Figure 3.4 a).

In contrast to Group 1 (Lineage I), there is a higher degree of genetic diversity within Group 2 (Lineages II, III, IV), with 22 haplotypes found in 25 grasshoppers. Furthermore, the Group 2 lineages were spatially more structured than Lineage I; three separate network branches each represent different geographic areas (Figure 3.4 b). Additionally, Lineage II could be further split into two geographic lineages (Figure 3.4 b). In addition to the higher genetic diversity found amongst Group 2 sequences most of the haplotypes were represented by a single individual.

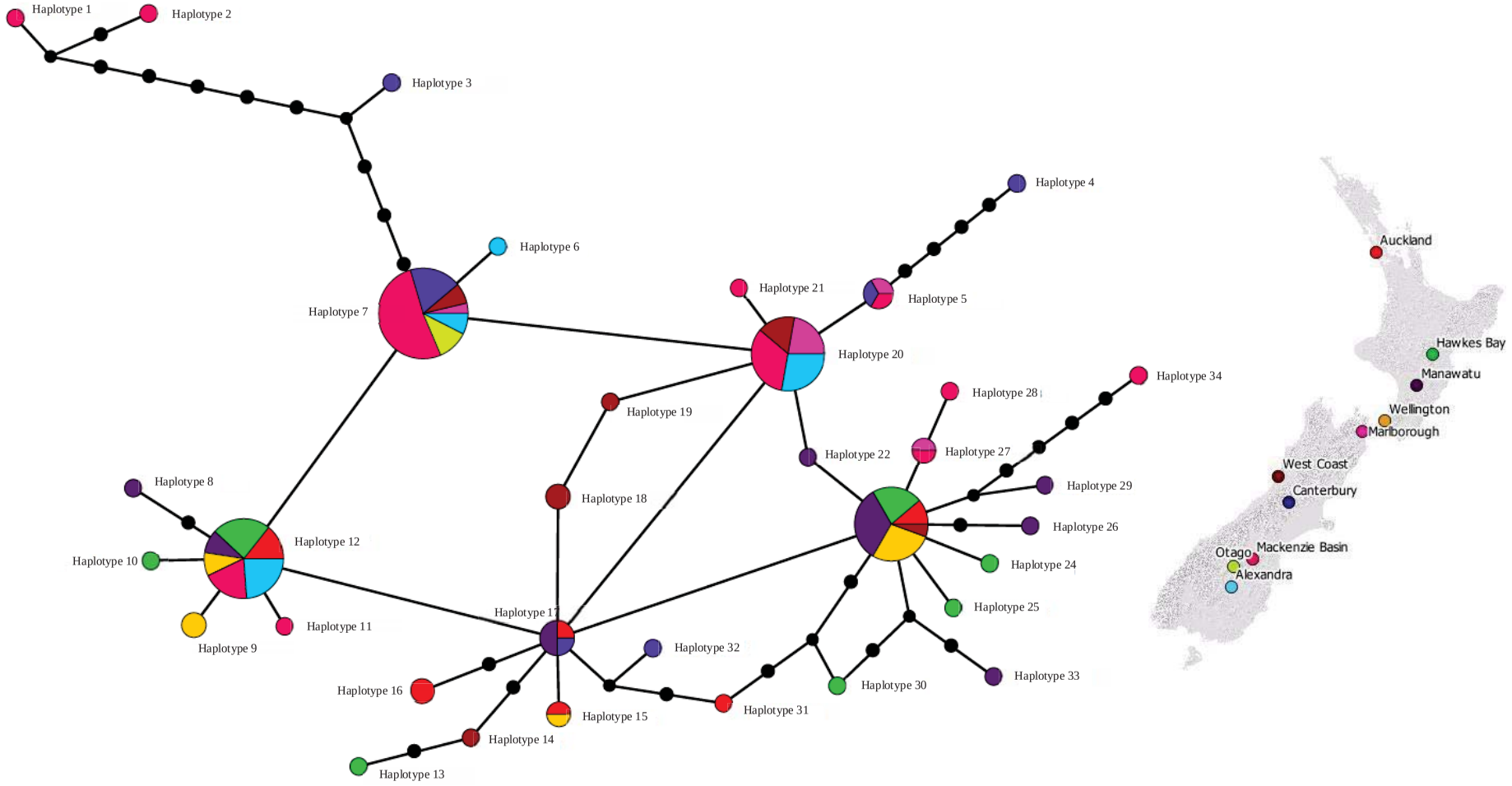

Figure 3.4 a: Distribution of mtDNA COI diversity among *Phaulacridium* grasshoppers from Lineage I (Group 1). Colour corresponds to the general region population samples are located; see Table 3.1 for population sample location details.

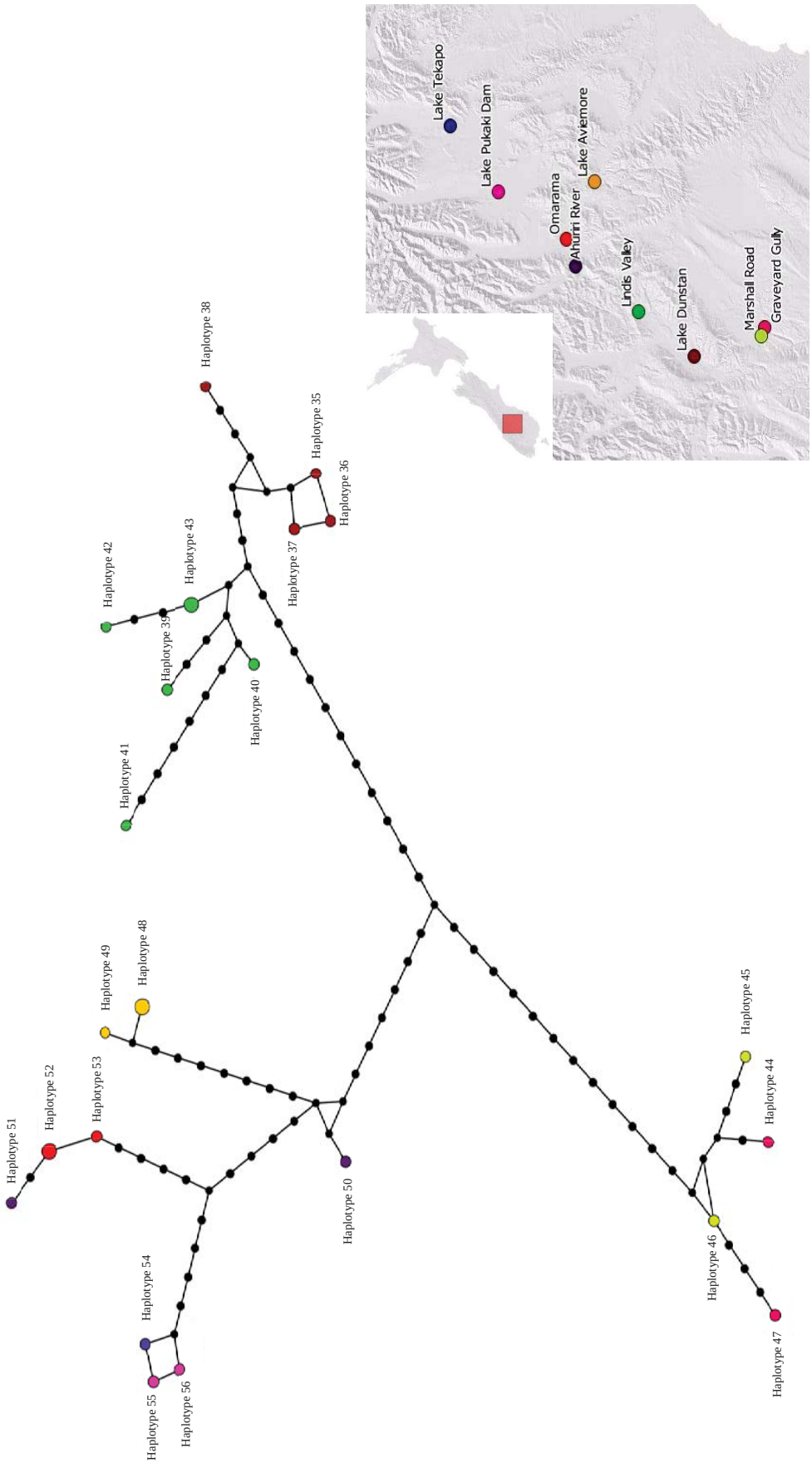

Figure 3.4 b: Distribution of mtDNA COI diversity among *Phaulacridium* grasshopper haplotypes in Group 2 (Lineages II, III and IV). Colours correspond to sampling locations.

Group 1 displayed lower levels of haplotype and nucleotide diversity than Group 2 (Table 3.2), although haplotype diversity was still relatively high. Within Group 2, Lineage IV had the highest nucleotide diversity. Of the population samples containing individuals from Lineage I, Lindis Valley was the only one where all individuals of lineage I had the same haplotype. Ngarururoro River had the lowest nucleotide and haplotype diversity (Table 3.3). Locations that contained individuals representing both haplotype groups had on average higher nucleotide diversity than locations containing individuals only from Lineage I (Table 3.3).

Table 3.2: MtDNA COI sequence variation and haplotype diversity within four New Zealand *Phaulacridium* lineages. Sample size (n), number of observed haplotypes (N_{haps}), average number of nucleotide differences (k), nucleotide diversity (π), number of polymorphic sites (S) and haplotype diversity (h) are given.

	n	N_{haps}	k	π	S	h
Lineage I (Group 1)	124	34	2.690	0.00356	43	0.884
Lineage II	10	9	8.267	0.01095	25	0.978
Lineage III	4	4	5.833	0.00773	11	1.000
Lineage IV	11	9	11.964	0.01585	28	0.964
Group 2	25	22	22.480	0.02977	76	0.990

Table 3.3: MtDNA COI sequence variation and haplotype diversity within population sample locations. Showing sample size for each location (n), number of observed haplotypes (N_{haps}), average number of nucleotide differences (k), nucleotide diversity (π), number of polymorphic sites (S) and haplotype diversity (h).

	Lineage	**n**	N_{haps}	k	π	S	h
Muriwai Beach	Lineage I	10	6	2.333	0.00309	8	0.889
Wharangi Rd	Lineage I	8	6	2.500	0.00331	10	0.893
Ngarururoro River	Lineage I	6	3	1.000	0.00132	3	0.600
Pohangina Valley	Lineage I	7	5	3.143	0.00416	11	0.857
Palmerston North	Lineage I	8	4	1.250	0.00166	3	0.821
Titahi Bay	Lineage I	10	4	1.644	0.00218	4	0.733
Whites Bay	Lineage I	7	4	1.429	0.00189	5	0.714
Greymouth	Lineage I	10	6	1.956	0.00259	6	0.889
Mt Fitzwilliam	Lineage I	10	6	3.600	0.00477	14	0.778
Lake Tekapo	Lineage I and IV	6	4	9.133	0.01508	26	0.800
Lake Pukaki Dam	Lineage I and IV	9	7	11.222	0.01486	28	0.944
Omarama	Lineage I and IV	8	4	14.857	0.01968	28	0.750
Lake Aviemore	Lineage I and IV	10	6	13.178	0.01745	29	0.844
Ahuriri River	Lineage I and IV	10	8	13.578	0.01798	43	0.956
Lindis Valley	Lineage I and II	9	6	14.583	0.01932	34	0.889
Lake Dunstan	Lineage II	4	4	3.833	0.00508	7	1.000
Graveyard Gully	Lineage I and III	9	4	11.000	0.01457	31	0.694
Marshall Rd	Lineage I and III	8	4	11.393	0.01509	29	0.643

Lineages II, III and IV had higher levels of genetic diversity (Table 3.2) than the more widespread Lineage I, which would suggest that their narrow modern range does not equate to a recent small population size. In contrast, the low genetic diversity of the now widespread Lineage I could suggest that this lineage has recently undergone a population expansion.

For New Zealand *Phaulacridium*, the Mantel test revealed a significant association between geographical and genetic distances for Group 1 (Lineage I) (r = 0.361, P < 0.001) and Group 2 (Lineages II, III and IV) populations (r = 0.387, P < 0.001) (Figure 3.5 a, b). However, there was no significant isolation by distance association when Lineage I population samples were considered separately for the North Island and South Island (Figure 3.5 c, d). The relationship was different for each island with North Island Lineage I population samples showing a decrease in genetic distance as geographic distance increased, but the opposite trend being seen in the South Island (Figure 3.5 c, d).

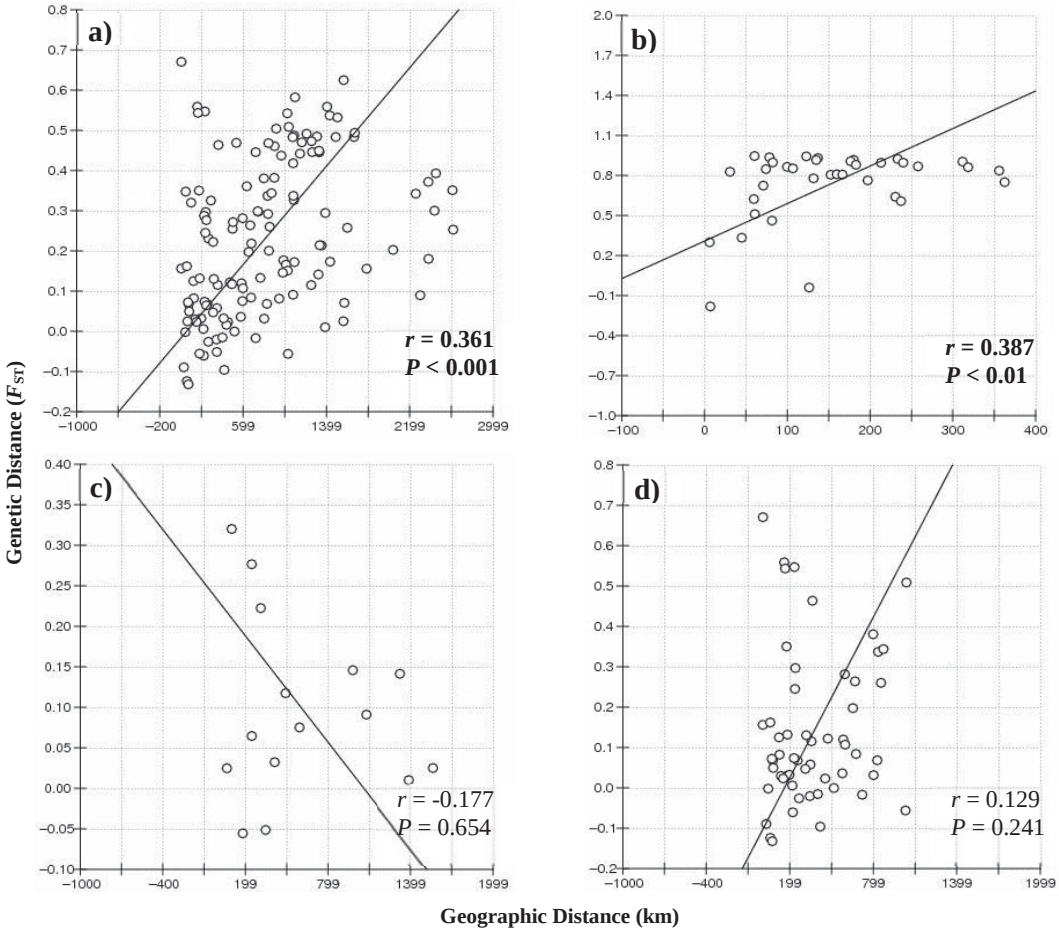

Figure 3.5: The relationship between linear geographic distance (km) and pairwise genetic distance (θ_{ST}) among mtDNA COI sequences from New Zealand *Phaulacridium* population samples. a) Group 1 (Lineage I) populations, b) Group 2 (Lineages II, III and IV) populations, c) Lineage I North Island populations, and d) Lineage I South Island populations. Significance of the correlation was tested using a Mantel test (1,000 iterations).

Demographic history

Inferred demographic histories of New Zealand *Phaulacridium* revealed noticeable differences between the two haplotype groups. All neutrality tests for Group 2 sequences were non-significant. For Group 1, however, Fu's F_S and Tajima's D test for Lineage I were significantly negative, and also had a significantly low R_2 value (Table 3.4). These results are indicative of population expansion of Lineage I (Group 1).

Table 3.4: Results of neutrality tests for mtDNA COI sequences from New Zealand *Phaulacridium.*

	Tajima's *D* (*P*)	**Fu's F_S (*P*)**	**Ramos-Onsins and Rozas's R_2 (*P*)**
Group 1 (Lineage I)	-2.063 (0.001**)	-25.291 (0.000***)	0.031 (0.006**)
Croup 2 (Lineages II, III and IV)	0.041 (0.563)	-4.037 (0.056)	0.138 (0.714)

***P* < 0.01, ****P* < 0.001

The distribution of pairwise nucleotide differences (mismatch distribution) for Group 1 (Lineage I) exhibited a smooth and unimodal shape consistent with a model of demographic expansion (Figure 3.6 a). In contrast, Group 2 haplotypes (Lineages II, III and IV) revealed a more ragged and multimodal distribution that is typical of stable population size (Figure 3.6 b). According to the mismatch distribution analysis, the hypothesis of population expansion could not be rejected for Lineage I (Group 1) (Harpending raggedness index = 0.0294, P = 0.658; SSD = 0.002, P = 0.413). Statistical tests also returned non-significant results for Group 2 data although P values were smaller. Harpending raggedness index was smaller (0.0161, P = 0.189) than for Group 1, and SSD larger (0.016, P = 0.094). Non-significant results for Group 2 could be a result of a smaller sampling size and the effect of pooling the three lineages together.

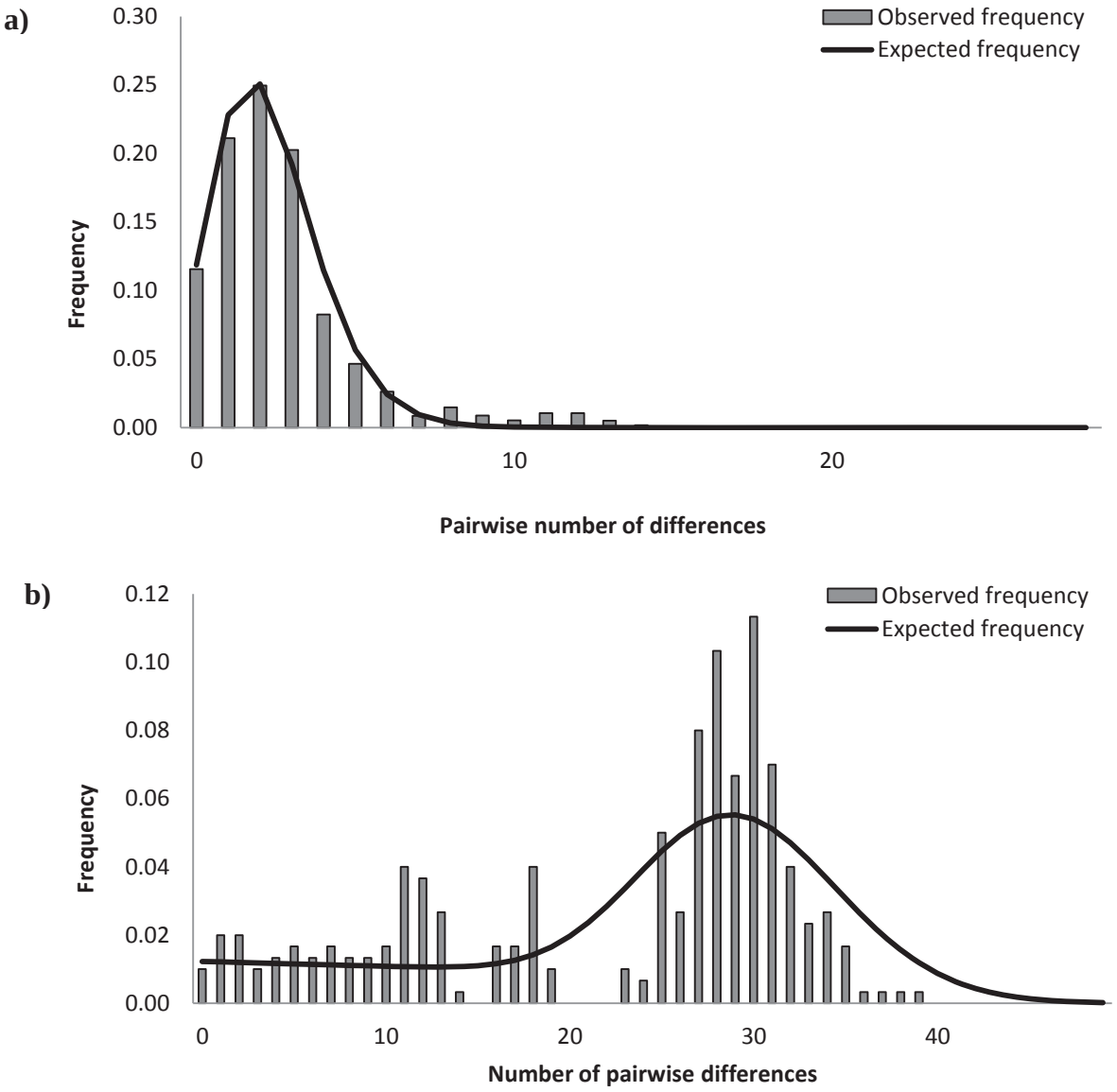

Figure 3.6: Frequency distribution of observed pairwise nucleotide differences among mtDNA COI sequences for two *Phaulacridium* haplotype groups a) Group 1 (Lineage I) and b) Group 2 (Lineages II, III, and IV). Expected frequencies were generated under a sudden population expansion model.

The estimated time since expansion ($t = \tau/2u$) suggested a more recent expansion of Group 1 haplotypes than Group 2 (Table 3.5). The later date estimates for this expansion coincides with a Pleistocene stadial period (97–88 ka) while earlier dates fall within the Aurora Interstadial (61–43 ka). The timing of New Zealand's Last Glacial Maximum (nzLGM; 72–62 ka) coincided with the estimated time of expansion (Williams *et al.* 2015).

Table 3.5: Parameters from the mismatch distribution and the estimated time since expansion for Group 1 (Lineage I) and Group 2 (Lineages II, III and IV). The estimated time since expansion are given for three different substitution rates equivalent to (a) 2.3% (b) 2.6% (c) 3.2% per million years.

	Group 1 (Lineage I)	**Group 2 (Lineages II, III and IV)**
Tau	1.934	29.648
θ_0	0.446	0.000
θ_1	38.883	80.449
a) $1.15 \times 10^{-8} \pm 0.14 \times 10^{-8}$	84 ka (96–75 ka)	1,289 ka (1,468–1,149 ka)
b) $1.33 \times 10^{-8} \pm 0.13 \times 10^{-8}$	73 ka (81–66 ka)	1,115 ka (1,235–1,015 ka)
c) $1.60 \times 10^{-8} \pm 0.19 \times 10^{-8}$	60 ka (69–54 ka)	927 ka (1,051–828 ka)

Historical demographic reconstructions (BSP's) of the Group 2 grasshopper lineage indicates a phase of demographic stability, followed by a steady increase in population size starting around 450,000 years ago for Group 2 (Figure 3.7 b). In contrast, Group 1 had a brief period of demographic stability, followed by population expansion around the time of the nzLGM until the global Last Glacial Maximum (gLGM; 33–26.5 ka) where the population size increase plateaued (Figure 3.7 a; Williams *et al.* 2015).

