Biological Journal of the Linnean Society, 2004, **81**, 119–133. With 3 figures

Population genetic structure of New Zealand's endemic corophiid amphipods: evidence for allopatric speciation

MARK I. STEVENS* and IAN D. HOGG

Centre for Biodiversity and Ecology Research, Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, New Zealand

Received 2 January 2003; accepted for publication 28 July 2003

Allozyme electrophoresis was used to examine population genetic structure at inter- and intraspecific levels for the New Zealand endemic corophiid amphipods, *Paracorophium lucasi* and *P. excavatum*. Individuals were collected from estuarine and freshwater habitats from North, South and Chatham Islands. Analyses of genetic structure among interspecific populations indicated clear allelic differentiation between the two *Paracorophium* species (Nei's genetic distance, $D = 1.62$), as well as considerable intraspecific substructuring ($D = 0.15$–0.65). These levels of divergence are similar to interspecific levels for other amphipods and it is proposed that at least two groups from the *P. lucasi* complex and three from the *P. excavatum* complex correspond to sibling species. In most cases allopatry can account for the differentiation among the putative sibling species. For populations that share a common coastline we found low levels of differentiation and little or no correlation with geographical distance, suggesting that gene flow is adequate to maintain homogeneous population genetic structure. By contrast, populations on separate coastlines (i.e. isolated by land) showed moderate levels of geographical differentiation indicating restricted gene flow. The juxtaposition of population genetic and biogeographical data for *Paracorophium* in conjunction with the geological record infers past histories of glacial extirpation, and possible isolating effects of sea-level and landmass changes that have occurred throughout the Plio-Pleistocene.

ADDITIONAL KEYWORDS: allozyme – glaciation – isolation-by-distance – *Paracorophium* – Pleistocene – Pliocene – sibling species.

INTRODUCTION

The isolation of populations, both geographically and genetically, has long been recognized as a potential mechanism conducive to speciation (Kimura, 1953; Mayr, 1954; Avise, 1992). Geographically isolated taxa with limited dispersal capabilities are particularly susceptible to microevolutionary processes (Mayr, 1954; Templeton, 1980). This is especially evident on islands where populations tend to become isolated from the main distributions, both in terrestrial and aquatic systems (Slatkin, 1993). For aquatic invertebrates large genetic divergences and/or a positive relationship between geographical and genetic distances often occur, albeit with little morphological variation (e.g. Knowlton *et al.*, 1993; Väinölä, 1995; Taylor, Finston & Hebert, 1998; Dawson, 2001). Fortunately, molecular techniques have in recent years made it possible to investigate how distributions of morphologically similar populations may be linked to geographical isolation and/or a taxon's dispersal capability (e.g. Avise, 1992; Hellberg, 1996; Parker *et al.*, 1998).

For taxa with limited dispersal, small or temporary geographical barriers may be sufficient to isolate populations. For example, the emergence of the Isthmus of Panama has been considered a major isolating barrier for the marine shrimp *Alpheus*, leading to the evolution of sibling species by the isolation of populations between the Caribbean and eastern Pacific (Knowlton *et al.*, 1993). In addition, ocean circulation has been found to correspond to phylogeographical breaks among populations of marine taxa between the Californian and Oregonian coastal regions (Dawson, 2001;

*Corresponding author. Current address: Allan Wilson Centre for Molecular Ecology and Evolution, Massey University Private Bag 11-222, Palmerston North, New Zealand. E-mail: M.I.Stevens@massey.ac.nz

