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Phylogeographic genetic analysis of the alpine weta, *Hemideina maori*: evolution of a colour polymorphism and origins of a hybrid zone

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Abstract Body colour is an important distinguishing feature in a New Zealand alpine weta hybrid zone and is strongly correlated with mitochondrial DNA haplotype variation. To assess the broader geographic pattern of this association, an intraspecific phylogeny was generated using mtDNA sequence. Both parsimony and likelihood analyses separated the colour morphs into two clades. One grouped the Rock and Pillar Range "yellow" haplotypes with other Central Otago populations and the other grouped the "black" Rock and Pillar haplotypes as a monophyletic group. The black body colour seen in the south of the Rock and Pillar Range appears to have evolved multiple times across the species. Application of a molecular clock estimated that the Rock and Pillar yellow and black lineages were separated approximately 2 million years ago. The Rock and Pillar yellow lineage split from other Central Otago populations approximately 1 million years later, possibly the result of sequential range contraction and expansion.

Keywords weta; *Hemideina maori*; hybrid zone; colour polymorphism; phylogeography; mtDNA

INTRODUCTION

Body colour is the most obvious distinguishing feature in a hybrid zone of the alpine weta, *Hemideina maori*, on the Rock and Pillar Range, South Island, New Zealand (King et al. 1996). A black and yellow striped variant ("yellow") is found in the higher (above 1100 m) northern parts of the mountain range. To the south (1000–1200 m), the yellow colour is replaced by a dark brown, that alternates with the black stripes ("black"). In allopatry there is complete association between colour and mitochondrial DNA variation. This relationship breaks down somewhat, presumably through hybridisation and introgression, where the forms are sympatric (King et al. 1996). A range of intermediate body coloration can also be found across the range. While there is significant colour variation in the species throughout its South Island distribution, the majority of populations comprise individuals of yellow to intermediate coloration.

There are two broadly different hypotheses that can explain the origins of this hybrid zone. First, the Rock and Pillar Range may straddle an extensive secondary contact zone of widespread northern (yellow) and southern (black) forms. The linkage between body colour and mtDNA seen on the Rock and Pillars would therefore be expected to be a global feature of the species. If body colour were mapped onto a phylogeny of haplotypes from several geographic populations of the species, yellow and black forms should be reciprocally

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monophyletic groups. Second, events leading to the formation of the Rock and Pillar hybrid zone could have occurred locally, independent of other populations. Here, the association between body colour and mtDNA haplotype may be limited to the Rock and Pillar Range. While black and yellow haplotypes would show local reciprocal monophyly, black and yellow coloration may have arisen independently elsewhere, and, therefore, colour would exhibit polyphyly.

Colour polymorphisms, particularly those involving melanisation, are common in insects and their occurrences are many and varied (Kettlewell 1973; Majerus 1998). Specific explanations include thermal melanism (Digby 1955; Brakefield & Willmer 1985; deJong & Brakefield 1998; Forsman et al. 2002), crypsis, either as a predator or as warning or camouflage in prey species (Harris 1988), protection from UV (Kettlewell 1973), immune responses to disease or parasites (Gillespie et al. 1997; Wilson et al. 2001), environmentally induced pigmentation changes (Burtt 1951; Heal 1989), or a response to social stimulus (Kennedy 1961; Sword 1999). The cause of the colour polymorphism in this hybrid zone is unknown but we can speculate on possible explanations.

If the cline were a response to a temperature gradient, one would expect to find darker animals at higher altitude, the opposite of what is observed. The fact that all weta species are nocturnal also limits the selective influence of UV. Native nocturnal predators of alpine weta are most likely to be reptiles such as geckos (*Hoplodactylus* spp.) and skinks (*Oligosoma* spp.), or birds such as ruru (morepork; *Ninox*). Although we do not know what factors presently limit the distribution of these species, on the Rock and Pillar Range, weta are only found co-occurring with geckos where their respective lower and upper altitudinal limits overlap. If geckos were historically important predators, it is difficult to imagine how their predatory activities could have driven changes in weta body colour in some populations while not in others.

Aposematic species are typically brightly coloured, in this case represented by the yellow body morph. Since other *Hemideina* species most closely resemble the Rock and Pillar yellow body morph, yellow is most likely the ancestral body coloration. There is no evidence that tree weta are unpalatable, being readily eaten by a variety of native predators (Ussher 1999; Haw et al. 2001). Although we have no specific information on the palatability of black weta, it would be unusual for a distasteful form to be less brightly coloured than its palatable counterpart.

One can argue hypothetical explanations for the evolution of the colour polymorphism but the difficulty in identifying any such influences is that they may no longer exist. As such, it is perhaps more important to identify how the zone itself arose, rather than attempting to explain the function, if any, of the colour polymorphism.

