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# Phylogeography of two New Zealand lizards: McCann's skink (*Oligosoma maccanni*) and the brown skink (*O. zelandicum*)

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ABSTRACT

The New Zealand skink fauna has proven to be an ideal taxonomic group in which to examine the impact of climatic and geological processes on the evolution of the New Zealand biota since the Pliocene. Here we examine the phylogeography of McCann's skink (*Oligosoma maccanni*) in order to gain insight into the relative contribution of Pliocene and Pleistocene processes on patterns of genetic structure in the South Island biota, and investigate the phylogeography of the brown skink (*O. zelandicum*) to examine whether Cook Strait landbridges facilitated geneflow between the North and South Islands in the late-Pleistocene. We obtained mitochondrial DNA sequence data (ND2 and ND4; 1282 bp) from across the range of both species. We examined the phylogeographic patterns evident in each species using Neighbour-Joining, Maximum Likelihood and Bayesian methods. We found substantial phylogeographic structure within *O. maccanni*, with seven distinct clades identified. Divergences among clades are estimated to have occurred during the Pliocene. Populations in the Otago/Southland region (south of the Waitaki River valley) formed a well-supported lineage within *O. maccanni*. A substantial genetic break was evident between populations in east and west Otago, either side of the Nevis-Cardrona fault system, while north-south genetic breaks were evident within the Canterbury region. Within-clade divergences in *O. maccanni* appear to have occurred during the mid- to late-Pleistocene. Shimodaira–Hasegawa topology tests indicated that the 'Garston' skink is not genetically distinct from *O. maccanni*. There was only relatively minor phylogeographic structure within *O. zelandicum*, with divergences among populations occurring during the mid- to late-Pleistocene. Our genetic data supports a single colonisation of the North Island by *O. zelandicum* from the South Island, with the estimated timing of this event (0.46 mya) consistent with the initial formation of Cook Strait.



## 1. Introduction

Intense tectonic activity, glacial cycles and dramatic fluctuations in sea-level have shaped the New Zealand archipelago and its landscape throughout the Pliocene and Pleistocene (Cooper and Millener, 1993; Markgraf et al., 1995; Worthy and Holdaway, 2002). The New Zealand skink fauna has proven to be an ideal taxonomic group in which to examine the impact of these climatic and geological processes on the evolution of the New Zealand biota since the Pliocene (Berry and Gleeson, 2005; Greaves et al., 2007, 2008; Hare et al., 2008; Chapple et al., in press a). New Zealand supports a diverse endemic skink fauna, comprising 32 described species in two genera, *Oligosoma* (23 species) and *Cyclodina* (9 species), and several undescribed species (Gill and Whitaker, 2001; Chapple and Patterson, 2007; Chapple et al., in press b,2008). Several New Zealand skink species have widespread distributions that span recognised biogeographic barriers (Hardy, 1977; Towns et al., 1985). Lizards typically exhibit substantial phylogeographic structuring due to their low dispersal abilities (Avise, 2000). Since skinks have been present in New Zealand since at least the early Miocene (Hickson et al., 2000; Worthy et al., 2006; Smith et al., 2007), they represent an ideal taxonomic group in which to examine the influence of historical processes during the Pliocene and Pleistocene on gene flow and pattens of genetic structuring.

Two ongoing debates in New Zealand biogeography have focused on: (i) whether Pliocene tectonism was a more important factor than Pleistocene glaciation in shaping the distribution and patterns of genetic structure in the South Island biota (McGlone, 1985; Wardle et al., 1988; McGlone et al., 2001; Trewick and Wallis, 2001; Greaves et al., 2007); and (ii) whether landbridges that formed intermittently during glacial maxima in the late-Pleistocene enabled the interchange of terrestrial taxa between the North and South Islands (Lewis et al., 1994; Worthy and Holdaway, 2002; Greaves et al., 2007). In this study we examine the phylogeography of McCann's skink (*Oligosoma maccanni*) in order to gain insight into the relative contribution of Pliocene and Pleistocene processes

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on patterns of genetic structure in the South Island biota, and investigate the phylogeography of the brown skink (*O. zelandicum*) to examine whether Cook Strait landbridges facilitated geneflow between the North and South Islands in the late-Pleistocene.

The Southern Alps that characterize the South Island of New Zealand were formed by tectonic activity along the alpine fault line (boundary of the Australasian and Pacific plates) that commenced during the Miocene, and intensified during the Pliocene and early-Pleistocene (Gage, 1980; Suggate, 1982; Stevens et al., 1995). The presence of mountainous regions in the South Island has facilitated extensive glaciation during the Pleistocene, created an expansive alpine zone, and fundamentally altered climatic conditions and prevailing weather patterns (Suggate, 1990; Pillans, 1991; Worthy and Holdaway, 2002). During Pleistocene glacial maxima, the merging of glaciers resulted in large ice sheets along the Canterbury Plains (Fig. 1). Ice from Fiordland spilled out across the landscape of Southland and Otago, scouring out valleys as they passed (Stevens et al., 1995). Glaciation was patchy in the northernmost region of the South Island, with the Nelson region consisting mainly of valley glaciers (Stevens et al., 1995). Early authors tended to emphasise the importance of Pleistocene processes on shaping the South Island biota (e.g. Hardy, 1977; Wardle et al., 1988). Recent molecular studies suggest that patterns of genetic structure in the South Island fauna are attributable to Pliocene mountain building (Trewick, 2000; Trewick, 2001; Buckley et al., 2001; Arensburger et al., 2004; Trewick and Morgan-Richards, 2005; Buckley and Simon, 2007). However, several studies highlight a role for both Pliocene and Pleistocene processes in shaping patterns of genetic diversity (Lloyd, 2003; Smissen et al., 2003; Chinn and Gemmell, 2004; Neiman and Lively, 2004; Winkworth et al., 2006).