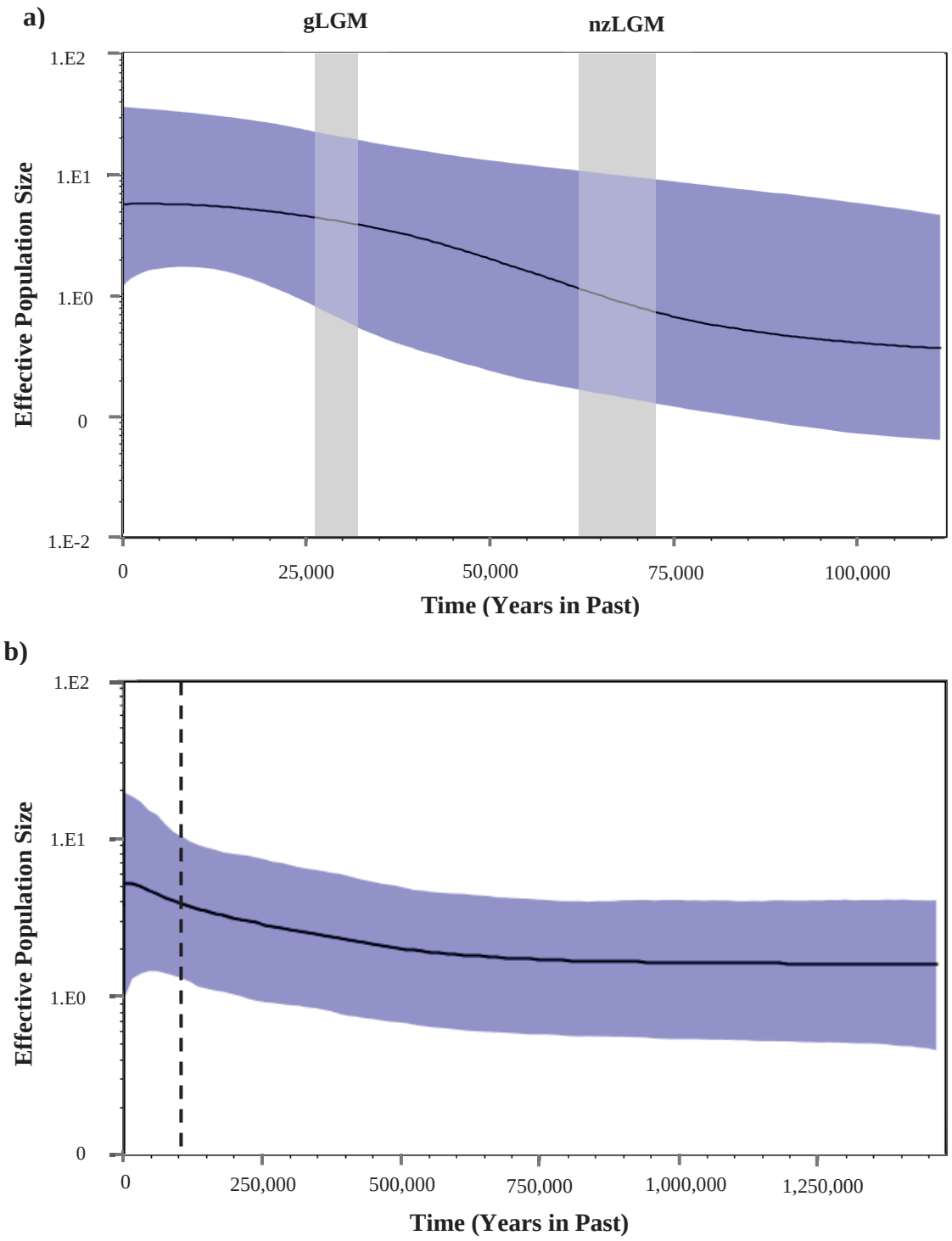

Figure 3.7: Historical demographic trends of a) Group 1 (Lineage I) and b) Group 2 (Lineages II, III and IV), represented by a Bayesian skyline plot (BSP) based on mtDNA COI haplotype data using the substitution rate equivalent to 2.3% per million years. The time scale on x-axis is in years before present (note difference in scale between groups), and the y-axis is the estimated effective population size. Estimates of medians are joined by a solid line, while the shaded range delineates the 95% HPD limits. The grey vertical gLGM bar represents ending of the global Last Glacial Maximum (33–26.5 ka), and the grey vertical nzLGM bar represents the New Zealand Last Glacial Maximum (72–62 ka) (Williams *et al.* 2015). Vertical dashed line in (b) indicates relative maximum time in (a).

3.4 Discussion

New Zealand's *Phaulacridium* grasshoppers provide a striking example of contrasting range size in sister lineages. The widespread lineage (Lineage I) has lower genetic diversity than the more geographically restricted lineages (Lineages II, III, and IV). Group 2 lineages (Lineages II, III, and IV) are more geographically structured and are restricted to three different areas in southern South Island, Otago (Lindis Valley/Lake Dunstan), Alexandra, and Mackenzie Basin respectively (Figure 3.3). Group 2 has a restricted range today, but high genetic diversity suggests that these lineages were recently represented in large populations. Larger population is suggestion of a bigger spatial range than the current distribution. Current observations do not demonstrate significantly denser populations of Group 2 grasshoppers. Similar patterns have been observed in other organisms including the giant mayfly *Palingenia longicauda* (Bálint *et al.* 2012) and aquatic plant *Sagittaria natans* (Chen *et al.* 2007). In contrast, the other lineage (Lineage I) is today more widespread than Group 2; demographic history analysis suggests that is the result of recent population expansion because low genetic diversity is best explained by small population size.

The estimated time of population expansion for Lineage I was between about 100 ka and 50 ka years ago. The wide errors around this estimation are due to the different substitution rates for mtDNA. A rapid rate for COI results in a younger expansion of Lineage I population, but it is unclear how realistic this substitution rate is. During this period (100–50ka) there were significant periods of climatic and vegetation change in New Zealand (Williams *et al.* 2015). The dates are aligned to Marine Isotope Stage (MIS) 5b (97–88 ka) through to the MIS 4/3 transition (MIS 4, 73–59 ka; MIS 3, 59–29 ka; Williams *et al.* 2015). Relatively cool conditions during MIS 5b resulted in glacial advances in the South Island, and subalpine shrubland and grassland were likely the dominant vegetation types (Vandergoes *et al.* 2005; Williams *et al.* 2015). This was followed by the Otamangaku Interstadial that coincides with MIS 5a (87–73 ka; Williams *et al.* 2015). The climate was wetter and cooler than today with temperatures on average 2°C below present levels, but still warmer than conditions during MIS 5b (McGlone and Topping 1983). A rapid change in vegetation from grassland and subalpine shrubland to tall

forests has been inferred, although interregional differences in vegetation composition are indicated by from palynological samples. Podocarp forest developed in Taranaki and Wairapapa, while *Nothofagus* forest was prominent in central and southern North Island and the West Coast of the South Island (Moar and Suggate 1996; Shulmeister *et al.* 2001; Newnham and Alloway 2004).

The transition from late MIS 5a to early MIS 4 was a period of rapid environment change from tall forests of podocarps and/or *Nothofagus* to grassland or grass/shrubland. This transition occurred throughout the country including in the Canterbury (Moar and Gage 1973) and Greymouth (Burge and Shulmeister 2007) regions of the South Island, and southern Wellington (Mildenhall 1994), Wairapapa (Carter and Lian 2000), central Tongariro (McGlone and Topping 1983), and eastern Hawkes Bay (Okuda *et al.* 2002) regions of the North Island. A mosaic of vegetation types during MIS 4 consisted of patches of forest growing in areas protected from climate extremes, but the dominant vegetation consisted of grassland and/or shrubland (Midenhall 1994; Williams *et al.* 2015). Climatic conditions were cool to cold and although wet in westerly windward locations it was probably dry in the east of both islands (Midenhall 1994; Williams *et al.* 2015). The coldest phase in this time frame was during the peak of the nzLGM 67 to 62 ka and has been referred to as the nzLGM (Williams *et al.* 2015). Although climate conditions were somewhat more severe during the gLGM (31–26.5 ka), the main difference between the two LGM's appears to have been in the levels of precipitation. Speleothem $\delta^{13}C_c$ records suggest that there was a longer duration of sustained water surplus during the nzLGM, implying the most extensive glacier advance in New Zealand occurred during MIS 4 (Williams *et al.* 2015).

It is possible that today's most widespread *Phaulacridium* lineage (Lineage I) started to expand its range during MIS 4 coinciding with an increase in their grassland habitat. During MIS 3 the milder Aurora (61–43 ka) and Moerangi (37–33 ka) Interstadials would have limited grasshopper range as forests increased (Williams *et al.* 2015). Grasshopper expansion could still have occurred during the stadial (42–38 ka) as conditions became dry and cool with glacial advances resulting in vegetation change to grassland and/or shrubland (Williams *et al.* 2015). The onset of the gLGM (31–26.5 ka) would have further allowed

Phaulacridium grasshoppers in Lineage I to expand as the cool, drier climate led to an increase in open grassland and tussock and forests being restricted to northern North Island and isolated patches of northern South Island (Figure 3.8; Newnham *et al.* 2013). However, by 18 ka the vegetation of New Zealand altered as forests expanded, and by 8000 years ago an estimated 85% of the country was covered in tall podocarp forest (Figure 3.8; McGlone *et al.* 1995; Perry *et al.* 2014). Grasslands were limited and mostly restricted to areas above treeline or disturbed environments around rivers and frost flats (Perry *et al.* 2014; Goldberg *et al.* 2015). This near complete coverage of non-alpine New Zealand by forest since the gLGM would have severely limited habitat availability for *Phaulacridium* in most regions.

However, the North Island populations of *Phaulacridium* do not follow a model of isolation by distance, as expected if gene flow was limited by geographic distance. Thus, the spatial distribution of genetic diversity does not support a population expansion as early as 60,000 years ago. Departure from isolation by distance in the North Island could be consistent with northern populations being a product of more recent and rapid northward range expansion. Since the Holocene major change to vegetation in New Zealand has occurred since the arrival of people (~1000 years ago). Widespread deforestation and conversion of native forest to open grassland for agriculture (Figure 3.8; McWethy *et al.* 2014; Perry *et al.* 2014) has vastly altered the New Zealand landscape (Trewick and Morgan-Richards 2014). This change in vegetation cover may have enabled the Lineage I to expand its range. Range expansion as the result of recent change in New Zealand's vegetation cover has been suggested in a number of other cases including the short-tailed bat *Mystacina tuberculate* (Lloyd 2003) and cicada *Kikihia subalpine* (Marshall *et al.* 2009), although range decreases have occurred in other species like the landsnail *Placostylus hongii* (Brook and McArdle 1999) and Northland brown kiwi *Apteryx mantelli* (Miller and Pierce 1995).

Extensive studies on the genus *Phaulacridium* in New Zealand during the 1970's recorded the distribution of two species. Apart from in the Lindis River Valley and the Stoney River Valley, a clear spatial separation of the two species was documented (Figure 3.9, Westerman and Ritchie 1984). The current study has

however shown that in all southern South Island sampling locations, apart from Lake Dunstan, individuals with Group 1 and Group 2 haplotypes co-occur (Figure 3.9). This data suggests that within a period of 40 years one lineage (Lineage I) has expanded its range to the majority of sites that were formerly occupied only by *P. otagoense* during the 1970's and before (Westerman and Ritchie 1984).

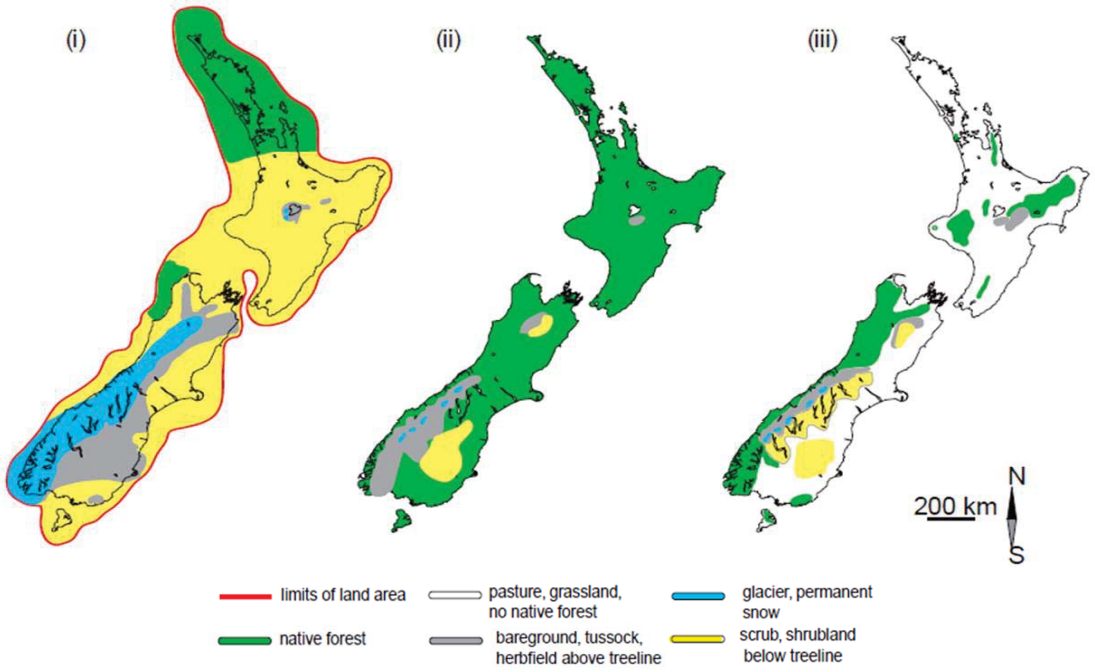

Figure 3.8: Approximate distribution of vegetation zones in New Zealand at (i) gLGM, (ii) pre-human, (iii) modern (post human settlement) New Zealand (modified from Goldberg *et al.* 2015 and Alloway *et al.* 2007).

The one widespread lineage and two smaller but more diverse lineages discovered by Goldberg *et al.* (2015) were previously inferred to represent *P. marginale* and *P. otagoense* respectively. This inference was based on morphological character differences (e.g. body size and sculpturing of pronotum) described by Bigelow (1967), Westerman and Ritchie (1984), and Key (1992). In the current study Group 1 (Lineage I) is considered to represent *P. marginale* and Group 2 (Lineages II, III, IV) as *P. otagoense* based on these earlier inferences (Goldberg *et al.* (2015), but there are notable differences in the geographic distribution of the lineages. Within the Alexandra area, *P. otagoense* was previously the only recorded species (Goldberg *et al.* 2015), however the present study has shown that grasshoppers

with the *P. marginale* mtDNA lineage now also occupy this region, and appear to be more common. This pattern of *P. marginale* being the more common species is also apparent in the Mackenzie Basin, and only the Lake Dunstan/Lindis Valley area (Otago) is the only region left where putative *P. otagoense* (Lineage II) is the more common mtDNA lineage.

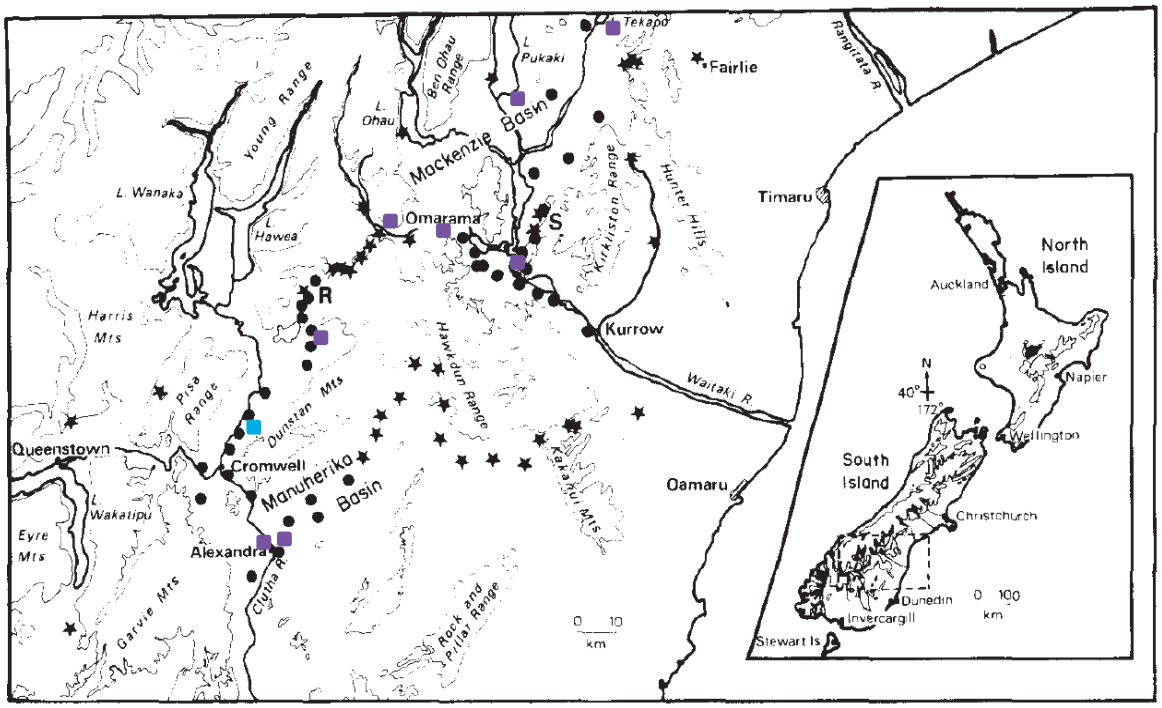

Figure 3.9: Recorded distributions of *Phaulacridium otagoense* (circles) and *P. marginale* (stars) in Central Otago and Canterbury, South Island, New Zealand (Modified from Westerman and Ritchie 1984). Sampling locations used in the present study are shown by squares. Population samples containing individuals from both haplotype groups are represented by purple squares and comprising only individuals from Group 2 haplotypes (inferred to be *P. otagoense*) are shown in blue.

Although both species of *Phaulacridium* are found in the same region, populations of each species were considered to be isolated, with transects showing the changeover from one species to the other in contiguous populations as being abrupt and absolute, even within a matter of metres (Westerman and Ritchie 1984). Furthermore, the ecological requirements of the two species were describe as distinct (Westerman and Ritchie 1984). No interspecific matings have ever been observed in the field and limited cage experiments have not been able to produce cross-matings or to yield hybrids (Westerman and Ritchie 1984). The results of the

present study demonstrate that within the southern South Island region, the mitochondrial lineages attributed to *P. marginale* (Group 1) and *P. otagoense* (Group 2) co-occur within a single location. Therefore, comparing the mitochondrial results from this study with both traditional and geometric morphometrics could enable us to distinguish whether introgression is occurring or not.

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Chapter 4

Morphological variation of New Zealand *Phaulacridium* grasshoppers

Chapter 4: Morphological variation of New Zealand *Phaulacridium* grasshoppers

Abstract

Traditionally the taxonomic classification of organisms was based on the description of their morphology. Advancing technology has allowed for morphological variation between species to be tested through geometric morphometric techniques, along with the more traditional morphometric methods. The present study explored the morphological variation of New Zealand *Phaulacridium* grasshoppers using traditional and geometric methods.

Two distinct morphotypes were apparent among New Zealand *Phaulacridium* grasshoppers. The smaller and geographically restricted Type 2 was inferred to be *P. otagoense*, while the larger and geographically widespread Type 1 represented *P. marginale*. Additionally, *P. marginale* has expanded its range to the majority of sites that were formally only occupied by *P. otagoense* before and during the 1970's.

Both *Phaulacridium* morphotypes co-occurred within a single location within the southern South Island region, therefore it is possible that introgression could occur. Furthermore, several individuals could not be classified into discreet morphotype, suggesting that these individuals had a mixture of morphological features, as expected of a hybrid.

4.1 Introduction

For centuries, biologists have compared the anatomical features of organisms. The taxonomic classification of organisms was historically based on the descriptions of morphology. Morphometrics is the quantitative study of biological shape, shape variation and its covariation with other variables within and among organisms (Rohlf and Marcus 1993; Adams *et al.* 2004; Webster and Sheets 2010). Traditional morphometric methods typically apply multivariate statistical analyses to sets of quantitative morphological variables such as linear distance measurements (length, width, and height), ratios, and angles (Rohlf and Marcus 1993; Adams *et al.* 2004; Mitteroecker and Gunz 2009; Webster and Sheets 2010). Geometric morphometrics, a new and more advanced methodology, studies shape variation of landmark coordinates that can be two- or three-dimensional (Adams *et al.* 2004; Mitteroecker and Gunz 2009; Webster and Sheets 2010). Landmarks are points of correspondence across biologically homologous anatomical structures on each specimen within the study (Webster and Sheets 2010).

Since the late 1980s geometric morphometrics has become increasingly popular in a number of biological fields (Adams *et al.* 2004). The fundamental difference between the two morphometric methods is that traditional morphometrics is better suited for capturing size information rather than for shape (Bookstein *et al.* 1985; Rohlf and Marcus 1993), whereas geometric morphometrics can eliminate all non-shape variation from the data through superimposition, while capturing all shape variability of the raw landmark data. Although geometric morphometrics is still the best method for obtaining shape variation information among organisms, combining this approach with additional traditional morphometric analysis has been implemented in a number of studies (e.g. grasshoppers *Sigaus australis* and *S. childi*, Dowle *et al.* 2014; populations of cichlids, *Tropheus moorii*, Maderbacher *et al.* 2008; orchid seeds from the Chloraeeae tribe, Chemisguy *et al.* 2008; wild mice *Mus macedonicus* and *M. cypriacus*, Macholan *et al.* 2008).

Within the Australasian grasshopper genus *Phaulacridium* (Key 1992), two endemic New Zealand species provide an excellent opportunity to compare morphological variation using traditional and geometric morphometric methods. *Phaulacridium marginale* (Walker 1870) occurs in mesic habitats throughout

mainland New Zealand and many off-shore islands (Westerman and Ritchie 1984). In contrast, *P. otagoense* (Westerman and Ritchie 1984) is restricted to more arid areas in the Central Otago and Central Canterbury regions of the South Island (Westerman and Ritchie 1984). Morphological studies in the 1970s and 1980s described in detail the diagnostic morphological characters of the two species (Westerman 1974, 1983; Westerman and Ritchie 1984).

Of the twelve species distinguishing traits identified by Westerman and Ritchie (1984) (Table 4.1), body length, lateral carinae pronotum angle, hind femur length and hind tibiae colour are most commonly used (Key 1992; Morris 2002). The larger *P. marginale* also exhibit more colouration polymorphism with green individuals being more common, in comparison to *P. otagoense.* *Phaulacridium otagoense* individuals are usually smaller and more cryptic in colour, which is suggested to be associated with their preference of drier, more open habitats with bare ground (Westerman and Ritchie 1984). Because the two *Phaulacridium* species differ in size (Key 1992; Scott 1997), it would be interesting to study shape differences using geometric morphometrics. As in many Acrididae, sexual dimorphism is apparent with females being larger than males.

Here, I examine in detail the morphological variation of New Zealand *Phaulacridium* grasshoppers by analysing dense population samples. For this study, traditional and geometric morphometric methods were used. More specifically, I have focused on the following questions: Are there still two morphologically distinct species? What are the morphometric differences between the grasshoppers? Does size variation (captured by traditional morphometrics) and shape variation (captured by geometric morphometrics) reveal the same separation of taxa? In southern South Island where the two species meet does grasshopper phenotype provide any evidence of genetic introgression?

Table 4.1: Body shape and colour characters identified by Westerman and Ritchie (1984) for New Zealand *Phaulacridium* grasshopper species.

	Phaulacridium marginale		***Phaulacridium otagoense***	
	Male	Female	Male	Female
Body length	≥ 10 mm	≥ 12.2 mm	≤ 10 mm	≤ 12.3 mm
Lateral carinae pronotum angle with first transverse sulcus	> 155°	> 155°	< 150°	< 150°
Anterior sections of carinae	Parallel (or nearly so)	Parallel (or nearly so)	Markedly divergent	Markedly divergent
Hind femur Length	> 6.5 mm	> 8.5 mm*	< 6.5 mm	< 8. 0 mm
Femur length/width	> 3.5 times		< 3.5 times	
Frontal ridge	Slightly or not at all indented		Distinctly sulcate	
Vertex lateral carinulae forms an obtuse angle anterior to eyes	< 125°	≤ 110°	≥ 140°	> 115°
Anterior margin of fastigium	Shallowly or not at all	Shallowly or not at all	Distinctly sulcate	Shallowly or not at all
Ratio least to greatest width fastigium	≥ 3.2	≥ 3.3	≤ 3.0	≤ 3.1
Pronotal hind margin	Margin accurate	Margin accurate	Accurate-medially indented	Accurate-medially indented
Tegmina	Larger		Smaller	
Supra-anal plate	More elongated		Less elongated	
Green morphs	Common	Common	Absent	Absent
Hind tibiae colour	Reddish, light to dark brown, grey	Reddish, light to dark brown, grey	Greyish	Greyish

* Key (1992) found a *P. marginale* female with a femur length of 8.38 mm.

4.2 Methods

Morphological data was collected for 148 adult *Phaulacridium* grasshoppers (see Chapter 3, Table 3.1 and Figure 3.1 for population samples location and sample size information) in two ways: using traditional morphometric species diagnostic characteristics, and testing for pronotum shape differences among species using geometric analysis. Adults were distinguished from juvenile individuals by their vestigial wings and tegmina lying dorsally, elongated anteroposteriorly with the

wings completely covered by the tegmina (Northcroft 1967). One juvenile grasshopper (GH1465 (LT)) was excluded from morphometric analysis. Females were separated from males by the presence of an ovipositor.

Traditional morphometrics

A total of 10 morphometric characters were measured in both sexes. These characters are listed in Table 4.2 and depicted in Figure 4.1. For each individual grasshopper, the hind femur and tibia, and the body (top of pronotum to the tips of the tegmina) was examined separately using an Olympus SZX7 stereo microscope. Images of both the hind femur and tibia, and body were taken using the camera Olympus SC100, all measurements were made using the Olympus cellSens Dimension 1.6 program. Eight morphometric traits were used to distinguish the two species and also the variation between and within the species: femur length, femur length/femur width (FL/FW), pronotum angle 1, pronotum angle 2, tibia length, tegmina length, pronotum width 3/pronotum length (PW3/PL), and pronotum width 1/pronotum width 2 (PW1/PW2).

Table 4.2: Description of the ten morphometric characters measured through traditional morphometric techniques.

Name	Code	Description
Femur Length	FL	Length of the hind femur
Femur Width	FW	Width of the hind femur across the third muscle band
Tibia Length	TL	Length of the hind tibia
Pronotum Angle 1	PA1	Lateral carinae pronotum angle with vertical sulcus
Pronotum Angle 2*	PA2	Lateral carinae pronotum angle with first transverse sulcus
Pronotum Length	PL	Length of pronotum
Pronotum Width 1	PW1	Width of pronotum
Pronotum Width 2	PW2	Width of pronotum at the narrowest point
Pronotum Width 3	PW3	Width of pronotum at the widest point
Tegmina Length	TeL	Mean length of the tegmina

* The lateral carinae pronotum angle is formed with the first transverse sulcus, but Westerman and Ritchie (1984) refer to this sulcus as the “second” transverse sulcus although this is not the correct terminology (Key 1992).

