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PHYLOGEOGRAPHY OF THE NEW ZEALAND CICADA *MAORICICADA CAMPBELLI* BASED ON MITOCHONDRIAL DNA SEQUENCES: ANCIENT CLADES ASSOCIATED WITH CENOZOIC ENVIRONMENTAL CHANGE

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Abstract.—New Zealand's isolation, its well-studied rapidly changing landscape, and its many examples of rampant speciation make it an excellent location for studying the process of genetic differentiation. Using 1520 base pairs of mitochondrial DNA from the cytochrome oxidase subunit I, ATPase subunits 6 and 8 and $tRNA^{Asp}$ genes, we detected two well-differentiated, parapatrically distributed clades within the widespread New Zealand cicada species *Maoricicada campbelli* that may prove to represent two species. The situation that we uncovered is unusual in that an ancient lineage with low genetic diversity is surrounded on three sides by two recently diverged lineages. Using a relaxed molecular clock model coupled with Bayesian statistics, we dated the earliest divergence within *M. campbelli* at 2.3 ± 0.55 million years. Our data suggest that geological and climatological events of the late Pliocene divided a once-widespread species into northern and southern components and that near the middle of the Pleistocene the northern lineage began moving south eventually reaching the southern clade. The southern clade seems to have moved northward to only a limited extent. We discovered five potential zones of secondary contact through mountain passes that will be examined in future work. We predict that, as in North American periodical cicadas, contact between these highly differentiated lineages will exist but will not involve gene flow.

Key words.—Biogeography, cicadas, insect mitochondrial DNA, *Maoricicada campbelli,* molecular clock, New Zealand, phylogeography.

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Biogeographic hypotheses make explicit predictions regarding the relationships among species or populations and the timing of divergences among these lineages. Therefore, an understanding of the effects of geological changes on biogeographic patterns requires the joint application of phylogenetic reconstruction and the molecular clock to multiple species, rather than distributional data alone (Wallis and Trewick, in press). Recent studies that coupled phylogenetic analysis with the molecular clock have been highly successful in testing alternative biogeographic hypotheses (e.g., Emerson et al. 2000; Ingman et al. 2000; Trewick et al. 2000; Nielsen et al. 2001).

Like many Pacific islands, New Zealand is an excellent place to study biogeographic and evolutionary processes because of its wide variety of habitats packed into a small geographic area and its dramatic and well-documented history of geological change (Cooper and Millener 1993; Newnham et al. 1999). New Zealand broke from Gondwanaland prior to the origin of many modern genera of plants and animals and reached its present location by the end of the Cretaceous approximately 65 million years ago (Cooper and Millener 1993). Since that time, New Zealand has been isolated from other large land areas by at least 1000 km of ocean. Because of this isolation, New Zealand is home to many endemic species, especially insects (Daugherty et al. 1993). Among the best studied of the insects from a natural history point of view are the New Zealand cicadas (Fleming 1971, 1975, 1984; Dugdale 1972; Dugdale and Fleming 1978). More than 40 species (five genera) are found, inhabiting a wide variety of habitats from mountain tops to sea coasts (Fleming 1975). The New Zealand cicada genus *Maoricicada* is unique among the world's cicada species in being almost entirely restricted to rocky mountain tops, cliff faces, or stream beds (Fleming 1971; Dugdale and Fleming 1978). Only two *Maoricicada* species are commonly found in trees or shrubs. The majority of the 14 species are found on the South Island and have restricted geographic ranges. The only widespread species in the genus is *Maoricicada campbelli.* It is found throughout the South Island from low elevation to subalpine regions and has a disjunct population on the central volcanic plateau of the North Island (Fleming 1971). Because of this broad geographic and altitudinal distribution, it is an excellent species for examining the effects of landscape changes on the genetic structure of populations.

The North Island is more volcanically but less tectonically active than the South Island and was separated from it only recently. The Pliocene period marked the beginning of climatic changes (2.5 million years ago) ushering in a cooler period with cold temperate forests on the North Island and glaciers and tundra covering much of the South Island. A Pliocene sea strait covered large areas of the southern North Island and disappeared as sea level fell during the Pleistocene. Following this marine transgression, the two main islands were connected by a broad low plain allowing the interchange of the two previously isolated biotas.

The South Island of New Zealand displays a number of interesting biogeographic patterns. In particular, many species have disjunct distributions between the north and the south of the South Island. This pattern has been explained in terms of Pliocene-Pleistocene glaciation (e.g., Wardle 1963; Burrows 1965), mountain building over the last 5 million years (McGlone 1985), and disjunction along the Alpine Fault during the last 25 million years (e.g, Heads 1998).

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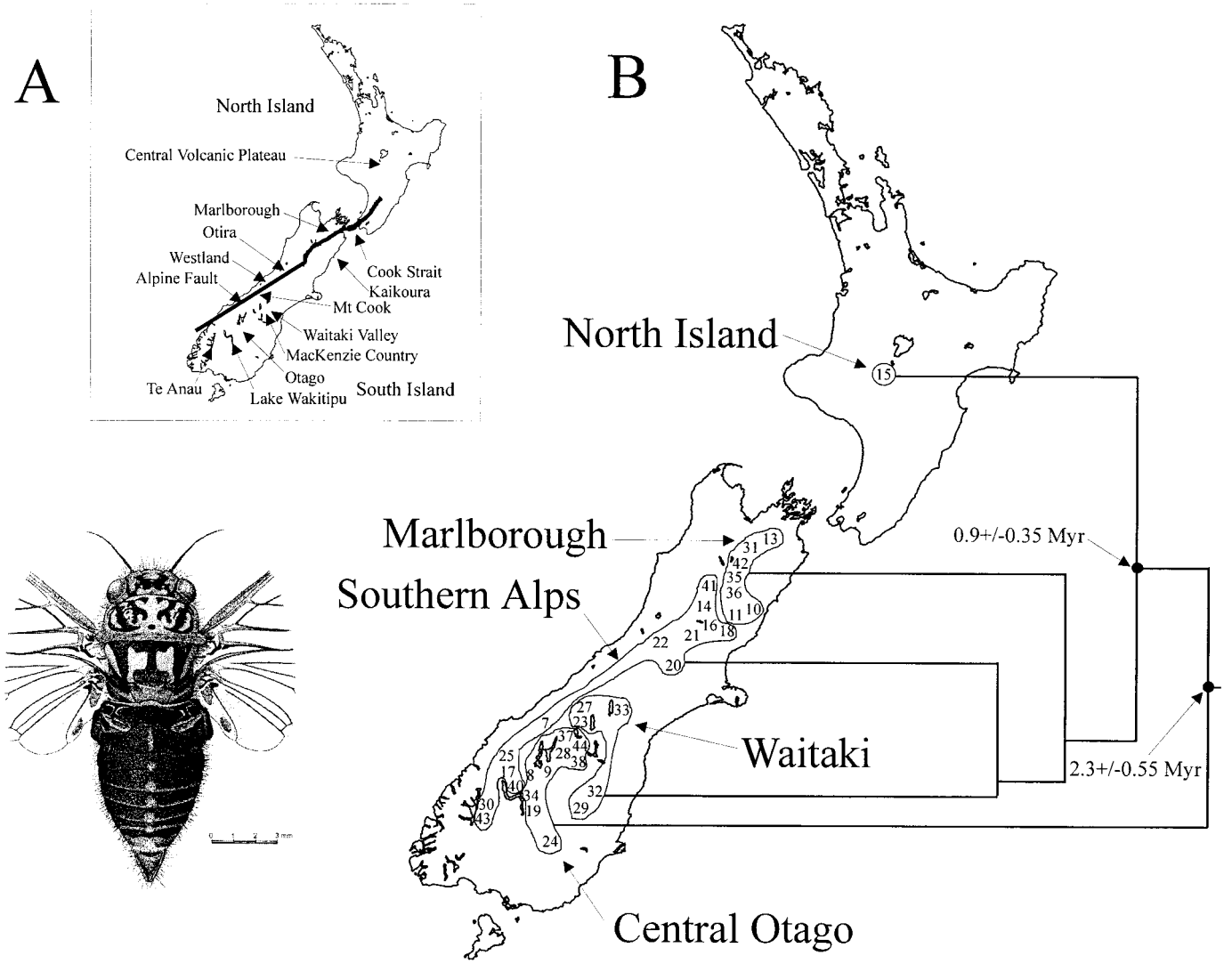

FIG. 1. (A) Map of New Zealand with place names mentioned in the text indicated. The Southern Alps, the main axial mountain range of the South Island, runs the length of the western side of the South Island. The Alpine Fault is marked as a bold line running the length of the South Island. (B) Map of New Zealand showing the locations of *Maoricicada campbelli* populations sampled. Numbered populations containing haplotypes belonging to each of the five major clades are circled and labeled. Further details regarding population localities are given in Table 1. The superimposed tree indicates phylogenetic relationships among the five clades. The estimated timing of divergences is given for two of the nodes. See text and Figure 4 for bootstrap support for each of the nodes. Inset is drawing of *M. campbelli* taken with permission from Fleming (1971).

