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Mitochondrial DNA sequences support allozyme evidence for cryptic radiation of New Zealand *Peripatoides* (Onychophora)

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Abstract

A combination of single-strand conformation polymorphism analysis (SSCP) and sequencing were used to survey cytochrome oxidase I (COI) mitochondrial DNA (mtDNA) diversity among New Zealand ovoviviparous Onychophora. Most of the sites and individuals had previously been analysed using allozyme electrophoresis. A total of 157 peripatus collected at 54 sites

displays a distinctive, if depauperate, biota with many unique taxa (e.g. Moa, tuatara and Leiopelmid frogs) as well as typical southern hemisphere taxa, including Onychophora. There is also ample evidence of various taxa recently colonizing New Zealand by dispersal so that the modern assemblage has elements typical of both continental and oceanic biotas (Fleming 1979; Daugherty *et al*. 1993). Thus, the origins and phylogeographical structure of the New Zealand biota provide an interesting model system in which to explore the roles of vicariance and dispersal in the evolution of species and species assemblages.

Within New Zealand, three live-bearing (ovoviviparous) species of Onychophora can be distinguished by leg number: *Peripatoides indigo* Ruhberg (1985), *P. novaezealandiae* (Hutton 1876) and *P. suteri* (Dendy 1894), having 14, 15 and 16 pairs of legs, respectively. However, *P. novaezealandiae* consists of a species complex (Trewick 1998). Four new species (*P. morgani*, *P. aurorbis*, *P. kawekaensis* and *P. sympatrica*) were described on the basis of allozyme data, although unfortunately no morphological characters distinguishing them were apparent (Trewick 1998). The present work is a survey of mitochondrial sequence variation among the *Peripatoides* individuals used in that allozyme study, plus additional specimens extending the sample to include most regions of New Zealand. These mitochondrial DNA (mtDNA) data are here used to further explore the origins, phylogeographical structure and ecology of the endemic *Peripatoides* in light of the turbulent geological history of New Zealand.

Materials and methods

Specimens of *Peripatoides* were collected from decaying logs and other moist, dark habitats throughout South Island and North Island, New Zealand (Fig. 1, Appendix 1). Of the specimens previously studied using allozyme electrophoresis, those from Whakapapa, Takaka and Pelorus were not available for the present study, but new material was obtained from Pelorus. DNA was extracted from whole tissue derived either from frozen body sections remaining from allozyme research or, for specimens collected subsequently, from one or two legs dissected from frozen or alcohol-preserved specimens. Extractions used a simple, solvent-free proteinase K and salting-out method (Sunnucks & Hales 1996).

Peripatus individuals were screened for haplotypic variation using isotopic labelling and single-stranded conformation polymorphism analysis (SSCP) according to the method described by Trewick (1999). SSCP utilizedD -0.005 Tc i

Results

Sequence data

Through a combination of SSCP and sequencing, 62 COI haplotypes were identified from 157 *Peripatoides* collected at 54 sites. Aligned sequences consisted of 540 bp from the 3′ end of COI corresponding to positions 754–1293 of the insect COI sequence described by Lunt *et al*

Table 2 Average genetic distances within and between taxa

	K2P	GTR	*O. viridimac.*	*P. suteri*	*P. aurorbis*	*P. sympat.*	*P. kaweka.*	*P. novaezea.*	*P. morgani*	'Catlins'	'Piano'
				0.01	0.04	0.09	0	0.07	0.01		
O. viridimaculatus											
P. suteri	3.1	3.6	12.4 20.8		1.75	1.23	1.25	2.66	1.72		
P. aurorbis	3.8	4.6	13.3 23.6	8.7 12.1		1.02	0.95	0.73	0.97		
P. sympatrica	2.4	2.7	12.8 20.8	7.4 9.7	11.3 16.9		0.36	0.63	0.85		
P. kawekaensis	0.8	0.4	12.6 20.5	7.9 10.5	10.5 15.9	6.5 8.9		0.44	0.48		
P. novaezealandiae	2.9	3.2	13.2 22.4	7.4 9.7	10.5 16.1	6.0 7.8	6.2 8.2		0.24		
P. morgani	1.9	4.6	13.3 21.9	7.6 10.2	10.7 16.5	6.3 8.4	6.7 7.9	3.2 4.3			
'Catlins'	2.0	2.2	14.1 24.9	9.2 11.9	7.9 10.5	9.9 13.3	9.9 13.5	9.7 12.8	9.7 13.1		
'Piano'	1.7	1.8	15.9 32.4	10.8 15.9	9.7 14.0	10.8 15.6	11.4 17.5	11.1 16.5	11.1 17.3	6.9 8.9	
'Dunedin'	2.8	3.2	14.0 28.0	9.1 12.9	8.5 11.9	10.4 15.2	10.5 15.6	11.2 17.5	11.1 16.8	7.7 13.3	9.3 9.1

Nei's D from allozyme data (Trewick 1998) is presented above the diagonal, Kimura 2-parameter (K2P) and general time-reversible (GTR + I+ Γ) mitochondrial DNA (mtDNA) cytochrome oxidase I (COI) distances are presented below the diagonal.
Within-taxon distances are given in the first row for Nei's D, and in the first and second columns for K2P and GTR + I + Γ, respectively5.545 83.8817 TTf 0.598C95(1)56s Dso9 eE35 1e

