Using New Zealand examples to teach Darwin's 'Origin of Species': Lessons from molecular phylogenetic studies of cicadas

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In the two hundredth anniversary year of the birth of Charles Darwin and the one hundred and fiftieth year of the publication of his most influential book, *On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life* (Darwin 1859, 1876, hereafter termed *Origin of Species*) it seems appropriate to discuss how some of Darwin's main theses can be supported using data from New Zealand organisms. My research group has been studying the evolution, biogeography, and systematics of the New Zealand cicadas since 1992 and in the process has published a number of studies that nicely illustrate some of Darwin's major points. These examples can be used to simultaneously teach Darwinian principles and New Zealand natural history and evolution.

Although Darwin's *Origin of Species* was published in 1859, it had been more than twenty years in the making. Stimulated by his teachers, his studies at Cambridge, and his own natural history observations – especially those made during his five-year round-the-world voyage aboard the *HMS Beagle* – Darwin began to amass a huge collection of data. He corresponded with most of the leading naturalists of the day including scientists in other countries. He synthesised ideas from economics, geology, paleontology, botany, zoology, comparative anatomy, embryology, and animal and plant breeding. He formed hypotheses and attempted to test them through observations and experiments. Using all of this knowledge, he formulated and refined his theory of natural selection and developed his ideas on the origin of species. His first essay on these subjects was written in 1844 but, not wishing to offend his more religious relatives, it was hidden in an envelope in a cabinet under the stairs to be published in the event of his death. Darwin occupied himself with writing geological books and his two-volume treatise on recent and fossil barnacles. The ideas in that essay were, however, published in Darwin's lifetime because in 1858, he received a manuscript containing very similar ideas from the young naturalist, Alfred Russell Wallace. Darwin's and Wallace's papers were read before a scientific audience that year, and the following year, Darwin published the *Origin of Species*. After publishing this, Darwin wrote a dozen other books. All of these used natural selection as a central theme. A complete listing of Darwin's works, searchable copies, and a list of scholarly books about Darwin can be found at http://darwin-online.org.uk/.

While the mechanism of 'natural selection' was Darwin's most original contribution to the topic of evolutionary biology, it was not the only subject he covered in the *Origin of Species*; in fact, it was the main subject of only half of the chapters. In the second half of this book, Darwin tackled difficult questions related to speciation, hybridisation, and biogeography. For this short essay, I have chosen six statements made by Darwin in the *Origin of Species* and have illustrated them using published work from my laboratory on New Zealand cicadas. The six statements (translated from Victorian into modern English) are:

(1) The similarity of organisms within different regions of the world is due to common inheritance and barriers to dispersal;

(2) The biota of isolated islands is unique and related to that of the nearest mainland;

(3) Recognisable varieties within species are potential species;

(4) Species that are distributed over a large geographic area give rise to the most new varieties or species;

(5) Hybridisation can occur between species without destroying the original species;

(6) Climate change results in changes in species ranges and altered evolutionary interactions among populations.

1. The similarity of organisms within different regions of the world is due to common inheritance and barriers to dispersal

In Chapter II of the *Origin of Species* entitled, 'Geographical Distributions,' Darwin said, 'Neither the similarity nor the dissimilarity of the inhabitants of various regions can be wholly accounted for by … physical conditions…The bond [within

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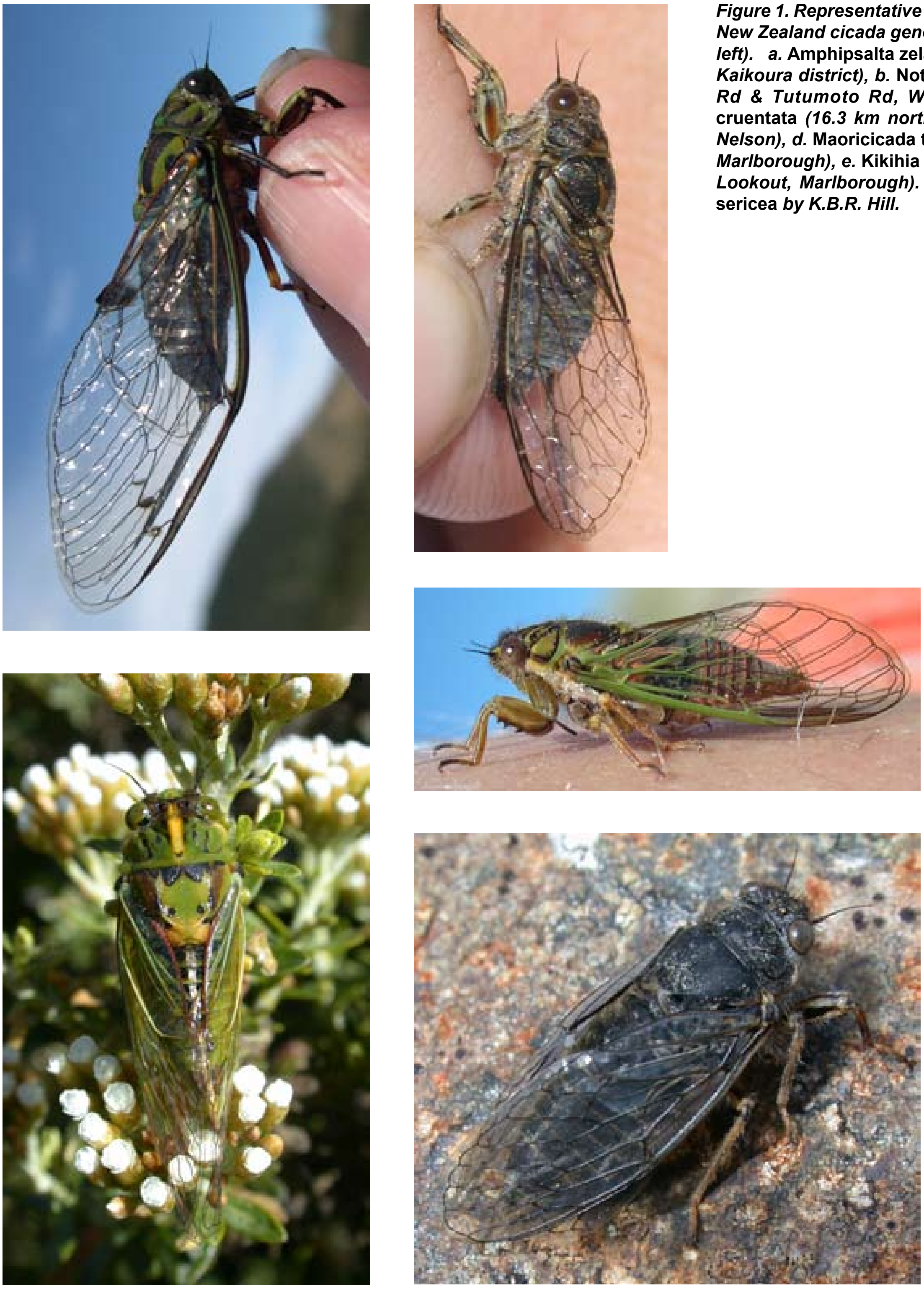


