Molecular Ecology (2000) **9**, 657–666

Phylogeographical pattern correlates with Pliocene mountain building in the alpine scree weta (Orthoptera, Anostostomatidae)

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Abstract

Most research on the biological effects of Pleistocene glaciation and refugia has been undertaken in the northern hemisphere and focuses on lowland taxa. Using single-strand conformation polymorphism (SSCP) analysis and sequencing of mitochondrial cytochrome oxidase I, we explored the intraspecific phylogeography of a flightless orthopteran (the alpine scree weta, *Deinacrida connectens*) that is adapted to the alpine zone of South Island, New Zealand. We found that several mountain ranges and regions had their own reciprocally monophyletic, deeply differentiated lineages. Corrected genetic distance among lineages was 8.4% (Kimura 2-parameter [K2P]) / 13% (GTR + I + Γ), whereas within-lineage distances were only 2.8% (K2P) / 3.2% (GTR + I + Γ). We propose a model to explain this phylogeographical structure, which links the radiation of *D. connectens* to Pliocene mountain building, and maintenance of this structure through the combined effects of mountain-top isolation during Pleistocene interglacials and ice barriers to dispersal during glacials.

Keywords: alpine, COI, mitochondrial DNA, New Zealand, phylogeography, Pleistocene glaciation

Received 23 September 1999; revision received 12 November 1999; accepted 12 November 1999

Introduction

Biologists live and work in what is in effect an interglacial. The 10 000 years or so since the end of the last glacial is a period similar in duration to each of the 20 or so interglacials of the Pleistocene (Stevens 1981; Cooper & Millener 1993). This brings an immediacy to the study of the evolutionary effects of the Pleistocene glacial epoch, which has stimulated considerable research interest in the field. Studies of the impact of such widespread and recent events reveal not only the nature of the world today but provide an insight into more ancient evolutionary processes.

Although the physical effects of the Pleistocene glacials were global, they were expressed in different ways regionally (Webb & Bartlein 1992; Cox & Moore 1993). Biological response to glacial–interglacial cycling appears to vary with locality and taxa (Jaarola & Tegelström 1995; Hewitt 1996; Zink 1996; Roy 1997; Taberlet *et al*. 1998; and references therein). Most studies of the biological effects of glaciation have been concerned with northern hemisphere regions where the last glacial epoch involved southward extension of continuous ice sheets. This ice erased biota from many regions of northern Europe and North America and forced southward range movement as climate patterns shifted (Comes & Kadereit 1998). In western Europe, for instance, several studies have indicated the existence of refugia where the biota became isolated between the advancing ice front and the Mediterranean Sea (see for examples: Comes & Kadereit 1998; Lunt *et al*. 1998; Taberlet *et al*. 1998). The final recession of ice sheets (≈ 14 000 years ago) opened up extensive areas that were recolonized from the refugia. The phylogeographical effects of Pleistocene glaciation were, however, more complex owing to the cycling of multiple glacial and interglacial episodes.

In the southern hemisphere, the distance of the southern continents from the south pole and the circumpolar current prevented extension of ice sheets from Antarctica to other

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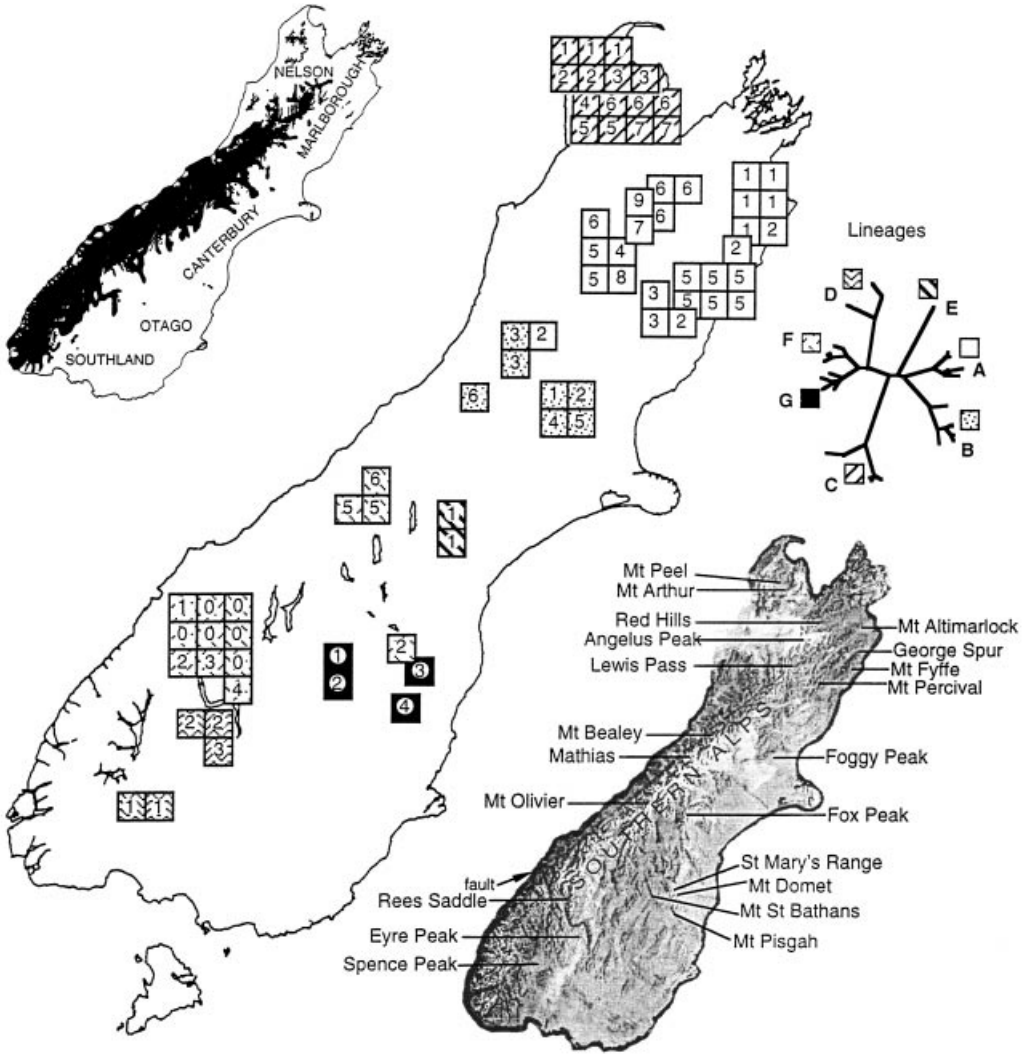

