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Chromosome races with Pliocene origins: evidence from mtDNA

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There are eight distinct chromosomal races of the New Zealand weta *Hemideina thoracica*. We used mtDNA sequence data to test the hypothesis that these races originated on islands during the early Pliocene (7–4 million years ago). Nine major mitochondrial lineages were identified from 65 cytochrome oxidase I sequences. Phylogenetic analysis of these lineages suggests that they arose at approximately the same time. The geographical distribution of some lineages coincides with areas that were islands during the Pliocene. Overall, hierarchical AMOVA analysis shows that chromosomal races and Pliocene islands describe only 28% and 24%, respectively, of the total current mtDNA variation. However, removing one widespread (A) and one putatively introgressed (F) lineage increases these estimates to 65% and 80%, respectively. Intraspecific sequence divergence was very high, reaching a maximum of 9.5% (uncorrected distance) and GC content was high compared to other insect mtDNA sequences. Average corrected distance among mtDNA lineages supports the Pliocene origins of this level of genetic diversity. In the southern part of the species range there is reduced mtDNA variation, probably related to local extinction of *H. thoracica* populations from recent volcanic activity and subsequent re-colonization from a leading edge. In contrast, in this southern part there are five chromosome races, suggesting that chromosome races here may be younger than those in the north.

Keywords: chromosome evolution, insect, mtDNA, New Zealand, phylogeography, Pliocene islands.

Introduction

Although many animal groups are karyotypically rather conserved, others, in particular small mammals (Searle, 1993) and orthopterans (White, 1978), are renowned for high intraspecific karyotypic diversity. Members of these groups often exist as geographically contiguous chromosome races bounded by sharp hybrid zones or stepped clines (Searle, 1993). The distributions of these races pose historical biogeographic questions about their origins, and their regions of narrow sympatry provide natural laboratories for analysis of gene flow between genetically differentiated forms (Barton & Hewitt, 1985; Kocher & Sage, 1986; Hewitt, 1988). Given a number of chromosome races with several contact zones, one can examine the level of gene flow in relation to the nature and extent of karyotypic differentiation. One of the potential confounding factors in this approach is that races and zones could be of quite different ages, and any reinforcement (Howard, 1993) or reproductive character displacement (Butlin, 1989) of barriers to gene flow, or indeed their weakening through assimilation and selection, could disparately alter the levels of gene flow across zones. In this paper we aim to determine the origins of numerous chromosome races in an orthopteran (the weta, *Hemideina thoracica*) and determine whether they have approximately contemporaneous vicariant origins (Mayr & O'Hara, 1986).

Weta are nocturnal flightless Orthoptera of the family Anostostomatidae (Johns, 1997). Endemic to New Zealand is a genus of arboreal, herbivorous weta (*Hemideina*) whose species are common and well studied. These species are sexually dimorphic; adult males use elongated mandibles in male–male combat for possession of tree cavities in which many adult females shelter during the day (Moller, 1985; Gwynne & Jamieson, 1998). The Auckland weta *H. thoracica* White is found throughout much of the North Island of New Zealand, a range of approximately 1800 km^2. Within this range eight karyotypically distinct 'races' are known (Morgan-Richards, 1997). A study of nuclear markers among populations of *H. thoracica* found little evidence of genetic differentiation, suggesting that chromosome races may have arisen quite recently. Mitochondria provide rapidly evolving

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haploid maternally inherited markers with a smaller effective population size, giving the ability to detect recent barriers to gene flow where nuclear markers fail (Avise, 1992). Thus a survey of mitochondrial markers has the potential to reveal genetic differentiation and provide an historical biogeographic framework to the analysis of gene flow through contact zones.

Greater diversity of both allozyme and chromosome markers was found in the northern part of the range of *H. thoracica* and led to the suggestion that a chain of early Pliocene (7–4 million years (Ma) ago) islands in the north of New Zealand may have facilitated the fixation of chromosome rearrangements (Fleming, 1979; Morgan-Richards, 1997). The Pliocene archipelago has since been connected by sand, volcanic activity and sea level changes. Some areas that were probably islands during the Pliocene have weta with unique karyotypes (North Cape, Karikari Peninsula). However, some weta populations on younger islands that were recently connected to the mainland (~20 000 years ago), also have unique karyotypes (Cuvier Island, Double Island). If isolation on islands during the Pliocene did result in the formation of genetically distinct populations within this species then we predict that: (1) Areas that are considered to have been islands during the Pliocene would have distinct mitochondrial lineages. (2) Mitochondrial diversity would have arisen at approximately the same time due to divergence during a period of geographical isolation resulting in a number of lineages of approximately equal divergence. (3) The sequence divergence among mitochondrial lineages would match estimates based on the molecular-clock hypothesis. Both recent (Pleistocene) and older (Pliocene) isolation events may have facilitated fixation of novel chromosome rearrangements, and thus the possibility that the chromosomal races are of varying age will be considered.