These hypotheses can now be tested with phylogenetic analysis and molecular dating (Trewick et al. 2000; Waters and Wallis 2000; Wallis and Trewick in press). Molecular clocks are notoriously difficult to apply because of many well-recognized problems (reviewed in Rambaut and Bromham 1998), the most difficult of which is that different lineages evolve at different rates. As part of a larger study of two New Zealand cicada genera (C. Simon unpubl. data), we applied a relaxed-clock dating technique that avoids the assumption of rate constancy (Thorne et al. 1998). Our analysis suggests that the initial separation of the two major *M. campbelli* lineages took place less than 2.3 million years ago and was associated with Pliocene climate changes and geological activity. These analyses also suggest that the northern lineage began moving south less than 0.9 million years ago, during the Pleistocene. We present a biogeographic scenario for the well-structured *M. campbelli* gene tree that can be tested with future data.

The study organism, *M. campbelli,* is a small (13–17 mm in length) darkly colored cicada commonly found on dry riverbeds, exposed subalpine surfaces, and other eroded areas (see inset in Fig. 1). Originally described by Myers (1923), *M. campbelli* was synonymized with *Pauropsalta maorica* (Myers 1923) by Salmon (1950) and was later revised by Fleming (1971). Fleming's description was based on extensive collections of *M. campbelli* made throughout its range. He noted that geographic variation in coloration existed in *M. campbelli,* and that this variation was partially correlated with climate. For example, Fleming observed that specimens from the high rainfall areas of Westland were more brilliantly

colored than the specimens from parts of Otago, MacKenzie Country, and the Waitaki Valley that were covered in short silvery hairs. Fleming (1971) also noted that geographic variation existed in tymbal morphology and song structure. Although among-population differentiation was sometimes extreme, he considered the variation to be clinal and was reluctant to designate subspecies (as was later done by Dugdale and Fleming [1978] for other *Maoricicada* species as part of a complete revision of the genus).

Fleming (1971) went on to propose an evolutionary scenario for the origin and evolution of the species. Under his hypothesis the ancestor of *M. campbelli,* ''proto-*campbelli,*'' arose in an ice-free area of the South Island such as a coastal region in North Westland or Otago. Proto-*campbelli* eventually spread into habitats with porous and sandy soils, such gravel beds and river terraces. As such habitats became more common throughout the South Island, the fully modern *M. campbelli* was slowly able to obtain its contemporary widespread distribution. During the Pleistocene, *M. campbelli* spread across the emergent western area of Cook Strait and up the western side of the North Island into the mountains of the central volcanic plateau. The return of the forests to the North Island following the last glaciation, some 10,000 years ago, left relict populations on the North Island volcanoes. Here we demonstrate that contrary to Fleming's (1971) scenario of gradual spread from the south, the major genetic division between *M. campbelli* populations is between the southern most populations on the South Island and the northernmost populations. The central area of the South Island seems to have been devoid of *M. campbelli* populations during the Pliocene and early Pleistocene and has been recolonized from the north. The southern populations may be distinct enough to warrant specific status. We make several predictions about secondary contact between the most derived and the oldest lineage that will be testable with future data.

Materials and Methods

Molecular Techniques

Populations of *M. campbelli* were sampled over most of its known range because preliminary analyses suggested that mtDNA haplotypes were geographically structured rather than being randomly scattered with respect to geography (Table 1, Fig. 1B). Voucher individuals have been lodged at the School of Biological Sciences, Victoria University of Wellington. One individual from each sampled population was analyzed. Total genomic DNA was extracted from thoracic muscle or ovarian tissue following the salting-out method of Sunnucks and Hales (1996). Two target mtDNA gene regions were amplified from genomic DNA using the polymerase chain reaction (PCR). We amplified the 3′ end of the cytochrome oxidase subunit I (COI) gene using PCR primers C1-J-2195 and TL2-N-3014 (Simon et al. 1994) and a region of the mitochondrial genome encompassing part of $tRNA^{Lys}$, $tRNA^{Asp}$, and ATPase subunit 8 (A8) and most of ATPase subunit 6 (A6) using PCR primers designed from conserved *Maoricicada* mtDNA sequences: TK-J-3799 (GGCTGAAAGTAAGTAATGGTCTCT) and A6-N-4570 (AAGACTGAATTATACAAACGGCTA) named using the conventions set out for insect mtDNA primers in Simon et al. (1994). Thermal cycling conditions for amplification of either target were denaturation at 94°C for 45 sec, annealing at 56–57°C for 45 sec, and extension at 72°C for 75 sec for 30 cycles. Double stranded DNA products were purified by excision from a low-melting-point gel and cycle sequenced using the Perkin Elmer Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Inc., Foster City, California) following the manufacturer's instructions. Fluorescently labeled cycle sequencing products were analyzed on a Prism 377 DNA Sequencer (Applied Biosystems, Inc., Foster City, California). Target DNA sequences were aligned manually using ESEE 3.2 (Cabot and Beckenbach 1989). This task was trivial due to the lack of length variation among different haplotypes.

Phylogenetic Analyses

Phylogenetic analyses were performed using the maximum parsimony (MP; Fitch 1971) and maximum likelihood (ML; Felsenstein 1981) optimality criteria as implemented in PAUP* version 4.0b1 (Swofford 1998). All phylogenetic trees were rooted using the congeneric species *M. clamitans* and *M. phaeoptera* as outgroups. These two species were chosen as outgroups based on phylogenetic analyses of a wider range of *Maoricicada* species (Buckley et al. 2001a,b).

An initial uniformly weighted MP search yielded 48 equally parsimonious trees (see Results). Next, we comparatively evaluated the relative fits of a range of substitution models to one of the most parsimonious trees following methods described in Frati et al. (1997), Sullivan et al. (1997), and Buckley et al. (2001a). For the site-specific rates models (e.g., Swofford et al. 1996), we partitioned the data into 10 rate classes, three for each codon position for each of the three protein-coding genes and one for the $tRNA^{Asp}$ sites (referred to as the SSR_{10} model).

We used likelihood-ratio tests (e.g., Frati et al. 1997; Huelsenbeck and Crandall 1997) and χ^2-tests to quantitatively compare nested substitution models and to determine the most appropriate model for phylogenetic analysis. We constrained the most likely tree found under the HKY85 + SSR_{10} and HKY85 + I models to conform to a molecular clock. The likelihood of the tree under this model was compared to the likelihood of the unconstrained tree using likelihood-ratio and χ^2-tests to determine if the sequences are evolving in a clocklike manner (Felsenstein 1988). We used χ^2-tests, as implemented in PAUP* version 4.0b1 (Swofford 1998) to determine whether the assumption of base frequency stationarity (equal base frequencies among sequences) was violated on all sites and parsimony sites only. We implemented the *DT*-test of Tajima (1989) and the *D*- and *F*-tests of Fu and Li (1983), as implemented in DnaSP 3.0 (Rozas and Rozas 1999) to test the hypothesis that the sequences represent neutral markers.

Point estimates for substitution model parameters were calculated from the data and fixed in ML tree searches. Empirical base frequencies were used in all analyses. For the ML tree searches, we constructed an initial tree using stepwise addition followed by TBR branch swapping. Statistical support for internal nodes was estimated using the nonparametric

TABLE 1. Collecting locality, accession numbers, and haplotype designation of sampled *Maoricicada campbelli* individuals and outgroup species.