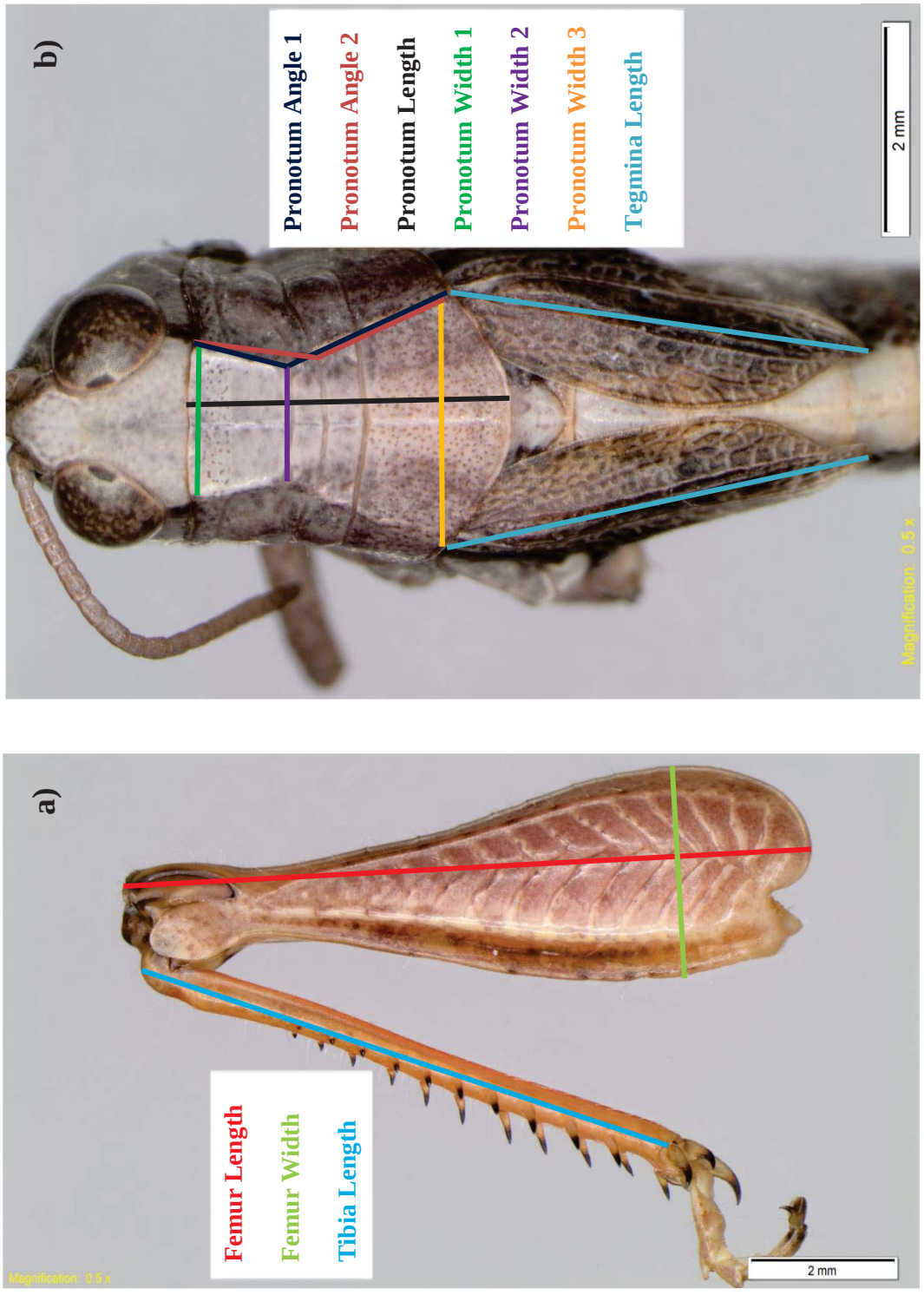


Figure 4.1: Location of the morphometric characters measured through traditional morphometric techniques at a) the hind leg (individual GH894(WB)) and b) at the thorax (individual GH1476(LP)).

Classifying individuals

Where possible, individuals were assigned to either species based on the trait differences described by Westerman and Ritchie (1984). Some individuals were phenotypically *P. marginale* for one trait, and *P. otagoense* for another trait, or fell between the maximum of one species and the minimum of the other. This problem involved three diagnostic morphometric traits (femur length, femur length/femur width and pronotum angle 2), used by Westerman and Ritchie (1984). For example, there were individuals who had a pronotum angle 2 value between the diagnostic trait values of 150° and 155° used by Westerman and Ritchie (1984). Likewise, a number of females had a femur length between 8.00mm and 8.50mm (Westerman and Ritchie 1984). These three morphometric traits were then examined together to determine if all three traits could be used to classify an individual according to the diagnostic criteria determined by Westerman and Ritchie (1984). Many individuals could not be accurately classified as a particular species because one of these three traits was classed as one species while the other two traits as the other species.

I used a model-based clustering approach and Bayesian Information Criterion (BIC) to select the best model with the software Mclust. Model-based clustering was used to classify individual grasshoppers as it does not require any prior information about specimen identity to classify individuals (Fraley and Raftery 1999, 2002, 2003). The Mclust algorithm, available in the Mclust "R" package (Fraley *et al.* 2012), is built from a general model, where the total data set is considered as a mixture of multivariate normal data sets, with a selection of covariance structures and vectors of expectation (Nanova 2014). The Mclust analysis allows for a total of 90 models to be examined (10 different models with various combinations of parameterization and 1 to 9 clusters/components; Fraley and Raftery 2003). The best model is then selected based on the BIC, which is the value of the maximized log likelihood, with a penalty for the number of parameters in the model (Fraley and Raftery 1999, 2002, 2003; Cordeiro-Estrela *et al.* 2008; Nanova 2014). The higher the BIC score, the lower the global average and median classification uncertainty, the better the model fits the data set (Fraley and Raftery 1999; Cordeiro-Estrela *et al.* 2008).

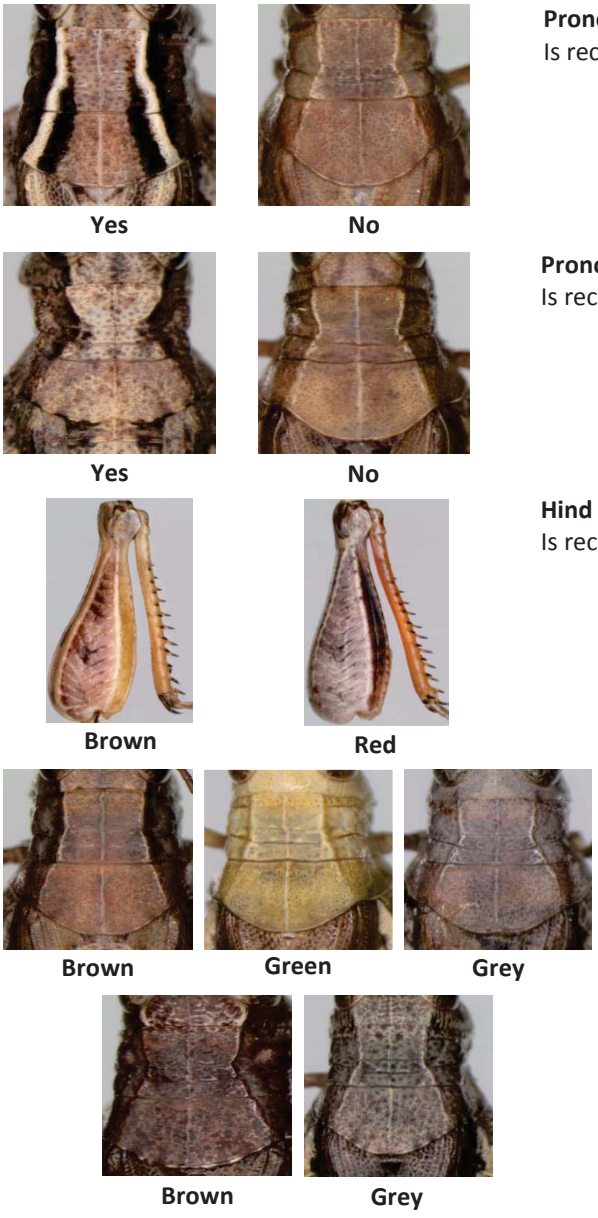
The input data, the measurements of eight morphometric traits for each grasshopper, was used for the Mclust algorithm v5.0.2 (see Appendix 3.1 for R code). The two sexes were considered separately as the species are sexually dimorphic. To select the optimal combination of morphometric traits for classification a series of trial datasets were analysed with different combinations of morphometric traits, including the three morphometric traits used by Westerman and Ritchie (1984) in all trials. The four models with the highest BIC score were selected as the optimal models to use for species classification purposes.

Morphological and colouration variation between morphotypes

The following statistical parameters were analysed based on the eight morphometric traits measured from the morphotype groupings (see Results) of New Zealand *Phaulacridium* grasshoppers: mean, standard error of the mean, range, and coefficients of variation (%). These eight morphometric traits were also visualised by boxplots for the morphotype groupings. The statistical parameters and boxplots were all computed using the program R v3.1.0 (R Core Team 2014).

For the eight morphometric traits, a multivariate analysis of variance (MANOVA) was used to determine whether the morphotype groupings exhibited differences in the morphometric traits within each sex, and also whether there was sexual dimorphism within a major cluster grouping. The two separate one-way MANOVA's were conducted with the program R v3.1.0 (R Core Team 2014).

Other morphological and colouration features were also compared between the morphotypes (Figure 4.2). The photographs used to measure the morphometric characters were also used to analyse these morphological and colouration features. A reference photo was used to determine which morphological/colouration feature an individual was most similar too. These morphological and colouration features were visualised by pie charts using the program R v3.1.0 (R Core Team 2014).



Pronotum Stripes
Is recorded as most similar to reference photo where:
Yes = colour changes marked by white line bounded by black
No = no defined colour change at pronotum lateral carinae

Pronotum Hind Margin Indentation
Is recorded as most similar to reference photo where:
Yes = markedly medially indented pronotum hind margin
No = no pronotum hind margin indentation

Hind Tibia Colour
Is recorded as most similar to reference photo where:
Brown = hind tibia is a brown colour (includes light brown to dark brown)
Red = hind tibia is a red colour

Pronotum Colour
Is recorded as most similar to reference photo where:
Brown = clear, uniform brown colour (includes light brown to dark brown)
Green = clear, uniform green colour
Grey = clear, uniform grey colour
Brown Patterned = mottled appearance, brown colour (includes light brown to dark brown)
Grey Patterned = mottled appearance, grey colour

Figure 4.2: Morphological and colouration features measured within New Zealand *Phaulacridium* grasshoppers. Morphological and colouration features were measured for each grasshopper, with the reference photo as guidance to what the individual was most similar to. Note that the red colour for the hind tibia and the green colour for the pronotum have faded as a resulted of being preserved in ethanol.

Geometric morphometrics

Geometric morphometric analysis was based on the pronotum of female and male New Zealand *Phaulacridium* grasshoppers from the morphotype groupings (see Results), as this body feature has been used to distinguish the two New Zealand *Phaulacridium* species (Westerman and Ritchie 1984). Geometric morphometrics studies the shape variation of objects, where the morphology of an object is represented by coordinates of sets of landmarks (Bookstein 1991). Digital images

of the pronotum obtained from the traditional morphometrics were used to test whether shape variation could be detected between New Zealand *Phaulacridium* morphotypes. Using tspDIG 2.17 (Rohlf 2013) on a Wacom Cintiq 22HD digitizing tablet, 12 landmarks (Figure 4.3) were identified and measured around the perimeter of the dorsal surface of the pronotum of each grasshopper. The landmark points were chosen for their ease of identification, homology among the grasshoppers, and the ability to capture the general shape of each morphological structure.

Figure 4.3: The 12 pronotum shape landmarks (black circles) used for geometric morphometric analysis of New Zealand *Phaulacridium* grasshoppers.

Measurement error was minimized by careful mounting, photography, and landmark selection. It was also verified that the variation due to measurement error was lower than the biological variation. Measurement error was divided between two potential sources of variation: the preparation of the mounting of the grasshopper pronotum and the accuracy of the digitization. Preparation of the mounting of the grasshopper pronotum was measured by preparing the pronotum of one grasshopper (GH 1705.4 (MB)) ten times, and each preparation was photographed and digitized separately. The accuracy of the digitization of the grasshopper pronotum was measured by digitizing the image of one grasshopper pronotum image (GH 1705.4 (MB)) ten separate times. A Procrustes analysis of variance (Procrustes ANOVA) was performed, which compared the variation in landmark location among grasshopper pronotums for the morphotype groupings. The scale of the measurement error was assessed by comparing the mean squares

of pronotum shape variation. As the variation between grasshopper pronotums clearly exceeded that of the measurement error, I considered the effect of the measurement error in the landmark location process to be negligible (see Results).

Pronotum shape variation was examined using MORPHOJ (Klingenberg 2011). A Procrustes fit aligned by principal axes was performed to eliminate size differences. Pronotum shape was assessed using principal component analysis (PCA) across all individuals and all landmarks, with grasshoppers grouped by clusters obtained from Mclust (see Results). Canonical variate analysis (CVA) of the clusters was used to describe differences among the pronotum shape for these clusters.

4.3 Results

Traditional Morphometrics

Cluster Analysis

A total of 32 combination trials ranging from three to eight morphometric traits were performed (see Appendix 3.2). The addition of more morphometric traits to the first three traits (femur length, femur length/femur width, and pronotum angle 2) did not decrease the BIC score. However, it was generally noted that when the morphometric trait pronotum angle 1 was included in the combination most BIC, scores notably decreased when compared to other combinations. For males, 12 of the 32 combination trials resulted in only one cluster grouping, which could be due to their smaller size which results in less variation in their morphometric traits measured. On the other hand, in females there were commonly two clusters, 64 to 69 individuals in one cluster and 16 to 21 individuals in the other cluster.

The four best Mclust models ranged from four to six combinations of morphometric traits, with the morphometric trait pronotum width 3/pronotum length being common in all four models along with the three morphometric traits used by Westerman and Ritchie (1984) (Table 4.3). Of the four models for male individuals, two of them (Models II and IV) only formed one cluster and could not be used for species classification (Figure 4.4 b, d), the other two models (Models I and III) formed two clusters (Figure 4.4 a, c). Males from Cluster A for the Models I

and III were found only in the southern/central South Island, with males from Cluster B being sampled across New Zealand (Table 4.3; see Appendix 3.3).

In contrast, Models I, II and III for female individuals generated two clusters (Figure 4.5 a, b, c), with Model IV producing three clusters (Figure 4.5 d). Females in Cluster A for Models I, II and III were only sampled in the southern/central South Island, with females from Cluster B found across New Zealand (Table 4.3; see Appendix 3.4). For Model IV, all females in Cluster A were sampled from the southern/central South Island, individuals in Cluster B were also mostly (79%) located in the southern/central South Island, and the majority (97%) of females in Cluster C were from northern South Island (Whites Bay, Greymouth and Mt. Fitzwilliam) and all North Island locations (Table 4.3; see Appendix 3.4). The addition of the third cluster in Model IV can be accounted for by the morphometric trait tegmina length, as Model II has all the same character traits as Model IV expect for the tegmina length.

Table 4.3: The four best Mclust models for New Zealand *Phaulacridium* grasshoppers with varying combinations of morphometric traits, based upon the highest BIC score. See Table 4.2 for codes of the morphometric traits.

	Model I		**Model II**		**Model III**		**Model IV**	
	Male	Female	Male	Female	Male	Female	Male	Female
Morphometric Traits	FL, FL/FW, PA2, PW3/PL		FL, FL/FW, PA2, PW3/PL, PW1/PW2		FL, FL/FW, PA2, PW3/PL, TL		FL, FL/FW, PA2, PW3/PL, PW1/PW2, TeL	
Model Type*	EEI	EVI	XXX	EVE	EVE	EVE	XXX	VEI
BIC	-347.075	-516.806	-236.370	-370.490	-341.605	-524.795	-317.865	-513.512
Log Likelihood	-146.607	-222.862	-76.754	-118.605	-108.656	-195.758	-103.000	-194.559
ICL	-348.262	-517.339	-236.370	-372.086	-342.291	-526.426	-317.865	-518.940
Number of clusters	2	2	1	2	2	2	1	3
Number of individuals in each clusters	Cluster A = 7, Cluster B = 56	Cluster A = 16, Cluster B = 69	Cluster A = 63	Cluster A = 16, Cluster B = 69	Cluster A = 5, Cluster B = 58	Cluster A = 18, Cluster B = 67	Cluster A = 63	Cluster A = 18, Cluster B =29, Cluster C = 38

* Description of model type: EEI (diagonal, equal volume and shape), EVI (diagonal, equal volume, varying shape), XXX (ellipsoidal multivariate normal – for single components/clusters), EVE (ellipsoidal, equal volume and orientation), and VEI (diagonal, equal shape).

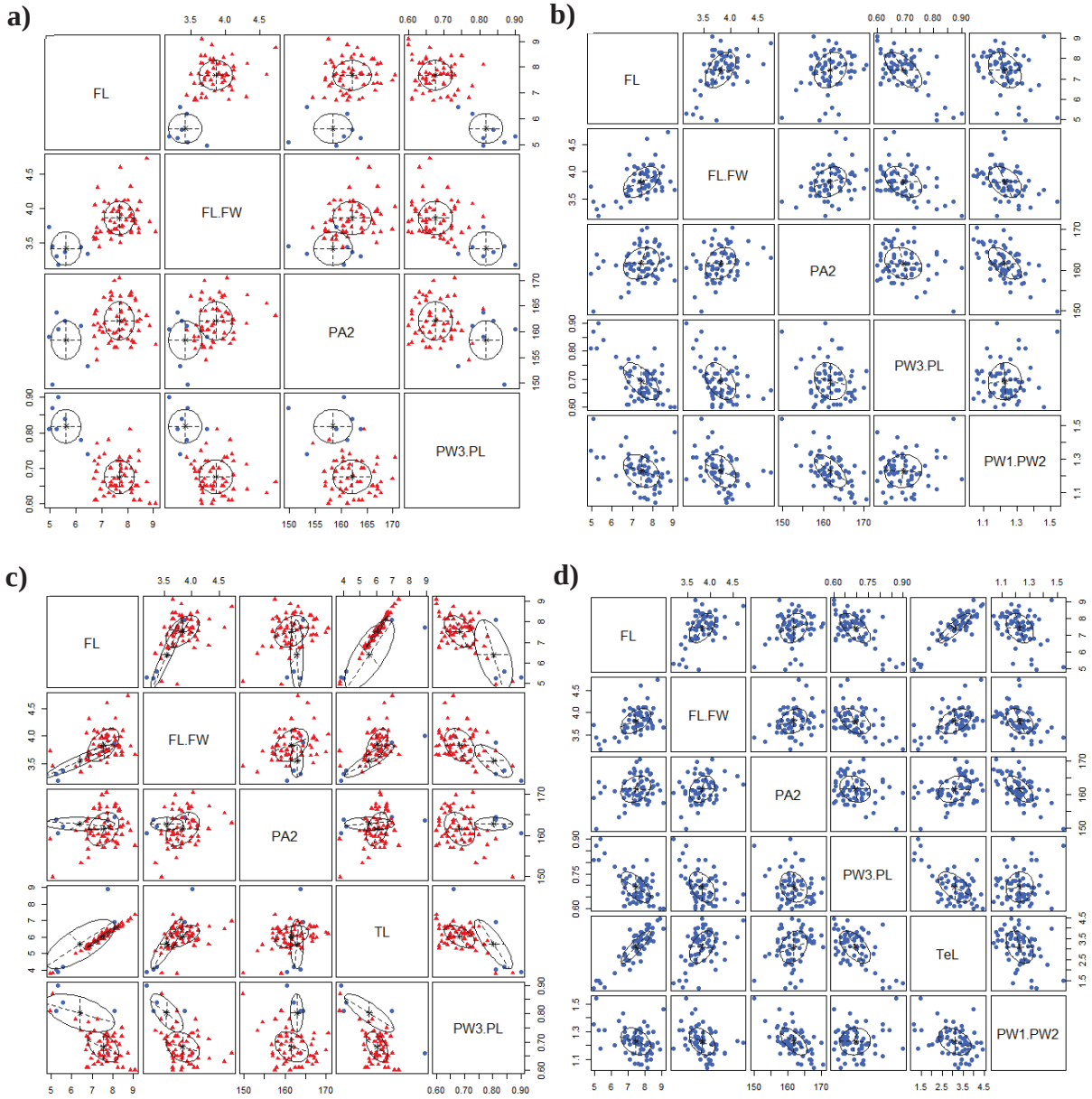


Figure 4.4: Mclust classification plots of the four best models for male *Phaulacridium* grasshoppers a) Model I b) Model II c) Model III, and d) Model IV. Models II and IV were not used for classification of male *Phaulacridium* grasshoppers. For Models I and III blue circles represent individuals in Cluster A and red triangles as from Cluster B. The ellipses correspond to the covariances of the components. See Table 4.2 for codes of the morphometric traits.

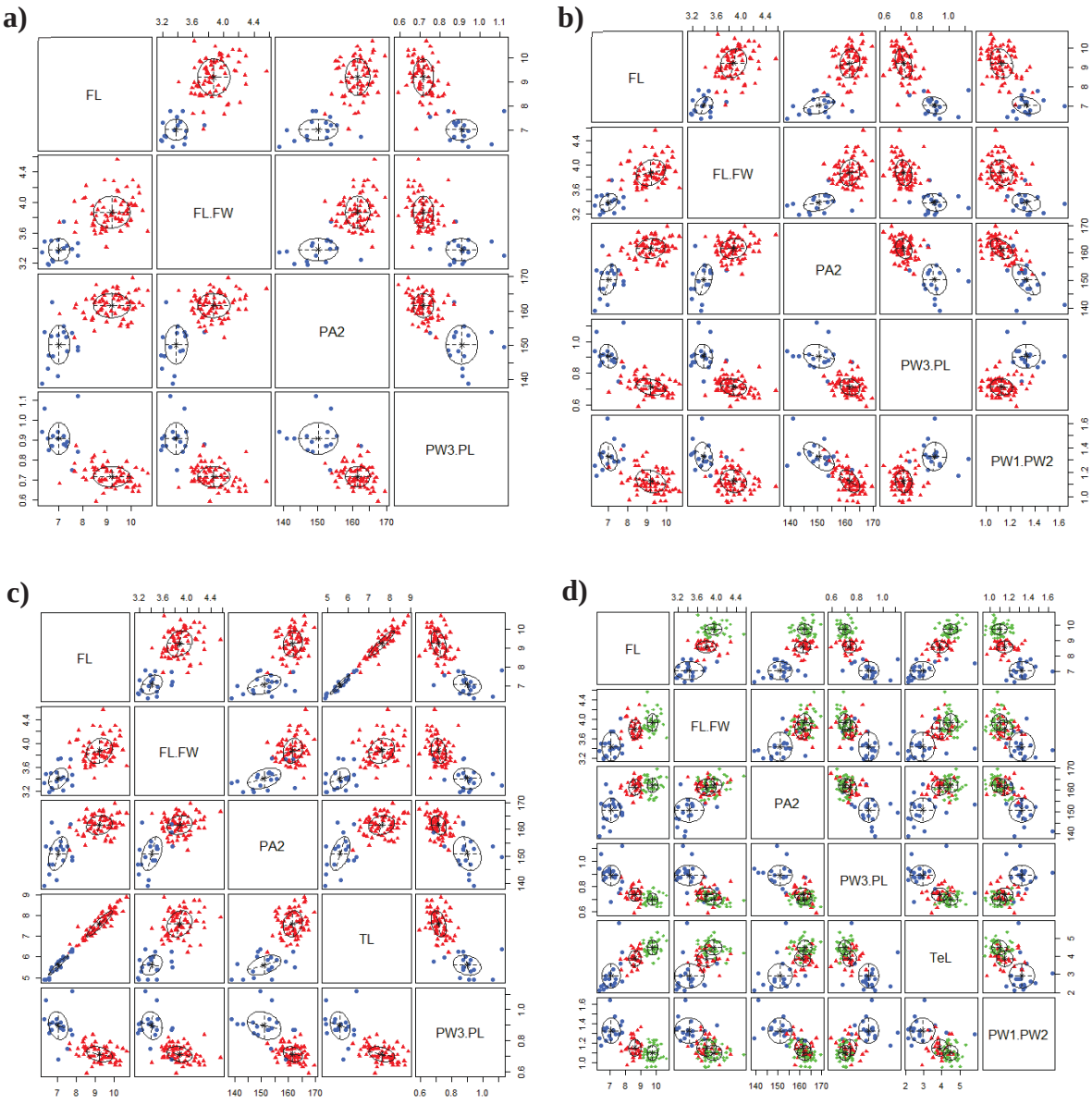


Figure 4.5: Mclust classification plots of the four best models for female *Phaulacridium* grasshoppers a) Model I b) Model II c) Model III, and d) Model IV. For Models I, II, III, and IV blue circles represent individuals in Cluster A and red triangles as from Cluster B. For Model IV, green diamonds represent individuals from Cluster C. The ellipses correspond to the covariances of the components. See Table 4.2 for codes of the morphometric traits.