Materials and methods

Weta

Weta were collected from the entire range of the species (Fig. 1). Some individuals were part of an earlier study that described the chromosome variation within this species. In this current study there are 32 new locations and 191 weta in total (Table 1). All weta collected were cytogenetically examined as described in Morgan-Richards (1997).

mtDNA

DNA was extracted from frozen muscle using a salting-out method (Sunnucks & Hale, 1996). To avoid sequencing every individual, single stranded conformational polymorphism (SSCP) was used to identify haplotype variants (Sunnucks *et al.*, 2000). Because mtDNA is inherited as a single non-recombining unit it was possible to use different regions of the genome for sorting and sequencing haplotypes. Universal insect mitochondrial primers SR-J-14233 and SR-N-14588 (Simon *et al.*, 1994) were used to amplify a 355 base pair (bp) fragment of the small ribosomal subunit (12S) gene for SSCP screening. The 3′ end of cytochrome oxidase I (*COI*), which has been shown to be appropriate for intraspecific studies (Lunt *et al.*, 1996; Trewick *et al.*, 2000), was used for the majority of the sequence analysis. We expected to detect less variation in 12S compared to that within *COI* due to the smaller size of the 12S fragment used and its slower rate of evolution compared to *COI* (Simon *et al.*, 1994). By selecting individuals to sequence based on the SSCP data we were maximizing the diversity detected within our sample and minimizing the number of identical or very similar haplotypes that we sequenced. The SSCP method followed Trewick (2000). A representative of each 12S-haplotype (as detected by SSCP) was then sequenced for a 550-bp fragment of the *COI* gene. Universal insect mitochondrial primers C1-J-2195 and L2-N-3014 (Simon *et al.*, 1994) were used to amplify an 800-bp fragment. PCR products were gel-purified and cleaned using Qiaquick spin columns. DNA was sequenced using Bigdye chemistry (Perkin Elmer) following the manufacturer's protocols using primer C1-J-2195, to give a 550-bp fragment of the 3′ end. Sequences were aligned by eye using SeqEd. v1.0.3 (ABI, PE).

Phylogenetic analysis

PAUP*4.0b (Swofford, 1998) was used to implement various methods of phylogenetic reconstruction: Maximum Likelihood (ML), Maximum Parsimony (MP) and Neighbour Joining (NJ). Tests for homogeneity and clock-like evolution of the sequences using a likelihood difference test (Felsenstein, 1988) were also performed in PAUP*. Transversion/transition ratio and number of invariant sites were calculated using MacClade 3.07 (Maddison & Maddison, 1992) and a NJ tree.

Population genetics

The AMOVA method of Excoffier *et al.* (1992) was implemented by ARLEQUIN 1.0 (Schneider *et al.*, 1997). The SSCP 12S-haplotype frequency data was used with a distance matrix based on *COI* sequences. Hierarchical analysis was performed across chromosomal races and