Figure 1. Representative species of each of the five New Zealand cicada genera (clockwise from upper left). a. *Amphipsalta zelandica* **(Kekerengu Valley, Kaikoura district), b.** *Notopsalta sericea* **(Waitomo Rd & Tutumoto Rd, Waikato), c.** *Rhodopsalta cruentata* **(16.3 km north of Karamea, Northwest Nelson), d.** *Maoricicada tenuis* **(Rainbow Ski Field, Marlborough), e.** *Kikihia horologium* **(Mt. Patriarch Lookout, Marlborough). Photos by C. Simon;** *N. sericea* **by K.B.R. Hill.**

regions] is simply inheritance, that cause which alone, as far as we positively know, produces organisms quite like each other.' He continued, 'Barriers … or obstacles to free migration, are related … to the differences between the productions [species] of various regions.' This idea, that the similarity of organisms within different regions of the world was due to common inheritance was used by Darwin as one of his arguments against creationism. He used desert organisms as an example, pointing out that although desert animals look superficially similar the world over and possess similar adaptations for arid conditions, a closer examination will show that in each region the desert species are related to nearby non-desert species. Darwin argued that if a creator had made these desert organisms and placed them in the world's deserts, he would have made just one set and this desert species set would be the same worldwide rather than resembling local non-desert relatives. In fact, Wallace and other biologists before and since have recognised regional biota and divided the world into approximately eight to ten biogeographic

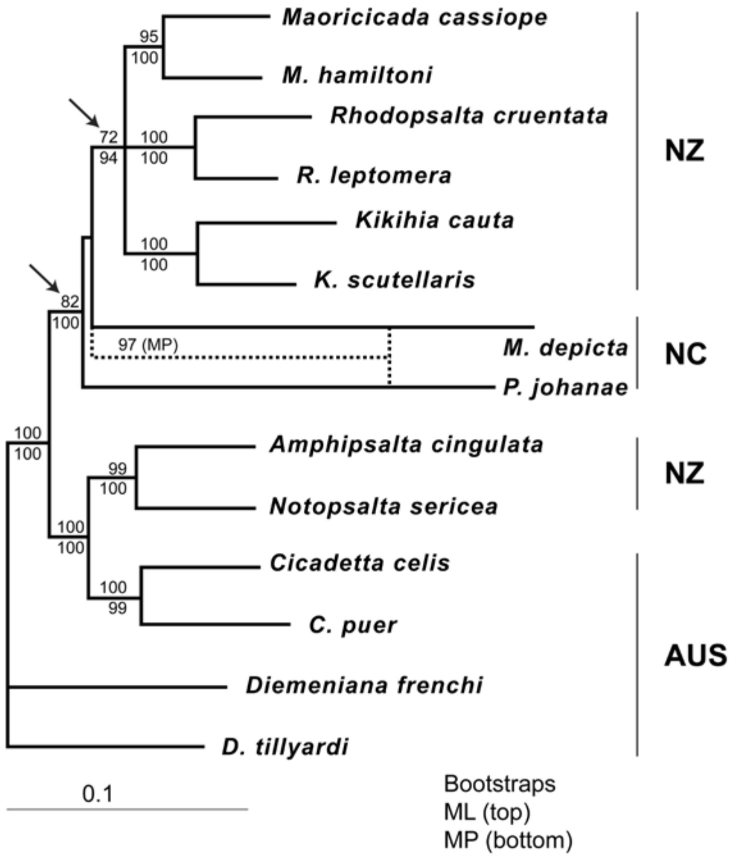


Figure 2. Maximum likelihood (ML) phylogenetic tree based on mitochondrial (12S, 16S, COI, COII) DNA segments and nuclear (EF1-alpha) DNA for the five genera of New Zealand (NZ) cicadas and relatives from Australia (AUS) and New Caledonia (NC). Phylogenetic trees were constructed using the program PAUP*; ML analysis was conducted using the best-fitting HKY85 + gamma model. Both ML and maximum parsimony (MP) bootstrap supports are shown above and below the branches, respectively. **M. depicta = Myersalna depicta, P. johanae = Pauropsalta johanae.** ***Gene abbreviations: 12S = small subunit ribosomal RNA; 16S = large subunit ribosomal RNA; COI = cytochrome C oxidase subunit I; COII = cytochrome C oxidase subunit II; EF1-alpha = elongation factor 1-alpha. Arrows indicate the location of the clade containing the New Caledonian and New Zealand subclades and the split of the three NZ genera,*** **Maoricicada, Rhodopsalta,** ***and*** **Kikihia.** ***The scale bar is estimated number of substitutions per site/branch lengths.***

provinces (Proches 2005). In each province, organisms show greater affinities to related organisms inside their province than to those outside. Species of one province do cross into other biogeographic provinces but are slowed by barriers to dispersal. To illustrate this I bring in our first New Zealand data. These data are discussed below because they also support his related point, again from Chapter XI of the *Origin of Species.*

2. The biota of isolated islands is unique and related to that of the nearest mainland

Darwin said: the 'proportion of endemic species on an island is particularly high when the opportunity for dispersal to the island is low' and he provided many examples of island species 'clearly related to species on the nearest mainland'. Darwin's argument here is similar to the first statement – if species were created, endemic island species would be similar to each other worldwide. Instead they tend to be more similar to organisms from the closest continent than to organisms of other islands. As an island group, New Zealand illustrates this statement very well. New Zealand supports a rich array of endemic species. Each group of endemics has evolved or 'radiated' from a single colonising ancestor into anywhere from two to hundreds of related species and the majority of these colonising species come from Australia or New Caledonia (Winkworth *et al*. 2002, 2005; Goldberg *et al*. 2008; Wallis & Trewick 2009). New Zealand cicadas are no exception...