Collecting locality (latitude, longitude)	Population number	Haplotype designation/ species	GenBank accession numbers
Twelve-Mile Delta, Lake Wakatipu (45°03′S, 168°50′E)	34	S	AF248796, AF248845
Rastus Burn, The Remarkables, Queenstown (45°05′S, 168°81′E)	19	S	AF248793, AF248851
Symes Road, Old Man Range, Otago (45°40′S, 169°21′E)	24	T	AF248794, AF248852
Lake Ohau Ski Field Road, Lake Ohau, MacKenzie Country (44°22′S, 169°80′E)	37	V	AF248791, AF248849
Little Omarama River, Omarama, MacKenzie Country (44°60′S, 169°88′E)	38	U	AF248792, AF248850
Ahuriri River, MacKenzie Country (44°23′S, 169°61′E)	44	U	AF248788, AF248846
Lindis Pass, MacKenzie Country (44°59′S, 169°64′E)	28	U	AF247611, AF248798
Albert Town, Lake Wanaka (44°68′S, 169°19′E)	9	W	AF248789, AF248847
NW of Lake Wanaka (44°44′S, 169°21′E)	8	W	AF248790, AF248848
Whakapapa Ski Field, Mt. Ruapehu (39°25′S, 175°55′E)	15	R	AF247610, AF248797
Wandle River, North Canterbury (42°49′S, 173°13′E)	11	M	AF248785, AF248840
Oaro River, Kaikoura (42°46′S, 173°44′E)	10	L	AF248784, AF248839
No Catchem Stream, Rainbow-Wairau Divide, Marlborough (41°81′S, 172°91′E)	42	Q	AF248787, AF248844
Kowhai Scenic Reserve, Wairau River, Marlborough (41°60′S, 173°37′E)	31	O	AF248786, AF248842
Lake Sedgemere, Wairau-Rainbow Divide, Marlborough (42°13′S, 172°91′E)	36	P	AF247609, AF248843
Pine Valley Stream, Wairau River, Marlborough (41°49′S, 173°50′E)	13	N	AF248795, AF248841
Kye Burn, South of Danseys Pass (45°08′S, 170°25′E)	29	K	AF248783, AF248838
Awakino River, St. Marys Range (44°75′S, 170°40′E)	32	J	AF248782, AF248837
Birch Stream, Mt. Cook National Park (43°74′S, 170°07′E)	27	I	AF248780, AF248835
Mount Cook Road, Mt. Cook National Park (43°73′S, 170°10′E)	23	I	AF248779, AF248834
Boundary Creek, Lake Tekapo (43°15′S, 171°18′E)	33	I	AF248781, AF248836
Dart Valley, Barrier Range, Otago (44°76′S, 168°34′E)	25	G	AF248774, AF248829
South of Airstrip, Lake Wakatipu (45°21′S, 168°72′E)	40	G	AF248775, AF248830
Invincible Mine, Rees River, Lake Wakatipu (44°72′S, 168°47′E)	17	G	AF248776, AF248831
Lake Tennyson, North of Hanmer Springs, Marlborough (42°19′S, 172°70′E)	41	C	AF248770, AF248825
Hurunui River, South Branch, North Canterbury (42°77′S, 172°18′E)	21	A	AF248766, AF248821
Serpentine Creek, Rainbow-Wairau Divide, Marlborough (42°19′S, 172°77′E)	35	A	AF248767, AF248822
East of Glynn Wye, Waiau River (42°59′S, 172°40′E)	18	A	AF248769, AF248824
West of Glynn Wye, Waiau River (42°59′S, 172°50′E)	16	A	AF248765, AF248820
St. James Walkway, Lewis Pass, North Canterbury (42°45′S, 172°40′E)	14	B	AF248768, AF248823
Arthurs Pass, Arthurs Pass National Park (42°90′S, 171°54′E)	22	H	AF248778, AF248833
Peg Leg Creek, Porters Pass (43°29′S, 171°73′E)	20	H	AF248777, AF248832
Haast Pass, Mt. Aspiring National Park (43°88′S, 169°03′E)	7	F	AF248773, AF248828
Henrys Creek, Eglinton Valley, Fiordland (42°29′S, 171°55′E)	30	D	AF248771, AF248826
Boundary Creek, Eglinton Valley, Fiordland (43°68′S, 169°43′E)	43	E	AF248772, AF248827
Lake Ohau Ski Field, South Island (44°23′S, 169°85′E)	—	*M. clamitans*	AF247617, AF248804
Lake Ohau Ski Field, South Island (44°23′S, 169°85′E)	—	*M. phaeoptera*	AF247615, AF248802

bootstrap (Felsenstein 1985). For the MP analyses, we used 500 pseudoreplicates. For the ML analysis, we used 200 pseudoreplicates.

Dating of Divergences

To date the origin of haplotypic diversity within *M. campbelli*, we applied the relaxed-clock method of Thorne et al. (1998) that allows evolutionary rates to vary across a tree. This model assumes that rates are autocorrelated and that these rates can change at any internode with the rates of the descendant lineages drawn from a log-normal distribution. We estimated the dates of the divergence of the three haplotypes from Central Otago (haplotype U, see below), Mount Ruapehu (haplotype R), and Marlborough (haplotype P) in addition to a range of other *Maoricicada* species (Buckley et al. 2001a,b; C. Simon unpubl. data). The method of Thorne et al. (1998) requires an internal calibration point; therefore, we used the dates of 4.9–5.1 million years for the radiation of the alpine *Maoricicada* species (C. Simon unpubl. data). This calibration value was used because the New Zealand alpine habitat is only 5 million years old (Batt et al. 2000; Chamberlain and Poage 2000), providing an upper limit on the origin of the alpine taxa. The software described in Thorne et al. (1998) uses the HKY85 + γ; (Hasegawa et al. 1985; Yang 1994) model to estimate branch lengths.

RESULTS

Patterns of Nucleotide and Protein Evolution

We aligned 1520 homologous sites for each of the 35 individuals sequenced. Excluding the outgroups, 23 unique *M. campbelli* haplotypes were identified (Table 1, Fig. 1B). The final dataset used in the phylogenetic analysis contained 753 bp from the 3′ end of the COI gene, the complete $tRNA^{Asp}$ (63 bp) and A8 (156 bp) genes, and 546 bp from the 5′ end of the A6 gene. As in other insect mitochondrial genomes (e.g., Flook et al. 1995), the A8 and A6 genes overlap by 7 bp. These overlapping sites have been included in the A8 data partition; however, all are unvaried in the sequences presented here.

A total of 121 varied nucleotide sites were observed, 83 of these being informative under the MP optimality criterion (Fig. 2). A total of 26 varied amino acid sites were identified, 13 of these being informative under the MP optimality cri-

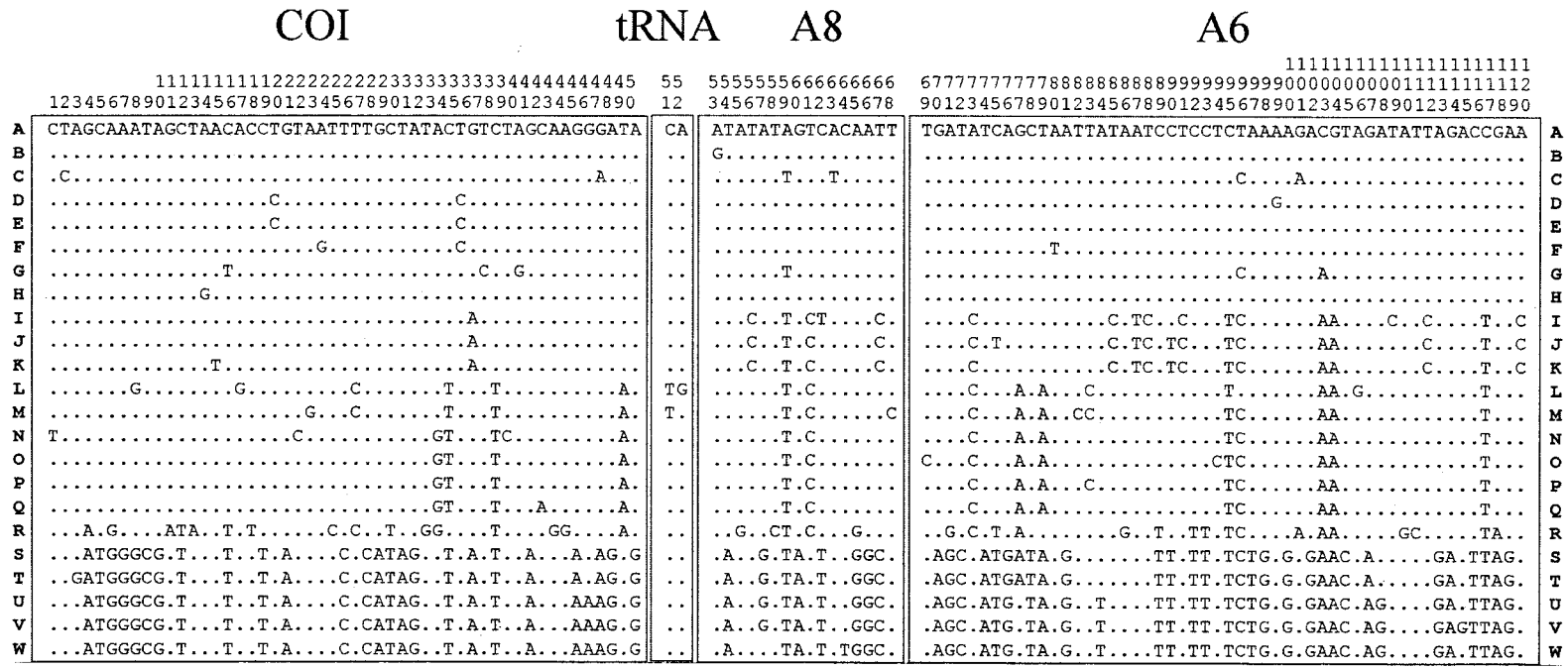

FIG. 2. Polymorphic nucleotide sites among *Maoricicada campbelli* haplotypes. The most common haplotype, A, is used as a reference sequence. Haplotype designations are given along the left and right edges. Identical character states are indicated by dots.