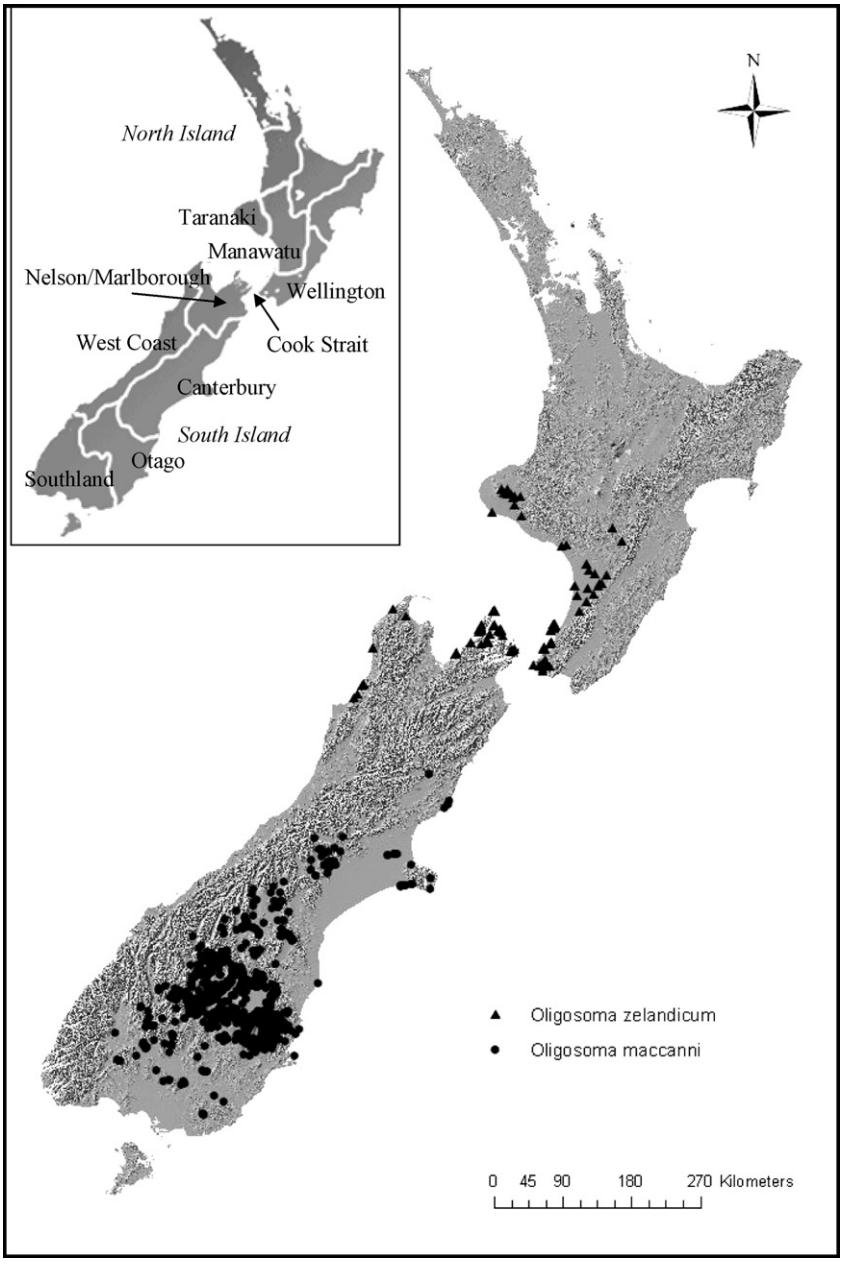


**Fig. 1.** Distributional range of *Oligosoma maccanni* (black circles) and *O. zelandicum* (black triangles). Distributional data were obtained from the New Zealand Department of Conservation's BioWeb Herpetofauna database (2006). (Figure adapted with permission from Geographx (NZ) Ltd.). Inset: Major geographic regions in New Zealand.

*Oligosoma maccanni* (maximum snout-vent length [SVL], 73 mm) occurs in the southern South Island, east of the divide from Canterbury to Southland at altitudes from sea-level to 1500 m (Patterson and Daugherty, 1990; Gill and Whitaker, 2001; Fig. 1). It lives in open habitats, usually occupying dry, rocky environments and is especially abundant in montane grassland (Gill and Whitaker, 2001; Whitaker et al., 2002). Given the substantial levels of phylogeographic structure evident in other taxa in the southern South Island, we predict that *O. maccanni* will exhibit patterns of genetic structuring consistent with both Pliocene mountain building (e.g. an east-west split in Otago similar to that observed in *O. grande*; Berry and Gleeson, 2005) and Pleistocene glacial cycles. Since there is substantial morphological variation within *O. maccanni* (Daugherty et al., 1990), we will examine the potential for unrecognised species within *O. maccanni*. In particular, we will assess the taxonomic status of the 'Garston' skink, known only from a single subadult specimen collected from the Garston region of Otago (R. Hitchmough, pers. comm.). Initial allozyme and morphological analysis of this specimen suggests that it may be a melanistic colour morph of *O. maccanni* (R. Hitchmough, pers. comm.), but we further assess its taxonomic status using mitochondrial DNA sequence data and topology tests.

The lower North Island was inundated throughout the Pliocene, with the Manawatu Strait occurring between present-day Taranaki/Hawkes Bay and Wellington, although geological evidence exists for intermittent landbridges between the North Island and South Island during the Pliocene (Lewis et al., 1994; King, 2000; Worthy and Holdaway, 2002). Tectonic activity in the late-Pliocene and early-Pleistocene resulted in the uplift of the lower North Island and draining of the Manawatu Strait (Lewis et al., 1994; Worthy and Holdaway, 2002). Present-day Cook Strait (separating the North Island and South Island) was formed during the late-Pleistocene (~0.45 mya; Lewis et al., 1994). Sea-level fluctuations (up to 130 m below present) associated with Pleistocene glacial cycles are believed to have resulted in intermittent landbridges across Cook Strait during glacial maxima of the late-Pleistocene (Lewis et al., 1994; Stevens et al., 1995; Worthy and Holdaway, 2002). However, it has been suggested that there was no landbridge (or no landbridge suitable for dispersal by terrestrial taxa) present during the last glacial maximum (LGM; Fig. 2), with the most recent landbridge occurring ~0.13 mya (Worthy and Holdaway, 1994, 2002). Despite geological evidence for repeated Cook Strait landbridges during the late-Pleistocene, few molecular studies have revealed empirical evidence consistent with geneflow or dispersal across these landbridges. Divergences across Cook Strait have been dated between the Pliocene and mid-Pleistocene, prior to the formation of Cook Strait in the late-Pleistocene (Baker et al., 1995; Buckley et al., 2001; Lloyd, 2003; Efford et al., 2002; Greaves et al., 2007). These studies support the suggestion that Cook Strait landbridges were not sufficiently long-lived to facilitate dispersal of terrestrial species between the North Island and South Island (Worthy and Holdaway, 1994, 2002).

