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Phylogeography and ecological niche modelling implicate coastal refugia and trans-alpine dispersal of a New Zealand fungus beetle

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Abstract

The Last Glacial Maximum (LGM) severely restricted forest ecosystems on New Zealand's South Island, but the extent of LGM distribution for forest species is still poorly understood. We used mitochondrial DNA phylogeography (COI) and ecological niche modelling (ENM) to identify LGM refugia for the mycophagous beetle *Agyrtodes labralis* (Leiodidae), a forest edge species widely distributed in the South Island. Both the phylogenetic analyses and the ENM indicate that *A. labralis* refuged in Kaikoura, Nelson, and along much of the South Island's west coast. Phylogeography of this species indicates that recolonization of the largely deforested east and southeast South Island occurred in a west–east direction, with populations moving through the Southern Alps, and that the northern refugia participated little in interglacial population expansion. This contradicts published studies of other New Zealand species, in which recolonization occurs in a north–south fashion from many of the same refugia.

Keywords: ecological niche model, fungus beetle, glacial refugia, Leiodidae, Maxent, Pleistocene

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Introduction

As public awareness of the impacts of modern climate change on global ecosystems grows, scientists are looking at past warming and cooling cycles to predict response to future climate fluctuations (Waltari *et al.* 2007). Of particular interest is where biota from a variety of habitats survived the Last Glacial Maximum (LGM) and how they colonized their modern ranges in the current interglacial. Phylogeographic studies of northern hemisphere temperate species indicate that this survival often took place in a combination of northern and southern refugia (Canestrelli *et al.* 2007; Dépraz *et al.* 2008; Gratton *et al.* 2008; Centeno-Cuadros *et al.* 2009) outside of the traditional southern retreat paradigm (Hewitt 1996).

New Zealand's LGM consisted of three major ice advances at 34 000–28 000, 24 500–21 500 and 20 500–19 000 cal. yr BP, and terminated at 18 000 cal. yr BP (Suggate & Almond 2005; Alloway *et al.* 2007). During the LGM, piedmont and valley glaciers extended 700 km along the Southern Alps (Vandergoes & Fitzsimons 2003), snowlines were depressed 600–800 m (Newnham *et al.* 1999), and global sea levels dropped by 120 m, connecting the North, South and Stewart Islands (Naish 2005). Mean annual temperatures were depressed 4–5 °C (Pillans 1991), precipitation declined, and the combination of expanded coastline and glacial height is thought to have shifted the rain shadow significantly west (Drost *et al.* 2007). Other climatic consequences included increased seasonality and weather extremes and, on the east coast, icy subantarctic winds (Drost *et al.* 2007).

An important question for assessing the impact of the LGM on New Zealand's biota is where temperate forest species survived and how they were able to expand into their modern range at the end of the LGM. Pollen records, fossils, ice cores and marine sediments from *c.* 22 000 years ago indicate that most of the South

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Island and lower North Island were covered in shrubs and grassland, with woody vegetation diminishing to the southeast and continuous podocarp-broadleaf and beech forest restricted to Northland and formerly emergent land along the west coast of Nelson (Alloway *et al.* 2007). Temperature depression and the extent of glaciation were not sufficient to explain such limited forest cover, although severe winds would have would have stunted tree growth and restricted forests in exposed areas (McGlone 1985). However, southern beech (*Nothofagus* spp.) was consistently present on the South Island's west coast, and scattered small tree pollen indicates that forest patches survived in sufficient numbers to rapidly expand during warmer periods (Suggate & Almond 2005). Insect fossils indicate that forest patches may have been even more widely distributed, including on the dry east coast (Marra & Leschen 2004; Marra *et al.* 2006). Evidence for widespread use of the Cook Strait land bridge by the terrestrial fauna during the mid- to late Pleistocene is conflicting (Worthy & Holdaway 1994; Marra *et al.* 2009), suggesting that North Island refugia were unavailable to many species.

Phylogeography is well-suited to determining the nature of glacial refugia in different locations (Provan & Bennett 2008). In New Zealand, if forest species survived primarily in and migrated from northern refugia, we would expect greater genetic diversity in the long-established northern populations, in a Southern Hemisphere reversal of the 'southern richness, northern purity' pattern (Hewitt 1996). Here, only some of the diversity preserved in refugia would participate in dispersal, and rapid colonization of large areas would further reduce this diversity through repeated bottlenecks in the founding genome (Hewitt 1996). Alternatively, if forest species survived in several South Island refugia, they could be identified as multiple hotspots of genetic diversity, differentiated from each other through genetic drift and evolution of unique genetic lineages (Provan & Bennett 2008). Several refugia within close proximity would give rise to colonized habitats with higher genetic diversity than those repopulated from a single refugium, but could still be distinguished from actual refugia by the absence of private refugial haplotypes (Canestrelli *et al.* 2007).

Phylogeographic studies of the New Zealand forest flora show conflicting refugia patterns. Isozyme studies of the hard beech *Nothofagus truncata* (Colenso) inferred genetic differentiation separating the southwest South Island from all other populations (Haase 1992), consistent with long-term survival in this region. While not a strict forest obligate, Shepherd *et al.* (2007) found rare chloroplast DNA haplotypes of the fern *Asplenium hookerianum* Colenso (Aspleniaceae) along the west coast of the South Island and near heavily glaciated areas. In contrast, Gardner *et al.* (2004) found a northern refugia pattern for *Metrosideros* Banks (Myrtaceae), with the South Island outside of Nelson represented by a single haplotype (Gardner *et al.* 2004). This apparently conflicts with *Metrosideros* pollen in late-LGM cores from Fiordland (Pickrill *et al.* 1992), but was attributed by Gardner *et al.* (2004) to rapid range expansion or failure of a Fiordland refugium to withstand the entire LGM and leave its genetic signature.

While most phylogeographic studies of the terrestrial fauna (reviewed by Wallis & Trewick 2009) have not focused on identifying LGM refugia, they agree that South Island refugia for a variety of habitats must have existed for older patterns to persist. Several have focused on the Biotic Gap, a broad swathe of the central South Island across which several biotic elements have disjunct ranges (Wardle 1963; Burrows 1965) for which elimination of taxa through glacial scour and erosion is one hypothesized origin (e.g. Trewick & Wallis 2001; Leschen *et al.* 2008). Relevant to our study are the absence of *Nothofagus* spp. and the forest litter beetle *Brachynopus scutellaris* Redtenbacher (Staphylinidae) from much of the Biotic Gap (Leschen *et al.* 2008). These species had restricted LGM ranges, and their failure to close the Biotic Gap while expanding widely through the rest of the South Island is not fully understood (Leathwick 1998; Leschen *et al.* 2008).

Relatively few studies feature obligate forest-dwellers, and fewer are of widely distributed species, due to naturally limited ranges (e.g. Boyer *et al.* 2007) or modern extirpation (e.g. Lloyd 2003). Of the widespread forest species, *B. scutellaris* persisted on the South Island's west coast north and south of the Biotic Gap (Leschen *et al.* 2008), while the forest edge cicada *Kikihia subalpina* (Hudson) was restricted to the northern South Island and most likely refuged in Nelson and Marlborough (Marshall *et al.* 2009). These deviating patterns indicate the need for further studies of widely distributed species to elucidate the impact of the LGM on New Zealand's forest communities.