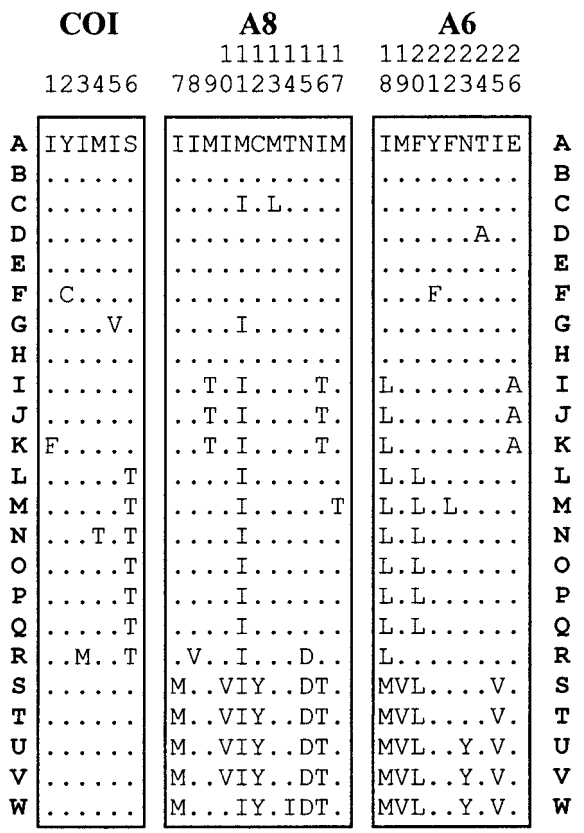

FIG. 3. Variable amino acid sites among *Maoricicada campbelli* haplotypes. Haplotype A is used as the reference sequence. Identical character states are indicated by dots.

terion (Fig. 3). The A8 gene has the greatest proportion of varied amino acid residues, followed by the A6 gene, and finally by the COI gene (Table 2, Fig. 3), consistent with other studies of insect mtDNA (see table 1 from Simon et al. 1994).

The lack of ambiguous sites in electropherograms, the absence of stop codons, and atypical nucleotide deletions suggest that the sequences obtained are of genuine mitochondrial origin (see Zhang and Hewitt 1996). The observed patterns of nucleotide and amino acid substitutions (see Table 2) are in agreement with this interpretation. The nucleotide composition of the sequences was highly rich in A and T (A = 33%, T = 43%, C = 12%, G = 12%) as is typical of insect mtDNA (Simon et al. 1994). Using χ^2-tests, we were unable to reject the hypothesis of base frequency stationarity (homogeneity) among sequences for all sites ($\chi^2_{72} = 5.980$, $P > 0.05$) and for parsimony sites only ($\chi^2_{25} = 85.320$, $P > 0.05$).

The likelihood-ratio tests indicate that the HKY85 + I (Hasegawa et al. 1985) and HKY85 + SSR_{10} models are the best-fit models for this dataset. Using both Tajima's (1989) and Fu and Li's (1993) tests, we were unable to reject the hypothesis that the sequences are evolving under neutral expectations. For Tajima's (1989) method, the test statistic D_T was not significantly positive ($D_T = 0.57041$, $P > 0.10$). Fu and Li's (1993) *D*-statistic was not significantly negative ($D = -0.18103$, $P > 0.10$) and the *F*-statistic was not significantly positive ($F = 0.09427$, $P > 0.10$).

TABLE 2. Summary of mitochondrial DNA variation within *Maoricicada campbelli*. Relative substitution rates are estimated under the HKY85 + SSR_{10} model and include the outgroup species. MP, maximum parsimony.

	No. of sites	No. of variable sites	% of sites variable	No. of MP sites	% of sites MP	Relative rate
$tRNA^{Asp}$	64	2	3.1	1	1.6	0.3711
COI 1st	251	4	1.6	2	0.8	0.1758
COI 2nd	251	2	0.8	0	0	0.0363
COI 3rd	251	45	17.9	29	11.6	2.4017
COI all	753	51	6.8	31	4.1	—
A8 1st	52	5	9.6	3	5.8	1.4170
A8 2nd	52	5	9.6	3	5.8	0.6570
A8 3rd	52	6	11.5	4	7.7	1.4397
A8 all	156	16	10.3	10	6.4	—
A6 1st	182	11	6.0	9	5.0	0.5832
A6 2nd	183	2	1.1	1	0.6	0.0527
A6 3rd	182	39	21.4	31	17.0	2.9764
A6 all	547	52	9.5	41	7.5	—
Total	1520	121	8.0	83	5.5	—

Geographical Distribution of Haplotypes and Among-Population Genetic Divergence

The heuristic MP tree search recovered 48 equally parsimonious topologies with a tree length of 212 steps (not shown). The bootstrap consensus tree from the MP analysis is presented in Figure 4A. The ML tree under the HKY85 + SSR_{10} model (Fig. 4B) was identical to one of the 48 most parsimonious trees. The ML tree under the HKY85 + I model differed from this only in the placement of the North Island haplotype (R), which was sister group to the Mount Cook population and the Waitaki and Southern Alps subclades (see below). All genetic distances were estimated using ML under the HKY85 + I substitution model.

Overall, *M. campbelli* displays relatively high levels of mtDNA variation among populations. From the phylogenetic analyses, we are able to identify two major clades, the Central Otago clade and the Northern clade. The Northern clade can be further subdivided into four subclades. We have labeled these subclades as North Island, Marlborough, Waitaki, and Southern Alps (Fig. 1B). Table 1 identifies the grouping of numbered localities into lettered haplotypes.

The most obvious pattern to emerge from the phylogenetic analysis is the clear break between populations from the Central Otago clade and the remaining four subclades. The clade of haplotypes from Central Otago (S, T, U, V, and W) is supported by a bootstrap value of 100% from both optimality criteria (Fig. 4). Corrected genetic distances between the Central Otago clade and all other *M. campbelli* haplotypes range from 0.051 to 0.065. Within Central Otago inferred phylogenetic relationships between haplotypes vary slightly among optimality criteria. However, all methods support a grouping of haplotypes U, V, and W (83–95% bootstrap support). These three haplotypes are all found in the northern area of Central Otago (Fig. 1B). The remaining two haplotypes are found around the Queenstown area (S) and the Old Man Range (T). Evidence for a sister group relationship between haplotypes S and T is weak, with only 46% bootstrap support in the MP analysis and no support under ML with the HKY85 + I model. On the shores of Lake Wakatipu, the sampled