For the purposes of this study, each grasshopper was classified into one of the two morphotype groupings. Individuals were regarded as Morphotype 1 (hence forward referred to as Type 1) if they were found in Cluster B for all four Mclust models, with the exception of Models II and IV for males. Females were also considered to be in Type 1 if they were in Cluster B for Models I, II, and III and in Cluster C for Model IV. An individual was considered to be in Morphotype 2 (hence forward referred to as Type 2) if it was found in Cluster A for all four Mclust models, with the exception of Models II and IV for males. Grasshoppers were classified as mixed if one or more of the four models were not identical with one another in terms of which cluster an individual was placed into.

A total of 120 (54 males and 66 females) *Phaulacridium* grasshoppers were considered to be in Type 1, with 19 individuals (3 males and 16 females) in Type 2, and 9 grasshoppers (6 males and 3 females) with a mixed morphotype classification (see Appendix 3.3 and 3.4). The northern South Island locations (Whites Bay, Greymouth, and Mt. Fitzwilliam) and all of the North Island populations only contained individuals that are classified as Type 1. The remaining nine locations were areas where both species had previously been described (Westerman and Ritchie 1984; Key 1992), and both morphotypes occurred together in some of these locations (Figure 4.6). Lindis Valley was the only location that was inhabited exclusively by Type 2 individuals. Lake Tekapo and Lake Dunstan had individuals in Type 2 and a few individuals with mixed morphotypes. Lake Pukaki and Graveyard Gully areas had grasshoppers of both morphotypes, and a few individuals of a mixed morphotype. The Ahuriri River individuals were of Type 1, apart from one individual which could not be classified. Lake Aviemore, Marshall Road, and Omarama only had Type 1 grasshoppers.

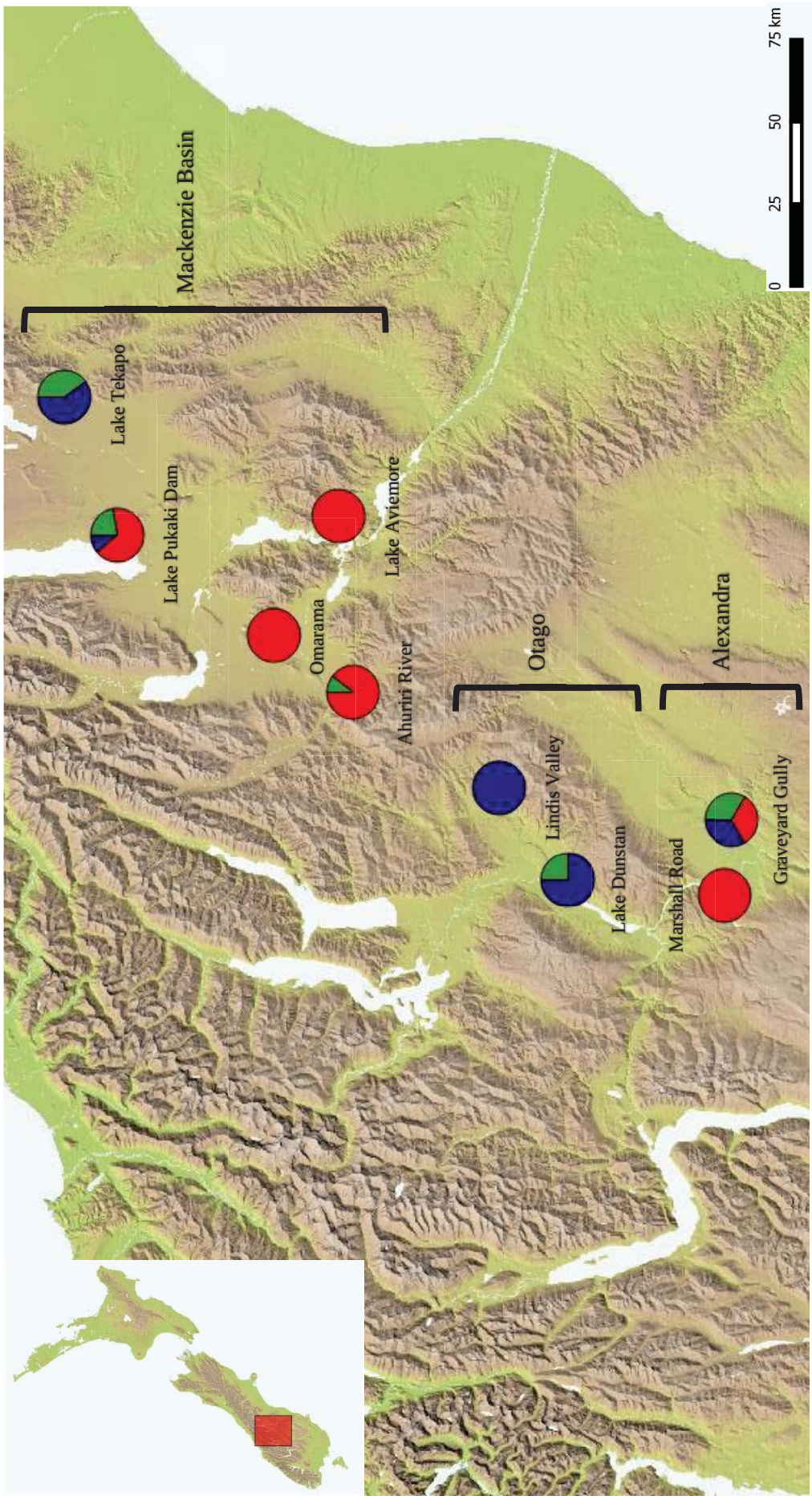


Figure 4.6: Geographic distribution of New Zealand *Phaulacridium* morphotypes around the southern South Island. The two morphotypes and individuals with an unknown morphotype are grouped by colour: Type 1 (red), Type 2 (blue), and mixed individuals (green).

Morphological and Colouration Variation between Morphotypes

Table 4.4 lists the descriptive statistics for each of the eight morphometric traits in the two morphotypes. Comparing the morphometric trait measurements of the grasshoppers from Type 1 to those from Type 2, it is evident that the femur length, femur length/femur width, tibia length, and tegmina length of the grasshoppers in Type 1 were larger than those from Type 2 (Figure 4.7 a). Furthermore, within a morphotype, males are smaller than females for femur length, tibia length, and tegmina length (Figure 4.7 a). Type 2 individuals had on average a larger pronotum width 3/pronotum length and pronotum width 2/pronotum width 1 than individuals from Type 1 (Figure 4.7 b). Type 1 females had a larger pronotum angle 1 and 2 when compared to Type 2; however, the pronotum angles for males from each cluster could not be discriminated (Figure 4.7 b). The differences in body size would suggest that the larger grasshoppers in Type 1 are equivalent to *P. marginale* described by Westerman and Ritchie (1984), whereas the smaller individuals from Type 2 correspond to *P. otagoense*.

Table 4.4: Descriptive statistics for the morphometric traits investigated in a) female and b) male New Zealand *Phaulacridium* grasshoppers, based on the morphotype groupings from Mclust.

	Morphometric Trait	Mean ± S.E.	Range	C.V. %
a) Female *Phaulacridium* Grasshoppers				
Type 1 (n = 66)	FL	9.28 ± 0.010	7.67–10.69	7.30
	FL/FW	3.87 ± 0.003	3.41–4.56	5.48
	TL	7.64 ± 0.009	6.51–8.88	7.62
	TeL	4.24 ± 0.007	2.90–5.34	11.43
	PW3/PL	0.71 ± 0.001	0.59–0.84	6.90
	PW1/PW2	1.12 ± 0.001	0.95–1.36	8.05
	PA1	155.87 ± 0.083	143.14–168.13	3.50
	PA2	161.74 ± 0.052	154.07–169.56	2.10
Type 2 (n = 16)	FL	7.00 ± 0.029	6.31–7.79	6.66
	FL/FW	3.38 ± 0.009	3.18–3.75	4.49
	TL	5.55 ± 0.030	4.90–6.39	8.75
	TeL	2.91 ± 0.059	2.13–5.84	32.30
	PW3/PL	0.91 ± 0.005	0.75–1.12	9.37
	PW1/PW2	1.33 ± 0.007	1.17–1.64	8.13
	PA1	140.06 ± 0.390	129.79–151.59	4.45
	PA2	150.04 ± 0.368	138.98–162.50	3.92
b) Male *Phaulacridium* Grasshoppers				
Type 1 (n = 54)	FL	7.67 ± 0.011	6.69–9.08	7.69
	FL/FW	3.86 ± 0.005	3.44–4.73	6.68
	TL	6.16 ± 0.009	5.26–7.35	7.93
	TeL	3.29 ± 0.010	2.03–4.45	17.18
	PW3/PL	0.67 ± 0.001	0.60–0.78	6.70
	PW1/PW2	1.22 ± 0.002	1.04–1.46	7.40
	PA1	155.89 ± 0.091	144.06–164.05	3.15
	PA2	162.02 ± 0.069	154.53–170.47	2.29
Type 2 (n = 3)	FL	5.38 ± 0.057	5.24–5.57	3.20
	FL/FW	3.28 ± 0.032	3.18–3.37	2.93
	TL	4.04 ± 0.052	3.89–4.20	3.83
	TeL	1.35 ± 0.055	1.18–1.51	12.24
	PW3/PL	0.85 ± 0.015	0.81–0.90	5.39
	PW1/PW2	1.27 ± 0.025	1.18–1.31	5.93
	PA1	155.39 ± 0.906	152.94–158.31	1.75
	PA2	162.19 ± 0.540	160.56–163.80	1.00

FL, Femur Length; FL/FW, Femur Length/Femur Width; TL, Tibiae Length; TeL, Tegmina Length; PW3/PL, Pronotum Width 3/Pronotum Length; PW1/PW2, Pronotum Width 1/Pronotum Width 2; PA1, Pronotum Angle 1; PA2, Pronotum Angle 2.

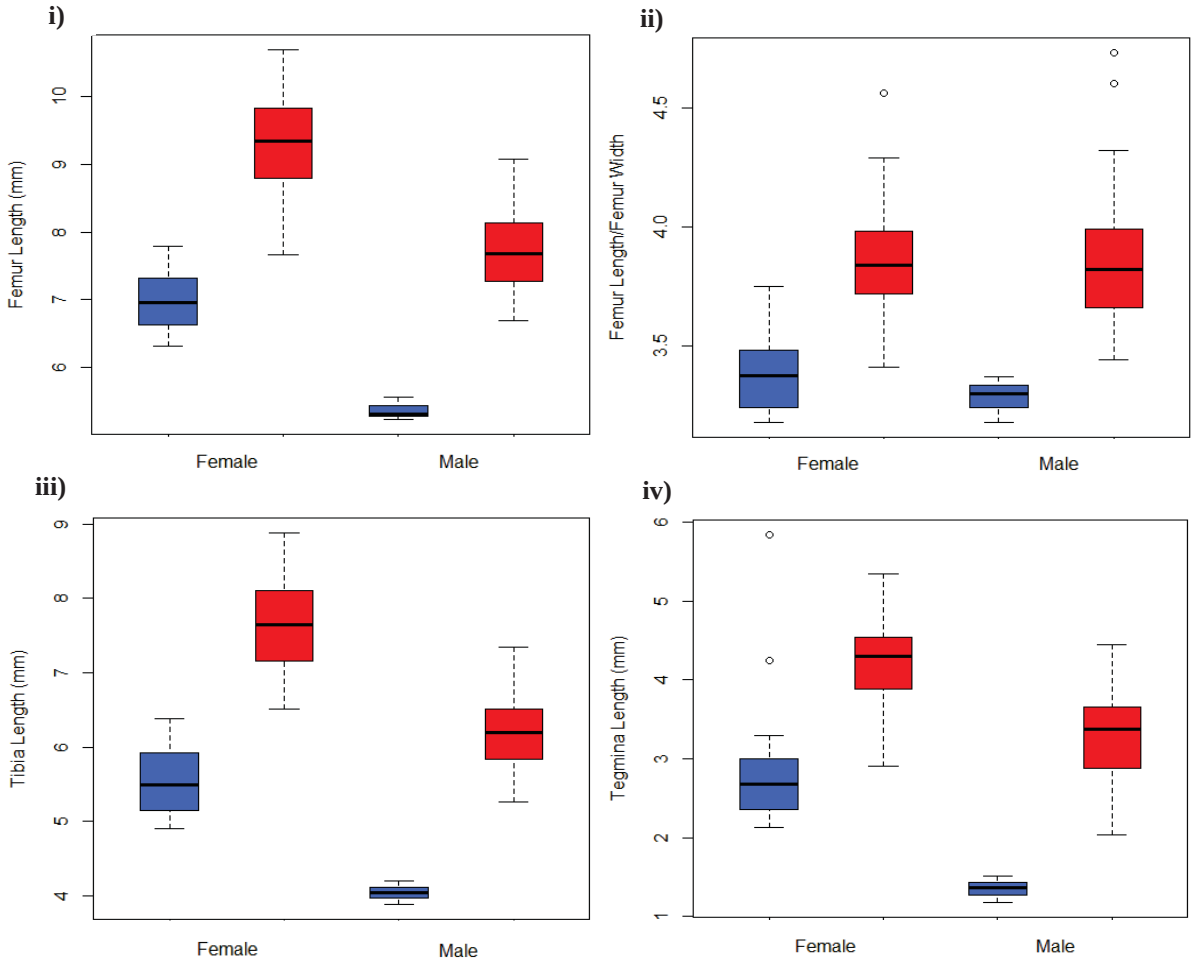


Figure 4.7a: Morphometric variation of each sex and morphotype of New Zealand *Phaulacridium* grasshoppers. Box plots show mean value (line), S.D. (boxes), and range (bars). Type 1 in red and Type 2 in blue. i) Femur Length, ii) Femur Length/Femur Width, iii) Tibia Length, and iv) Tegmina Length.

The results of the coefficient of variation for Type 1 males are higher than those obtained from males in Type 2. However, the coefficient of variation for female Type 2 grasshoppers are higher than those obtained from females in Type 1, with the exception of femur length and femur length/femur width. Based on the values of coefficient of variation, the tegmina length had the greatest heterogeneity out of the eight morphometric traits.

Although there are noticeable differences in the eight morphometric traits within sex comparisons for the two morphotypes, there are still overlaps in these ranges. Tibia length was the only morphometric trait within females where there was no overlap between the ranges for each morphotype. In contrast, five of the eight morphometric traits within males showed no overlaps between each morphotype, this could be due to the smaller sample size of Type 2 males.

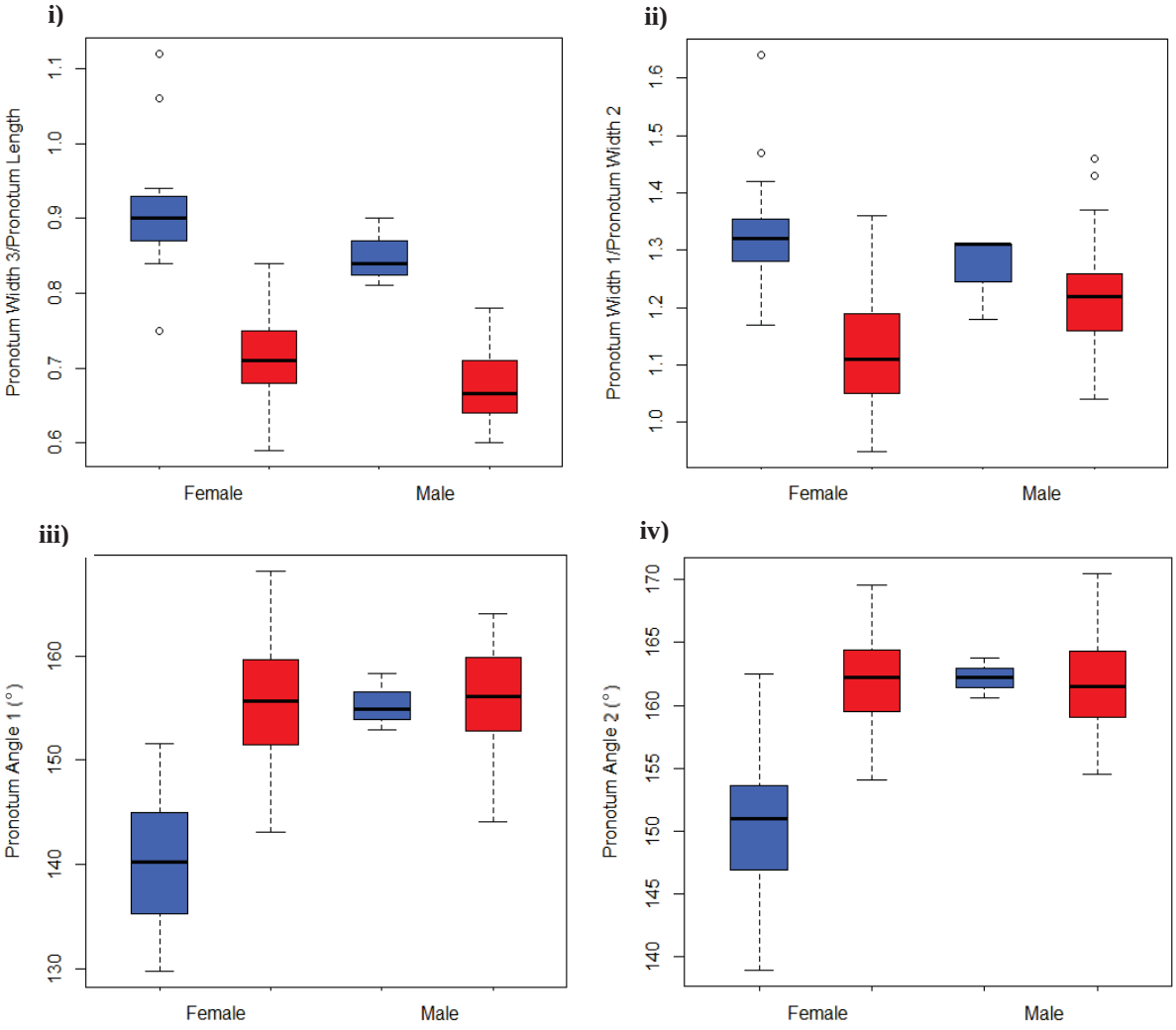


Figure 4.7b: Morphometric variation of each sex and morphotype of New Zealand *Phaulacridium* grasshoppers. Box plots show mean value (line), S.D. (boxes), and range (bars). Type 1 in red and Type 2 in blue. i) Pronotum Width 3/Pronotum Length, ii) Pronotum Width 1/Pronotum Width 2, iii) Pronotum Angle 1, and iv) Pronotum Angle 2.

The MANOVA of all eight morphometric traits revealed highly significant differences between the two *Phaulacridium* morphotypes for both females and males (Table 4.5 a). All eight morphometric traits showed highly significant differences between female Type 1 and 2. However, for males the two pronotum angles and the pronotum width 2/pronotum width 1 ratio were not considered significantly different (Table 4.6 a). Significant sexual dimorphism was observed within each morphotype (Table 4.5 b). Femur length, tibia length, and tegmina length was significantly between sexes within both morphotypes, however, the two pronotum angles were significantly different only between sexes for Type 2, with the two pronotum ratios being significant between the sexes for Type 1 (Table 4.6 b).

Table 4.5: Multivariate analysis of variance (MANOVA) of New Zealand *Phaulacridium* grasshopper morphometric traits quantifying a) between morphotype morphological variation, and b) within morphotype morphological variation.

	Pillai's Test	**Approximate *F***	**df**	***P***	
a) Between Morphotypes					
Females	0.815	40.184	8 and 73	<0.001	***
Males	0.662	11.764	8 and 48	<0.001	***
b) Within Morphotypes					
Type 1	0.741	39.737	8 and 111	<0.001	***
Type 2	0.836	6.376	8 and 10	0.004	**

** $P < 0.01$, *** $P < 0.001$

Eight character traits used as dependent variables, between-subject effects listed in Table 4.5.

Table 4.6: Morphological variation a) within and b) between morphotypes of New Zealand *Phaulacridium* grasshoppers.

	Dependent Variable	**df**	***F***	***P***	
a) Within Morphotypes					
Morphotype 1	Femur Length	1	187.88	<0.001	***
	Femur Length/Femur Width	1	0.118	0.732	
	Tibia Length	1	221.500	<0.001	***
	Tegmina Length	1	98.586	<0.001	***
	Pronotum Width 3/Pronotum Length	1	20.162	<0.001	***
	Pronotum Width 2/Pronotum Width 1	1	34.347	<0.001	***
	Pronotum Angle 1	1	<0.001	0.982	
	Pronotum Angle 2	1	0.671	0.672	
Morphotype 2	Femur Length	1	34.252	<0.001	***
	Femur Length/Femur Width	1	0.996	0.332	
	Tibia Length	1	27.095	<0.001	***
	Tegmina Length	1	7.854	0.012	*
	Pronotum Width 3/Pronotum Length	1	1.312	0.268	
	Pronotum Width 2/Pronotum Width 1	1	1.049	0.320	
	Pronotum Angle 1	1	16.886	<0.001	***
	Pronotum Angle 2	1	12.084	0.003	**
b) Between Morphotypes					
Female	Femur Length	1	160.660	<0.001	***
	Femur Length/Femur Width	1	78.246	<0.001	***
	Tibia Length	1	176.160	<0.001	***
	Tegmina Length	1	64.116	<0.001	***
	Pronotum Width 3/Pronotum Length	1	147.880	<0.001	***
	Pronotum Width 2/Pronotum Width 1	1	66.885	<0.001	***
	Pronotum Angle 1	1	102.280	<0.001	***
	Pronotum Angle 2	1	110.980	<0.001	***
Male	Femur Length	1	44.431	<0.001	***
	Femur Length/Femur Width	1	14.653	<0.001	***
	Tibia Length	1	55.044	<0.001	***
	Tegmina Length	1	34.616	<0.001	***
	Pronotum Width 3/Pronotum Length	1	42.982	<0.001	***
	Pronotum Width 2/Pronotum Width 1	1	0.858	0.358	
	Pronotum Angle 1	1	0.031	0.861	
	Pronotum Angle 2	1	0.006	0.937	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Two basic types of pronotum patterns were found within the morphotypes, a striped form and an unstriped form, with the unstriped form being more common (Figure 4.8 a). However, pronotum colouration varied in both morphotypes, Type 2 individuals tended to have duller colours when compared to Type 1 grasshoppers, which had more phenotypic variation in pronotum colour (Figure 4.8 d). Although individuals with red tibias were found in each morphotype, it was more common in Type 1 (Figure 4.8 c). The most obvious difference between the two morphotypes was the pronotum hind margin. The majority of individuals from Type 1 had a pronotum hind margin considered to be arcuate (no indentation) (Figure 4.8 b). In contrast, Type 2 individuals have a pronotum hind margin that is medially indented, with some individuals having a more pronounced pronotum margin indentation than others (Figure 4.8 b).

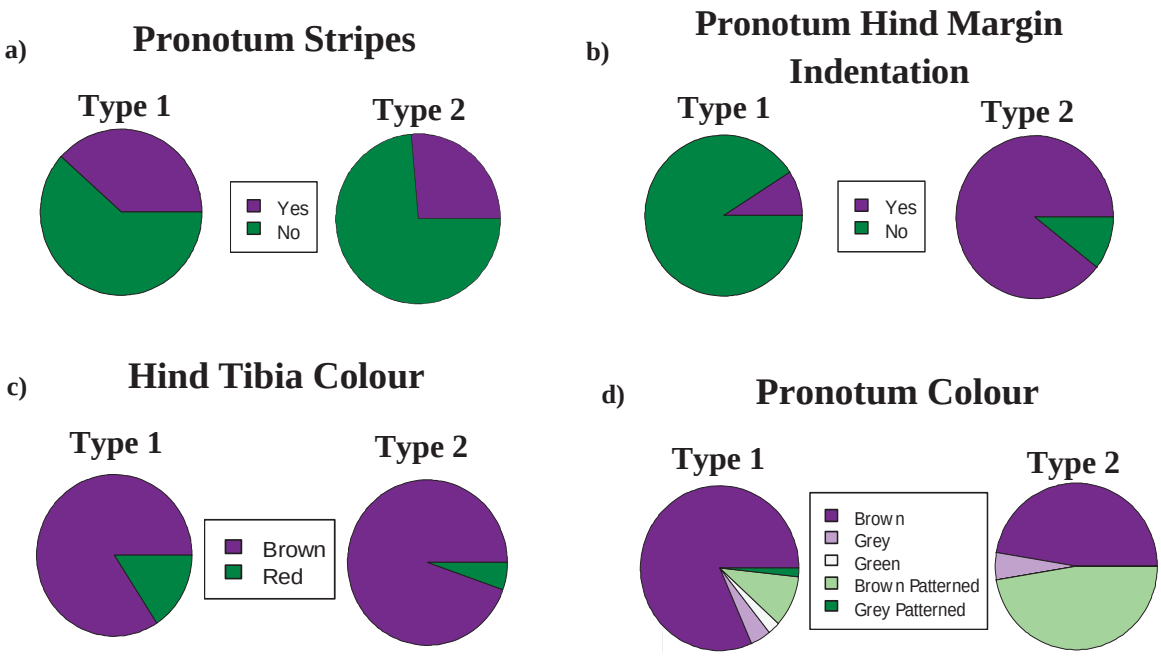


Figure 4.8: Comparison of morphological and colouration features between New Zealand *Phaulacridium* grasshoppers from Type 1 (n=120) and Type 2 (n=19). a) Presence of pronotum stripes, b) Presence of a pronotum hind margin indentation, c) Hind tibia colour, and d) Pronotum colour.