Within phylogeography is a growing movement to explicitly incorporate environmental data in phylogeographic interpretation (Hugall *et al.* 2002; Richards *et al.* 2007). The goal of phylogeography is to unravel the history of a species in space as well as time (Avise 2009), yet until recently geographical and ecological information have mostly been invoked descriptively during tree interpretation (Richards *et al.* 2007). One way to integrate these data more objectively is with ecological niche models (ENMs), which use collection localities and Geographic Information System (GIS) maps of environmental data to develop spatial predictions of a species' historical and current range (Hugall *et al.* 2002; Richards *et al.* 2007). This technique has yet to be

widely adopted in New Zealand phylogeography, although species distribution modelling has been implemented in other ecological studies (Leathwick *et al.* 2005; Hall & McGlone 2006; Overton *et al.* 2009).

We aim to identify the locations of South Island forest refugia using mitochondrial DNA phylogenies, coalescent dating and ENMs for the mycophagous beetle *Agyrtodes labralis* (Broun) (Leiodidae: Camiarinae). Specifically, we will investigate whether *A. labralis* was (i) restricted to northern South Island refugia, particularly the putative Nelson refugium, from which it expanded south, or (ii) survived in multiple scattered refugia outside of Nelson, particularly in the southern half of the South Island, during the LGM. *Agyrtodes labralis* is an ideal focal species for this study because it is widely distributed throughout the South Island, which is of primary interest for glacial studies, but is absent in the North Island (Seago 2009). *Agyrtodes labralis* is found in beech and podocarp forests, feeding (apparently exclusively) on *Schizopora radula* Hallenb. (Schizoporaceae), a resupinate polypore fungus that encrusts moist, dead wood.

Methods

Taxon sampling

Agyrtodes labralis was collected throughout the South Island and on Stewart Island, from polypore fungi growing under dead logs and smaller woody debris in beech and podocarp forests with well-developed leaf litter deposits. Most specimens were collected by hand directly from over-turned pieces of wood, but occasionally woody debris was crumbled, sifted, and subjected to Berlese or Winkler funnel extraction. All specimens were collected directly into 95% ethanol and stored at −20 °C. GIS locality information was recorded for each collection site (Table 1).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from entire beetles using the DNeasy Tissue Kit (Qiagen), incorporating a 3-h digest, with the DNA resuspended in 50 μL DNA hydration solution and stored at 4 °C. The 3′ end of the mitochondrial cytochrome oxidase subunit I (COI) gene region was amplified using the primers C1-J-2195 and TL2-N-3014 (Simon *et al.* 1994). Polymerase chain reaction (PCR) was performed in 25 μL reactions containing 2 μL DNA template, 2.5 μL Faststart *Taq* DNA Polymerase PCR Buffer with $MgCl_2$ (Roche), 2.5 μL dNTPs, 1 μL each of 10 μM primers, 1 μL of 10 μM BSA, and 1.5 U Faststart *Taq* DNA Polymerase (Roche). PCRs were conducted on a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) using the following protocol: 5 min at 95 °C; 40 cycles of 1 min at 94 °C, 1 min at 50 °C and 1.5 min at 72 °C; and 10 min at 72 °C.

Polymerase chain reaction products were purified using either a High Pure PCR Product Purification Kit (Roche Diagnostics), eluting in 50 μL elution buffer, or the MinElute 96 UF Plate (Qiagen), eluting in 50 μL water. Sequencing of the purified PCR templates was conducted in both directions using BigDye™ Terminator Version 3.1 (Applied Biosystems), with unincorporated dye labels removed by ethanol precipitation. Sequence data were collected on a 3100-Avant Genetic Analyzer (Applied Biosystems) and visualized using Sequencher 4.6 (Gene Codes). Sequences were aligned and edited using BioEdit v. 7.0.5.3 (Hall 1999). Summary statistics were calculated using DnaSp 4.5 (Rozas *et al.* 2003).

Phylogenetic analyses and molecular clock

The best-fit model of sequence evolution for maximum likelihood (ML) analyses was determined using the Akaike Information Criterion (AIC) implemented in ModelTest 3.7 (Posada & Crandall 1998) and PAUP*4.0b10 (Swofford 2003). We conducted an ML tree search on the resulting best-fit AIC model, GTR+I+Γ, in RAxML 7.0.3 (Stamatakis 2006; Stamatakis *et al.* 2008). Rapid bootstrap analysis (100 bootstrap replicates) was followed by 10 ML tree searches. This was repeated 10 times, and a final 'best' tree was chosen based on the optimized likelihood scores for each run. Trees were rooted using two individuals of *Agyrtodes nebulosus* (Broun), a North Island species which differs slightly from *A. labralis* in colour and genitalia (Seago 2009).

Bayesian analyses using Markov chain Monte Carlo (MCMC) were implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) under the GTR+I+Γ model, using the following prior distributions: Dirichlet prior on the substitution rate matrix (1,2,1,1,2,1), uniform prior on tree topology, uniform prior on proportion of invariant sites, exponential prior on the alpha parameter for among-site rate variation (mean = 5), and an exponential prior on branch lengths (unconstrained; mean = 10). Four MCMC chains, one heated (temperature = 0.05) and three unheated, ran for 5 million generations, sampling every 1000 generations. The analysis was repeated 10 times, and the resulting parameter files were inspected for chain convergence in Tracer 1.4 (Rambaut & Drummond 2008) before ending each run. Approximately half of the runs yielded a bimodal marginal likelihood, despite regular swapping between heated and unheated chains and apparent chain convergence, and repeating the runs for 10 million generations did not alleviate this issue. Sullivan *et al.* (1999) observed that

Table 1 Collecting localities, haplotypes and geographic references for *Agyrtodes labralis* specimens used in DNA analysis. Specimen codes are referenced in GenBank