Fig. 1 South Island, New Zealand. The main map shows the number, based on single-strand conformation polymorphism (SSCP) analysis, and haplotypic lineage of alpine scree weta (*Deinacrida connectens*) individuals at each site. At Rees Saddle (lineage F), eight individuals had an identical SSCP signal, but two sequences represented different haplotypes (1 and 4), the remaining individuals are indicated by '0'. The inset satellite image (lower right of the figure) shows the position of named collecting sites and regions. Mountain ranges, the Alpine fault (running southwest to northeast), lakes, rivers and the Canterbury plains are visible. The inset map (top left of the figure) shows the approximate maximal extent of Pleistocene glaciers (redrawn from Pillans *et al*. 1992) and named regions.

landmasses. However, where terrain, temperature and precipitation allowed, glaciers formed that had considerable local impact. Glaciers were almost absent from Australia and southern Africa but were present in South America (Flint 1957; Denton & Hughes 1981).

For most of its history, New Zealand has had a low topography and a warm climate (Fleming 1979), but during the Pliocene (5–2 million years ago [Ma]) an episode of extensive mountain building began. In South Island, mountains extend across some 60% of the island (Whitehouse & Pearce 1992), and uplift continues today at a rate of ~ 15 mm/year (Wellman 1979). All of the major axial mountain ranges, including the South Islands Southern Alps, were formed at this time (Stevens 1981; Whitehouse & Pearce 1992), and their development provided conditions for the formation of extensive glaciers. These were particularly widespread in the southern and central parts of this axial range but were also present northward to Mt Arthur (Fig. 1). The absence of mountains until recent geological times means that few, if any, alpine-adapted taxa existed in New Zealand prior to the late Pliocene/Pleistocene.

The modern flora of South Island includes a high number of endemic alpine species (Mark & Adams 1995). The extent of this diversity has caused some researchers to consider that recent evolution *in situ* is improbable. They have proposed alternative hypotheses of earlier origins from other sources, e.g. coastal dispersal and

subantarctic land-bridging (Fleming 1963, 1979; Wardle 1963). The alpine invertebrate fauna is diverse, highly endemic and specialized (Mark & Dickinson 1997), and several groups show well-developed freeze tolerance (Block *et al*. 1998; Sinclair *et al*. 1999). The evidence of the impact of the Pleistocene glacial epoch, both physical (e.g. glacier erosion surfaces, moraines, valleys and fjords) (Stevens 1981; Pillans *et al*. 1992) and biological (extinction, zonation, alpine specialization) is abundant (Willet 1950; Fleming 1963; Burrows 1965; Dumbleton 1970; McGlone 1985, 1988). Fleming (1979) proposed a Pleistocene glaciation model of forced adaptation for speciation in New Zealand parrots (*Nestor*) and suggested that this would be a 'plausible mechanism for the occupation of the alpine zone by many plants and insects (e.g. *Hemideina* and *Deinacrida*)'. Yet to date there have been few phylogeographical studies exploring the molecular evidence for the biological effects of these events (but see Emerson & Wallis 1995; King *et al*. 1996; Buckley *et al*. 1998).

We have used a molecular approach to examine the intraspecific phylogeography of one of New Zealand's largest and most notable alpine invertebrates, the scree weta *Deinacrida connectens* (Orthoptera: Anostostomatidae). Scree weta are widespread in South Island but absent from North Island. The species inhabits alpine scree slopes 1200–3600 m above sea level and is limited to this zone by as yet undetermined factors. The range cut-off at the lower altitudinal limit on each mountain is not consistent with any outwardly apparent features and exists despite the extension of apparently suitable scree habitat below this altitude. The scree weta appears to survive freezing without ill effects (B. Sinclair, personal communication). The physiological restriction to high altitude and flightlessness of this insect mean that in current climatic conditions, populations can be isolated from one another, even on mountain tops within the same ranges.

Studies of the processes governing the geographical distributions of genealogical lineages (Riddle 1996) are particularly well served in the intraspecific context by the use of mitochondrial DNA (mtDNA) analysis (Avise *et al*. 1987; Avise 1994). The lack of recombination and maternal inheritance allow historical information to be retained intact even where a complex glacial history may have fostered repeated dispersal events that lead to blending of the nuclear genome through recombination.

We have two alternative broad hypotheses relating to various possible patterns that we might find. We refer to these as: (i) alpine radiation and (ii) recent isolation models. The alpine radiation model proposes that the origin of the species and its special adaptations coincided with major alpine uplift and onset of the Pleistocene glacial epoch. If this model is correct, then coalescence should fall within the time of initial alpine uplift (2–5 Ma), with marked phylogeographical structuring. Adaptation to the alpine environment could have occurred either in parallel on the different ranges, or at an early stage in one location with subsequent spread to multiple emerging ranges. Alternatively, our recent isolation model proposes that all populations were effectively in contact until either: (i) modern populations became isolated in alpine regions as the climate warmed at the end of the Pleistocene (< 14 000 years ago); or (ii) habitat modification and introduction of mammalian predators (e.g. rats, Holdaway 1996) within the last 2000 years led to a more fragmented distribution. Under these circumstances, coalescence time would depend on effective population size, but would probably be quite recent, and there should be little, if any, phylogeographical structure. Population isolation as a result of predation seems less probable because the species appears to be physiologically restricted to altitudes well above the point at which predators are thought to impact (Gibbs & Richards 1990).

Thus, the timings of our two alternative hypotheses differ by more than two orders of magnitude, and should be distinguishable using the methods we propose. In fact, genetic differentiation will be minimal on a timescale of at least an order of magnitude greater than that predicted by our recent isolation models, so cessation of pre-existing gene flow at any time during the Holocene would probably be undetected.

