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Sympatric cryptic species in New Zealand Onychophora

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Allozyme electrophoresis was used to examine genetic diversity within live-bearing Peripatopsid Onychophora from the North Island of New Zealand. Specimens of two previously described morpho-species that differ in leg number (*Peripatoides suteri* and *P. novaezealandiae*) were found to be genetically diverse. *P. suteri* showed little intraspecific genetic variation but were very distinct from specimens assignable to *P. novaezealandiae*. Within *P. novaezealandiae* five genetically differentiated species were identified although none showed any consistent morphological differentiation, thus *P. novaezealandiae* (Hutton) is a species complex. All of these species occur in sympatry or parapatry (in one instance) with other cryptic species of the *P. novaezealandiae* group or with *P. suteri*. Four new species are described on the basis of this genetic evidence, they are ***P. morgani*, *P. aurorbis*, *P. kawekaensis* and *P. sympatrica***. Other genotypes encountered indicate further cryptic species remain unrecognized. Among the North Island species, *P. suteri* and ***P. aurorbis* sp. nov.** are both more closely related to undescribed species from the South Island than to others examined from the North Island. ***P. sympatrica* sp. nov.** exists in sympatry with at least three other species in different parts of its range. The complexity of relationships and distributions probably arose through the interaction of low vagility in peripatus and the active geophysical history of the region. How these cryptic species persist in sympatry is not known but may be linked to differences in ecology not evident in their morphology, and/or may indicate recent dispersal from allopatry.

ADDITIONAL KEY WORDS—peripatus – allozyme – speciation – biogeography.

CONTENTS

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INTRODUCTION

In the study of evolution it is important to distinguish between morphologic change and speciation (Larson, 1989). The traditions of taxonomy, set as they are in morphology, have tended to underestimate actual biological diversity in many groups and the use of molecular techniques has led to the discovery of numerous discrete genetic groupings within recognized species boundaries (Hebert *et al.*, 1990; Eldridge & Close, 1992; Briscoe & Tait 1995). An empirical framework for the identification of species therefore exists, within which corollaries of subtle morphology and perhaps more importantly ecology can be explored (Mayr, 1942). The existence of morphologically cryptic species has tested theories about competition (Hardin, 1960; MacArthur & Levins, 1967; Abrams, 1983) and has highlighted a need for ecological determinants that explain the persistence of such species in sympatry (Carapelli *et al.*, 1995; Bruna, Fisher & Case, 1996). The further exploration of cryptisism/morphological stasis among diverse animal groups promises to facilitate the development of generalized theories in speciation by providing more accurate estimates of diversity.

The Onychophora (or peripatus) have been considered to be a morphologically (and functionally) conservative group (Ghiselin, 1984). This view has arisen because of the strong similarities between extant terrestrial species and fossil Cambrian marine taxa, and the perceived consistency of form among living species. Paradoxically, one of the few features that does vary among species of living Onychophora is leg number, a phenomenon that would be considered remarkable in most other animals. Leg number has until recently been one of the key features in peripatus taxonomy (Ruhberg, 1985). As a result of genetic research, peripatus have been shown to be far more diverse than previously indicated by morphology (Hebert *et al.*, 1990; Briscoe & Tait, 1995). The world species count for this group has more than doubled as a result of studies in Australia alone (Briscoe & Tait, 1995). Correlated with much of the genetic diversity, morphological variation has subsequently also been identified (Reid *et al.*, 1995; Reid, 1996). In many taxa, morphological structures are of a bizarre and sophisticated nature, apparently associated with reproductive behaviour (Tait & Briscoe, 1990).

Only five species of peripatus within two genera are described from New Zealand (Ruhberg, 1985; Gleeson, 1996), although evidence from allozyme electrophoresis has indicated the existence of greater numbers (Tait & Briscoe, 1995). Live-bearing and egg-laying species have been identified (as in Australia), but little is known about the New Zealand taxa beyond this. Of the live-bearing species, *Peripatoides novaezealandiae* (Hutton, 1876) was, until recently considered the most widely distributed and was described from both main islands. Representatives of the species found in the south of the South Island are, however, genetically distinct from those in the North Island at a level suggestive of generic distinction, and those in the North Island are also genetically differentiated (Tait & Briscoe, 1995). A second live-bearing species *P. suteri* is described from the North Island alone (Dendy, 1894).

These two species cannot be distinguished by colour or size as these traits show considerable and overlapping intraspecific variation. They are however, easily separated on leg number: 15 and 16 pairs in *Peripatoides novaezealandiae* and *P. suteri* respectively. Egg laying peripatus are also known from the North Island but are seldom found (Fletcher, 1900; Watt, 1960; Tait & Briscoe, 1995).

This paper reports on an allozyme study of the two viviparous species of peripatus present in the North Island of New Zealand, and addresses the significance of cryptic species in the systematics of the endemic Onychophoran fauna.

MATERIAL AND METHODS

Collection

Peripatus were collected during 1995 and 1996 in the North Island of New Zealand (Fig. 1 inset). With the exception of one individual from Mangatutara in the Raukumara Range, which was found under a stone, all specimens were collected from within or beneath decomposing logs. Additional material, including tissue from two specimens collected in the north of the South Island, was included from a previous study (Tait & Briscoe, 1995). Two morphologically distinct species were encountered and collected: *Peripatoides novaezealandiae* and *P. suteri* with 15 and 16 pairs of legs respectively. Where possible, individuals were collected from a number of different logs at each location to increase the chances of encountering different genotypes. A total of 108 individuals from 31 locations, 29 localities in the North Island and two (Pelorus Bridge and Takaka) in the north of the South Island were used (Fig. 1 inset).

Material used, numbers of logs (L) collected from, and individuals (I) collected, are followed by details of longitude and latitude of sites.

P. novaezealandiae (15 pairs of legs): Kawau Island [3L, 9I, two sites separated by >2 km] (36°30′:174°40′); Waiwawa Coromandel Range [1L, 2I] (36°59′:175°37′); Kaueranga, Coromandel Range [1L, 2I] (37°06′:175°38′); Forthbranch, Coromandel Range [2L, 4I] (37°08′:175°44′); Waitomo Caves [1L, 1I] (38°15′:175°06′); Mangatutara, Raukumara Range [2L, 3I] (37°55′:177°55′); Lake Tikitapu [1L, 1I] (38°11′:176°20′); Opepe historic reserve, Taupo [3L, 5I] (38°42′:176°10′); Rangataiki [3L, 3I] (38°59′:175°39′); Kakaho, Pureora forest [3L, 4I] (38°33′:175°43′); Balls Clearing reserve [7L, 9I] (39°16′:176°39′); Hutchinson reserve [3L, 3I] (39°16′: 176°32′); Oueroa [3L, 3I] (40°06′:176°41′); Mohi Bush reserve [5L, 5I] (39°35′: 177°05′); Monckton reserve [2L, 5I] (39°57′:176°16′); Miller reserve [1L, 2I] (40°42′: 175°39′); ANZAC reserve, Norsewood [4L, 8I] (40°52′:176°13′); Saddle Road [3L, 4I] (40°17′:175°49′); Bideford [1L, 2I] (40°51′:175°52′); Bideford south [3L, 9I] (40°50′:175°52′); Perry's Road, Carterton [2L, 4I] (41°00′:175°35′); Pahiatua [3L, 5I] (40°26′:175°47′); Waiohine reserve [2L, 3I] (40°59′:175°23′); Akatarawa [1L, 2I] (40°57′: 175°06′); Otari plant museum [3L, 5I] (41°6′:174°45′); Takaka scenic reserve [1L, 1I] (40°45′:172°55′); Pelorus Bridge [1L, 1I] (41°05′:173°30′).

P. suteri (16 pairs of legs): Waiwawa, Coromandel Range [1I in log with peripatus with 15 pairs of legs as above] (36°59′:175°37′); Whakapapa village [1L, 1I] (39°12′: 175°32′); Dawson Falls [1L, 1I] (39°19′:174°06′); Lake Rotokare [1L, 2I] (39°27′: 174°24′).