Locality	Haplotypes	Specimen code	Latitude	Longitude
Arthurs Pass, Bealey River	Hap 13 (2)	AM05, AM46	−43.0214	171.5958
Asbestos Track	Hap 12, Hap 82	AM28, AM193	−41.1053	172.7194
Ashley Gorge	Hap 41	AM149	−43.2275	172.2239
Blue Duck Scientific Reserve	Hap 86, Hap 85	AM172, AM173	−42.2361	173.7878
Bullock Creek Rd, Punakaiki	Hap 63	AM113	−42.1003	171.3436
Burke Flat, Haast River	Hap 03 (2)	AM31, AM180	−44.0214	169.3672
Canavans Knob	Hap 19, Hap 37	AM15, AM59	−43.3808	170.1619
Catlins, Tahakopa Valley Road	Hap 02	AM157	−46.4761	169.1761
Chinamans Bluff	Hap 09 (5)	AM18, AM20, AM44, AM47, AM61	−44.6569	168.3178
Coal Creek, Runanga	Hap 18, Hap 36	AM13, AM60	−42.4083	171.2578
Cowshed Bay Campground	Hap 75	ASP07	−41.2022	174.0319
Craigieburn	Hap 13 (2)	ASP1, ASP2	−43.1389	171.7361
Dolamore Park, Hokonui Hills	Hap 02 (3)	AM98, AM99, ASP3	−46.0594	168.8278
Dundas Creek	Hap 09	AM24	−44.6803	168.3442
Dunsdale, Hokonui Hills	Hap 02	AM80	−46.1317	168.6025
Eglinton Valley, Cascade Creek	Hap 08 (2)	AM23, AM39	−44.8947	168.0839
Flora Saddle, Lodestone Track	Hap 81, Hap 82	AM162, AM163	−41.1922	172.7478
Garden Mound Track, Stewart Island	Hap 61	AM118	−46.8675	168.1217
Glentui Reserve	Hap 41	AM95	−43.2003	172.2531
Glitter Burn, Haast River	Hap 09	AM178	−43.9375	169.1333
Glory Track, Bluff	Hap 49, Hap 02, Hap 65	AM92, AM93, AM135	−46.6139	168.3528
Governors Bush, Mt Cook	Hap 16 (5), Hap 69, Hap 08	AM09, AM21, AM50, AM51, AM66, AM137, AM145	−43.7389	170.0947
Gunns Bush, Hunters Hills	Hap 17 (2), Hap 44	AM77, AM78, AM146	−44.6647	170.9725
Haast Pass	Hap 03 (2), Hap 22, Hap 66	AM04, AM42, AM140, AM141	−44.1069	169.3553
Haast River mouth	Hap 97	AM181	−43.8078	169.0914
Harwoods Hole	Hap 87, Hap 89	AM166, AM167	−40.9450	172.8894
Jackson Bay	Hap 96 (2)	AM182, AM183	−43.9789	168.6164
Jum Michel	Hap 25	AM06	−42.7917	170.9008
Kelceys Bush, Hunters Hills	Hap 17 (2)	AM89, AM90	−44.6994	170.9653
Klondyke Corner, Arthurs Pass	Hap 13, Hap 33	AM11, AM67	−43.0217	171.5903
Klondyke Spur Track, Rahu	Hap 11 (3), Hap 62, Hap 60, Hap 51	AM111, AM112, AM116, AM117, AM119, AM120	−42.3136	172.1175
Kohaihai	Hap 04,Hap 52, Hap 68	AM126, AM127, AM144	−41.1089	172.1075
Lake Matheson	Hap 03, Hap 97	AM184, AM185	−43.4439	169.9681
Lewis Pass	Hap 11 (3), Hap 47 (2)	AM08, AM49, AM62, AM129, AM130	−42.3744	172.3956
Lyell Walkway	Hap 20, Hap 41	AM22, AM52	−41.7958	172.0500
Matukituki Valley, Mt Aspiring NP	Hap 21	AM25	−44.4925	168.7758
Moria Gate Track, Oparara Gorge	Hap 57	AM123	−41.1514	172.1975
Mt Arthur, Summit Track	Hap 40, Hap 38, Hap 29, Hap 07	AM10, AM16, AM55, AM58	−41.1900	172.7372
Mt Fyffe, Hinau Loop	Hap 30 (2), Hap 85	AM26, AM164, AM165	−42.3500	173.5678
Mt Lyford Ski Field	Hap 93	AM179	−42.4786	173.1489
Mt Nimrod, Hunters Hills	Hap 17	AM153	−44.4342	170.8703
Mt Robert	Hap 15, Hap 39	AM14, AM57	−41.8206	172.8078
Ohau Stream	Hap 79, Hap 80	AM160, AM161	−42.2447	173.8306
Onamalutu Scenic Reserve	Hap 31	AM36	−41.4583	173.7050
Oparara Gorge, Fenian Track	Hap 27, Hap 28, Hap 34	AM12, AM43, AM65	−41.2078	172.1561
Palmers Road, Mt Alexander	Hap 72	AM156	−42.3497	172.1483
Papatowai	Hap 67, Hap 02	AM73, AM142	−46.5531	169.4753
Pelorus Bridge	Hap 76	ASP05	−41.2986	173.5728

Table 1 *Continued*

Locality	Haplotypes	Specimen code	Latitude	Longitude
Piano Flat	Hap 02 (6), Hap 08, Hap 46, Hap 70	AM07, AM56, AM82, AM91, AM102, AM147, AM148, AM154, AM155	−45.5547	169.0175
Pigeon Saddle, Abel Tasman NP	Hap 74, Hap 73, Hap 32, Hap 26, Hap 24, Hap 23	AM03, AM32, AM48, AM68, AM176, AM177	−40.8325	172.9689
Pororari River Track 1	Hap 58, Hap 59	AM121, AM122	−42.1167	171.3633
Pororari River Track 2	Hap 53, Hap 64, Hap 18	AM107, AM108, AM132	−42.1164	171.3611
Port Underwood Saddle	Hap 77	ASP04	−41.2836	174.1064
Pudding Hill	Hap 41 (3)	AM86, AM87, AM94	−43.5578	171.5283
Puhi Puhi River	Hap 83 (2)	AM170, AM171	−42.2703	173.7381
Pukekura Scenic Reserve	Hap 94 (2)	AM188, AM189	−43.0367	170.6428
Puketiro, Table Hill Scenic Reserve	Hap 02 (2), Hap 48	AM100, AM101, AM143	−46.4933	169.5114
Rahu Scenic Reserve	Hap 11 (2)	AM109, AM110	−42.2517	171.9647
Rainbow Ski Field Road	Hap 01 (3), Hap 42	AM02, AM53, AM71, AM139	−41.8386	172.9292
Rarangi Track	Hap 78, Hap 92	AM174, AM175	−41.3794	174.0636
Riwaka Resurgence	Hap 88 (2), Hap 84	AM158, AM159, AM168	−41.0328	172.9003
Robinson Creek, Haast River	Hap 03 (4), Hap 08	AM01, AM41, AM70, AM133, AM134	−44.0114	169.3806
Seaward Bush	Hap 02, Hap 49, Hap 50	AM103, AM104, AM138	−46.4306	168.4194
Sharplin Falls	Hap 43 (2), Hap 41, Hap 06	AM74, AM75, AM96, AM97	−43.6269	171.4158
Shenandoah Saddle	Hap 05, Hap 11	AM105, AM106	−42.0186	172.2400
Ship Creek	Hap 22	AM30	−43.7589	169.1503
St James Walkway, Boyle Creek	Hap 11	AM35	−42.5183	172.3917
Takitimu Mountains, Princhester Hut	Hap 14, Hap 35 (2)	AM17, AM45, AM63	−45.5933	167.9519
Temple Valley	Hap 17 (3)	AM19, AM54, AM69	−44.1067	169.8169
Toms Creek Park	Hap 45, Hap 02	AM79, AM88	−45.9433	169.4731
Tuatapere Scenic Reserve	Hap 08, Hap 10	AM27, AM33	−46.1258	167.6828
Ulva Island, Stewart Island	Hap 02 (2), Hap 54	AM114, AM115, AM128	−46.9294	168.1267
Waihi Gorge	Hap 06, Hap 41 (2)	AM84, AM85, AM131	−44.0011	171.1503
Waipori Falls 1	Hap 71	AM76	−45.9258	170.0294
Waipori Falls 2	Hap 02 (2)	AM81, AM150	−45.9061	169.9861
Washbourn Scenic Reserve	Hap 90	ASP06	−40.7689	172.7067
Whanganui Inlet	Hap 91	AM169	−40.6239	172.5008
Woods Creek	Hap 55, Hap 56	AM124, AM125	−42.5531	171.3486
Xmas Flat, Haast River	Hap 95 (2)	AM186, AM187	−43.9411	169.1414

correlation between estimates of the proportion of invariant sites and the gamma shape parameter can affect the likelihood surface, possibly due to problems with parameter identifiability (Rannala 2002). We used the less complex GTR+Γ model (priors otherwise as above), which ran 10 times, for 20 million generations each, sampling every 1000 generations and with the first 5000 sampled trees from each run discarded as burnin. Bimodality was greatly reduced in the marginal likelihood and alpha shape parameter for three of the 10 runs, and was absent in the other seven. Following examination of the results in Tracer, all 10 tree files were combined in MrBayes to yield a consensus tree.