Weta are Gondwanan in origin and have been present in New Zealand since the Mesozoic 200–140 m.y. ago (Stevens 1980). Many of the mountain ranges of South Island did not exist until the Pleistocene period (5–2 m.y. ago) when they began to form during the Kaikoura Orogeny. Until that time, ancestors of the present-day alpine fauna would have occurred over relatively low-relief land (Fleming 1979). Weta previously living in lowland forests were raised with the mountains to become part of the present-day alpine fauna. During the last ice age, a series of glacial and interglacial periods meant that the alpine fauna experienced serial changes in range as temperatures fell and rose (Trewick et al. 2000). Divergence in body colour could have occurred, by whatever means, during any one of those range contractions and the hybrid zone formed as a result of subsequent range expansion.

By analysing the phylogeographic relationships of South Island weta populations, we can help to resolve the question of the evolution of body colour variation and the origins of the hybrid zone.

Fig. 1 South Island of New Zealand showing sampling sites (modified from McKenzie 1987).

MATERIALS AND METHODS

Hemideina maori were obtained from 10 South Island localities (Fig. 1; Table 1). Five sites were from the Central Otago region (Harwich and Crescent Islands, Mt Ida, Dunstan and Rock and Pillar Ranges) and five from Canterbury and the West Coast. *H. ricta* and *H. femorata* were chosen as outgroup species as they are the most closely related weta to *H. maori* (Morgan-Richards & Townsend 1995). Femur muscle was stored in 70% ethanol or frozen at –80°C.

DNA was extracted using a standard phenol:chloroform method (Kocher et al. 1989). An approximately 670 bp fragment of mtDNA comprising part of the genes for tRNA leucine and cytochrome oxidase subunit II was amplified using primers A-tLeu (modified) (5′ AATATGGCAGATTAGTGCA 3′; Simon et al. 1993) and COIIB-605 (5′ GCTCCACAAATTTCTGAGCA 3′) (C. Simon pers. comm.). Reaction volumes of 100 µl were used. Each reaction contained 0.5 µ*M* each primer, 1.5 m*M* $MgCl_2$, and 0.2 m*M* each dNTP. Annealing temperatures of 45–48°C were used. Amplified products were gel-purified from 2% low-melt agarose gels using GELase (Epicentre Technologies) following the "high activity" protocol of the manufacturer.

The same primers used for PCR amplifications were used for sequencing. Manual sequencing using chain termination incorporating ^{35}S-labelled nucleotides (Sanger et al. 1977) was carried out using a USB Sequenase version 2.0 sequencing kit. Completed sequencing reactions were run on 6% denaturing polyacrylamide gels. Dried gels were exposed for at least 3 days to Kodak X-OMAT-AR autoradiography film. Because overlapping sequence could not always be read using primers A-tLeu and COIIB-605, two internal

primers were designed from weta sequence to allow more sequence to be read. The first internal primer (C2-J-3089 (alias "180") 5′ ACATGATCTGACGTTAATTTACAA 3′) showed moderate similarity between weta sequence and species from other insect orders (Liu & Beckenbach 1992). The second primer (C2-N-3488 (alias "083") 5′ TTCGATTATCTACATCTAG 3′), although more similar to sequence from other insect orders than the internal forward primer, did not give good sequence in weta, although it may prove useful in other insect species.

Sequence was read and entered directly into MacClade (Maddison & Maddison 1992) and aligned by eye with reference to *Drosophila yakuba* (Clary & Wolstenholme 1985) and *Schistocerca gregaria* (Liu & Beckenbach 1992). Variation between individuals from the same location was double-checked against the autoradiograph. Identical sequences were combined. All sequences have been submitted to GenBank (accession numbers: AY377443-AY377463) and the aligned dataset and resultant trees placed in TreeBASE (www.treebase.org).

Unless otherwise stated, analyses were conducted in PAUP* 4.0b4a or 4.0b10 (Swofford 2002). Phylogenetic trees were constructed using both maximum parsimony and maximum likelihood. For all analyses, the two *Hemideina femorata* haplotypes were designated as the outgroup. We used the PTP test (Faith 1991; Faith & Cranston 1991) and g_1 statistic (Hillis & Huelsenbeck 1992) to investigate whether the data contained significant phylogenetic signal. The parsimony trees were estimated using heuristic searches with 1000 random additional sequences and TBR branch swapping. For the maximum likelihood heuristic searches, one of the equally weighted parsimony trees was used as the starting point for branch swapping (TBR). The maximum likelihood model was selected using the Akaike information criterion of Modeltest (Posada & Crandall 1998).

Table 1 Collecting localities of *Hemideina maori*, *H. ricta*, and *H. femorata* samples sequenced in this study. Latitude, longitude, and the number of individuals analysed from each site are shown. A range of samples were collected from the Rock and Pillar Range. Two individuals of each previously identified haplotype were collected (see King (1997) for details).