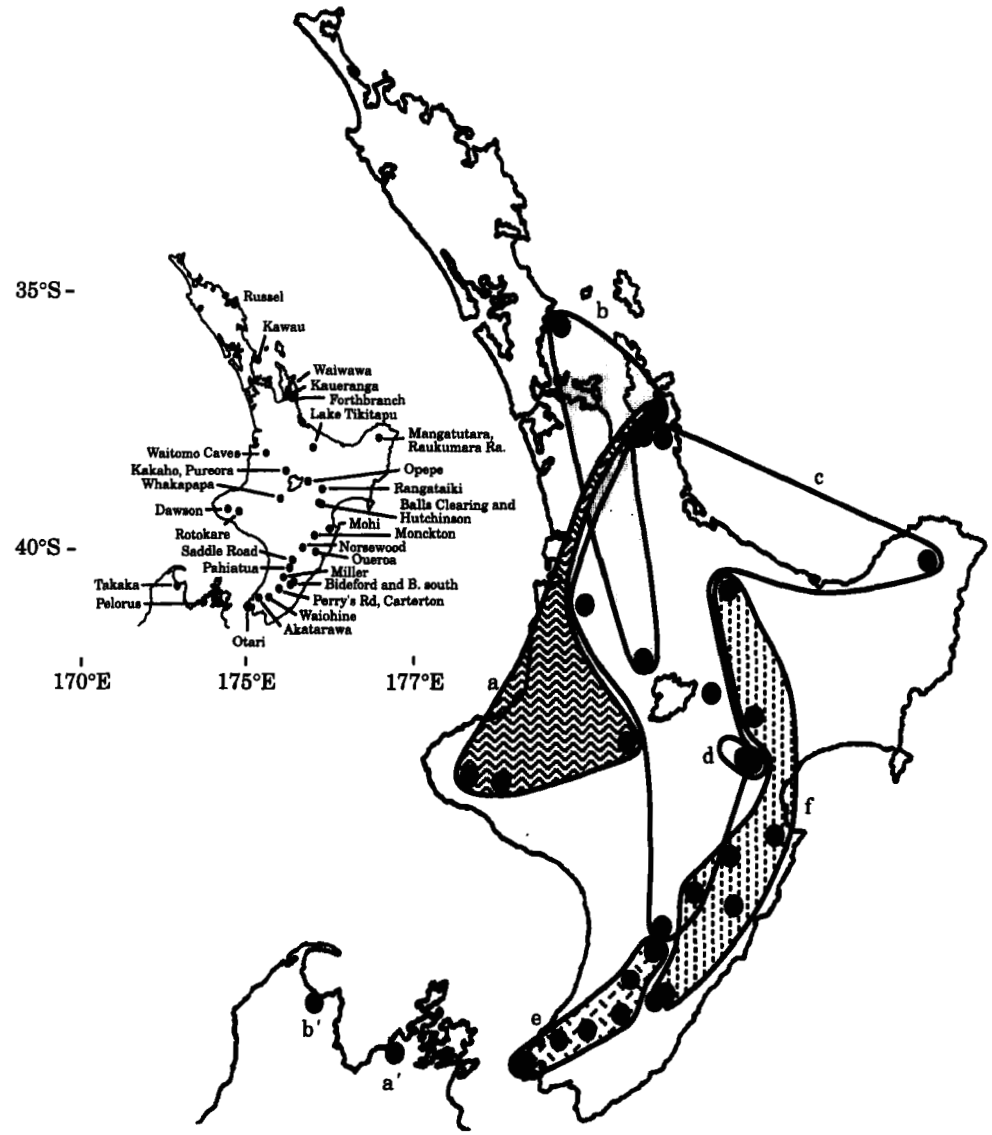

Figure 1. New Zealand localities from where peripatus were collected for this study. Names of locations are given on inset map. Main map shows sites grouped according to Neighbour-Joining clades a–f in Fig. 2. Sites clustered by species identity are grouped by minimum area polygon, but in each case this is drawn to exclude locations where species were absent in the present analysis.

Allozyme electrophoresis

In the laboratory, specimens were killed using ether and morphologically examined. Sex was determined by the presence of two posterior lateral anal glands in males. Females were dissected and embryos where present removed and stored separately. From all but the smallest individuals representative tissue including two to three pairs of legs was dissected from each end and stored in alcohol. The remaining

portion was frozen at −80° for genetic analysis. Protein extraction and electrophoresis followed the methods detailed in Briscoe & Tait (1995). Cellulose acetate media and Spencer's buffer were used for all loci (Spencer, Hopkinson & Harris, 1964).

Allozyme phenotypes were determined at 17 loci as follows (full name, EC number, abbreviation): Aspartate aminotransferase, 2.6.1.1, *Aat* (2 loci); Aconitase, 4.2.1.3, *Acon*; Adenylate kinase, 2.7.4.3, *Ak*; Aldolase, 4.1.2.13, *Aldol*; Enolase, 4.2.1.11, *Enol*; Fructose 1,6 Diphosphatase, 3.1.3.11, *Fdp*; Glyceraldehyde-3-phosphate dehydrogenase, 1.2.1.12, *Gapd*; Glycerol-3-phosphate dehydrogenase, 1.1.1.8, *alphaGpd*; Hexokinase, 2.7.1.1, *Hk*; Isocitrate dehydrogenase, 1.1.1.42, *Idh*; Malate dehydrogenase, 1.1.1.37, *Mdh*; Mannose phosphate isomerase, 5.3.1.8, *Mpi*; Phosphoglycerate mutase, 2.7.5.3, *Pgam*; 6-Phosphogluconate dehydrogenase, 1.1.1.44, *6Pgd*; Phosphoglycerate kinase, 2.7.2.3, *Pgk*; Pyruvate kinase, 2.7.1.40, *Pk* (Richardson, Baverstock & Adams, 1986).

The BIOSYS-1 programme (Swofford & Selander, 1981) was used to calculate allele frequencies and genetic distances; Nei's (1978) unbiased Distance and Cavalli-Sforza and Edwards' (1967) arc distance. Nei's D is widely quoted in the literature and is therefore presented here for comparison, analyses use Cavalli-Sforza and Edwards' arc distance which is a mathematically more rigorous metric (Swofford & Olsen, 1990). Tree construction used the Neighbour-Joining method of Saitou & Nei (1987) invoked by PHYLIP (Felsenstein, 1993), and SplitsTree (Huson & Wetzel, 1994) which incorporates a split decomposition algorithm (Bandelt & Dress, 1992). Mantel tests were performed with Genepop v1.2 (Raymond & Rousset, 1995).

RESULTS

Collection

Specimens assignable to *P. novaezealandiae* and *P. suteri* were found to have limited ranges within the North Island as previously noted (Ruhberg, 1985). *P suteri* appears to be restricted to the mid-western region but specimens have now been obtained further east at Whakapapa (Tait & Briscoe, 1995) and north in the Coromandel Peninsula (Waiwawa). This extends the range significantly and brings *P. suteri* into sympatry with *P. novaezealandiae* at both of those sites. Considerable search effort during the present study failed to find any peripatus in the region immediately north and east of Rotokare (Fig. 1 inset). *P. novaezealandiae* was generally more abundant and certainly more widespread than *P. suteri*, although sparse in eastern areas such as Waitomo. The local density of peripatus appeared to be extremely variable, demanding search times from 30 minutes to several hours for the recovery of a single specimen. In most situations sites requiring longer search times than this were abandoned in this study.

Allozyme electrophoresis

Seventeen presumptive genetic loci were scored for individuals discussed in the present analysis, and all were found to be polymorphic. However, only 26 heterozygotes were encountered across all loci and individuals (a total of approximately

1830 genotypes). Geographic sample sizes ranged from one to nine (Table 1). At most sites, loci were monomorphic, with the exceptions of Waiwawa, Kakaho, Balls Clearing, Norsewood and Rangataiki where polymorphic loci were common. At Waiwawa, both *P. suteri* and *P. novaezealandiae* were collected (in the same log) and their taxonomic difference is reflected in the allozyme data. At the remaining sites where all peripatus had 15 pairs of legs it was apparent that distinct multi-locus genotypes were present and the data are organized accordingly (Table 1). At Bideford the *Idha* locus was exceptional in that three alleles were encountered in two individuals collected from the same log. At Norsewood individuals with different genotypes were found in the same log on two occasions. At other sites where more than one genotype occurred these were present in separate but nearby logs (within 20 m).

P. suteri was almost genetically constant throughout its range (Rotokare, Dawson Falls, Waiwawa and Whakapapa) with just two instances of polymorphism across all loci and individuals in this species. Although morphologically and genetically distinct, this species had only two loci fixed for alleles different from any found in peripatus with 15 pairs of legs (*Mdha* C and *6pgd* D). It was identical to the specimen from Pelorus in the South Island at 12 loci (Table 2). Genetic distances (Nei's D) between *P. suteri* and peripatus with 15 pairs of legs ranged from 0.293 (Pelorus) to 2.833 (Miller reserve) (Table 2).

Among peripatus with 15 pairs of legs currently assignable to *P. novaezealandiae*, several distinct genetic groups were evident (Table 2). The five dominant clusters of populations indicated by Neighbour-Joining analysis (Fig. 2) have high among-group genetic distances compared to within-group distances. However, genetic distances between samples collected at some locations were high (Nei's D 0.323 to 0.887) and beyond those normally encountered within animal species. Sympatry between genetically distinct peripatus bearing 15 pairs of legs was evident at four locations, Kakaho, Balls Clearing, Norsewood and Rangataiki.