Molecular clock analyses were conducted in BEAST 1.4.8 (Drummond & Rambaut 2007) under a Bayesian coalescent framework. We used Brower's (1994) rate of invertebrate mitochondrial DNA evolution of 0.0115 substitutions/site from the root to the tips of the tree. MCMC simulations were performed under the GTR+I+Γ model using a strict molecular clock, under the exponential growth rate model. The MCMC started from a UPGMA tree due to the large number of sequences being analysed. The final BEAST profile incorporated gamma prior distributions (α, $\beta = 1$) for all parameters except the coalescent growth rate (uniform, 0.0115) and clock (normal; mean 0.0115, SD 0.00115) settings, and the final BEAST profile ran five times for 20 million generations. Tree files from all runs were combined using LogCombiner 1.4.8 and TreeAnnotator 1.4.8 (from the BEAST package) to yield a consensus tree.

A Bayesian Skyline Plot (Drummond *et al.* 2005) was also generated to examine the timing and magnitude of changes in effective population size. Prior settings were as described for the final BEAST profile above, with the addition of a gamma prior on skyline population size (α, $\beta = 1$) and 10 skyline groups, and the analysis ran three times at 200 million generations, sampling every 1000. Results were visualized in Tracer to ensure that all parameters had effective sample sizes >200 and to generate skyline plots.

Ecological niche modelling

Climate data used to construct the ENM were limited to those for which LGM counterparts are also available, including mean annual rainfall (mm), mean February rainfall (mm), mean annual solar radiation (kJ/day/m^2), mean annual temperature (°C), mean February temperature (°C), minimum temperature of the coldest month (°C, July), and October vapour pressure deficit (when persistent westerly winds result in strong geographic variation in vapour pressure deficit; Leathwick *et al.* 2003) (kPa). Present-day 100-m resolution climate surfaces were derived from observed meteorological data (1950–1980) fitted by thin-plate spline regression to a New Zealand digital elevation model (DEM) (Leathwick *et al.* 1998; Leathwick *et al.* 2003) using ANUSPLIN 4.1 (Hutchinson 2000). The same methods were used to fit observed meteorological data (1950–1980) to the LGM (*c.* 22 000 cal. yr BP) based on estimates of temperature depression from marine isotope stages and estimates of LGM topography obtained by extending the modern DEM down to the 120-m bathymetry (J. R. Leathwick unpublished data). These climate data are held in a GIS as ESRI (Environmental Systems Research Institute, Redlands, California) grids using New Zealand Map Grid (NZMG) coordinates.

Ecological niche models were generated with Maxent 3.3.1 (Phillips *et al.* 2006; Phillips & Dudík 2008), which uses the maximum entropy method to model presence-only species distribution data by distinguishing presence from random. Present-day and LGM niche models for *A. labralis* were generated using 79 localities from the molecular analysis, plus 12 localities listed by Seago (2009). In Maxent, the default convergence threshold (10^{-5}) was used, maximum iterations were increased to 2000, and regularization values and functions of environmental variables were selected automatically by the program. For each run, 75% of the localities were used to train the model and 25% were randomly selected to test the model. We performed 10 replicate runs without projecting the data, using 'random seed' to generate a different 75%/25% partition each time, to ensure consistency in the statistical output between runs. We then ran four separate projections of the data onto present-day and LGM surfaces, with a different random 75%/25% partition for each projection pair, using the 'fade by clamping' setting for the output grids.

Model performance was evaluated using the area under the (receiver operating characteristic) curve (AUC) and the threshold-dependent binomial omission tests calculated by Maxent. The AUC varies between 0.5, where localities are equally likely to be predicted 'presence' or 'absence', to 1, indicating perfect assignment of 'presence' and 'absence', although in practice the maximum possible AUC is <1 (Phillips *et al.* 2006). Values ≥0.7 indicate adequate discrimination (Swets 1988). Model predictions were visualized in ArcGIS 9.2 (ESRI) using the 10th percentile training presence threshold calculated by Maxent for each run, meaning the 10% of data with the lowest predicted probabilities fall into the 'absence' region of the thresholded model, and 'presence' regions include the 90% of distribution records with the highest model values. The threshold is the highest probability value of that lowest 10%.

Analyses of molecular variance

We used analyses of molecular variance (AMOVA) (Excoffier *et al.* 1992) to test the amount and significance of variance in a priori hypotheses on the locations of glacial refugia. Specifically, we looked for evidence of a Nelson refugium, broader survivorship in the northern vs. southern South Island (SSI), and Biotic Gap subdivision (boundaries after Wardle 1963). We imposed an east–west division using the Alpine Fault, along which the Southern Alps are being continuously uplifted (King 2000); this also approximately corresponds with the northwestern glacial face (Newnham *et al.* 1999). We also tested the distribution of refugia predicted by the Maxent ENM. For comparison, we performed AMOVA using the major mtDNA clades from the ML and Bayesian coalescent analyses. All AMOVAs were performed using Arlequin 3.1 (Excoffier *et al.* 2005), using 10 000 permutations. Areas included in hypothesis tests are shown in Fig. 1.

Results

Data summary

We obtained mitochondrial COI sequence data (788 bp) for 187 *Agyrtodes labralis* from 79 localities (Fig. 2a), with most populations (55) represented by ≤2 individuals (GenBank Accession nos GU017127–GU017315). We observed 97 unique haplotypes with 75 parsimony-informative sites (Haplotype diversity 0.975; Tajima's *D* and Fu and Li's *F** and *D** not significant), with 71

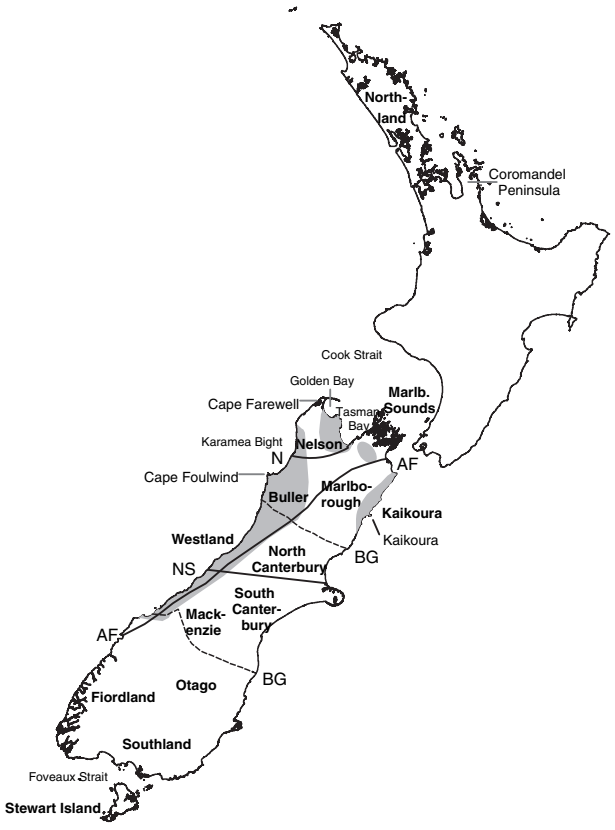

Fig. 1 New Zealand locations and AMOVA hypotheses. Region names are in bold, and specific locations in regular type. Lines transecting the South Island are the boundaries for AMOVA hypotheses: N = Nelson refugium, AF = Alpine Fault, BG = Biotic Gap and NS = northern and southern halves. Shaded areas are considered in/adjacent to Maxent refugia.

haplotypes being private to individuals and a further 11 shared among individuals within single populations. Single-individual haplotypes were distributed throughout the length of the South Island, from both coasts, and from Stewart Island (Fig. 2b; Table 1). Only 15 haplotypes were shared among different localities, with the most widely distributed haplotype (Hap 02) detected in 12 locations (21 individuals).