There are more than 50 species and subspecies of New Zealand cicadas. All are part of the tribe Cicadettini and are unevenly distributed in five genera (Figure 1; Dugdale 1971). Two of these genera, *Amphipsalta* (3 species, Figure 1a) and *Notopsalta* (1 sp., Figure 1b), have not radiated substantially and have their closest relatives in Australia. The other three genera, *Rhodopsalta* (≥ 3 spp., Figure 1c), *Maoricicada* (> 18 spp./subspp., Figure 1d), and *Kikihia* (> 30 spp./subspp. Figure 1c) have radiated extensively in New Zealand and have their closest relatives in New Caledonia (Buckley *et al.* 2001a, 2002, 2006; Arensburger *et al.* 2004a, b; Buckley & Simon 2007; Marshall *et al.* 2008) (Figure 2). This New Caledonian lineage is ultimately related to cicadas in Australia that are also members of the tribe Cicadettini. DNA sequence data from both the mitochondrial and nuclear genomes of these cicadas analysed using a relaxed molecular clock analysis suggest that both of the original colonising ancestors arrived in New Zealand about 14 million years (Ma) ago (Buckley *et al.* 2002; Arensburger *et al.* 2004a, b). Colonists from Australia would have arrived via the wind, but there is evidence that the New Caledonian connection could have been made via a chain of long low islands that became submerged about this time and now form the undersea Norfolk Ridge (Herzer *et al.* 1997).

3. Recognisable varieties within species are potential species

In Chapter II of the *Origin of Species*, 'Variation Under Nature,' Darwin said: 'I believe a well-marked variety may be justly called an incipient species… It need not be supposed that all varieties or incipient species necessarily attain the rank of species. They may whilst in this incipient state become extinct, or they may endure as varieties for long periods…' Today, in agreement with Darwin, we recognise that species are part of a continuum of differentiation from populations through species to genera and higher taxonomic groups. Geographic studies of genetic variation often reveal subgroups, which may or may not be morphologically distinguishable from each other. Morphologically distinguishable subgroups are termed subspecies and are officially recognised taxonomically. Subspecies are groups of populations. Even today it is often difficult to decide whether to call a recognisable group of populations a species or subspecies. The recognition of a species must be done in the context of a particular species concept. There are many species concepts in the evolutionary literature (Coyne & Orr 2004) and different species boundaries can be applied to groups of populations depending on the species concept used.

The presence of subgroups within groups is well illustrated in the evolutionary tree of the New Zealand cicada genus *Kikihia* (Figure 3, Marshall *et al.* 2008, Marshall *et al.* in prep.) This phylogenetic tree is composed of subtrees and each of those subtrees has multiple subtrees within it. This picture of trees within trees illustrates Darwin's idea of incipient species and geographic variation. These trees or groups of populations may some day go on to be groups of species, as populations are isolated over long periods of time and develop greater and greater genetic differences in isolation.

Luckily in New Zealand cicadas, we have help in recognising species. New Zealand cicadas have species-specific songs sung by the males (Figure 3). The females respond to the males in a very specific gap in the male's song (Lane 1984, 1995; Marshall & Hill 2009). Because the species-specific songs facilitate the location of the most appropriate mates, natural selection favours males and females who do not deviate from the norm in signal and preference, respectively. Over time, presumably in isolation, the single song of the ancestral *Kikihia* has become differentiated into the songs of the descendant *Kikihia* species.

In our example (Figure 3), *Kikihia muta*, *K.* 'aotea,' and *K.* 'westlandica' are presently classified under the one species name, *K. muta*. However, there are subtle differences between the songs of these different groups. Most song differences are not detectable by the untrained human ear but they can easily be identified by detailed analysis of oscillograms. The Kikihia phylogenetic tree shows that the song-types are well differentiated genetically and that within each song-type, there are clear genetic subdivisions and sub-subdivisions. It is not absolutely clear when a song becomes different enough to prevent genetic exchange between incipient species, nor is it clear how much genetic exchange can take place between two diverging populations and still have them continue to diverge. Because of this, there are still some taxa within *Kikihia* where it is difficult to draw the line between species and subspecies designation (Marshall *et al.* in prep.).

Finally, Darwin used this pattern of subtrees within trees to counter creationism. He pointed out in Chapter II of the *Origin of Species* that, 'if species have once existed as varieties and have thus originated…these analogies' make sense; but they 'are utterly inexplicable if each species has been independently created.'

4. Species that are distributed over a large geographic area give rise to the most new varieties or species

Darwin said in Chapter IV of the *Origin of Species*, entitled 'Natural Selection', '…those [species that are] spread most widely will give rise to [the] most new varieties and species, and will thus play an important part in the changing history of the organic world.'

The cicada species *Maoricicada campbelli* is the most widespread species of its genus in New Zealand. Throughout its geographic range, it is morphologically variable in colour, colour pattern, and the presence of dense setae or hairs. Our genetic work (Buckley *et al.* 2001a, b; Hill *et al.* 2009) suggests that *M. campbelli* is divided into six very distinct genetic/geographic subgroups or clades (Figure 4 a,b), but that these clades are not correlated with any specific morphological traits. Five of the clades seem to have originated at the same time, approximately 1.0 to 0.7 Ma. These clades have been named Canterbury, Waitaki, Southern Alps, Marlborough/Nelson/Kaikoura, and North Island, respectively. The sixth clade is found in Otago and appears to have split from the ancestor of the other five approximately 1.4 to 2.5 Ma (Figures 4, 5).

The Otago *M. campbelli* seems to be forming a new species. Not only is it very different in its mitochondrial DNA from the other clades, it has a consistent difference in song pattern compared to all other clades (Figure 6) and appears to inhabit drier areas. Interestingly, we have discovered five secondary contact zones between the Otago clade and two of the other clades (Figure 4a). We are currently sequencing nuclear genes to look for hybridisation and gene flow at these contact zones.

Other New Zealand cicada taxa that show the tendency for widespread species to give rise to new species are the South Island *Maoricicada mangu* complex which currently has four recognised subspecies that are genetically, morphologically and acoustically distinguishable (Buckley *et al.* 2006; Dugdale & Fleming 1978) and the North Island *Kikihia muta* complex discussed in example 3, above.

5. Hybridisation can occur between species without destroying the original species

In the eighth chapter of the *Origin of Species,* entitled 'Hybridism,' Darwin said: '…the degree of fertility, both of first crosses [between species] and of [their] hybrid [offspring] graduates from zero to perfect fertility.' He continued, 'No one has been able to point out what kind, or what amount, of difference in any recognisable character is sufficient to prevent two species crossing.'