*Oligosoma zelandicum* (maximum SVL 73 mm) occurs in the west of the North Island from Taranaki to Wellington, and in the South Island in Marlborough, Nelson and northern Westland (Gill and Whitaker, 2001; Fig. 1). It lives in a variety of densely-vegetated, and usually damp, habitats from supralittoral scrubs and grassland to lowland forest (Gill, 1976; Gill and Whitaker, 2001). Its present-day distribution either side of Cook Strait makes *O. zelandicum* an ideal species in which to examine whether Cook Strait landbridges (between Nelson/Marlborough and Taranaki; Fig. 2) during the late-Pleistocene facilitated geneflow and dispersal between the North and South Islands. Recent molecular studies have indicated that grassland (or open habitat) *Oligosoma* species (*O. lineoocellatum*, *O. infrapunctatum*) have not utilised late-Pleistocene Cook Strait landbridges (Greaves et al., 2007, 2008). Hardy (1977) hypothesized that some New Zealand skink species retreated to forested refugial areas in the Nelson/Marlborough region during glacial maxima. A recent allozyme study revealed only minor genetic divergence across the range of *O. zelandicum* (Miller, 1999). *Oligosoma zelandicum* lives in forested areas, therefore we hypothesise that it was present in Nelson/Marlborough refugia during glacial maxima, and dispersed across Cook Strait landbridges to the North Island during the late-Pleistocene.

Here we use mitochondrial DNA sequence data (ND2, ND4; 1282 bp) to examine the phylogeography of *O. maccanni* and *O. zelandicum*. We use patterns of genetic structuring and divergence time estimates (ND2 molecular clock) to test specific hypotheses for the impact of historical processes during the Pliocene and Pleistocene on *O. maccanni* and *O. zelandicum*. In addition, we complete Shimodaira–Hasegawa topology tests to examine the taxonomic status of the 'Garston' skink.

## 2. Materials and methods

### 2.1. Taxonomic sampling

We obtained tissue samples from across the entire distribution of *O. maccanni* (32 samples) and *O. zelandicum* (except the Taranaki region; 17 samples) (Table 1, Figs. 1 and 2). Samples were obtained primarily from the National Frozen Tissue Collection (NFTC; Victoria University of Wellington, New Zealand) and ethanol-preserved specimens housed at Te Papa (National Museum of New Zealand, Wellington). Four additional *O. maccanni* samples were collected for this study (OMA5-8; Table 1). We included two New Caledonian *Eugongylus*-lineage skinks (*Nannoscincus mariei*, *Marmorosphax tricolor*) as outgroups in our study (Table 1).

### 2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from liver, toe or tail samples using a modified phenol-chloroform extraction protocol (Sambrook et al., 1989). For each sample we targeted portions of the mitochondrial ND2 (~600 bp) and ND4 genes (~850 bp; incorporating most of the flanking 3′ tRNA cluster, including the histidine and serine tRNA genes). These regions were chosen because work at comparable taxonomic levels in other squamate reptile groups have indicated useful levels of variability (e.g. Chapple et al., 2004, 2005; Greaves et al., 2007; Hare et al., 2008). Several primers were used to amplify and sequence ND2 (L4437, H4980, L4221, Macey et al., 1997; ND2r102, Sadlier et al., 2004) and ND4 (ND4I, tRNA-Leu, Forstner et al., 1995; ND4R-NZ, Greaves et al., 2007). PCR and sequencing were conducted as outlined in Greaves et al. (2007). GenBank accession numbers for all sequences are provided in Table 1.

### 2.3. Phylogenetic analyses

Sequence data were edited using ContigExpress version 10.0.1 (Invitrogen), and aligned using the default parameters of Clustal X (Thompson et al., 1997). The aligned sequences were translated into amino acid sequences using the vertebrate mitochondrial code. This was performed to determine if these data were truly mitochondrial in origin. As no premature stop codons were observed, we conclude that all sequences obtained are true mitochondrial copies.

MEGA 3.1 (Kumar et al., 2004) was used to calculate Tamura-Nei (TrN) corrected genetic distances for the concatenated dataset (ND2 + ND4) and pairwise uncorrected genetic distances for the ND2 dataset. The number and frequency of all unique mtDNA haplotypes, as well as summary statistics for estimates of molecular diversity, were obtained using DNAsp 4.1 (Rozas et al., 2003).