Collection site	Lat. S	Long. E	Number
Hemideina maori			
Maukuratawhai (Clarence Valley)	42°26′	172°51′	1
Mt Percival (Hanmer Range)	42°29′	172°56′	1
Foggy Peak (Torlesse Range)	43°17′	171°45′	2
Mt Somers	43°43′	171°34′	2
Mt Cook	43°36′	178°07′	2
Harwich Island, Lake Wanaka (Mou Waho)	44°34′	169°04′	2
Crescent Island, Lake Wanaka (Mou Tapu)	44°37′	169°03′	2
Mt Ida	45°56′	170°05′	1
Dunstan Range	44°53′	169°39′	2
Rock and Pillar Range	45°29′–45°32′	170°01–170°02′	12
Hemideina ricta			
Banks Peninsula	44°15′	173°07′	2
Hemideina femorata			
Kaikoura	42°23′	173° 44′	1
Banks Peninsula	44°15′	173° 07′	1

To investigate the support for our trees and the phylogenetic signal in our dataset we used bootstrap analysis (Felsenstein 1985) and spectral analysis (Hendy & Penny 1993). For the bootstrap analyses, fast heuristic searches with 10 000 replicates were performed for parsimony, while 1000 replicates were performed for likelihood. The fast heuristic search has been shown to provide similar (though lower) values than the full heuristic search, with the greatest discrepancy when the bootstrap values are relatively low (Mort et al. 2000). Thus, the fast heuristic search provides a conservative estimate of the bootstrap support. We used the program Spectrum 2.3 (Charleston 1998), which implements spectral analysis, to further investigate the phylogenetic signal in the data. In spectral analysis, support for the bipartitions of the dataset (possible branches in the resultant trees) is related to the number of character state changes that correspond to that bipartition or split (i.e., the expected number of substitutions per site). The conflict for a bipartition is the sum of the support for those bipartitions that conflict with it. For discussions of spectral analysis and its use see Charleston & Page (1999), Lento et al. (1995), Kennedy & Spencer (2000), and Kennedy et al. (2000). We computed the spectrum from distance matrices that had been calculated using the Tamura-Nei model to correct for superimposed changes (this model allows for unequal base frequencies, and a transition/transversion bias with two transition classes (Tamura & Nei 1993)). In addition to calculating the spectrum for the best supported bipartitions in the dataset, Spectrum can calculate the support and conflict values for any bipartition of interest. Conflict values were normalised following Lento et al. (1995).

To evaluate which biogeographic hypothesis is best supported by our data, the colour of weta from each site was mapped onto the reduced maximum likelihood tree topology. The body colour of weta collected from each site was classified into one of three classes: black, intermediate, and yellow (see King et al. (1996) and King (1997) for definitions of colour categories).

Mean distances between pairs of populations (i.e., sequence divergence) were calculated using the Tamura-Nei model (see Table 2). The Tamura-Nei model was used for consistency with the spectral analyses. We tested for the presence of a molecular clock (and, thus, whether it is valid to use the distances to estimate divergence times) using a likelihood difference test (Felsenstein 1995). We computed our likelihood tree both with and without imposing a molecular clock constraint. We found that we could reject the molecular clock hypothesis (log likelihood difference = 104.84, with 19 d.f. $\chi^2 = 30.144$, $P < 0.05$) for the full dataset, but could not reject the clock hypothesis with a smaller, 16 taxa, version of the dataset (log likelihood difference = 13.37, with 14 d.f. $\chi^2 = 23.685$, $P > 0.05$). The result for the reduced dataset should be treated with some caution as relatively short sequences may lack the power to reject a molecular clock (Bromham et al. 2000). The 16 taxa chosen for further analyses excluded populations from outside Central Otago, whose positions were not well resolved in the initial analyses. We used the Tamura-Nei distances calculated for the reduced dataset for our divergence calculations. Because of the variety of sources of error associated with dating divergence times, however, these estimates should be treated as approximations (Hillis et al. 1996). The estimated times since divergence (Table 2) were calculated using a rearrangement of the formula in Brower (1994):

$$\text{age of clade (years)} = \frac{\text{\% sequence divergence} + 0.19241}{2.3435 \times 10^{-6}}$$

This formula offers a reasonable calibration as it is based on arthropod data and has been shown to be appropriate over short divergence times (<3–4 Ma), which encompasses a time frame appropriate for *H. maori*.

Table 2 Percentage sequence divergence calculated using the Tamura-Nei model (above diagonal) and divergence time in m.y. ago (below diagonal) for mtDNA sequences from Central Otago populations of *Hemideina maori*. Outgroup taxa (*H. ricta* (*Hr*) and *H. femorata* (*Hf*)) are shown for comparison. For details of collecting sites and samples, see Fig. 1 and Table 1.