Darwin corresponded with leading botanists and plant breeders of his day and gathered many examples of hybridisation between species (as well as examples of disagreements as to what populations constituted species). He noted fewer examples of hybridisation in animals. Today there is growing evidence that hybridisation is more common than previously thought in both plants and animals, that it is unevenly distributed among taxa, that in some animal groups it can be even more common than in plants, and that with the help of natural selection some genes can cross species boundaries more easily than others (Grant & Grant 1992; Mallet 2005; Hey 2006; Rieseberg *et al.* 2006; Reyer 2008). A recent review paper in the *Journal of the Royal Society of New Zealand* (Morgan-Richards *et al.* 2009)

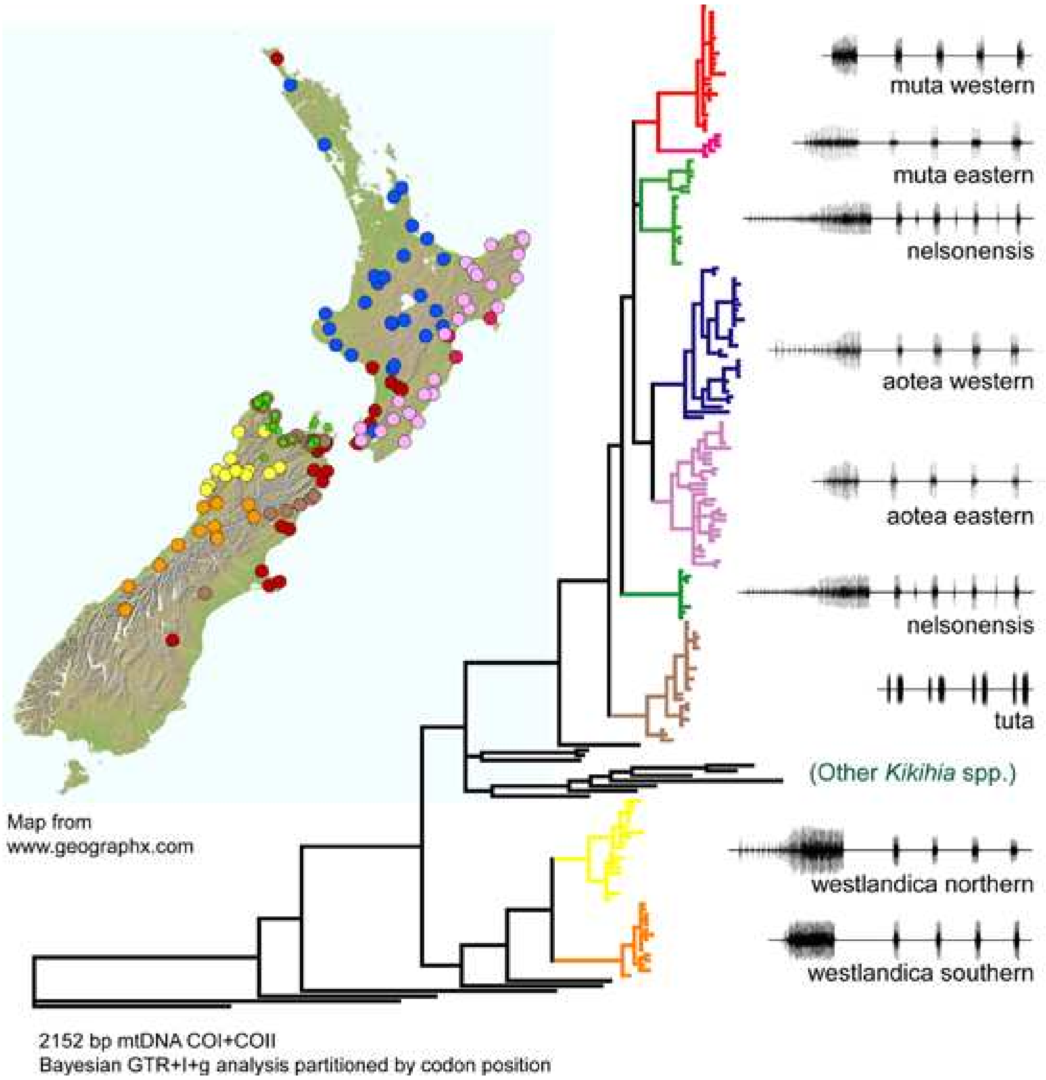


Figure 3. Evolutionary tree, geographic distributions, and songs of selected Kikihia *species. Names are names of song types rather than names of described species; the taxonomy is currently under review. The tree was constructed using Bayesian analysis of mitochondrial COI and COII genes partitioned by codon position and modeled using a GTR+I+gamma model (Marshall* et al. *in prep.). Taxa designated "muta", "aotea", and "westlandica" are currently all grouped under the species name* Kikihia muta *but exhibit clear song and genetic differences. Colours of dots on map correspond to colours of song-type clades on the tree. Within each song-type, genetic subgroups and sub-subgroups are obvious. Gene abbreviations as in figure 2. Map base from www.geographix.com*

describes how hybridisation has been important in the evolution of the New Zealand biota.

In New Zealand cicadas, hybridisation has been documented in both the genus *Kikihia* (Marshall *et al.* 2008) and in the genus *Maoricicada* (Buckley *et al.* 2006). In *Kikihia* numerous contact zones have been identified between parapatric species, and in many of them hybrids have been detected through a combination of DNA and acoustic data (Marshall *et al.* 2008). Phylogenetic analysis and molecular clock dating has established that species that are separated in time by more than 2 million years (My) may be sympatric (overlapping in distribution) or parapatric (adjacent in distribution) with no sign of hybridisation while species separated for less time are never sympatric and when parapatric can hybridise (Figure 7). This 2-million-year 'sympatry threshold' lies squarely in the middle of the average time to speciation in *Drosophila* (1.0–3.0 My) calculated by Coyne & Orr (1997) from experimental data.

In *Maoricicada,* there are several instances of presumed hybridisation. These have been detected by phylogenetic analysis of multiple genes followed by tests to distinguish hybridisation from random sorting of ancestral lineages into descendant species. These cases of hybridisation appear to have taken place in the distant past because, unlike the hybrids in *Kikihia,* no hybrids exist that have the song of one species and the exact mitochondrial DNA haplotype of another extant species. The putative hybrids were originally suspected based on the fact that different genes place them in different parts of the evolutionary tree and some of these placements do not group them with the morphologically and acoustically most similar taxa.