Materials and methods

Sampling

Specimens of *Deinacrida connectens* were collected from alpine scree slopes throughout South Island, New Zealand, in 1991 (Morgan-Richards & Gibbs 1996) and 1998 (Fig. 1). Populations at the following locations were sampled (name with abbreviation underlined, longitude/latitude, sample size): Mt Peel, 41°08′S 172°35′E, $n = 7$; Mt Arthur, 41°13′S 172°42′E, $n = 8$; Spence Peak, 44°52′S 167°51′E, $n = 2$; Eyre Peak, 45°18′S 168°27′E, $n = 3$; Fox Peak, 43°41′S 170°48′E, $n = 2$; Rees Saddle, 44°33′S 168°34′E, $n = 10$; St Mary's Ra., 44°47′S 170°19′E, $n = 1$; Mt Olivier, 43°43′S 170°04′E, $n = 3$; Mt St Bathans, 44°46′S 169°47′E, $n = 2$; Mt Domet, 44°52′S 170°22′E, $n = 1$; Mt Pisgah, 45°05′S 170°23′E, $n = 1$; Mathias, 43°08′S 171°05′E, $n = 1$; Foggy Peak, 43°17′S 171°45′E, $n = 4$; Angelus Peak, 41°54′S, 172°44′E, $n = 2$; Mt Fyffe, 42°19′S 173°37′E, $n = 6$; Lewis Pass, 42°23′S 172°21′E, $n = 5$; Mt Bealey, 42°57′S 171°33′E, $n = 3$; Red Hills Ridge, 41°41′S 173°03′E, $n = 3$; Mt Percival, 42°29′S 172°56′E, $n = 3$; George Spur, 42°08′S 173°43′E, $n = 1$; Mt Altimarlock, 41°45′S 173°42′E, $n = 6$.

Following euthanasia with ether, muscle tissue was removed from hind femora and stored at −80 °C. DNA was extracted using a salting-out method (Sunnucks & Hales 1996). Tissue was macerated and incubated with

5 μL of 10 mg/mL proteinase K in 600 μL of TNES buffer (20 mM EDTA, 50 mM Tris, 400 mM NaCl, 0.5% sodium dodecyl sulphate [SDS]) at 50 °C. Ten per cent 5 M NaCl was added and the extractions were shaken vigorously for 20 s followed by centrifugation at 16 060 *g* for 5 min. The supernatant was removed and precipitated with an equal volume of cold 100% ethanol. DNA was collected by centrifugation and washed with 70% ethanol before being dried and resuspended in water.

Our molecular analysis used primers that target regions of the mtDNA cytochrome oxidase I gene (COI). These primers are known to be highly conserved and applicable to a wide range of invertebrate taxa (Lunt *et al*. 1996), and the COI gene has been successfully utilized in intra- and interspecific studies (Zhang & Hewitt 1996; Funk 1999).

Single-stranded conformation polymorphism analysis

Mitochondrial primers C1-J-1718 and C1-N-2191 (Simon *et al*. 1994) were used to amplify a short (≈ 400 bp) fragment of the 5′ end of the COI. Single-stranded conformation polymorphism (SSCP) analysis was used to screen for variant haplotypes. Polymerase chain reaction (PCR) products were isotopically labelled by incorporation of [α^{33}P]-dATP. Ten-microlitre reaction mixtures (200 μM dNTPs, 2.5 mM $MgCl_2$, 0.25 U of Qiagen *Taq*) were amplified as follows: an initial denaturation at 94 °C for 60 s followed by 40 cycles of 94 °C for 15 s, 50 °C for 30 s and 72 °C for 90 s. Following PCR, products were denatured for 5 min at 95 °C in the presence of an equal volume (10 μL) of 95% formamide loading buffer. Denatured products were loaded from ice into vertical, nondenaturing polyacrylamide gels consisting of 6% 37.5 : 1 *bis*acrylamide, 5% glycerol and 0.5 × Tris-borate buffer (TBE). Gels were electrophoresed at 4 °C for 200 W/h at ≈ 13 W. Gels were lifted on blotting paper, dried and exposed with Biomax (Kodak) autorad film for 24–48 h. Individuals were scored for haplotype by comparison of renatured single-strand DNA migration patterns.

Sequencing

One to four representatives (Table 1) of each haplotype resolved by SSCP were sequenced for a longer fragment towards the 3′ end of COI using the primers, C1-N-2195 and C1-J-3014 (Simon *et al*. 1994). PCR reactions were performed in 25-μL reaction volumes and the products were gel-purified in 2% agarose stained with ethidium bromide. Bands of expected molecular weight were excised and the DNA extracted from the agarose using Prepagene (BioRad). Purified DNA fragments were quantified by eye using agarose electrophoresis with a molecular weight marker. Cycle sequencing used Bigdye chemistry (Perkin-Elmer) following the manufacturer's protocols. Sequences were aligned manually using SEQED, version 1.0.3 (ABI, PE). Distance estimation and phylogenetic analysis was performed using PAUP 4.0 (Swofford 1998). Population genetic analysis followed the method of Excoffier *et al*. (1992) as implemented by ARLEQUIN 1.0 (Schneider *et al*. 1997). All primers used were sourced from the insect mtDNA primer set (John Hobbs, UBC).

Results

Sequence

Seventy-four weta from 21 locations were included in the study. Thirty-six different 3′ COI sequence haplotypes were detected by a combination of SSCP and sequencing ($N = 50$). The 540 bp of aligned sequence contained 96 variable sites, of which 76 were phylogenetically informative. The transition : transversion ratio was 6.5 : 1, as reconstructed from the neighbour-joining (NJ) (Kimura 2-parameter [K2P]) tree, and the distribution of variable sites across first, second and third codon positions was 10.5, 1.5 and 88%, respectively. The vast majority of substitutions were synonymous although two amino acid variants were found in sequences in lineage C. These were relatively conservative changes positioned in a region of the gene that is variable within the family. An Ala–Ile change was present in all clade C weta (i.e. those from Mt Arthur and Mt Peel) while an Ala–Ser substitution was present only in individuals from Mt Arthur. Sequences were deposited in GenBank (acc. nos: AF202586–AF202621).

