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ORIGINAL ARTICLE

After the deluge: mitochondrial DNA indicates Miocene radiation and Pliocene adaptation of tree and giant weta (Orthoptera: Anostostomatidae)

Steven A. Trewick* and Mary Morgan-Richards

Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Palmerston North, New Zealand

*Correspondence: Steven A. Trewick, Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Private Bag 11 222, Palmerston North, New Zealand.
E-mail: s.trewick@massey.ac.nz

ABSTRACT

Aim New Zealand broke away from the margins of Gondwana *c.* 75 Ma. Since then, New Zealand taxa derived from the Gondwanan biota are thought to have been exposed first to a subtropical climate on a low lying terrain, then severe land reduction during the Oligocene marine transgression, followed by much cooler climates of the Pliocene and Pleistocene, at which time mountain ranges emerged. The biological consequence of New Zealand's geological and climatic history is not well understood, in particular the extent to which the Oligocene acted as a biological bottleneck remains unresolved.

Methods We used mitochondrial cytochrome oxidase I and 12S DNA sequences to examine the extent of diversity and inferred timing of speciation of New Zealand weta (Anostostomatidae), a group of Orthoptera with a Gondwanan distribution generally thought to be ancient inhabitants of New Zealand.

Main conclusions We hypothesize that at least three distinct groups of weta survived the Oligocene marine transgression and radiated subsequently. Speciation followed during the Miocene and radiation into new habitats occurred during the Pliocene when mountain building created novel environments. Patterns of genetic diversity within species reflect, in some instances, geographical subdivision in the Pliocene, and in other cases, Pleistocene range changes resulting from climate change.

Keywords
Adaptive radiation, biogeography, cytochrome oxidase I, insects, New Zealand, Oligocene, phylogeography, Pliocene, 12S.

INTRODUCTION

New Zealand has an unusual and distinctive biota, much of which is thought to have evolved in isolation since the Cretaceous (Stevens, 1980; Cooper & Millener, 1993). Although a relatively large island archipelago (*c.* 269,000 km^2), New Zealand is small by continental standards (a little smaller than the state of California), and because of its position on the juncture of the Indian–Australian and Pacific plates, has been subjected to extensive geological disturbance since its oceanic isolation from the rest of Gondwana some 75 Ma. Periods of volcanism, tectonics and marine inundation have, in addition to climate change, produced a dynamic evolutionary environment. New Zealand's continental origin means that the history of its biota contrasts with that of volcanic island systems that have been the focus of many evolutionary studies (e.g. Hawaii – Fleischer *et al.*, 1998; Roderick & Gillespie, 1998). To what extent the biodiversity of New Zealand, at both ends of the taxonomic scale, reflects the vicariate and dynamic history is poorly understood. For instance, New Zealand lacks mammals (except for two bats), but the existence of mammalian fossils in Australia that predate separation of New Zealand (Archer *et al.*, 1985; Rich *et al.*, 1988; Flannery *et al.*, 1995) suggests they probably were an initial component of the biota. Absences from the modern biota may simply be explained by extinction but did the geological and climatic disturbances stimulate speciation too? We study the evolution of New Zealand's weta (Orthoptera:

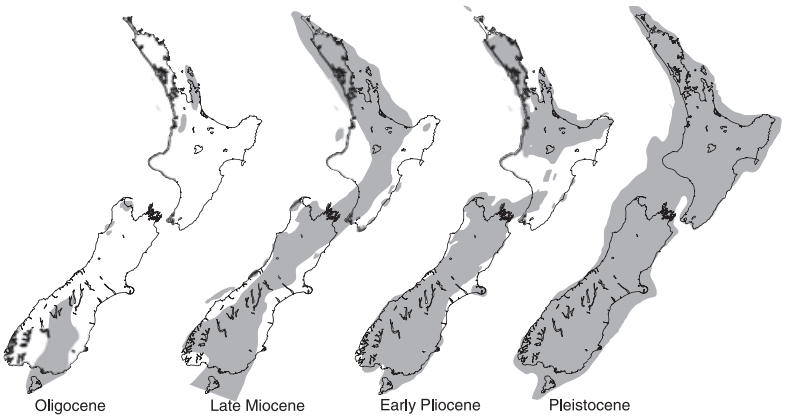

Figure 1 Palaeogeographical reconstructions indicating the historic extent of land (in grey) in New Zealand. Oligocene land area at times of high sea level after Cooper & Cooper (1995), others after Stevens (1980). The position of land is shown relative to the modern shape of New Zealand, but this has altered since the Oligocene because of tectonic activity.

Anostostomatidae) fauna in the context of this geological history.

By the Oligocene (35 Ma), extensive erosion and associated marine inundation had reduced New Zealand to a fragmented, low lying plain or peneplain, the largest drowned continent of the world (Stevens *et al.*, 1988). The emergent land surface probably amounted to *c.* 18% of its modern area (Cooper & Cooper, 1995) (Fig. 1). Indeed, some have speculated that more if not all of New Zealand was submerged at this time, but the geological evidence for this is equivocal (Pole, 1994; LeMasurier & Landis, 1996). The impact on the biota must have been considerable (Fleming, 1979), and Stevens (1980) was prompted to write emotively: 'On this exhausted rocky stump, this island prison, only the hardiest of plants and animals lingered on in the most stressful and difficult conditions'.

The Miocene climate of New Zealand was comparatively warm and wet, even subtropical, and the land low lying, but with increasing land area. During the Pliocene, the Kaikoura orogeny formed the Southern Alps in South Island, and elevated sea levels generated a low-lying archipelago in North Island. The epoch was marked by general climate cooling (McGlone, 1988 and references therein). The Pleistocene saw the extension and retraction of montane glaciers in the Southern Alps, changing sea level that altered the composition of the archipelago and shifting vegetation patterns responding to climate cycling (Fleming, 1979; McGlone, 1988) (Fig. 1).

One consequence of the dynamic geology is a poor fossil record for terrestrial fauna (Cooper & Millener, 1993; Daugherty *et al.*, 1993). However, genetic based methods have provided some much-needed insights into the age and patterns of diversification. For instance, marine inundation during the Oligocene (36–24 Ma) has been implicated in extinction (and subsequent radiation) of some bird groups including moa (Cooper & Cooper, 1995). Mountain-building and island formation during the Pliocene (7–2 Ma), and climate fluctuations in the Pleistocene (< 2 Ma) were apparently important stimulants of radiation in some plant genera (Lockhart *et al.*, 2001; Winkworth *et al.*, 2002; Heenan & Mitchell, 2003) and invertebrates (Chambers *et al.*, 2001; Morgan-Richards *et al.*, 2001; Trewick & Wallis, 2001).

The Anostostomatidae (Orthoptera) have a classic Gondwanan distribution (including South America, Australasia, South Africa), and are thought to have arrived in New Zealand prior to its isolation during the Cretaceous (Stevens, 1980). The group is comparatively speciose in New Zealand, where they are referred to as weta. Features such as gigantism and flightlessness are cited as adaptations consistent with long isolation from mammalian predators found elsewhere, and weta have been interpreted as occupying niches utilized by rodents elsewhere in the world (Fleming, 1977; Ramsay, 1978). While many of the apparently distinctive features are actually common to the group throughout its range in the southern hemisphere, the predominantly herbivorous diet of some New Zealand weta is unusual.

To understand better the pattern and timing of speciation and place morphological, ecological and behavioural characteristics of weta in a phylogenetic context we used mitochondrial DNA sequences from four genera of weta. We address questions relating to the monophyly of the so-called tree, giant and tusked weta; the depth/age of taxonomic diversity; biogeographical relationships of this diversity; and patterns of ecological diversification. Determining the extent of interspecific distances in this group might allow us to identify in which epochs diversification occurred. In particular we examine alternative scenarios for the timing and stimulus of species radiation: Miocene increase in land area, Pliocene habitat diversification/fragmentation, Pleistocene vegetation shifts associated with climate change.

Background to the systematics and ecology of weta

Four groups of anostostomatid weta are recognized in New Zealand: tree (*Hemideina*), giant (*Deinacrida*), ground (*Hemiandrus*) and tusked weta (*Anisoura*, *Motuweta*). *Hemideina* and *Deinacrida* comprise most (Johns, 1997) or all of the species of the small subfamily Deinacridinae (Gorochov, 2001). Johns (1997) also tentatively placed *Anisoura* (small tusked weta) in Deinacridinae but put *Motuweta* (other tusked weta) in Anostostomatinae (tribe Anostostomatini). Weta from Africa, Australia, south and central America, and elsewhere are placed within the Anostostomatinae, although Johns (1997) included

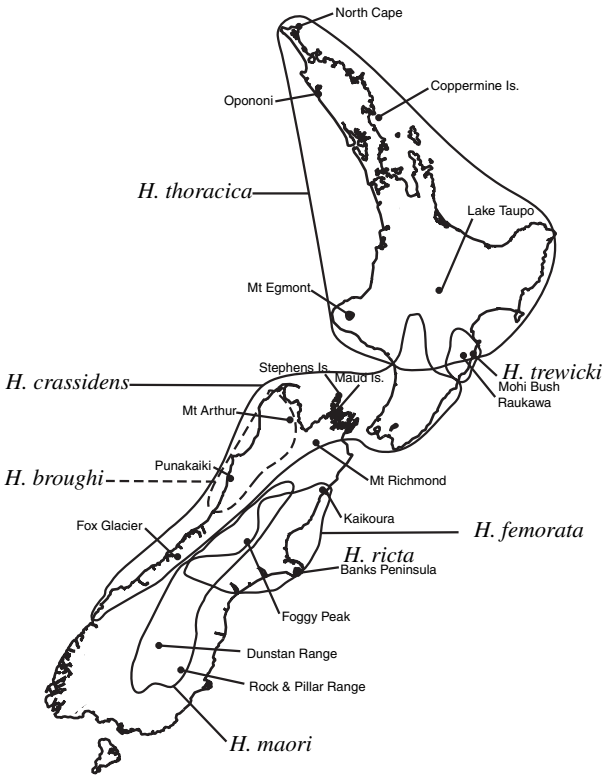

Figure 2 Extant ranges of seven tree weta (*Hemideina*) in New Zealand; the locations from which individuals used in this study were collected are indicated (range information from Trewick & Morgan-Richards, 1995; Field & Bigelow, 2001; Gibbs, 2001).

a taxon from India within the Deinacridinae. Molecular analysis indicates that the New Zealand tusked weta are allied to New Caledonian taxa (S.A. Trewick & M. Morgan-Richards, unpubl. data). *Hemideina* and *Deinacrida* are closely allied, and indeed finding support for the reciprocal monophyly of the two genera has so far proven difficult (Morgan-Richards & Gibbs, 2001). One species in particular (*H. broughi*) shares characteristics of both and it has been suggested that it represents the nearest form to the deinacridine common ancestor (Field, 1993; Gibbs, 2001), and that *H. broughi* might appropriately be placed in a separate, new genus (Field, 2001).