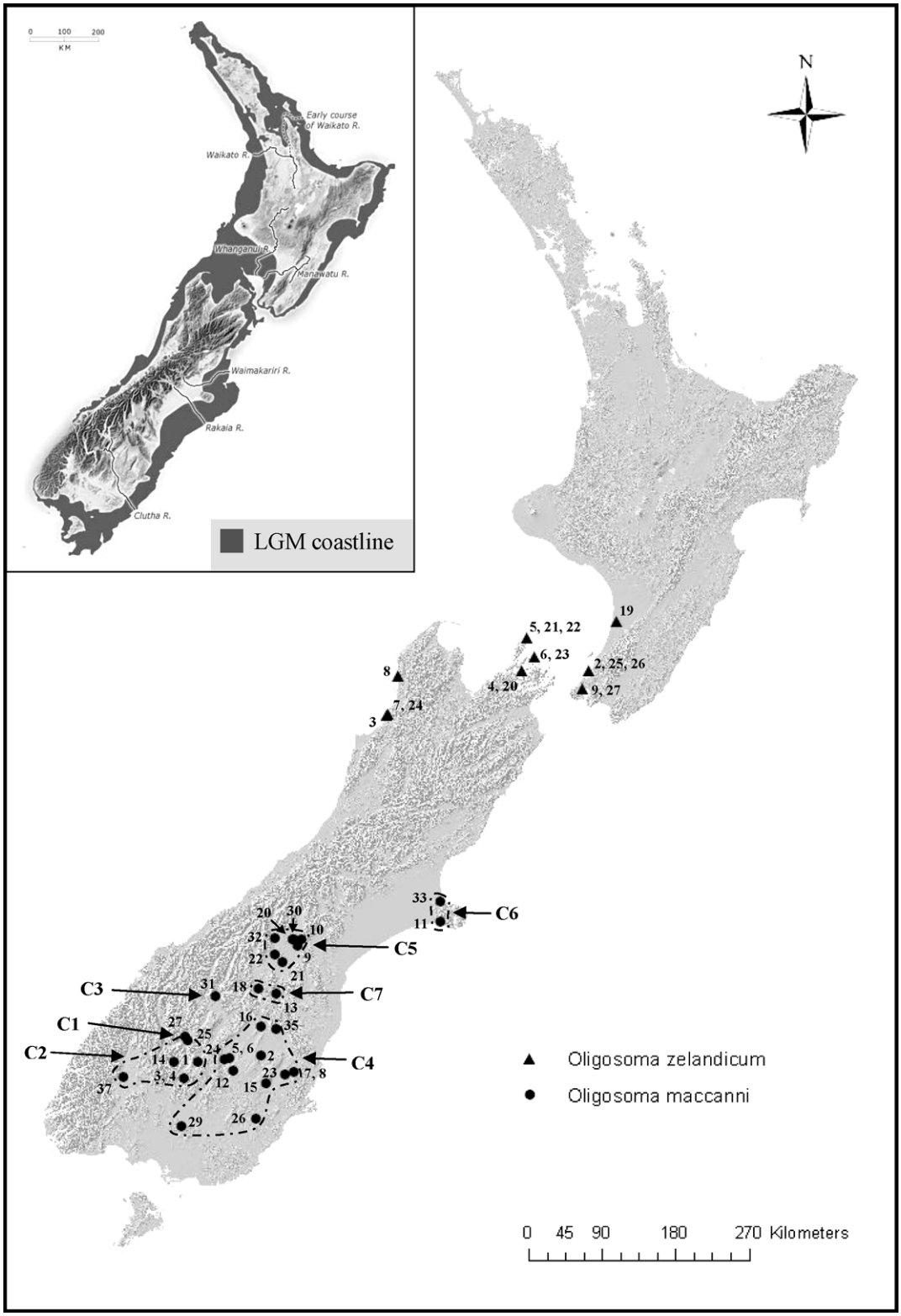


**Fig. 2.** Location of *O. maccanni* (black circles) and *O. zelandicum* (black triangles) tissue samples used in this study. The approximate distribution of *O. maccanni* clades identified in Fig. 3 are shown. Note that clade 2 (OMA27) and clade 4 (OMA31) are each represented by a single sample. (Figure adapted with permission from Geographx (NZ) Ltd.). Inset: Map indicating the coastline (dark shading) of New Zealand during the last glacial maximum (LGM). The map highlights the landbridge that connected the Taranaki region with the Nelson region during the LGM. (Figure reproduced with permission from Te Ara, Encyclopedia of New Zealand).

We used ModelTest 3.7 and the hierarchical likelihood test (hLRT) (Posada and Crandall, 1998) to select the best model of sequence evolution for Maximum Likelihood (ML) analyses. Base frequencies, gamma distribution (G), substitution rates of invariant sites (I) and the among-site substitution rate were estimated. Due to the size of the dataset, search of the best ML was done with PAUP* v4.0b10 (Swofford, 2002) by heuristic searching using the tree-bisection-reconnection (TBR) method, with random addition

**Table 1**
Locality information and GenBank accession numbers for samples used in this study

| Species | Museum Code | Tissue Code | GenBank Accession No. | | Locality |
|---|---|---|---|---|---|
| | | | ND2 | ND4 | |
| *Oligosoma maccanni* | CD930 | OMA1 | EF081195 | EF081222 | Nevis Range, central Otago |
| *O. maccanni* | CD1261 | OMA2 | EF447115 | EF447148 | Puketoi, Otago |
| *O. maccanni* | FT3213 | OMA3 | EF447116 | EF447149 | Garston, Southland/Westland |
| *O. maccanni* | FT3039 | OMA4 | EF447117 | EF447150 | Garston, Southland/Westland |
| *O. maccanni* | — | OMA5 | EF447118 | EF447151 | Alexandra, central Otago |
| *O. maccanni* | — | OMA6 | EF447119 | EF447152 | Alexandra, central Otago |
| *O. maccanni* | — | OMA7 | EF447120 | EF447153 | Macraes Flat, Otago |
| *O. maccanni* | — | OMA8 | EF447121 | EF447154 | Macraes Flat, Otago |
| *O. maccanni* | CD956 | OMA9 | EF447122 | EF447155 | Edwards Creek, Canterbury |
| *O. maccanni* | CD804 | OMA10 | EF447123 | EF447156 | Mount Hay Station, Canterbury |
| *O. maccanni* | CD615 | OMA11 | EF447124 | EF447157 | Birdlings Flat, Canterbury |
| *O. maccanni* | CD1254 | OMA12 | EF447125 | EF447158 | Gordon Peak, Knobby Range, Otago |
| *O. maccanni* | CD1119 | OMA13 | EF447126 | EF447159 | Otematata, Canterbury |
| *O. maccanni* | CD1106 | OMA14 | EF447127 | EF447160 | Gorge Burn, Eyre Mountains, Southland |
| *O. maccanni* | CD778 | OMA15 | EF447128 | EF447161 | Burgan Stream Hut, Otago |
| *O. maccanni* | CD1255 | OMA16 | EF447129 | EF447162 | Hills Creek, Otago |
| *O. maccanni* | CD837 | OMA18 | EF447130 | EF447163 | Tara Hills, Canterbury |
| *O. maccanni* | CD635 | OMA20 | EF447131 | EF447164 | Mount John Station, Canterbury |
| *O. maccanni* | CD634 | OMA21 | EF447132 | EF447165 | Simons Hill, Canterbury |
| *O. maccanni* | CD627 | OMA22 | EF447133 | EF447166 | Mount Mary, Canterbury |
| *O. maccanni* | CD626 | OMA23 | EF447134 | EF447167 | Taieri Ridge, Otago |
| *O. maccanni* | CD567 | OMA24 | EF447135 | EF447168 | Conroys Dam, Otago |
| *O. maccanni* | CD428 | OMA25 | EF447136 | EF447169 | Remarkables, Otago |
| *O. maccanni* | CD426 | OMA26 | EF447137 | EF447170 | 10 km North of Millers Flat, Otago |
| *O. maccanni* | CD425 | OMA27 | EF447138 | EF447171 | Frankton, Otago |
| *O. maccanni* | CD10 | OMA29 | EF447139 | EF447172 | Hokonui Hills, Southland |
| *O. maccanni* | RE4990 (S1353) | OMA30 | EF447140 | EF447173 | Lake Tekapo, Canterbury |
| *O. maccanni* | RE4890 (S1253) | OMA31 | EF447141 | EF447174 | Near outlet, Lake Hawea, Otago |
| *O. maccanni* | RE4888 (S1251) | OMA32 | EF447142 | EF447175 | Tekowai Island, Lake Pukaki, Canterbury |
| *O. maccanni* | RE4860 (S1223) | OMA33 | EF447143 | EF447176 | Port Hills, Lyttleton, Canterbury |
| *O. maccanni* | RE4449 (S807) | OMA35 | EF447144 | EF447177 | Ida Range, 8 miles north of Naseby, Otago |
| *O. maccanni* | RE3917 (S275) | OMA37 | EF447145 | EF447178 | Te Anau, Southland |
| *Oligosoma zelandicum* | FT6516 | OZE2 | EF447181 | EF447201 | Pukerua Bay, Wellington |
| *O. zelandicum* | FT3814 | OZE3 | EF447182 | EF033070 | Mokihinui River Mouth, West Coast |
| *O. zelandicum* | CD331 | OZE4 | EF447183 | EF447202 | Maud Island, Nelson/Marlborough |
| *O. zelandicum* | CD542 | OZE5 | EF447184 | EF447203 | Stephens Island, Nelson/Marlborough |
| *O. zelandicum* | CD1951 | OZE6 | EF447185 | EF447204 | Outer Chetwode Island, Nelson/Marlborough |
| *O. zelandicum* | FT3743 | OZE7 | EF447186 | EF447205 | Gentle Annie, West Coast |
| *O. zelandicum* | FT3773 | OZE8 | EF447187 | EF447206 | Scotts Beach, Heaphy Track, West Coast |
| *O. zelandicum* | FT6525 | OZE9 | EF447188 | EF447207 | Johnsonville, Wellington |
| *O. zelandicum* | RE1594 | OZE19 | EF447189 | EF447208 | Foxton, Manawatu |
| *O. zelandicum* | CD335 | OZE20 | EF447190 | EF447209 | Maud Island, Nelson/Marlborough |
| *O. zelandicum* | CD543 | OZE21 | EF447191 | EF447210 | Stephens Island, Nelson/Marlborough |
| *O. zelandicum* | CD544 | OZE22 | EF447192 | EF447211 | Stephens Island, Nelson/Marlborough |
| *O. zelandicum* | CD1952 | OZE23 | EF447193 | EF447212 | Outer Chetwode Island, Nelson/Marlborough |
| *O. zelandicum* | FT3744 | OZE24 | EF447194 | EF447213 | Gentle Annie, West Coast |
| *O. zelandicum* | FT6517 | OZE25 | EF447195 | EF447214 | Pukerua Bay, Wellington |
| *O. zelandicum* | FT6518 | OZE26 | EF447196 | EF447215 | Pukerua Bay, Wellington |
| *O. zelandicum* | FT6526 | OZE27 | EF447197 | EF447216 | Johnsonville, Wellington |
| *Nannoscincus mariei* | NR9808 | EUG1 | EU423132 | EU423130 | New Caledonia |
| *Marmorosphax tricolor* | NR9800 | EUG2 | EU423133 | EU423131 | New Caledonia |