	Har	Cres	Ida	Dun	Y1	Y2	Y3	Y4	B1	B2	B3	B4	B5	*Hr*	*Hf*1	*Hf*2
Har		0.00	1.91	1.53	2.02	2.17	2.13	1.71	4.94	5.01	4.93	5.15	5.21	4.60	20.75	20.01
Cres	0.08		1.88	1.49	2.33	2.46	2.44	1.85	5.02	4.99	5.05	5.24	5.28	4.61	21.35	21.03
Ida	0.90	0.89		1.67	1.93	2.05	2.01	1.46	3.74	3.82	3.96	3.94	3.99	2.84	20.68	20.38
Dun	0.74	0.72	0.79		2.48	2.59	2.55	1.99	5.26	4.84	4.70	5.45	5.52	4.73	21.19	20.89
Y1	0.95	1.08	0.91	1.14		0.18	0.00	1.52	4.46	4.65	4.49	4.66	4.51	4.28	21.25	20.32
Y2	1.01	1.13	0.96	1.19	0.16		0.18	1.63	4.49	4.52	4.52	4.71	4.54	4.33	21.67	20.70
Y3	0.99	1.12	0.94	1.17	0.08	0.16		1.60	4.40	4.61	4.44	4.61	4.45	4.31	21.11	20.17
Y4	0.81	0.87	0.71	0.93	0.73	0.78	0.77		4.21	4.60	4.24	4.41	4.45	4.10	19.61	19.34
B1	2.19	2.22	1.68	2.33	1.98	2.00	1.96	1.88		0.18	0.36	0.00	0.72	4.91	20.48	20.48
B2	2.22	2.21	1.71	2.15	2.07	2.01	2.05	2.04	0.16		0.18	0.36	0.75	5.09	20.11	19.74
B3	2.18	2.24	1.77	2.09	2.00	2.01	1.97	1.89	0.23	0.16		0.53	1.09	5.37	20.52	20.52
B4	2.28	2.32	1.76	2.41	2.07	2.09	2.05	1.97	0.08	0.24	0.31		0.90	5.14	20.74	20.73
B5	2.31	2.33	1.78	2.44	2.00	2.02	1.98	1.98	0.39	0.40	0.55	0.47		5.17	20.04	19.77
Hr	2.04	2.05	1.30	2.10	1.91	1.93	1.92	1.83	2.18	2.25	2.37	2.28	2.29		22.45	22.63
*Hf*1	8.93	9.19	8.91	9.13	9.15	9.33	9.09	8.45	8.82	8.66	8.84	8.93	8.63	9.66		1.76
*Hf*2	8.62	9.05	8.78	9.00	8.75	8.91	8.69	8.34	8.82	8.51	8.84	8.93	8.52	9.74	0.83	

RESULTS

Sequence was obtained for the first 571 bp of the cytochrome oxidase II gene. Duplicate sequences within each site were identical and were treated as a single sequence. Four different haplotypes were found among the six yellow-bodied weta collected and five from the six black weta. Of the 571 sites, 112 of the characters were parsimony informative. Both the significantly skewed tree-length distribution ($g_1 = -2.81$ from 10 000 random trees, $P < 0.01$; Hillis & Huelsenbeck 1992) and the PTP test (1000 replicates, $P = 0.001$) showed that the data contained significant signal. There was an overall excess of nucleotides A (32.1%) and T (32.0%) over G (13.6%) and C (22.3%), a common feature of insect mitochondrial DNA (Crozier et al. 1989).

Equal weighting parsimony analysis found 102 most parsimonious trees. In the equally weighted strict consensus tree (Fig. 2A) the black morph Rock and Pillar weta (B1–5) are monophyletic with 99% bootstrap support. In this analysis, three of the four yellow morph Rock and Pillar weta group together (Y1–3) with 98% bootstrap support and the two Rock and Pillar colour groups fall within different parts of the tree. Given that transitions occur more commonly than transversions, a more realistic assumption is to downweight transitions, lessening the effect of homoplasy caused by multiple transitions at a site. With the optimality criterion set to maximum likelihood, the transition to transversion ratio estimated on one of the equally weighted parsimony trees was approximately 6:1. With the weight of transversions increased by this ratio we again found 102 most parsimonious trees. The strict consensus of these 102 trees has the same topology as the equally weighted strict consensus tree (Fig. 2A). The bootstrap support for the monophyly of the Rock and Pillar black morph is again 99% in the weighted analysis, and the grouping of Y1–3 is seen with 98% bootstrap support. The weighted parsimony analysis provides 77% support for grouping the rest of the *H. maori* populations (including *H. ricta*) separate from the Rock and Pillar black morph. *H. ricta* is placed as sister taxon to the Mt Somers population of *H. maori* in both forms of parsimony.

In some circumstances maximum likelihood performs better than parsimony at estimating phylogeny, because it is better at dealing with unobserved substitutions (Swofford et al. 1996). The maximum likelihood tree (Fig. 2B) shows similar structure to the strict consensus of the parsimony trees, with those branches that are unresolved in the parsimony analysis having very short branch lengths. The Rock and Pillar black morph weta group together, but with only one branch receiving bootstrap support and with very low levels of divergence between the haplotypes. The Rock and Pillar yellow morph weta are monophyletic in the likelihood tree, with three of the haplotypes (Y1–3) grouping with 98% bootstrap support (as in the parsimony analyses). As with the parsimony analyses, likelihood strongly supports the sister relationship of the two populations from islands in Lake Wanaka (Harwich and Crescent) and their sister taxa relationship with the population from the Dunstan Range (81% bootstrap support). As in the parsimony analysis, this clade groups with the yellow morph Rock and Pillar weta (58% bootstrap support). Likelihood also supports grouping *H. ricta* with the Mt Somers population of *H. maori* (83% bootstrap support), and the grouping separating the black morph Rock and Pillar weta from the rest of the *H. maori* populations and *H. ricta* (69% bootstrap support).