6. Climate change results in changes in species ranges and altered evolutionary interactions among populations

In Chapter XII of the *Origin of Species* entitled, 'Geographical Distribution,' Darwin wrote, 'As the cold came on, …the mountains would become covered by snow and ice, and their former Alpine inhabitants would descend to the plains.' …as each more southern zone became… 'ill-fitted for their former … inhabitants, [these] would be supplanted ….[by] arctic productions…'

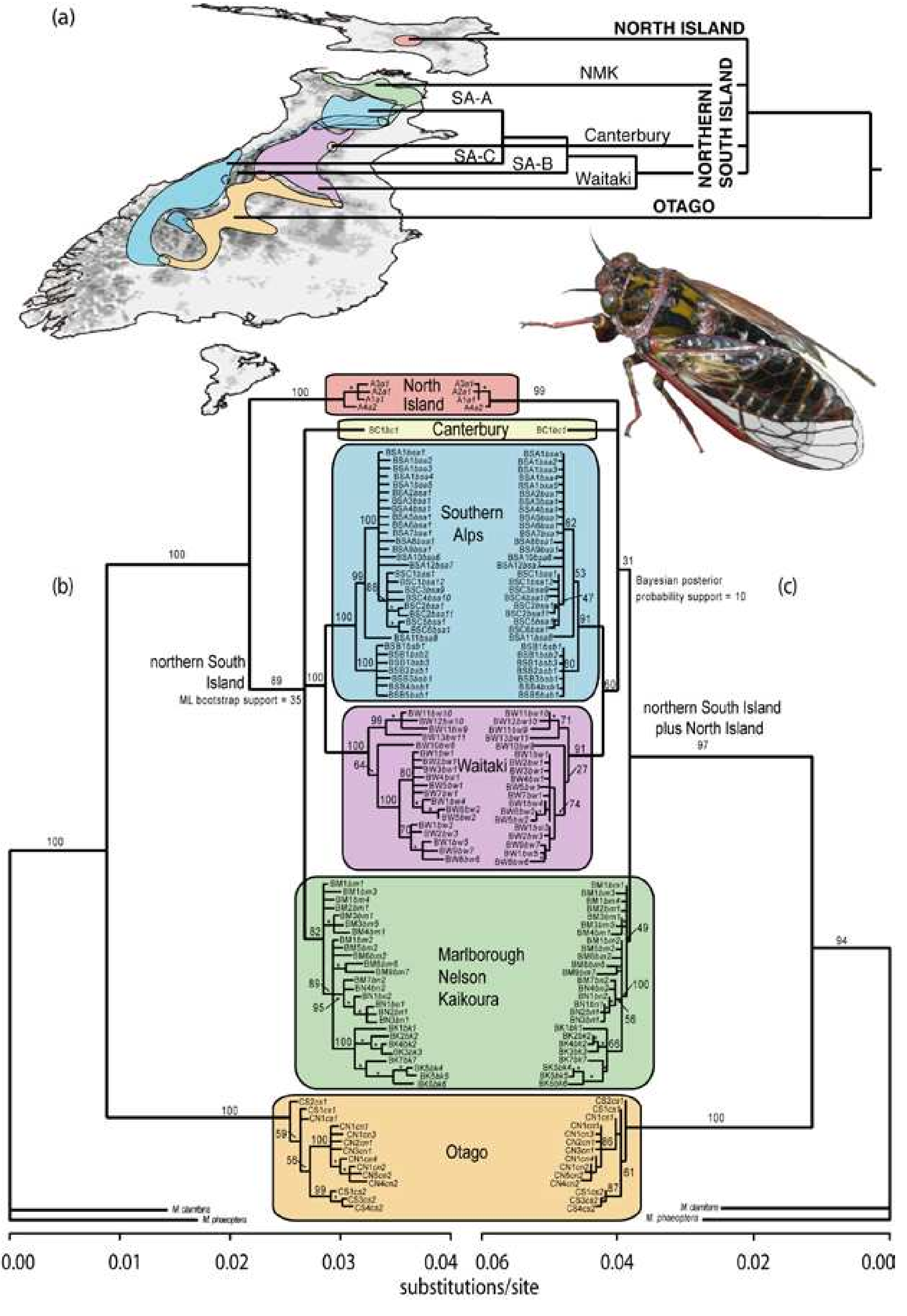


Figure 4. Phylogeography of Maoricicada campbelli *based on mitochondrial DNA (COI, A6, and A8 genes); (a) Cladogram showing the geographical locations of the main clades in New Zealand. Some branches are collapsed to show that speciation events of northern South Island and North Island lineages are so close together in time as to be indistinguishable. Note that clade SA-B is divided into three parts connected by dashed lines. Abbreviations include: COI (cytochrome C oxidase subunit 1 gene), A6/A8 (ATPase subunits 6 and 8, respectively), NMK (Nelson + Marlborough + Kaikoura), SA-A (Southern Alps A), etc. (b) Bayesian phylogenetic tree, three partitions, 20 m generations, 10% burn-in. (c) Maximum likelihood phylogenetic tree, HKY+I, 100 bootstrap pseudoreplicates. *, Nodes with > 50% support where support is not otherwise given. Clade colours in (a) correspond to those in (b) and (c). Inset photo is of a male of M. campbelli from Fox Glacier. Reprinted with permission from Hill, K.B.R.; Simon, C.; Marshall, D.C.; Chambers, G.K. 2009. Surviving Glacial Ages within the Biotic Gap: Phylogeography of the New Zealand cicada* Maoricicada campbelli. Journal of Biogeography 36*: 675-692.*