The tree weta (*Hemideina*) are the best known and most frequently encountered species because they are large, often frequent human habitats, and possess impressive spines on their hind legs that are used in defensive displays. The adult males of most species of *Hemideina* have an enlarged head and mandibles (Field & Deans, 2001). Most tree weta species are abundant and have wide distributions (Fig. 2). They conceal themselves by day in tree holes or (in one instance) beneath stones. The giant weta (*Deinacrida*) include some very large species, and all but one of the 11 species are the subject of conservation efforts having suffered the effects of introduced mammalian predators (Gibbs, 1998; McGuinness, 2001; Sherley, 2001). Tree and giant weta will scavenge invertebrate food (usually dead) but they are, unusually among anostostomatids, primarily herbivores that feed on the leaves, flowers and fruit of trees and shrubs (Table 1). In contrast, the ground (*Hemiandrus*) and tusked weta (*Motuweta*, *Anisoura*) are predators, primarily active on the ground, and sheltering by day in burrows.

MATERIALS AND METHODS

Sampling

We sampled all species of tree (seven species), giant (11 species), plus the three tusked weta and four ground weta for comparison. A number of species have been sampled fairly extensively throughout their respective ranges, and their population genetics reported in detail (Trewick *et al.*, 2000; Morgan-Richards *et al.*, 2001; Trewick, 2001; Morgan-Richards, 2002; King *et al.*, 2003). For the present study we obtained data from individuals of the remaining *Hemideina* and *Deinacrida* and tusked species utilizing, wherever possible, specimens collected for studies of morphology, allozymes and cytogenetics (Morgan-Richards, 1995, 1997, 2000; Morgan-Richards & Townsend, 1995; Cameron, 1996; Morgan-Richards & Gibbs, 1996, 2001; Gibbs, 1999; Trewick & Wallis, 2001). Other taxa were collected in the field during the course of the present study, or were provided to us by researchers engaged in ecological studies (including captive rearing) of the rare species. Samples sizes are for the most part small, reflecting the rarity and conservation status of many of the species. Where possible we obtained specimens and data from several individuals to provide an indication of minimum within-species genetic diversity across geographical ranges, but this was not a priority of the study. In all instances, appropriate authority to collect and use specimens was obtained from the New Zealand Department of Conservation.

Molecular methods

Muscle tissue from freshly killed, frozen or alcohol preserved specimens was removed from hind femora or other leg element for DNA extraction. Whole genomic DNA was extracted using a salting-out method (Sunnucks & Hale, 1996). Tissue was macerated and incubated with 5 μL of 10 mg mL^{-1} proteinase-K in 600 μL of TNES buffer (20 mM ethylenediaminetetraacetic acid, 50 mM Tris, 400 mM NaCl, 0.5% sodium dodecyl sulphate) at 50 °C for 1–4 h. 10% 5 M NaCl was added and the extractions shaken vigorously for 20 s followed by spinning at 16,000 g for 5 min. The supernatant was removed and precipitated with an equal volume of cold 100% ethanol. DNA was collected by spinning and washed with 70% ethanol, then dried and dissolved in water.

Molecular analysis used mitochondrial DNA sequences obtained using primers that target the 3′ end of cytochrome oxidase I (COI) (C1-J-2195 and L2-N-3014, Simon *et al.*, 1994), and the third domain of the small ribosomal subunit (12S) plus 5′ end of the 16S gene and tRNA valine (SR-N-14588 and LR-J-13417, Simon *et al.*, 1994). Polymerase chain reaction (PCR) was performed in 25 μL reactions (200 μM

Table 1 Summary of ecological features of weta included in study

Species	Group	Current range	Region	NI/SI	Altitude	Habitat type	Source Refs.
H. trewicki Morgan-Richards	Tree	Narrow	Hawkes Bay (mideast)	NI	Lowland	Arboreal in tree holes	1
H. crassidens (Blanchard)	Tree	Wide	Southern NI, northwest SI	NI + SI	Lowland	Arboreal in tree holes	2
H. maori Pictet & Saussure	Tree	Wide	Southern & central	SI	Sub/alpine	Terrestrial under stones	3
H. ricta Hutton	Tree	Narrow	Bank's Peninsula	SI	Lowland	Arboreal under rocks/in tree holes	3, 4
H. femorata Hutton	Tree	Wide	North eastern	SI	Lowland	Arboreal in tree holes	3
H. thoracica (White)	Tree	Wide	Central & north North Island	NI	Lowland	Arboreal in tree holes	5
H. broughi (Buller)	Tree	Narrow	Northwest South Island	SI	Subalpine	Arboreal in tree holes	3
D. talpa Gibbs	Giant	Narrow	Paparoa Range	SI	Subalpine	Terrestrial excavates burrows	3
D. pluvialis Gibbs	Giant	Narrow	West coast mountains	SI	Subalpine	Terrestrial under stones	3
D. connectens Ander	Giant	Wide	Southern Alps	SI	Alpine	Terrestrial lives in scree	6
D. tibiospina Salmon	Giant	Narrow	Nelson area	SI	Subalpine	Terrestrial in vegetation	3
D. carinata Salmon	giant	Narrow relict	Foveaux Strait islands	SI	Lowland	Terrestrial in vegetation	
D. heteracantha White	Giant	Narrow relict	Little Barrier Island	NI	Lowland	Arboreal in vegetation	3
D. mahoenui Gibbs	Giant	Narrow relict	Mahoenui (midwest)	NI	Lowland	Arboreal in vegetation	3
D. fallai Salmon	Giant	Narrow relict	Poor Kniqhts Islands	NI	Lowland	Arboreal in vegetation	3
D. parva Buller	Giant	Narrow	Seaward Kaikoura Ra.	SI	Subalpine	Terrestrial in vegetation/ under rocks	3
D. rugosa Buller	Giant	Narrow relict	Cook Strait islands	Between	Lowland	Terrestrial in vegetation	3
D. elegans Gibbs	Giant	Narrow	Central and northeast (disjunct)	SI	Subalpine	Terrestrial rock crevices	3
Anisoura nicobarica Ander	Tusk		Northland	NI		Terrestrial excavates burrows	
Motuweta isolata Johns	Tusk		Middle Mercury Is.	NI		Terrestrial excavates burrows	
M. riparia Gibbs	Tusk		North-east	NI		Arboreal in tree holes	

Source references for details of location sampling are as follows (Source Refs): 1, Morgan-Richards (1995); 2, Morgan-Richards *et al.* (1995), Morgan-Richards (2002); 3, Morgan-Richards & Gibbs (2001); 4, Morgan-Richards & Townsend (1995); 5, Morgan-Richards (1997), Morgan-Richards *et al.* (2001); 6, Morgan-Richards & Gibbs (1996), Trewick *et al.* (2000), Trewick (2001).

dNTPs, 2.5 mM $MgCl_2$, 0.25 U Qiagen Taq), treated to 40 cycles of 94 °C for 15 s, 50 °C for 30 s, 72 °C for 90 s with an initial denaturation of 94 °C for 60 s. Amplification products were either gel-purified using Qiaquick spin columns (Qiagen, Hilden, Germany) or cleaned directly using High Pure purification columns (Roche, Indianapolis, IN). Cycle sequencing employing primers C1-J-2195 and SR-N-14588 used Bigdye chemistry (Perkin Elmer, Boston, MA) following the manufacturer's protocols. Sequences were aligned manually using SeqEd. v1.0.3 (ABI, PE).

One species (*H. femorata*) consistently yielded COI sequences with ambiguous calls indicating the presence of one or more nuclear copies (Bensasson *et al.*, 2001). PCR with primers that amplify a larger fragment comprising adjacent parts of the COI and COII genes produced products of two sizes that were separable by gel electrophoresis. Sequencing of the product of expected size produced unambiguous sequence that aligned appropriately with COI and COII genes.

Phylogenetic analysis employed neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) methods implemented by PAUP* v.4.0b10 (Swofford, 2002). NJ trees were initially obtained using observed distances and compared with results produced with more complex distance estimations. MP analysis used either unweighted and unordered data or one of a range of transition/transversion weightings, and we explored the effects of weighting among codon positions for the COI data. For ML analyses, permutations of alternative nucleotide substitution and among-site rate variation models (I – invariable sites and Γ – gamma distribution) were first assessed by comparing likelihood scores for a suite of models in order to achieve the best compromise between parameter richness and likelihood scores (Sullivan *et al.*, 1997). We used either the shortest unweighted MP tree or an NJ tree as starting points for these searches and compared the resulting log likelihood scores using χ^2 tests. All ML analyses used empirical base frequencies and in ML tree searches we obtained an initial tree by stepwise addition followed by TBR branch-swapping. We examined support for nodes using nonparametric bootstrapping with 1000 replications for MP and NJ searches, and 200 or 500 replications for ML searches depending on the number of taxa involved.

Molecular clock

To obtain estimates of the age of haplotype diversity among the weta studied we applied a molecular clock calibration of 2% sequence divergence per million years. This rate is derived from studies of arthropod taxa using mitochondrial data (Brown *et al.*, 1979; Brower, 1994; Juan *et al.*, 1995, 1996; Fleischer *et al.*, 1998) calibrated using geological evidence.

Such a rate appears to be applicable to mtDNA data from a broad range of arthropod groups. But, the relationship between time and observed genetic dissimilarity is not linear; observed genetic distances tend to plateau as actual genetic divergence (and time) increases. This is the expected masking effect of repeated mutations at some nucleotide sites. To compensate for this 'multiple hit' effect we used parameter-rich models of molecular evolution that take into account among site rate variation and more complex patterns of nucleotide substitution. Distance estimations using such methods are very similar to observed genetic distances (or distance from simpler models) at shallow divergences, but the extent of the disparity between observed and 'corrected' distance tends to increase with increasing distance. It is assumed that this pattern reflects the tendency of observed distances or simple models to underestimate true genetic divergence with increasing time.