Within the groups defined by Neighbour-Joining (Fig. 2) there was generally some low degree of genetic differentiation although one individual from Rangataiki was indistinguishable at 17 loci from Mohi Bush specimens (Table 2, group f). Similarly, an individual from Waiwawa was identical to the Kawau Island population (Table 2, group b) and in *P. suteri*, Dawson Falls and Whakapapa specimens were identical (Table 2, group a). The populations in these three comparisons were separated by minimum geographic distances of 90, 94 and 127 km respectively. Although there appears to be evidence of isolation by distance in some groups (Fig. 3), Mantel tests indicated slight support for positive correlation in group f alone ($P=0.042$). The arrangement of populations within clades does not suggest clines of genetic relatedness are ordered in any geographically consistent way (Figs 1, 2). However, the geographic ranges of species (populations in clades) do appear to have a general north-south alignment. Clades a and b are western, e and f eastern and c central.

The western North Island peripatus with 16 pairs of legs, *P. suteri* (clade a) and one clade of peripatus with 15 pairs of legs (clade b) were each apparently more closely related to South Island populations (a′ and b′) than to each other or other peripatus in the North Island (Fig. 2). In both instances, Pelorus (a′) and Takaka (b′) were nevertheless genetically distinct from their nearest relatives in the North Island, Nei's D 0.238 and 0.333 respectively (Table 2).

Genetic distances between populations in sympatry varied among locations (Fig. 4). For instance, Norsewood 1 was found to be more closely related to Balls Clearing 1 (Nei's D 0.027), than Norsewood 2 and Balls Clearing 2 were to each other (Nei's

TABLE 1. Allele frequencies within populations of *Peripatoides novaezealandiae* and *P. suteri* for 17 polymorphic loci. Alleles are labelled alphabetically from A, closest to origin. Maximum sample size is given as *n*, and the average sample size over all loci by *n* mean. Where more than one allele at a locus was encountered per population the number of individuals that were heterozygotes and the total number of individuals sampled at that locus are given in parentheses (-/-)

Population	Roto	Daws	Waiw1	Whak	Pelo	Taka	Kaka2	Waiw2	Kawa	Wait	Ball1	Nors1
n												
n mean	2	1	1	1	1	1	2	1	8	1	6	5
	1.9	1	1	1	1	1	2	1	7.9	1	5.9	5
Aata												
A	–	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	1	1	1
C	1	1	1	1	1	–	–	–	–	–	–	–
D	–	–	–	–	–	1	1	1	1	–	–	–
Aalc												
A	–	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	–	–	–	–	–	–	–
C	–	–	–	–	–	1	1	1	1	–	–	–
D	–	–	–	–	–	–	–	–	1	1	1	
E	–	–	–	–	–	–	–	–	–	–	–	–
Acon											(0/6)	
A	–	–	–	–	–	–	–	–	–	1	0.833	1
B	–	–	–	–	–	–	–	–	–	–	0.167	–
C	1	1	1	1	1	1	1	1	1	–	–	–
Ak												
A	–	–	–	–	–	1	1	1	1	–	–	–
B	1	1	1	1	1	–	–	–	–	1	1	1
Aldol												
A	1	1	1	1	1	–	–	–	–	1	1	1
B	–	–	–	–	–	1	1	1	1	–	–	–
alphaGpd					(1/1)							
A	–	–	–	–	0.5	1	1	1	1	1	1	1
B	1	1	1	1	0.5	–	–	–	–	–	–	–
Enol												
A	1	1	1	1	–	–	–	–	–	1	1	1
B	–	–	–	–	–	1	1	1	1	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	1	–	–	–	–	–	–	–
Fdp												
A	1	1	1	1	1	1	1	1	1	–	–	–
B	–	–	–	–	–	–	–	–	–	1	1	1
C	–	–	–	–	–	–	–	–	–	–	–	–
Gapd												
A	1	1	1	1	1	–	–	–	–	–	–	–
B	–	–	–	–	–	1	1	1	1	1	1	1
Hk												
A	–	–	–	–	–	–	1	1	1	1	–	–
B	1	1	1	1	1	1	–	–	–	–	1	1
Idha	(1/2)											(1/5)
A	–	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	0.1
D	0.25	–	–	–	–	1	–	–	–	–	–	–
E	0.75	1	1	1	1	–	1	1	1	1	1	0.9
Mdha						(1/1)						
A	–	–	–	–	–	0.5	–	1	1	–	–	–
B	–	–	–	–	1	–	1	–	–	1	1	1
C	1	1	1	1	–	–	–	–	–	–	–	–
D	–	–	–	–	–	0.5	–	–	–	–	–	–
Mpi												
A	–	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	1	1	1
D	–	–	–	–	–	–	1	1	1	–	–	–
E	1	1	1	1	1	1	–	–	–	–	–	–
Pgam			(1/1)									
A	–	–	0.5	–	1	–	–	–	–	–	–	–
B	1	1	0.5	1	–	–	–	–	–	–	–	–
C	–	–	–	–	–	1	1	1	1	1	1	1
D	–	–	–	–	–	–	–	–	–	1	–	–
Pgk												
A	1	1	1	1	1	–	–	–	–	–	–	–
B	–	–	–	–	–	1	1	1	1	1	1	1
Pk												
A	–	–	–	–	–	1	1	1	1	1	1	1
B	1	1	1	1	1	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–
6pgd											(0/6)	
A	–	–	–	–	–	1	–	–	–	1	0.667	–
B	–	–	–	–	1	–	–	–	–	–	0.333	1
C	–	–	–	–	–	–	1	1	1	–	–	–
D	1	1	1	1	–	–	–	–	–	–	–	–

continued

TABLE 1. *Continued*

Population	Fort	Kaue	Opep	Mang	Kaka1	Sadd	Rang1	Hutch	Ball2	Otar	Waio
n	4	2	5	3	2	4	2	3	3	5	3
n mean	4	1.9	5	2.9	2	3.9	2	2.9	2.9	4.4	2.9
Aata											
A	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	1	1
C	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	–	–
Aatc									(2/3)		
A	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–
D	1	1	1	1	1	1	1	–	0.333	1	1
E	–	–	–	–	–	–	–	1	0.667	–	–
Acon											
A	1	1	1	1	1	1	–	–	–	–	–
B	–	–	–	–	–	–	1	1	1	1	1
C	–	–	–	–	–	–	–	–	–	–	–
Ak											
A	–	–	–	–	–	–	–	–	–	1	1
B	1	1	1	1	1	1	1	1	1	–	–
Aldol											(1/3)
A	1	1	1	1	1	1	1	1	1	–	0.333
B	–	–	–	–	–	–	–	–	–	1	0.667
alphaGpd											
A	1	1	1	1	1	1	1	1	1	1	1
B	–	–	–	–	–	–	–	–	–	–	–
Enol											
A	1	1	1	1	1	1	1	1	1	1	1
B	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	–	–
Fdp										(0/3)	
A	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	0.667	–
C	–	–	–	–	–	–	–	–	–	0.333	1
Gapd											
A	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	1	1
Hk											
A	–	–	–	–	–	–	–	1	1	1	1
B	1	1	1	1	1	1	–	–	–	–	
Idha											
A	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	1	1
E	1	1	1	1	1	1	1	1	1	–	–
Mdha											
A	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	1	1
C	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	–	–
Mpi	(0/4)										
A	0.75	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	1	1	1	1	1
C	0.25	1	1	1	1	1	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	–	–
E	–	–	–	–	–	–	–	–	–	–	–
Pgam	(1/4)			(1/3)							
A	–	1	–	0.167	–	–	1	–	–	–	–
B	0.875	–	–	–	–	–	–	1	1	–	–
C	0.125	–	1	0.833	1	1	–	–	–	1	1
D	–	–	–	–	–	–	–	–	–	–	–
Pgk											
A	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	1	1
Pk											
A	1	1	1	1	1	1	1	1	1	1	1
B	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–
6pgd	(0/4)		(0/5)								
A	0.75	1	0.8	1	1	–	–	–	–	–	–
B	0.25	–	0.2	–	–	1	1	1	1	1	1
C	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	–	–