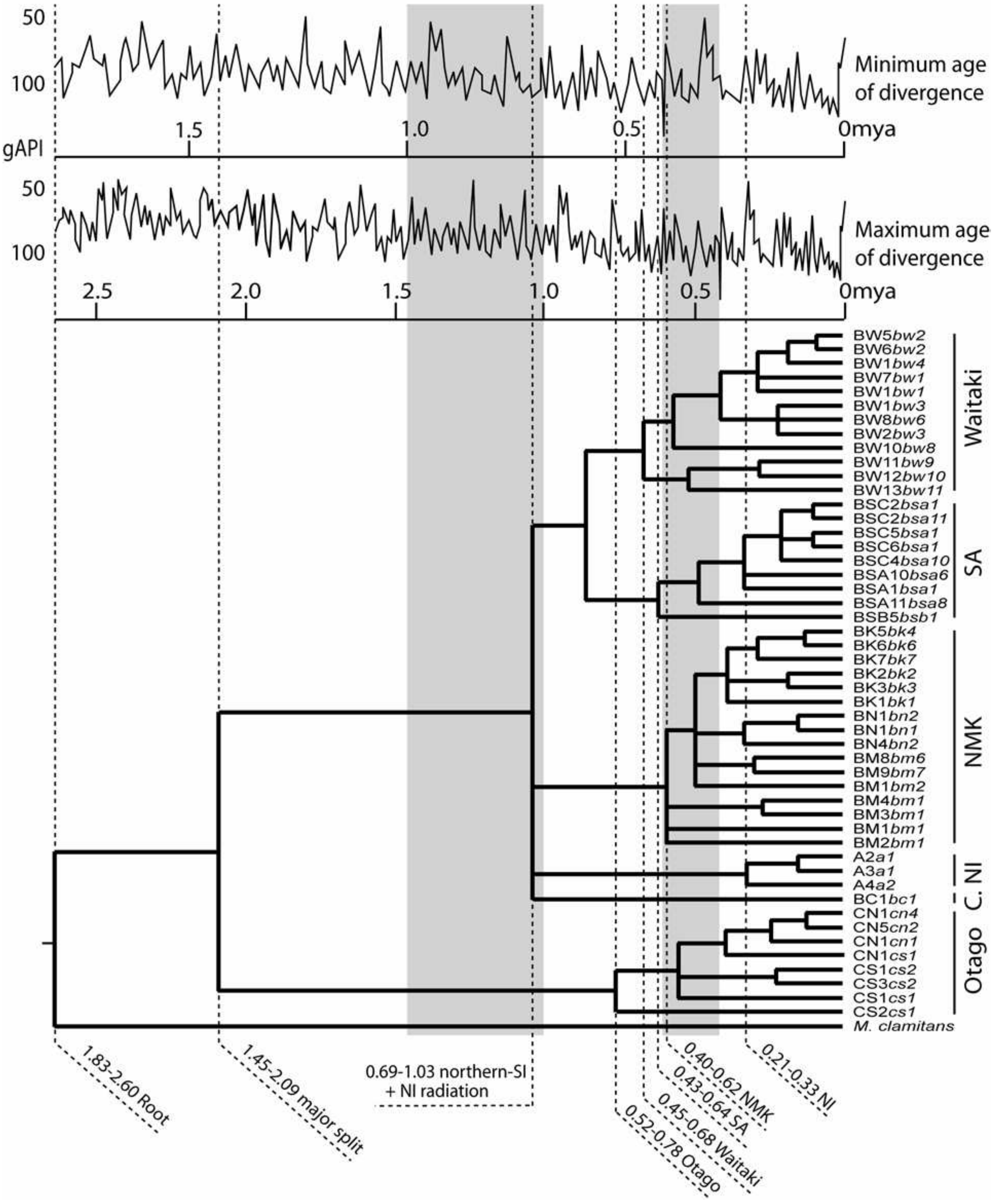
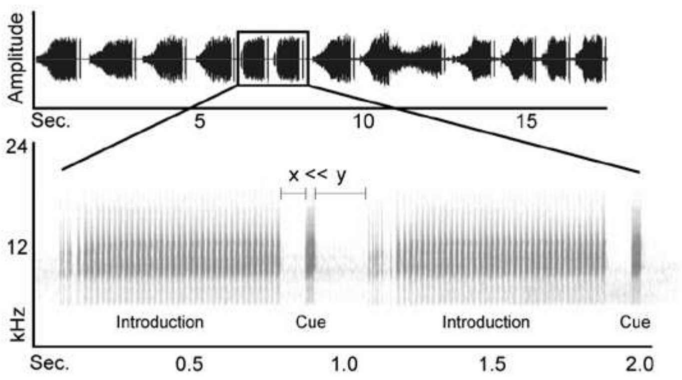


Figure 5. Molecular clock tree for Maoricicada campbelli *showing maximum and minimum estimated dates of divergence of main clades in millions of years. Graphs above the tree show a representation of the glacial-interglacial cycling during the Quaternary [adapted from figure 5F in Carter (2005): composite natural gamma curve (API gamma units, y-axis reversed) plotted against age; upper and lower graphs, respectively, correspond to minimum and maximum age of splits estimated in our analyses (shown at the bottom of the tree)]. Shaded areas represent the start of the increased glacial-interglacial cycling amplitude at mid-Pleistocene, c. 1 Ma, and the extremely cold glacial maximum at c. 0.43 Ma, respectively. Abbreviations: C. (Canterbury); NI (North Island); SI (South Island); NMK (Nelson + Marlborough + Kaikoura); SA (Southern Alps). Reprinted with permission from Hill, K.B.R.; Simon, C.; Marshall, D.C.; Chambers, G.K. 2009. Surviving Glacial Ages within the Biotic Gap: Phylogeography of the New Zealand cicada* Maoricicada campbelli. Journal of Biogeography 36*: 675-692.*

a) northern-South Island and North Island *M. campbelli*



b) Otago *M. campbelli*

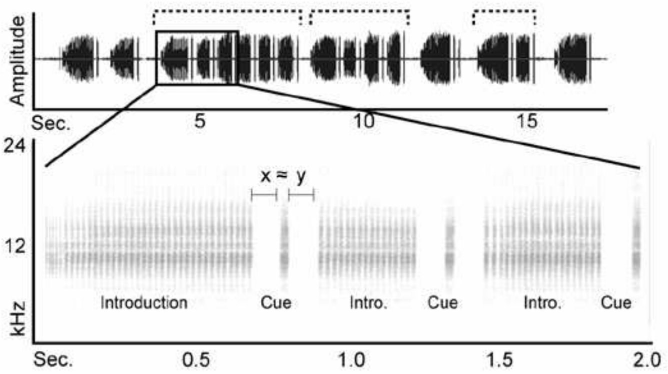


Figure 6. Song divergence between (a) northern South Island + North Island and (b) Otago populations of* Maoricicada campbelli. *Otago populations commonly exhibit more cue notes per unit time and 'phrase grouping' [indicated by dotted lines in (b)] and their pre-cue gaps (x) are about equal in duration to the post-cue gaps (y). The non-Otago song has longer post-cue gaps. The song cue is known to trigger the wing-flick response in sexually receptive females and may be important in species recognition. Reprinted with permission from Hill, K.B.R.; Simon, C.; Marshall, D.C.; Chambers, G.K. 2009. Surviving Glacial Ages within the Biotic Gap: Phylogeography of the New Zealand cicada* Maoricicada campbelli. Journal of Biogeography 36*: 675-692.

Darwin began his scientific career as a geologist and some of his earliest observations concern the effects of glaciation on the landscape of Great Britain. As he began to think more and more about the evolution of species, his background knowledge of geology made it obvious to him that in glacial times, species ranges would necessarily change.

This statement could be directly applied to New Zealand *Maoricicada.* All but one of the extant species exist in tundra-like or arid habitats and the majority are found on mountains. Undoubtedly, these mountain cicadas must have been forced from their isolated mountaintops onto a common lowland tundra during the last glacial maximum (LGM) and those that preceded it. This may account for the ancient hybridisation events discussed above.