Geometric Morphometrics

Grasshopper pronotums differ in both size and shape as revealed by the Procrustes ANOVA (Table 4.7). The mean squares for pronotum size variation (MS pronotum = 166809.51) exceeded the mean squares for the measurement error (MS error =

0.005677) by 29,383,301-fold. For shape, mean squares for pronotum variation (MS pronotum = 0.0041481066) exceeded the mean squares for the measurement error (MS error = 0.0000265062) by 156.50-fold. These results indicate low measurement error and consequently strong repeatability of the landmark location of the pronotum.

Table 4.7: Results of the Procrustes ANOVA computed for a) size and b) shape for the variation among pronotums in New Zealand *Phaulacridium* grasshoppers.

	Sum of Squares	Mean of Squares	DF	F value	P value
a) Size					
Individuals	500428.54	166809.51	3	2938407.01	**0.0001**
Measurement Error	0.005677	0.005677	1	-	-
b) Shape					
Individuals	0.24888640	0.0041481066	60	156.50	**<0.0001**
Measurement Error	0.00053012	0.0000265062	20	-	-

Both sexes are combined in the geometric morphometric analysis as size is removed through superimposition of the raw data. Principal component analysis (PCA) of all individuals and all landmarks showed that 75% of observed morphological variance was explained by the first two principal components (PC1 = 47.81%, PC2 = 27.54%). Individuals were found to group with others of the same morphotype (based on traditional traits) with no overlap between Type 1 and Type 2. The exception to this were two grasshoppers (GH1586 (GG) and GH1476 (LP)) from Type 2 that had a pronotum shape that was more similar to individuals in Type 1 (Figure 4.9 a).

Type 2 individuals have a generally wider pronotum than grasshoppers from Type 1. The lateral carinae pronotum angle with the vertical sulcus (referred to pronotum angle 1 in the traditional morphometrics section) and the apex angle of the pronotum hind margin are considerably smaller in Type 2 individuals. Individuals with an unknown morphotype were scattered across both morphotypes (Figure 4.9). Canonical variate analysis of all individuals and all landmarks shows a clear strong separation in the pronotum shapes between morphotypes with little overlap between the morphotypes (Figure 4.9 b).

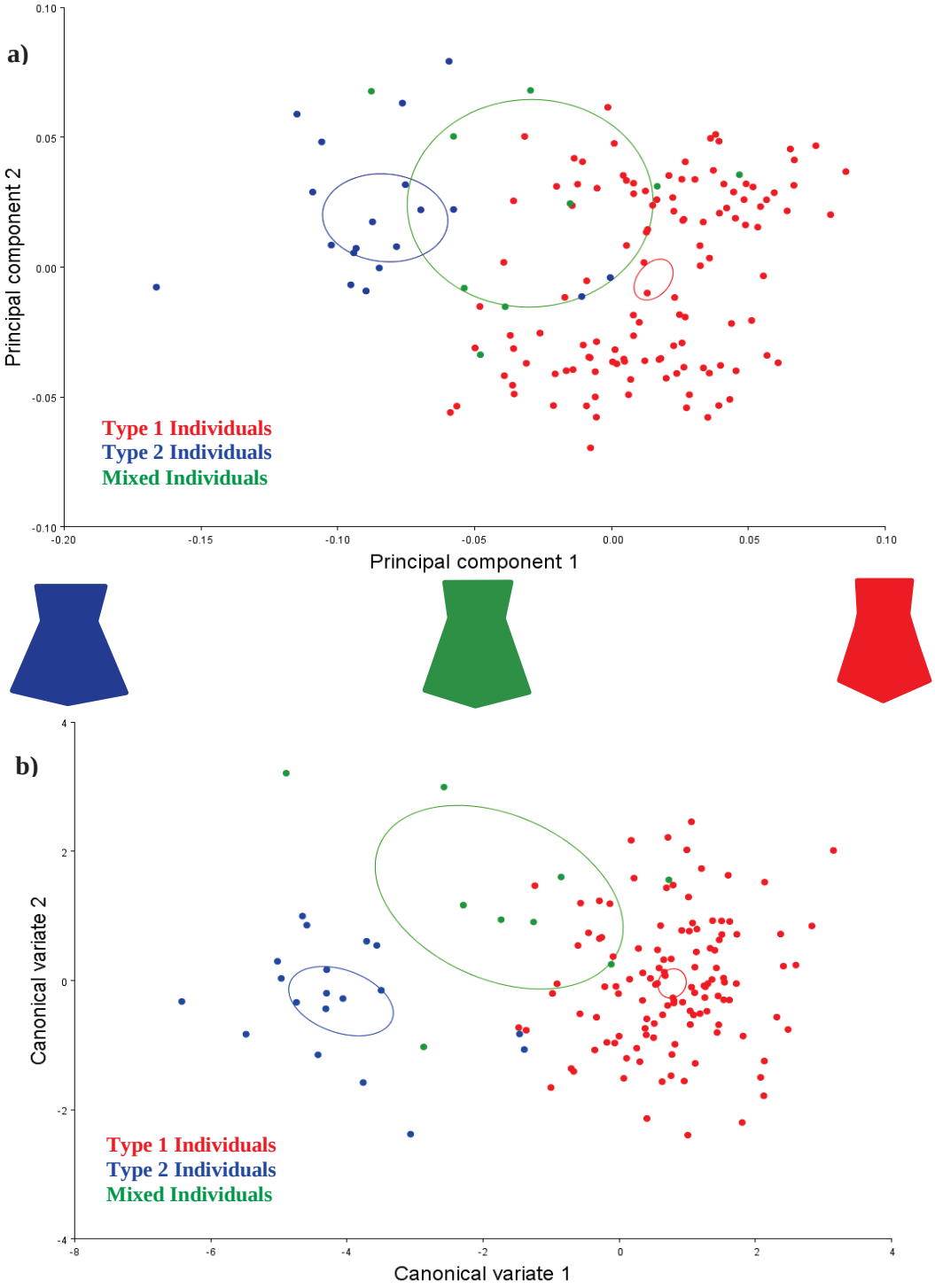


Figure 4.9: Variation in the shape of the pronotum using digital imagery of New Zealand *Phaulacridium* grasshopper pronotum shape: a) PCA for both morphotypes and individuals with a mixed morphotype; and b) CVA for both morphotypes and individuals with a mixed morphotype. Pronotum shapes of individuals from Type 1 (red), Type 2 (blue), and with a mixed morphotype (green) indicated along the x axis.

4.4 Discussion

Two distinct morphotypes were apparent among New Zealand *Phaulacridium* grasshoppers using traditional and geometric morphometric methods. Individuals of Type 2 were restricted to southern South Island locations compared to the more widespread Type 1. Traditional morphometrics revealed that grasshoppers assigned to Type 1 were significantly larger than grasshoppers from Type 2. However, individuals in Type 2 had a wider pronotum, and females of Type 2 had smaller pronotum angles than females from Type 1. Furthermore, traditional morphometrics also illustrated that Type 2 grasshoppers had less colour variation in pronotum colours than the Type 1 individuals that exhibited more variation. The majority of Type 2 individuals also had a medial indentation on the hind margin of the pronotum compared to the arcuate pronotum hind margin of most individuals from Type 1. Considerable pronotum shape variation was apparent from the geometric morphometric analysis. Males and females of the same morphotype had similar pronotum shapes despite overall differences in size. Type 2 grasshoppers generally had a wider pronotum and a smaller lateral carinae pronotum angle with the vertical sulcus and a smaller apex angle of the pronotum hind margin in comparison to individuals from Type 1.

Westerman and Ritchie (1984) found *P. marginale* to be the larger species with lateral carinae of pronotum forming a shallowly obtuse exterior angle (larger angle) at the second (technically the first) transverse sulcus. In contrast, in the smaller *P. otagoense*, the lateral carinae of pronotum forms a less shallowly obtuse exterior angle (smaller angle) at the second (technically the first) transverse sulcus (Westerman and Ritchie 1984). Furthermore, *P. otagoense* is suggested to be found only in the central/southern South Island, whereas *P. marginale* is more widespread (Westerman and Ritchie 1984). Based on Westerman and Ritchie's (1984) description of the New Zealand *Phaulacridium* species, I inferred that Type 1 and Type 2 individuals represent *P. marginale* and *P. otagoense*, respectively.

The morphological and colouration species discrimination characteristics described by Westerman and Ritchie (1984) were inconsistent with the present study (Table 4.8). Pronotum angle 2 had similar dimensions for *P. marginale* and

Type 1 in both studies, but *P. otagoense* (Type 2) had a larger angle in the current study compared to earlier data (Westerman and Ritchie 1984). *Phaulacridium marginale* females examined in this study tended to have shorter hind femura than specimens examined by Westerman and Ritchie (1984). Additionally, one *P. otagoense* individual from the current study had red hind tibiae, a colour thought previously to be absent in this species (Westerman and Ritchie 1984). Westerman and Ritchie (1984) found no overlap of morphological dimensions expressed by male and female *Phaulacridium*. However, in the current study all morphological characters overlap between the sexes of both species with the exception of tibia length, and in males the femur length, femur length/femur width, and tegmina length; which could be due to the smaller sample size of *P. otagoense* males. The morphotypes apparent in the current study do not perfectly match the two *Phaulacridium* species described by Westerman and Ritchie (1984).

However, further comparison of this study with Westerman and Ritchie (1984) suggests otherwise. A comparison of morphometrics between the current study and Westerman and Ritchie (1984) revealed that the means of the seven morphological parameters are the same or very similar for the sexes of both *Phaulacridium* species (Table 3.9). Generally the *Phaulacridium* specimens from the present study are slightly smaller than those from Westerman and Ritchie (1984). Therefore, although there are some notable differences between the present study and Westerman and Ritchie (1984), it is highly likely that the morphotypes produced by the Mclust analysis represents the two New Zealand *Phaulacridium* species. There are a number of reasons as to why there are some notable differences between the results obtained in the current study and those from Westerman and Ritchie (1984). These could include: the time difference between studies (1970s-1980s versus 2015), difference in techniques used for traditional morphometrics measurements (callipers versus microscope), specimen sampling, and environmental differences. Furthermore, introgression between the two species may contribute to the differences in traditional morphometric measurements.

Table 4.8: Comparison of morphometrics and colouration diagnostic characters of New Zealand grasshopper *Phaulacridium* species, a) *P. marginale* and b) *P. otagoense*, from Westerman and Ritchie (1984) (W & R 1984) and the current study (2015).

	Morphometric Traits	W & R (1984)	Current Study
a) *P. marginale* (Type 1)			
Male	Pronotum Angle 2	> 155°	> 154.53°
	Femur Length	> 6.5 mm	> 6.69 mm
	Femur Length/ Femur Width	> 3.5 times	> 3.44 times
	Tegmina	Larger	> 2.03mm
	Pronotum margin	Margin accurate	Arcuate-medially indented
	Hind tibiae colour	All colours	All colours
Female	Pronotum Angle 2	> 155°	> 154.07°
	Femur Length	> 8.5 mm*	> 7.67 mm
	Femur Length/ Femur Width	-	> 3.41 times
	Tegmina	-	> 2.90 mm
	Pronotum margin	Margin accurate	Arcuate-medially indented
	Hind tibiae colour	All colours	All colours
b) *P. otagoense* (Type 2)			
Male	Pronotum Angle 2	< 150°	< 163.80°
	Femur Length	< 6.5 mm	< 5.57 mm
	Femur Length/ Femur Width	< 3.5 times	< 3.37 times
	Tegmina	Smaller	< 1.51 mm
	Pronotum margin	Arcuate-medially indented	Medially indented
	Hind tibiae colour	Never red	Never red
Female	Pronotum Angle 2	< 150°	< 162.50°
	Femur Length	< 8.0 mm	< 7.79 mm
	Femur Length/ Femur Width	-	< 3.75 times
	Tegmina	-	< 5.84 mm
	Pronotum margin	Arcuate-medially indented	Arcuate-medially indented
	Hind tibiae colour	Never red	One red

* Key (1992) found a *P. marginale* female with a femur length of 8.38 mm.

Table 4.9: Comparative morphometrics (including the mean and range) of New Zealand *Phaulacridium* species, a) *P. marginale* and b) *P. otagoense*, from two separate studies, Westerman and Ritchie (1984) (W & R 1984) and the current study (2015).

	Morphometric Traits	W & R (1984)*	Current Study
a) *P. marginale* (Type 1)			
Male	Tegmen Length (mm)	3.8 (2.9–4.6)	3.3 (2.0–4.5)
	Hind Femur Length (mm)	8.1 (7.5–8.7)	7.7 (6.7–9.1)
	Femur Length/ Femur Width (depth)	3.9 (3.5–4.2)	3.9 (3.4–4.7)
	Pronotum Length (mm) †	3.2 (2.8–3.6)	2.9 (2.4–3.5)
	Hind Femur Width (mm) (depth) †	2.2 (2.0–2.4)	2.0 (1.6–2.5)
	Tegmen Length/ Pronotum Length †	1.2 (1.0–1.4)	1.1 (0.7–1.5)
	Tegmen Length/ Femur Length †	0.5 (0.4–0.6)	0.4 (0.3–0.6)
Female	Tegmen Length (mm)	4.4 (2.8–5.3)	4.4 (2.9–5.3)
	Hind Femur Length (mm)	9.7 (8.6–11.0)	9.3 (7.7–10.7)
	Femur Length/ Femur Width (depth)	3.8 (3.5–4.1)	3.9 (3.4–4.6)
	Pronotum Length (mm) †	4.2 (3.5–4.8)	3.9 (3.1–4.5)
	Hind Femur Width (mm) (depth) †	2.5 (2.1–2.8)	2.4 (1.9–3.0)
	Tegmen Length/ Pronotum Length †	1.1 (0.7–1.3)	1.1 (0.8–1.4)
	Tegmen Length/ Femur Length †	0.5 (0.3–0.6)	0.4 (0.4–0.5)
b) *P. otagoense* (Type 2)			
Male	Tegmen Length (mm)	1.9 (1.4–2.6)	1.4 (1.2–1.5)
	Hind Femur Length (mm)	5.6 (5.0–6.4)	5.4 (5.2–5.6)
	Femur Length/ Femur Width (depth)	3.1 (2.7–3.4)	3.3 (3.2–3.4)
	Pronotum Length (mm) †	2.0 (1.8–2.2)	1.9 (1.8–1.9)
	Hind Femur Width (mm) (depth) †	1.8 (1.6–2.0)	1.6 (1.6–1.7)
	Tegmen Length/ Pronotum Length †	0.9 (0.6–1.2)	0.7 (0.6–0.8)
	Tegmen Length/ Femur Length †	0.3 (0.2–0.4)	0.3 (0.2–0.3)
Female	Tegmen Length (mm)	3.0 (2.3–3.8)	2.9 (2.0–4.5)
	Hind Femur Length (mm)	6.8 (6.1–7.8)	7.0 (6.7–9.1)
	Femur Length/ Femur Width (depth)	3.4 (3.1–3.7)	3.4 (3.4–4.7)
	Pronotum Length (mm) †	2.8 (2.5–3.2)	2.7 (2.2–3.1)
	Hind Femur Width (mm) (depth) †	2.0 (1.9–2.2)	2.1 (1.8–2.4)
	Tegmen Length/ Pronotum Length †	1.1 (0.8–1.4)	1.1 (0.8–2.0)
	Tegmen Length/ Femur Length †	0.4 (0.3–0.6)	0.4 (0.3–0.8)

* *P. otagoense* were from Omarama and Lindis Pass, *P. marginale* were from various places.
† Were not part of the eight morphometric traits; however are some of the morphometric characters that made up the morphometric traits used in the current study.

If *P. marginale* and *P. otagoense* were reproductively isolated, distinct morphological traits between the species would be expected (Orr *et al.* 1994). Although no conclusive evidence of introgression has been discovered between the two species (Westerman and Ritchie 1984; Scott 1997), the results from the present morphological analysis could be explained by introgression. Firstly, nine grasshoppers could not be classified into a morphotype as the cluster the individual was placed into was inconsistent across the four models. This suggests that these individuals had a mixture of morphological features, with some features being more similar to one grasshopper species then the other. Pronotum shape of five of these individuals was also intermediate. Secondly, a number of the traditional morphometric characters measured between species of the same sex showed some overlap in the ranges of values, which could be a sign of on-going introgression. Thirdly, the samples from two locations comprised both species (Figure 4.10); therefore the two *Phaulacridium* species are occurring in close proximity. Furthermore, within these two sampling locations individuals with a mixed classification were also present. The mixed individuals were only found at sites in southern South Island, either where both species occur, or adjacent to one species. Additionally, *P. marginale* (Type 1) has expanded its range to the majority of sites that were formerly only occupied by *P. otagoense* before and during the 1970's (Figure 4.10, Westerman and Ritchie 1984). This further supports the idea that introgression may be occurring.

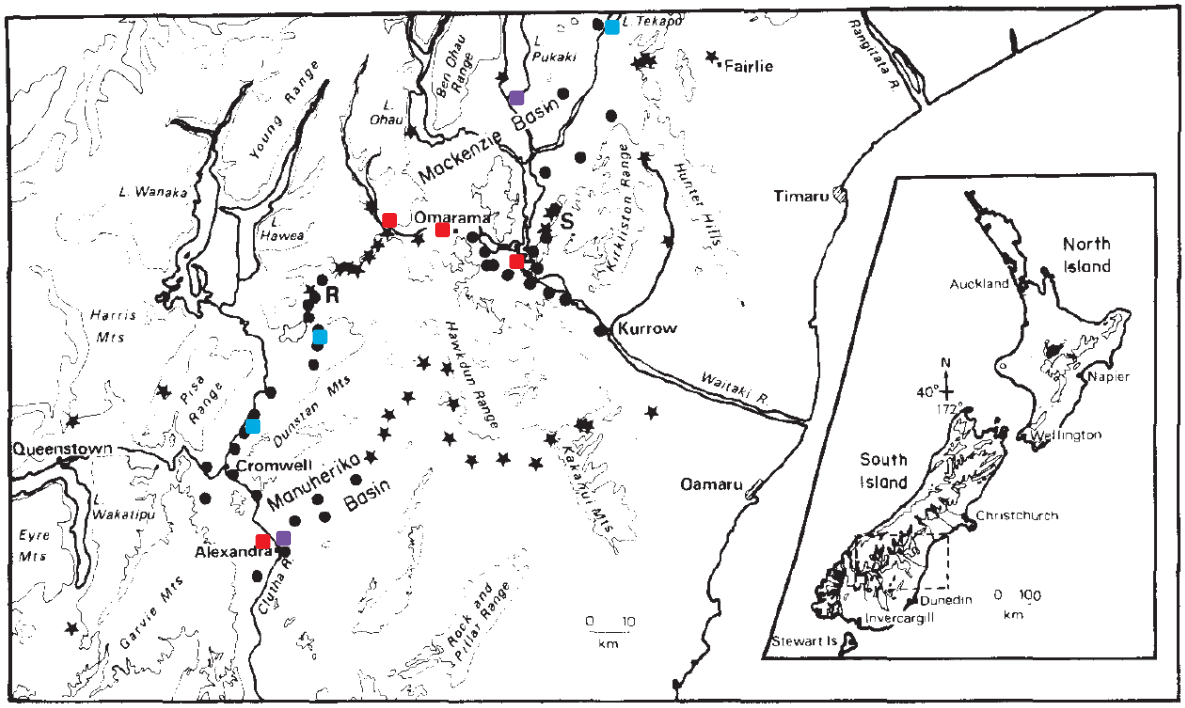


Figure 4.10: Location records of *Phaulacridium otagoense* (black dots) and *P. marginale* (black stars) in southern South Island, New Zealand (Modified from Westerman and Ritchie 1984). Sampling locations used in the present study are shown with coloured squares. Population samples containing individuals of both morphotypes (purple squares), population samples with individuals only of Type 1 (inferred to be *P. marginale*) (red square), and populations samples individuals only of Type 2 (inferred to be *P. otagoense*) (blue squares).

The present study demonstrates that the morphology of the two morphotypes represent the two described New Zealand *Phaulacridium* species. There are some differences between the current and former studies in diagnostic traits but this could be a result of recent introgression. Gene flow between *P. marginale* and *P. otagoense* would generate hybrids in the field that exhibit a mixture of characteristics. Evidence for introgression among New Zealand *Phaulacridium* could come from a mismatch of morphology and genetic data. Furthermore, comparing the morphological results acquired from the current study with environmental data would help identify whether there is an association between the morphology of a species and its preferred environmental conditions.

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Chapter 5

General discussion and future directions

Chapter 5: General discussion and future directions

5.1 Key findings

This study examined the distribution, spatial genetics, and morphology of two New Zealand *Phaulacridium* species; the widespread *P. marginale* and the restricted *P. otagoense*. I focused on populations of *Phaulacridium* from the southern South Island where the two species ranges overlap to examine evolutionary and ecological interactions of them. The research was separated into three main parts.

Chapter 2 examined the geographic distribution of the two species by analysing the recorded and potential modern distribution of the *Phaulacridium* grasshoppers. Models of environmental envelopes for each species demonstrated that the potential distribution of *P. marginale* covered the majority of New Zealand. In contrast, the potential distribution of *P. otagoense* is restricted to patches of land primarily in the central/southern South Island where this species is known to occur. Like *P. marginale*, there was a very high probability of *P. otagoense* being present in the Cromwell and Alexandra areas. Suitable habitat for *P. otagoense* is present in the Awatere Valley of northern South Island, however, this species has only been recorded as far as north as the Mackenzie Basin. The preferred temperature of *P. marginale* was explored using a temperature gradient experiment in the lab. *Phaulacridium marginale* exhibited a bimodal distribution of temperature preference. Some individual grasshoppers changed their preferred temperature between observations. It is possible that the observed bimodal distribution detected for this species is the result of the difference in optimal temperatures required for feeding and digestion.

In **Chapter 3** the phylogeographic structure of *Phaulacridium* species was analysed using dense population samples. Two main mtDNA COI sequence groups were found; the shallow but geographically widespread Group 1 (Lineage I) is considered to represent *P. marginale*, while the more diverse but geographically restricted Group 2 (Lineages II, III, IV) are inferred to be *P. otagoense*. The results also demonstrated that within the southern South Island region, the mitochondrial lineages attributed to *P. marginale* and *P. otagoense* co-occur within a single

location. Additionally, the prediction of recent population expansion in *P. marginale* was also tested. Demographic history analysis suggested that the widespread range of *P. marginale* is the result of recent population expansion because low genetic diversity is best explained by small population size. In contrast, *P. otagoense* has a restricted range today, but their high genetic diversity suggests that this species was recently represented in large populations.

Chapter 4 explored the morphological variation of *Phaulacridium* grasshoppers using traditional and geometric methods. Two distinct morphotypes were apparent among New Zealand *Phaulacridium* grasshoppers. The smaller and geographically restricted Type 2 was inferred to be *P. otagoense*, while the larger and geographically widespread Type 1 represented *P. marginale*. Both *Phaulacridium* morphotypes co-occurred within a single location within the southern South Island region. Furthermore, several individuals could not be classified into discreet morphotype, suggesting that these individuals had a mixture of morphological features, as expected of a hybrid.

The overlapping recorded and potential distributions of *Phaulacridium* grasshoppers, along with both species occurring in the same southern South Island populations for genetic and morphometric analyses suggest that there is the potential for introgression. Evidence for introgression among *P. marginale* and *P. otagoense* could come from the mismatch between morphology and genetic data.