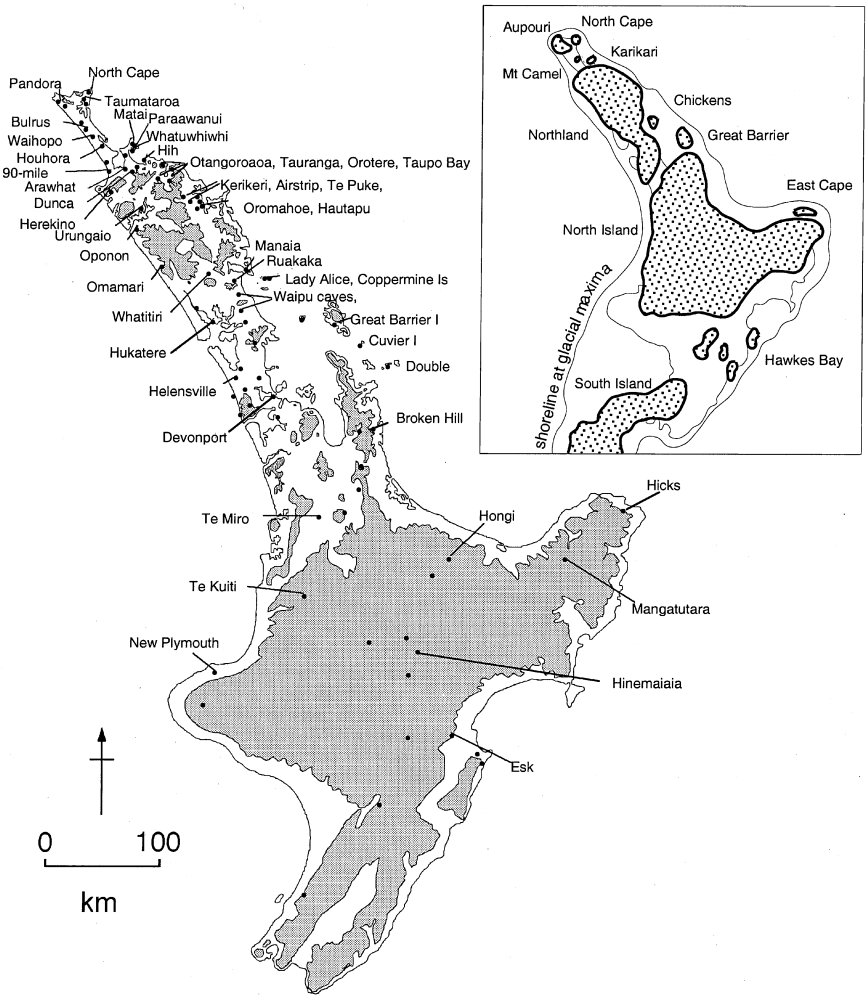

Fig. 1 Collecting locations for *Hemideina thoracica* from North Island, New Zealand. Unlabelled spots indicate locations where weta have only been cytogenetically examined. Shaded areas indicates land above 150 m. Inset shows the hypothesized distribution of land during the early Pliocene and the shoreline during glacial maxima (Fleming, 1979; Stevens, 1981; Ballance & William, 1992).

across Pliocene Islands based on geological evidence (Fig. 1; Fleming, 1979; Stevens, 1981; Ballance & Williams, 1992).

Isolation by distance was tested using geographical distances between locations and pairwise K2P genetic distances. One representative of each haplotype from each location was used. Sample sizes range from 1 to 14 weta per location while numbers of haplotypes per location range from 1 to 4. A Mantel test was implemented in GENEPOP (Raymond & Rousset, 1995) using 1000 iterations.

Results

The known geographical distribution of the eight chromosomal races of *H. thoracica* has been extended and refined (Table 1, Fig. 2). The 13-karyotype previously known only from Double Island, has now been recorded at four sites adjacent to each other on North Island, 120 km west of Double Island. This is only the second instance of a chromosome race within *H. thoracica* having a disjunct distribution. The known range of the 19-karyotype race has been extended west, the range of 17′-karyotype extended south and the 15′-karyotype range extended north.

Fifty-eight unique 12S-SSCP haplotypes were identified. Fifteen of 31 locations from which more than one weta were studied showed only a single haplotype (Table 1). The maximum number of haplotypes in any sample was four (Orotere, $n = 5$). A representative of each 12S-SSCP haplotype was sequenced for *COI*. Additionally, five 12S-SSCP haplotypes were sequenced in duplicate and one haplotype in triplicate, so sequence information was obtained for 65 weta. In five cases, the same 12S-SSCP haplotypes gave an identical *COI* sequence, and two other *COI* sequence comparisons differed by 1 bp and 2 bp.