TABLE 1. *Continued*

Population	Akat	Cart	Pahi	Mill	Bides	Nors2	Mohi	Ouer	Bide	Rang2	Tiki	Monc
n												
n mean	2	4	5	2	9	4	5	3	2	1	1	4
	2	4	4.9	2	8.2	3.9	4.7	3	2	1	1	4
Aata						(1/4)			(1/2)			
A	–	1	–	–	–	–	–	–	0.25	–	–	–
B	1	–	1	1	1	0.375	–	–	–	–	–	–
C	–	–	–	–	–	0.625	1	1	0.75	1	1	1
D	–	–	–	–	–	–						
Aatc						(1/4)					(1/1)	(1/4)
A	–	–	–	–	–	0.125	–	–	–	–	0.5	0.125
B	–	–	–	–	–	–	–	–	–	–	–	–
C	–	–	–	–	–	–	–	–	–	–	–	–
D	1	1	1	1	1	0.875	1	1	1	1	0.5	0.875
E	–	–	–	–	–	–	–	–	–	–	–	–
Acon	(0/2)											
A	0.5	–	–	–	–	–	–	–	–	–	–	–
B	0.5	1	1	1	–	1	1	1	1	1	1	1
C	–	–	–	–	1	–	–	–	–	–	–	–
Ak												
A	1	1	1	1	–	–	–	–	–	–	–	–
B	–	–	–	–	1	1	1	1	1	1	1	1
Aldol					(2/9)							
A	–	–	–	–	0.889	–	–	–	–	–	–	–
B	1	1	1	1	0.111	1	1	1	1	1	1	1
alphaGpd												
A	1	1	1	1	1	1	1	1	1	1	1	1
B	–	–	–	–	–	–	–	–	–	–	–	–
Enol	(1/2)							(1/3)				
A	0.25	1	1	1	1	1	1	0.833	1	1	1	1
B	–	–	–	–	–	–	–	0.167	–	–	–	–
C	0.75	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	–	–	–
Fdp												
A	–	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–
C	1	1	1	1	1	1	1	1	1	1	1	1
Gapd												
A	–	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	1	1	1
Hk												
A	1	1	1	1	1	1	1	1	1	1	1	1
B	–	–	–	–	–	–	–	–	–	–	–	–
Idha		(1/4)							(2/2)			
A	–	–	–	–	–	–	–	–	0.25	–	–	–
B	–	–	–	–	–	–	–	–	0.5	–	–	–
C	–	0.625	–	–	–	–	–	–	–	–	–	–
D	1	0.375	1	1	1	1	1	1	0.25	1	1	1
E	–	–	–	–	–	–	–	–	–	–	–	–
Mdha												
A	–	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	1	1	1
C	–	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	–	–	–
Mpi												
A	–	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	1	1	1
C	–	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	–	–	–
E	–	–	–	–	–	–	–	–	–	–	–	–
Pgam								(0/3)				
A	–	–	–	–	–	–	–	–	–	–	–	–
B	–	–	–	–	–	–	–	0.333	–	–	–	–
C	1	1	1	1	1	1	1	0.667	1	1	1	1
D	–	–	–	–	–	–	–	–	–	–	–	–
Pgk												
A	–	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	1	1	1
Pk			(1/5)		(2/9)				(1/2)			
A	–	1	0.9	1	0.111	–	–	–	0.25	–	–	–
B	1	–	0.1	–	–	–	–	–	–	–	–	–
C	–	–	–	–	0.889	1	1	1	0.75	1	1	1
6pgd												
A	–	–	–	–	–	–	–	–	–	–	–	–
B	1	1	1	1	1	1	1	1	1	1	1	1
C	–	–	–	–	–	–	–	–	–	–	–	–
D	–	–	–	–	–	–	–	–	–	–	–	–

TABLE 2. Pairwise genetic distances between 35 populations of peripatus. Cavalli-Sforza and Edwards' (1967) arc distance below the diagonal and Nei's (1978) unbiased genetic distance above the diagonal. Populations are distinguished as having 16 pairs of legs (16) or 15 (no suffix). Dark bordered boxes indicate genetic distances among populations grouped according to Neighbour-Joining analysis of Cavalli-Sforza and Edwards' arc distance, and labelled a–f as in Fig. 2. Single-cell boxes highlight genetic distances between sympatric species at four sites

	Roto	Daws	Waiw1	Whak	Pelo	Taka	Kaka2	Waiw2	Kawa	Wait	Ball1	Nors1	Fort	Kaue	Opep	Mang	Kaka1	Sadd
Lake Rotokare (16)	a	0.000	0.015	0.000	0.298	1.356	1.807	1.807	1.497	1.236	1.270	1.057	1.260	1.250	1.250	1.260	1.260	1.260
Dawson falls (16)	0.081		0.015	0.000	0.293	1.432	1.735	1.735	1.735	1.447	1.200	1.238	1.029	1.224	1.213	1.214	1.224	1.224
Waiwawa 1 (16)	0.146	0.121		0.015	0.238	1.417	1.720	1.720	1.720	1.432	1.185	1.223	1.091	1.114	1.198	1.182	1.209	1.209
Whakapapa (16)	0.081	0.000	0.121		0.293	1.432	1.735	1.735	1.735	1.447	1.200	1.238	1.029	1.224	1.213	1.214	1.224	1.224
Pelorus Bridge	0.506	0.500	0.454	0.500		1.299	1.314	1.566	1.566	1.314	1.031	0.956	1.035	0.946	1.067	1.074	1.114	0.946
Takaka	0.856	0.874	0.874	0.874	0.849		0.333	0.293	0.293	1.209	0.897	1.021	1.014	1.027	0.891	0.887	0.872	1.027
Kakaho 2	0.911	0.907	0.907	0.907	0.849	0.542	b	0.061	0.061	0.887	0.864	0.896	0.987	1.041	0.887	0.901	0.887	0.887
Waiwawa 2	0.911	0.907	0.907	0.907	0.883	0.500	0.243		0.000	1.041	1.018	1.052	1.165	1.224	1.031	1.060	1.041	1.041
Kawau Island	0.911	0.907	0.907	0.907	0.883	0.500	0.243	0.000		1.041	1.018	1.052	1.165	1.224	1.031	1.060	1.041	1.041
Waitomo Caves	0.878	0.874	0.874	0.874	0.849	0.840	0.767	0.804	0.804	c	0.135	0.195	0.160	0.125	0.128	0.115	0.125	0.194
Balls Clearing 1	0.844	0.840	0.840	0.840	0.790	0.773	0.767	0.804	0.804	0.362		0.027	0.082	0.069	0.000	0.006	0.006	0.027
Norsewood 1	0.845	0.842	0.842	0.842	0.778	0.804	0.769	0.806	0.806	0.423	0.169		0.118	0.126	0.038	0.062	0.061	0.000
Forthbranch	0.810	0.806	0.815	0.806	0.793	0.794	0.789	0.826	0.826	0.388	0.256	0.299		0.091	0.079	0.075	0.083	0.117
Kaueranga	0.844	0.840	0.813	0.840	0.776	0.804	0.804	0.840	0.840	0.343	0.268	0.347	0.302		0.063	0.040	0.061	0.125
Opepe	0.844	0.840	0.840	0.840	0.795	0.770	0.767	0.804	0.804	0.350	0.069	0.178	0.247	0.253		0.001	0.001	0.038
Mangatutara	0.844	0.840	0.828	0.840	0.797	0.770	0.770	0.807	0.807	0.343	0.132	0.256	0.264	0.178	0.097		0.000	0.061
Kakaho 1	0.844	0.840	0.840	0.840	0.813	0.767	0.767	0.804	0.804	0.343	0.115	0.248	0.260	0.243	0.072	0.065		0.061
Saddle Road	0.844	0.840	0.840	0.840	0.776	0.804	0.767	0.804	0.804	0.420	0.161	0.050	0.295	0.343	0.171	0.251	0.243	
Rangataiki 1	0.844	0.840	0.813	0.840	0.738	0.840	0.804	0.840	0.840	0.542	0.413	0.423	0.450	0.420	0.454	0.456	0.485	0.420
Hutchinson	0.844	0.840	0.849	0.840	0.813	0.874	0.767	0.804	0.804	0.542	0.537	0.545	0.514	0.594	0.569	0.594	0.594	0.542
Balls Clearing 2	0.844	0.840	0.849	0.840	0.813	0.874	0.767	0.804	0.804	0.507	0.502	0.509	0.477	0.562	0.535	0.562	0.562	0.507
Otari	0.953	0.970	0.970	0.970	0.916	0.728	0.686	0.728	0.728	0.649	0.597	0.602	0.650	0.693	0.625	0.652	0.649	0.602
Waiohine	0.934	0.951	0.951	0.951	0.895	0.734	0.693	0.734	0.734	0.658	0.608	0.612	0.660	0.702	0.636	0.662	0.658	0.612
Akatarawa	0.936	0.953	0.953	0.953	0.883	0.767	0.728	0.767	0.767	0.715	0.680	0.673	0.717	0.755	0.694	0.718	0.715	0.673
Carterton	0.959	0.970	0.970	0.970	0.916	0.741	0.686	0.728	0.728	0.728	0.682	0.673	0.729	0.767	0.707	0.730	0.728	0.686
Pahiatua	0.942	0.959	0.959	0.959	0.904	0.729	0.688	0.729	0.729	0.688	0.639	0.644	0.689	0.729	0.666	0.691	0.688	0.644
Miller reserve	0.953	0.970	0.970	0.970	0.916	0.728	0.686	0.728	0.728	0.686	0.637	0.642	0.688	0.728	0.664	0.689	0.686	0.642
Bideford south	0.857	0.876	0.876	0.876	0.815	0.776	0.737	0.776	0.776	0.626	0.596	0.577	0.628	0.671	0.602	0.629	0.626	0.577
Norsewood 2	0.895	0.913	0.913	0.913	0.855	0.804	0.767	0.804	0.804	0.703	0.655	0.659	0.704	0.743	0.681	0.706	0.703	0.659
Mohi Bush	0.889	0.907	0.907	0.907	0.849	0.804	0.767	0.804	0.804	0.728	0.682	0.686	0.729	0.767	0.707	0.730	0.728	0.686
Oueroa	0.871	0.889	0.895	0.889	0.849	0.793	0.755	0.793	0.793	0.730	0.692	0.696	0.714	0.770	0.716	0.739	0.737	0.696
Bideford	0.901	0.911	0.911	0.911	0.853	0.800	0.745	0.784	0.784	0.705	0.658	0.662	0.706	0.745	0.683	0.708	0.705	0.662
Rangataiki 2	0.889	0.907	0.907	0.907	0.849	0.804	0.767	0.804	0.804	0.728	0.682	0.686	0.729	0.767.	0.707	0.730	0.728	0.686
Lake Tikitapu	0.889	0.907	0.907	0.907	0.849	0.804	0.767	0.804	0.804	0.738	0.693	0.697	0.739	0.776	0.717	0.740	0.738	0.697
Monckton res.	0.889	0.907	0.907	0.907	0.849	0.804	0.767	0.804	0.804	0.730	0.684	0.688	0.731	0.769	0.709	0.733	0.730	0.688