Phylogenetic analyses

Maximum likelihood (RAxML), Bayesian (MrBayes), and Bayesian coalescent (BEAST) analyses yielded slightly different tree topologies (Fig. 3), with broad agreement on clade memberships for the northern South Island, and disagreement on lineage affinities from the SSI. All three trees identified three monophyletic northern lineages which are spatially parapatric and have moderate to strong posterior probability (pp) and bootstrap support. They were the Kaikoura–Marlborough Sounds (hereafter KA-SD) clade, the only lineage recovered in all three trees endemic to the east coast, the Buller–Canterbury (BR-CA) clade, which crossed the Southern Alps at Lewis Pass and occurred both east and west of the divide, and the Nelson–Marlborough (NN-MB) clade, limited to the northwestern South Island. KA-SD was sister to the rest of the tree, and diverged from BR-CA [NN-MB(remaining lineages)] *c.* 2.69 Ma (95% CI 1.86–3.61 Ma, pp 1). Within KA-SD, the Marlborough Sounds and coastal Kaikoura populations diverged from each other at 1.31 Ma (0.80–1.88 Ma, pp 0.8707). Sites within KA-SD consisted almost entirely of private haplotypes.

Buller–Canterbury diverged from NN-MB(remaining lineages) next, *c.* 2.24 Ma (1.54–3.02 Ma, pp 0.7618). Within BR-CA, the west coast populations diverged from the Lewis Pass and Canterbury ones at 0.83 Ma (0.47–1.22 Ma, pp 1). Haplotype diversity within BR-CA was not evenly distributed spatially, and seven of BR-CA's 18 unique haplotypes were identified from west coast Oparara and Kohaihai populations alone. Most individuals from the populations around Lewis Pass shared a single haplotype, from which the eastern populations differed by a maximum of three base pair substitutions, and the eastern populations possessed only three haplotypes in total, indicating relatively recent origins. Lewis Pass and Canterbury populations diverged from each other *c.* 0.31 Ma (0.16–0.48 Ma, pp 1).

Similar in age to BR-CA was the NN-MB clade, which diverged from all remaining lineages *c.* 1.98 Ma (1.34–2.67 Ma, pp 0.6549). With the exception of one individual from Whanganui Inlet, west of Cape Farewell, all populations in this clade occurred north or east of central Nelson's Tasman Mountains, while Nelson populations in BR-CA occurred west of the Tasmans.

The remaining sequences, most of which were from the SSI, grouped into either two (Bayesian, Bayesian coalescent) or three (ML) clades (Fig. 3). Both analyses with no clock assumption (ML and Bayesian) had a separate clade along the west coast and Southern Alps, which extended east of the mountains only at Arthur's Pass in Northern Canterbury and the Takitimu Mountains in Southland. In both trees, the northern (north Westland and Buller; NW) and southern (south Westland; SW) halves of this clade were reciprocally monophyletic, with a high posterior probability (0.93) but low bootstrap support, and with the division geographically corresponding to the centre of the Biotic Gap (Fig. 1).

This west coast clade formed a trichotomy with the Mackenzie–South Canterbury (MK-SC) and Otago–Southland (O-S) clades in the ML tree, while the Bayesian analysis placed it as sister to MK-SC + O-S (SSI). Clade O-S contained the single most abundant haplotype detected, and all other O-S haplotypes were within three base pair substitutions of it (half were only one

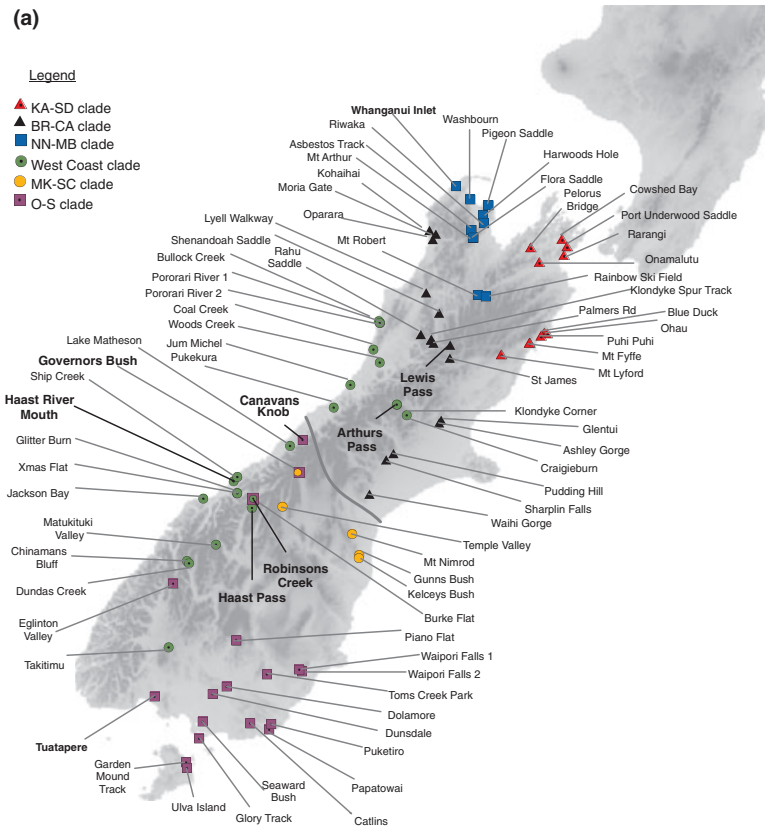

Fig. 2 Distributions of populations and haplotypes of *Agyrtodes labralis*. Map shading indicates topographic relief. (a) Localities sampled. Clade membership is according to the ML tree, with the grey line indicating the northern boundary of the (Bayesian coalescent) Southern clade and the division between NW and SW in the (ML & Bayesian) West Coast clade. Localities mentioned in the discussion are shown in bold. (b) Distribution of mitochondrial haplotypes. Populations with at least one private haplotype are coloured, and those with only shared haplotypes are grey. Populations sharing haplotypes are connected by shaded areas, and labels for shared haplotypes are printed in bold.