Phylogenetic analysis

The 36 haplotype sequences were analysed using NJ and maximum parsimony (MP) analyses. MP with transversions : transitions weighted 5 : 1 gave four shortest trees that differed only with respect to placement of some terminal edges within each clade. The internal topology of these trees was identical to that of the NJ tree.

We used the NJ tree based on K2P distances to test for the most appropriate nucleotide substitution model by comparing likelihood scores for a suite of models: JC (Jukes & Cantor 1969), K2P (Kimura 1980), HKY85 (Hasegawa *et al*. 1985) and GTR (Yang 1994) with a combination of among-site rate variation models: I (invariable sites) and Γ (gamma distribution). There was a substantial improvement in likelihood scores for models incorporating among-site rate variation, with GTR + I + Γ having the lowest score (–lnL 1630.267). With the estimated proportion of invariant sites as 0.55, the α-shape parameter was 0.75. These parameter estimates were used to recalculate the distance matrix (Table 1). NJ analysis using GTR + I + Γ distances produced a tree of the same topology as K2P.

Table 1 Pairwise GTR + I + Γ (below diagonal) and Kimura 2-parameter (K2P) (above diagonal) genetic distances among 36 sequenced cytochrome oxidase (COI) mitochondrial DNA (mtDNA) haplotypes in the alpine scree weta, *Deinacrida connectens*. Haplotype labels (as described in Figs 1 and 2) and abbreviated location names are given. Boxes group pairwise genetic distances of haplotypes associated with the principal lineages (A–G). The numbers of each haplotype sequenced are given (*n*). Where single-strand conformation polymorphism (SSCP) analysis indicated that a haplotype was present at more than one location, at least one representative from each of those locations was sequenced