TABLE 2. *Continued*

	Rang1	Hutch	Ball2	Otar	Waio	Akat	Cart	Pahi	Mill	Bides	Nors2	Mohi	Ouer	Bide	Rang2	Tiki	Monc
Lake Rotokare (16)	1.260	1.260	1.244	2.579	2.343	2.377	2.713	2.512	2.595	1.385	1.739	1.640	1.563	1.727	1.640	1.625	1.632
Dawson falls (16)	1.224	1.224	1.208	2.817	2.530	2.575	2.817	2.732	2.833	1.463	1.844	1.735	1.654	1.765	1.735	1.720	1.727
Waiwawa 1 (16)	1.114	1.314	1.298	2.802	2.515	2.560	2.802	2.717	2.818	1.448	1.830	1.720	1.694	1.750	1.720	1.705	1.712
Whakapapa (16)	1.224	1.224	1.208	2.817	2.530	2.575	2.817	2.732	2.833	1.463	1.844	1.735	1.654	1.765	1.735	1.720	1.727
Pelorus Bridge	0.803	1.114	1.098	1.886	1.761	1.530	1.886	1.857	1.902	1.121	1.378	1.314	1.288	1.314	1.314	1.299	1.307
Takaka	1.209	1.432	1.416	0.723	0.765	0.837	0.804	0.746	0.739	0.978	1.003	1.027	1.029	1.056	1.027	1.012	1.019
Kakaho 2	1.041	0.887	0.871	0.620	0.658	0.718	0.620	0.641	0.636	0.844	0.864	0.887	0.885	0.795	0.887	0.872	0.880
Waiwawa 2	1.224	1.041	1.026	0.738	0.780	0.852	0.738	0.760	0.754	0.993	1.018	1.041	1.043	0.944	1.041	1.027	1.034
Kawau Island	1.224	1.041	1.026	0.738	0.780	0.852	0.738	0.760	0.754	0.993	1.018	1.041	1.043	0.944	1.041	1.027	1.034
Waitomo Caves	0.348	0.348	0.305	0.549	0.584	0.750	0.738	0.641	0.636	0.518	0.699	0.754	0.749	0.666	0.754	0.803	0.762
Balls Clearing 1	0.207	0.367	0.323	0.474	0.508	0.684	0.653	0.563	0.558	0.462	0.617	0.669	0.704	0.583	0.669	0.715	0.677
Norsewood 1	0.195	0.351	0.307	0.444	0.476	0.623	0.607	0.529	0.525	0.417	0.579	0.630	0.661	0.546	0.630	0.672	0.637
Forthbranch	0.216	0.304	0.261	0.582	0.619	0.800	0.785	0.680	0.674	0.549	0.744	0.801	0.764	0.711	0.801	0.857	0.811
Kaueranga	0.194	0.435	0.390	0.658	0.697	0.888	0.871	0.760	0.754	0.624	0.829	0.887	0.885	0.795	0.887	0.946	0.898
Opepe	0.242	0.407	0.361	0.518	0.552	0.714	0.703	0.608	0.603	0.488	0.665	0.719	0.755	0.631	0.719	0.766	0.726
Mangatutara	0.246	0.425	0.380	0.556	0.592	0.762	0.749	0.650	0.645	0.525	0.710	0.765	0.797	0.676	0.765	0.816	0.774
Kakaho 1	0.268	0.435	0.390	0.549	0.584	0.750	0.738	0.641	0.636	0.518	0.699	0.754	0.792	0.666	0.754	0.803	0.762
Saddle Road	0.194	0.348	0.305	0.450	0.482	0.629	0.620	0.535	0.531	0.423	0.585	0.636	0.667	0.552	0.636	0.678	0.643
Rangataiki 1		0.194	0.155	0.361	0.390	0.629	0.515	0.439	0.435	0.423	0.470	0.531	0.488	0.449	0.531	0.516	0.523
Hutchinson	0.420	d	0.004	0.361	0.390	0.629	0.515	0.439	0.435	0.423	0.470	0.531	0.488	0.449	0.531	0.516	0.523
Balls Clearing 2	0.373	0.095		0.316	0.345	0.575	0.466	0.392	0.390	0.377	0.426	0.482	0.440	0.401	0.482	0.483	0.479
Otari	0.551	0.551	0.516	e	0.029	0.137	0.113	0.024	0.024	0.275	0.185	0.227	0.239	0.208	0.227	0.250	0.229
Waiohine	0.562	0.562	0.528	0.175		0.113	0.090	0.004	0.004	0.204	0.161	0.202	0.213	0.183	0.202	0.225	0.204
Akatarawa	0.673	0.673	0.645	0.348	0.330		0.207	0.094	0.106	0.271	0.209	0.252	0.256	0.256	0.252	0.277	0.255
Carterton	0.642	0.642	0.612	0.317	0.296	0.422		0.085	0.084	0.354	0.210	0.224	0.236	0.151	0.224	0.247	0.226
Pahiatua	0.596	0.596	0.564	0.156	0.107	0.279	0.285		0.000	0.234	0.147	0.188	0.198	0.169	0.188	0.210	0.190
Miller reserve	0.594	0.594	0.562	0.148	0.095	0.316	0.280	0.050		0.239	0.153	0.194	0.204	0.173	0.194	0.216	0.196
Bideford south	0.577	0.577	0.544	0.460	0.403	0.489	0.518	0.437	0.436		0.140	0.182	0.192	0.194	0.182	0.203	0.183
Norsewood 2	0.613	0.611	0.581	0.403	0.387	0.426	0.447	0.375	0.375	0.347	f	0.006	0.011	0.022	0.006	0.014	0.004
Mohi Bush	0.642	0.642	0.612	0.445	0.431	0.466	0.443	0.420	0.420	0.396	0.116		0.004	0.019	0.000	0.015	0.000
Oueroa	0.645	0.616	0.585	0.460	0.446	0.478	0.458	0.436	0.436	0.412	0.163	0.115		0.025	0.004	0.020	0.004
Bideford	0.616	0.616	0.585	0.438	0.423	0.493	0.386	0.414	0.412	0.425	0.227	0.198	0.229		0.019	0.037	0.020
Rangataiki 2	0.642	0.642	0.612	0.445	0.431	0.466	0.443	0.420	0.420	0.396	0.116	0.000	0.115	0.198		0.015	0.000
Lake Tikitapu	0.653	0.642	0.620	0.461	0.447	0.482	0.459	0.437	0.437	0.414	0.121	0.121	0.167	0.232	0.121		0.008
Monckton res.	0.644	0.642	0.614	0.449	0.434	0.470	0.447	0.424	0.424	0.400	0.102	0.056	0.128	0.206	0.056	0.065	