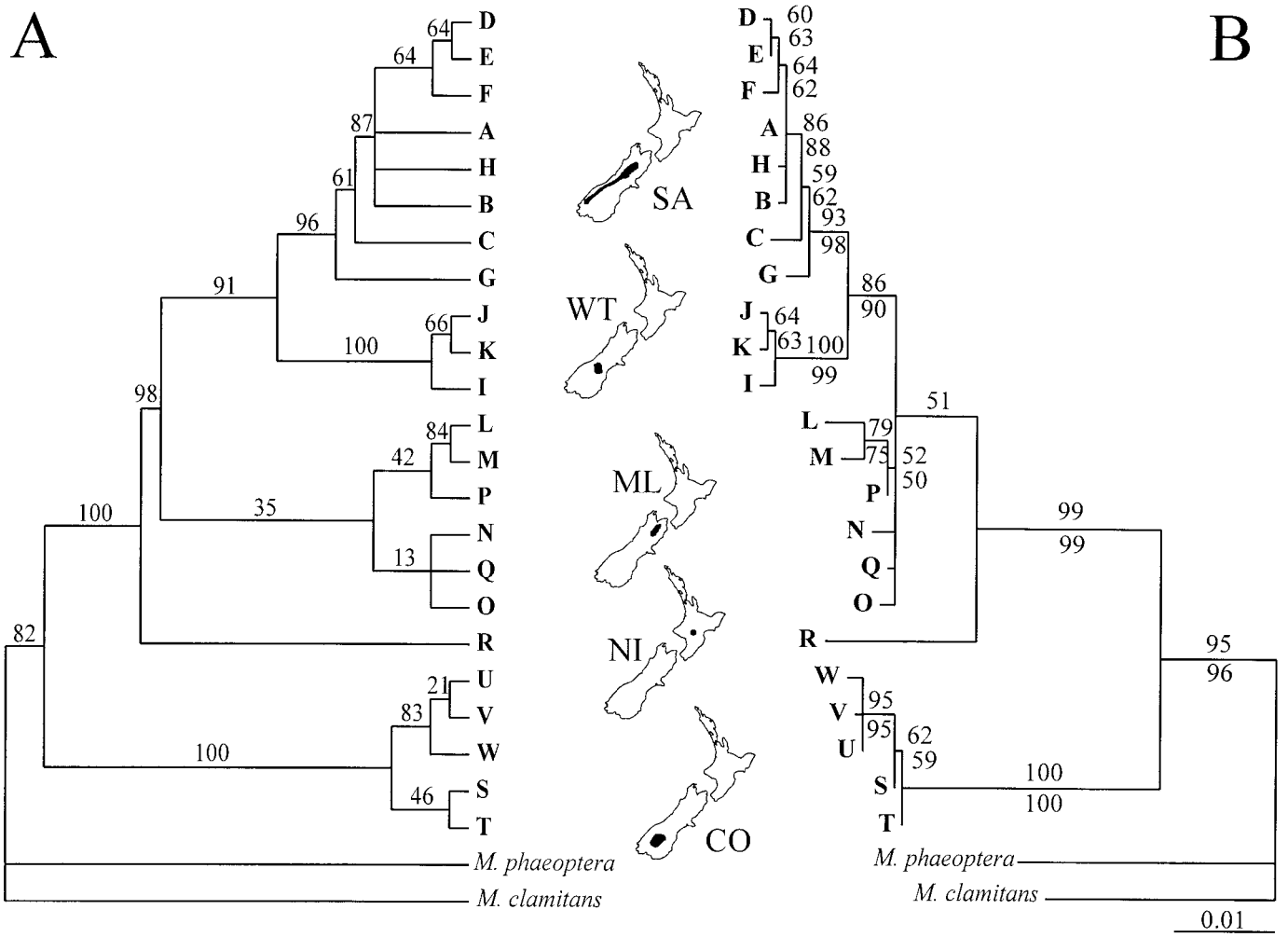

FIG. 4. (A) Majority rule bootstrap consensus maximum parsimony tree from 500 pseudoreplicates. Branch lengths are not drawn in proportion to the amount of evolutionary change. Maps of New Zealand with shaded areas indicate the approximate geographic distribution of monophyletic clades of haplotypes. (B) Topology and branch lengths are optimized under the HKY85 + SSR_{10} model. Numbers above nodes represent bootstrap values estimated (200 pseudoreplicates) under the HKY85 + SSR_{10} model. Numbers below nodes are bootstrap estimates from the HKY85 + I model. The scale lines indicate the number of substitutions per site estimated by maximum likelihood under the HKY + SSR_{10} model. The five major clades are indicated in both figures as follows: Southern Alps, SA (A, B, C, D, E, F, G, and H); Waitaki, WT (I, J, and K); Marlborough, ML (L, M, N, O, P, and Q); North Island, NI (R); Central Otago, CO (S, T, U, V, and W).

populations of the Central Otago clade and the Southern Alps subclade are separated by only 14 km (Fig. 1B). Despite this narrow geographical separation between the Twelve-Mile Delta (haplotype S) and the Lake Wakatipu Airstrip populations (haplotype G), a surprisingly large genetic distance of 0.054 differentiates the two haplotypes. Among the Central Otago haplotypes, corrected genetic distances range from 0.0048 to 0.0007.

The single North Island haplotype (R) is well differentiated from the Central Otago clade (0.063–0.056) and Marlborough, Waitaki, and Southern Alps subclades (0.033–0.022). However, support for the split between the North Island and the Southern Alps, Waiktaki, and Marlborough haplotypes is weak in the ML bootstrap analysis under the HKY + SSR_{10} (51%) and HKY85 + I (< 50%) models (Fig. 4A).

Corrected genetic distances among all haplotypes from the Marlborough, Waitaki, and Southern Alps subclades range from 0.0176 to 0.0007. The six haplotypes, from the Marlborough region of the South Island (L, M, N, O, P, and Q) form a distinct but internally poorly resolved radiation with the Waitaki and Southern Alps haplotypes as sister group. Although both ML and MP analyses recover the Marlborough haplotypes as a monophyletic assemblage, bootstrap support is very low (< 50%). The only well-supported grouping within this radiation is between haplotypes L (population 10, Oaro River) and M (population 11, Wandle River). Both of these localities in the Kaikoura region are close to the northeast coast of the South Island (Fig. 1B). Corrected genetic distances within the Marlborough clade range from 0.013 to 0.082.

The Waitaki subclade (I, J, and K) is well supported in the bootstrap analyses (Fig. 4). These haplotypes were all sampled in the central to southern region of the South Island, east of the Southern Alps (Fig. 1B). Three localities, two in

Mount Cook National Park (populations 23 and 27) and the other near Lake Tekapo (population 33), share the same haplotype (I). Haplotypes J (population 32, Awakino River) and K (population 29, Kye Burn Stream), which are also geographically proximate to each other, form a clade with moderate bootstrap support (Fig. 4).

Haplotypes belonging to the Southern Alps subclade (A, B, C, D, E, F, G, and H) were sampled from the central and southwestern regions of the South Island. Haplotype G, from three populations near the head of Lake Wakatipu, is basal to the remaining haplotypes in this clade, with moderate bootstrap support (59–62%). The three southwestern haplotypes, D, E, F, are recovered as a monophyletic group (Fig. 4). Haplotypes D and E, found near Lake Te Anau, form a clade with moderate support (60–64%) to the exclusion of haplotype F, which is found further north near Haast Pass.

Dates of Divergence

Using the likelihood-ratio test of a molecular clock, we are forced to reject the null hypothesis that the sequences are evolving under a clocklike process. The results of this test are the same for both the HKY85 + SSR_{10} ($\chi^2_{23} = 43.844$, $P = 0.0055$) and HKY85 + I ($\chi^2_{23} = 40.104$, $P = 0.015$) models. Because the likelihood-ratio clock tests indicated that the rate of evolution is variable across the phylogeny, we implemented the relaxed-clock model of Thorne et al. (1998) to obtain estimates of dates for the divergences of the major splits within *M. campbelli.* We have estimated the date of divergence of the Central Otago clade from the Northern clade at 2.3 ± 0.55 million years. The date for the divergence of the North Island clade from the Waitaki, Marlborough, and Southern Alps clades was estimated to be 0.9 ± 0.35 million years. The time of divergence of *M. campbelli* from the outgoup species was estimated at 3.50 ± 0.52 million years.

Discussion

Genetic Variation within Maoricicada campbelli

Our analyses of mtDNA sequences show relatively high levels of genetic variation within *M. campbelli.* The maximum divergence between the two major clades is 0.0398 for the COI gene and 0.054 for the COI + A6 + A8 + $tRNA^{Asp}$ genes. Langor and Sperling (1997) have summarized levels of inter- and intraspecific variation for the 3′ region of the COI gene in insect species. This taxonomic sampling included one coleopteran, one dipteran, three lepidopteran, and one hemipteran species. Levels of uncorrected intraspecific sequence divergence within each species never exceeded 0.023 substitutions per site. Other insect taxa also show similar levels of intraspecific divergence calculated from other mitochondrial gene regions including *Apis mellifera* subspecies (Arias and Sheppard 1996) and the grasshopper *Chorthippus parallelus* (Syzmura et al. 1996). Still other insect species display higher levels of intraspecific differentiation. For example, the deepest divergence within the lepidopteran *Heliconius erato* for the 3′ region of the COI gene, the 5′ region of the COII gene, and intervening $tRNA^{Leu}$ gene was 0.034 (uncorrected, Brower 1994). Brown et al. (1994) observed that intraspecific divergences among haplotypes within another lepidopteran genus, *Greya,* ranged from 0.01 to 0.057. The value of 0.057 within *G. obscura* exceeded the maximum estimated sequence divergence among three other closely related *Greya* species. Landry et al. (1999) observed uncorrected divergences as high as 0.038 within the *Argyrotaenia franciscana, A. citrana,* and *A. insulana* (Lepidoptera: Tortricidae) species group. Although this level of variation is relatively high for a single species, Landry et al. (1999) concluded that the observed variation could be accommodated within a highly variable species due to the lack monophyly of mtDNA haplotypes among the three species.

Other studies have shown that even higher levels of intraspecific genetic variation can exist in some insect species. For example, Juan et al. (1998) observed a maximum genetic distance of 0.1385 among COI haplotypes from the Canary Island darkling beetle, *Hegeter politus,* and hypothesized that this unusually high value may reflect cryptic species. In Collembola, Frati et al. (1997) recorded the highest levels of intraspecific mtDNA sequence variation observed in any insect species. In their study, uncorrected sequence divergence values for the *Isotomurus palustris* COII gene reached a maximum of 0.188 between two populations. However, Frati et al. (1997) interpreted this to indicate the presence of cryptic species with *I. palustris,* in accord with previous allozyme studies (Frati et al. 1995). Model-corrected genetic distances were even higher, but cannot be compared to other studies that did not correct their sequences in this way. Such corrections are advisable because they accommodate known biases in DNA sequence evolution and produce more realistic estimates of genetic distance (Yang 1996; Buckley et al. 2001a).

Of course, there is no magic value of genetic distance above which species status can be postulated. However, three lines of evidence suggest to us that the Northern and Central Otago clades may prove to be cryptic species. First, the level of sequence divergence between the Central Otago clade and other *M. campbelli* haplotypes exceeds that between other *Maoricicada* species (Buckley et al. 2001b) that are well established on morphological and behavioral criteria (Dugdale and Fleming 1978). Second, our phylogenetic analyses, in fact, recognize two distinct evolutionary lineages, one that arose in the north and another that arose in the south. Third, a strong phylogeographic boundary is evident between the two lineages.