		1	4	1	1	2	3	1	1	1	1	1	1	1	1	1	3	2	2	1	1	2	1	2	2	1	2	1	1	1	1	1	1	1	1	1
	n	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**D1**	**D2**	**D3**	**E1**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**G1**	**G2**	**G3**	**G4**
Alt	**A1**	**A**	0.2	0.4	1.5	1.3	1.5	1.1	1.3	1.7	3.7	4.6	4.7	4.9	5.1	4.9	5.9	6.3	5.5	6.5	6.7	6.7	6.5	7.1	6.7	7.4	4.4	4.6	4.6	4.8	4.8	4.4	4.4	4.6	4.4	4.0	4.8
Alt, Geo, Per, Bea	**A2**	0.2		0.2	1.3	1.1	1.3	0.9	1.1	1.5	3.5	4.4	4.5	4.6	4.8	4.7	5.7	6.1	5.3	6.3	6.5	6.5	6.3	6.9	6.5	7.1	4.2	5.0	4.8	5.0	5.0	4.2	4.2	4.8	4.2	3.8	4.6
Per	**A3**	0.4	0.2		1.5	1.3	1.5	1.1	1.3	1.7	3.3	4.6	4.7	4.9	5.1	4.9	5.9	6.3	5.5	6.5	6.7	6.7	6.5	6.7	6.3	6.9	4.0	4.8	4.6	4.8	4.8	4.0	4.0	5.0	4.4	4.0	4.8
Lew	**A4**	1.7	1.4	1.6		0.2	0.4	0.4	0.6	0.9	3.6	4.6	4.7	4.8	5.1	5.1	5.9	6.3	5.5	6.5	6.7	6.7	6.5	6.7	6.3	6.9	4.4	4.8	4.6	4.8	4.8	4.0	4.0	4.6	4.0	3.6	4.4
Lew, Fyf	**A5**	1.4	1.2	1.4	0.2		0.2	0.2	0.4	0.7	3.9	4.8	4.9	5.1	5.3	5.1	6.1	6.5	5.7	6.7	6.9	6.9	6.7	6.9	6.5	7.2	4.6	5.0	4.8	5.0	5.0	4.2	4.2	4.8	4.2	3.8	4.6
Lew, Red	**A6**	1.6	1.4	1.6	0.4	0.2		0.4	0.6	0.9	4.0	5.0	5.1	5.2	5.5	5.3	6.3	6.7	5.9	6.9	7.1	7.1	6.9	7.1	6.7	7.3	4.8	5.2	5.0	5.1	5.2	4.4	4.4	5.0	4.4	4.0	4.8
Ang	**A7**	1.2	1.0	1.2	0.4	0.2	0.4		0.2	0.6	3.6	4.6	4.7	4.8	5.1	4.9	5.9	6.3	5.5	6.5	6.7	6.7	6.5	6.7	6.3	6.9	4.4	4.8	4.6	4.8	4.8	4.0	4.0	4.6	4.0	3.6	4.4
Lew	**A8**	1.4	1.2	1.4	0.6	0.4	0.6	0.2		0.7	3.8	4.8	4.9	5.1	5.3	5.1	5.7	6.1	5.3	6.3	6.5	6.5	6.3	6.5	6.1	6.7	4.6	4.6	4.4	4.6	4.6	3.8	3.8	4.4	3.8	3.4	4.2
Ang	**A9**	1.8	1.6	1.8	1.0	0.8	1.0	0.6	0.8		4.2	5.2	4.9	5.0	5.2	5.0	6.3	6.7	5.9	6.9	7.1	7.1	6.9	7.1	6.9	7.5	4.8	5.4	5.2	5.4	5.4	4.6	4.6	5.0	4.4	3.8	4.6
Fog	**B1**	4.5	4.2	4.0	4.4	4.8	5.0	4.4	4.8	5.2	**B**	2.1	1.7	1.9	2.1	2.1	6.5	6.9	6.1	6.3	6.5	6.5	6.3	6.1	5.7	6.3	5.4	5.0	4.8	5.0	5.0	4.2	4.6	5.6	5.0	4.6	5.4
Fog	**B2**	5.6	5.3	5.5	5.5	5.9	6.1	5.5	5.9	6.3	2.0		1.1	0.9	1.1	1.5	7.5	7.5	7.1	7.7	7.5	7.9	8.2	6.7	6.3	6.9	6.2	5.8	5.4	5.2	5.6	5.6	5.2	5.8	5.2	5.2	6.0
Bea	**B3**	6.0	5.6	5.9	5.9	6.3	6.5	5.9	6.2	6.1	1.9	1.0		0.2	0.7	0.8	7.0	7.4	6.6	6.5	6.3	6.7	7.0	6.5	6.3	7.0	5.7	6.1	5.7	5.9	5.9	5.7	5.7	5.5	4.9	4.9	5.7
Fog	**B4**	6.3	5.9	6.2	6.2	6.5	6.8	6.2	6.5	6.4	2.1	0.8	0.2		0.6	0.9	7.1	7.5	6.7	6.9	6.7	7.1	7.3	6.3	6.1	6.7	5.8	6.0	5.6	5.8	5.8	5.4	5.4	5.4	4.8	4.8	5.6
Fog	**B5**	6.7	6.3	6.6	6.5	6.9	7.2	6.5	6.9	6.8	2.3	1.0	0.8	0.6		0.4	7.3	7.7	6.9	7.1	6.9	7.3	7.5	6.1	6.3	6.9	6.0	5.8	5.4	5.6	5.6	5.6	5.6	6.0	5.4	5.4	6.3
Mat	**B6**	6.2	5.8	6.1	6.4	6.5	6.7	6.1	6.5	6.3	2.3	1.4	0.8	1.0	0.4		7.3	7.7	6.9	6.9	6.7	7.1	7.3	6.3	6.5	7.1	6.1	6.3	5.9	6.1	6.1	6.1	6.1	6.5	5.9	5.9	6.7
Pee	**C1**	9.0	8.4	8.7	8.7	9.2	9.5	8.7	8.2	9.2	9.8	11.2	10.8	11.3	12.0	11.6	**C**	0.4	0.2	2.1	2.3	2.3	2.5	6.9	7.3	8.0	6.9	6.2	5.8	6.0	6.0	6.5	6.0	6.3	5.2	5.7	6.5
Pee	**C2**	9.6	9.0	9.3	9.2	9.8	10.1	9.2	8.7	9.8	10.4	11.9	11.5	12.0	12.7	12.3	0.2		0.6	2.5	2.7	2.7	2.8	7.3	7.7	8.4	7.3	6.7	6.2	6.0	6.4	6.9	6.5	6.7	5.6	6.1	6.9
Pee	**C3**	8.2	7.6	7.9	7.9	8.4	8.7	7.9	7.4	8.4	8.9	10.4	9.9	10.4	11.0	10.7	0.2	0.4		1.9	2.1	2.1	2.3	6.7	6.9	7.6	6.7	5.8	5.4	5.6	5.6	6.1	5.7	6.1	5.0	5.5	6.3
Art	**C4**	9.8	9.2	9.5	9.5	10.0	10.3	9.5	9.0	10.0	8.7	11.1	9.0	9.9	10.4	9.9	2.3	2.6	2.1		0.2	0.2	0.4	6.7	7.1	7.7	7.1	6.4	6.0	6.2	6.2	6.2	6.2	6.5	5.4	5.9	6.7
Art	**C5**	10.4	9.7	10.1	10.0	10.6	10.9	10.0	9.5	10.6	9.1	10.6	8.6	9.4	9.9	9.4	2.6	2.9	2.3	0.2		0.4	0.6	6.9	7.3	8.0	7.3	6.7	6.2	6.4	6.4	6.5	6.5	6.7	5.6	6.1	6.9
Art	**C6**	10.4	9.8	10.1	10.1	10.6	10.9	10.1	9.5	10.6	9.2	11.7	9.5	10.4	11.0	10.4	2.6	2.9	2.3	0.2	0.4		0.6	6.9	7.3	8.0	7.3	6.7	6.2	6.4	6.4	6.5	6.5	6.7	5.6	6.1	6.9
Art	**C7**	9.8	9.2	9.5	9.5	10.0	10.3	9.5	9.0	10.0	8.7	12.3	10.0	11.0	11.6	11.0	2.9	3.2	2.6	0.4	0.6	0.6		7.1	7.5	8.2	7.1	6.7	6.4	6.7	6.7	6.2	6.7	6.9	5.8	5.9	6.7
Spe	**D1**	10.4	9.9	9.5	9.3	9.8	10.1	9.3	8.9	9.9	8.4	8.8	9.0	8.5	8.1	8.4	10.5	11.1	9.9	9.7	10.2	10.2	10.8	**D**	1.9	2.5	6.9	4.6	4.2	4.4	4.4	4.8	4.4	4.2	4.4	4.9	4.9
Eyr	**D2**	9.7	9.2	8.9	8.6	9.1	9.4	8.6	8.2	9.6	7.8	8.1	8.7	8.2	8.5	8.8	10.7	11.2	9.9	10.0	10.5	10.5	11.0	2.1		0.6	7.3	4.5	4.2	4.4	4.4	4.1	3.7	4.6	4.4	4.9	4.9
Eyr	**D3**	11.4	10.7	10.4	10.0	10.5	10.8	10.0	9.5	11.0	9.3	9.4	10.1	9.5	9.8	10.2	12.4	13.0	11.4	11.5	12.1	12.1	12.7	2.8	0.6		7.9	5.1	4.9	5.1	4.6	4.7	4.3	4.8	4.7	5.3	5.1
Fox	**E1**	5.7	5.3	5.1	5.5	5.9	6.2	5.5	5.9	6.0	7.2	8.0	7.5	7.8	8.2	8.1	11.7	12.5	11.0	11.2	11.8	11.9	11.2	10.0	10.6	12.2	**E**	5.6	5.2	5.4	5.4	5.4	5.0	5.6	5.0	5.0	5.8
Ree	**F1**	6.0	6.3	6.1	5.8	6.2	6.5	5.8	5.5	6.6	6.2	6.6	7.4	7.3	7.0	7.6	8.1	8.6	7.4	8.0	8.4	8.5	8.9	5.1	4.9	5.8	6.7	**F**	0.4	0.6	0.6	1.3	1.1	2.7	2.8	3.0	3.1
Ree, StM	**F2**	6.0	6.3	6.1	5.8	6.2	6.5	5.8	5.5	6.6	6.2	6.6	7.4	7.3	7.0	7.6	8.1	8.6	7.3	8.0	8.4	8.5	8.9	5.1	5.2	6.1	6.7	0.0		0.2	0.2	1.3	0.9	2.3	2.5	2.9	2.9
Ree	**F3**	6.0	6.3	6.1	5.8	6.2	6.5	5.8	5.5	6.6	6.2	6.6	7.4	7.3	7.0	7.6	8.1	8.6	7.4	8.0	8.4	8.5	8.9	5.1	5.2	6.1	6.7	0.0	0.0		0.4	1.5	1.1	2.5	2.7	3.0	3.0
Ree	**F4**	6.4	6.7	6.5	6.2	6.6	6.9	6.2	5.8	7.0	6.6	6.9	7.8	7.6	7.4	8.0	8.6	9.1	7.8	8.5	8.9	8.9	9.4	5.4	5.5	5.8	7.1	0.2	0.2	0.2		1.5	1.1	2.1	2.3	2.9	2.7
Oli	**F5**	6.0	5.6	5.4	5.0	5.4	5.7	5.0	4.7	5.8	5.2	7.0	7.4	6.9	7.3	8.0	9.2	9.8	8.5	8.3	8.7	8.7	8.3	6.1	4.9	5.8	7.2	1.2	1.4	1.4	1.6		0.4	2.5	2.3	2.3	2.3
Oli	**F6**	6.0	5.5	5.4	5.0	5.4	5.7	5.0	4.7	5.8	5.9	6.3	7.3	6.9	7.3	7.9	8.2	8.7	7.5	8.3	8.7	8.7	9.1	5.5	4.4	5.2	6.4	0.8	1.0	1.0	1.2	0.4		2.1	1.9	2.3	2.3
StB	**G1**	6.1	6.4	6.7	5.9	6.3	6.5	5.9	5.5	6.4	7.3	7.1	6.9	6.9	7.9	8.6	9.2	9.8	8.7	9.0	9.5	9.5	10.0	5.2	5.7	6.0	7.6	2.5	2.5	2.5	2.2	2.8	2.3	**G**	0.9	1.5	1.3
StB	**G2**	6.0	5.6	5.8	5.0	5.4	5.7	5.0	4.7	5.5	6.4	6.2	6.0	6.0	6.9	7.5	7.2	7.6	6.7	7.1	7.5	7.5	7.9	5.6	5.5	5.8	6.7	2.7	2.7	2.7	2.5	2.6	2.1	1.0		0.9	1.1
Dom	**G3**	5.1	4.7	5.0	4.3	4.6	4.9	4.3	4.0	4.5	5.7	6.2	6.0	5.9	6.9	7.5	7.9	8.4	7.4	7.8	8.2	8.3	7.8	6.2	6.1	6.8	6.5	3.2	3.2	3.2	3.2	2.5	2.5	1.6	1.0		0.9
Pis	**G4**	6.5	6.1	6.3	5.5	5.9	6.2	5.5	5.2	5.8	7.0	7.5	7.4	7.3	8.3	9.0	9.8	10.4	9.3	9.5	10.0	10.1	9.5	6.1	6.0	6.4	8.2	3.2	3.2	3.2	3.0	2.6	2.6	1.4	1.2	1.0	