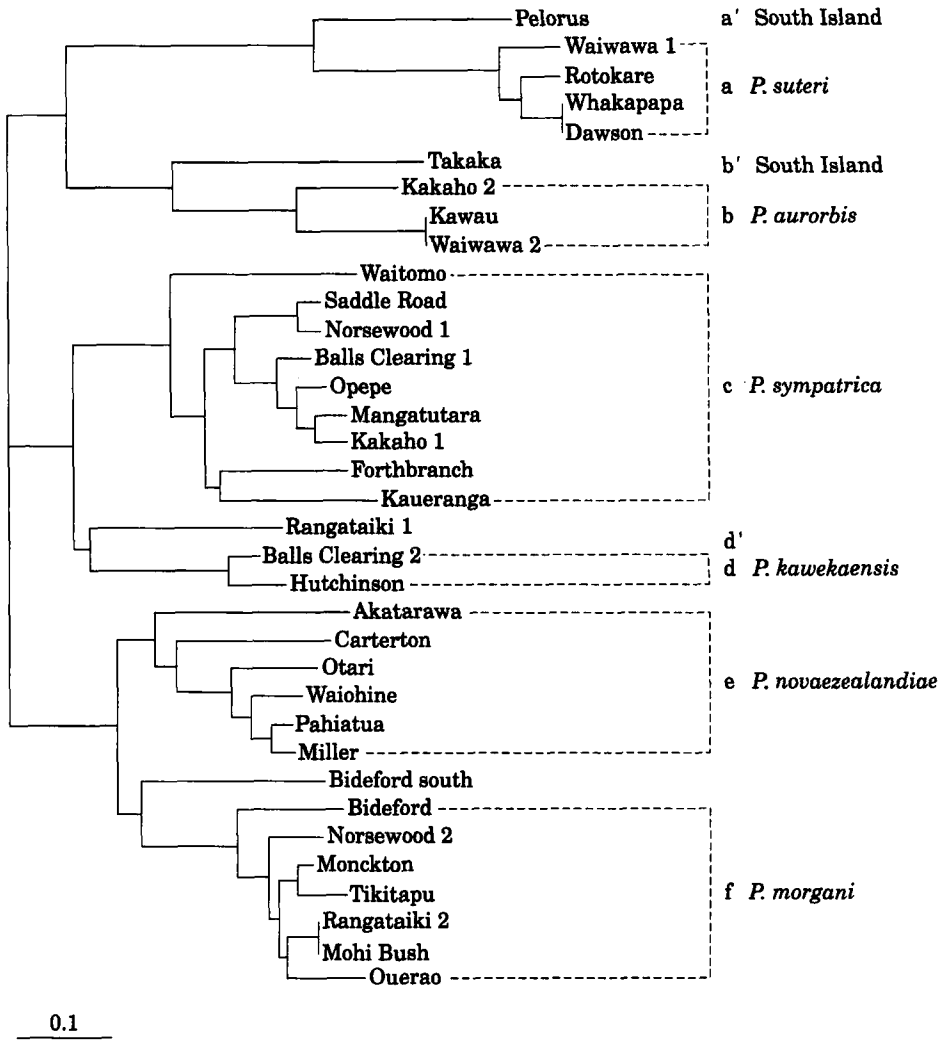

Figure 2. Neighbour-Joining tree (Saitou & Nei 1987) using Cavalli-Sforza and Edwards' (1967) arc distance from 17 loci for 35 populations of peripatus. Prominent clades are labelled a to f. Single populations that appear associated with a particular clade but are genetically and geographically isolated are indicated by '.

D 0.426). In fact Norsewood 1, Balls Clearing 1 and Kakaho 1 are members of the same clade (Figs 2, 4 - clade c). The genetic populations with which each of these is sympatric (Norsewood 2, Balls Clearing 2 and Kakaho 2) are each members of separate clades (Figs 2, 4 - clades b, d, f). Rangataiki 1 is closest to, but apparently distinct from clade d. Rangataiki 2, however, is genetically close to Norsewood 2 (clade f).

Species descriptions

Six distinct genetic groupings can be identified within this sample of peripatus (Fig. 2 clades a–f). One of these, clade a, is consistent with the existing species *P.*

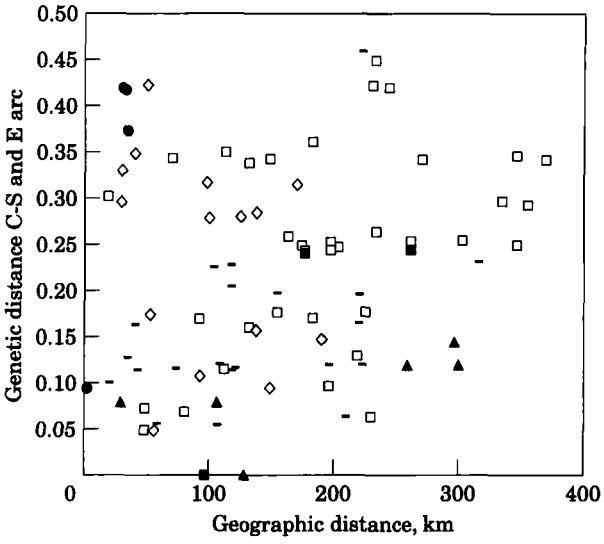

Figure 3. Plot of Cavalli-Sforza and Edwards' (1967) arc distance against minimum geographic distance (km) for populations within each of six clades indicated by Neighbour-Joining analysis of genetic distance (Fig. 2). (—) clade a; (▲) clade b; (□) clade c; (●) clade d; (■) clade e; (◇) clade f.

suteri. Five genetic groupings therefore exist within *P. novaezealandiae* in the North Island. In all instances genetic distance among clades is high. Furthermore, most clades include at least one population that is sympatric with a distinct genotype. Thus, clade b (Fig. 2) includes Waiwawa 2 which is sympatric with Waiwawa 1 (clade a = *P. suteri*), and Kakaho 2 which is sympatric with Kakaho 1 (clade c). Clade c has sympatry at Norsewood (with clade f), Balls Clearing (with clade d) as well as Kakaho.

Clade e has no sympatric populations in this study but is the most genetically divergent from *P. suteri* (Tables 2, 3). Populations in clade e are apparently parapatric with members of clade f (east) and d (north). In fact adjacent populations of clades e and d are separated by a significant geological barrier—the Manawatu Gorge. Genetic distances among populations in clades e and f, ranging from Nei's D 0.147 to 0.277 (Table 2). Bideford South is positioned genetically and geographically between clades e and f but is distinguished from both by the presence of the *Acon* C allele not found in any populations in clades e and f. *Acon* C is however present in *P. suteri* and clade b suggesting that it is ancestral in this data set (Table 1).

Four new species are therefore proposed to divide *P. novaezealandiae* in the North Island, and it is evident that distinct species with 15 pairs of legs are present in the South Island too (Fig. 2 a′ and b′). Further subdivision of North Island taxa might be appropriate and some populations such as Rangataiki 1 and Bideford South are of uncertain affinity. External macroscopic examination of morphological specimens from individuals used in genetic analysis failed to reveal any characters in respect of coloration and number of claws, leg-pads and papillae that consistently distinguished members of genetic clades. These characteristics are often diagnostic in peripatus taxonomy.

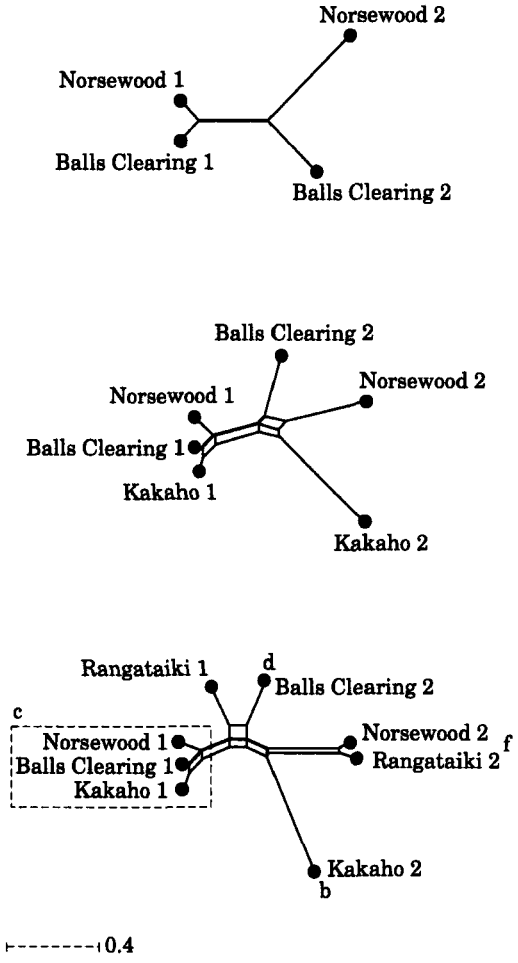

Figure 4. SplitsTree (Huson & Wetzel, 1994) analyses of Cavalli-Sforza and Edwards' (1967) arc distance from 17 loci for 2, 3 and 4 pairs of sympatric peripatus with 15 pairs of legs. Polygonal internal edges indicate support for alternative nodes. Clades from Neighbour-Joining analysis (Fig. 2) to which these populations belong are indicated on the lower tree. Three populations from different locations and belonging to clade c are indicated by the dashed box. Clade c is assigned to the species ***P. sympatrica***, clade d to ***P. kawekaensis***, clade f to ***P. morgani***, clade b to ***P. aurorbis***.