An alternative approach is to adjust the rate calibration rather than the distance estimation. Juan *et al.* (1995, 1996) used a relatively simple substitution model (Kimura 2 parameter) with DNA sequence data from beetles in the Canary Islands (dated from geological evidence) and estimated that genetic divergences of 8–10% corresponded to *c.* 5 Ma, 13–15% to 10 Ma, 16–18% to *c.* 15 Ma and 20% to *c.* 20 Ma (Sandoval *et al.*, 1998).

Spatial distribution of haplotype diversity within two species (*D. connectens*, *H. thoracica*) correlates with habitat subdivision in the Pliocene (Trewick *et al.*, 2000; Morgan-Richards *et al.*, 2001). These examples provide useful points of reference in the present analyses indicating minimum coalescence times among taxa (Sandoval *et al.*, 1998). Prior to applying molecular clock calibrations to our data, we first checked the validity of assuming that sequences in our analyses evolved in a clocklike manner by comparing log likelihood scores for trees resulting from analyses with and without a molecular clock enforced using χ^2 tests.

RESULTS

Our analyses utilized 52 COI and 25 12S sequences with aligned lengths of 510 and 440 bp respectively. We sequenced 64 individuals from 21 ingroup taxa and four outgroup *Hemiandrus* for COI, and 23 individuals of the same ingroup taxa (with each species represented once except *H. maori* and *H. femorata*) plus two *Hemiandrus* for 12S. Our data therefore comprise one or more representatives of each of 11 giant weta (*Deinacrida*), seven tree weta (*Hemideina*), three tusked weta (*Anisoura*, *Motuweta*) and four ground weta (*Hemiandrus*). The provenance of ingroup samples is indicated in Figs 2 and 3, with details referenced in Table 1. A combined COI/12S data set was created that comprised 25 sequences of 950 bp each representing all the species in our analysis.

As is typical for insects, these sequences were AT rich (Fig. 4). The COI sequences comprised 28.5% A, 35.5 % T, 19% C and 16.6% G overall, but third codon positions were the most heavily AT biased (41.6% A, 35% T, 17.8% C, 5.4% G). The set of 25 combined COI/12S sequences had a mean composition of 30.4% A, 35.6% T, 12.4% C, 21.7% G. Using

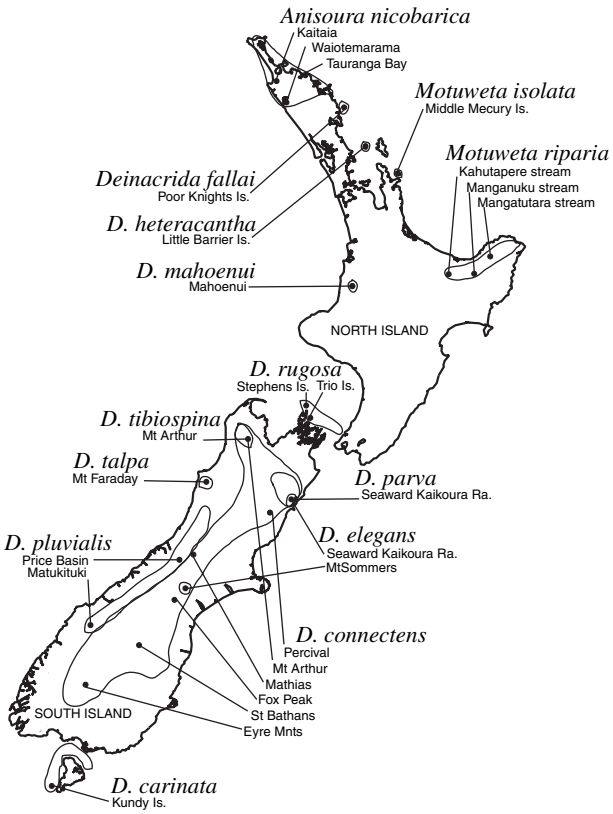

Figure 3 Approximate extant ranges of giant (*Deinacrida*) and tusked (*Motuweta*, *Anisoura*) weta in New Zealand; the locations from which individuals used in this study were collected are indicated (range information from Field & Bigelow, 2001; Gibbs, 2001).

χ^2 tests implemented by PAUP* we accepted the hypothesis of base frequency homogeneity among all sequences for all sites. However, we had to reject this hypothesis for COI third codon sites alone ($\chi^2 = 293.7$, d.f. $= 165$, $P < 0.001$) and because most informative sites are at third codon positions, we had to reject the hypothesis of base homogeneity for parsimony informative sites too ($\chi^2 = 304.5$, d.f. $= 165$, $P < 0.001$). We also found that for the combined COI/12S data set including all taxa and all sites we could accept the hypothesis of base homogeneity ($\chi^2 = 42.3$, d.f. $= 72$, $P > 0.05$), although this was not the case for informative sites only ($\chi^2 = 142.6$, d.f. $= 72$, $P < 0.0001$). Comparisons of base frequencies for each sequence revealed that the tusked wetas (in particular *M. riparia*) have the highest AT content (Fig. 4). Among the tree and giant weta with these data, base composition at parsimony informative sites was apparently less heterogenous: COI – $\chi^2 = 159.7$, d.f. $= 129$, $P = 0.035$, COI/12S – $\chi^2 = 84.55$, d.f. $= 57$, $P > 0.01$. Compared with the other weta, *Hemideina thoracica* has the lowest AT content (58% vs. the next lowest 62%) and the disparity is much more marked at third codon positions (61% vs. 70%). When *Hemideina thoracica* was excluded from our COI data we could accept the hypothesis of base homogeneity for informative sites ($\chi^2 = 75.3$, d.f. $= 117$, $P > 0.05$).

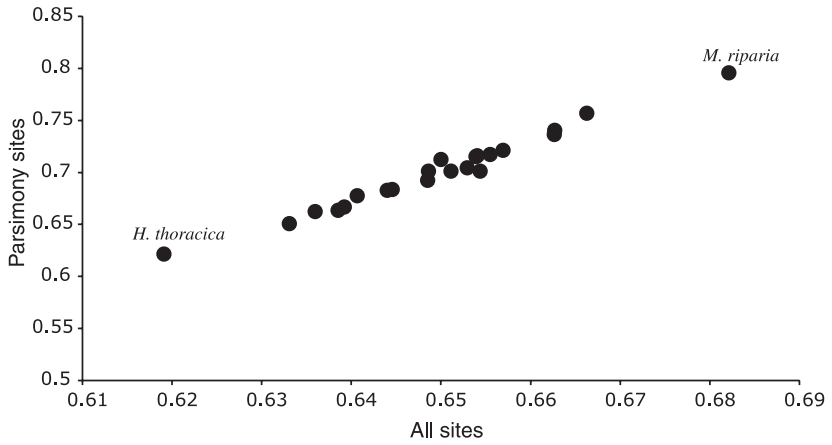

Figure 4 Nucleotide composition of mtDNA sequences in New Zealand weta AT content, at parsimony sites versus all sites. Each point represents composition of a single COI/12S sequence per species.

Phylogenetic relationships

All NJ and MP analyses of our total COI data set resulted in trees with similar topologies, irrespective of substitution model and character weighting used (Fig. 5). The three tusked weta species (*Motuweta isolata*, *M. riparia*, *Anisoura nicobarica*) always formed a monophyletic group, with ground weta (*Hemiandrus*) basal to these (see below). Similarly, the tree and giant weta formed a monophyletic group with respect to the tusked weta (*Motuweta* and *Anisoura*) and ground weta (*Hemiandrus*).

Although *Hemideina* and *Deinacrida* were not reciprocally monophyletic, several species associations were evident and supported in all our analyses: Clade I – *Deinacrida tibiospina*, *D. carinata* and *D. connectens*; II – *D. fallai*, *D. heteracantha* and *D. mahoenui*; III – *D. rugosa* and *D. parva*; IV – *H. crassidens* and *H. trewicki*; V – *H. maori* and *H. ricta*; VI – *D. talpa* and *D. pluvialis* (Fig. 5). These same groupings were found in our analysis of the 12S data, although we additionally found support for nodes grouping clades II and III (MP bootstrap support with unweighted and Tv : Ti weighted 5 : 1 was 71% and 78% respectively), and for clade V with *H. thoracica* (69% and 75% respectively, not shown).

Haplotypes from individuals of the same species formed monophyletic clades, with the exception of two instances of paraphyly. As previously reported, *Deinacrida pluvialis* sampled from two sites was paraphyletic with respect to *D. talpa* (Trewick & Wallis, 2001), and *Hemideina maori* was paraphyletic with respect to *H. ricta* (King *et al.*, 2003).

For a subset of weta representing all tree, giant and tusked weta, plus two ground weta we combined COI and 12S data for further analysis in an attempt to resolved deeper level relationships. Analysis of 25 sequences using NJ and MP supported our findings from separate analysis of COI and 12S. We subsequently divided this data set into two parts comprising the tree and giant weta, and the tusked and ground weta.

With the combined COI/12S data for the tree and giant weta group reduced to one representative per species, all analyses resulted in polyphyletic trees as before (not shown). We found support for a clade comprising *H. broughi* and Clade VI (*D. talpa*, *D. pluvialis*), which had also been indicated in other trees (e.g. Fig. 5). We also found support for the grouping of *D. elegans* and clades II and III in MP analysis using a Tv : Ti weighting of 5 : 1. We examined the effect of constraining tree topology to make tree and giant weta monophytic using MP. In all cases constrained trees were longer than unconstrained trees. For instance, using 18 taxa MP with unordered characters yielded one tree of length 898, constraining the search to make *Hemideina* monophyletic results in two trees of 907 steps. However, constraining the search to keep *Hemideina* without *H. broughi*, monopyletic results in a single tree just three steps longer (901) than the unconstrained tree (mean random tree length 1240).

We further reduced the size of the COI/12S data set by including only a single representative of each clade supported in other analyses. This resulted in a set of 10 taxa (five *Hemideina* and five *Deinacrida*). Tree searches using these data with NJ, MP and ML returned similar topologies. Nonparametric bootstrapping with MP (Tv : Ti 5 : 1), NJ (p distance, K2p, LogDet) and ML (HKY, GTR + I + G) all yielded support for the same four nodes within this topology and resolution of three clades (*H. broughi* with *D. talpa*; *D. elegans* with *D. fallai* and *D. parva*; *H. thoracica* with *H. maori*). Internal edges between these clades were short implying little signal at this level (Fig. 6).