COI sequences showed 159 variant nucleotide sites in the 550 bp observed. Nucleotide composition was

Table 1 Collecting locations for *Hemideina thoracica* from North Island, New Zealand, showing sample sizes, number of SSCP-haplotypes, mtDNA lineage, karyotype and Pliocene land mass closest to the location

Location	South latitude	East longitude	No. of weta (SSCP)	No. haplo (*COI*)	No. of sequences	mtDNA lineage	Karyotype	Closest Pliocene Island
Esk	39 21	176 42	2	1	1	A	17	North
Hinemaiaia	38 51	176 01	14	1	1	A	17	North
Hongi	38 44	176 10	1	1	1	A	15	North
Te Kuiti	38 21	175 10	1	1	1	A	15	North
Mangatutara	37 55	174 51	1	1*	1	A	17	North
Te Miro	37 49	175 33	1	1*	1	A	15	North
Broken Hill	37 07	175 43	1	1	1	A	17	North
Devonport	36 49	174 50	1	1	1	A	15′	Northland
Helensville	36 42	174 29	1	1	1	A	13	Northland
Cuvier I	36 26	175 46	1	1	1	A	11	North
Hukatere	36 11	174 10	1	1	1	A	15′	Northland
Ahuroa	35 59	174 25	12	3*	3	A	15′	Northland
Waipu caves	35 56	174 22	8	2*	1	A	15′	Northland
Ruakaka	35 52	174 24	6	2*	1	A	17	Northland
Manaia	35 49	174 30	1	1*	1	A	17	Northland
Omamari	35 49	173 44	1	1	1	A	15′	Northland
Whatitiri	35 47	174 09	3	1	1	A	17	Northland
Hautapu	35 23	174 00	4	2*	1	A	17	Northland
Oromahoe	35 19	174 07	4	1*	1	A	17	Northland
Paihia	35 18	174 05	4	1*	1	A	17	Northland
Airstrip	35 16	173 55	4	3*	2	A	19	Northland
Te Puke	35 15	174 02	10	1	1	A	19	Northland
Kerikeri	35 13	173 58	1	1	1	A	19	Northland
New Plymouth	39 04	174 04	6	1	1	B	15	North
Double I	36 37	175 53	1	1	1	C	13	North
Hicks	37 35	178 27	1	1	1	C	15	East Cape
Lady Alice I	35 54	174 43	1	1	1	D	15′	Chickens
Coopermine I	35 54	174 46	1	1	1	D	15′	Chickens
Great Barrier I	36 18	175 30	1	1	1	D	17	Great Barrier
Orotere	35 07	173 52	5	4*	4	E	19	Northland
Otangoroaoa	35 04	173 40	2	2*	1	E	19	Northland
Tauranga	35 00	173 47	6	3*	3	E	19	Northland
Taupo Bay	34 59	173 42	8	2*	1	E	19	Northland
Waihopo	34 46	173 04	3	1	1	F	17′	Mt Camel
Hauhora	34 50	173 09	3	1	1	F	17′	Mt Camel
90-mile	35 01	173 15	6	1	1	F	19	Northland
Herekino	35 10	173 16	3	1*	1	F	19	Northland
Arawhata	35 02	173 19	2	1	1	F	19	Northland
Duncan	35 03	173 21	12	3	3	F	19	Northland
Urungaio	36 18	173 31	2	2*	1	F	19	Northland
Opononi	35 31	173 24	1	1	1	F	19	Northland
Hihi	34 58	173 32	9	1	1	F	19	Northland
Whatuwhiwhi	34 53	173 24	12	1*	2	F	23	Karikari
Matai	34 51	173 25	4	1*	2	G	23	Karikari
Paraawanui	34 50	173 26	2	2*	2	G & F	23	Karikari
Bulrush	34 43	173 01	4	2	2	H & F	17′	Mt Camel
Pandora	34 29	172 46	8	2	2	I	17′	Aupouri
North Cape	34 25	173 02	2	2*	2	I	17′	North Cape
Taumataroa	34 27	172 58	4	2*	1	I	17′	North Cape
49			192	58	65			

* Some haplotypes observed at adjacent sites.