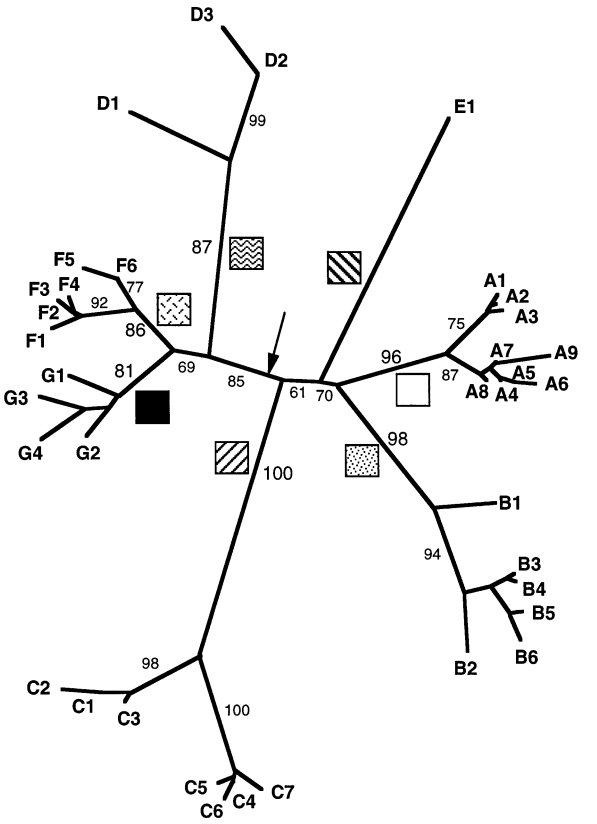

Fig. 2 Neighbour-joining (NJ) tree of Kimura 2-parameter (K2P) distances among alpine scree weta cytochrome oxidase (COI) haplotypes. Principal lineages are coded with a filled square and an alphabetic label (A–G). Terminal edges (haplotypes) have numeric labels (1–9). Numbers on branches show percentage support from 500 bootstrap replicates using maximum parsimony (MP) analysis and 5 : 1 transversion : transition weighting. An arrow indicates the position of root in analyses that include outgroup *Deinacrida* spp.

Internal structure of trees was generally well supported; 13 nodes had bootstrap values in excess of 80% (Fig. 2). Using a combination of edge length and bootstrap support, we identified seven major lineages (A–G; Fig. 2), although several of these (A, B, C, D and F) were resolved into two branches.

Estimates of molecular diversity (π) for each geographical site with more than one weta were low (mean 0.005); intraspecific variation was largely caused by differences among populations (Φ_{ST} 0.92). K2P distances among the seven deep lineages reached 8.4% (mean 5.6%), and GTR + I + Γ reached 13.0% (mean 8.0%) (Table 1). Genetic distances among terminal edges within each lineage were small, up to 2.8% (K2P) and 3.2% (GTR + I + Γ).