Samples with CD or FT codes were obtained from the National Frozen Tissue Collection (NFTC) housed at Victoria University of Wellington, New Zealand. Samples with RE codes were obtained from ethanol-preserved specimens housed at Te Papa, National Museum of New Zealand, Wellington (S codes refer to specimens from the former Ecology Division collection, now housed at Te Papa).

of sequences to the dataset. Ten replicate searches were conducted. Only trees within 5% of the target likelihood score were subject to reiteration. Neighbour-Joining (NJ) analyses were done using the TrN distance correction. A Maximum Parsimony (MP) tree was generated in PAUP* using the heuristic search option.

Bayesian analyses were completed using the computer program MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). We used the default value of four Markov chains per run, and ran the analysis for five million generations. To ensure that the analyses obtained a sampling of the full tree space rather than becoming trapped in local optima, the analysis was run twice. Chains were sampled every 100 generations to obtain 50,000 sampled trees. The program Tracer 1.3 (Rambaut and Drummond, 2003) was used to check for chain convergence. The first 12,500 sampled trees were discarded as the burn-in phase, with the last 37,500 trees used to estimate the Bayesian posterior probabilities. We used both bootstrap values and Bayesian posterior probabilities to assess branch support. Due to the size of the dataset, Maximum-Parsimony (MP) bootstraps (1000 replicates) were generated in PAUP*. We consider branches supported by bootstrap values greater than or equal to 70% (Hillis and Bull, 1993), and posterior probability values greater than or equal to 95% (Wilcox et al., 2002) to be well-supported by our data. We also constructed a haplotype network for *O. zelandicum* using the method of Templeton et al. (1992) in TCS v1.21 (Clement et al., 2000).

### 2.4. Estimating divergence times

To estimate the divergence time of lineages, we calibrated the evolutionary rate of ND2 by re-analysing the data from Macey

et al. (1998) for the agamid genus *Laudakia*. Specifically, we re-calculated the evolutionary rate for *Laudakia* using only the 507 bp fragment of ND2 used in the present study (e.g. Smith et al., 2007). The ND2 evolutionary rate has been demonstrated to be consistent (~1.2–1.4%) across several vertebrate groups (fish, amphibians, reptiles; Weisrock et al., 2001), and the ND2 molecular clock is suitable for estimating divergence times that are under 10 million years. We calculated average between-group nucleotide differences across each of the calibrated nodes from Macey et al. (1998) (1.5, 2.5, 3.5 mya), plotted them against time and then used the slope of the linear regression to calculate a rate of evolution for our 507 bp fragment of ND2. This resulted in an evolutionary rate of 1.3% per myr (0.65% per lineage, per myr), the same as calculated by Macey et al. (1998).

### 2.5. Hypothesis testing

We completed Shimodaira–Hasegawa tests in PAUP* (Shimodaira and Hasegawa, 1999; Goldman et al., 2000) using full optimization and 1000 replicates to examine to taxonomic status of the Garston skink. Allozyme evidence previously suggested that the Garston skink was a melanistic colour morph (darker colouration and glossier scales) of *O. maccanni* in the Garston region of Otago (CHD, unpublished data). We tested the significance of the log-likelihood difference between our optimal ML tree (using the ML $-\ln L$) and the alternative hypothesis that the Garston skink (OMA4) represents a distinct species.

## 3. Results

The edited alignment comprised 1282 characters (507 bp ND2, 775 bp ND4 + tRNAs), of which 425 (33%) were variable and 306 (24%) were parsimony-informative. For the ingroup only, the alignment contained 304 (24%) variable characters, of which 264 (21%) were parsimony-informative. Base frequencies were unequal (A = 0.3510, T = 0.2397, C = 0.3040, G = 0.1054), but a $\chi^2$ test confirmed the homogeneity of base frequencies among sequences (df = 150, $P = 1.0$).

The hLRT from ModelTest supported the TrN + I + G substitution model as the most appropriate for our dataset. Parameters estimated under this model were: relative substitution-rates (A↔C = 1.0, A↔G = 33.55, A↔T = 1.0, C↔G = 1.0, C↔T = 14.01, G↔T = 1.00), gamma shape parameter (1.9090) and proportion of invariable sites (0.5972). The topologies of the NJ, MP, ML (optimal ML tree, $-\ln L = 5475.96442$) and Bayesian trees were very similar, therefore we present the mean consensus Bayesian tree with MP bootstrap values and posterior probabilities indicating branch support (Fig. 3). The Bayesian tree strongly supports the monophyly of both *O. maccanni* and *O. zelandicum* (100% bootstrap and 1.0 posterior probability in both cases).