Genetic distance and age

We estimated genetic diversity using observed distance, and Kimura 2 parameter (K2p) and ML GTR + I + Γ models. In all instances genetic distances using the same model were higher for COI data compared with the combined COI/12S data (Table 2). In the COI data set, which comprised more individuals than the combined COI/12S data, the genetically closest pair of tree weta (*H. ricta* and Foggy Peak *H. maori*, and giant weta (*H. heteracantha* and *H. fallai*) had observed distances of 0.031 and 0.014 respectively (Table 2). Within species K2p distances for COI were highest in *H. thoracica* (0.097). Haplotypes representing the two disjunct populations of *D. elegans* (Fig. 3) differed by 0.039 (Table 2).

The K2p distances between combined COI/12S species haplotypes reached 0.19 (mean = 0.14) among tree and giant

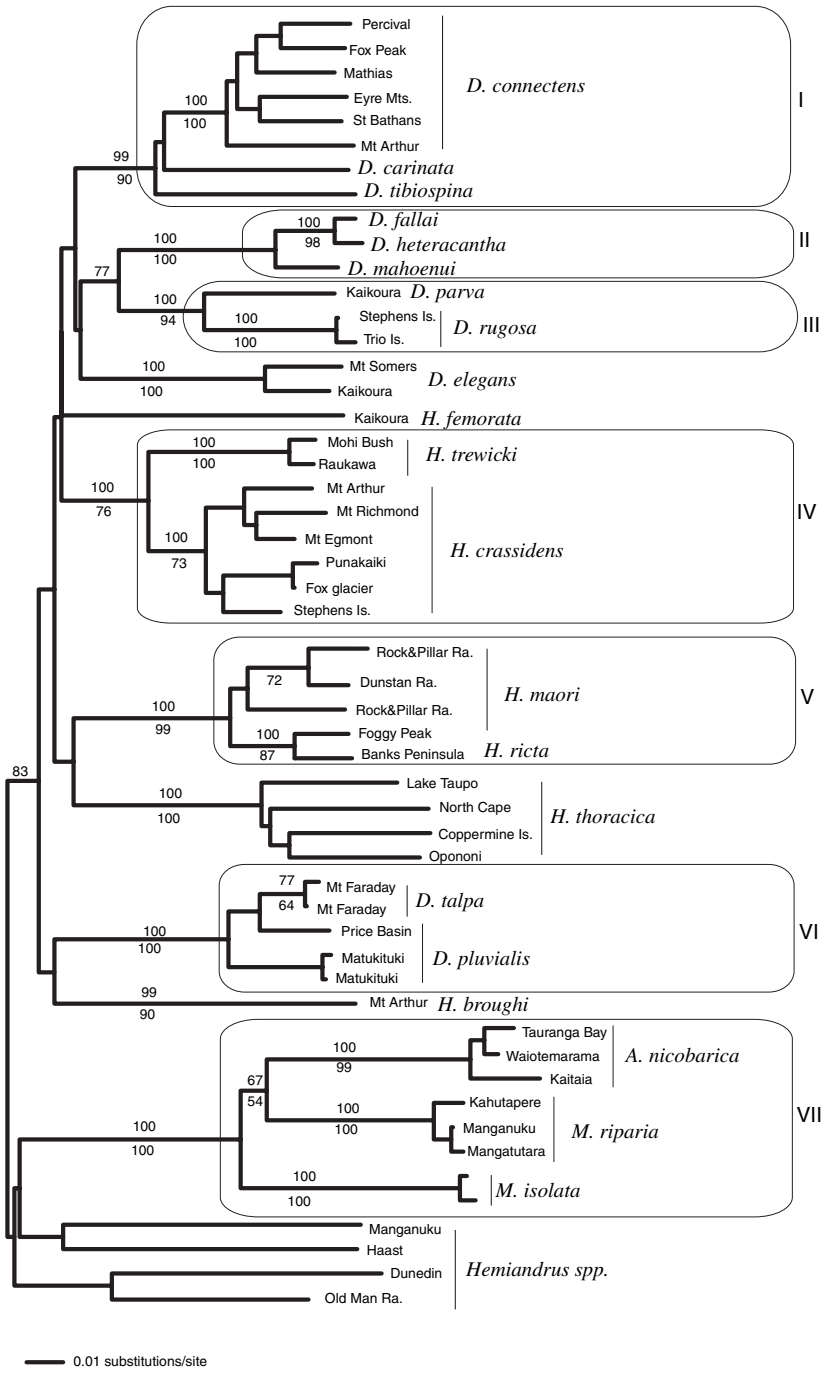

Figure 5 Neighbour-joining (NJ) tree using observed distances, comprising weta sequenced for COI included in the present study. Numbers above and below edges indicate bootstrap support using NJ (observed distance) and maximum parsimony (fast search option, unordered characters) respectively.

weta species (*H. thoracica* and *H. broughi*), and had a maximum of 0.25 (mean = 0.22) in comparisons of tusked weta and tree/giant weta (Table 3). K2p distance among the tusked weta species averaged 0.11. Estimates using methods that incorporate more complex nucleotide substitution and among site variation models were, as expected, much higher. For example GTR + I + Γ distance among tree and giant weta reached 0.27, and between tree/giant and tusked weta 0.72 (Table 3).

We found better phylogenetic resolution among the more species rich giant weta (*Deinacrida*) than tree weta (*Hemideina*), and we found evidence of base frequency variation among tree weta species. Therefore, using our COI/12S dataset for only the *Deinacrida* species we compared ML tree lengths with and without a molecular clock enforced. Using a χ^2 test we found that these sequences were evolving in a clocklike manner (Fig. 7). Likelihood genetic distances (GTR + I + Γ) were used to estimate the age of divergences. This revealed coalescence events within the genus over a long time range, dating back to the early Miocene. The shallowest phylogenetic splits in these data are among members of the northern, arboreal giant weta (*D. heteracantha* complex), which appear

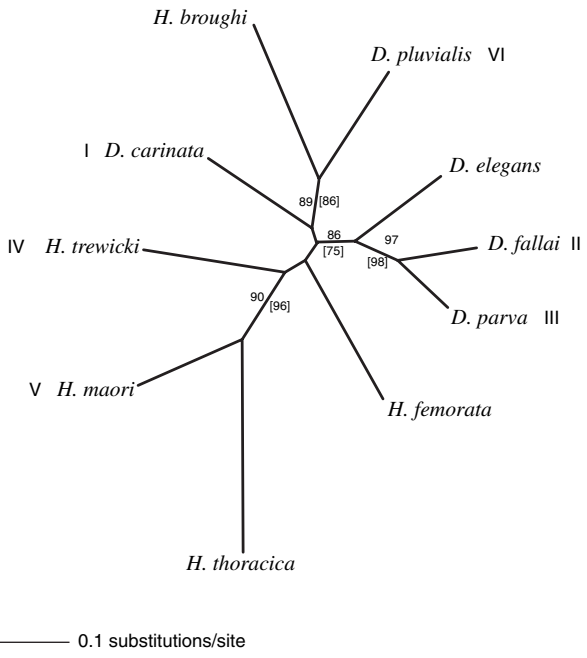

Figure 6 Maximum likelihood tree from COI/12S data using GTR + I + Γ for species representative of *Deinacrida* and *Hemideina* clades/lineages. Clade labels from Fig. 5 are shown. Numbers on edges indicate bootstrap support under ML GTR + I + Γ model and [MP with Tv : Ti 5 : 1].

to have speciated during the Pleistocene. Clades comprising species adapted to distinct habitats appear to have evolved during the Pliocene (e.g. *D. connectens* group).

The alternative approach of applying a sliding age calibration scale as estimated for COI sequences using K2p distances from beetles and calibrated using geological evidence (Juan *et al.*, 1995, 1996) yielded similar estimates to those obtained using GTR + I + Γ (Fig. 7). K2p distances among COI sequences from representatives of tree and giant weta species reached 0.24 (0.22 excluding *H. broughi*) and 0.23 respectively (Table 3), indicating radiation within the group dating back > 20 Ma. Within species diversity among COI haplotypes was substantial (0.055 in *D. talpa* to 0.097 in *H. thoracica*) in most species sampled for several individuals (Table 2), indicating coalesence in the Pliocene (7–2 Ma) or early Pleistocene (< 2 Ma). Coalesence of haploytpes of close species (e.g. K2p 0.032 between *H. ricta* and neighbouring *H. maori*) indicate speciation after the mid-Pleistocene. Deeper divergences among species in clades and among clades date to the Pliocene and before. For example, K2p among members of clade I range from 0.10 to 0.13 indicating divergence 5–10 Ma, and between *D. elegans* and clades II/III 0.16–0.17 indicating divergence *c.* 15 Ma.

DISCUSSION

Phylogenetics

In our analyses, the tree and giant weta are monophyletic with respect to the ground and tusked weta, and we found strong support for monophyly of the tusked weta. This is consistent with many morphological and behavioural traits that group tree and giant weta as the Deinacridinae (Gorochov, 2001). Clades I–VI revealed in our analyses of COI sequence data are consistent with those indicated by allozyme and morphological data (Field, 2001; Morgan-Richards & Gibbs, 2001). We did not find strong support for the reciprocal monophyly of the tree and giant weta with the present data set. This is interesting because in terms of morphology and ecology the tree weta (*Hemideina*), with the exception of *H. broughi*, form a homogenous group with little variation among species, and so we expected their mtDNA to form a monophyletic group too. Analysis of morphological and nuclear genetic markers was consistent with monophyly of the genera although it did not strongly support this split (Morgan-Richards & Gibbs, 2001). The group comprises generalist, arboreal, allopatric species.

Resolution of *Hemideina* relationships was poor. Three species pairings were revealed, and two of these (*H. crassidens* with *H. trewicki* and *H. maori* with *H. ricta*) are consistent with evidence from morphology and allozymes (Morgan-Richards & Gibbs, 2001). *Hemideina maori* is paraphyletic with respect to *H. ricta* which, on the basis of mtDNA sequences, is a very recent isolate of the more widespread species. It is interesting to note therefore that *H. maori* is, among tree weta, unusual in seeking refuge beneath rocks in its alpine habitat. The use of tree holes (as well as rocks) by *H. ricta* appears to be a reversal to the typical state. *Hemideina crassidens* and *H. trewicki* are morphologically similar species with parapatric ranges.