Most peripatus collected from a single site shared the same COI haplotype. At all sites where sympatric species had previously been found using allozymes (Trewick 1998), more than one haplotype was detected. Individuals assigned to a particular species from allozyme data also had mtDNA haplotypes consistent with this (see phylogenetic analysis). This was true even for those rare peripatus individuals that were heterozygous or homozygous for allozyme alleles characteristic of a different species. Thus, at Balls Clearing, two *P. kawekaensis* individuals were heterozygous for an AATc allele characteristic of *P. sympatrica*, and a third *P. sympatrica* individual was homozygous for an aconitase (ACON) allele characteristic of *P. kawekaensis*. At Norsewood, two *P. morgani* had an aspartate aminotransferase (AATa) allele typical of *P. sympatrica* (Trewick 1998). In all these individuals their mtDNA haplotype was concordant with the majority of their nuclear markers.

Of the locations not surveyed for allozyme variability, only one (Piano Flat, South Island) had two haplotypes. Sequences from individuals collected at three locations in North Island (Ngaiotonga, Ball's Clearing and Opepe), which were otherwise unambiguous, contained some sites with ambiguous nucleotides. These suspect nucleotide substitutions were coded as N.

Genetic distance

Genetic distances were initially calculated using the Kimura 2-parameter (K2P) model. A NJ tree derived from these distances was then used to test for the most appropriate nucleotide substitution model by comparing likelihood scores for a suite of models: Jukes–Cantor (JC) (Jukes & Cantor 1969), K2P (Kimura 1980), Hasegawa–Kishino–Yang (HKY85) (Hasegawa *et al*. 1985) and general time-reversible (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 a significantly better score (–lnL 3612.344) than other models. With the estimated proportion of invariant sites of 0.524, the α-shape parameter was 0.863. These parameter estimates were used to recalculate the distance matrix.

Pairwise genetic distances among the ingroup taxa (*Peripatoides*) calculated from COI sequences using K2P and GTR + I + Γ, reached a maximum of 13.4% and 22.4%, respectively. Mean distance between clades ranged from 3.2 to 11.4% using the K2P model, and 4.3 to 17.5% using the GTR + I + Γ models (Table 2). The highest within-taxon mean distance was 3.8% using the K2P model, in the *P. aurorbis* clade (maximum 6.6%). Mean genetic distances between *Ooperipatellus viridimaculatus and Peripatoides* clades ranged from 12.4 to 15.9% using the K2P model, and 20.8 to 32.4% using the GTR + I + Γ models. The greatest single distance was between *O. viridimaculatus* and a Piano peripatus (16.3% using the K2P model, 34.2% using the GTR + I + Γ models). The two outgroup *Ooperipatellus* differed by 9.7% using the K2P. The greatest linear geographical distance between sample sites with identical haplotypes was ≈ 190 km (*P. morgani*, Monckton—Tikitapu).

Phylogenetic analysis

Phylogenetic analysis of all haplotypes using NJ with K2P weighting produced the tree shown in Fig. 3.

Discussion

Sequence evolution

A two-dimensional model of the insect COI gene has been proposed that consists of a number of structural regions associated with membrane-spanning helices, external loops and internal loops (Lunt *et al*. 1996). The pattern of amino acid variability among these various regions is constrained by the function of the regions. Comparison of among-region amino acid variability of insects and *Peripatoides* revealed a very similar pattern (Fig. 2). A + T composition of peripatus COI DNA sequence overall, and at third-codon positions in particular, was also similar to that found in insects (Table 1), and together these features suggest that COI composition and structure have changed little during the evolutionary history of these invertebrates.

Interspecific sequence and allozyme divergences within *Peripatoides* were higher than recorded for many insects (Fig. 7). In fact, the genetic diversity of the *Peripatoides* is more similar to that found in intergeneric studies of insects. This highlights the extreme level of morphological conservatism th s

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Appendix 1 Details of sampling sites: location names, latitude/longitude and other notes of interest

North Island

Ngaiotonga reserve (35°18′: 174°15′); Waipoua forest (35°38′: 173°33′); Herekino (35°09′: 173°16′); Puketi forest (35°16′: 173°40′); Waitakere forest (36°54′: 174°32′); Kawau Island [two sites separated by > 2 km] (36°30′: 174°40′); Waiwawa, Coromandel Range (36°59′: 175°37′); Kaueranga, Coromandel Range (37°06′: 175°38′); Forthbranch, Coromandel Range (37°08′: 175°44′); Waitomo Caves reserve (38°15′: 175°06′); Mangatutara, Raukumara Range (37°55′: 177°55′); Lake Tikitapu (38°11′: 176°20′); Opepe historic reserve, Taupo (38°42′: 176°10′); Rangataiki (38°59′: 175°39′); Kakaho, Pureora forest (38°33′: 175°0.38suTc 0 2 lnSla0a8 0 T9i(1ilearingF3 1 Tf 0.3958 09D -0.005 Tc (39955)4(¢)Tj /F3 1 Tf 0.24

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