### 3.1. Oligosoma maccanni

Substantial genetic structuring was evident within *O. maccanni*, with a mean pairwise uncorrected ND2 genetic distance (GD) of 4.1% and seven clades identified (Fig. 3). Estimates of genetic diversity for *O. maccanni* are shown in Table 2. Haplotypic diversity was extremely high, with virtually every sample having a different haplotype (Table 2). Clade 1 is represented by a single sample (OMA27) from Frankton, near Queenstown in western Otago (Fig. 2). Clade 2 (100% bootstrap, 1.0 posterior probability, GD = 0.2%, 0.15 mya) encompasses samples from western Otago and western Southland, from the Remarkables to just south of Lake Te Anau (Fig. 2). The 'Garston skink' (OMA4) is part of Clade 2, with our Shimodaira–Hasegawa topology test clearly rejecting ($P < 0.0001$) the hypothesis that it is distinct from *O. maccanni*. Clade 3 is represented by a single sample (OMA31) from Lake Hawea in northwestern Otago (Fig. 2). Clade 4 (0.79 posterior probability, but well-supported [86% bootstrap] in the NJ and ML trees, GD = 1.2%, 0.92 mya) comprises samples from eastern Otago and eastern Southland, from the Ida Range south to the Hokonui Hills (Fig. 2). Clade 5 (100% bootstrap, 1.0 posterior probability, GD = 0.8%, 0.62 mya) incorporates samples from central Canterbury, from Lake Tekapo south to Lake Pukaki (Fig. 2). Clade 6 (100% bootstrap, 1.0 posterior probability, GD = 1.6%, 1.2 mya) is represented by two samples from the Banks Peninsula in Canterbury (Fig. 2). Clade 7 (100% bootstrap, 1.0 posterior probability, GD = 0.2%, 0.15 mya) encompasses two samples from south Canterbury, north of the Waitaki River (Fig. 2).

Considerable genetic differentiation is present among *O. maccanni* clades, with genetic divergences ranging from 0.2% (0.15 mya) to 6.2% (4.77 mya) (Table 3). There is support for a close relationship between Clade 1 and Clade 2 (56% bootstrap, 1.0 posterior probability), with relatively little genetic divergence evident between these two clades (0.2%, 0.15 mya; Table 3). Although the subdivision of clades is generally north-south, there is a substantial east-west split in Otago and Southland (Fig. 2). Substantial genetic differentiation exists (~4.7%, 3.6 mya) between populations in western Otago/Southland (Clades 1 and 2) and eastern Otago/Southland (Clade 4). There is strong support for the monophyly (82% bootstrap, 1.0 posterior probability) of the Otago/Southland clades (Clades 1–4), but there is no support for the Canterbury populations representing a well-supported group.

### 3.2. Oligosoma zelandicum

Haplotypic diversity in *O. zelandicum* was extremely high, with 12 unique haplotypes (from 17 samples) distinguished by 34 segregating sites and 34 mutations (Table 2). Relatively minor levels of phylogeographic structuring were evident across the range of *O. zelandicum* (maximum pairwise uncorrected ND2 genetic distance = 1.2%) (Fig. 3). Levels of genetic divergence among North Island populations (GD = 0.2%) was lower than that evident among South Island populations (GD = 0.4%). However, there was strong support for the monophyly of the North Island populations (85% bootstrap, 1.0 posterior probability; Fig. 3). The haplotype network supported this result (haplotype network not shown), indicating the presence of several mtDNA lineages in South Island, but only a single mtDNA lineage in the North Island, suggesting a single colonisation of the North Island from the South Island. The level of genetic differentiation between the North Island and South Island populations (GD = 0.6%), suggests that *O. zelandicum* reached the North Island ~0.46 mya.

## 4. Discussion

### 4.1. The relative contribution of Pliocene and Pleistocene processes in shaping genetic structure in Oligosoma maccanni

Substantial phylogeographic structure is evident within *O. maccanni*, with seven distinct clades identified. The seven *O. maccanni* clades appear to have diverged during the early-Pliocene to late-Pleistocene (4.77–0.15 mya), although the majority exhibit Pliocene divergence (Table 3). Our evidence for Pliocene divergence among *O. maccanni* clades is consistent with studies of other South Island taxa (e.g. Trewick, 2000; Trewick, 2001; Buckley et al., 2001; Arensburger et al., 2004; Chinn and Gemmell, 2004; Trewick and Morgan-Richards, 2005; Buckley and Simon, 2007; Greaves et al., 2007), and is generally attributed to increased tectonic activity along the alpine fault line during the Miocene (Gage, 1980; Suggate, 1982; Stevens et al., 1995).