The full dataset does not evolve in a clock-like manner (i.e., we were able to reject the molecular clock using a likelihood difference test). In order to make valid estimates of the divergence times of the taxa of interest we excluded five non-Central Otago populations of *H. maori* (i.e., those with the least direct relevance to this study) from the analysis. For this reduced dataset we found that the molecular clock hypothesis could not be rejected. We repeated both parsimony and likelihood to estimate the phylogeny for this reduced dataset (i.e., with 16 taxa included in the analyses). This dataset had 108 parsimony informative

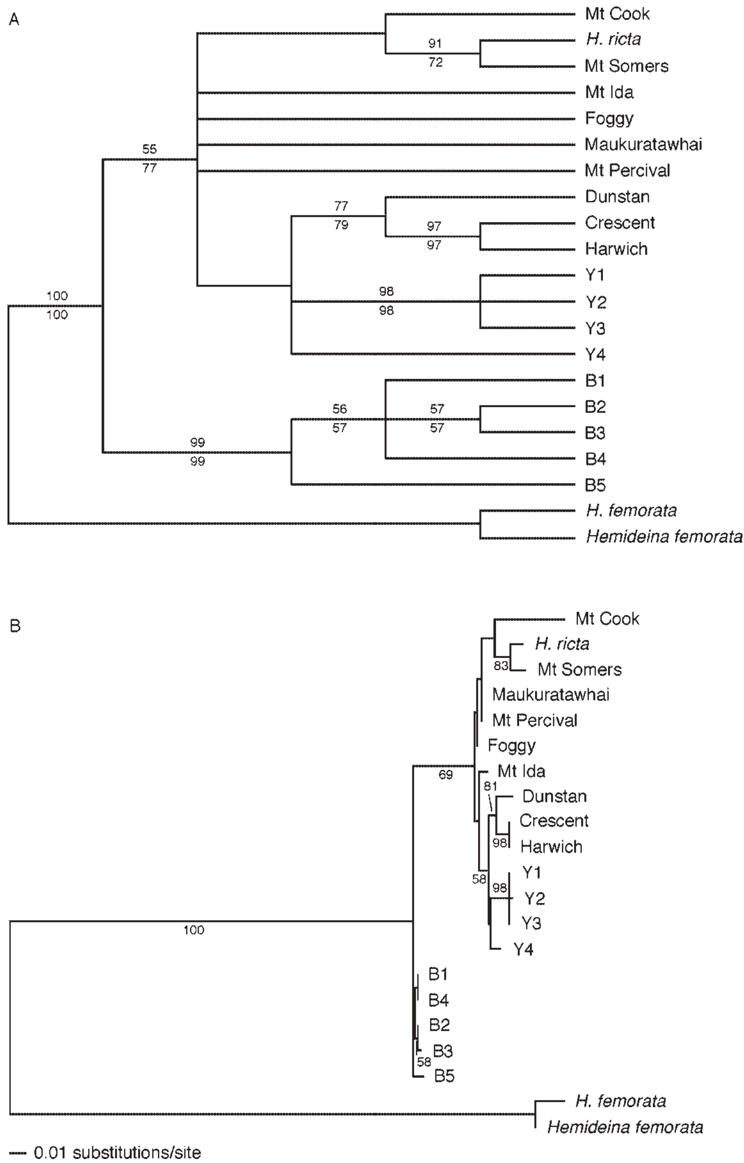

Fig. 2 Parsimony and likelihood trees for the full dataset. **A**, The strict consensus topology for both equally weighted and weighted parsimony analyses (102 trees each) with bootstrap values (10 000 fast heuristic replicates) for equally weighted analysis above branches and values for weighted analysis below branches. For equally weighted analysis, tree length = 187, CI = 0.818, and RI = 0.865. For weighted parsimony analysis, tree length = 422, CI = 0.848, and RI = 0.892; **B**, Maximum likelihood phylogram with bootstrap values (1000 fast heuristic replicates). Branch lengths represent the estimated proportion of substitutions per site. One of the equally weighted parsimony trees was used as the starting tree for branch swapping. The –Ln likelihood is 1630.28483. For likelihood analysis, a general-time-reversible model (selected using Modeltest) was used with invariable sites and among-site rate heterogeneity. Nucleotide frequencies, gamma shape parameter for rate heterogeneity (with four rate categories), substitution rate-matrix (with six substitution types), and proportion of invariable sites were all estimated by maximum likelihood.