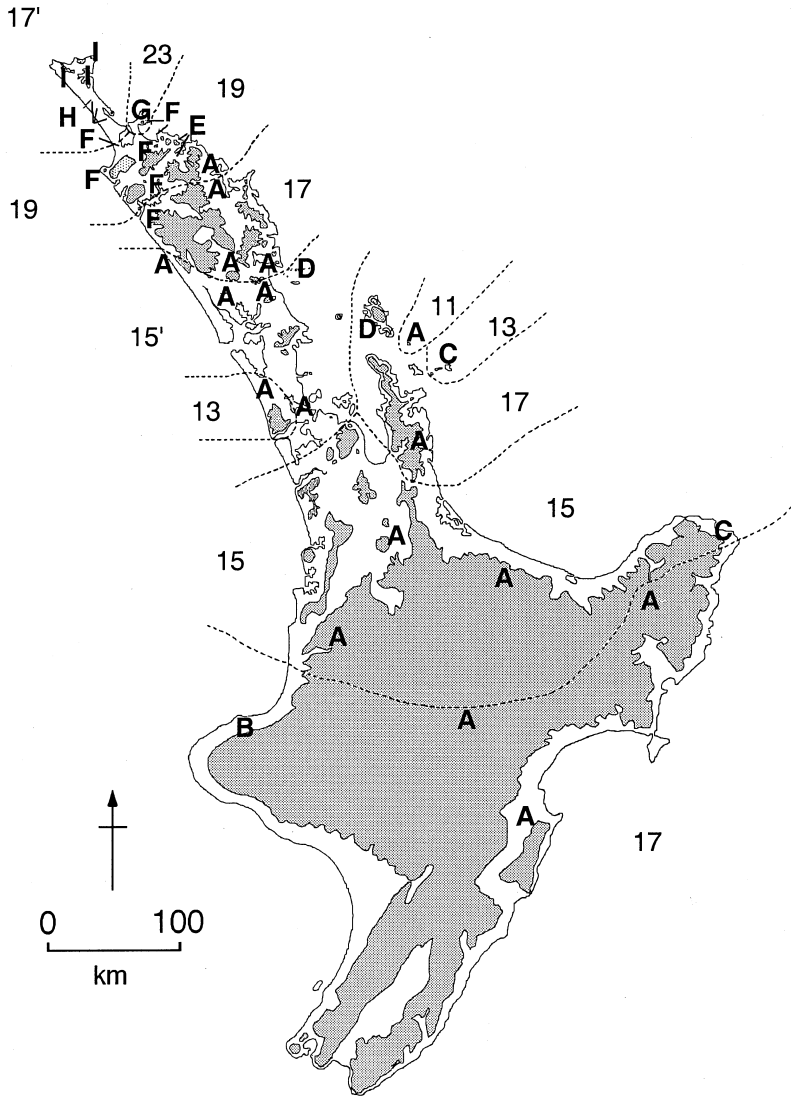

Fig. 2 The distribution of eight chromosome races (11–23; dotted lines) and nine mitochondrial lineages (A–I) of *Hemideina thoracica* in North Island, New Zealand.

marginally AT biased (A + T = 58%), lower than most values for insect mtDNA and more typical of a vertebrate (Frati *et al*., 1997; Langor & Sperling, 1997; Saccone *et al*., 1999; Trewick, 2000). A transition: transversion ratio of 4.2:1 was obtained using the NJ tree.

Genetic distances

An appropriate nucleotide substitution model for estimating genetic distances was calculated using the 65 *COI* sequences. An initial NJ tree based on K2P was used as a starting point for Maximum Likelihood analysis and the likelihood scores for a suite of models were compared: 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) using four rate categories (increasing the rate categories to 8 did not affect which model performed best). HKY + I + Γ was the simplest model that was not significantly worse than any of the more parameter-rich models, therefore this model was used to calculate the pairwise genetic distances and used in NJ analyses. The maximum uncorrected distance (p) between any two sequences was 9.5%. With HKY + I + Γ correction the maximum pairwise distance was 16.3% (data available from authors on request).

Phylogenetics

NJ with 65 *COI* sequences resolved nine major lineages (A–I, Fig. 3). These lineages were obtained with a fast-bootstrap MP search (2000 iterations). At only two locations (Paraawanui and Bulrush) was more than one