We found signal in our data indicating a close association of the aberrant tree weta *H. broughi* and *Deinacrida* species (Fig. 6), and this is consistent with at least some morphological characteristics. For instance, most tree weta (*Hemideina*) show secondary sexual dimorphism with males having enlarged heads and mandibles as in many genera of the Anostostomatidae (Field & Deans, 2001). *Hemideina broughi* does not express this dimorphism and in this respect is more like *Deinacrida*. Such inconsistencies have long been recognized, and the species has been referred to as the stem species of living Deinacridinae (Field, 1993; Gibbs, 2001; Morgan-Richards & Gibbs, 2001).

In contrast to the tree weta, the giant weta (*Deinacrida*) comprise an ecologically diverse group with most of that diversity in South Island (Fig. 3). The geographical bias in diversity reflects a dichotomy between the generalist, arboreal species that predominate in North Island (in many respects similar to the tree weta), and the specialist, alpine taxa of South Island. The largest deinacridine clade in our analysis includes South Island and North Island taxa (*D. elegans*, *D. rugosa*, *D. parva*, *D. mahoenui*, *D. fallai*, *D. heteracantha*) and was also apparent in a combined analysis of allozyme and morphological characters (Morgan-Richards & Gibbs, 2001).

The six deep lineages indicated in our analyses (*H. broughi* and clade VI; *D. elegans* and clades II and III; *H. femorata*;

Table 2 Pairwise genetic distances among weta species based on combined COI/12S sequence data. GTR + I + Γ above diagonal, observed distance below diagonal (COI &12S/COI). Column headings are abbreviated species names given in left column. Clades indicated by phylogenetic analysis of full COI data set and COI/12S data are indicated by roman numbering (I–VII) (from Fig. 5), and boxes respectively. Bold boxes indicate clades in ML analyses (Figs 6 and 7)

		H. tre	*H. cras*	*H. mao*	*H. ric*	*H. fem*	*H. tho*	*H. bro*	*D. tal*	*D. plu*	*D. con*	*D. tib*	*D. car*	*D. het*	*D. fal*	*D. mah*	*D. par*	*D. rug*	*D. ele*	*A. nic*	*M. rip*	*M. iso*
MOHI522	*H. trewicki*		0.07	0.27	0.32	0.28	0.37	0.32	0.29	0.30	0.25	0.31	0.31	0.26	0.27	0.26	0.26	0.26	0.26	0.78	0.67	0.71
SI55	*H. crassidens*	0.06/0.09	**IV**	0.26	0.30	0.26	0.34	0.29	0.26	0.27	0.21	0.27	0.23	0.23	0.24	0.23	0.22	0.23	0.21	0.73	0.61	0.65
DUN1	*H. maori*	0.14/0.17	0.14/0.16		0.06	0.31	0.28	0.33	0.34	0.35	0.29	0.36	0.31	0.33	0.32	0.30	0.28	0.31	0.33	0.77	0.77	0.68
HR320	*H. ricta*	0.16/0.18	0.15/0.17	0.05/0.07	**V**	0.32	0.29	0.36	0.31	0.33	0.32	0.38	0.33	0.31	0.31	0.30	0.26	0.29	0.34	0.71	0.70	0.63
Hf181	*H. femorato*	0.14/0.17	0.14/0.17	0.15/0.18	0.16/0.18		0.43	0.36	0.33	0.32	0.25	0.27	0.26	0.35	0.35	0.31	0.29	0.28	0.27	0.74	0.71	0.70
TA440	*H. thoracica*	0.17/0.19	0.16/0.18	0.16/0.19	0.15/0.18	0.18/0.22		0.45	0.37	0.36	0.36	0.37	0.34	0.37	0.38	0.39	0.34	0.39	0.35	0.89	0.79	0.68
HB254	*H. broughi*	0.16/0.19	0.15/0.18	0.16/0.18	0.17/0.19	0.17/0.20	0.19/0.24		0.26	0.27	0.31	0.37	0.31	0.34	0.33	0.33	0.34	0.32	0.32	0.89	0.80	0.78
PAP822	*D. talpa*	0.14/0.17	0.13/0.15	0.16/0.21	0.15/0.19	0.15/0.19	0.17/0.21	0.14/0.18		0.05	0.25	0.29	0.30	0.24	0.25	0.25	0.27	0.28	0.32	0.79	0.66	0.70
MTK844	*D. pluvialis*	0.15/0.17	0.14/0.17	0.17/0.22	0.16/0.20	0.15/0.18	0.17/0.20	0.14/0.19	0.05/0.06	**VI**	0.26	0.30	0.31	0.25	0.27	0.27	0.28	0.30	0.28	0.77	0.65	0.72
TKT959	*D. connectens*	0.13/0.16	0.12/0.15	0.15/0.19	0.15/0.19	0.13/0.18	0.16/0.20	0.15/0.20	0.13/0.18	0.13/0.18		0.10	0.08	0.27	0.27	0.26	0.23	0.21	0.25	0.71	0.68	0.67
Dtib160	*D. tibiospina*	0.15/0.18	0.14/0.17	0.16/0.20	0.17/0.20	0.14/0.18	0.16/0.19	0.17/0.21	0.14/0.17	0.14/0.18	0.08/0.12		0.11	0.31	0.31	0.30	0.25	0.26	0.28	0.79	0.73	0.78
Kundy	*D. carinata*	0.15/0.19	0.12/0.15	0.15/0.18	0.16/0.19	0.13/0.18	0.16/0.18	0.15/0.19	0.15/0.19	0.15/0.20	0.06/0.10	0.08/0.13	**I**	0.28	0.28	0.26	0.23	0.24	0.26	0.79	0.72	0.74
220.00	*D. heteracantha*	0.13/0.16	0.12/0.15	0.16/0.20	0.15/0.20	0.17/0.21	0.17/0.20	0.16/0.21	0.13/0.17	0.13/0.18	0.14/0.20	0.14/0.18	0.14/0.19		0.02	0.03	0.15	0.14	0.21	0.72	0.72	0.69
DF620	*D. fallai*	0.14/0.16	0.13/0.15	0.16/0.20	0.15/0.19	0.16/0.20	0.17/0.20	0.16/0.20	0.13/0.17	0.14/0.18	0.14/0.19	0.14/0.18	0.14/0.18	0.01/0.01		0.03	0.14	0.14	0.20	0.74	0.73	0.71
180	*D. mahoenui*	0.13/0.16	0.12/0.15	0.15/0.18	0.15/0.18	0.15/0.18	0.17/0.20	0.16/0.20	0.13/0.17	0.14/0.18	0.13/0.19	0.14/0.18	0.14/0.17	0.03/0.04	0.03/0.04	**II**	0.13	0.14	0.20	0.71	0.72	0.67
DP15	*D. parva*	0.14/0.17	0.12/0.15	0.14/0.17	0.13/0.16	0.14/0.18	0.16/0.19	0.16/0.21	0.13/0.17	0.14/0.18	0.12/0.17	0.13/0.16	0.12/0.16	0.10/0.15	0.10/0.14	0.09/0.14		0.06	0.20	0.66	0.65	0.66
DR29	*D. rugosa*	0.13/0.17	0.13/0.16	0.15/0.20	0.15/0.18	0.14/0.17	0.17/0.22	0.16/0.20	0.14/0.17	0.14/0.18	0.12/0.16	0.13/0.17	0.12/0.16	0.09/0.13	0.09/0.13	0.09/0.14	0.05/0.08	**III**	0.20	0.67	0.70	0.71
SO611	*D. elegans*	0.14/0.16	0.12/0.15	0.16/0.18	0.16/0.20	0.14/0.17	0.16/0.19	0.16/0.20	0.16/0.23	0.14/0.19	0.13/0.18	0.14/0.17	0.13/0.17	0.12/0.17	0.12/0.16	0.12/0.16	0.12/0.17	0.12/0.16		0.76	0.70	0.70
NTW300	*A. nicobarica*	0.23/0.28	0.22/0.26	0.23/0.30	0.22/0.28	0.22/0.27	0.25/0.31	0.24/0.30	0.22/0.29	0.22/0.27	0.22/0.29	0.22/0.28	0.23/0.29	0.22/0.29	0.22/0.29	0.22/0.28	0.20/0.27	0.20/0.28	0.22/0.28		0.18	0.22
RTW100	*M. riparia*	0.21/0.23	0.20/0.24	0.23/0.26	0.22/0.25	0.21/0.25	0.24/0.29	0.23/0.27	0.21/0.25	0.21/0.26	0.22/0.27	0.22/0.26	0.22/0.25	0.21/0.28	0.22/0.28	0.21/0.28	0.20/0.24	0.20/0.25	0.21/0.26	0.11/0.14		0.16
MI100	*M. isolata*	0.22/0.25	0.21/0.24	0.22/0.26	0.21/0.25	0.22/0.28	0.21/0.25	0.23/0.26	0.22/0.27	0.22/0.27	0.22/0.27	0.23/0.28	0.22/0.26	0.22/0.29	0.22/0.29	0.22/0.29	0.21/0.27	0.22/0.28	0.22/0.27	0.12/0.17	0.10/0.13	**VII**

Table 3 Summary of within group genetic diversity in weta based on COI and combined COI/12S sequence data

		COI			12S/COI	
Clade	Species grouped	Min. p distance	Max. p distance	Max. K2p	Max. p distance	Max. GTR + I + G
I	*D. connectens, D. tibiospina, D. carinata*	0.088	0.114		0.072	0.106
II	*D. heteracantha, D. fallai, D. mahoenui*	0.014	0.041		0.028	0.033
III	*D. rugosa, D. parva*	0.079	0.079		0.046	0.06
IV	*H. crassidens, H. trewicki*	0.074	0.106		0.055	0.074
V	*H. maori, H. ricta* paraphyletic	0.031	0.078		0.048	0.065
VI	*D. pluvialis, D. talpa* paraphyletic	0.033	0.063		0.043	0.054
VII	*M. isolata, M. riparia, A. nicobarica*	0.116	0.156		0.112	0.218
	Forced monophyly					
	Hemideina excluding *H. broughi*		0.189		0.159	0.429
	Hemideina including *H. broughi*		0.204		0.165	0.445
	Deinacrida		0.192		0.139	0.317
	Deinacrida plus *H. broughi*		0.192		0.147	0.366
	Within species					
	Hemideina thoracica		0.091	0.097		
	Hemideina crassidens		0.071	0.077		
	Hemideina maori		0.068	0.073		
	Deinacrida connectens		0.076	0.082		
	Deinacrida talpa		0.053	0.055		
	Deinacrida elegans		0.039	0.04		