Weta (N = 26) from seven localities in the northeast area (Marlborough), all carried haplotypes from a single lineage (A) (Fig. 1). Nearby, in the northwest (Nelson), all weta (N = 15) from two sites carried lineage C haplotypes, and pairwise genetic distances between A and C ranged from 5.9 to 6.9% (K2P) or from 7.4% to 10.9% (GTR + I + Γ) (Table 1). The geographically most distant regions (Southland vs. Nelson) were also the most genetically distinct (lineages C and D: K2P mean 7.2%, GTR + I + Γ mean 11%).

Deep lineages were restricted to distinct geographical areas, with few sites bearing individuals with haplotypes found elsewhere (Fig. 1, Table 1). Outside Marlborough, only one site (St Mary's Range) had a haplotype (F2) found at another site (Rees Saddle).

Based on the widely used global mitochondrial sequence divergence rates of 2–2.4% per million years (Myr) (Brown *et al*. 1979; Brower 1994) and the mean among-lineage GTR + I + Γ distance of 8%, these data indicate divergences beginning 3.3–4.0 Ma. The highest genetic distance among haplotypes (GTR + I + Γ 13%) would indicate coalescence as early as 6.5 Ma.

Discussion

We encountered high levels of genetic variation and diversity within the alpine scree weta, *Deinacrida connectens*. In fact, the highest genetic distance found between two COI haplotypes (7.6% uncorrected *P*, 8.4% K2P, 13% GTR + I + Γ) is more typical of interspecific divergence in insects (Funk *et al*. 1999). Studies using DNA sequences from similar fragments of COI from beetles (Funk *et al*. 1995) and moths (Brown *et al*. 1994) have revealed intraspecific distances as high as 3.8% and 5.7% (K2P), respectively, but values closer to 2% are more typical (Langor & Sperling 1997). Although COI exhibits a high level of functional constraint (Lunt *et al*. 1996) and begins to shows evidence of saturation beyond 13% sequence divergence in intergeneric studies (Szymura *et al*. 1996), our within-species distances are not expected to experience loss of phylogenetic information. Nuclear markers (morphology, karyology and allozymes) present no evidence that *D. connectens* is anything other than a single species (Field 1980; Morgan-Richards & Gibbs 1996).

Three levels of phylogenetic structure are discernible from analysis of these COI data. The primary, well-supported divisions are deep, perhaps polytomous, edges apparently derived from isolation some 3.3–4.0 Ma (lineages A–G). Shallow divisions are present at the termini of most of these edges (haplotypes 1–9) but their branching order is generally not well resolved. Most lineages also have some additional, well-supported subdivisions that generally reflect an intermediate level of geographical structure (e.g. lineage C separated into Mt Peel and Mt Arthur haplotypes).

The phylogeographical structure encountered is consistent with our alpine radiation model. It is clearly not associated with environmental changes (climate warming

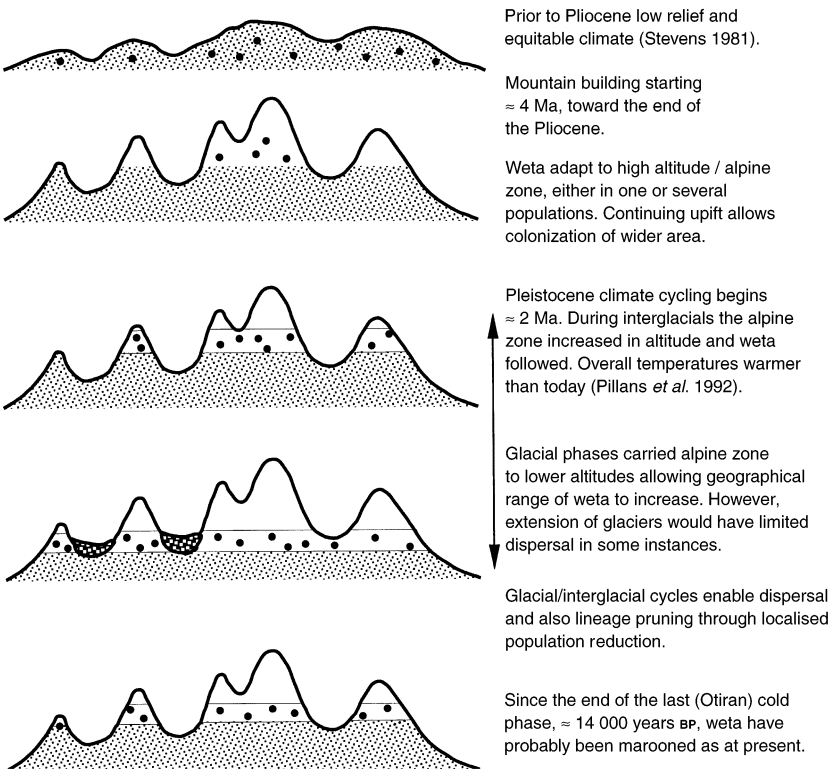

Fig. 3 Hypothesis of the role of Pliocene orogenics and Pleistocene glacial/interglacial cycling in the evolution and phylogeography of the alpine scree weta, *Deinacrida connectens*. Dots indicate weta.

and predator introduction). The estimated mean coalescence time of ~ 4 Myr falls within the time range estimated for the emergence of the main axial range of South Island mountains, and the polytomous structure of the gene tree can be explained by isolation of populations on separate mountain ranges. If correct, this timing indicates that *D. connectens* speciated prior to Pleistocene climate cooling and therefore under the influence of new, altitudinally structured habitats generated by Pliocene mountain building.