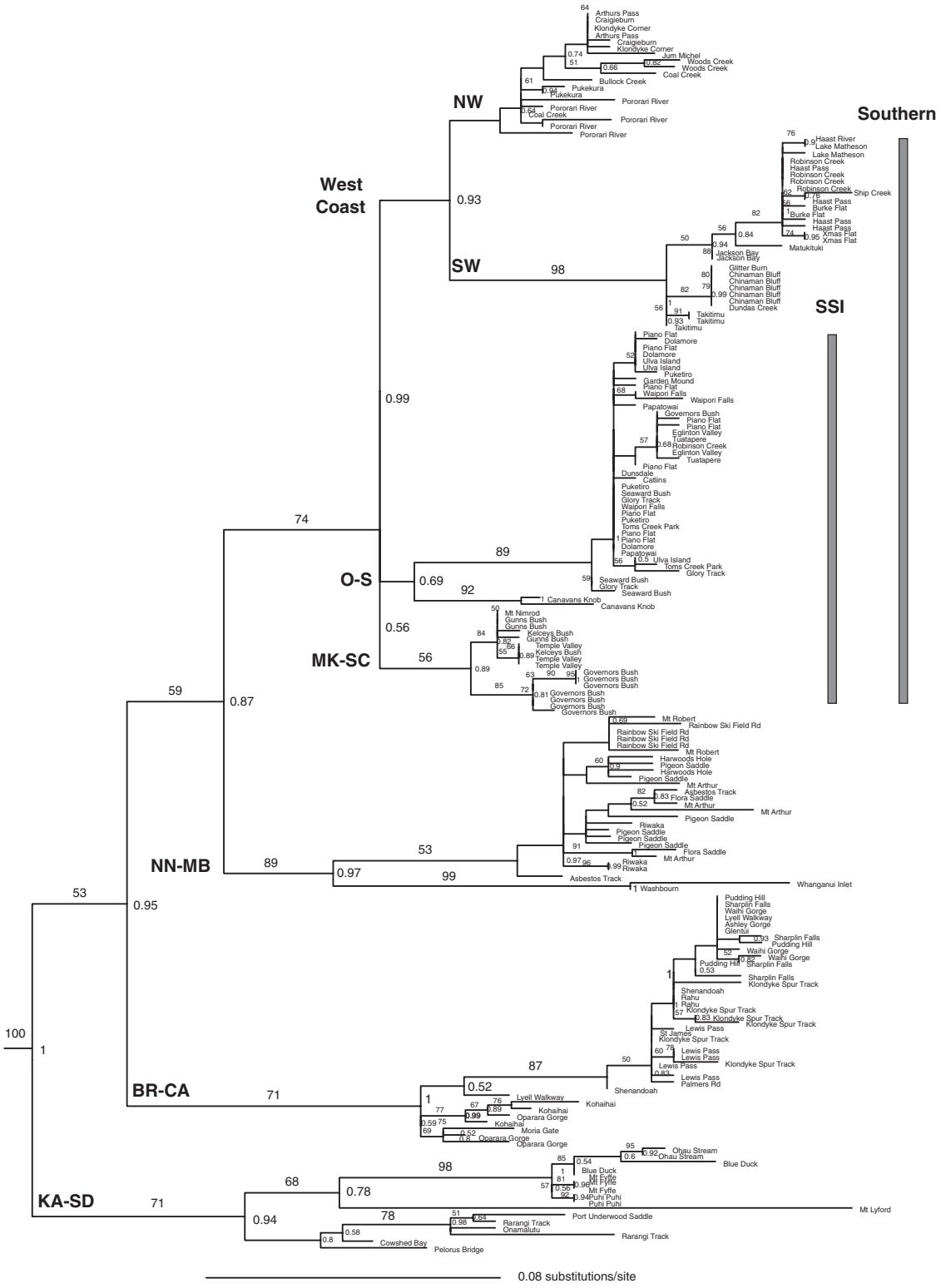

Fig. 3 GTR+I+Γ maximum likelihood tree with branch lengths drawn proportional to the number of substitutions per site as indicated by the scale bar. Bootstrapped nodal support values are labelled above the branches. Posterior probability nodal support values from the Bayesian consensus tree are given in italics at the nodes. Clade abbreviations are KA-SD (Kaikoura–Marlborough Sounds); BR-CA (Buller–Canterbury); NN-MB (Nelson–Marlborough); MK-SC (Mackenzie–South Canterbury); O-S (Otago–Southland); and West Coast, including NW (north Westland–Buller) and SW (south Westland). Side bars indicate membership in the Bayesian SSI (Southern South Island) and Bayesian coalescent Southern (SSI) clades.

substitution away). At this sampling scale, spatial distributions of O-S and SW were interdigitated along the Southern Alps and sympatric at one locality along the Haast River (Robinson Creek), while O-S and MK-SC are sympatric at Governors Bush (Fig. 2). Posterior support for reciprocally monophyletic West Coast and SSI clades was strong (pp 0.9), but bootstrapping only recovered MK-SC (74%) and failed to support O-S, NW, SW, or larger SSI or West Coast clades above 50%.

The strict molecular clock (Bayesian coalescent) analysis combined SSI with SW as a well-supported (pp 1.0) Southern clade (SW + MK-SC + O-S), diverging from NW *c.* 1.32 Ma (0.87–1.82 Ma). Within this Southern clade, the MK-SC, O-S and SW clades had low posterior probabilities as separate lineages. The two unique haplotypes from Canavans Knob, the northernmost population in the Southern clade, grouped with O-S in both trees with no clock assumptions but were outside O-S in the Southern clade, albeit with low posterior support as a separate lineage.

Bayesian Skyline Analysis indicated a sharp population increase *c.* 0.10 Ma (0.05–0.20 Ma), but did not indicate any change in population demographics during the LGM (Fig. 4).

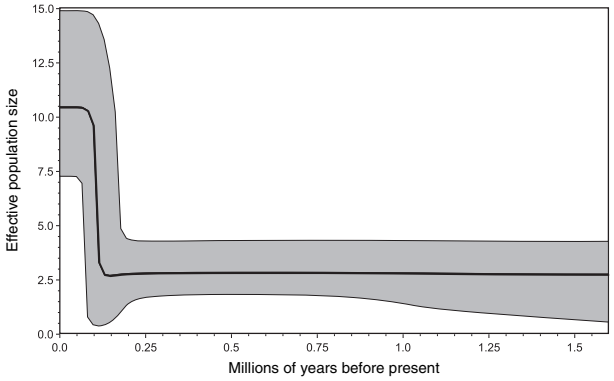

Fig. 4 Bayesian skyline plot for *Agyrtodes labralis*. The *x*-axis measures time in millions of years and the *y*-axis is the scaled effective population size. The 95% confidence interval is indicated in grey.

Ecological niche model

Bioclimatic modelling of the distribution of *A. labralis* yielded a moderately high test AUC score for the replicated runs (mean 0.847, range 0.777–0.883), and predictions were significantly different from random for all runs at all thresholds measured by the binomial omission tests. Heuristic estimation of the relative contributions of the environmental variables to the model indicated that mean February temperature (26.4%) and October vapour pressure deficit (25.0%) were the top two predictors of the species' presence. The least informative variable was minimum temperature of the coldest month (3.7%). The modelled current distribution of *A. labralis* (Fig. 5a) included most of the South Island's west coast, Southland, Nelson, Marlborough, the Kaikoura coastline, and parts of central Canterbury, as well as some regions of the North Island, where *A. labralis* is absent but other *Agyrtodes* species are found (Seago 2009). Areas of presence were similar across the four projections.

Modelling indicated a substantial reduction in the range of *A. labralis* during the LGM, with almost no suitable habitat predicted in the central and SSI (Fig. 5b). South Island refugia were predicted for four main regions: a small area north of Kaikoura, Tasman Bay and northern Marlborough, along the west coasts of Nelson, Buller and Westland, and a small area along the southern coast of Fiordland. Small inland patches of refugia were also predicted for the northwestern Southern Alps, but the majority of hospitable habitat was projected onto the expanded LGM continent in areas currently submerged. The four models predicted similar refugia for Kaikoura, northern Marlborough and Tasman Bay, but differed in the length and connectivity of the west coast refugia and the presence of a Fiordland refugium. For the North Island, suitable habitat was predicted for the coastal northeast and inland of the present-day west coast, and absent from areas which would have abutted the species' range, such as on or immediately north of the Cook Strait land bridge.

Hypothesis testing

Analysis of molecular variance revealed a high level of geographic structuring in *A. labralis*, but in all tests the majority of variation (65–66%) was within individual populations, reflecting the high level of haplotype diversity relative to the number of specimens sampled (Table 2). Even between the mtDNA clades, the among-group variance only accounted for 11.24% (ML clades) or 5.6% (Bayesian coalescent clades) of the molecular variation. There was significant geographical partitioning in the data, between north and south, around the Biotic Gap, and across the Alpine Fault. Partitioning into the refugia projected by Maxent was also significant. Of these, the Biotic Gap hypothesis explains the largest amount of phylogeographic structure (6.09%). The Nelson refugium hypothesis did not significantly explain the molecular variance in the data.