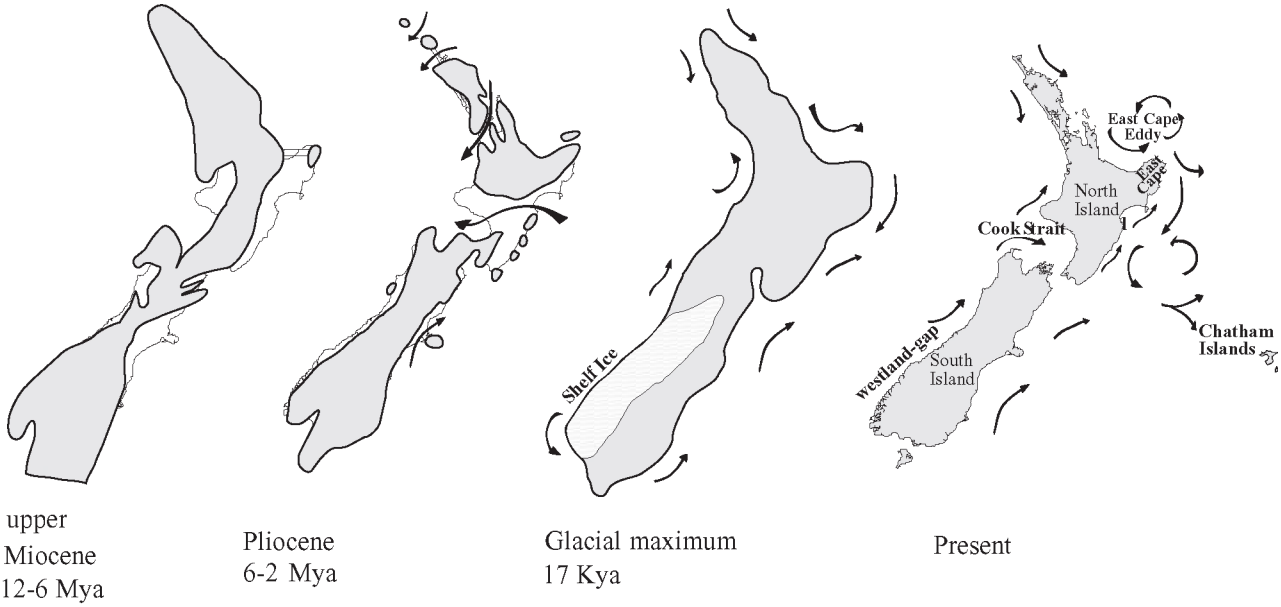

Figure 1. The changing outline of the New Zealand archipelago over the last 12 Myr. Land above sea level shaded grey. Arrows represent prevailing ocean circulation. The Pliocene (6–2 Mya) landmass provided few barriers for aquatic dispersal between the east and west coasts, in contrast to the upper Miocene (12–6 Mya), the last glacial maximum (approx. 17 Kya) and the present. Figures adapted from Fleming (1979) and Stevens *et al.* (1995).

Edmands, 2001; Wares, Gaines & Cunningham, 2001). Such isolating oceanographic processes have also been invoked to explain high levels of genetic substructuring and cryptic species among populations of the amphipod *Talitrus saltator* in the Mediterranean Sea (De Matthaeis *et al.*, 2000). Similarly, genetic subdivision of the greenshell mussel *Perna canaliculus* (Apte & Gardner, 2002) and of the corophiid amphipod *Paracorophium excavatum* (Schnabel, Hogg & Chapman, 2000) also suggest that coastal currents may be barriers to present-day gene flow in New Zealand. In addition, the turbulent geological history of New Zealand has been implicated as a potential agent for morphological as well as genetic differentiation of taxa (Craw, 1988; Pole, 1989; Trewick, 2000a; Trewick & Wallis, 2001; Wallis *et al.*, 2001).

The New Zealand archipelago (Fig. 1) has undergone considerable geological change during the Cenozoic (*c.* 65 Mya–present; Stevens, McGlone & McCulloch, 1995). For example, marine intrusions occurred throughout the upper Miocene (*c.* 12–6 Mya) and Pliocene (*c.* 6–2 Mya) until uplift of the landmass separated the east and west coasts of North Island, and its present landmass was only attained towards the beginning of the Pleistocene (*c.* 2 Mya; Fleming, 1979; Cooper & Millener, 1993; Stevens *et al.*, 1995). In the Pleistocene the isolation of regions has been influenced by the glacial/interglacial oscillations with sea-level changes and the advance and retreat of glaciers (Fleming, 1979; Stevens *et al.*, 1995). In New Zealand such geological and climatic effects are frequently associated with the inability of poorly dispersing organisms to recolonize denuded regions (Craw, 1988; Main, 1989; Pole, 1989; Trewick & Wallis, 2001).

Despite this association, there has been little investigation into patterns of diversification and dispersal of New Zealand aquatic taxa. Here, we assessed the population genetic structures of two New Zealand endemic corophiid amphipods, *Paracorophium lucasi* and *P. excavatum*. Both reproduce sexually and offspring hatch in the mothers brood pouch as free-living juveniles (i.e. have an adult morphology). They therefore lack a specific dispersal stage and may be exposed to present-day geographical barriers. However, population genetic structure may also be a consequence of landmass alterations over time (see Fig. 1). Accordingly, we tested the hypothesis that two closely related species would exhibit similar population genetic structures due to common geographical barriers. We also examined whether patterns of divergence would correspond to geological changes and climatic fluctuations that occurred throughout the Plio-Pleistocene.