Scree weta populations have apparently experienced differing degrees of isolation throughout their range, presumably as a result of different barrier characteristics. For example, despite similar linear geographical distances, genetic distances between lineages F and G are lower than those between lineages A and C. Lineages F and G form a southern partition with D in the phylogenetic analysis, but D is distinguished by a long edge. This is despite the geographical proximity of Rees Saddle and Eyre Peak with lineages F and D, respectively. Isolation of these two lineages may have been maintained by the presence of a long lake (Wakatipu), which is known to have contained and been formed by a substantial glacier system during the Pleistocene. Mt Arthur and Mt Peel in Nelson are only ≈ 10 km apart, yet show a significant intermediate level split (e.g. 2.0–2.6% GTR + I + Γ) of two reciprocally monophyletic lineages, C1–C3 and C4–C7. This probably reflects isolation during interglacials as there is little scope for glacier development in the intervening area.

A single major mitochondrial lineage (A) consisting of nine haplotypes ($N = 26$) is present in the Marlborough region, indicating a single colonization event or multiple extinction events. The branching pattern within this lineage suggests extensive lineage sorting (prior to recent times) and subsequent dispersal among populations within the region. Given their close proximity, it is remarkable that there is no evidence of exchange between Marlborough (lineage A) and Nelson (lineage C).

Interestingly, these two regions (Marlborough and Nelson) are bisected by the Alpine Fault (with the exception of Red Hills, Marlborough, which lies just to the west, Fig. 1 inset). Land movement along this fault has been proposed as a vicariant explanation for disjunct distributions of many taxa (Heads 1998). However, despite an apparent fit of the geographical patterns of distribution (i.e. inconsistency among populations across the fault line), this model is not compatible with the depth of genetic structure pattern observed here. The majority of lateral movement along the fault took place 10–20 Ma and was therefore considerably prealpine.

The cyclical nature of the Pleistocene glaciation consisting of some 20 separate glacial and interglacial episodes (Stevens 1981; Cooper & Millener 1993) probably provided repeated opportunities for genetic exchange (Fig. 3). Indeed, the alternating raising and lowering of the alpine zone

would presumably have forced the geographical range of alpine organisms to fluctuate accordingly. Two features of glaciation will, however, have had subtly different effects. A colder climate will have lowered the alpine zone and allowed *D. connectens* to colonize lower altitudes with potentially greater habitat area. At the same time, however, glacier extension would have prevented colonization of many valley systems and maintained lowland barriers between scree populations (Fig. 3). Populations in the vicinity of Eyre Peak and Rees Saddle, for instance, would have remained separated by ice (see Fig. 1 inset). In areas where ice was sparse, such as Marlborough, exchange between populations by dispersal across valleys would have been feasible. The presence of glaciers at the northern end of the Southern Alps may have prevented gene flow between weta populations in Marlborough and Nelson, as has been proposed for alpine grasshoppers in the region (Peterson 1968). It would certainly have limited exchange between Nelson and the other populations to the south. Warm interglacials will have restricted the range of *D. connectens* to higher altitudes and may even have led to local extinction.

Although the phylogeographical structure of mtDNA haplotypes is very clear, it is only vaguely paralleled by morphological and chromosome variation among populations. Colour variation shows some north-to-south clustering (Field 1980), but colour variation within regions such as Marlborough (lineage A) is high (Morgan-Richards & Gibbs 1996). Southern populations had karyotype numbers (XO males) of 17 or 19, whereas those from Fox Peak northwards had 21 (Morgan-Richards & Gibbs 1996). There is good bootstrap support (85%) for a split between the mtDNA lineages (D, F, G vs. A, B, C, E) that coincides with this karyotypic differentiation (Fig. 2). Within these two (north and south) groups, the distribution of other, more subtle, karyotype variation appears to overlay mtDNA structure. Notably, weta on Mt Arthur (lineage C) have a similar karyotype to populations in Marlborough (lineage A), and at St Mary's Range haplotype F2 was found in an individual with 17 chromosomes whilst the same haplotype was present among individuals with 19 chromosomes at Rees Saddle. This level of intraspecific karyotype variation is a feature of Orthoptera (White 1978) and typical of the family (Morgan-Richards 1997).

Quaternary biogeography is dominated by postglacial range expansion from glacial age refugia that were positioned away from cold and arid areas (e.g. Cwynar & MacDonald 1987; Bennett *et al*. 1991; Hewitt 1993; Cooper *et al*. 1995; Joseph *et al*. 1995; Roy 1997; Taberlet *et al*. 1998). In a pleasing inversion of this trend, *D. connectens* may have experienced its most severe refugiation during the warm interglacials rather than the glacials (Fig. 3). This amelioration of the effects of glacial/interglacial climate extremes through altitudinal migration was suggested by Hewitt (1996) as a possible explanation for the retention of genetic diversity in the European, nemoral meadow grasshopper (*Chorthippus parallelus*). In the case of *D. connectens*, however, the population structuring scenario has taken place within a relatively small (but geologically diverse) island in the absence of a polar ice sheet. The general applicability of the alpine radiation model can be tested by looking for similar phylogeographical patterns in ecologically similar species.

Acknowledgements

We are grateful to George Gibbs, Peter Johns and Brian Patrick for their collecting efforts. The Department of Conservation provided collection permits. Johns Hobbs (Nucleic Acid—Protein Service, NAPS Unit, University of British Columbia, Vancouver, BC, Canada) supplied insect mitochondrial DNA primers, based on sequences compiled by Dr B. Crespi (Simon Fraser University, Burnaby, BC, Canada) and described on postings to bug-net@sfu.ca. Jack Sullivan assisted with ML analysis. The map inset was based on National Oceanic and Atmospheric Administration data received and processed by Landcare Research, New Zealand. This work was supported by a fellowship to S. A. T. (contract UOO704) from the Foundation for Research, Science and Technology, New Zealand. M. M.-R. was in receipt of funding under the Ministry for Research, Science and Technology Marsden scheme (contract no.: PVT601).

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This work is part of S. A. T.'s programme, studying terrestrial invertebrate biogeography, ecology and systematics, primarily with the aid of molecular genetic techniques. The research is exploring intra- and interspecific phylogeography among New Zealand endemic invertebrates, their relatives in Australia and further afield. M. M. R is currently exploring the role of intraspecific karyotype variation in speciation using tree weta as a model organism. Work by G. P. W.'s research team centres around phylogeography of New Zealand endemic fish and insects, genetic analysis of hybridization and molecular analysis of co-speciation.