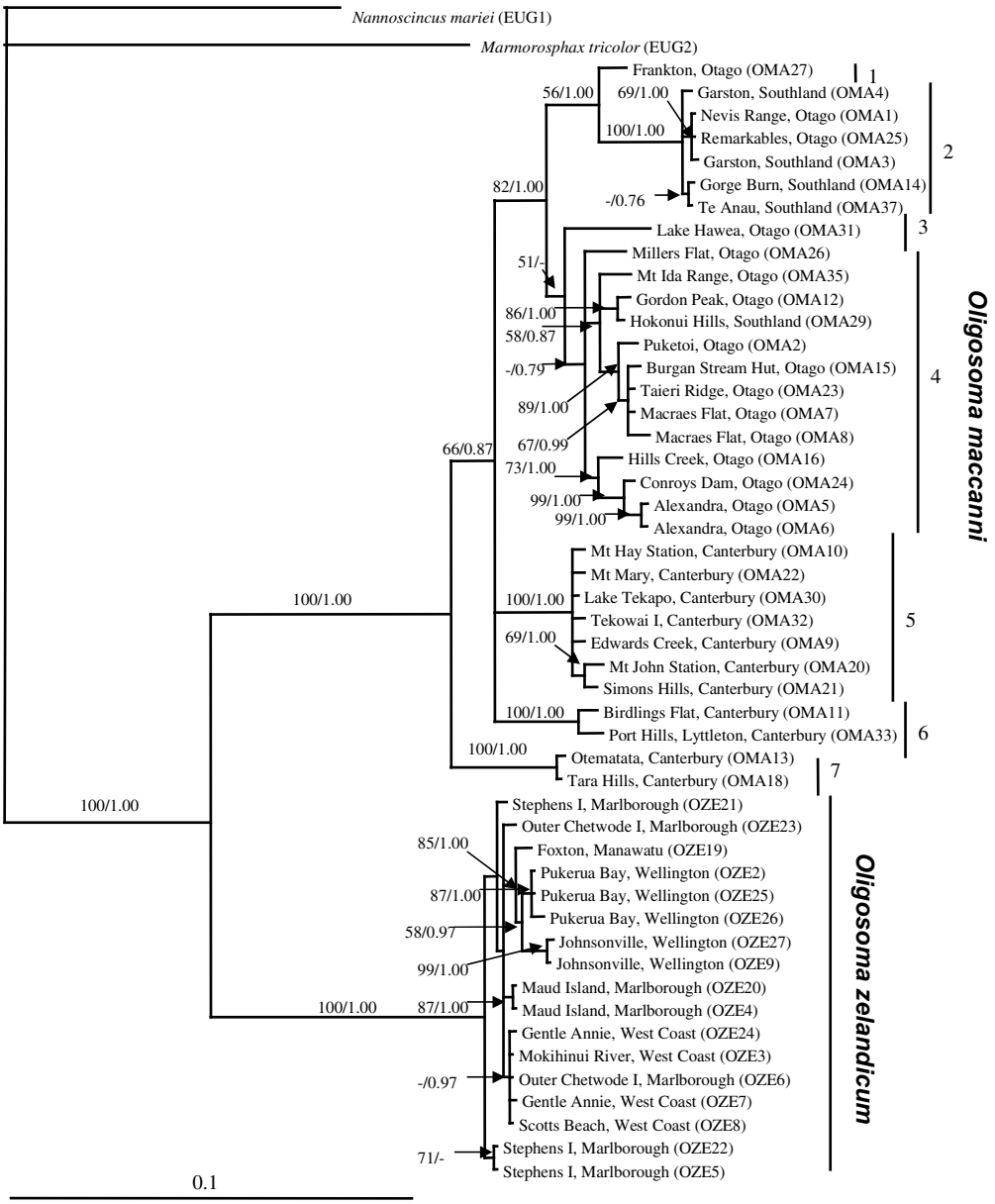


**Fig. 3.** Bayesian consensus tree for *Oligosoma maccanni* and *O. zelandicum* based on 1282 bp of mitochondrial DNA (507 bp ND2, 775 bp ND4). The topologies of the Neighbour-Joining, Maximum Parsimony (MP), and Maximum Likelihood trees were almost identical to the Bayesian tree shown. Two measures of branch support are indicated with MP bootstraps on the left and Bayesian posterior probabilities on the right (only values over 50 and 0.7, respectively, are shown). The seven main clades within *O. maccanni* are indicated.

The four clades from the Otago and Southland regions of the South Island (Clades 1–4) form a well-supported lineage within *O. maccanni* (Fig. 3). The Otago and Southland clades appear to have diverged from those in Canterbury (Clades 5–7) in the early- to mid-Pliocene (4.77–3.69 mya; Table 3). The genetic break between the Otago/Southland lineage and the Canterbury clades occurs approximately 20 km north of Oamaru along the Waitaki River. The Waitaki River designates the border between the Canterbury and Otago regions of New Zealand (Fig. 1), and also delineates the boundary between the central and southern biogeographic regions of the South Island (Emerson et al., 1997; Wallis et al., 2001). The Waitaki River valley has the potential to represent a significant biogeographic barrier as it follows a set of active faults (Darby et al., 2003), and is surrounded to the southwest by the Hawkdun range and Kakanui Mountains, and the Two Thumbs range, Hunters Hills and Canterbury Plains (grassland) in the northeast (Nordmeyer, 1981). In addition, the Waitaki River is the drainage for a large catchment area (~11,826 km$^2$; including the three natural lakes of the Mackenzie Basin and several glaciers) in the Southern Alps (Nordmeyer, 1981; Darby et al., 2003). Indeed, several other South Island taxa exhibit genetic breaks concordant with the Waitaki River valley (weta, *Deinacrida connectens*, Trewick, 2000; cicadas, *Maoricicada campbelli*, Buckley et al., 2001; skinks, *O. lineoocellatum-chloronoton*, Greaves et al., 2007; *O. nigriplantare polychroma*, L. Liggins, DGC, CHD, PAR, unpublished data). Interestingly, *O. maccanni* populations either side of the Waitaki River valley exhibit colour pattern differences (e.g. mid-dorsal stripe, speckling under chin, ventral pattern, dorsal pattern, stripe on anterior surface of forelimbs; Daugherty et al., 1990), supporting the genetic results that there has been restricted geneflow between the Canterbury and Otago regions since the Pliocene.

**Table 2**
Estimates of mitochondrial DNA (ND2, ND4) genetic diversity for *Oligosoma maccanni* (including 7 clades) and *O. zelandicum* (North Island and South Island samples)

| Species/clade | *N* | *h* | H*d* | *S* | *n* | $\pi$ |
|---|---|---|---|---|---|---|
| *Oligosoma maccanni* | 32 | 31 | 0.998 | 198 | 210 | 0.0390 |
| Clade 1 | 1 | 1 | NA | NA | NA | NA |
| Clade 2 | 6 | 5 | 0.933 | 6 | 6 | 0.0023 |
| Clade 3 | 1 | 1 | NA | NA | NA | NA |
| Clade 4 | 13 | 13 | 1 | 68 | 78 | 0.0150 |
| Clade 5 | 7 | 7 | 1 | 23 | 23 | 0.0057 |
| Clade 6 | 2 | 2 | 1 | 12 | 12 | 0.0094 |
| Clade 7 | 2 | 2 | 1 | 2 | 2 | 0.0016 |
| *Oligosoma zelandicum* | 17 | 12 | 0.956 | 34 | 34 | 0.0061 |
| North Island | 6 | 5 | 0.933 | 18 | 18 | 0.0062 |
| South Island | 11 | 7 | 0.909 | 15 | 15 | 0.0032 |

*N* is the number of individual sequences obtained. *h* is the number of haplotypes in each clade/population. H*d* is the haplotype diversity. *S* is the number of polymorphic (segregating) sites. *n* is the number of mutations (substitutions) for each clade/population. $\pi$ represents the nucleotide diversity within each clade/population.

**Table 3**
Mean genetic distances among *Oligosoma maccanni* clades identified in Fig. 3

| | Clade 1 | Clade 2 | Clade 3 | Clade 4 | Clade 5 | Clade 6 | Clade 7 |
|---|---|---|---|---|---|---|---|
| Clade 1 | — | 0.027 | 0.042 | 0.027 | 0.053 | 0.050 | 0.066 |
| Clade 2 | 0.002 | — | 0.047 | 0.048 | 0.052 | 0.051 | 0.063 |
| Clade 3 | 0.055 | 0.057 | — | 0.034 | 0.056 | 0.051 | 0.061 |
| Clade 4 | 0.046 | 0.047 | 0.038 | — | 0.050 | 0.052 | 0.062 |
| Clade 5 | 0.054 | 0.056 | 0.060 | 0.048 | — | 0.045 | 0.060 |
| Clade 6 | 0.053 | 0.055 | 0.059 | 0.052 | 0.046 | — | 0.062 |
| Clade 7 | 0.062 | 0.062 | 0.058 | 0.054 | 0.059 | 0.056 | — |

ND2 uncorrected genetic distances are shown below the diagonal and Tamura-Nei (TrN) corrected genetic distances for the concatenated dataset (ND2 + ND4) are shown above the diagonal.