Kikihia may have survived on the South Island during the LGM at lower elevations in coastal areas. At this time, the Southern Alps were covered by glaciers; pollen and macrofossil evidence suggest that the surrounding mid-elevation landscape was a tundra-like biome with shrubs, tussock, herbfields, and no trees (Alloway *et al.* 2007). At lower elevations in the lee of the alps in the southeast, dry open grasslands with rare shrubs predominated and there is some evidence of rare forest near hills. In the windward lowlands on the northwest coast of the South Island, there is evidence for a shrubland–grassland habitat with patches of *Nothofagus* and rare podocarps. Adjacent to the current northwest coast, on lowlands revealed by lowered sea levels, tall podocarp, broadleaf, and beech forests have been reconstructed (Alloway *et al.* 2007).

There has been debate on the extent of forest refugia on the southern half of the South Island during the LGM (Leschen *et al.* 2008, Marshall *et al.* 2009). To help determine whether there were forests large enough and temperate enough to serve as refugia, Marshall *et al.* (2009) examined phylogeographic patterns of *Kikihia subalpina,* the only forest-specialist *Kikihia* on the South Island. Evolutionary trees constructed from mitochondrial DNA data (Figure 8) suggest that the northern half of the South Island has many old and diverse lineages while the southern two-thirds of the South Island was colonised rapidly just after the LGM in two waves. One wave moved quickly down the west coast and the other swept south on the east side of the main divide. Although there is no evidence for glacial refugia suitable for *Kikihia subalpina,* studies in progress suggest otherwise for small bark- and litter-dwelling beetles (K. Marske, T. Buckley, R. Leschen *pers. comm.*) and for a dry-forest fern (Shepherd *et al.* 2007).

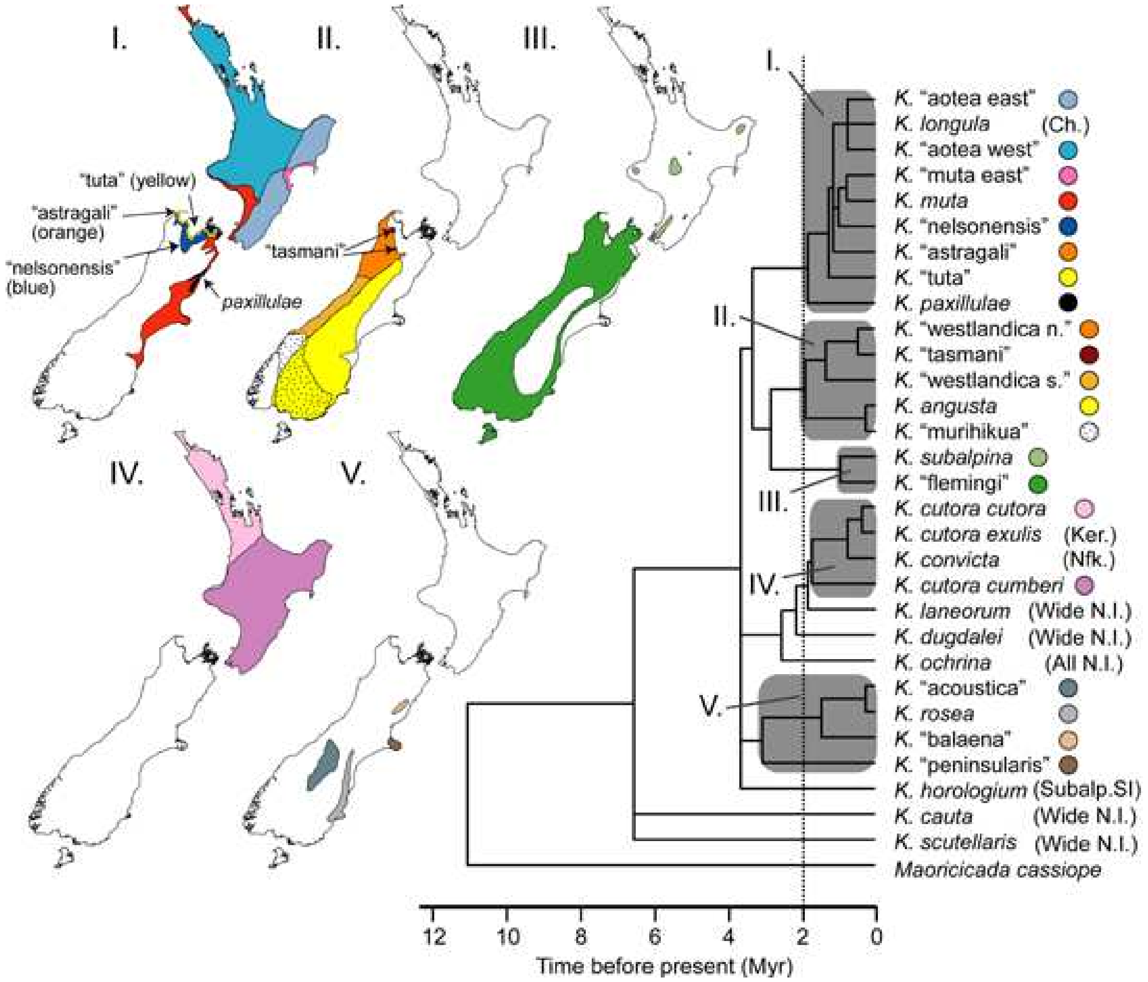


Figure 7. Restriction of geographic overlap (sympatry) to* Kikihia *lineages with divergence times greater than approximately 2 Ma. Taxa grouped within a given grey shaded box are non-overlapping or adjacent and form hybrid zones upon contact. Taxa from different shaded boxes or unboxed lineages are commonly overlapping or sympatric in distribution. The vertical dotted line approximates the 'sympatry threshold'. Simplified geographic distributions of species within each boxed group I-V are shown in accompanying maps. Distributions of the remaining species are summarised verbally as follows:* K. horologium *is found in subalpine areas of South Island.* K. longula *is found only on the Chatham Islands.* K. cutora exulis *is found only on the Kermadec Islands.* K. convicta *is found only on Norfolk Island. Species labeled "Wide N.I." are widely distributed on North Island and frequently found in sympatry. The chronogram was obtained from relaxed-clock dating using the* Kikihia *mtDNA dataset. Reprinted from Marshall, D.C.; Slon, K.; Cooley, J.R.; Hill, K.B.R.; Simon, C. 2008. Steady Plio-Pleistocene diversification and a 2-million-year sympatry threshold in a New Zealand cicada radiation.* Molecular Phylogenetics and Evolution 48(3)*: 1063. Copyright 2008 with permission from Elsevier.