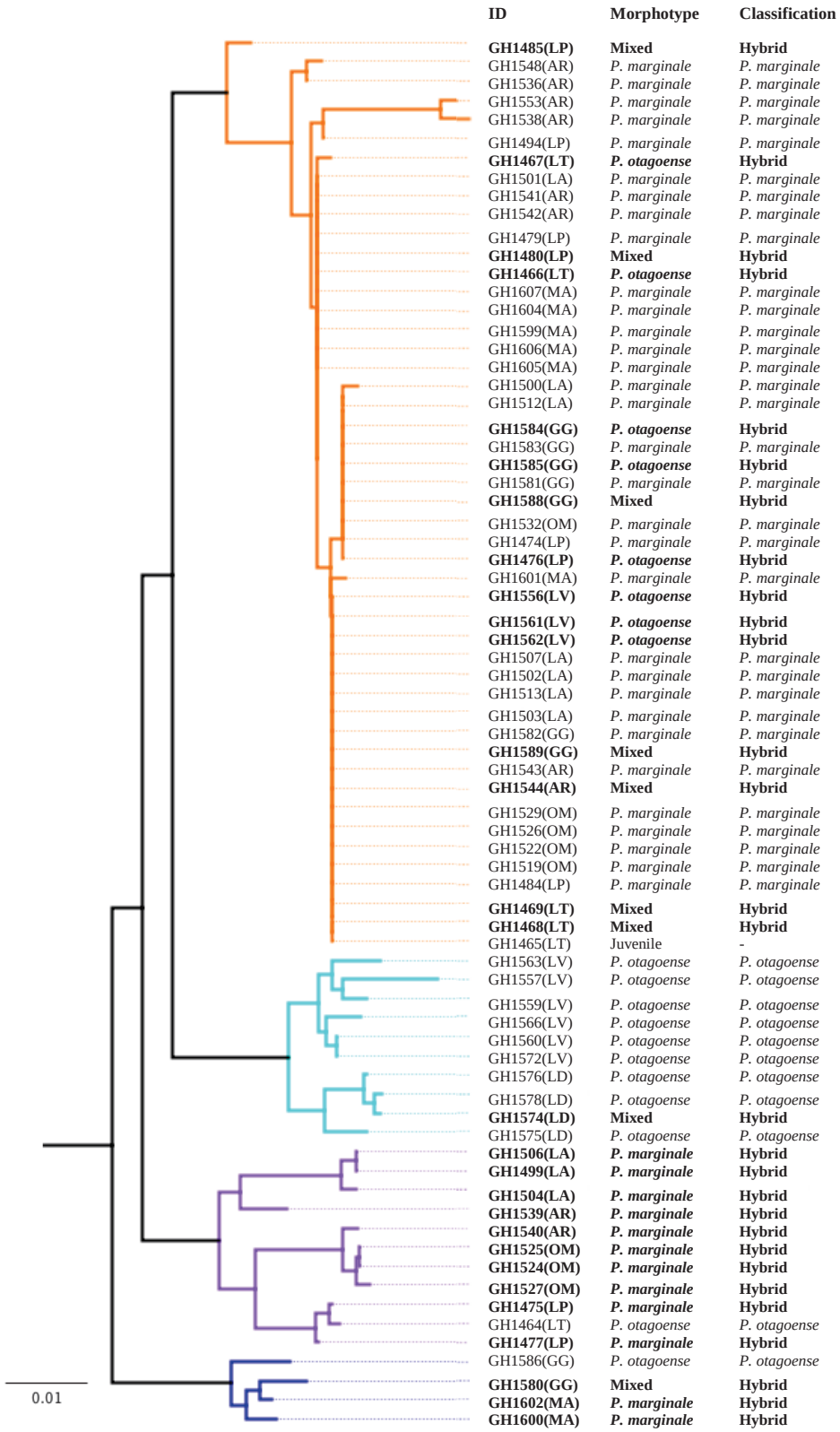
5.2 Evidence of introgression?

All North Island and northern South Island population samples (see Figure 3.1) consisted of individuals inferred to be *P. marginale* (Genetics = Group 1, Morphology = Type 1). However, within the samples from nine southern South Island locations there was a mixture of *Phaulacridium* classifications. Three overall classification categories were used to identify the southern South Island individuals (Figure 5.1). Individuals were considered to be *P. marginale* if they were from the Group 1 mtDNA lineage and a morphotype consisting of Type 1, while grasshoppers were inferred to be *P. otagoense* if they were from the Group 2 mtDNA lineage and Type 2 phenotype. Individuals that had a mismatch between morphology and genetic data (Group 1 and Type 2 or Group 2 and Type 1) were

classified as hybrids. Grasshoppers were also considered as hybrids if they had mixed morphotype (displayed a mixture of morphological features) regardless of the mtDNA lineage group they came from.

Within each southern South Island location, a mixture of individuals with different classification co-occur (Figure 5.2). Graveyard Gully was the only location that had individuals from all classifications. Lake Aviemore, Omarama, Lake Pukaki, Ahuriri River, and Marshall Road individuals were either *P. marginale* or hybrids. Lake Tekapo, Lindis Valley, and Lake Dunstan had grasshoppers with a *P. otagoense* or hybrid classification. Furthermore, the majority of individuals in Lineages III and IV (Group 2) were classified as hybrids. I repeated the species environmental distribution models for both *P. marginale* and *P. otagoense* based on these revised classification but they showed no significant difference to those obtained earlier (Chapter 2; data not shown).

Evidence of introgression has been documented in a number of grasshopper species (e.g. *Chorthippus parallelus* and *C. montanus*, Rohde *et al.* 2015; *Melanoplus sanguinipes* and *M. devastator*, Orr *et al.* 1994; *Stenobothrus rubicundus* and *S. clavatus*, Vedenina *et al.* 2012). Comparing the morphological and genetic data from the current study demonstrates the first reported case of introgression between *P. marginale* and *P. otagoense*. It is evident that *Phaulacridium* F_1 hybrids exist in the wild, however it is unknown whether these F_1 hybrids are fertile and also if F_2 hybrids (backcrossed from parental species or F_1 hybrids) are viable and fertile. Several studies have shown male grasshopper F_1 hybrids often have reduced fitness and/or are sterile (Hewitt *et al.* 1987; Vedenina *et al.* 2007). However, there are other cases where there is no significant reduction in hybrid fitness in F_1 hybrids relative to the parental species (Saldamando *et al.* 2005). Future work would need to be performed to determine if F_1 hybrids of *P. marginale* and *P. otagoense* are sterile.



ID	Morphotype	Classification
GH1485(LP)	**Mixed**	**Hybrid**
GH1548(AR)	*P. marginale*	*P. marginale*
GH1536(AR)	*P. marginale*	*P. marginale*
GH1553(AR)	*P. marginale*	*P. marginale*
GH1538(AR)	*P. marginale*	*P. marginale*
GH1494(LP)	*P. marginale*	*P. marginale*
GH1467(LT)	***P. otagoense***	**Hybrid**
GH1501(LA)	*P. marginale*	*P. marginale*
GH1541(AR)	*P. marginale*	*P. marginale*
GH1542(AR)	*P. marginale*	*P. marginale*
GH1479(LP)	*P. marginale*	*P. marginale*
GH1480(LP)	**Mixed**	**Hybrid**
GH1466(LT)	***P. otagoense***	**Hybrid**
GH1607(MA)	*P. marginale*	*P. marginale*
GH1604(MA)	*P. marginale*	*P. marginale*
GH1599(MA)	*P. marginale*	*P. marginale*
GH1606(MA)	*P. marginale*	*P. marginale*
GH1605(MA)	*P. marginale*	*P. marginale*
GH1500(LA)	*P. marginale*	*P. marginale*
GH1512(LA)	*P. marginale*	*P. marginale*
GH1584(GG)	***P. otagoense***	**Hybrid**
GH1583(GG)	*P. marginale*	*P. marginale*
GH1585(GG)	***P. otagoense***	**Hybrid**
GH1581(GG)	*P. marginale*	*P. marginale*
GH1588(GG)	**Mixed**	**Hybrid**
GH1532(OM)	*P. marginale*	*P. marginale*
GH1474(LP)	*P. marginale*	*P. marginale*
GH1476(LP)	***P. otagoense***	**Hybrid**
GH1601(MA)	*P. marginale*	*P. marginale*
GH1556(LV)	***P. otagoense***	**Hybrid**
GH1561(LV)	***P. otagoense***	**Hybrid**
GH1562(LV)	***P. otagoense***	**Hybrid**
GH1507(LA)	*P. marginale*	*P. marginale*
GH1502(LA)	*P. marginale*	*P. marginale*
GH1513(LA)	*P. marginale*	*P. marginale*
GH1503(LA)	*P. marginale*	*P. marginale*
GH1582(GG)	*P. marginale*	*P. marginale*
GH1589(GG)	**Mixed**	**Hybrid**
GH1543(AR)	*P. marginale*	*P. marginale*
GH1544(AR)	**Mixed**	**Hybrid**
GH1529(OM)	*P. marginale*	*P. marginale*
GH1526(OM)	*P. marginale*	*P. marginale*
GH1522(OM)	*P. marginale*	*P. marginale*
GH1519(OM)	*P. marginale*	*P. marginale*
GH1484(LP)	*P. marginale*	*P. marginale*
GH1469(LT)	**Mixed**	**Hybrid**
GH1468(LT)	**Mixed**	**Hybrid**
GH1465(LT)	Juvenile	-
GH1563(LV)	*P. otagoense*	*P. otagoense*
GH1557(LV)	*P. otagoense*	*P. otagoense*
GH1559(LV)	*P. otagoense*	*P. otagoense*
GH1566(LV)	*P. otagoense*	*P. otagoense*
GH1560(LV)	*P. otagoense*	*P. otagoense*
GH1572(LV)	*P. otagoense*	*P. otagoense*
GH1576(LD)	*P. otagoense*	*P. otagoense*
GH1578(LD)	*P. otagoense*	*P. otagoense*
GH1574(LD)	**Mixed**	**Hybrid**
GH1575(LD)	*P. otagoense*	*P. otagoense*
GH1506(LA)	***P. marginale***	**Hybrid**
GH1499(LA)	***P. marginale***	**Hybrid**
GH1504(LA)	***P. marginale***	**Hybrid**
GH1539(AR)	***P. marginale***	**Hybrid**
GH1540(AR)	***P. marginale***	**Hybrid**
GH1525(OM)	***P. marginale***	**Hybrid**
GH1524(OM)	***P. marginale***	**Hybrid**
GH1527(OM)	***P. marginale***	**Hybrid**
GH1475(LP)	***P. marginale***	**Hybrid**
GH1464(LT)	*P. otagoense*	*P. otagoense*
GH1477(LP)	***P. marginale***	**Hybrid**
GH1586(GG)	*P. otagoense*	*P. otagoense*
GH1580(GG)	**Mixed**	**Hybrid**
GH1602(MA)	***P. marginale***	**Hybrid**
GH1600(MA)	***P. marginale***	**Hybrid**

Figure 5.1: Classification of 73 New Zealand *Phaulacridium* grasshoppers from populations in central/southern South Island. The two main mtDNA COI lineages are Group 1 (Lineage I (orange)) considered to be *P. marginale* and Group 2 (Lineage II (light blue), Lineage III (dark blue), and Lineage IV (purple)) considered to be *P. otagoense* (see Chapter 3). Morphotype is obtained from cluster analysis of the traditional morphology where Type 1 is *P. marginale* and Type 2 as *P. otagoense* (see Chapter 4). Classification is determined by the combined result of the genetic and morphotype data. Individuals classified as hybrids are in bold.

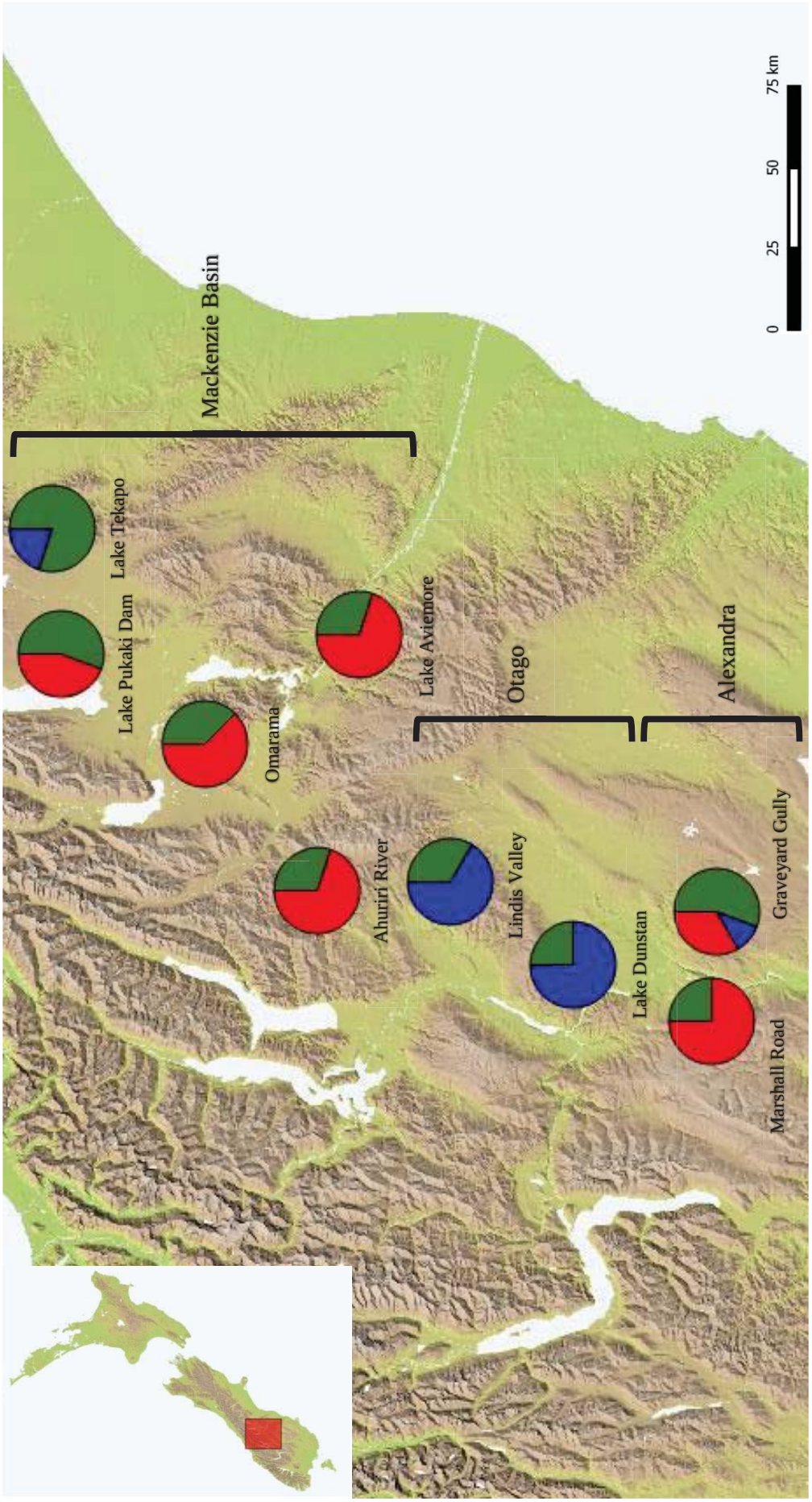


Figure 5.2: Geographic distribution of New Zealand *Phaulacridium* classifications around the southern South Island. The three classifications are grouped by colour: *P. marginale* (red), *P. otagoense* (blue), and hybrid individuals (green).

If *Phaulacridium* male F_1 hybrids are sterile, and female F_1 hybrids remain unaffected, with no preference for the parental species, they might still be able to backcross in both directions. Therefore, there is the possibility for F_2 generations (from backcross with parental species). Previous studies have shown in grasshoppers that F_2 generations can either be viable with good relative fitness relative to the parental generations (Saldamando *et al.* 2005), or there could be the complete breakdown of the F_2 generation (Moran *et al.* 1980). It is possible that *Phaulacridium* F_1 hybrids may have been able to backcross with parental species and/or other F_1 hybrids due to some grasshoppers from the current study showing no distinct morphotype (Type 1 or Type 2) instead these individuals possess a mixture of characteristics (individuals with a mixed morphotype). Backcrossing may result in the loss of distinct morphotypes.

Furthermore, Lindis Valley and Lake Dunstan areas appear to be the last stronghold of *P. otagoense* (Figure 5.2). Based on historical records of the distribution of *Phaulacridium* (Figure 5.3) and the results of the current study (Figure 5.2), it seems that *P. marginale* has begun to steadily replace *P. otagoense*. This scenario of introgression and advancing replacement has been documented in several other species (e.g. katydids *Orchelimum nigripes* and *O. pulchellu*, Shapiro 1998; warblers *Vermivora pinus* and *V. chrysoptera*, Gill 1980; freshwater minnows *Pseudorasbora pumila* and *P. parva*, Konishi and Takata 2004). It is possible that human interference through habitat change has facilitated the expansion of *P. marginale* into habitats previously occupied only by the other species. *Phaulacridium otagoense* has more specialized habitat requirements than the more generalist *P. marginale*, and the conversion of forest to pasture would have opened up a corridor, allowing *P. marginale* to reach areas previously only occupied by *P. otagoense*.

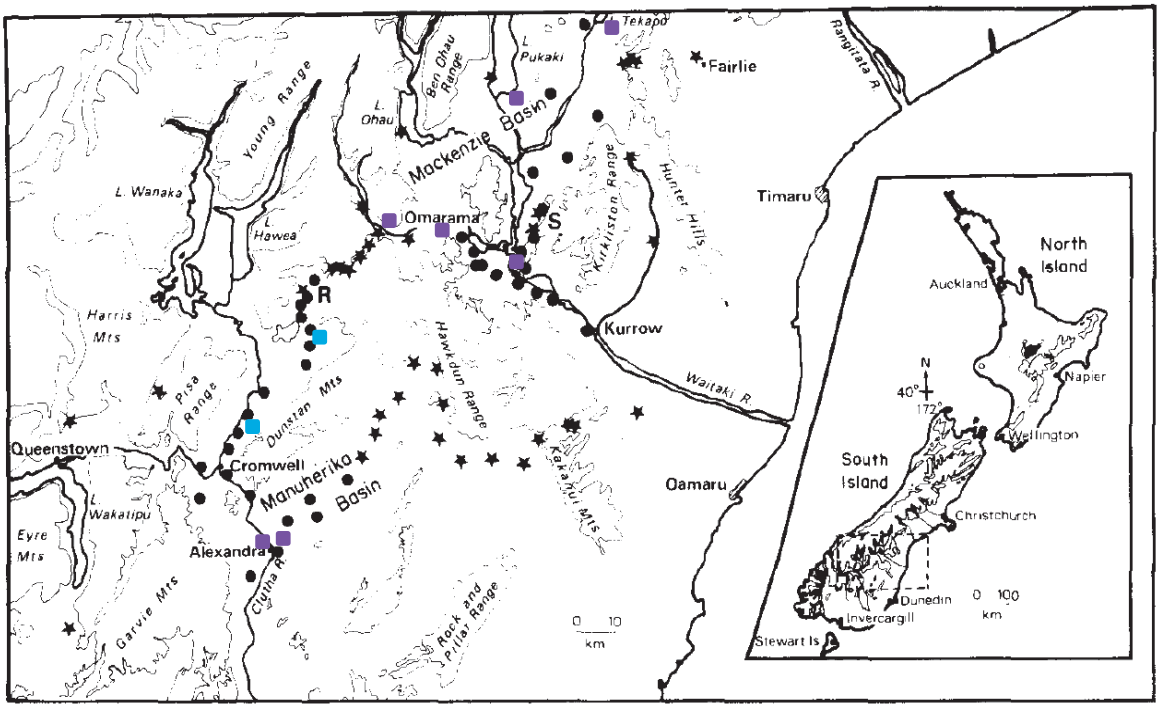


Figure 5.3: Recorded distributions of *Phaulacridium otagoense* (circles) and *P. marginale* (stars) in Central Otago and Canterbury, South Island, New Zealand (Modified from Westerman and Ritchie 1984). Sampling locations used in the present study are shown by squares. Population samples containing individuals that are predominantly classified as either *P. marginale* or hybrid are represented by purple squares and comprising only individuals predominantly classified as *P. otagoense* are shown in blue.

5.3 Summary of future directions

Although two independent forms of data were used in this study (mtDNA and morphology) to identify genetic introgression between two New Zealand *Phaulacridium* species, it should be noted that these data does not fully provide a picture of the processes behind the introgression of these species. Mitochondria DNA only shows the maternal inheritance, and therefore it alone cannot identify hybrid origin and introgression (Wilson *et al.* 1985). However, it can still be used to identify the maternal species in F_1 hybrids. Nuclear DNA is more useful as hybrids and their backcrosses carry this from both parental species, and hybridisation and introgression can be detected by nuclear markers (Michalczuk *et al.* 2014).

The use of nuclear markers for the identification of hybrids has been shown for introns (e.g. Daguin *et al.* 2001; Mettler and Spellmen 2009), single nucleotide

polymorphisms (SNP; e.g. Talbot *et al.* 2011; Pujolar *et al.* 2014), and microsatellites (e.g. Hanfling *et al.* 2005; Fowler *et al.* 2009). A combined set of nuclear and mitochondrial markers, with the addition of morphological data, may be the best option for hybrid identification within New Zealand *Phaulacridium*. For example, a multilocus study was performed on two sympatric short-horned grasshopper species in the South Island of New Zealand; one (*Sigaus australis*) widespread and the other (*S. childi*) a narrow endemic (Dowle *et al.* 2014). This study used six types of data; morphology, mtDNA sequencing, microsatellite genotyping, multi-copy nuclear sequencing, SNPs, and spatial position. When performing further multilocus analyses on New Zealand *Phaulacridium*, it should be taken into account that most acridid grasshoppers have larger genomes (5,950-20,600 Mb) than other insects (98-8,900 Mb) and larger than all mammals (1,420-5,680 Mb) (Bensasson *et al.* 2001).

Additionally, observations of mating pairs in the wild and breeding experiments between *P. marginale* and *P. otagoense* individuals could be performed. From these experiments we could establish the fitness of hybrids in terms of hatching success, physical abnormalities, and male sterility. Backcrossing experiments with the hybrids could verify whether there is the complete breakdown of F_2 generations or are they viable and how is their fitness affected. Furthermore, if these F_2 generations are viable the backcrossing experiments might determine if there is mate choice preferences, and therefore asymmetric introgression, such as performed by Hochkirch and Lemke (2011).

A combination of multilocus analyses and breeding experiments with *P. marginale* and *P. otagoense* would allow for a better understanding of the nature and extent of hybridisation in these sibling species with overlapping distributions. In doing so, it may elucidate information on the future of these two grasshopper species.

5.4 References

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Appendix 1

Localities details of known New Zealand *Phaulacridium* grasshopper locations in Chapter 2

Appendix 1: Localities details of known New Zealand *Phaulacridium* grasshopper locations in Chapter 2

Appendix 1.1 Localities details of known *Phaulacridium marginale* locations

Appendix 1.1: Localities details of known New Zealand *Phaulacridium marginale* grasshopper locations from published sources and collections.