METHODS

Collection of samples

Between September 1998 and August 2000 we examined a total of 53 sites throughout New Zealand. At each site we sampled approximately 50 m^2 of fine mud

and sand by passing a meshed (2 mm) net through the superficial sediment (upper 30–50 mm), and live-sorting for *Paracorophium* spp. In addition to the two endemic *Paracorophium* (*P. lucasi* and *P. excavatum*) we included the exotic *P. brisbanensis* as an outgroup taxon collected from a single site in Tauranga Harbour (N8) (Fig. 2). Species determination used the diagnostic characters suggested by Chapman *et al.* (2002), and for *P. brisbanensis* we used Chapman (2002) and Stevens, Hogg & Chapman (2002). All individuals used for allozyme analyses were flash-frozen in liquid nitrogen and stored at −76°C. Sites were coded according to geographical location to indicate common coastline or habitat type, for example NE = North

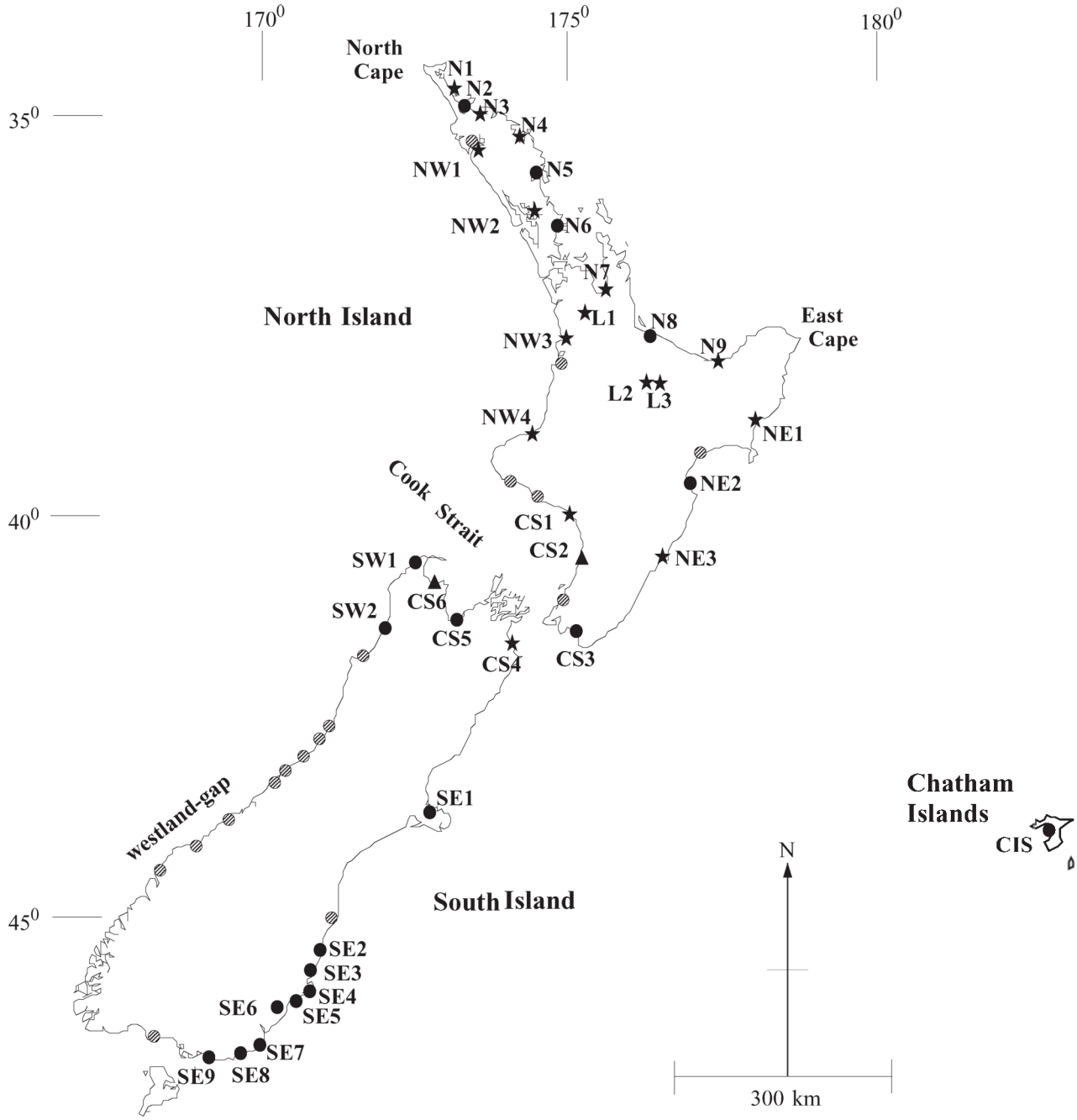

Figure 2. Distribution of *Paracorophium lucasi* (stars) and *P. excavatum* (solid circles) in New Zealand. Triangles show sympatric occurrences and hatched circles indicate sites where *Paracorophium* was not found. *P. brisbanensis* was found at N8.

Island, east coast; SW = South Island, west coast; CS = Cook Strait; and L = Lake.

Allozyme electrophoresis

We used cellulose acetate electrophoresis to assess levels of genetic differentiation among populations (Richardson, Baverstock & Adams, 1986; Hebert & Beaton, 1993). Individual specimens were homogenized in 14 μL of distilled water and 10 enzyme systems were used that revealed sufficient activity and resolution to be reliably scored for *Paracorophium* (Stevens *et al.*, 2002): aldehyde oxidase (AO: EC 1.2.3.1); arginine kinase (ARK: EC 2.7.3.3); glyceraldehyde-3-phosphate dehydrogenase (G3PDH: EC 1.2.1.12); isocitrate dehydrogenase (IDH: EC 1.1.1.42); lactate dehydrogenase (LDH: EC 1.1.1.27); mannose-6-phosphate isomerase (MPI: EC 5.3.1.8); malate dehydrogenase $NADP^+$ (ME: EC 1.1.1.40); peptidase (PEP: EC 3.4.11/13); phosphoglucomutase (PGM: EC 5.4.2.2); 6-phosphogluconate dehydrogenase (6PGDH: EC 1.1.1.44). Two enzymes (IDH, LDH) were coded by two loci designated numerically in order of increasing electrophoretic mobility (e.g. *IDH-1*, *IDH-2*). Alleles were designated by the relative differences in anodal mobility of the respective gene products, i.e. the 'fastest' allele was designated 'A', the next fastest allele 'B', and so on. We detected between 3 and 7 alleles for the loci examined. Two individuals from previous runs were re-run on subsequent gels to control for any variation in mobility between gel plates, and putative novel alleles were verified using gel line-ups (*sensu* Richardson *et al.*, 1986).

Data analyses

BIOSYS-1 (Swofford & Selander, 1981) was used to calculate descriptive and hierarchical population statistics. Genotypic frequencies were determined for each population, and polymorphic loci (95% criterion) were examined for agreement of genotypes with Hardy–Weinberg (H–W) equilibrium using Fisher's exact test, followed by sequential Bonferroni corrections (Rice, 1989). Divergence was assessed among populations using Wright's (1978) F_{ST}, and among individuals in a single population (F_{IS}). Significance of pairwise comparisons of F_{ST} (bootstrapping across loci with 5000 replicates) was used to pool some populations for *P. excavatum* only when homogeneous population genetic structure could be inferred. Preliminary analyses of the phenetic relationships among populations used several algorithms (e.g. Roger's similarity, Cavalli-Sforza & Edwards arc and cord distances, Nei's distance and identity) and various methods (e.g. Wagner network, neighbour-joining, multidimensional scaling) which all revealed identical topologies. Here, we present a hierarchical cluster analysis performed using UPGMA (Sneath & Sokal, 1973) calculated using Nei's (1978) unbiased genetic distance (D) chosen on the basis of goodness of fit statistics (i.e. cophenetic correlation = 0.904).