Concluding comments

New Zealand is a treasure trove of evolutionary examples and an excellent place to study evolutionary biology. This review illustrates how one group of organisms arising from just two ancestral colonisation events has spread across the landscape and diversified and provided evidence to support many of Darwin's evolutionary hypotheses from the *Origin of Species*. Many other New Zealand organisms illustrate these same phenomena and have been reviewed comprehensively in this anniversary year (Wallis & Trewick 2009, Morgan-Richards *et al.* 2009). By studying the evolution, genetics, and systematics of New Zealand organisms we can, in Darwin's own words (Darwin 1845, pg. 378), 'be brought somewhat near to that great fact – that mystery of mysteries – the first appearance of new beings on this earth.'

Acknowledgements

This paper summarises work on New Zealand cicadas conducted in my laboratory since 1992. It benefited tremendously from the hard work and imagination of my students, postdocs, and colleagues who are coauthors on the papers reviewed here and who participated in all aspects of this research. None of this work would have been possible without the pioneering studies of Charles Fleming and John Dugdale who left a valuable body of published work spanning more than 20 years. John Dugdale provided helpful advice on cicada biology and New Zealand natural and geological history, as did David Lane, George Gibbs, Mary McEwen, and many biologists from Victoria University of Wellington, Otago University, Massey University, Landcare Research, Geological and Nuclear Sciences, and the National Institute of Water and Atmospheric Research. Kathy Hill, Dave Marshall, and Steve Chiswell provided comments on the manuscript. The New Zealand Department of Conservation (Te Papa Atawhai) via Ian Millar provided valuable assistance and collecting permits; DOC biologists in the field provided local expertise. This work benefited from support from the University of Connecticut, the Royal Society of New Zealand Marsden Fund (LCR0502) and the National Science Foundation (NSF DEB 04-22386, DEB 00-29679, DEB06- 19012(REU) and DEB 07-20664.

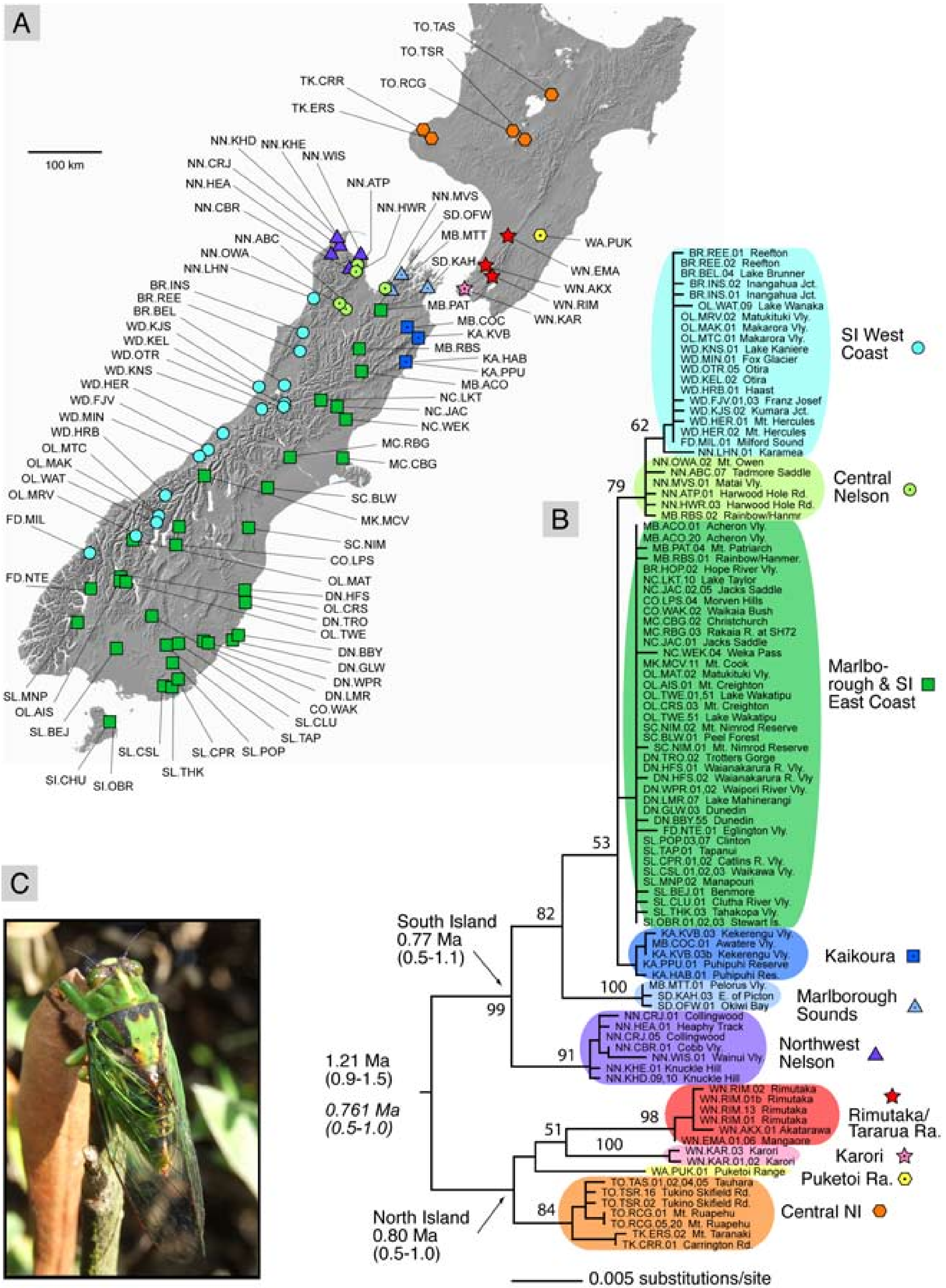


Figure 8. (a) **Kikihia subalpina** ***sampling localities. Colours correspond to haplotype associations noted on the phylogenetic tree. For locality codes see the original publication. (b) HKY85+I maximum-likelihood phylogenetic tree with nodal support values from 100 bootstrap replicates and geographic haplotype associations grouped by colour-shaded boxes. A molecular clock analysis found the root position to lie between the North Island and South Island clades. Strict molecular clock date estimates and 95% confidence intervals are shown for the most recent common ancestor of the root, the North Island (NI) clade, and the South Island (SI) clade. Also shown (in italics) is the date of the North Island/South Island population split, estimated from the gene tree root divergence time by correcting for ancestral genetic polymorphism. (c) K. subalpina male on native vegetation at Waimate, South Island. Reprinted with permission from Marshall, D. C.; Hill, K. B. R.; Fontaine, K.; Buckley, T.; Simon, C. 2009. Glacial refugia in a maritime temperate climate: Cicada (*****Kikihia subalpina* **complex) mtDNA phylogeography in New Zealand.** ***Molecular Ecology*** **18*****: 1995–2009.***

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