Phylogeographic Patterning

The hypothesis that the northern and southern lineages of *M. campbelli* represent distinct species is reinforced by the strong geographic population structuring (Figs. 1B, 4) and the observation of a distinct mtDNA break over a very narrow geographical distance in the Lake Wakatipu area. A genetic break of this magnitude, over such a small geographic range, conforms to the type II pattern of genetic divergence described by Avise et al. (1987). The type II pattern is characterized by phylogeographically discontinuous variation with a lack of spatial separation. Such large mtDNA breaks over short distances can be interpreted as evidence for either sympatric divergence or historical population fragmentation

followed by secondary contact (Avise et al. 1987; Kidd and Friesen 1998; Simon et al. 2000). This type II pattern can also be interpreted as evidence for a barrier to gene flow and is perhaps indicative of a species boundary. A very similar pattern was observed in North American periodical cicadas (Martin and Simon 1988, 1990; Simon et al. 2000), where two mitochondrial lineages of the *-decim* species complex differed in genetic distance by an estimated 0.026 average substitutions per site based on restriction digests of the entire mitochondrial genome. Evidence from nuclear-encoded traits (allozyme, abdominal color, and song) strongly suggests that these two lineages diverged in allopatry (and allochrony) and came into secondary contact via life-cycle switching. In the zone of contact, strong reproductive character displacement (Marshall and Cooley 2000) and lack of intermediate genotypes (Cooley et al. 2001) suggests that reproductive isolation may be complete.

Contemporary patterns of mtDNA variation may not faithfully reflect population history. Alternative explanations for the observed distribution of *M. campbelli* mtDNA haplotypes (both within the trees obtained and geographically) include departures from neutrality of mtDNA alleles (Ballard and Kreitman 1995), differential dispersal of mtDNA and nuclear alleles (Baker et al. 1993), retention of ancestral polymorphisms (Niegel and Avise 1986), hybridization (Besansky et al. 1994), and errors in phylogenetic accuracy (e.g., Swofford et al. 1996). We have attempted to reduce the possibility of errors in phylogenetic reconstruction by using realistic models of nucleotide substitution and optimality criteria and by comparing results of MP and ML analyses to check for artifacts due to long branch attraction. In addition, the relatively low levels of corrected sequence divergence in this dataset (0.08 substitutions per site for all taxa) and the high degree of geographic structure in the data suggests that homoplasy has not yet reached levels that would be likely to severely distort inferred phylogenetic relationships.

Other factors that may confound the phylogeographic analysis of population history also seem to be unlikely explanations for our data. It is possible that the retention of ancestral polymorphisms may have misled the population-level interpretations of the mtDNA variation presented here (Neigel and Avise 1986; Pamilo and Nei 1988; Avise 1994). Although in most instances mtDNA (because of its faster coalescence rate) is less likely than nuclear DNA to experience lineage sorting (Moore 1995, 1997), there are conditions that can slow the loss of ancestral polymorphisms. Hoelzer (1997) pointed out that gender-biased dispersal and extremely polygynous breeding systems can increase the coalescence time of mtDNA to levels similar to that of nuclear DNA. Although we cannot formally discount the possibility of gender-biased dispersal or polygyny in *M. campbelli*, dispersal is a rare phenomenon in cicadas generally (Itô and Nagamine 1981; Williams and Simon 1995; deBoer and Duffels 1996). In the few instances when it does occur, it tends to involve female rather than male dispersal (Williams and Simon 1995). If female-biased dispersal were occurring in *M. campbelli*, then we would expect homogenization of mtDNA variation, rather than geographic structuring, as is our actual observation.

Hybridization is common in insects (Roderick 1996). If hybridization was occurring between *M. campbelli* and other *Maoricicada* species, we would expect that some of the mtDNA haplotypes of *M. campbelli* would be identical or very similar to that of another *Maoricicada* species. In a phylogenetic analysis including all known *Maoricicada* species, we consistently observe that the *M. campbelli* haplotypes are all well differentiated (0.053–0.136 GTR + I + Γ–corrected number of substitutions per site) from that of all known *Maoricicada* species (Buckley et al. 2001b). Therefore, we believe that introgression of mtDNA from some other *Maoricicada* species is most unlikely to have produced the patterns observed here.

Finally, it is also possible that some form of natural selection is responsible for maintaining the high levels of observed mtDNA variation in *M. campbelli.* We do not believe that selection is capable of maintaining such high levels of mtDNA variation over such a small geographic range (i.e., in the Lake Wakatipu region). Neither Tajima's (1989) nor Fu and Li's (1993) tests are able to reject the hypothesis that the observed mtDNA substitutions are selectively neutral, supporting our contention. We also note that acceptance of the null hypothesis—that the sequences are evolving in a neutral fashion—make surprising the rejection of the molecular clock hypothesis. This apparent disparity may in fact reflect a lack of power, sensitivity, or violation of one or more of the assumptions inherent in the neutrality tests (see Wayne and Simonsen 1998). For example, Tajima's (1989) test is based on the infinite sites model of molecular evolution, and thus the test statistic may be invalidated by the presence of among-site rate variation in the sequences (Bertorelle and Slatkin 1995; Aris-Brosou and Excoffier 1996; Yang 1996) that characterizes our data.

We suggest that the most likely explanation for the deep phylogeographic split between the Central Otago clade and the North Island, Marlborough, Waitaki, and Southern Alps clades is an ancient cladogenetic event (Avise 1994). The type locality for *M. campbelli* is at Otira in Westland (Fig. 1A). Although we were unable to sample from this area, it does not lie within the Central Otago region. The geographically closest population is from Arthurs Pass (population 22, haplotype H), only 8 km from Otira. Thus, if taxonomic changes are required, the Central Otago haplotypes may have to be placed into a new species. Future research into geographic variation in song structure and morphology will be particularly informative with respect to identifying correct taxonomic boundaries within *M. campbelli.*

Testing Biogeographic Hypotheses Using Dates of Divergence

Regardless of the taxonomic status of the Central Otago clade, the occurrence of a deep genetic divergence within *M. campbelli* requires a historical explanation. The Central Otago clade is almost completely surrounded by populations of the Northern clade containing haplotypes from the Southern Alps and Waitaki subclades. Within the Northern clade, a phylogeographic split occurs between the North Island haplotype and the Waitaki, Southern Alps, and Marlborough clades, with the later three subclades occurring progressively to the south. Thus, it appears the Southern Alps, Marlbor-

ough, and Waitaki clades have migrated down from the north of the South Island and surrounded the Central Otago clade (Fig. 1B). The Central Otago region is generally well known for its high proportion of endemic taxa (Climo 1975; Craw 1988) and genetically divergent lineages (e.g., Trewick et al. 2000). Therefore, the occurrence of highly divergent haplotypes in *M. campbelli* and possibly a unique species in the Central Otago region is congruent with the distributions of other taxa.

The observation that many New Zealand taxa share congruent geographic distributions led some authors (e.g., Wardle 1963; Burrows 1965; McGlone 1985; Heads 1998) to propose common historical explanations. Wardle (1963) and Burrows (1965) argued that glacial activity and temperature fluctuations over the last 2.6 million years (Newnham et al. 1999) have been responsible for disjunctions in the distributions of species and other higher taxa. Conversely, Heads (1998) emphasized the role of tectonic activity, and in particular rifting of the crust along the Alpine Fault of the South Island (Fig. 1A). The Alpine Fault began its most active period in the latest Oligocene or early Miocene (Smale 1991; King 1998; Sutherland 1999), approximately 25–20 million years ago, causing 480 km of lateral displacement of the ancestral South Island landmass. The most recent geological analyses suggest that this displacement has increased over time (Sutherland 1999). As late as 10 million years ago, the northwestern region of the South Island lay adjacent to Otago (King 1998). In addition, McGlone (1985) has discussed the role of rapid environmental change caused by recent mountain building. McGlone (1985) hypothesized that the rising Southern Alps drastically altered the environment in the center of the South Island. This hypothesis is supported by pollen cores of Cenozoic floras from Central Otago (Mildenhall 1999). Altered environments in the central South Island could have caused the splitting of once continuously distributed taxa into vicariant northern and southern populations. Evaluation of these biogeographic hypotheses will be contingent on obtaining reliable phylogenetic relationships among species and populations and in dating these divergences (Wallis and Trewick in press).

We dated the divergence of the Central Otago clade from the remaining *M. campbelli* haplotypes at 2.3 ± 0.55 million years. We note that this date is likely to be an overestimate of the time of population divergence because of haplotype diversity within the ancestral *M. campbelli* population (Nei and Li 1979; Edwards 1997). The confidence limits on the date for the earliest split within *M. campbelli* encompass the onset of late Pliocene temperature fluctuations and associated glaciations and the recent acceleration in the rate of uplift of the Southern Alps. Our data are consistent with a widespread, ancestral population that was subdivided by environmental instability caused by uplift of the Southern Alps that began to accelerate some 5 million years ago and early glacial activity that began approximately 2.6–2.4 million years ago (Newnham et al. 1999). Mildenhall (1999) reports that at this time, the vegetation in the Mackenzie Basin (the potential area of secondary contact between the northern and southern *M. campbelli* lineages) underwent a dramatic shift from cool temperate beech-dominated forest to grass and shrublands now characteristic of alpine areas. We suggest that disturbance caused by early glaciation and/or mountain building was the barrier between the northern clade (at this time possibly located in northwest Nelson) and the southern clade. We cannot reconcile the earliest date of divergence within *M. campbelli* with rifting along the Alpine Fault, a process that began to accelerate 10 million years ago.