TABLE 3. Summary of number of diagnostic loci (below diagonal) and average Nei's genetic distance among members of the *Peripatoides novaezealandiae* complex. A+ is inserted where most populations within a species are apparently fixed for additional diagnostic loci in the relevant pairwise comparison

	P. nov	*P. mor*	*P. kaw*	*P. aur*	*P. sym*	*P. sut*
P. novaezealandiae	0.074	0.237	0.438	0.730	0.631	2.664
P. morgani	1+	0.012	0.478	0.969	0.849	1.716
P. kawekaensis	3+	4+	0.004	0.982	0.358	1.248
P. aurorbis	8	8+	10	0.041	1.020	1.750
P. sympatrica	6	7	3	9	0.085	1.228
P. suteri	14	13	13	14	11	0.007

Peripatoides novaezealandiae (Hutton, 1876)

Fifteen pairs of legs, three spinous pads on the underside of the legs, three distal papillae on the feet. Length variable, 2.5–5 cm. Colour variable, dependent principally on pigmentation of epidermal papillae which ranges from orange brown to purple black. Arrangement of pigmented papillae are sometimes suggestive of lateral stripes or other patterns but is variable within populations. Ventral surface smoother and usually paler than dorsal. Genital opening circular, tumid, wrinkled, usually grey sometimes pale, almost white. Differs from all other peripatus studied here except *P. aurorbis* at the *Ak* locus.

Original description based on material collected first in Wellington then also Dunedin and Nelson, but evidently consisting of a number of different species. The name can be applied to the species with populations in the Wellington region in the present analysis. No type material was proposed by Hutton (1876) so material from Otari in the present study will be identified as the neotype.

Material. Neotype Otari.

Distribution. Southern North Island, Wairarapa, southern Hawkes Bay; Otari, Carterton, Akatarawa, Waiohine, Pahiatua, Miller reserve. Represented by clade e in the present analysis (Fig. 2).

***Peripatoides morgani* sp. nov.**

Etymology. Named for the collector Mary Morgan Richards.

Diagnosis. As for *P. novaezealandiae.* Five and six populations of the seven examined were found to be fixed for different alleles compared to other peripatus with 15 pairs of legs at the *Pk* and *Aat-a* loci respectively. The *Pk* allele in this species has only otherwise been found at Bideford South (a population of unresolved affinity), and the *Aat-a* allele has only been found in *P. suteri* and the Pelorus population. Sympatric with *P. sympatrica* at Norsewood from which it is distinct at seven allozyme loci: *Acon, Aldol, Fdp, Hk, Idh-a, Mpi, Pk* , and parapatric with *P. novaezealandiae* from which it is distinct at the *Ak* locus (Tables 1, 3). Represented by clade f in the present analysis (Table 2, Fig. 2).

Material. Holotype Mohi Bush.

Distribution. Eastern North Island in a narrow coastal strip including southern and central Hawkes Bay and north to Lake Tikitapu. Present at Bideford, Oueroa, Monckton reserve, ANZAC scenic reserve at Norsewood, Mohi Bush, Lake Tikitapu.

***Peripatoides kawekaensis* sp. nov.**

Etymology. Kaweka mountain, Hawkes Bay, which overlooks the small range of this species.

Diagnosis. As for *P. novaezealandiae.* Distinguished by a unique fast allele at the *Aat-c* locus (Table 1). Sympatric with *P. sympatrica* sp. nov. at Balls Clearing where it is distinct at three loci: *Hk, Mpi* and *Pgam* (Tables 1, 3). Represented by clade d in the present analysis (Table 2, Fig. 2).

Material. Holotype Hutchinson Reserve.

Distribution. Local, Hutchinson and Balls Clearing Reserves, Hawkes Bay. Possibly also Rangataiki where sympatric with *P. morgani* sp. nov.

Peripatoides aurorbis sp. nov.

Etymology. Latin *aurum* gold, *orbis* round or circular; a reference to the bright yellow genital opening.

Diagnosis. As for *P. novaezealandiae.* Locally identifiable by bright yellow genital opening, but this character has also been observed rarely in specimens of other species from other areas (e.g. one specimen at Lake Tikitapu). Sympatric with *P. sympatrica* sp. nov. at Kakaho from which it is distinct at 10 loci: *Aat-a, Aat-c, Acon, Ak, Aldol, Enol, Fdp, Hk, Mpi, 6Pgd,* and yellow rather than grey genital opening. Sympatric with *P. suteri* at Waiwawa from which it is distinct at 14 loci: *Aat-a, Aat-c, Ak, Aldol, alphaGpd, Enol, Gapd, Hk, Mdh, Mpi, Pgam, Pgk, Pk, 6Pgd*) (Table 1) and 15 rather than 16 pairs of legs (Tables 1, 3). Represented by clade b in the present analysis (Table 2, Figure 2).

Material. Holotype Kawau Island.

Distribution. Central and mid-northern North Island, Kakaho, Waiwawa, Kawau Island.

Peripatoides sympatrica sp. nov.

Etymology. Occurs in sympatry with at least three other species.

Diagnosis. As *P. novaezealandiae.* The *Acon* A allele is almost exclusive to this species throughout its wide range, the allele occurs at low frequency in *P. kawekaensis* at Balls Clearing and *P. novaezealandiae* in the Akatarawas. The species is fixed for separate alleles from those in sympatric species at Balls Clearing (*P. kawekaensis*), Kakaho (*P. aurorbis*) and Norsewood (*P. morgani*), as detailed above (Tables 1, 3).

Material. Holotype Norsewood.

Distribution. Widespread in northern central and mid-eastern central North Island, Waitomo Caves, Saddle Road, Norsewood, Balls Clearing, Opepe, Mangatutara, Kakaho, Forthbranch and Kaueranga.

Sympatric with *P. kawekaensis* sp. nov. at Balls Clearing, *P. morgani* sp. nov. at Norsewood, *P. aurorbis* sp. nov. at Kakaho. Represented by clade c in the present analysis (Table 2, Figure 2).

DISCUSSION

Molecular taxonomy

Molecular techniques can challenge traditional taxonomy where genetic subdivisions are encountered within accepted species. In some instances, subsequent detailed morphological examination has revealed characters considered suitable for the diagnosis of new species (e.g. among Onychophora; Reid, 1996). Where reliable

morphological characters have not been immediately apparent there is reluctance to formally define new species. This is despite the fact that genetic characters are powerful indicators of species identity under the terms of any popular definition, including the biological species concept (Mayr, 1970) when such species are encountered in sympatry (Buth, 1984; Avise, 1994). The benefits of having characters that enable identification of species in the field is undoubted, but the absence of such characters should not be allowed to retard the recognition of discrete biological units. Where genetically distinct units exist in allopatry, determination of species is at its most problematic (Ponder, Eggler & Colgan, 1995; Ruedi, 1996) although not insoluble (e.g. Avise & Ball, 1990). In the present situation however, the existence of genetically distinct groups with representative populations in sympatry provides sound evidence that full species are present (Richardson *et al.*, 1986).

The utility of multiple polymorphic nuclear loci for observing gene flow among populations of sexual organisms is well established (Murphy *et al.*, 1996). The use of allozymes in studies such as this provide an opportunity to rapidly assess the exchange of alleles among sympatric taxa even where sample sizes are fairly small (Avise, 1975) and to identify species boundaries among populations (Davis & Nixon, 1992). The approach has proven especially useful in revealing geographic patterns in morphologically conservative taxa (Wake, Roth & Wake, 1983; Larson, 1989).

Low levels of heterozygosity among and within clades encountered in this study and Tait & Briscoe (1995) are indicative or extremely low levels of gene flow and are consistent with notions of low vagility of peripatus. In fact in the present study, the presence within clades of individuals that are homozygous for alleles not otherwise expressed in their clade, and the existence of a unique allele (*Pgam* D) at Waitomo suggests further subdivisions may exist (e.g. *Acon* B in Balls Clearing 1; *Fdp* B in Otari). Further splitting may be appropriate but will require additional evidence to support recognition of allopatric species. Four new species have been described so that the processes that led to their evolution, and the ecological and behavioural characteristics that distinguish them can subsequently be explored and discussed without ambiguity (Stern, Aoki & Kurosu, 1997). Molecular based taxonomy has been adopted with a small but diverse range of fauna including wallabies (Eldridge & Close, 1992), springtails (Carapelli *et al.*, 1995) and snails (Ponder *et al.*, 1995). The merit of morphological descriptions are not devalued by this approach, rather the resolving power of the alternative techniques in the context of morphologically conservative taxa is recognized.

Of the two morphospecies examined in this study, one has been revealed to be a complex of several discrete groups (*P. novaezealandiae* Hutton) whilst the other (*P. suteri*) shows little genetic variation throughout its known range. However, despite the distinction of having an extra pair of legs *P. suteri* is genetically quite close to one member of the *P. novaezealandiae* complex (Pelorus). The characteristic (16 pairs of legs) upon which it was originally named is a reliable taxonomic character but is not phylogenetically informative, being uniquely derived. The genetic relationships of the animals promise to reveal much more about the biogeographic processes that led to their speciation than can be expected from morphology.