Isolation-by-distance (*I-D*) analyses were performed to examine geographical differentiation among populations (Wright, 1943; Kimura & Weiss, 1964; Slatkin, 1993). We performed a regression of log transformed pairwise genetic and geographical (aquatic) distances (log$D$ and logkm, respectively), and calculated ordinary least squares regression coefficients (R^2) (Kimura & Weiss, 1964; Felsenstein, 1976; Slatkin, 1993; Hellberg, 1996). Spearman's rank correlation index (R) was used to test how much of the allelic variance among populations could be explained by geographical distance alone (De Matthaeis *et al.*, 2000). SPSS (ver. 10) for Windows was used for these analyses.

RESULTS

Geographic distribution

Paracorophium was found throughout New Zealand waters (Fig. 2). *P. lucasi* was collected from 18 sites, *P. excavatum* from 21; we were unable to find either species at a further 17. Both species were found along the east coast of North Island and the coastal regions of Cook Strait. Only *P. lucasi* was found on the west coast of North Island, and *P. excavatum* on the west and east coasts of South Island and Chatham Island. We did not find either species on most of the west coast of South Island.

Allozyme variation

Several diagnostic alleles were identified that could reliably distinguish between the three *Paracorophium* species (see Appendix): A and B (*AO* locus) were diagnostic for *P. lucasi*, C, D and F (*AO*) and A (*LDH-1*) for *P. excavatum*, and E (*AO*), A (*G3PDH*) and B (*IDH-1*) for *P. brisbanensis* (only a single population). The mean number of alleles per locus/polymorphic locus was 1.5/2.6 for *P. lucasi*, and 1.6/2.8 for *P. excavatum*, while the percentage of polymorphic loci (95% criterion) was 23.5% and 31.6%, respectively; mean heterozygosities were similar (Table 1). For *P. lucasi*, significant deviations from H–W equilibrium were detected at *ARK* for N9, at *6PGDH* for NE1 and NE3 and at *PEP* for NE1 and L2 (all heterozygote deficiencies). For *P. excavatum*, genotypic frequencies deviated from H–W at *ARK* for N8, at *MPI* for N6, NE2, CS3, SE8 and CIS, and at *LDH-1* for CS6, SE5, SW1 and SW2 of Cook Strait and South Island. Chatham Islands (CIS) had genotypic frequencies deviating from H–W at *LDH-2* and *6PGDH* and one Cook Strait population (CS5) at *IDH-1*.

Table 1. Genetic variability at 12 loci in all populations of *Paracorophium lucasi*, *P. excavatum* and *P. brisbanensis*. Notation preceding locations refers to that used in the text, figures, tables, and appendix. N = mean sample size per locus; P = percentage of polymorphic loci (95% criterion); A = mean number of alleles per locus; Ap = mean number of alleles per polymorphic locus; H_{obs} = observed heterozygosity; H_{exp} = expected heterozygosity; *significant ($P < 0.05$) deviation at one or more loci (see text), Superscripts [1–4] indicate proximate populations pooled for UPGMA only when F_{ST} among populations was not significantly different to zero (determined from bootstrap analyses)