Regardless of the cause of the basal divergence within *M. campbelli,* our data do suggest that the various populations have recently undergone range expansion and have migrated into regions where they now form potential zones of secondary contact. For example, in the Lake Wakatipu area we observed a situation where two of the most divergent halpotypes (S and G) are in close proximity, similar to the situation seen in ring species (e.g., Moritz et al. 1992; Jackman and Wake 1994; Wake 1997; Irwin et al. 2001), where highly derived lineages come into contact. Although the northern invaders appear to have traveled the length of the South Island, expansion from the Otago population has been more limited (Fig. 1B). It remains to be seen which populations are reproductively isolated. Other potential contact zones are located in the Waitaki Valley east of Omarama, south of Lake Pukaki near Twizel, and along the Hopkins and Dobson Rivers north of Lake Ohau.

The second oldest split within *M. campbelli* is between the disjunct population in the North Island and the remaining South Island haplotypes. We have estimated the date for this divergence to be 0.9 ± 0.35 million years ago. The pattern and timing of this divergence is not completely consistent with the predictions of Fleming (1971), who hypothesized that the North Island populations were derived from the very recent (≈20,000 years ago) migration of ancestors from the north of the South Island. Our results indicate that the separation of the North Island population from the Waitaki, Marlborough, and Southern Alps clades predates the divergence of the later three South Island clades. Additionally, the estimated date of 0.9 ± 0.35 million years ago indicates a more ancient Pleistocene origin for the North Island populations than predicted by Fleming (1971).

Although geographic variation exists among the haplotypes from the Marlborough, Waitaki, and Southern Alps subclades, this variation (<0.012 uncorrected for the COI gene) is easily accommodated within a single species. Such fine-grained intraspecific structure is likely to be of recent origin. Pleistocene-associated glacial activity is certainly likely to have had a dramatic effect on species structure and the geographical distribution of mtDNA haplotypes within Central Otago, Waitaki, and Southern Alps clades. More intensive within-population sampling is required to further investigate the magnitude and significance of this variation.

Conclusion

We obtained robust estimates of phylogenetic relationships among sampled *M. campbelli* haplotypes and have dated the timing of divergences among the major lineages. These phylogenetic relationships and dates of divergence revealed previously unsuspected patterns of population differentiation and allowed us to critically evaluate several biogeographic hypotheses (e.g., Wardle 1963; Burrows 1965; McGlone 1985; Heads 1998). Further studies on other widespread spe-

cies will facilitate a greater understanding of the role of past environmental change on phylogeographic patterns of New Zealand organisms.

The question of the species status of the two oldest *M. campbelli* lineages awaits further study. Certainly there are two distinct evolutionary lineages, but the questions of whether these two lineages are currently exchanging genes and if so, to what extent, remains unanswered. Genetic and behavioral studies of populations at contact zones and beyond (in progress) will bear directly on the species question.

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Literature Cited

Arias, M. C., and W. S. Sheppard. 1996. Molecular phylogenetics of honey bee subspecies (*Apis mellifera* L.) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 5:557–566.

Aris-Brosou, S., and L. Excoffier. 1996. The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Mol. Biol. Evol. 13:494–504.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, London.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18: 489–522.

Baker, C. S., A. Perry, J. L. Bannister, M. T. Weinrich, R. B. Abernathy, J. Calambokidis, J. Lien, R. H. Lambersten, J. Urban Ramirez, O. Vasquez, P. J. Clapham, A. Alling, S. J. O'Brien, and S. R. Palumbi. 1993. Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. Proc. Natl. Acad. Sci. 90:8239–8243.

Ballard, J. W. O., and M. Kreitman. 1995. Is mitochondrial DNA a strictly neutral marker? Trends Ecol. Evol. 10:485–488.

Batt, G. E., J. Braun, B. P. Kohn, and I. McDougall. 2000. Thermochronological analysis of the dynamics of the Southern Alps, New Zealand. Geol. Soc. Am. Bull. 112:250–266.

Bertorelle, G., and M. Slatkin. 1995. The number of segregating sites in expanding human populations, with implications for estimates of demographic parameters. Mol. Biol. Evol. 12: 887–892.

Besansky, N. J., J. R. Powell, A. Caccone, D. M. Hamm, J. A. Scott, and F. H. Collins. 1994. Molecular phylogeny of the *Anopheles gambiae* complex suggests genetic introgression between principal malaria vectors. Proc. Natl. Acad. Sci. 91:6885–6888.

Brower, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. 91:6491–6495.

Brown, J. M., O. Pellmyr, J. N. Thompson, and R. G. Harrison. 1994. Phylogeny of *Greya* (Lepidoptera: Prodoxidae), based on nucleotide sequence variation in mitochondrial cytochrome oxidase I and II: congruence with morphological data. Mol. Biol. Evol. 11:128–141.

Buckley, T. R., C. Simon, and G. K. Chambers. 2001a. Exploring among-site rate variation models in a maximum likelihood framework using empirical data: effects of model assumptions on estimates of topology, branch lengths and bootstrap support. Syst. Biol. 50:67–86.

Buckley, T. R., C. Simon, H. Shimodaira, and G. K. Chambers. 2001b. Evaluating hypotheses on the origin and evolution of the New Zealand alpine cicadas (*Maoricicada*) using multiple comparison tests of tree topology. Mol. Biol. Evol. 18:223–234.

Burrows, J. C. 1965. Some discontinuous distributions of plants within New Zealand and their ecological significance. II. Disjunctions between Otago-Southland and Nelson Marlbrough and related distribution patterns. Tuatara 13:9–29.

Cabot, E. L., and A. T. Beckenbach. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Comput. Appl. Biosci. 5:233–234.

Chamberlain, C. P., and M. A. Poage. 2000. Reconstructing the paleotopography of mountain belts from the isotopic composition of authigenic minerals. Geology. 28:115–118.

Climo, F. M. 1975. The land snail fauna. Pp. 459–489 *in* G. Kuschel, ed. Biogeography and ecology in New Zealand. Junk, The Hague.

Cooley, J. R., C. Simon, D. C. Marshall, K. Slon, and C. Ehrhardt. 2001. Allochronic speciation, secondary contact, and reproductive character displacement in periodical cicadas (Hemiptera: *Magicicada* spp.): genetic, morphological, and behavioral evidence. Mol. Ecol. *In press.*

Cooper, R. A., and P. R. Millener. 1993. The New Zealand biota: historical background and new research. Trends Ecol. Evol. 8: 429–433.

Craw, R. 1988. Continuing the synthesis between panbiogeography, phylogenetic systematics and geology as illustrated by empirical studies on the biogeography of New Zealand and the Chatham Islands. Syst. Zool. 37:291–310.

Daugherty, C. H., G. W. Gibbs, and R. A. Hitchmough. 1993. Megaisland or micro-continent? New Zealand and its fauna. Trends Ecol. Evol. 8:437–442.

deBoer, A. J., and J. P. Duffels. 1996. Historical biogeography of the cicadas of Wallacea, New Guinea and the West Pacific: a geotectonic explanation. Paleogeogr. Paleoclimatol. Paleoecol. 124:153–177.

Dugdale, J. S. 1972. Genera of New Zealand Cicadidae (Homoptera). N. Z. J. Sci. 14:856–882.

Dugdale, J. S., and C. A. Fleming. 1978. New Zealand cicadas of the genus *Maoricicada* (Homoptera: Tibicinidae). N. Z. J. Zool. 5:295–340.

Edwards, S. V. 1997. The relevance of microevolutionary processes to higher level molecular systematics. Pp. 251–278 *in* D. Mindell, ed. Avian molecular evolution and systematics. Academic Press, London.

Emerson, B. C., P. Oromí, and G. M. Hewitt. 2000. Tracking colonization and diversification of insect lineages on islands: mitochondrial DNA phylogeography of *Tarphius canariensis* (Coleoptera: Colydiidae) on the Canary Islands. Proc. R. Soc. Lond. B 267:2199–2205.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17:368–376.

———. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

———. 1988. Phylogenies from molecular sequences: inference and reliability. Annu. Rev. Genet. 22:521–565.

Fitch, W. M. 1971. Toward defining the course of evolution: minimal change for a specific tree topology. Syst. Zool. 20:406–416.