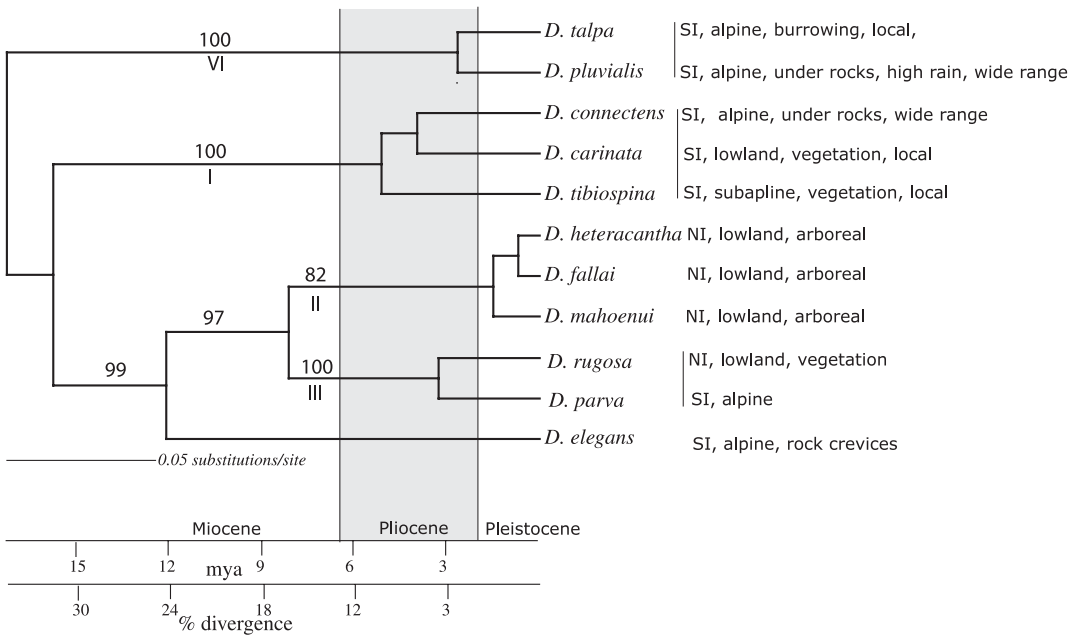

Figure 7 Maximum likelihood tree from COI/12S data using GTR + I + Γ with molecular clock enforced for all species of *Deinacrida*. Genetic distances are estimated using the model GTR + I + Γ with COI/12S data, and age estimated using calibration of 2% sequence divergence per million years. Clade labels from Fig. 5 are indicated below relevant edges.

H. thoracica and clade V; clade I; clade IV) may represent the early lineages of the tree/giant (Deinacridinae) radiation. Inconsistency in the placement of *H. thoracica* with respect to *H. maori*, i.e. tree weta not forming southern and northern clades as indicated by morphological and allozyme evidence (Morgan-Richards & Gibbs, 2001) is, we believe, a true discordance of the mitochondrial gene tree and the organismal tree. Together with lack of monophyly of the genera, this is consistent with a rapid radiation of the core Deinacridine lineages. A discussion of the systematic implications of these analyses is presented elsewhere (Trewick & Morgan-Richards, in press).

Biogeography, ecology and age of radiation

Oligocene and Miocene

The species we studied evidently fall into three, not four, main groups (Deinacridinae, tusked weta, *Hemiandrus*) differing only slightly from the existing taxonomy, and representing three ancient New Zealand lineages. However, we have by no means sampled to include all the diversity known to exist within the ground weta (*Hemiandrus*; Johns, 2001). Diversity within the Deinacridinae (tree and giant weta) probably dates to the Miocene and we have no evidence of New Zealand radiation prior to or during the Oligocene when New Zealand had its smallest land area. The lack of resolution of the basal nodes of the Deinacridinae tree might be due to rapid radiation following the Oligocene marine transgression. Our data are consistent with the Oligocene drowning hypothesis whereby much, but not all, of New Zealand was submerged at this time. At least three weta lineages appear to have survived in New Zealand through this event. The oldest splits between North Island and South Island are evident in the giant weta, and apparently date to the middle or late Miocene. The weta radiation we date to the Miocene includes three tree weta lineages comprising species with little morphological or ecological diversity, indicating allopatric rather than adaptive radiation.

Pliocene

In contrast to North Island, mountainous South Island presents a more diverse environment with respect to climate (especially rainfall), vegetation and surface geology. Such diversity constitutes alternative adaptive environments, and dispersal barriers. Fleming (1979) proposed that in many groups, including *Deinacrida* and *Hemideina*, alpine species evolved from lowland relatives during episodes of Pleistocene climate cooling. However, we find species radiation in weta and in particular *Deinacrida*, predate this. Even within alpine species there is strong phylogeographical evidence that population structuring dates to the Pliocene (Trewick *et al.*, 2000). Pliocene diversification is indicated for other animal groups including wren (Cooper & Cooper, 1995) and cicada (Buckley *et al.*, 2001). We suggest that the Pliocene emergence of mountains in South Island was the principal stimulus for diversification in many invertebrate groups (Trewick *et al.*, 2000; Trewick, 2001; Trewick & Wallis, 2001).

Within *Deinacrida* there are three well-supported, geographically distinct clades comprising species adapted to specific habitats. These are the South Island west coast montane species plus *H. broughi*, the Southern Alps group, and eastern South Island and North Island group (Fig. 8). The Southern Alps group is particularly striking, as it comprises the wide-ranging alpine *D. connectens*, the subalpine species *D. tibiospina* and the lowland *D. carinata*. The existence of a species gap (notably in *Deinacrida*), through lower North Island, and a phylogenetic split between essentially South Island taxa and the northern North Island *D. heteracantha* group in the late Miocene (Fig. 8) are consistent with an absence of land in the lower North Island until quite recently. Although a landmass bridging areas that now comprise the North and South islands is thought to have existed during the Miocene, the Manawatu Strait was open through lower North Island during the Plicocene. Terrestrial surfaces of modern lower North Island for the most part did not emerge until the second half of the Pleistocene, and the Manawatu Strait itself probably persisted longer than that (Heerdegen & Shepherd, 1992).

Pleistocene

The impact of Quaternary climates on the distribution of vegetation has been inferred from fossil pollen data (Wardle, 1963; McGlone, 1988). It is assumed that changes following the last glacial maxima are indicative of events during interglacials through the Pleistocene (McGlone *et al.*, 1993; Newnham *et al.*, 1999). Some information on the response of the biota has also been gleaned from deposits of Quaternary sub-fossil bones deposited in caves, dunes and swamps, although this is largely restricted to vertebrate taxa (e.g. Worthy & Holdaway, 1996;

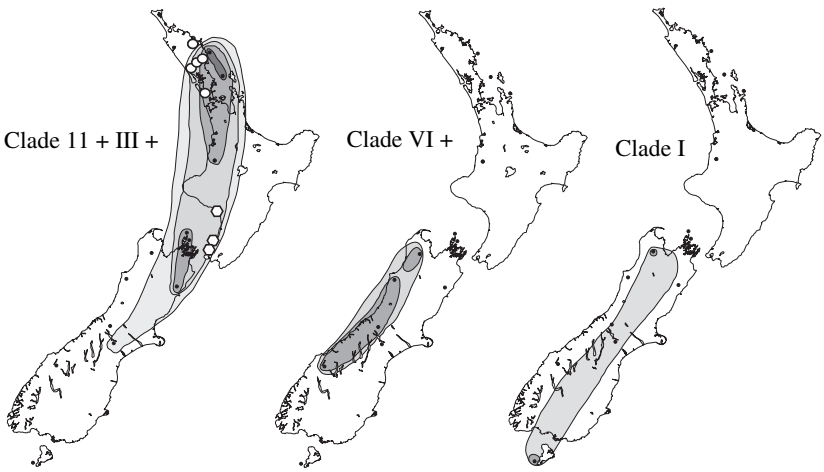

Figure 8 Biogeographical contour plots for *Deinacrida* clades. Locations of early records for *D. heteracantha* and *D. rugosa* (clade II+III+) are indicated by open circles and hexagons, respectively (after Watt, 1963).