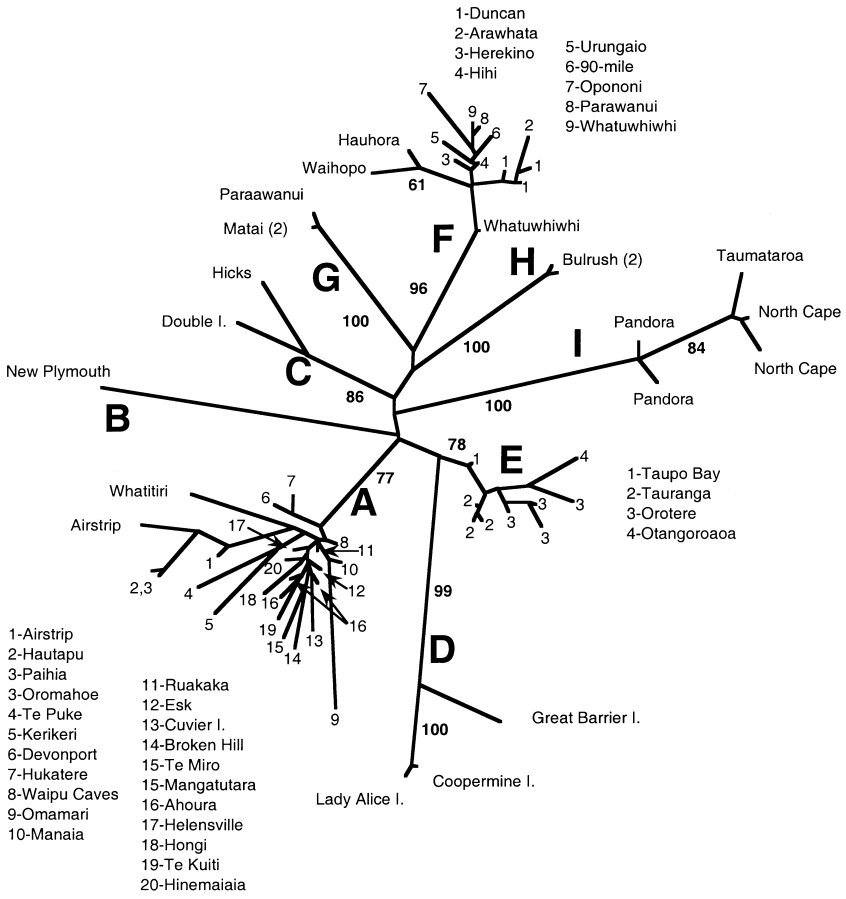

Fig. 3 Neighbour-Joining tree of HKY + I + Γ distances of *COI* from 65 *Hemideina thoracica*. Principal lineages are coded with a letter (A–I). Numbers on branches show percentage support from 2000 bootstrap replicates using MP and 4:1 tv:ts weighting.

Table 2 Average pairwise genetic distances (HKY + I + Γ) within and among the nine major mtDNA lineages in *Hemideina thoracica*

	A	B	C	D	E	F	G	H	I
A	0.030								
B	0.096								
C	0.082	0.090	0.030						
D	0.106	0.112	0.110	0.022					
E	0.066	0.096	0.057	0.072	0.013				
F	0.105	0.085	0.088	0.101	0.089	0.017			
G	0.086	0.975	0.081	0.104	0.075	0.069	0.001		
H	0.084	0.976	0.062	0.098	0.061	0.073	0.062	0.000	
I	0.106	0.134	0.095	0.139	0.099	0.125	0.119	0.102	0.022

major lineage observed. Average genetic distances (HKY + I + Γ) among the nine major lineages were all above 5% (Table 2).

Due to the computational time required for a data set of this size, a subset of sequences was chosen for further analysis. One member of each major mtDNA lineage (A–I) was randomly selected, plus an additional sequence for lineage A, C, D, F and I. For these 14 weta the 12S fragment previously used for SSCP was sequenced. A partition homogeneity test ($P = 0.140$) was performed before combining *COI* and 12S data. One hundred and five nucleotide sites were variable over these 840 bp. MP (weighting: tv:ts 4:1) gave a single tree with each major lineage well supported (>80% bootstrap support), but the highest inter-lineage bootstrap value was only 56% for A + B.

To test whether the sequences had evolved in a clock-like fashion we used the subset of 14 taxa. We

constrained the most likely tree found under ML to conform to a molecular clock and found using a χ^2 test that this did not differ significantly in likelihood scores. Neither the combined data set (*COI* and 12S) nor *COI* analysed separately differed significantly from a clock-like model.