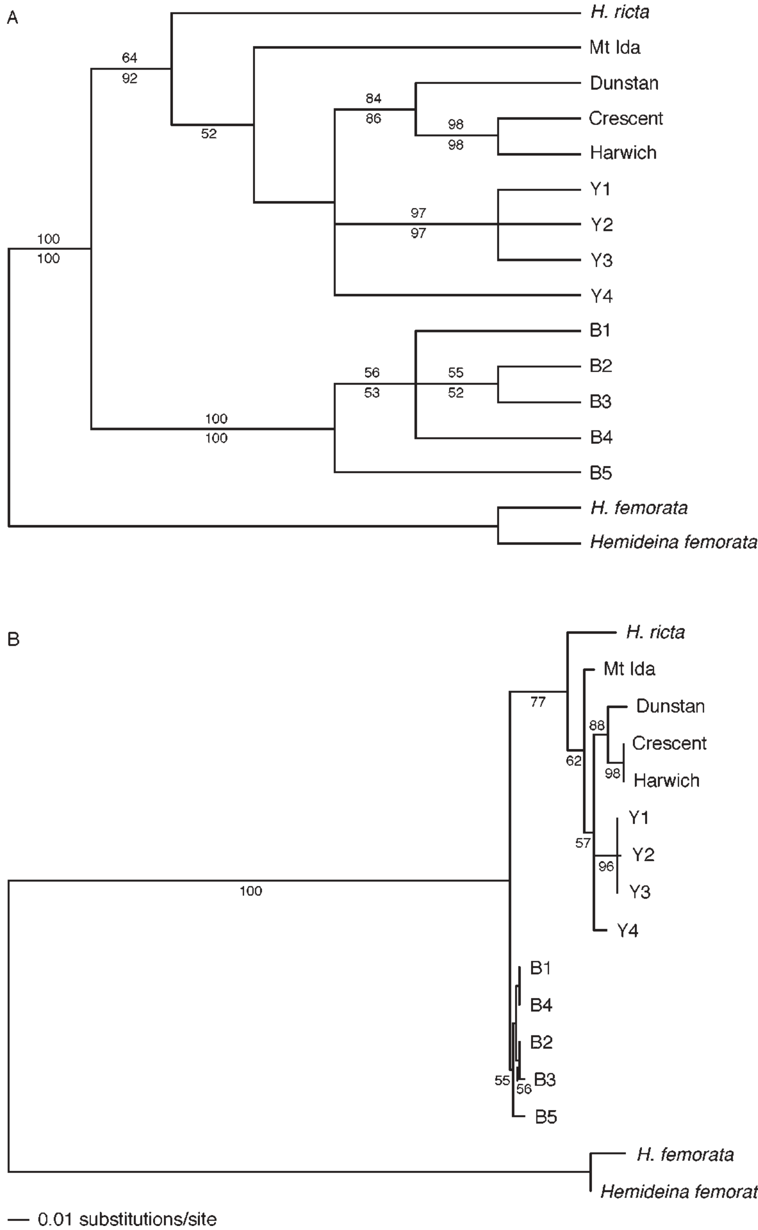

Fig. 3 Parsimony and likelihood trees for the reduced dataset. **A**, Strict consensus topology for weighted parsimony analysis (12 trees). As in Fig. 2A, bootstrap values (10 000 fast heuristic replicates) for equally weighted analysis are above branches and values for weighted analysis below the branches. The tree length = 365, the CI = 0.914, and the RI = 0.945; **B**, Maximum likelihood phylogram with bootstrap values shown (1000 fast heuristic replicates). Branch lengths represent estimated proportion of substitutions per site. One of the equally weighted parsimony trees was used as the starting tree for branch swapping. The –Ln likelihood is 1491.48479. For likelihood analysis, a general-time-reversible model (selected using Modeltest) was used with among-site rate heterogeneity. Nucleotide frequencies, gamma shape parameter for rate heterogeneity (with four rate categories), and substitution rate-matrix (with six substitution types) were all estimated by maximum likelihood.

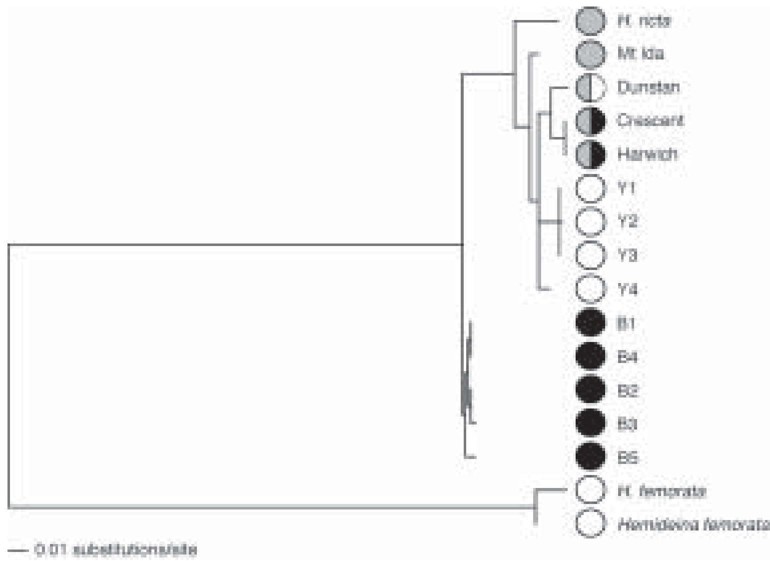

Fig. 4 Body colours of individuals sequenced at each site mapped onto the reduced maximum likelihood tree topology (see Fig. 3B). Open symbols, yellow; dotted, intermediate; solid, black.