Fleming, C. A. 1971. A new species of cicada from rock fans in

Southern Wellington, with a review of three species with similar songs and habitat. N. Z. J. Sci. 14:443–479.
———. 1975. Adaptive radiation in New Zealand cicadas. Proc. Am. Philos. Soc. 119:298–306.
———. 1984. The cicada genus *Kikihia* Dugdale (Hemiptera, Homoptera). I. The New Zealand green foliage cicadas. Natl. Mus. N. Z. Rec. 2:191–206.
Flook, P., C. H. F. Rowell, and G. Gellissen. 1995. The sequence, organisation, and evolution of the *Locusta migratoria* mitochondrial genome. J. Mol. Evol. 41:928–941.
Frati, F., A. Carapelli, P. P. Fanciulli, and R. Dallai. 1995. The genus *Isotomurus*: where molecular markers help to evaluate the importance of morphological characters for the diagnosis of species. Pol. Pismo Entomol. 64:41–51.
Frati, F., C. Simon, J. Sullivan, and D. L. Swofford. 1997. Evolution of the mitochondrial cytochrome oxidase II gene in Collembola. J. Mol. Evol. 44:145–158.
Fu, Y.-X., and W.-H. Li. 1993. Statistical tests of neutrality of mutations. Genetics 133:693–709.
Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape split by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22:160–174.
Heads, M. 1998. Biogeographic disjunction along the alpine fault, New Zealand. Biol. J. Linn. Soc. 63:161–176.
Hoelzer, G. A. 1997. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees revisited. Evolution 51:622–626.
Huelsenbeck, J. P., and K. A. Crandall. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annu. Rev. Ecol. Syst. 28:437–466.
Ingman, M., H. Kaessmann, S. Pääbo, and U. Gyllensten. 2000. Mitochondrial genome variation and the origins of modern humans. Nature 408:708–713.
Irwin, D. E., S. Bensch, and T. D. Price. 2001. Speciation in a ring. Nature 409:333–337.
Itô, Y., and M. Nagamine. 1981. Why a cicada, *Mogannia minuta* Matsumura, became a pest of sugarcane: an hypothesis based on the theory of ''escape.'' Ecol. Entomol. 6:273–283.
Jackman, T. R., and D. B. Wake. 1994. Evolutionary and historical analysis of protein variation in the blotched forms of salamanders of the *Ensatina* complex (Amphibia: Plethodontidae). Evolution 48:876–897.
Juan, C., K. M. Ibrahim, P. Oromí, and G. Hewitt. 1998. The phylogeny of the darkling beetle, *Hegeter politus*, in the eastern Canary Islands. Proc. R. Soc. Lond. B 265:135–140.
Kidd, M. G., and V. L. Friesen. 1998. Analysis of mechanisms of microevolutionary change in *Cepphus guillemonts* using patterns of control region variation. Evolution 52:1158–1168.
King, P. R. 1998. Palegeographic reconstructions of New Zealand. Geological Society of New Zealand Misc. Publ. no. 97.
Landry, B., J. A. Powell, and F. A. H. Sperling. 1999. Systematics of the *Argyrotaenia franciscana* (Lepidoptera: Tortricidae) species group: evidence from mitochondrial DNA. Ann. Entomol. Soc. Am. 92:40–46.
Langor, D. W., and F. A. H. Sperling. 1997. Mitochondrial DNA sequence divergence in weevils of the *Pissodes stobi* species complex (Coleoptera: Curculionidae). Insect. Mol. Biol. 6: 255–265.
Marshall, D. C., and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim.* Evolution 54:1313–1325.
Martin, A. P., and C. Simon. 1988. Anomalous distribution of nuclear and mitochondrial DNA markers in periodical cicadas. Nature 336:237–239.
———. 1990. Differing levels of among-population divergence in the mitochondrial DNA of periodical cicadas related to historical biogeography. Evolution 44:1066–1080.
McGlone, M. S. 1985. Plant biogeography and the late Cenozoic history of New Zealand. N. Z. J. Boty. 23:723–749.
Mildenhall, D. 1999. Pollen analysis of the Plio-Pleistocene Kowhai formation (Kurow Group), Mackenzie Basin, Central Otago. Institute of Geological and Nuclear Sciences Science Report no. 99/17. IGNS Ltd, Lower Hutt, New Zealand.
Moore, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49:718–726.
———. 1997. Mitochondrial-gene trees versus nuclear-gene trees, a reply to Hoelzer. Evolution 51:627–629.
Mortiz, C., C. J. Schneider, and D. B. Wake. 1992. Evolutionary relationships within the *Ensatina escholtzii* complex confirm the ring species interpretation. Syst. Biol. 41:273–291.
Myers, J. G. 1923. New species of New Zealand Cicadidae. Trans. N. Z. Inst. 54:430–431.
Nei, M., and W.-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76:5269–5273.
Neigel, J. E., and J. C. Avise. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pp. 515–534 *in* S. Karlin and E. Nevo, eds. Evolutionary processes and theory. Academic Press, Orlando, FL.
Nielsen, M., K. Lohman, and J. Sullivan. 2001. Phylogeography of the tailed frog (*Ascaphus truei*): implications for the biogeography of the Pacific Northwest. Evol. 55:147–160.
Newnham, R. M., D. J. Lowe, and P. W. Williams. 1999. Quaternary environmental change in New Zealand: a review. Prog. Physol. Geogr. 23:567–610.
Pamilo, P., and M. Nei. 1988. Relationships between gene trees and species trees. Mol. Biol. Evol. 5:568–583.
Rambaut, A., and L. Bromham. 1998. Estimating divergence dates from molecular sequences. Mol. Biol. Evol. 15:442–448.
Roderick, G. K. 1996. Geographic structure of insect populations: gene flow, phylogeography, and their uses. Annu. Rev. Entomol. 41:325–352.
Rozas, J., and R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.
Salmon, J. T. 1950. Notes on synonymy among New Zealand insects. Trans. R. Soc. N. Z. 78:1–2.
Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.
Simon, C., J. Tang, S. Delwadi, G. Stanley, J. Deniega, and T. R. Unnasch. 2000. Genetic evidence for assortive mating between 13-year cicadas and sympatric ''17-year cicadas with 13-year life cycles'' provides support for allochronic speciation. Evolution 54:1326–1336.
Smale, D. 1991. Provenance changes and movement on the Alpine Fault indicated by heavy minerals from Cretaceous-Cenozoic sediments in south Westland. J. Roy. Soc. N. Z. 21:151–160.
Sullivan, J., J. A. Markert, and C. William Kilpatrick. 1997. Phylogeography and molecular systematics of the *Peromyscus aztecus* species group (Rodentia: Muridae) inferred using parsimony and likelihood. Syst. Biol. 46:426–440.
Sunnucks, P., and D. F. Hales. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). Mol. Biol. Evol. 13: 510–524.
Sutherland, R. 1999. Cenozoic bending of New Zealand basement terranes and Alpine Fault displacement: a brief review. N. Z. J. Geol. Geophys. 42:295–301.
Swofford, D. L. 1998. PAUP*: phylogenetic analysis using parsimony (* and other methods). Vers. 4. Sinauer Associates, Sunderland, MA.
Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pp 407–514 *in* D. M. Hillis, C. Moritz, and B. K. Mabee, eds. Molecular systematics. Sinauer Associates, Sunderland, MA.
Szymura, J. M., D. H. Lunt, and G. M. Hewitt. 1996. The sequence and structure of the meadow grasshopper (*Chorthippus parallelus*) mitochondrial srRNA, ND2, COI, COII, ATPase8 and 9 tRNA genes. Insect. Mol. Biol. 5:127–139.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.
Thorne, J. L., H. Kishino, and I. S. Painter. 1998. Estimating the rate of evolution of the rate of molecular evolution. Mol. Biol. Evol. 15:1647–1657.
Trewick, S. A., G. P. Wallis, and M. Morgan-Richards. 2000. Phylogeographical pattern correlates with Pliocene mountain building in the alpine scree weta (Orthoptera, Anostostomatidae). Mol. Ecol. 9:657–666.
Wake, D. B. 1997. Incipient species formation in salamanders of the *Ensatina* complex. Proc. Natl. Acad. Sci. 94:7761–7767.
Wallis, G. P., and S. A. Trewick. In press. Finding fault with vicariance: a critique of Heads (1998). Syst. Biol.
Wardle, P. 1963. Evolution and distribution of the New Zealand flora as affected by Quaternary climate. N. Z. J. Boty. 1:3–17.
Waters, J. M., and G. P. Wallis. 2000. Across the Southern Alps by river capture? Freshwater fish phylogeography in South Island, New Zealand. Mol. Ecol. 9:1577–1582.
Wayne, M. L., and K. L. Simonsen. 1998. Statistical tests of neutrality in the age of weak selection. Trends Ecol. Evol. 13: 236–240.
Williams, K. S., and C. Simon. 1995. The ecology, behavior and evolution of periodical cicadas. Annu. Rev. Entomol. 40: 269–295.
Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable substitution rates over sites: approximate methods. J. Mol. Evol. 39:306–314.
———. 1996. Among-site rate variation and its impact on phylogenetic analyses. Trends Ecol. Evol. 11:367–372.
Zhang, D., and G. M. Hewitt. 1996. Nuclear intergrations: challenges for mitochondrial DNA markers. Trends Ecol. Evol. 11: 247–251.

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