Population genetics

The majority of sites from which weta were collected had unique mtDNA haplotypes (Table 1). In 20 samples, haplotypes were shared by adjacent sites. Distances between these sites ranged from 2 to 212 km. The nine major mtDNA lineages showed some geographical structure, with adjacent locations generally sharing the same lineage (Fig. 2). The range of the nine mtDNA lineages varied considerably, with six lineages (B, C, D, G, H and I) detected at three or fewer locations. Lineage A was found at 23 locations and F at 12. The most widespread lineage (A) was found throughout much of the southern 3/4 of the species' range, and the greatest distance between any two locations having A was 525 km. No evidence of isolation by distance was found using all haplotypes (Mantel test, $P = 0.912$). The distribution of genetic variation was analysed with hierarchical Φ-statistics using AMOVA (Excoffier *et al.*, 1992); Φ_{SC} quantifies the proportion of genetic variation within groups (chromosome races or Pliocene islands), Φ_{ST} is the variation among all populations and Φ_{CT} is the variation among groups. Two groupings were explored. Populations were grouped according to their karyotypes and according to the hypothesized Pliocene island they were closest to. These levels of division described relatively little of the total genetic diversity within the species; the majority of genetic diversity being attributed to comparisons among samples within groups (with eight chromosome races $\Phi_{SC} = 0.94$, $\Phi_{ST} = 0.96$, $\Phi_{CT} = 0.28$; with nine Pliocene islands $\Phi_{SC} = 0.94$, $\Phi_{ST} = 0.96$, $\Phi_{CT} = 0.24$). Rather than rejecting the significance of the Pliocene islands and chromosome races as potential causal factors of the major lineages, we performed some exploratory data analyses, omitting two selected lineages that we felt to be atypical. The first, selected lineage was lineage A, which is considerably more widespread that any other lineage, encompassing several Pliocene island regions and chromosomal races. This is in keeping with its southern distribution and the eradication of much of the biota of central North Island by repeated eruptions of Taupo, the largest 20 000 years ago (see Discussion). The second selected lineage was F; although lineage F is found only in Northland, it does occur peripherally on adjacent Mt Camel and Karikari areas, and may well indicate more recent gene flow. When the hierarchical Φ-statistics analysis was repeated without one or other of these lineages, the picture did not change greatly, but with both removed the proportions of variation described increased to 65% for chromosomes and 81% for islands (Table 3).

Table 3 Hierarchical Φ-statistics (Excoffier *et al.*, 1992) for *Hemideina thoracica*

Φ_{CT}	All lineages	Without A	Without F	Without A & F
Chromosome races	0.28	0.23	0.36	0.65
Pliocene Islands	0.24	0.37	0.37	0.81
No. locations	49	26	39	16
No. weta	192	109	139	56

Φ_{CT}, genetic variation among races or islands.

Discussion

Paraawanui and Bulrush had weta with mtDNA haplotypes that differed by 5.8% and 7.6% (lineages F, G and F, H). Although these distances are more typical of interspecific comparisons in insects (Funk, 1999), these weta had identical karyotypes and were morphologically indistinguishable. Four mtDNA lineages were found in more than one chromosomal race (A, C, D, F). Thus, the distribution of the mtDNA markers suggests that gene flow between chromosomal races is possible and supports the idea that *H. thoracica* is a single interbreeding species. The distribution of haplotypes and the level of divergence are indicative of a species that has extremely low dispersal. Gene flow is apparently low enough to allow differentiation of populations, as sampled at this scale, as almost every location has a novel haplotype.

Pliocene islands with distinct mitochondrial lineages

There is varying concordance between mtDNA lineages and Pliocene islands. For example, the distributions of lineages G, H, and I coincide exactly with islands in the far north. The rare presence of two deep lineages at a single location is strongly suggestive of recent gene flow. Range expansion of haplotypes is suggested by the distribution of lineage F which is found on the boundaries of the 19-karyotype and has apparently crossed into the range of the 17′-, 23- and 17-karyotypes. Removal of this lineage from the AMOVA analysis increased the proportion of total

genetic variation that was described by both the chromosomal races and the Pliocene island partitioning of the data. The distribution of lineages A, C and D also implies dispersal over longer distances from Pliocene refugia. The occurrence of lineage B on the Mt Taranaki volcano cannot be explained by Pliocene islands or chromosome races as currently hypothesized.

Lineages of equal divergence

The topology of the phylogenetic hypothesis for the mtDNA of *H. thoracica* is star-like, with no resolution of the relationships among the major lineages. Although nine clades have bootstrap values of 78% or above (A–I) no edges connecting these lineages have good bootstrap support. Even with fewer taxa and a longer sequence (840 bp) the relationships between major lineages remain unresolved. There are two possible explanations; either the data set is saturated at this level or these lineages began to diverge at approximately the same time. Other studies have resolved relationships using this region of *COI* and with similar levels of divergence (Langor & Sperling, 1997). Similar studies of insect *COI* frequently find lower tv/ts ratios within species and at levels of divergence lower than we observed (Caterino & Sperling, 1999). Our estimation of I (invariant sites) suggests that there are sites still free to vary and the fact that other studies have recorded divergences of 30% between insect *COI* sequences (Stauffer *et al.*, 1997) also indicates that saturation is not a feature of our data.