characters and weighted parsimony analysis found 12 most parsimonious trees. The strict consensus of these 12 most parsimonious trees is almost identical to the topology found for the full dataset (Fig. 3A). Weighted parsimony supports the monophyly of the black morph Rock and Pillar weta (100% bootstrap support) and grouping the remaining populations of *H. maori* (and *H. ricta*) together (92% bootstrap support). The maximum likelihood tree (Fig. 3B) shows similar structure to the strict consensus of the weighted parsimony trees. The likelihood tree also supports the monophyly of the black morph Rock and Pillar weta (55% bootstrap support), as well as grouping the rest of the *H. maori* (and *H. ricta*) populations together (77% bootstrap support). It also groups the yellow morph Rock and Pillar weta with the Dunstan, Crescent, and Harwich populations (57% bootstrap support).

For computational reasons, spectral analysis is currently restricted to fewer than the 21 taxa in the full dataset. Therefore, we performed the spectral analyses with the reduced dataset. We evaluated the level of support and conflict for the monophyly of each of the Rock and Pillar colour morphs and contrasted those with the support and conflict for the monophyly of the Rock and Pillar weta (i.e., grouping the two colour morphs). The monophyly of the yellow morph Rock and Pillar weta is not well supported and has relatively substantial conflict (support = 0.0009, conflict = 0.0040). The monophyly of Y1+Y2+Y3 is comparatively well supported and has no conflict (support = 0.0094, conflict = 0), confirming that the position of Y4 is difficult for the data to resolve. The monophyly of the black morph Rock and Pillar weta is well supported by the data and has relatively low conflict (support = 0.0149, conflict = 0.0017). Within this group the monophyly of B1+B2+B3+B4 is relatively well supported and has no conflict (support = 0.0036, conflict = 0), while the sister taxon groupings of B1+B4 and B2+B3 both receive some support (0.0015 and 0.0012, respectively) and no conflict. When the bipartition that groups the two Rock and Pillar colour morphs as monophyletic was evaluated we found no support and very high conflict (support = 0.0000, conflict = 0.0284). Thus, the separation of the two Rock and Pillar weta colour morphs is well supported by the spectral analysis.

When body colour is mapped onto the maximum likelihood tree (Fig. 4) we can see that black morphs occur in both major clades of *H. maori*. This tree topology suggests that either the black morph evolved two or more times (from yellow, the ancestral state suggested by the outgroup *H. femorata*) or has been lost in some populations. Although intermediate body coloration is present across the species as a whole, we do not know how this relates to either black or yellow colour as it represents a continuum between the two.

A matrix of mean sequence divergence between pairs of taxa was calculated and subsequently used to estimate pairwise divergence times (Table 2). This shows that the Rock and Pillar yellow and black lineages last shared a common ancestor approximately 2.0–2.1 m.y. ago, whereas the estimated time of divergence of the Rock and Pillar yellow haplotype from other Central Otago populations is 0.7–1.2 m.y. ago.

DISCUSSION

The reconstruction of evolutionary relationships within or among species using one set of characters can be used to inform us about the evolution of other characters. In this case, our analyses show that patterns of relationships among populations of the alpine tree weta, *Hemideina maori*, (generated using mitochondrial COII sequence) do not support global monophyly of either yellow or black body colour (see Fig. 4). In particular, black and yellow weta from the hybrid zone on the Rock and Pillar Range do not form a monophyletic group. This result suggests that the hybrid zone on the Rock and Pillar Range formed through secondary contact, not *in situ*. However, dark body coloration occurs repeatedly on the tree, so although Rock and Pillar blacks are clearly monophyletic, dark body coloration has evolved multiple times across the species as a whole.

Focusing on the Rock and Pillar Range, the majority of our analyses support the monophyly of the Rock and Pillar black colour morph, but the branches separating these haplotypes are very short (e.g., Fig. 2B) and, thus, difficult to resolve. Both parsimony bootstrapping and spectral analysis strongly support the monophyly of the Rock and Pillar black morph, while likelihood bootstrapping of the reduced data set weakly supports this group. The monophyly of three of the four Rock and Pillar yellow colour morph haplotypes (Y1–3) is strongly supported in all our analyses (>96% bootstrap support) while the position of the fourth yellow haplotype is equivocal. The Y4 haplotype may form a monophyletic group with the other Rock and Pillar yellow morph haplotypes (e.g., Fig. 2B) but its position is difficult to resolve with certainty. Across the species as a whole, both black and yellow weta are found in all groupings that are well supported.