Discussion

Timing population divergence

New Zealand's South Island has had a turbulent history, leaving an indelible mark on the biota. The Plio-

Fig. 5 Ecological niche models for *Agyrtodes labralis*. Dark grey to black indicates the species' projected distribution after thresholding, with darker areas indicating presence in multiple (1–4) Maxent runs, while light grey indicates regions of predicted absence. (a) Current distribution. (b) LGM distribution. (c) LGM detail: south Fiordland.

cene (5–2 Ma) saw accelerated uplift and mountain-building along the Alpine Fault (King 2000), with the Southern Alps blocking the prevailing westerly winds, resulting in a significant southeastern rain shadow (Chamberlain *et al.* 1999). Heightened orogeny coincided with major global climate transitions, with progressive cooling during the late Pliocene (3.0–2.5 Ma) establishing 40-ka glacial–interglacial cycles (Naish 2005). These were replaced by 100-ka cycles when cooling intensified around 0.9 Ma (Mildenhall *et al.* 2004), the last of which (Otira; 74–11.5 ka) consisted of at least five glacial advances including the LGM (Woodward & Shulmeister 2007).

The presence of three endemic clades in the northern South Island (Kaikoura–Southland, Buller–Canterbury and Nelson–Marlborough; KA-SD, BR-CA and NN-MB) suggests *Agyrtodes labralis* was widely distributed across this region prior to isolation in refugia, and the Bayesian coalescent dating analyses indicated that they diverged in the late Pliocene/early Pleistocene, as the mountain-driven weather regime was exacerbated by climate cooling. These changes would have especially affected the northeast South Island, which also experienced tectonic activity along Marlborough's four active faults (Craw *et al.* 2008), which may indicate why KA-SD, the only clade endemic to the eastern South Island, diverged from the remaining lineages earliest, around the end of the Pliocene. As cooling intensified, lower tree lines and snow-filled mountain ranges may have segregated BR-CA, NN-MB and NW (north Westland-Buller). NW diverged from the (Bayesian coalescent) Southern Clade, which included SW (south Westland), near the mid-Pleistocene Climate Transition and beginning of the Otira (Mildenhall *et al.* 2004), possibly as a result of the developing glacial activity implicated in forming the Biotic Gap (Leschen *et al.* 2008). Although NW and SW formed a single clade in both the ML and Bayesian trees, and NW had a higher proportion of sin-

Table 2 Analysis of molecular variance (AMOVA) for five a priori and two a posteriori hypotheses. Population refers to individual localities, and region refers to the division being tested (e.g. inside vs. outside the Nelson refugium)

Hypotheses	Source of variation	d.f.	Sum of squares	Variance components	% variation	*P*-value
A priori						
Nelson refugium	Among regions	1	0.960	0.00153	0.31	0.31782
	Populations within regions	75	54.219	0.16818	34.27	0.00000
	Within populations	109	34.988	0.32099	65.41	0.00000
North vs. south	Among regions	1	2.501	0.01769	3.55	0.00000
	Populations within regions	75	52.678	0.15959	32.03	0.00000
	Within populations	109	34.988	0.32099	64.42	0.00000
Biotic gap	Among regions	2	5.346	0.03041	6.09	0.00000
	Populations within regions	74	49.833	0.14831	29.68	0.00000
	Within populations	109	34.988	0.32099	64.24	0.00000
Alpine fault	Among regions	1	1.831	0.01085	2.19	0.00099
	Populations within regions	75	53.348	0.16324	32.97	0.00000
	Within populations	109	34.988	0.32099	64.84	0.00000
Maxent refugia	Among regions	1	1.765	0.01018	2.06	0.00119
	Populations within regions	75	53.413	0.16359	33.06	0.00000
	Within populations	109	34.988	0.32099	64.88	0.00000
A posteriori						
ML clades	Among clades	5	12.097	0.05616	11.24	0.00000
	Populations within clades	71	43.081	0.12243	24.51	0.00000
	Within populations	109	34.988	0.32099	64.25	0.00000
Bayesian coalescent clades	Among clades	4	6.709	0.02788	5.60	0.00000
	Populations within clades	72	48.469	0.14854	29.86	0.00000
	Within populations	109	34.988	0.32099	64.53	0.00000

gle-locality haplotypes, eleven base pair substitutions separated the closest members of each lineage, indicating that SW did not recently descend from NW ancestors, and that these populations remained separated through the LGM.

A central issue in any single-locus study is the ability to estimate divergence dates among populations, and we estimated gene coalescence between and within clades that well predate the LGM. The most striking example is between Oparara, Lewis Pass and Canterbury (BR-CA), at ≥300 000 years ago, yet the detection of few closely related haplotypes in the eastern populations and LGM closure of Lewis Pass by glaciers (Newnham *et al.* 1999) are consistent with more recent population expansion. We cannot discount the possibility of accelerated substitution rate (e.g. Boyer *et al.* 2007), time-dependent mutation rates across population genetic timescales (e.g. Burridge *et al.* 2008), or the time discrepancy between gene and population divergence (Edwards & Beerli 2000), which are most influential when reconstructing recent events, yielding the observed patterns. The Bayesian skyline method assumes panmixia, but this is violated in species with high levels of geographic structuring like *A. labralis*, and can greatly influence date estimation (Edwards & Beerli 2000).

Our estimates are consistent with divergence and lineage sorting within refugia through multiple glacial cycles. This study aims to identify LGM refugia because the LGM was the last major geo-climatic bottleneck for temperate species, but it was only the most recent driver of population reduction and fragmentation in New Zealand's tectonically active, repeatedly glaciated landscape. Given the refugia, two divergence hypotheses are possible: Population divergence and contraction during the LGM and restriction into refugia (yielding coalescent dates approximately 25–35 000 years ago), or earlier divergence due to previous glacial cycles and older tectonic activity followed by survival in LMG refugia (yielding dates between 1.8 Ma and 35 000 years ago). That subdivided populations with limited migration have older coalescent times is known (Wakeley 2000), and Jesus *et al.* (2006) demonstrated the role of glacial cycles in driving this process through repeatedly fragmenting populations into multiple refugia. The deep and ancient clades of *A. labralis* suggest that it survived many glacial cycles in the refugia from which it emerged post-LGM.

South Island glacial refugia

For *A. labralis*, the scenario of multiple South Island refugia is more appropriate than the northern refugia/southward expansion model, although northern refugia were present and contributed to colonization of the east-central South Island. Provan & Bennett (2008)

reviewed the criteria for identifying glacial refugia, in which refugia themselves are characterized by high genetic diversity and many unique haplotypes, recolonized areas contain less diversity and a subset of refugial haplotypes, and contact zones between refugia have high diversity without the unique haplotypes. For *A. labralis*, being able to incorporate explanations from ENMs was particularly helpful because the South Island is littered with unique haplotypes and South Island refugia have been hypothesized to exist in coastal areas now submerged (Alloway *et al.* 2007), meaning we may not have sampled haplotypes from within the refugia themselves.

Geographic distributions of the clades were consistent with our LGM niche model, which indicated the existence of suitable habitat at Kaikoura, scattered through the Marlborough Sounds, in Nelson's Tasman Bay, along the west coasts of Nelson, Buller and Westland, and at southernmost Fiordland (Fig. 5b, c). Divergence dates between the major clades are also consistent with the geological processes described above resulting in the projected LGM distribution. No refugia were projected for southeast of the Southern Alps and Kaikoura ranges, though it is possible that *A. labralis* was present in the southeast prior to the Pleistocene, and subsequent glacial events have erased all trace of their existence.