Molecular-clock hypothesis

Both the combined sequences data set and a subset of *COI* sequences have apparently evolved in a clock-like fashion. For insect mtDNA, calibration has been based on geological events < 3.5 Ma ago and have used a mixture of uncorrected and corrected sequence divergences (Brower, 1994; Knowlton & Weigt, 1998). Although the levels of divergence observed in this study are somewhat higher, we have used evolutionary models in the correction of genetic distances that should compensate for the nonlinear relationship between time and observed rate. Rate estimations vary from 1.2 to 0.7% per Ma (Brower, 1994; Knowlton & Weigt, 1998). Average pairwise genetic distances (HKY + I + Γ) between mtDNA lineages of *H. thoracica* range from 13.9% to 5.7%. Using the range of rate estimates these values correspond to 5.8–9.9 and 2.4–4.1 Ma since the last common ancestor. The majority of estimates therefore place the divergence of these lineages in the early Pliocene.

Chromosomal races of varying age

In the extreme north of the range of *H. thoracica*, there is close correspondence of mtDNA lineages with chromosomal races and Pliocene islands. On the other hand, the widespread distribution of lineage A may be a result of more recent geological and climatic events. The central area of the North Island was greatly modified by volcanic eruptions. Approximately 20 000 years ago the largest of the Taupo eruptions destroyed all land surfaces in central North Island (Ballance & Williams, 1992) and more recent eruptions (2000 years ago) destroyed approximately 20 000 km^2 of forest in central North Island (Wilson & Walker, 1985). As the forest re-established a large area would have been available for re-colonization by the southward dispersal of *H. thoracica*. The consequence of recent range expansion is likely to be loss of genetic diversity through leading-edge re-colonization (Wallis & Arntzen, 1989; Phillips, 1994; Hewitt, 1999). In addition to volcanic activity, the fluctuations in climate during the Pleistocene may have caused range expansion and contraction of *H. thoracica* in lower North Island. There is good evidence that *H. thoracica* has recently expanded south and displaced a close relative, *H. crassidens*, from all but high altitude regions of central North Island (Trewick & Morgan-Richards, 1995). The pattern of reduced mtDNA diversity in the southern half of the range of *H. thoracica* is paralleled by the allozyme data (Morgan-Richards, 1997), although karyotypic diversity is less affected. This disparity could be explained by recent origins of the chromosomal races that have the A mtDNA lineage (11, 13, 15, 15′, 17) resulting from fixation during local extinction and re-colonization (Lande, 1979) or the retention of ancestral polymorphism. On the other hand, introgression of lineage A into the range of mtDNA lineages C and D (karyotypes 15, 15′, 17 and 13) cannot be excluded.

In conclusion, *H. thoracica* is a single species with an unprecedented level of mtDNA divergence. Support for the idea that this divergence arose during periods of population isolation on islands that existed during the Pliocene comes from the level of divergence, the apparent simultaneous origins of the lineages and the match between current range of some of the mtDNA markers and hypothesized islands. However, due to the intraspecific nature of this variation, introgression of lineages into neighbouring populations has reduced the correspondence of mtDNA with islands and with chromosomal races. The age of some of the northern chromosomal rearrangements (17′, 23 and 19) may date from the same period of Pliocene isolation, but southern chromosomal races probably have a more recent origin. The age of the chromosomal races within this species is

in contrast to many studies that have found evidence of extremely recent and rapid karyotype evolution. For example, in the shrew *Sorex araneus* mtDNA diversity suggests that chromosomal rearrangements have arisen within the last 1 Ma (Taberlet *et al*., 1994). In the house mouse (*Mus domesticus*) five chromosome races may have arisen on Madeira in 500 years (Britton-Davidian *et al*., 2000), and in an aphid (*Sitobion*) the rate of chromosome evolution exceeds the rate of microsatellite evolution (Sunnucks *et al*., 1996). The antiquity of the chromosome races of *H. thoracica* is more remarkable given that these populations have apparently survived as distinct units and yet remained as part of a single species for millions of years, especially when one considers the climate changes during the Pleistocene, and the continuity of land during glacial maxima.

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