A substantial genetic break was present between *O. maccanni* populations in east Otago (Clade 4) and west Otago (Clades 1 and 2). *Oligosoma maccanni* populations in west Otago occur in mountainous sub-alpine regions of the Southern Alps, while those in east Otago generally occur in grassland habitats that remained un-glaciated during Pleistocene glacial cycles (Stevens et al., 1995; Fig. 2). Indeed, the position of the east-west Otago break in *O. maccanni* is concordant with the Nevis-Cardrona fault system that has been active since the Miocene and delineates a topographic boundary between east Otago (gently-deformed erosion surface) and west Otago (deeply-eroded rugged mountains) (reviewed in Waters et al., 2001). Similar east-west splits are evident in other skink species, with divergence time estimates in *O. maccanni* (ND2: 4.7%, 3.6 mya), *O. grande* (control region: 11%, 3.8 mya; Berry and Gleeson, 2005), *O. otagense* (control region: 10%, 3.5 mya; Birkett, 2004), *O. chloronoton* (ND2: 7.4%, 5.3 mya; Greaves et al., 2007), and *O. inconspicuum* (ND2: 3.7%, 2.6 mya; DGC, S. Greaves & CHD, unpublished data) indicating that divergence between populations in east and west Otago occurred during the late Miocene-Pliocene. However, the east-west Otago split in some other taxa (e.g. alpine weta, *Hemideina maori*, COII: 0.8–1.2 mya; King et al., 2003) appears to have occurred more recently in the mid-Pleistocene.

Although there is an east-west split evident in the Otago region, the three clades in the Canterbury region (Clades 5, 6, and 7) are separated by north-south genetic breaks (Fig. 2). Divergence time estimates indicate that the differentiation among Canterbury clades occurred during the Pliocene. Concordant north-south breaks in the Canterbury region (of Pliocene origin) are evident in the *O. lineoocellatum-O. chloronoton* species complex (Greaves et al., 2007). However, this pattern has not been documented in other taxa as the majority of studies have focused on the alpine fauna of this region of the South Island (e.g. Buckley et al., 2001; Chinn and Gemmell, 2004). The Canterbury region is known to be depauperate in biodiversity as a result of repeated glaciation (periglacial), and glacial erosion/washout from the Southern Alps, during Pleistocene glacial cycles (Wardle, 1963; McGlone, 1985; Connor, 2002). It is possible that the north-south genetic breaks in the Canterbury region in *O. maccanni* originated as the result of Pliocene tectonism and have been maintained through the extirpation of intervening populations during Pleistocene glacial cycles.

Patterns of within-clade genetic divergences in *O. maccanni* appear to be the result of climatic and geological processes during the mid- to late-Pleistocene (1.2–0.15 mya). While Pliocene processes appear to have driven the diversification of the seven clades within *O. maccanni*, Pleistocene processes (predominately glacial cycles) appear to have acted to maintain divergences among these clades and shaped the pattern of within-clade divergences. Thus, although the pattern of genetic structure in *O. maccanni* is predominately shaped by Pliocene processes, the genetic imprint of Pleistocene processes is also evident. Studies on other New Zealand skink species have clearly indicated that both Pliocene and Pleistocene processes are responsible for patterns of genetic differentiation within species (Berry and Gleeson, 2005; Greaves et al., 2007, 2008; Hare et al., 2008; Chapple et al., in press a).

### 4.2. Taxonomic status of the 'Garston' skink

The 'Garston' skink is known only from a single subadult specimen collected from the Garston region of Otago (R. Hitchmough, pers. comm.). It was initially suggested to be distinct from sympatric *O. maccanni* due to its darker colouration and glossier scales. However, out topology test clearly rejects the hypothesis that the 'Garston' skink is genetically distinct from *O. maccanni*. This result supports previous allozyme and morphological analyses suggesting that the 'Garston' skink represents a melanistic colour morph of *O. maccanni* (R. Hitchmough, pers. comm.; CHD, unpublished data).

### 4.3. Did terrestrial taxa use Cook Strait landbridges?: insights from *Oligosoma zelandicum*

Although the distribution of *O. zelandicum* spans Cook Strait, a recognised biogeographic barrier, there was only relatively limited phylogeographic structure evident. This indicates that the divergences among the current *O. zelandicum* populations occurred during the mid- to late-Pleistocene. While there were several genetic lineages present in the South Island, the North Island populations formed a well-supported lineage within *O. zelandicum* (Fig. 3). Since the lower North Island was inundated throughout the Pliocene by the Manawatu Strait, it is possible that terrestrial taxa such as *O. zelandicum* only colonised the region from the South Island during the late-Pleistocene (~0.45 mya) with the formation of Cook Strait landbridges (Lewis et al., 1994; Worthy and Holdaway, 2002). Our genetic data supports a single colonisation of the North Island by *O. zelandicum* from the South Island, with the estimated timing of this event (0.46 mya) consistent with the initial formation of Cook Strait.

There has been some debate as to whether landbridges that formed intermittently during glacial maxima in the late-Pleistocene enabled the interchange of terrestrial taxa between the North Island and South Island (Lewis et al., 1994; Worthy and Holdaway, 1994, 2002). The majority of molecular studies that have examined species whose distribution spans Cook Strait have documented genetic divergences between the North Island and South Island that substantially pre-date the initial formation of Cook Strait landbridges: *O. lineoocellatum* (ND2: 4.6%, 3.3 mya; Greaves et al., 2007), *O. nigriplantare polychroma* (ND2: 2.9%, 2.1 mya; L. Liggins, DGC, CHD, PAR, unpublished data), land snails (*Wainuia umula*,

~4 mya; Efford et al., 2002), brown kiwi (*Apteryx australis*, 0.9 mya; Baker et al., 1995), cicadas (*M. campbelli*, 0.9 mya; Buckley et al., 2001), and short-tailed bats (*Mystacina tuberculata*, 0.89 mya; Lloyd, 2003).

In contrast, our data suggests that *O. zelandicum* might have used a Cook Strait landbridge to disperse from the South Island to the North Island. Geological evidence indicates that Cook Strait landbridges connected the Nelson/Marlborough region of the South Island to the lower North Island (Fig. 2). Hardy (1977) hypothesized that several skink species persisted in refugia in the Nelson/Marlborough region during Pleistocene glacial maxima. Thus, the presence of *O. zelandicum* in the Nelson/Marlborough region during glacial maxima may have enabled it to disperse into the North Island via a Cook Strait landbridge. However, it appears that *O. zelandicum* might have only used the initial Cook Strait landbridge and not landbridges in subsequent glacial maxima. This result supports the suggestion by Worthy and Holdaway (1994, 2002) that there was no Cook Strait landbridge (or no landbridge suitable for dispersal by terrestrial taxa) during the LGM, with the most recent landbridge occurring at least ~0.13 mya.

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