Combining the results of both analyses, we propose the following LGM refugia scenario: that KA-SD, which consists almost entirely of single-locality haplotypes, refuged at Kaikoura and in the Marlborough Sounds; that NN-MB refuged in Tasman Bay, the closest refugium to its modern range; and that BR-CA, NW and SW refuged along the west coast in areas adjacent to their current distributions (BR-CA in Karamea Bight, NW in Buller and north Westland, SW in central and south Westland) and/or scattered inland refugia in Buller. Assigning the other southern lineages to either the west coast or Fiordland refugia is more difficult, given the uncertain relationships among O-S (Otago–Southland), MK-SC (Mackenzie–South Canterbury) and Canavans Knob and the vague ENM support for a Fiordland refugium. This is complicated by the fact that much of Fiordland is difficult to access, so locally endemic haplotypes close to the putative refugium may have been missed.

If the ancestors of O-S and MK-SC refuged in south Westland, they would probably have crossed the Southern Alps at Haast Pass, which is relatively low at present (570 m), is currently forested, and has been kept open by river capture events since before the Quaternary (Craw *et al.* 2008). The population at Robinson Creek, 11 km north of the pass along Haast River, shares haplotypes with both O-S and SW, suggesting that this was an important dispersal route for multiple lineages. The O-S haplotype from Robinson Creek is shared with Governors Bush, which groups with both MK-SC and O-S, and is two substitutions away from the widespread haplotype in O-S, which is closely related to most other haplotypes in that clade. West coast survival, followed by persistence *in situ*, would best explain the two divergent haplotypes at Canavans Knob, included in O-S in the ML and Bayesian trees and treated as a separate lineage in the Bayesian coalescent analysis. Even today, although southernmost Fiordland is predicted to contain suitable habitat (Fig. 5a), it is bounded to the north and west by areas where *A. labralis* is predicted to be absent, and to the south by ocean. This would limit opportunities for dispersal from a Fiordland refugium, which is projected to be very small, and with less certainty than those on the west coast (Fig. 5b, c).

However, if the southern clades refuged on the west coast, it is unlikely that they would have been extirpated so completely as to be detected at only one site, although we cannot rule out the possibility of more extensive sampling near the coast recovering O-S and MK-SC haplotypes. Within the widely distributed O-S clade, all single-locality haplotypes except those from Canavans Knob are in Southland and Stewart Island, and although these are within a few substitutions of each other, they are more distantly linked to Canavans Knob. Further, one widespread (Hap 02) and two unique haplotypes are present on Stewart Island, which the ENM predicts were uninhabited by *A. labralis* during the LGM. Rapid glacial retreat began *c.* 16 000 years ago (Suggate & Almond 2005), and Foveaux Strait reopened between 14–10 000 years ago (Cullen 1967), so migration from the west coast all the way to Stewart Island would have had to be rapid indeed. In contrast, populations within the Southern Alps, potential links to the west coast, are represented by a single widely distributed haplotype (Hap 08) also present further south. Without more extensive sampling in remote areas of Fiordland and the Southern Alps, however, we cannot definitively choose between these scenarios, and given the unique and divergent haplotypes at Canavans Knob, it is possible that the diversity in Southland today is the result of descendants from both refugia.

Origins of the modern distribution

How do our findings relate to previous South Island refugia hypotheses? The Nelson region has long been posited as an important refugium for temperate species based on plant distribution patterns (Wardle 1963; Burrows 1965), and this is supported here. However, both our AMOVA and ENM show that Nelson was not the

only refugium for *A. labralis*, highlighting that refugia in the northern South Island played little role in colonizing the far south, and that southern populations probably descended from ancestors closer to their present ranges. At the end of the Pleistocene, habitats in Kaikoura, Marlborough and the Marlborough Sounds may have remained fragmented due to dryer weather in the northeast and continuing tectonic activity near Kaikoura (Craw *et al.* 2008). This would have given forest species expanding east through the Southern Alps a head start over their northern relatives at filling newly habitable regions in the east and south.

Only two clades have distributions far beyond their hypothesized refugia: O-S, described above, and BR-CA, which crosses the Southern Alps in the Lewis Pass area and stretches down their eastern flank to the central South Island. Lyell Walkway shares one haplotype with all of the Canterbury populations and is one to three substitutions away from most Lewis Pass area haplotypes. Lyell Walkway's unique haplotype is more similar to the cluster of unique haplotypes from the Oparara Basin, although the ML tree placed it as sister to the Lewis Pass–Canterbury populations. This pattern is consistent with eastward movement through Lewis Pass or some of its neighbouring passes and expansion south along the east coast to central Canterbury.

This pattern of west–east migration is repeated, to a lesser extent, in NW clade. Each population from NW contains at least one unique haplotype, and in most cases two, except for the populations east of the main divide at Arthur's Pass, which share one common haplotype. While it is more difficult to speculate about haplotype relationships in this small clade, it contributes to an expansion pattern of west–east colonization. Ancestors for NW probably refuged along the Buller coastline south of Cape Foulwind, based on the clade's proximity to that part of the western coastal refugium, or in the scattered inland refugia projected for the Buller region by the ENM.

While west–east expansion of BR-CA, NW, and the southern lineages makes sense from a phylogeographic perspective, trans-alpine movement of temperate forest species has never been discussed as a major mechanism for postglacial colonization and the Southern Alps are generally considered an important phylogeographic barrier (e.g. Liggins *et al.* 2008; Boyer & Giribet 2009; O'Neill *et al.* 2009), although lowland species have made limited use of mountain passes (e.g. Apte *et al.* 2007; Craw *et al.* 2008). Phylogeography of *Kikihia subalpina* (Marshall *et al.* 2009) illustrates an 'expected' pattern of southern expansion from northern refugia, with the west coast related to populations from Nelson and the east coast related to populations from Kaikoura, with little intermixing and seemingly unaffected by drought or tectonic activity in the northeast. However, most phylogeographic studies of terrestrial species, with the exception of Hill *et al.* (2009), Leschen *et al.* (2008) and Marshall *et al.* (2009), have incorporated very few sampling sites from the west coast, and Leschen *et al.* (2008) did not sample widely on the east coast, making it difficult to compare patterns between species.

Consistent among these is a lack of evidence for temperate forest refugia in the southeast South Island. Early authors (Wardle 1963; Burrows 1965) suggested that Central Otago may have served as a major regional ice age refugium. So far this has not been demonstrated for temperate lowland species, although Otago is an important distribution centre for alpine and generalist species (Hill *et al.* 2009; O'Neill *et al.* 2009). This does not mean that forest was entirely absent from the southeast—Alloway *et al.* (2007) indicated that Southland, Stewart Island, and central Canterbury could have contained small isolated patches of forest—but a growing consensus of phylogeographic studies suggest that these microrefugia failed to leave a significant genetic record in modern forest species.

Forest likely expanded in all directions from several refugia simultaneously (McGlone 1985), with attendant species following along several routes, highlighting variability among species which appear to have very similar habitat requirements. Relative contributions of the different environmental variables to our ENM indicated that winter temperatures were less limiting for *A. labralis* than moisture availability, and species which are more temperature-sensitive (cicadas, Marshall *et al.* 2009; stick insects, Buckley *et al.* 2009) should be expected to have refuged in different areas. Without understanding how different members of the forest community responded to past climate change, it will be extremely difficult to predict how these ecosystems will react to future climate perturbations. Although there is growing evidence across multiple disciplines that temperate forest species survived the Pleistocene ice ages in Nelson, Marlborough and/or Kaikoura (Marra & Leschen 2004; Alloway *et al.* 2007; Boyer *et al.* 2007; Marshall *et al.* 2009; herein), more studies of widely distributed taxa are needed, with particular emphasis on sampling the oft-neglected west coast and remote south, to more fully comprehend the impacts of glacial cycling on New Zealand's biota.

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