Location		Lat (S)	Long (E)	N	P	A	Ap	H_{obs}	H_{exp}
Paracorophium lucasi									
N1	Houhora Harbour	34°48′	173°06′	22.8	16.7	1.4	3.5	0.06	0.07
N3	Awanui River	35°01′	173°17′	26.1	16.7	1.3	3.0	0.06	0.06
N4	Taumarere	35°20′	174°06′	24.6	25.0	1.6	3.0	0.04	0.05
N7	Thames	37°05′	175°30′	25.8	8.3	1.4	3.0	0.05	0.05
N9	Whakatane	38°00′	177°06′	25.8	30.8	1.5	2.3	0.09	0.10*
NW1	Rawene	35°26′	173°31′	21.8	16.7	1.3	3.0	0.05	0.06
NW2	Topuni River	36°13′	174°28′	16.8	16.7	1.3	2.0	0.05	0.06
NW3	Raglan Harbour	37°48′	174°57′	35.7	33.3	1.8	3.0	0.06	0.07
NW4	Waitara	39°04′	174°03′	27.0	16.7	1.5	2.0	0.07	0.06
L1	Lake Waikare	37°26′	175°13′	37.0	8.3	1.1	2.0	0.03	0.04
L2	Lake Rotorua	38°02′	176°17′	30.1	33.3	1.4	2.0	0.05	0.07*
L3	Lake Rotoiti	38°01′	176°21′	26.0	8.3	1.2	2.0	0.05	0.05
CS1	Whanganui River	39°55′	175°02′	22.5	41.7	1.7	2.5	0.10	0.11
CS2	Foxton	40°18′	175°15′	11.0	25.0	1.5	2.5	0.06	0.07
CS4	Wairau River	41°29′	174°02′	24.3	33.3	1.8	3.3	0.05	0.06
CS6	Collingwood	40°41′	172°40′	23.5	33.3	1.8	3.3	0.07	0.09*
NE1	Gisborne	38°34′	177°56′	28.0	33.3	1.4	2.0	0.06	0.08*
NE3	Porangahau	40°38′	176°22′	18.0	25.0	1.3	2.0	0.07	0.09*
	Mean			24.8	23.5	1.5	2.6	0.06	0.07
Paracorophium excavatum									
N2	Rangauna Harbour	35°01′	173°15′	24.8	8.3	1.4	3.0	0.04	0.05
N5	Whangarei Harbour	35°43′	174°19′	23.5	16.7	1.6	3.0	0.05	0.05
N6	Omaha Bay	36°35′	174°76′	25.6	8.3	1.3	2.0	0.01	0.04*
N8	Tauranga Harbour	37°40′	176°10′	28.8	16.7	1.6	3.0	0.05	0.06*
NE2	Napier	39°30′	176°48′	30.0	33.3	1.4	2.3	0.04	0.07*
CS2	Foxton[1]	40°18′	175°15′	13.0	30.8	1.7	2.3	0.04	0.04
CS3	Lake Onoke[1]	41°25′	175°09′	29.7	38.5	2.0	3.0	0.07	0.04*
CS5	Nelson	41°17′	173°14′	23.3	25.0	1.7	3.0	0.02	0.09*
CS6	Collingwood[1]	40°41′	172°40′	13.1	38.5	1.6	2.4	0.07	0.04*
SW1	Whanganui Inlet	40°34′	172°38′	26.8	25.0	1.7	2.7	0.05	0.08*
SW2	Little Wanganui	41°23′	172°03′	24.3	33.3	1.5	2.0	0.06	0.13*
SE1	Christchurch	43°32′	172°43′	24.4	25.0	1.7	2.7	0.08	0.09
SE2	Shag River[2]	45°29′	170°47′	21.5	53.8	2.2	3.0	0.11	0.09*
SE3	Karitane[2]	45°38′	170°38′	11.5	38.5	1.5	2.0	0.07	0.04*
SE4	Tomahawk Lagoon[3]	45°51′	170°32′	5.7	46.2	1.7	2.5	0.07	0.06
SE5	Brighton River[3]	45°57′	170°20′	22.8	38.5	1.6	2.4	0.09	0.07*
SE6	Lake Waihola[3]	46°01′	170°05′	5.0	30.8	1.3	2.0	0.09	0.08
SE7	Waikawa Harbour[4]	46°38′	169°07′	5.0	38.5	1.4	2.0	0.08	0.07
SE8	Catlins Lake[4]	46°28′	169°38′	22.5	30.8	1.8	2.8	0.08	0.06*
SE9	Fortrose[4]	46°34′	168°47′	22.6	53.8	1.8	2.1	0.08	0.07
CIS	Chatham Island	43°57′	176°33′	73.4	33.3	1.9	3.3	0.04	0.08*
	Mean			34.3	31.6	1.6	2.8	0.05	0.08*
Paracorophium brisbanensis									
N8	Tauranga Harbour	37°40′	176°10′	30.8	0.0	1.1	–	0.01	0.01

Table 2. *F*-statistics (F_{ST}, F_{IS}) averaged over 12 loci. Ordinary least squares regression coefficient (R^2) and Spearman's rank correlation index (R) of log$D$ and logkm used to assess isolation-by-distance (*I-D*) for different clusters of *Paracorophium lucasi* and *P. excavatum* ($^{ns}P > 0.05$, $^{*}P < 0.01$, $^{**}P < 0.001$). For populations included in each cluster refer to Fig. 3

	F_{ST}	F_{IS}	R^2	R
Paracorophium lucasi				
Overall	0.66	0.15	0.10**	0.16^{ns}
PL2, PL3	0.51	0.13	0.11*	0.07^{ns}
PL2	0.21	0.10	0.70**	0.88**
PL2 without N4	0.04	0.07	0.34^{ns}	0.70*
PL3	0.27	0.15	0.35*	0.32^{ns}
Paracorophium excavatum				
Overall	0.70	0.48	0.17**	0.41*
PE2	0.09	0.42	0.01^{ns}	0.49^{ns}
PE3	0.47	0.47	0.14**	0.41*
PE3 without CIS	0.20	0.45	0.06^{ns}	0.23^{ns}