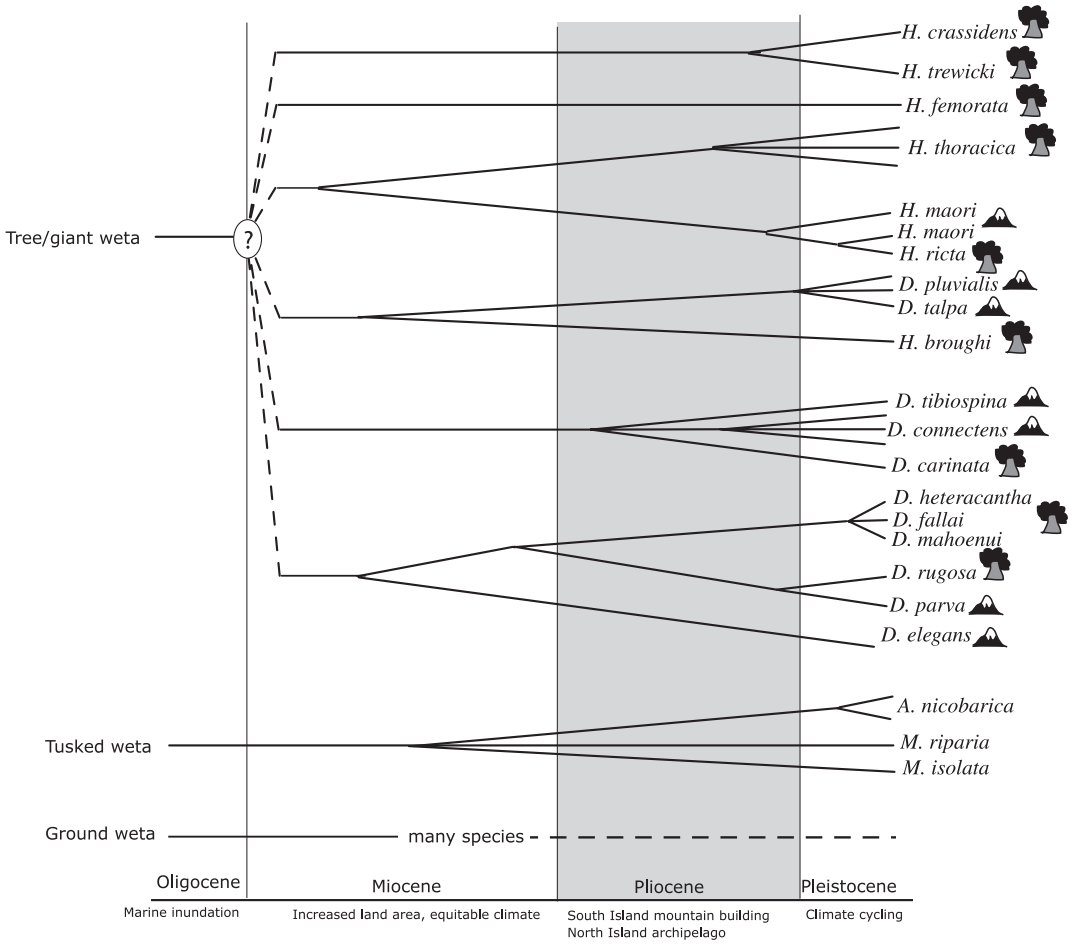

Figure 9 Phylogenetic hypothesis of weta diversity in New Zealand based on analysis of COI and 12S sequence data. Tree and mountain symbols indicate forest and alpine habitats respectively. Genera are *Deinacrida* (*D.*), *Hemideina* (*H.*), *Motuweta* (*M.*) and *Anisoura* (*A*).

Worthy, 1998; Trewick & Worthy, 2001). Few deposits include remains of invertebrates (but see Worthy & Holdaway, 1996).

Hemideina (without *H. broughi*) comprises a relatively homogenous group of generalist, arboreal species with allopatric or parapatric distributions. One instance of partial sympatry is observed between *H. trewicki* and *H. thoracica* (Morgan-Richards, 1995; Trewick & Morgan-Richards, 1995). Geographical ranges of tree weta species must have fluctuated in response to available habitat, which is in most instances forest, and molecular data are consistent with this. For instance, the isolation of *H. crassidens* on islands in Cook Strait, and on mountain tops in North Island within an area now occupied by *H. thoracica* probably resulted from glacial and post-glacial range shifts (Trewick & Morgan-Richards, 1995). Parapatry of these two species (by latitude and altitude) indicates a degree of competitive exclusion and specialization to differing climate regimes. Recent expansion of *H. crassidens* southwards (west coast of South Island) is indicated by low genetic diversity and a distinct karyotype among individuals in this area (Morgan-Richards, 2002). Of the more extensively studied species, *H. thoracica* appears to have recolonized central North Island from the north following the massive volcanic eruptions at Taupo < 2000 years ago (Morgan-Richards *et al.*, 2000). Similarly, although Banks Peninsula is derived from a late Miocene volcano (Stevens, 1980), the ancestors of *H. ricta* probably did not arrive until alluvial plains extended and lower sea levels provided a land bridge from South Island during the Pleistocene. Paraphyly of *H. maori* with respect to *H. ricta* is consistent with morphological and genetic evidence that the former is the recent ancestor of the latter (Field 1993; Morgan-Richards & Townsend, 1995).

The disparity between the taxonomic and genetic diversity of North and South Island giant weta (*Deinacrida*), and the species gap evident in lower North Island (Fig. 3), might be a reflection of differential extinction since the arrival of humans. However, specimens collected by early biologists show that the forest weta, *D. heteracantha* group and *D. rugosa* once had wider distributions in North Island (Fig. 8), and prior to recent clearance, North Island supported near continuous forests (McGlone, 1988; McGlone *et al.*, 1993; Newnham *et al.*, 1999). This is consistent with the pattern of few, generalist, arboreal, allopatric species of *Deinacrida* as seen in the tree weta (*Hemideina*). Genetic diversity within the *D. heteracantha* group is less than that recorded within other species surveyed, and consistent with coalesence during the Pleistocene. This level of diversity may indicate that each species in this group

represent populations of a once more widely distributed species that survived in separate North Island forest refugia during Pleistocene glacials.

CONCLUSIONS

It appears that several distinct groups of weta (ground, tusked, tree/giant) survived Oligocene inundation in New Zealand, although the genetic bottleneck resulting from extreme land reduction is likely to have reduced diversity within each lineage (Cooper & Cooper, 1995). The implication that several distinct weta lineages survived in New Zealand through the Oligocene contrasts with accumulating evidence that much of the New Zealand flora speciated following long-distance dispersal during and after the late Miocene (Lockhart *et al.*, 2001; McGlone *et al.*, 2001; Winkworth *et al.*, 2002). A gradual increase in land area in the Miocene, probably distributed among several islands, may have provided the stimulus for initial speciation. Although climate change in the Pleistocene must have affected weta ranges, especially the forest inhabitants, it does not explain speciation among alpine taxa. We find that much weta diversification appears to date to the Pliocene. Immediately prior to the Pliocene Kaikoura orogeny that resulted in the mountainous terrain of South Island seen today, it is thought that New Zealand had a rather low profile and equitable climate (Cooper & Millener, 1993), and by implication ecologically uniform environment. The Pliocene emergence of mountains generated heterogeneous environment, providing the impetus for allopatric (across mountain ranges) and adaptive (across altitude) speciation (Fig. 9). In comparison, molecular diversity dating to the Pleistocene is contained within species or among ecologically close species.

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REFERENCES

Archer, M., Flannery, T.F., Ritchie, A. & Molnar, R. (1985) First Mesozoic mammal from Australia – an Early Cretaceous monotreme. *Nature*, **318,** 363–366.

Bensasson, D., Zhang, D-X., Hartl, D.L. & Hewitt, G.M. (2001) Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends in Ecology and Evolution*, **16,** 314–321.

Brower, A.V.Z. (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences USA*, **91,** 6491–6495.

Brown, W.M., George, M. Jr & Wilson, A.C. (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences USA*, **76,** 1967–1971.

Buckley, T.R., Simon, C. & Chambers, G.K. (2001) Phylogeography of the New Zealand cicada *Maoricicada campbelli* based on mitochondrial DNA sequences: ancient clades associated with cenozoic environmental change. *Evolution*, **55,** 1395–1407.

Cameron, W. (1996) *A study of the taxonomic status of* Deinacrida parva *and* Deinacrida rugosa *(Orthoptera: Stenopelmatidae), two giant weta from central New Zealand*, Unpublished MSc thesis. Victoria University of Wellington, Wellington, New Zealand.

Chambers, G.K., Boon, W.M., Buckley, T.R. & Hitchmough, R.A. (2001) Using molecular methods to understand the Gondwanan affinities of the New Zealand biota: three case studies. *Australian Journal of Botany*, **49,** 377–387.

Cooper, A. & Cooper, R.A. (1995) The Oligocene bottleneck and New Zealand biota: genetic record of a past environmental crisis. *Proceedings of the Royal Society, London B Biological Sciences*, **261,** 293–302.

Cooper, R.A. & Millener, P.R. (1993) The New Zealand biota: historical background and new research. *Trends in Ecology and Evolution*, **8,** 429–433.

Daugherty, C.H., Gibbs, G.W. & Hitchmough, R.A. (1993) Mega-island or micro-continent? New Zealand and its fauna. *Trends in Ecology and Evolution*, **8,** 437–442.

Field, L.H. (1993) Structure and evolution of stridulatory mechanisms in New Zealand weta (Orthoptera: Stenopelmatidae). *International Journal of Insect Morphology and Embryology*, **22,** 163–183.

Field, L.H. (2001) Stridulatory mechanisms and associated behaviour in New Zealand wetas. *The biology of wetas, king crickets and their allies* (ed. by L.H. Field), pp. 271–295. CABI Publishing, Wallingford.

Field, L.H. & Bigelow, R.S. (2001) Morphometric analysis of *Hemideina* spp. in New Zealand. *The biology of wetas, king crickets and their allies* (ed. by L.H. Field), pp. 163–178. CABI Publishing, Wallingford.

Field, L.H. & Deans, N.A. (2001) Sexual selection and secondary sexual characters of wetas and kink crickets. *The biology of wetas, king crickets and their allies* (ed. by L.H. Field), pp. 179–204. CABI Publishing, Wallingford.

Flannery, T.F., Archer, M., Rich, T.H. & Jones, R. (1995) A new family of monotremes from the Cretaceous of Australia. *Nature*, **377,** 2647–2652.

Fleischer, R.C., McIntosh, C.E. & Tarr, C.L. (1998) Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K–Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Molecular Ecology*, **7,** 533–545.

Fleming, C.A. (1977) The history of life in New Zealand forests. *New Zealand Journal of Forestry*, **22,** 249–262.

Fleming, C.A. (1979) *The geological history of New Zealand and its life*. Auckland University Press, New Zealand.

Gibbs, G.W. (1998) Why are some weta (Orthoptera: Stenopelmatidae) vulnerable yet others are common? *Journal of Insect Conservation*, **2,** 161–166.

Gibbs, G.W. (1999) Four new species of giant weta, *Deinacrida* (Orthoptera: Anostostomatidae) from New Zealand. *Journal of the Royal Society of New Zealand*, **29,** 307–324.

Gibbs, G.W. (2001) Habitats and biogeography of New Zealands Deinacridine and tusked weta species. *The biology of wetas, king crickets and their allies* (ed. by L.H. Field), pp. 35–55. CABI Publishing, Wallingford.

Gorochov, A.V. (2001) The higher classification, phylogeny and evolution of the superfamily Stenopelmatiodea. *The biology of wetas, king crickets and their allies* (ed. by L.H. Field), pp. 3–33. CABI Publishing, Wallingford.

Heenan, P.B. & Mitchell, A.D. (2003) Phylogeny, biogeography and adaptive radiation of *Pachycladon* (Brassicacaeae) in the mountains of South Island, New Zealand. *Journal of Biogeography*, **30,** 1–13.