The absence of an association between the phylogenetic structure of the species and the colour of weta found in different populations does not support the hypothesis that the hybrid zone is part of a widespread contact. Although other Central Otago populations form a natural group, there is insufficient structure in the dataset to make statements about many other groupings. The one exception is that *H. ricta* clusters strongly with the Mt Somers population of *H. maori*.

Hemideina ricta is an endangered species found only around Banks Peninsula. It is distinguished from *H. maori* by minor morphological differences (Gibbs 2001). Our results do not separate it as a species distinct from *H. maori*, instead clustering it within *H. maori*. We can explain this observation in three ways. First, *H. ricta* may be a recently derived species. Second, it may be a distinct species into which *H. maori* mitochondrial DNA has introgressed, either completely or to an extent that it was not detected with our limited sampling. Finally, *H. ricta* may not in fact be a distinct species but may be better regarded as a population of *H. maori*.

Cross-species introgression of mitochondrial DNA is well documented (Ferris et al. 1983; Powell 1983; Spolsky & Uzzell 1984; Harrison et al. 1987; Marchant 1988; Wallis & Arntzen 1989) and it is possible that this is the explanation for our observation with *H. ricta*. However, studies of allozymes, chromosomes, coloration, stridulatory structures, and behaviour (Field 1982, 1993; Morgan-Richards 1995; Morgan-Richards & Townsend 1995) have all shown that *H. ricta* is either very similar to or indistinguishable from *H. maori*. It is highly unlikely that all of these markers would have introgressed from *H. maori* into *H. ricta* if they were distinct species. Given the similarity between these two species, it is perhaps more likely that *H. ricta* is either a recently derived species that has yet to diverge to any great extent from *H. maori* or a remnant population of *H. maori*. *H. ricta* is known to hybridise with *H. femorata* (Morgan-Richards & Townsend 1995), although only F_1 hybrids were produced. To determine whether *H. ricta* and *H. maori* were fully inter-fertile, one would need to examine the viability and fertility of F_1, F_2, and backcross animals. The taxonomic status of *H. ricta* may require re-examination.

Yellow haplotypes from the Rock and Pillar Range are more closely affiliated with other Otago populations than with black haplotypes from the Rock and Pillars (Fig. 2, 3; Table 2). This finding suggests that the isolation of the yellow and black Rock and Pillar lineages occurred before the yellows became completely isolated from other populations to the north-west. The black lineage on the Rock and Pillars could have derived from a large population that included future Rock and Pillar yellow lineages and other Otago populations. Divergence of the Rock and Pillar yellow lineage from the rest of the Otago populations would have occurred at a later stage. This scenario is consistent with estimates of divergence times using a molecular clock. The estimated timing of the split between the Central Otago group and Rock and Pillar yellows is between 0.8 and 1.2 m.y. ago (Table 2) and could have been caused by a combination of mountain formation and serial range contraction and expansion events during the last ice age. The same pattern may well be reflected among the Rock and Pillar black lineages and more southern populations such as the Lammermoor or Umbrella Ranges, where dark individuals have been reported but could not be found during the course of this study. Sequences of individuals from these populations may help resolve the timing of divergence among the southern populations.

The pattern of colour variation found in this isolated alpine tree weta species is similar to that found in the alpine scree weta, *Deinacrida connectens*. Like *H. maori*, the species is found on mountain ranges throughout South Island. Colour variation among populations has been noted in several studies (Ramsay 1961; Field 1980; Morgan-Richards 1995). Using analyses of colour, morphology, karyotypes, and allozymes, Morgan-Richards (1995) suggested several explanations for the evolution of the species. Although not able to distinguish definitively between the varying hypotheses, those best supported by the data were: (1) that a widespread cline was disrupted by the formation of South Island mountains 1–3 m.y. ago, (2) that separation was gradual such that geographically close populations retained some communication prior to becoming completely isolated, or (3) that isolation on mountain ranges was followed by periods of contact during the ice age. Hypothesis 1 was not well supported by the genetic data. Hypotheses 2 and 3 are the more likely options but could not be distinguished using present-day data.

The similarity of patterns seen in *D. connectens* and *H. maori* supports a recent (1–3 Ma) origin of New Zealand's alpine fauna. Geological events have played a major role in shaping the present-day distribution patterns of South Island's alpine fauna (Emerson & Wallis 1995). As a result, other species that could have become isolated on mountain ranges may be expected to show similar patterns. Results from this study can be used as a basis for hypotheses of distribution patterns in other species including insects, molluscs, and plants.

Focusing on the Rock and Pillar Range, it is unlikely that divergence between the two parental types occurred in parapatry (i.e., not a primary contact hybrid zone *sensu* Endler (1977)), unless yellow weta spread out from the zone subsequently. While it is impossible to identify a causal agent of divergence in body coloration, it is possible that *H. maori* exhibited a colour polymorphism prior to the separation of the two Rock and Pillar lineages. Following the split, genetic drift, selection, or a combination of both may have precipitated a change in the predominant colour in each population now found on the Rock and Pillar Range.

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