Island	Location	Latitude	Longitude	Source
North Island	Black Hill Jack	-36.7221	175.7327	Westerman & Ritchie 1984
North Island	Coromandel	-37.0753	175.5209	Goldberg *et al.* 2015
North Island	Days Bay	-41.2794	174.9072	Key 1992
North Island	East Island	-37.6895	178.5761	Goldberg *et al.* 2015
North Island	Foxton	-40.4650	175.2687	Cumber 1959
North Island	Great Barrier Island	-36.2688	175.4917	Goldberg *et al.* 2015
North Island	Hen Island	-35.1615	174.0245	Bigelow 1967
North Island	Hokio Beach	-40.5994	175.2002	Phoenix Collection
North Island	Kaimai Range	-37.8086	175.9063	Cumber 1959
North Island	Kaiparoro (Mt Bruce)	-40.7634	175.6123	Westerman & Ritchie 1984
North Island	Kawakawa Track	-38.6580	175.9054	Goldberg *et al.* 2015
North Island	Kaweka Range	-39.1998	176.4707	Bigelow 1967
North Island	KoroKoro	-41.2173	174.8639	Phoenix Collection
North Island	Kuripapango Lakes	-39.3702	176.3410	Phoenix Collection
North Island	Lake Rotorua	-38.0352	176.2939	Phoenix Collection
North Island	Lake Waikaremoana	-38.7481	177.1593	Goldberg *et al.* 2015
North Island	Levin	-40.6415	175.2672	Goldberg *et al.* 2015
North Island	Little Barrier Island	-36.2189	175.0550	Bigelow 1967
North Island	Little Bush Road	-39.2871	176.5505	Phoenix Collection
North Island	Mahia Beach	-39.0831	177.8732	Phoenix Collection
North Island	Makara	-41.2713	174.6893	Goldberg *et al.* 2015
North Island	Makara Beach	-41.2182	174.7053	Phoenix Collection
North Island	Makororo River	-39.7422	176.2300	Phoenix Collection
North Island	Mangakuri Beach	-39.9692	176.9162	Phoenix Collection
North Island	Martinbourough	-41.2266	175.4524	Cumber & Eyles 1961
North Island	Maungaharuru Ranges	-39.0958	176.8058	Phoenix Collection
North Island	Maungatautari	-37.9999	175.5772	Goldberg *et al.* 2015
North Island	Moanui Stream	-38.4252	177.3411	Phoenix Collection
North Island	Mohaka River	-39.1997	176.5099	Cumber 1959
North Island	Mohi Bush	-39.8564	176.8940	Phoenix Collection

Island	Location	Latitude	Longitude	Source
North Island	Mokoia Island	-38.0829	176.2924	Meads & Fitzgerald 2001
North Island	Mokopuna Island	-41.2520	174.8648	Grehan 1990
North Island	Mokoroa Bush Scenic Reserve	-37.9791	176.9886	Phoenix Collection
North Island	Motu-o-Kura	-39.8332	177.0277	Crosby 1996
North Island	Mt Hauhungatahi	-39.2207	175.4820	Phoenix Collection
North Island	Mt Hikurangi	-38.2935	178.2258	Bigelow 1967
North Island	Mt Karioi	-37.8491	174.7793	Phoenix Collection
North Island	Mt Kaukau	-41.2338	174.7795	Goldberg *et al.* 2015
North Island	Mt Piorongia	-38.0212	175.1131	Phoenix Collection
North Island	Mt Ruapehu	-39.3259	175.4979	Phoenix Collection
North Island	Muriwai Beach	-36.8241	174.4263	Phoenix Collection
North Island	Napier-Taupo Road	-39.1490	176.6017	Westerman & Ritchie 1984
North Island	Newlands	-41.2313	174.8272	Goldberg *et al.* 2015
North Island	Ngaruroro River	-39.3880	176.3313	Phoenix Collection
North Island	Ngatapa	-38.5875	177.7882	Bigelow 1967
North Island	Northland Oil Refinery	-35.8574	174.4318	Westerman & Ritchie 1984
North Island	Nuhaka	-39.0398	177.7189	Westerman & Ritchie 1984
North Island	Ocean Beach	-41.3770	175.0722	Phoenix Collection
North Island	Okere	-38.0163	176.3454	Cumber 1959
North Island	Opotiki	-38.0029	177.2956	Cumber 1959
North Island	Paekakariki	-40.9707	174.9643	Westerman & Ritchie 1984
North Island	Palmerston North	-40.4145	175.6617	Phoenix Collection
North Island	Pohangina Valley	-40.1834	175.8697	Phoenix Collection
North Island	Porangahau Beach	-40.2599	176.6689	Phoenix Collection
North Island	Pukerua Bay	-41.0355	174.8776	Westerman 1974
North Island	Puna Hokoi	-38.7163	177.1442	Brock 1980
North Island	Rangipo Desert	-39.3032	175.7426	Goldberg *et al.* 2015
North Island	Ruakituri Valley	-38.7724	177.4289	Phoenix Collection
North Island	Sandy's Bridge	-38.3323	177.4181	Westerman & Ritchie 1984
North Island	Sharp Bush Track	-36.9045	174.5623	Green 1982
North Island	Smith Russell Track	-39.3556	176.3390	Phoenix Collection
North Island	Somes Island	-41.2577	174.8654	Grehan 1990
North Island	Taihape-Napier Road	-39.4658	176.1398	Westerman & Ritchie 1984
North Island	Tairua	-37.0025	175.8377	Bigelow 1967
North Island	Takapau	-38.0165	178.2674	Bigelow 1967
North Island	Takarere Road	-39.0560	176.5710	Phoenix Collection
North Island	Tawa Station	-38.3905	177.6178	Bigelow 1967
North Island	Te Araroa	-37.6352	178.3626	Phoenix Collection
North Island	Te Kuiti	-38.3063	175.1364	Westerman & Ritchie 1984
North Island	Te Mata Park	-39.6985	176.9072	Phoenix Collection
North Island	Te Puke	-37.7794	176.3092	Goldberg *et al.* 2015
North Island	Te Tumu Kaituna	-37.7332	176.3735	Ecological monitoring for Te Tumu Kiatuna 7B2 ecological restoration project, 2013
North Island	Te Whaiti	-38.5840	176.7806	Bigelow 1967
North Island	Titahi Bay	-41.1076	174.8247	Phoenix Collection
North Island	Tolaga Bay	-38.3695	178.3051	Cumber 1959

Island	Location	Latitude	Longitude	Source
North Island	Tongariro National Park	-39.0501	175.5806	Bigelow 1967
North Island	Urewera National Park	-38.6004	176.9839	Westerman & Ritchie 1984
North Island	Uruti	-38.9435	174.5302	Cumber 1959
North Island	Wharangi Road	-39.0501	176.5780	Phoenix Collection
North Island	Whirinaki Forest	-38.6707	176.7082	Goldberg *et al.* 2015
North Island	Whitianga	-36.8373	175.6799	Goldberg *et al.* 2015
South Island	Albury Range	-44.0959	170.7253	Crown Pastoral Tenure Review - West Hills Conservation Resource Report (2002)
South Island	Ariki Junction	-41.7824	172.2171	Westerman 1973
South Island	Arthurs Pass	-42.9453	171.5650	Goldberg *et al.* 2015
South Island	Awakino	-44.7474	170.4181	Goldberg *et al.* 2015
South Island	Awaroa	-40.8621	173.0410	Goldberg *et al.* 2015
South Island	Awatere Valley	-41.7120	173.9084	Westerman & Ritchie 1984
South Island	Bankside Scientific Reserve	-43.7342	172.1709	Emberson *et al.* 2011
South Island	Birdwood Range	-43.1734	171.4293	Crown Pastoral Tenure Review - Glenthorne Conservation Resource Report (2002)
South Island	Blackball	-42.3679	171.4114	Bigelow 1967
South Island	Blumine Island	-41.1695	174.2338	Moeed & Meads 1987
South Island	Bold Peak	-44.8569	168.3334	Bigelow 1967
South Island	Borland Lodge	-45.7763	167.5400	Goldberg *et al.* 2015
South Island	Boyle River	-42.5138	172.4344	Crown Pastoral Tenure Review - The Poplars Conservation Resource Report, Part 1 (2006)
South Island	Brod's Bay	-45.4046	167.6759	Bigelow 1967
South Island	Bruce Bay	-43.6043	169.5900	Bigelow 1967
South Island	Burkes Pass	-44.0893	170.5971	Goldberg *et al.* 2015
South Island	Burleigh Road Bridge	-41.5280	173.9414	Key 1992
South Island	Caberfeidh	-44.6247	170.5605	Crown Pastoral Tenure Review - Caberfeidh Conservation Resource Report (2006)
South Island	Camp Stream	-43.7879	170.6446	Crown Pastoral Tenure Review - Richmond Conservation Resource Report (2005)
South Island	Cardrona Ski Field	-44.8746	168.9697	Phoenix Collection
South Island	Cardrona Valley	-44.8570	169.0297	Crown Pastoral Tenure Review - The Larches Conservation Resource Report, Part 2 (2002)
South Island	Carrick Range	-45.1101	169.0708	Bigelow 1967
South Island	Cascade River	-44.0998	168.5250	Crown Pastoral Tenure Review - Lower Cascade Conservation Resource Report, Part 1 & 2 (2005)
South Island	Cass	-43.0295	171.7487	Hilgendorf 1918
South Island	Cave Stream Scenic Reserve	-43.1963	171.7420	Goldberg *et al.* 2015
South Island	Clarence River	-42.4578	172.8389	Goldberg *et al.* 2015
South Island	Clayton	-43.9202	170.8879	Crown Pastoral Tenure Review - Clayton Conservation Resource Report (2005)
South Island	Clent Hills	-43.6279	171.2258	Crown Pastoral Tenure Review - Clent Hills Conservation Resource Report (2004)
South Island	Cora Lynn	-43.0314	171.6473	Crown Pastoral Tenure Review - Cora Lynn Conservation Resource Report (2006)
South Island	Coronet Peak Ski Field	-44.9294	168.7308	Goldberg *et al.* 2015
South Island	Craigeburn	-43.1033	171.8648	Phoenix Collection
South Island	Dee Stream	-42.0003	173.7572	Goldberg *et al.* 2015
South Island	Denniston Plateau	-41.7389	171.7953	Phoenix Collection
South Island	Dublin Bay	-44.6522	169.1665	Bigelow 1967
South Island	Duffers Gully	-45.1759	169.0746	Crown Pastoral Tenure Review - Happy Valley Conservation Resource Report, part 1 (2005)
South Island	Dunstan Mountains	-45.0860	169.3405	Bigelow 1967

Island	Location	Latitude	Longitude	Source
South Island	D'Urville Island	-40.8278	173.8162	Bigelow 1967
South Island	Earnscleugh Tailings Historic Reserve	-45.2449	169.3572	Jamieson 1998
South Island	Ewe Range	-44.5489	169.9182	Patrick 1984
South Island	Fairlie Airstrip	-44.1081	170.8315	Westerman & Fontana 1973
South Island	Fox's Peak	-43.8681	170.8234	Bigelow 1967
South Island	Glenrock	-43.3275	171.3997	Crown Pastoral Tenure Review - Glenrock, Holbrook, Rollesby Conservation Resource Report, Part 2 (2006)
South Island	Greymouth	-42.4981	171.1870	Phoenix Collection
South Island	Hawkdun Range	-44.7245	169.9278	Patrick 1984
South Island	Holbrook	-44.7272	170.7753	Crown Pastoral Tenure Review - Glenrock, Holbrook, Rollesby Conservation Resource Report, Part 1 (2006)
South Island	Horseshoe Lake	-42.5970	172.5242	Bigelow 1967
South Island	Ida Range	-44.9677	170.1000	Patrick 1984
South Island	Invercroy	-44.4966	170.6221	Crown Pastoral Tenure Review - Invercroy Conservation Resource Report (2003)
South Island	Irishman Creek	-44.1776	170.3451	Crown Pastoral Tenure Review - Irishman Creek Conservation Resource Report (2006)
South Island	Island Hill	-46.9129	167.8196	Bigelow 1967
South Island	Kaikoura	-42.4066	173.6767	Bigelow 1967
South Island	Kaiwarua	-44.6256	170.8215	Crown Pastoral Tenure Review - Kaiwarua Conservation Resource Report (2005)
South Island	Kawaunui Stream	-42.1177	173.9214	Phoenix Collection
South Island	Killermont	-44.5155	169.8013	Crown Pastoral Tenure Review - Killermont Conservation Resource Report (2004)
South Island	Kurinui Hampden	-45.3339	170.7670	Goldberg *et al.* 2015
South Island	Kyeburn-Palmerston	-45.2229	170.4284	Phoenix Collection
South Island	Lake Benmore	-44.4355	170.2859	Crown Pastoral Tenure Review - Black Forest and Stony Creek Conservation Resource Report (2007)
South Island	Lake Forsythe	-43.8122	172.7140	Bigelow 1967
South Island	Lake Mahinerangi	-45.8440	169.8849	Bigelow 1967
South Island	Lake Monowai	-45.8586	167.4067	Phoenix Collection
South Island	Lake Ohau	-44.2345	169.8180	Bigelow 1967
South Island	Lake Pukaki	-44.1940	170.1375	Westerman & Ritchie 1984
South Island	Lake Roe	-45.7048	167.1404	Phoenix Collection
South Island	Lake Rotoiti	-41.8331	172.8242	Bigelow 1967
South Island	Lake Sumner	-42.7246	172.2760	Bigelow 1967
South Island	Lake Waitaki	-44.6807	170.3944	Bigelow 1967
South Island	Lake Wakatipu	-45.0289	168.4415	Bigelow 1967
South Island	Landsborough River	-43.9537	169.4863	Morris 1998
South Island	Laurence	-45.9133	169.6928	Westerman 1973
South Island	Lewis Pass	-42.3786	172.3991	Bigelow 1967
South Island	Lindis Pass	-44.5797	169.6532	Bigelow 1967
South Island	Loch Linnhe	-45.2652	168.7713	Crown Pastoral Tenure Review - Loch Linnhe Conservation Resource Report, part 1 & 3 (2007)
South Island	Lochaber	-43.8421	171.0800	Crown Pastoral Tenure Review - Lochaber Conservation Resource Report (2002)
South Island	Lochy River	-45.2371	168.4956	Crown Pastoral Tenure Review - Halfway Bay Station Conservation Resource Report (1999)
South Island	Long Island	-41.1233	174.2713	Moeed & Meads 1987
South Island	Lyttleton Lookout	-43.5936	172.7078	Westerman & Ritchie 1984
South Island	Mackenzie Pass	-44.1922	170.6106	Goldberg *et al.* 2015
South Island	Manahune	-44.2101	170.7408	Crown Pastoral Tenure Review - Manahune Conservation Resource Report (2003)

Island	Location	Latitude	Longitude	Source
South Island	Manapouri	-45.5671	167.6052	Key 1992
South Island	Marble Hill Picnic Area	-42.3556	172.2097	Goldberg *et al.* 2015
South Island	Maryburn Station	-44.1806	170.3191	Crown Pastoral Tenure Review - Maryburn Conservation Resource Report (2002)
South Island	Mathias Valley	-43.1877	171.1315	Crown Pastoral Tenure Review - Manuka Point Conservation Resource Report (2006)
South Island	McKinlays Creek	-45.1208	168.5859	Crown Pastoral Tenure Review - Walter Peak Conservation Resource Report, Part 2 (2005)
South Island	Meyer's Pass	-44.6776	170.6871	Goldberg *et al.* 2015
South Island	Milford Sound	-44.6720	167.9253	Westerman & Fontana 1973
South Island	Moke Lake	-45.0050	168.5673	Bigelow 1967
South Island	Monty's Saddle	-44.5197	170.2498	Westerman & Ritchie 1984
South Island	Morven Hills	-44.6432	169.5158	Crown Pastoral Tenure Review - Morven Hills Conservation Resource Report, Part 3 (2011)
South Island	Mount Albert Station	-44.2293	169.2129	Westerman 1973
South Island	Mt Arthur	-41.1946	172.7585	Bigelow 1967
South Island	Mt Aspiring Station	-44.4953	168.8366	Crown Pastoral Tenure Review - Mt Aspiring Station Conservation Resource Report, part 1 (2005)
South Island	Mt Buster	-44.9441	170.2412	Patrick 1984
South Island	Mt Cook Village	-43.7345	170.0987	Goldberg *et al.* 2015
South Island	Mt Fitzwilliam	-43.1738	171.5249	Phoenix Collection
South Island	Mt Fyffe	-42.3238	173.5933	Goldberg *et al.* 2015
South Island	Mt Hutt	-43.5339	171.5468	Bigelow 1967
South Island	Mt Lyford	-42.4844	173.1539	Goldberg *et al.* 2015
South Island	Mt Patriarch	-41.5900	173.2600	Goldberg *et al.* 2015
South Island	Mt Robert	-41.8227	172.8103	Goldberg *et al.* 2015
South Island	Mt Studholme	-44.6434	170.9135	Phoenix Collection
South Island	Ngahere	-42.3952	171.4457	Westerman 1973
South Island	Old Dunstan Road	-45.5441	169.9451	Barratt & Patrick 1987
South Island	Old Man Range	-43.9937	170.3472	Goldberg *et al.* 2015
South Island	Omahau Downs	-44.2484	170.1154	Crown Pastoral Tenure Review - Ohamau Downs Conservation Resource Report (2002)
South Island	Otira	-42.8310	171.5642	Bigelow 1967
South Island	Oxford	-43.3069	172.1834	Bigelow 1967
South Island	Palmer Range	-43.3414	171.3756	Crown Pastoral Tenure Review - Glenfalloch Conservation Resource Report(2005)
South Island	Paradise	-44.7244	168.3655	Westerman 1973
South Island	Pelorus Sound	-41.2391	173.7846	Goldberg *et al.* 2015
South Island	Piano Flat	-45.5704	169.0076	Harris 1990
South Island	Picton	-41.2951	173.9948	Bigelow 1967
South Island	Pisa Range	-44.8689	169.0464	Bigelow 1967
South Island	Port Hills	-43.5900	172.6900	Bigelow 1967
South Island	Porters Pass	-43.2966	171.7418	Bigelow 1967
South Island	Quartz Reef Point	-45.0083	169.2304	Phoenix Collection
South Island	Round Hill	-44.7809	168.4825	Crown Pastoral Tenure Review - Temple Peak Station Conservation Resource Report (2003)
South Island	Sabine Forks	-42.0136	172.6756	Bigelow 1967
South Island	Sandhill Point	-46.9261	167.7952	Bigelow 1967
South Island	Scotsburn	-43.9302	171.2292	Crown Pastoral Tenure Review - Scotsburn Conservation Resource Report (2002)
South Island	Shortlands Station	-45.0442	170.2665	Crown Pastoral Tenure Review - Shortlands Conservation Resource Report, part 1 & 2 (2006)
South Island	Skippers Saddle	-44.9404	168.7056	Westerman 1973

Island	Location	Latitude	Longitude	Source
South Island	South Rough Ridge	-45.3996	169.7382	Bigelow 1967
South Island	Spec Gully	-45.0231	170.1892	Crown Pastoral Tenure Review - Kyeburn Conservation Resource Report, part 1 (2001)
South Island	St. Arnaud Range	-41.7984	172.8811	Phoenix Collection
South Island	St. Bathans	-44.8721	169.8167	Goldberg *et al.* 2015
South Island	Stirling Brook	-41.7240	174.0762	Key 1992
South Island	Sumner	-43.5751	172.7507	Westerman 1973
South Island	Taylors Pass	-41.6301	173.9645	Phoenix Collection
South Island	Timaru	-44.3908	171.2497	Bigelow 1967
South Island	Trio Island	-40.8370	173.9980	Bigelow 1967
South Island	Wairuna	-46.1684	169.3192	Bigelow 1967
South Island	West Wanaka Station	-44.6210	169.0030	Crown Pastoral Tenure Review - West Wanaka Station Conservation Resource Report (2002)
South Island	Westport	-41.7408	171.6116	Bigelow 1967
South Island	White Burn	-45.2195	168.3479	Crown Pastoral Tenure Review - Walter Peak Conservation Resource Report, Part 2 (2005)
South Island	Whites Bay	-41.3859	174.0586	Phoenix Collection
South Island	Winterslow	-43.6046	171.4545	Crown Pastoral Tenure Review - Winterslow Conservation Resource Report (2004)
South Island	Woodbank	-42.5266	172.7632	Crown Pastoral Tenure Review - Woodbank Conservation Resource Report (2003)
South Island	Woolshed Creek	-44.9438	169.7519	Crown Pastoral Tenure Review - Lauder Conservation Resource Report, part 1 & 4 (2006)

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Appendix 1.2 Localities details of known *Phaulacridium otagoense* locations

Appendix 2.2: Localities details of known New Zealand *Phaulacridium otagoense* grasshopper locations from published sources and collections.

Island	Location	Latitude	Longitude	Source
South Island	Benmore Range	-44.3191	170.1182	Goldberg *et al.* 2015
South Island	Big Spur Creek	-44.8329	169.5828	Crown Pastoral Tenure Review - Cluden Station Conservation Resource Report, Part 3 (2005)
South Island	Black Forest	-44.4458	170.2876	Westerman & Ritchie 1984
South Island	Bog Roy	-44.5655	170.1077	Crown Pastoral Tenure Review - Bog Roy Conservation Resource Report (2003)
South Island	Bridge Hill	-45.2645	169.3826	Goldberg *et al.* 2015
South Island	Camp Creek	-44.7064	169.3957	Crown Pastoral Tenure Review - Deep Creek Conservation Resource Report (2006)
South Island	Camp Stream	-43.7914	170.6470	Crown Pastoral Tenure Review - Richmond Conservation Resource Report (2005)
South Island	Cluden Stream	-44.8022	169.5663	Crown Pastoral Tenure Review - Cluden Station Conservation Resource Report, Part 3 (2005)
South Island	Coal River	-43.8241	170.6087	Crown Pastoral Tenure Review - Richmond Conservation Resource Report (2005)
South Island	Conroys Dam	-45.2825	169.3215	Goldberg *et al.* 2015
South Island	Earnscleugh Tailings Historic Reserve	-45.2359	169.3597	Jamieson 1998
South Island	Graveyard Gully	-45.2620	169.3973	Goldberg *et al.* 2015
South Island	Lake Tekapo	-44.0022	170.4995	Goldberg *et al.* 2015
South Island	Lindis Creek	-44.5084	169.5630	Westerman & Ritchie 1984
South Island	Lindis Pass	-44.6266	169.5221	Westerman & Ritchie 1984
South Island	Lowburn	-44.9984	169.2082	Westerman & Ritchie 1984
South Island	Manor Burn	-45.2163	169.4804	Goldberg *et al.* 2015
South Island	Morven Hills	-44.6389	169.5096	Crown Pastoral Tenure Review - Morven Hills Conservation Resource Report, Part 2 (2011)
South Island	Mt John	-43.9861	170.4637	Goldberg *et al.* 2015
South Island	Nevis Road	-45.1324	169.1502	Westerman & Ritchie 1984
South Island	Omarama	-44.4653	169.8991	Westerman & Ritchie 1984
South Island	Otematata	-44.6400	170.1650	Westerman & Ritchie 1984
South Island	Richmond Valley	-44.7667	169.6707	Crown Pastoral Tenure Review - Cluden Station Conservation Resource Report, Part 3 (2005)
South Island	Rugged Ridges	-44.6496	170.2947	Crown Pastoral Tenure Review - Rugged Ridges Conservation Resource Report, Part 1 (2002)
South Island	Simons Pass Hill	-44.1963	170.2853	Goldberg *et al.* 2015
South Island	Tekapo-Pukaki Road	-44.0773	170.3556	Westerman & Ritchie 1984

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Appendix 2

Lineage and haplotype number of New Zealand *Phaulacridium* grasshoppers in Chapter 3

Appendix 2: Lineage and haplotype number of New Zealand *Phaulacridium* grasshoppers in Chapter 3

Appendix 2: New Zealand *Phaulacridium* sample ID, with sampling locations, lineage and haplotype (Hap) number.

ID Code	Location	Lineage	Hap	ID Code	Location	Lineage	Hap
GH761(WR)	Wharangi Rd	I	23	GH1450(MF)	Mt Fitzwilliam	I	7
GH894(WB)	Whites Bay	I	7	GH1454(MF)	Mt Fitzwilliam	I	17
GH896(WB)	Whites Bay	I	20	GH1455(MF)	Mt Fitzwilliam	I	7
GH900(WB)	Whites Bay	I	27	GH1457(MF)	Mt Fitzwilliam	I	5
GH901(WB)	Whites Bay	I	20	GH1451(MF)	Mt Fitzwilliam	I	32
GH902(WB)	Whites Bay	I	5	GH1453(MF)	Mt Fitzwilliam	I	4
GH904(WB)	Whites Bay	I	20	GH1464(LT)	Lake Tekapo	IV	54
GH905(WB)	Whites Bay	I	20	GH1465(LT)	Lake Tekapo	I	7
GH923.1(NR)	Ngaruroro River	I	23	GH1466(LT)	Lake Tekapo	I	20
GH923.2(NR)	Ngaruroro River	I	12	GH1467(LT)	Lake Tekapo	I	21
GH923.3(NR)	Ngaruroro River	I	12	GH1468(LT)	Lake Tekapo	I	7
GH923.5(NR)	Ngaruroro River	I	12	GH1469(LT)	Lake Tekapo	I	7
GH923.6(NR)	Ngaruroro River	I	12	GH1474(LP)	Lake Pukaki Dam	I	12
GH923.7(NR)	Ngaruroro River	I	10	GH1475(LP)	Lake Pukaki Dam	IV	55
GH927.0(WR)	Wharangi Rd	I	24	GH1476(LP)	Lake Pukaki Dam	I	12
GH927.1(WR)	Wharangi Rd	I	30	GH1477(LP)	Lake Pukaki Dam	IV	56
GH927.2(WR)	Wharangi Rd	I	25	GH1479(LP)	Lake Pukaki Dam	I	20
GH927.3(WR)	Wharangi Rd	I	13	GH1480(LP)	Lake Pukaki Dam	I	20
GH927.4(WR)	Wharangi Rd	I	23	GH1484(LP)	Lake Pukaki Dam	I	7
GH927.6(WR)	Wharangi Rd	I	23	GH1485(LP)	Lake Pukaki Dam	I	34
GH927.7(WR)	Wharangi Rd	I	12	GH1494(LP)	Lake Pukaki Dam	I	5
GH939.1(TB)	Titahi Bay	I	23	GH1499(LA)	Lake Aviemore	IV	48
GH939.10(TB)	Titahi Bay	I	12	GH1500(LA)	Lake Aviemore	I	11
GH939.2(TB)	Titahi Bay	I	23	GH1501(LA)	Lake Aviemore	I	20
GH939.3(TB)	Titahi Bay	I	15	GH1502(LA)	Lake Aviemore	I	7
GH939.4(TB)	Titahi Bay	I	9	GH1503(LA)	Lake Aviemore	I	7
GH939.5(TB)	Titahi Bay	I	9	GH1504(LA)	Lake Aviemore	IV	49
GH939.6(TB)	Titahi Bay	I	23	GH1506(LA)	Lake Aviemore	IV	48
GH939.7(TB)	Titahi Bay	I	23	GH1507(LA)	Lake Aviemore	I	7
GH939.8(TB)	Titahi Bay	I	23	GH1512(LA)	Lake Aviemore	I	12
GH939.9(TB)	Titahi Bay	I	12	GH1513(LA)	Lake Aviemore	I	7
GH1444(MF)	Mt Fitzwilliam	I	7	GH1519(OM)	Omarama	I	7
GH1446(MF)	Mt Fitzwilliam	I	3	GH1522(OM)	Omarama	I	7
GH1447(MF)	Mt Fitzwilliam	I	7	GH1524(OM)	Omarama	IV	52
GH1448(MF)	Mt Fitzwilliam	I	7	GH1525(OM)	Omarama	IV	52