Heerdegen, R.G. & Shepherd, M.J. (1992) Manawatu landforms – product of tectonism, climate change and process. *Landforms of New Zealand*, 2nd edn (ed. by J.M. Soons & M.J. Selby), pp. 308–333. Longman Paul, Auckland.

Johns, P.M. (1997) The Gondwanaland weta: family Anostostomatidae (formerly Stenopelmatidae, Henicidae or Mimnermidae): nomenclatural problems, world checklist, new genera and species. *Journal of Orthopteran Research*, **6,** 125–138.

Johns, P.M. (2001) *Distribution and conservation status of ground weta,* Hemiandrus *species (Orthoptera: Anostostomatidae)*. Science for Conservation 180. Department of Conservation, Wellington, New Zealand.

Juan, C., Oromi, P. & Hewitt, G.M. (1995) Mitochondrial DNA phylogeny and sequential colonization of Canary Island by darkling beetles of the genus *Pimelia* (Tenebrionidae). *Proceedings of the Royal Society of London, B, Biological Sciences*, **261,** 173–180.

Juan, C., Oromi, P. & Hewitt, G.M. (1996) Phylogeny of the genus *Hegeter* (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from cytochrome oxidase I mitochondrial DNA sequences. *Heredity*, **76,** 392–403.

King, T.M., Kennedy, M. & Wallis, G.P. (2003) Phylogeographic genetic analysis of the alpine weta, *Hemideina maori*: evolution of a colour polymorphism and origins of a hybrid zone. *Journal of the Royal Society of New Zealand*, **33,** 715–729.

LeMasurier, W.E. & Landis, C.A. (1996) Mantle-plume activity recorded by low-relief erosion surfaces in West Antarctica and New Zealand. *Geological Society of America Bulletin*, **108,** 1450–1466.

Lockhart, P.J., McLenachan, P.A., Havell, D., Glenny, D., Huson, D. & Jensen, U. (2001) Phylogeny, radiation, and transoceanic dispersal of New Zealand alpine buttercups: molecular evidence under split decomposition. *Annals of the Missouri Botanic Gardens*, **88,** 458–477.

McGlone, M.S. (1988) New Zealand. *Vegetation history* (ed. by B. Huntley and T. Webb), pp. 557–602. Kluwer Academic Press, Dordrecht.

McGlone, M.S., Salinger, M.J. & Moar, N.T. (1993) Paleovegetation studies of New Zealand's climate since the last glacial maximum. *Global climate since the last glacial maximum* (ed. by H.E. Wright, J.E. Kutsback, T. Webb, W.F. Ruddiman, F.A. Street-Perrot and P.J. Bartlein), pp. 294–317. University of Minnesota Press, Minneapolis.

McGlone, M.S., Duncan, R.P. & Heenan, P.B. (2001) Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. *Journal of Biogeography*, **28,** 199–216.

McGuinness, C.A. (2001) *The conservation requirements of New Zealand's nationally threatened invertebrates*. Department of Conservation, Wellington, New Zealand.

Morgan-Richards, M. (1995) A new species of tree weta in the North Island of New Zealand (Orthoptera: Stenopelmatidae: *Hemideina*). *New Zealand Entomologist*, **18,** 15–23.

Morgan-Richards, M., Daugherty, C.H. & Gibbs, G.W. (1995) Taxonomic status of tree weta from Stephens Island, Mt Holdsworth and Mt Arthur, based on allozyme variation. *Journal of the Royal Society of New Zealand*, **25,** 301–312.

Morgan-Richards, M. (1997) Intraspecific karyotype variation is not concordant with allozyme variation in the Auckland tree weta of New Zealand, *Hemideina thoracica* (Orthoptera: Stenopelmatidae). *Biological Journal of the Linnean Society*, **60,** 423–442.

Morgan-Richards, M. (2000) Robertsonian translocations and B chromosomes in the Wellington tree weta, *Hemideina crassidens* (Orthoptera: Anostostomatidae). *Hereditas*, **132,** 49–54.

Morgan-Richards, M., Trewick, S.A. & Wallis, G.P. (2000) Characterization of a hybrid zone between two chromosomal races of the weta *Hemideina thoracica* following a geologically recent volcanic eruption. *Heredity*, **85,** 586–592.

Morgan-Richards, M. (2002) Fission or fusion? Mitochondrial DNA phylogenetics of the chromosome races of *Hemideina crassidens* (Orthoptera: Anostostomatidae). *Cytogenetics and Genome Research*, **96,** 217–222.

Morgan-Richards, M. & Gibbs, G.W. (1996) Colour, allozyme and karyotype variation in the New Zealand giant scree weta *Deinacrida connectens* (Orthoptera: Stenopelmatidae). *Hereditas*, **125,** 265–276.

Morgan-Richards, M. & Gibbs, G.W. (2001) A phylogenetic analysis of New Zealand giant and tree weta (Orthoptera: Anostostomatidae: *Deinacrida* and *Hemideina*) using morphological and genetic characters. *Invertebrate Taxonomy*, **15,** 1–12.

Morgan-Richards, M. & Townsend, J.A. (1995) Hybridisation of tree weta on Banks Peninsula, New Zealand, and colour polymorphism within *Hemideina ricta* (Orthoptera: Stenopelmatidae). *New Zealand Journal of Zoology*, **25,** 301–312.

Morgan-Richards, M., Trewick, S.A. & Wallis, G.P. (2001) Chromosome races with Pliocene origins: evidence from mtDNA. *Heredity*, **86,** 303–312.

Newnham, R.M., Lowe, D.J. & Williams, P.W. (1999) Quaternary environmental changes in New Zealand: a review. *Progress in Physical Geology*, **23,** 567–610.

Pole, M. (1994) The New Zealand flora – entirely long-distance dispersal? *Journal of Biogeography*, **21,** 625–635.

Ramsay, G.W. (1978) Invertebrate mice. *New Zealand Entomologist*, **6,** 400.

Rich, P.V., Rich, T.H., Wagstaff, B.E. & Mason, M.J. (1988) Evidence of low temperatures and biologic diversity in Cretaceous high latitudes of Australia. *Science*, **242,** 1403–1406.

Roderick, G.K. & Gillespie, R.G. (1998) Speciation and phylogeography of Hawaiian terrestrial arthropods. *Molecular Ecology*, **7,** 519–531.

Sandoval, C., Carmean, D.A. & Crespi, B.J. (1998) Molecular phylogenetics of sexual and parthenogenetic *Timema* walking-sticks. *Proceedings of the Royal Society of London, B, Biological Sciences*, **265,** 589–595.

Sherley, G. (2001) Conservation of threatened species of weta (Orthoptera: Anostostomatidae) in New Zealand. *The biology of wetas, king crickets and their allies* (ed. by L.H. Field), pp. 521–527. CABI Publishing, Wallingford.

Simon, C., Frati, F., Beckenbach, A., Crespi, B.J., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomolgical Society of America*, **87,** 651–701.

Stevens, G.R. (1980) *New Zealand adrift*. Reed, Wellington, New Zealand.

Stevens, G., McGlone, M. & McCulloch, B. (1988) *Prehistoric New Zealand*. Henemann Reed, Auckland, New Zealand.

Sullivan, J.J., Markert, A. & Kilpatrick, C.W. (1997) Phylogeography and molecular systematics of the *Peromyscus aztecus* species group (Rodentia: Muridae) inferred using parsimony and likelihood. *Systematic Biology*, **46,** 426–440.

Sunnucks, P. & Hale, D.F. (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, **13,** 510–524.

Swofford, D.L. (2002) *PAUP*. Phylogenetic analysis using parsimony (* and other methods)*, Version 4. Sinauer Associates, Sunderland, MA, USA.

Trewick, S.A. (2001) Scree weta phylogeography: surviving glaciation and implications for Pleistocene biogeography in New Zealand. *New Zealand Journal of Zoology*, **28,** 291–298.

Trewick, S.A. & Morgan-Richards, M. (1995) On the distribution of tree weta in the North Island, New Zealand. *Journal of the Royal Society of New Zealand*, **25,** 1–9.

Trewick, S.A. & Morgan-Richards, M. (in press) Phylogenetics of New Zealand's tree, giant and tusked weta (Orthoptera: Anostostomatidae): evidence from mitochondrial DNA. *Journal of Orthopteran Research*, in press.

Trewick, S.A. & Wallis, G.P. (2001) Bridging the 'beech-gap': New Zealand invertebrate phylogeography implicates Pleistocene glaciation and Pliocene isolation. *Evolution*, **55,** 2170–2180.

Trewick, S.A. & Worthy, T.H. (2001) Origins and prehistoric ecology of takahe based on morphometric, molecular, and fossil data. *The Takahe: fifty years of conservation management and research* (ed. by W.G. Lee and I.G. Jamieson), pp. 31–48. University of Otago Press, Wellington, New Zealand.

Trewick, S.A., Morgan-Richards, M. & Wallis, G.P. (2000) Phylogeographic pattern correlates with Pliocene mountain building in the alpine scree weta (Orthoptera: Anostostomatidae). *Molecular Ecology*, **9,** 657–666.

Wardle, P. (1963) Evolution and distribution of the New Zealand flora, as affected by Quaternary climates. *New Zealand Journal of Botany*, **37,** 17–39.

Watt, J.C. (1963) The rediscovery of a giant weta, *Deinacrida heteracantha*, on the North Island mainland. *New Zealand Entomologist*, **3,** 9–13.

Winkworth, R.C., Grau, J., Robertson, A.W. & Lockhart, P.J. (2002) The origins and evolution of the genus *Myosotis* L. (Boraginaceae). *Molecular Phylogenetics and Evolution*, **24,** 180–193.

Worthy, T.H. (1998) The Quaternary fossil avifauna of Southland, South Island, New Zealand. *Journal of the Royal Society of New Zealand*, **28,** 537–589.

Worthy, T.H. & Holdaway, R.N. (1996) Quaternary fossil faunas, overlapping taphonomies, and palaeofaunal reconstruction in north Canterbury, South Island, New Zealand. *Journal of the Royal Society of New Zealand*, **26,** 275–361.

BIOSKETCHES

Steve Trewick and **Mary Morgan-Richards** are research fellows at Massey University specializing in speciation, biogeography, phylogeography and systematics of invertebrates.

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