Implications of sympatry

Paradoxically, the existence of morphologically cryptic species in sympatry, so useful in confirming the genetic integrity of those species, presents something of a

challenge to ecological and evolutionary theory. How and why do some species remain apparently morphologically and ecologically identical in sympatry? One approach to this problem is to assume that the species have only recently become sympatric and are therefore presumably in the process of evolving distinguishing characteristics or becoming extinct (Bruna *et al.*, 1996). However, the number and taxonomic diversity of cryptic, sympatric animals suggests that alternative causes are likely (e.g. skinks – Daugherty, Patterson & Hitchmough, 1994; Bruna *et al.*, 1996; springtails – Carapelli *et al.*, 1995; marsupials – Dickman *et al.*, 1988; bats – Baker, 1984; rotifers – Gomez & Snell, 1996; snails – Emberton, 1995). A likely explanation for the apparent similarity of some species is simply deficiency of knowledge about the ecology of the species being studied (Chesson, 1985; Carapelli *et al.*, 1995). Differences in resource use in time as well as space probably ameliorate the similarities that seem to contradict the predictions of competition theory. In addition, external morphology may simply not be amenable to obvious changes or may respond to overriding selection favouring convergent or parallel evolution (Bruna *et al.*, 1996; Larson, 1989).

Among Onychophora in Australia it has been revealed that although many previously unrecognized species exist, most of these are allopatric. Where Australian peripatus are in sympatry each species tends to be a member of a morphologically distinct and genetically deeply diverged group (Briscoe & Tait, 1995; Tait & Briscoe, 1995; Reid *et al.*, 1995). In these situations taxa are differentiated by specialized reproductive strategies. Mechanisms that now serve to reproductively isolate Australian sympatric species may not have evolved in the present geographic circumstances as they appear to be of very ancient origin (i.e. Gondwanan). In contrast the newly discovered diversity within the New Zealand fauna (Tait & Briscoe, 1995; present study) exists within one morphologically homogeneous group. This diversity might relate to differences in reproductive behaviour which are not obviously reflected in their morphology as in some Australian species or may indicate very recent dispersal following speciation in allopatry.

This study has identified four locations in New Zealand where peripatus with 15 pairs of legs exist in sympatry but the actual extent of sympatry is very likely to be greater. As a biogeographic model the minimum area convex polygons (Fig. 5) are an over simplification but they do give an indication of the probable geographic complexity of peripatus diversity. Where the range of *P. suteri* overlaps with *P. aurorbis* and *P. sympatrica*, three species are likely to be occupying the same area. Although not included in the present study, peripatus with 15 pairs of legs are known to be present in sympatry with *P. suteri* at Whakapapa as predicted by this minimum area model (Tait & Briscoe, 1995). In a similar way it is feasible that in northern Hawkes Bay, *P. sympatrica*, *P. morgani* and *P. kawekaensis* are sympatric. In the vicinity of areas such as Norsewood, sympatry and parapatry of several species emphasizes the biogeographical complexity in the group and of the region.

Biogeography

There is an absence of genetic differentiation between *P. aurorbis* at Waiwawa (Coromandel Range) and Kawau Island, areas which are most likely to have last been separated at the end of the Otiran glaciation about 14 000 years bp. (Stevens, 1980). Therefore the genetic structure observed in this study is probably derived

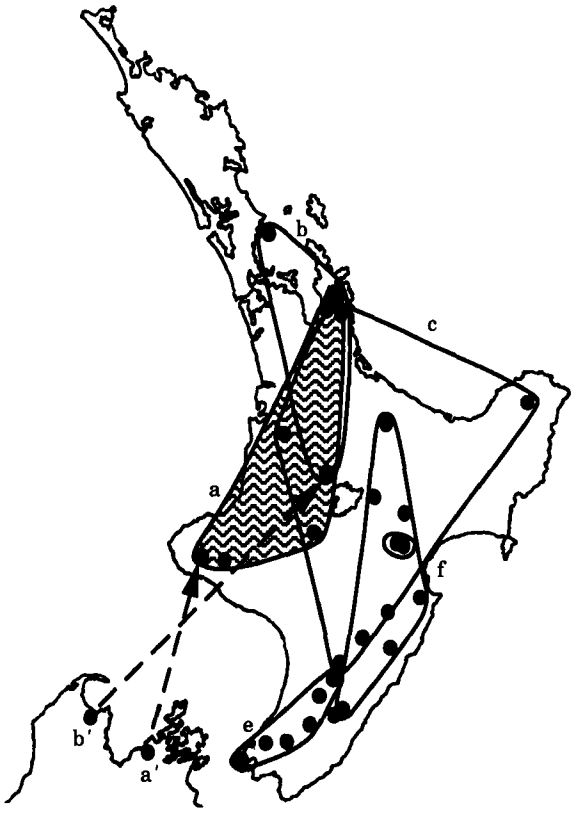

Figure 5. Hypothetical distribution of peripatus species in the North Island of New Zealand according to minimum area grouping of locations with populations that had been clustered by Neighbour-Joining analysis (Fig. 2). *P. suteri* is indicated by wavy fill. All groups are labelled as in Fig. 2. Dashed arrows indicate populations and clades that are geographically and genetically nearest to South Island populations at Pelorus (a') and Takaka (b').

from more ancient biogeographic events (Briscoe & Tait, 1995). However, it is probable that population fragmentation and dispersal was influenced by climate induced changes in habitat availability during the last glacial epoch. Some or all of the present sympatry may have resulted from this.

The apparent parapatry of *P. novaezealandiae* with *P. morgani* (east) and *P. kawekaensis* (north) is similar to that observed for tree weta in this region (Trewick & Morgan-Richards, 1995). The proximity of *P. novaezealandiae* (south) and *P. kawekaensis* (north) either side of the present day Manawatu Gorge suggests that this feature or an earlier (Pliocene) marine equivalent (Manawatu Strait) may be implicated in their isolation (Fleming, 1979). Conversely the close genetic relationship of *P. suteri* and *P. aurorbis* with peripatus in the South Island (Fig. 5) presumably derives from a time prior to the development of Cook Strait separating the island about 16 000 years bp. (Lewis, Carter & Davey, 1994).

If a model of allopatric speciation is assumed then at least two speciation phases can be proposed for the present distribution of peripatus in the North Island (Fig. 4). The first of these events would have involved geographic isolation leading to the formation of a number of new species including *P. sympatrica.* Subsequently, *P.*

sympatrica expanded its range so that it overlapped with other allopatric species at Norsewood, Balls Clearing and Kakaho. Such events may have resulted from this widely distributed species evolving rather different reproductive and dispersal characteristics than its sibling species, or that the speciation centre of *P. sympatrica* was geographically convenient for dispersal into the ranges of other species. Alternatively, the role of speciation in sympatry perhaps through the auspices of chromosomal rearrangements, cannot be excluded as a possible factor (Reid *et al.*, 1995). To what extent the complex and turbulent geophysical history of New Zealand has contributed to the evolution of endemic peripatus cannot yet be determined but differences in the nature of this diversity when compared to that seen in the geologically stable landmass of Australia points to an association between geophysiology and diversity.

Conservation

This study demonstrates that the New Zealand Onychophoran fauna is far more diverse than the existing taxonomy indicates. In conjunction with Tait & Briscoe (1995) it contributes considerably to our meagre knowledge of the diversity and patterns of distribution of these animals, features that will be relevant for their conservation (Gleeson, 1996). Several cryptic species are present in the North Island, some with localized distributions. The new species, *P. kawekaensis* in particular appears to have an extremely limited range and may merit special conservation consideration, but there are also many populations within clades and some that have remained unclassified in the present study are also genetically divergent and worthy of conservation (e.g. Rangataiki 1 and Bideford South). Further full species are likely to be present in the North Island and certainly exist in the South Island (Tait & Briscoe, 1995) and the evolutionary significance of these will need to be determined in order to define conservation strategies (Moritz, 1994).

Among-population genetic variability is high, but low variation is noted within most populations suggesting substantial subdivision (Avise, 1975, 1989). Low vagility and habitat tolerance characteristics make the group vulnerable to reduction of genetic diversity through local extinction. Habitat destruction and collection by biological suppliers is bound to have considerable impact on populations, although the extent of this threat cannot currently be gauged given the lack of basic biological information on these animals. It is likely that populations inhabiting logs in degraded and non-regenerating forests have a high risk of extinction. However, the discovery of peripatus in isolated logs in paddocks and small forest fragments suggests that their low vagility may act in their favour in buffering the expected effects of large scale habitat destruction on genetic diversity.

The identification of Onychophora as target taxa in the conservation of invertebrates might be a useful approach for New Zealand's endemic invertebrate fauna (New, 1995). The apparent concordance of patterns of distribution among taxa discussed above supports this. Continued molecular research with the material used in the present study will help in determining the vulnerability of peripatus populations by exploring population genetics and phylogenetics of these taxa. This research may also highlight aspects of peripatus ecology that are most likely to provide fruitful insights into their evolution.

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