ID Code	Location	Lineage	Hap	ID Code	Location	Lineage	Hap
GH1526(OM)	Omarama	I	7	GH1602(MA)	Marshall Road	III	46
GH1527(OM)	Omarama	IV	53	GH1604(MA)	Marshall Road	I	20
GH1529(OM)	Omarama	I	7	GH1605(MA)	Marshall Road	I	20
GH1532(OM)	Omarama	I	12	GH1606(MA)	Marshall Road	I	20
GH1536(AR)	Ahuriri River	I	27	GH1607(MA)	Marshall Road	I	20
GH1538(AR)	Ahuriri River	I	2	GH1652(PN)	Palmerston North	I	23
GH1539(AR)	Ahuriri River	IV	50	GH1653(PN)	Palmerston North	I	12
GH1540(AR)	Ahuriri River	IV	51	GH1655(PN)	Palmerston North	I	23
GH1541(AR)	Ahuriri River	I	20	GH1656(PN)	Palmerston North	I	17
GH1542(AR)	Ahuriri River	I	20	GH1658(PN)	Palmerston North	I	12
GH1543(AR)	Ahuriri River	I	7	GH1662(PN)	Palmerston North	I	22
GH1544(AR)	Ahuriri River	I	7	GH1663(PN)	Palmerston North	I	23
GH1548(AR)	Ahuriri River	I	28	GH1664(PN)	Palmerston North	I	17
GH1553(AR)	Ahuriri River	I	1	GH1669(PV)	Pohangina Valley	I	23
GH1556(LV)	Lindis Valley	I	7	GH1670(PV)	Pohangina Valley	I	8
GH1557(LV)	Lindis Valley	II	41	GH1671(PV)	Pohangina Valley	I	23
GH1559(LV)	Lindis Valley	II	40	GH1674(PV)	Pohangina Valley	I	33
GH1560(LV)	Lindis Valley	II	43	GH1675(PV)	Pohangina Valley	I	26
GH1561(LV)	Lindis Valley	I	7	GH1679(PV)	Pohangina Valley	I	23
GH1562(LV)	Lindis Valley	I	7	GH1681(PV)	Pohangina Valley	I	29
GH1563(LV)	Lindis Valley	II	39	GH1683(GM)	Greymouth	I	18
GH1566(LV)	Lindis Valley	II	42	GH1684(GM)	Greymouth	I	7
GH1572(LV)	Lindis Valley	II	43	GH1685(GM)	Greymouth	I	14
GH1574(LD)	Lake Dunstan	II	35	GH1686(GM)	Greymouth	I	23
GH1575(LD)	Lake Dunstan	II	38	GH1687(GM)	Greymouth	I	18
GH1576(LD)	Lake Dunstan	II	37	GH1688(GM)	Greymouth	I	20
GH1578(LD)	Lake Dunstan	II	36	GH1689(GM)	Greymouth	I	20
GH1580(GG)	Graveyard Gully	III	44	GH1690(GM)	Greymouth	I	20
GH1581(GG)	Graveyard Gully	I	12	GH1691(GM)	Greymouth	I	7
GH1582(GG)	Graveyard Gully	I	7	GH1692(GM)	Greymouth	I	19
GH1583(GG)	Graveyard Gully	I	12	GH1705.1(MB)	Muriwai Beach	I	23
GH1584(GG)	Graveyard Gully	I	12	GH1705.10(MB)	Muriwai Beach	I	16
GH1585(GG)	Graveyard Gully	I	12	GH1705.2(MB)	Muriwai Beach	I	12
GH1586(GG)	Graveyard Gully	III	47	GH1705.3(MB)	Muriwai Beach	I	17
GH1588(GG)	Graveyard Gully	I	12	GH1705.4(MB)	Muriwai Beach	I	15
GH1589(GG)	Graveyard Gully	I	7	GH1705.5(MB)	Muriwai Beach	I	12
GH1599(MA)	Marshall Road	I	20	GH1705.6(MB)	Muriwai Beach	I	23
GH1600(MA)	Marshall Road	III	45	GH1705.7(MB)	Muriwai Beach	I	12
GH1601(MA)	Marshall Road	I	6	GH1705.8(MB)	Muriwai Beach	I	31
				GH1705.9(MB)	Muriwai Beach	I	16

Appendix 3

Mclust results of New Zealand *Phaulacridium* grasshoppers in Chapter 4

Appendix 3: Mclust result of New Zealand *Phaulacridium* grasshoppers in Chapter 4

Appendix 3.1 R code used for morphotype classification in Mclust

Appendix 3.1: The R code used for morphotype classification in Mclust.

```
>filename<-read.table("filename.csv",header=T,sep=",",row.names="id")
    #to upload your file
    #id name = use the name that was used for the column that contains all your individual id's
>library(mclust)
>filenameMclust<-Mclust(filename)
>summary(filenameMclust,parameters=TRUE)
    #detailed summary including the estimated parameters (Note = if "Inf" occurs in the variances
    results then can only get graph 1)
>filename$CLUST<-filenameMclust$classification
>write.csv(filename,file="filename.csv",row.names=TRUE,quote=FALSE)
    #to obtain a csv file that contains information on the cluster an individual falls into
>plot(filenameMclust)
    #four graphs are produced (1.BIC, 2.classification, 3.uncertainty, 4.density)
>filenameBIC<-mclustBIC(filename)
>filenamesummary<-summary(filenameBIC,data=filename)
>filenamesummary
    #gives information on the best 3 BIC values
>filenameBIC
    #table of BIC scores from the 10 different models across 9 components (clusters)
```

Appendix 3.2 Mclust results of 32 morphometric trait combinations

Appendix 4.2: Mclust results of 32 morphometric trait combinations, ranging from three to eight morphometric traits (the four best morphometric trait combinations are in bold).

Number of Traits	Morphometric Traits	Sex	Model Type	BIC	Log Likelihood	ICL	Number of Clusters	Number of Individuals in Clusters
3	FL; FL/FW; PA2	Male	EEI	-537.9215	-248.2451	-541.4904	2	7; 56
		Female	EVI	-744.6540	-345.6711	-746.2823	2	17; 68
4	FL; FL/FW; PA2; PA1	Male	XXX	-891.5984	-416.7973	-891.5984	1	63
		Female	VEE	-1258.7380	-584.9425	-1261.5510	2	67;18
	FL; FL/FW; PA2; PW1/PW2	Male	XXX	-416.4763	-179.2362	-416.4763	1	63
		Female	EEE	-596.8169	-256.2033	-598.7402	2	18; 67
	FL; FL/FW; PA2; PW3/PL	**Male**	**EEI**	**-347.0753**	**-146.6073**	**-348.2621**	**2**	**7; 56**
		Female	**EVI**	**-516.8060**	**-222.8618**	**-517.3385**	**2**	**16; 69**
	FL; FL/FW; PA2; TeL	Male	EVE	-619.6807	-264.2659	-625.1276	2	39; 24
		Female	EEI	-890.0676	-405.0499	-897.3193	3	30; 40; 15
	FL; FL/FW; PA2; TL	Male	EVE	-514.1372	-211.4941	-514.5997	2	60; 3
		Female	EVE	-750.5673	-326.4145	-753.5673	2	19; 66
5	FL; FL/FW; PA2; PA1; PW1/PW2	Male	XXX	-761.5713	-339.3543	-761.5713	1	63
		Female	VEE	-1076.2750	-478.1618	-1080.2590	2	65; 20
	FL; FL/FW; PA2; PA1, PW3/PL	Male	XXX	-719.3616	-318.2494	-719.3616	1	63
		Female	VEE	-1039.3390	-459.6937	-1046.0990	2	58; 27
	FL; FL/FW; PA2; PA1; TeL	Male	XXX	-968.0230	-442.5802	-968.0230	1	63
		Female	VEE	-1397.6910	-638.8697	-1401.3890	2	66; 19
	FL; FL/FW; PA2; PA1, TL	Male	EVE	-871.4092	-373.5576	-873.0322	2	4; 59
		Female	EEE	-1258.8620	-571.6764	-1261.9850	2	67; 18
	FL; FL/FW; PA2; PW3/PL; PW1/PW2	**Male**	**XXX**	**-236.3699**	**-76.7536**	**-236.3699**	**1**	**63**
		Female	**EVE**	**-370.4900**	**-118.6053**	**-372.0855**	**2**	**16; 69**
	FL; FL/FW; PA2; TeL; PW1/PW2	Male	XXX	-494.2634	-205.7004	-494.2634	1	63
		Female	VEE	-739.3991	-309.7237	-743.4110	2	19; 66
	FL; FL/FW; PA2; TeL; PW3/PL	Male	EEI	-437.2559	-173.0535	-441.4986	3	20; 6; 37
		Female	VEI	-661.8981	-277.6372	-66.8978	3	19; 28; 38
	FL; FL/FW; PA2; TeL; TL	Male	EVE*	-590.4477	-233.0768	-590.4725	2	61; 2
		Female	EVE	-895.1023	-380.9114	-896.5454	2	68; 17

Number of Traits	Morphometric Traits	Sex	Model Type	BIC	Log Likelihood	ICL	Number of Clusters	Number of Individuals in Clusters
5	FL; FL/FW; PA2; TL; PW1/PW2	Male	EVE*	-388.4883	-132.0971	-389.4273	2	3; 60
		Female	EVE	-599.9600	-233.3402	-601.7812	2	67; 18
	FL; FL/FW; PA2; TL; PW3/PL	**Male**	**EVE**	**-341.6049**	**-108.6555**	**-342.2913**	**2**	**5; 58**
		Female	**EVE**	**-524.7952**	**-195.7578**	**-526.4258**	**2**	**67; 18**
6	FL; FL/FW; PA2; PA1; PW3/PL; PW1/PW2	Male	XXX	-584.9065	-236.5209	-584.9065	1	63
		Female	VEE	-856.1559	-350.3315	-861.0424	2	57; 28
	FL; FL/FW; PA2; PA1; TeL; PW1/PW2	Male	XXX	-841.7749	-364.9551	-841.7749	1	63
		Female	VEE	-1219.0640	-531.7856	-1224.3190	2	64; 21
	FL; FL/FW; PA2; PA1; TeL; PW3/PL	Male	XXX	-799.8509	-343.9931	-799.8509	1	63
		Female	VEE	-1178.2250	-511.3662	-1180.7630	2	65; 20
	FL; FL/FW; PA2; PA1; TeL; TL	Male	EEE*	-948.7737	-368.7269	-952.6595	3	36; 5; 22
		Female	VEE	-1408.3980	-626.4527	-1411.6900	2	66; 19
	FL; FL/FW; PA2; PA1; TL; PW1/PW2	Male	VVE	-742.5633	-288.4189	-743.4742	2	7; 56
		Female	VEE	-1088.1370	-466.3221	-1090.9090	2	64; 21
	FL; FL/FW; PA2; PA1; TL; PW3/PL	Male	VVE	-717.9510	-276.1128	-717.9837	2	7; 56
		Female	EVE	-1038.7250	-432.721	-1042.5830	2	64; 21
	FL; FL/FW; PA2; TeL; PW3/PL; PW1/PW2	**Male**	**XXX**	**-317.8652**	**-103.0003**	**-317.8652**	**1**	**63**
		Female	**VEI**	**-513.5116**	**-194.5587**	**-518.9402**	**3**	**18; 29; 38**
	FL; FL/FW; PA2; TeL; TL; PW1/PW2	Male	EVV	-487.8221	-132.0464	-487.8227	2	6; 57
		Female	EVE	-751.0537	-288.8952	-752.4417	2	68; 17
	FL; FL/FW; PA2; TeL; TL; PW3/PL	Male	EVV	-464.3138	-120.2922	-465.5585	2	9; 54
		Female	EVE	-675.4382	-251.0874	-676.4613	2	68; 17
	FL; FL/FW; PA2; TL; PW3/PL; PW1/PW2	Male	EVE*	-209.6854	-24.05157	-209.9601	2	3; 60
		Female	VVE	-377.7863	-100.0401	-382.8438	2	28; 57
7	FL; FL/FW; PA2; PA1; TeL; PW3/PL; PW1/PW2	Male	XXX	-668.9705	-261.9804	-668.9705	1	63
		Female	VEE	-1002.0680	-403.2956	-1006.1510	2	57; 28
	FL; FL/FW; PA2; PA1; TeL; TL; PW3/PL	Male	EVV	-819.4226	-264.7016	-820.4593	2	8; 55
		Female	VEE	-1193.7420	-499.1324	-1195.9940	2	65; 20
	FL; FL/FW; PA2; PA1; TeL; TL; PW1/PW2	Male	EVV	-852.8830	-281.4318	-852.8857	2	7; 56
		Female	VEE	-1234.9580	-519.7408	-1238.2610	2	64; 21
	FL; FL/FW; PA2; PA1; TL; PW3/PL; PW1/PW2	Male	EVE*	-569.0614	-569.1619	-183.0239	2	58; 5
		Female	EVE	-865.5469	-323.9285	-867.2032	2	67; 18
	FL; FL/FW; PA2; TL; TeL; PW3/PL; PW1/PW2	Male	EVE*	-319.7278	-29.35515	-324.6336	3	3; 41; 19
		Female	EEE	-531.6142	-170.2901	-532.2207	2	70; 15
8	FL; FL/FW; PA2; PA1; TL; TeL; PW3/PL; PW1/PW2	Male	EVV	-664.6308	396.8763	-664.3080	8	7; 8; 7; 8; 7; 12; 7; 7
		Female	VEE	-1018.0700	-389.0834	-1020.2390	2	66; 19

* The combination of morphometric traits resulted in "Inf" occurring in the variance output which meant not all of the graph outputs could be produced therefore these combinations of traits were not considered for the final four combinations.

Appendix 3.3 Morphotype classification for male New Zealand *Phaulacridium* grasshoppers

Appendix 4.3: Morphotype classification for male New Zealand *Phaulacridium* grasshoppers from the best four Mclust models. Models I and III were only used for classification purposes with individuals from Type 2 only located in the southern/central South Island and Type 1 individuals sampled across New Zealand.

Individual Codes	Location	Mclust Model Cluster Groupings				Morphotype Classification
		Model I	Model II†	Model III	Model IV†	
GH761(WR)	Wharangi Rd	B	A	B	A	Type 1
GH901(WB)	Whites Bay	B	A	B	A	Type 1
GH902(WB)	Whites Bay	B	A	B	A	Type 1
GH905(WB)	Whites Bay	B	A	B	A	Type 1
GH927.0(WR)	Wharangi Rd	B	A	B	A	Type 1
GH927.3(WR)	Wharangi Rd	B	A	B	A	Type 1
GH939.1(TB)	Titahi Bay	B	A	B	A	Type 1
GH939.2(TB)	Titahi Bay	B	A	B	A	Type 1
GH939.4(TB)	Titahi Bay	B	A	B	A	Type 1
GH939.9(TB)	Titahi Bay	B	A	B	A	Type 1
GH1447(MF)	Mt Fitzwilliam	B	A	B	A	Type 1
GH1451(MF)	Mt Fitzwilliam	B	A	B	A	Type 1
GH1453(MF)	Mt Fitzwilliam	B	A	B	A	Type 1
GH1454(MF)	Mt Fitzwilliam	B	A	B	A	Type 1
GH1455(MF)	Mt Fitzwilliam	B	A	B	A	Type 1
GH1469(LT)	Lake Tekapo	A	A	B	A	Mixed*
GH1475(LP)	Lake Pukaki Dam	B	A	B	A	Type 1
GH1484(LP)	Lake Pukaki Dam	B	A	B	A	Type 1
GH1485(LP)	Lake Pukaki Dam	B	A	A	A	Mixed*
GH1494(LP)	Lake Pukaki Dam	B	A	B	A	Type 1
GH1504(LA)	Lake Aviemore	B	A	B	A	Type 1
GH1506(LA)	Lake Aviemore	B	A	B	A	Type 1
GH1507(LA)	Lake Aviemore	B	A	B	A	Type 1
GH1512(LA)	Lake Aviemore	B	A	B	A	Type 1
GH1513(LA)	Lake Aviemore	B	A	B	A	Type 1
GH1519(OM)	Omarama	B	A	B	A	Type 1
GH1525(OM)	Omarama	B	A	B	A	Type 1
GH1529(OM)	Omarama	B	A	B	A	Type 1
GH1532(OM)	Omarama	B	A	B	A	Type 1
GH1540(AR)	Ahuriri River	B	A	B	A	Type 1
GH1541(AR)	Ahuriri River	B	A	B	A	Type 1
GH1542(AR)	Ahuriri River	B	A	B	A	Type 1
GH1543(AR)	Ahuriri River	B	A	B	A	Type 1
GH1544(AR)	Ahuriri River	B	A	A	A	Mixed*

Individual Codes	Location	Mclust Model Cluster Groupings				Morphotype Classification
		Model I	Model II†	Model III	Model IV†	
GH1566(LV)	Lindis Valley	A	A	A	A	Type 2
GH1572(LV)	Lindis Valley	A	A	A	A	Type 2
GH1578(LD)	Lake Dunstan	A	A	A	A	Type 2
GH1580(GG)	Graveyard Gully	A	A	B	A	Mixed*
GH1581(GG)	Graveyard Gully	B	A	B	A	Type 1
GH1588(GG)	Graveyard Gully	A	A	B	A	Mixed*
GH1589(GG)	Graveyard Gully	A	A	B	A	Mixed*
GH1600(MA)	Marshall Road	B	A	B	A	Type 1
GH1605(MA)	Marshall Road	B	A	B	A	Type 1
GH1606(MA)	Marshall Road	B	A	B	A	Type 1
GH1607(MA)	Marshall Road	B	A	B	A	Type 1
GH1656(PN)	Palmerston North	B	A	B	A	Type 1
GH1658(PN)	Palmerston North	B	A	B	A	Type 1
GH1662(PN)	Palmerston North	B	A	B	A	Type 1
GH1663(PN)	Palmerston North	B	A	B	A	Type 1
GH1664(PN)	Palmerston North	B	A	B	A	Type 1
GH1671(PV)	Pohangina Valley	B	A	B	A	Type 1
GH1679(PV)	Pohangina Valley	B	A	B	A	Type 1
GH1681(PV)	Pohangina Valley	B	A	B	A	Type 1
GH1684(GM)	Greymouth	B	A	B	A	Type 1
GH1688(GM)	Greymouth	B	A	B	A	Type 1
GH1689(GM)	Greymouth	B	A	B	A	Type 1
GH1690(GM)	Greymouth	B	A	B	A	Type 1
GH1691(GM)	Greymouth	B	A	B	A	Type 1
GH1705.1(MB)	Muriwai Beach	B	A	B	A	Type 1
GH1705.3(MB)	Muriwai Beach	B	A	B	A	Type 1
GH1705.5(MB)	Muriwai Beach	B	A	B	A	Type 1
GH1705.6(MB)	Muriwai Beach	B	A	B	A	Type 1
GH1705.9(MB)	Muriwai Beach	B	A	B	A	Type 1

† These models only created one cluster (63 individuals) and could not be used to morphotype classification.
* Could not be classified into a morphotype as the cluster the individual was placed into was inconsistent across the two models.

Appendix 3.4 Morphotype classification for female New Zealand *Phaulacridium* grasshoppers

Appendix 3.4: Morphotype classification for female New Zealand *Phaulacridium* grasshoppers from the best four Mclust models. Individuals from Type 2 were only located in the southern/central South Island, and Type 1 individuals were sampled across New Zealand.

Individual Codes	Location	Mclust Model Cluster Groupings				Morphotype Classification
		Model I	Model II	Model III	Model IV	
GH894(WB)	Whites Bay	B	B	B	C	Type 1
GH896(WB)	Whites Bay	B	B	B	C	Type 1
GH900(WB)	Whites Bay	B	B	B	C	Type 1
GH904(WB)	Whites Bay	B	B	B	C	Type 1
GH923.1(NR)	Ngaruroro River	B	B	B	C	Type 1
GH923.2(NR)	Ngaruroro River	B	B	B	C	Type 1
GH923.3(NR)	Ngaruroro River	B	B	B	B	Type 1
GH923.5(NR)	Ngaruroro River	B	B	B	C	Type 1
GH923.6(NR)	Ngaruroro River	B	B	B	C	Type 1
GH923.7(NR)	Ngaruroro River	B	B	B	B	Type 1
GH927.1(WR)	Wharangi Rd	B	B	B	C	Type 1
GH927.2(WR)	Wharangi Rd	B	B	B	C	Type 1
GH927.4(WR)	Wharangi Rd	B	B	B	C	Type 1
GH927.6(WR)	Wharangi Rd	B	B	B	C	Type 1
GH927.7(WR)	Wharangi Rd	B	B	B	C	Type 1
GH939.10(TB)	Titahi Bay	B	B	B	C	Type 1
GH939.3(TB)	Titahi Bay	B	B	B	B	Type 1
GH939.5(TB)	Titahi Bay	B	B	B	C	Type 1
GH939.6(TB)	Titahi Bay	B	B	B	C	Type 1
GH939.7(TB)	Titahi Bay	B	B	B	C	Type 1
GH939.8(TB)	Titahi Bay	B	B	B	C	Type 1
GH1444(MF)	Mt Fitzwilliam	B	B	B	C	Type 1
GH1446(MF)	Mt Fitzwilliam	B	B	B	B	Type 1
GH1448(MF)	Mt Fitzwilliam	B	B	B	C	Type 1
GH1450(MF)	Mt Fitzwilliam	B	B	B	C	Type 1
GH1457(MF)	Mt Fitzwilliam	B	B	B	B	Type 1
GH1464(LT)	Lake Tekapo	A	A	A	A	Type 2
GH1466(LT)	Lake Tekapo	A	A	A	A	Type 2
GH1467(LT)	Lake Tekapo	A	A	A	A	Type 2
GH1468(LT)	Lake Tekapo	B	B	A	A	Mixed*
GH1474(LP)	Lake Pukaki Dam	B	B	B	B	Type 1
GH1476(LP)	Lake Pukaki Dam	A	A	A	A	Type 2
GH1477(LP)	Lake Pukaki Dam	B	B	B	B	Type 1
GH1479(LP)	Lake Pukaki Dam	B	B	B	B	Type 1
GH1480(LP)	Lake Pukaki Dam	B	B	B	A	Mixed*

Individual Codes	Location	Mclust Model Cluster Groupings				Morphotype Classification
		Model I	Model II	Model III	Model IV	
GH1499(LA)	Lake Aviemore	B	B	B	B	Type 1
GH1500(LA)	Lake Aviemore	B	B	B	B	Type 1
GH1501(LA)	Lake Aviemore	B	B	B	B	Type 1
GH1502(LA)	Lake Aviemore	B	B	B	B	Type 1
GH1503(LA)	Lake Aviemore	B	B	B	B	Type 1
GH1522(OM)	Omarama	B	B	B	B	Type 1
GH1524(OM)	Omarama	B	B	B	B	Type 1
GH1526(OM)	Omarama	B	B	B	C	Type 1
GH1527(OM)	Omarama	B	B	B	B	Type 1
GH1536(AR)	Ahuriri River	B	B	B	B	Type 1
GH1538(AR)	Ahuriri River	B	B	B	B	Type 1
GH1539(AR)	Ahuriri River	B	B	B	B	Type 1
GH1548(AR)	Ahuriri River	B	B	B	B	Type 1
GH1553(AR)	Ahuriri River	B	B	B	B	Type 1
GH1556(LV)	Lindis Valley	A	A	A	A	Type 2
GH1557(LV)	Lindis Valley	A	A	A	A	Type 2
GH1559(LV)	Lindis Valley	A	A	A	A	Type 2
GH1560(LV)	Lindis Valley	A	A	A	A	Type 2
GH1561(LV)	Lindis Valley	A	A	A	A	Type 2
GH1562(LV)	Lindis Valley	A	A	A	A	Type 2
GH1563(LV)	Lindis Valley	A	A	A	A	Type 2
GH1574(LD)	Lake Dunstan	B	B	A	B	Mixed*
GH1575(LD)	Lake Dunstan	A	A	A	A	Type 2
GH1576(LD)	Lake Dunstan	A	A	A	A	Type 2
GH1582(GG)	Graveyard Gully	B	B	B	B	Type 1
GH1583(GG)	Graveyard Gully	B	B	B	B	Type 1
GH1584(GG)	Graveyard Gully	A	A	A	A	Type 2
GH1585(GG)	Graveyard Gully	A	A	A	A	Type 2
GH1586(GG)	Graveyard Gully	A	A	A	A	Type 2
GH1599(MA)	Marshall Road	B	B	B	B	Type 1
GH1601(MA)	Marshall Road	B	B	B	B	Type 1
GH1602(MA)	Marshall Road	B	B	B	B	Type 1
GH1604(MA)	Marshall Road	B	B	B	B	Type 1
GH1652(PN)	Palmerston North	B	B	B	C	Type 1
GH1653(PN)	Palmerston North	B	B	B	C	Type 1
GH1655(PN)	Palmerston North	B	B	B	C	Type 1
GH1669(PV)	Pohangina Valley	B	B	B	C	Type 1
GH1670(PV)	Pohangina Valley	B	B	B	C	Type 1
GH1674(PV)	Pohangina Valley	B	B	B	C	Type 1
GH1675(PV)	Pohangina Valley	B	B	B	C	Type 1
GH1683(GM)	Greymouth	B	B	B	C	Type 1
GH1685(GM)	Greymouth	B	B	B	B	Type 1
GH1686(GM)	Greymouth	B	B	B	C	Type 1
GH1687(GM)	Greymouth	B	B	B	C	Type 1
GH1692(GM)	Greymouth	B	B	B	C	Type 1
GH1705.10(MB)	Muriwai Beach	B	B	B	C	Type 1

Individual Codes	Location	Mclust Model Cluster Groupings				Morphotype Classification
		Model I	Model II	Model III	Model IV	
GH1705.2(MB)	Muriwai Beach	B	B	B	C	Type 1
GH1705.4(MB)	Muriwai Beach	B	B	B	C	Type 1
GH1705.7(MB)	Muriwai Beach	B	B	B	C	Type 1
GH1705.8(MB)	Muriwai Beach	B	B	B	C	Type 1

* Could not be classified into a morphotype as the cluster the individual was placed into was inconsistent across the four models.