Population genetic structure

Genetic differentiation (Wright's (1978) F_{ST}) averaged 0.66 among all *P. lucasi* populations and 0.70 among *P. excavatum*, indicating low levels of gene flow for both species. High levels of intrapopulation substructuring were indicated by high F_{IS} values for *P. excavatum* (mean = 0.48), but less so for *P. lucasi* (mean = 0.15) (Table 2).

The UPGMA (Fig. 3) showed that all *P. excavatum* populations formed a distinct cluster to *P. lucasi* ($D = 1.62$). *P. brisbanensis*, the only other Australasian *Paracorophium*, formed a sister group to *P. lucasi* ($D = 1.22$). The two most northern North Island *P. lucasi* populations (N1, N3) were fixed at the *PEP* and *ME* loci, and found to be genetically distinct ($D = 0.52$, cluster PL1). We identified two *P. lucasi* populations (cluster PL4: NE1, NE3) on the south-east coast of North Island fixed at *AO*, resulting in high genetic distance ($D = 0.41$) from the others. PL2 and PL3 included populations throughout North Island and Cook Strait and were fixed for alternate alleles at *AO, ME* or *PEP* when compared to populations from PL1 and PL4. In addition, PL2 and PL3 were separated genetically ($D = 0.15$) by an allelic frequency shift at *LDH-2*, with NW3 the only population with alleles common to both (Appendix). High levels of differentiation were also found among *P. excavatum* populations with three distinct clusters identified. The Cook Strait population CS5 (PE1) was morphologically identified as *P. excavatum*, but found to be genetically distinct from the northern populations in PE2 ($D = 0.51$). CS5 was also found to contain a few *P. lucasi* and *P. excavatum* individuals in low numbers (not included in analyses) identified using several diagnostic alleles (Appendix). PE3 was also genetically distinct with a genetic distance of 0.65 and grouped with Chatham Islands (CIS) ($D = 0.24$). CIS possessed common alleles with PE3 at three loci (*IDH-2, LDH-1, LDH-2*), and with PE2 at two loci (*AO, PEP*), which included populations from the north-east coast of North Island.

Isolation-by-distance analyses among all *P. lucasi* populations showed a significant relationship, but only 16% of the variance in allele frequencies could be explained by geographical separation (see Table 2). A similar significant relationship was found among all *P. excavatum* populations with 41% of the variance explained by geographical separation. The clusters from Figure 3 (corresponding to fixed allelic differences) were re-analysed to examine the degree of genetic correlation with geographical distance (Table 2). For *P. lucasi* we removed the four most divergent populations (clusters PL1, PL4), and analysed PL2 and PL3, which resulted in a small increase in the relationship. However, only 7% of the variance in allele frequencies was explained by geographical separation. Analysing PL2 separately revealed a highly significant relationship, but using only populations connected by ocean currents (i.e. without N4) showed limited support for this association. PL3 showed a significant relationship among populations that were geographically proximate for *P. lucasi*, and 32% of the variance in allele frequencies could be explained by geographical separation (Table 2). For *P. excavatum* we removed the genetically distinct CS5 population (PE1) and analysed the populations of PE2 which showed no genetic correlation with geographical distance. By contrast, PE3 revealed strong isolation-by-distance, but this relationship was not significant when CIS was removed from the analysis (Table 2).

DISCUSSION

We found fixed allelic differences corresponding to geographically isolated regions for both *P. lucasi* and *P. excavatum*. Genetic isolation of clusters PL3 and PL4 for *P. lucasi* and of PE2 and PE3 for *P. excavatum* (Fig. 3) suggest that the East Cape is a significant isolating boundary as a result of ocean currents moving from the north and south (Fig. 1). This potential barrier to gene flow may be particularly effective during upwelling events when potential dispersers may be lost to coastal systems either by transport offshore to the east or by being entrained in the permanent anticyclonic East Cape Eddy (Chiswell, 2000). Other isolated groups are not as clearly associated with a physical feature. The two east coast *P. lucasi* populations (PL4: NE1, NE3) were genetically distinct from

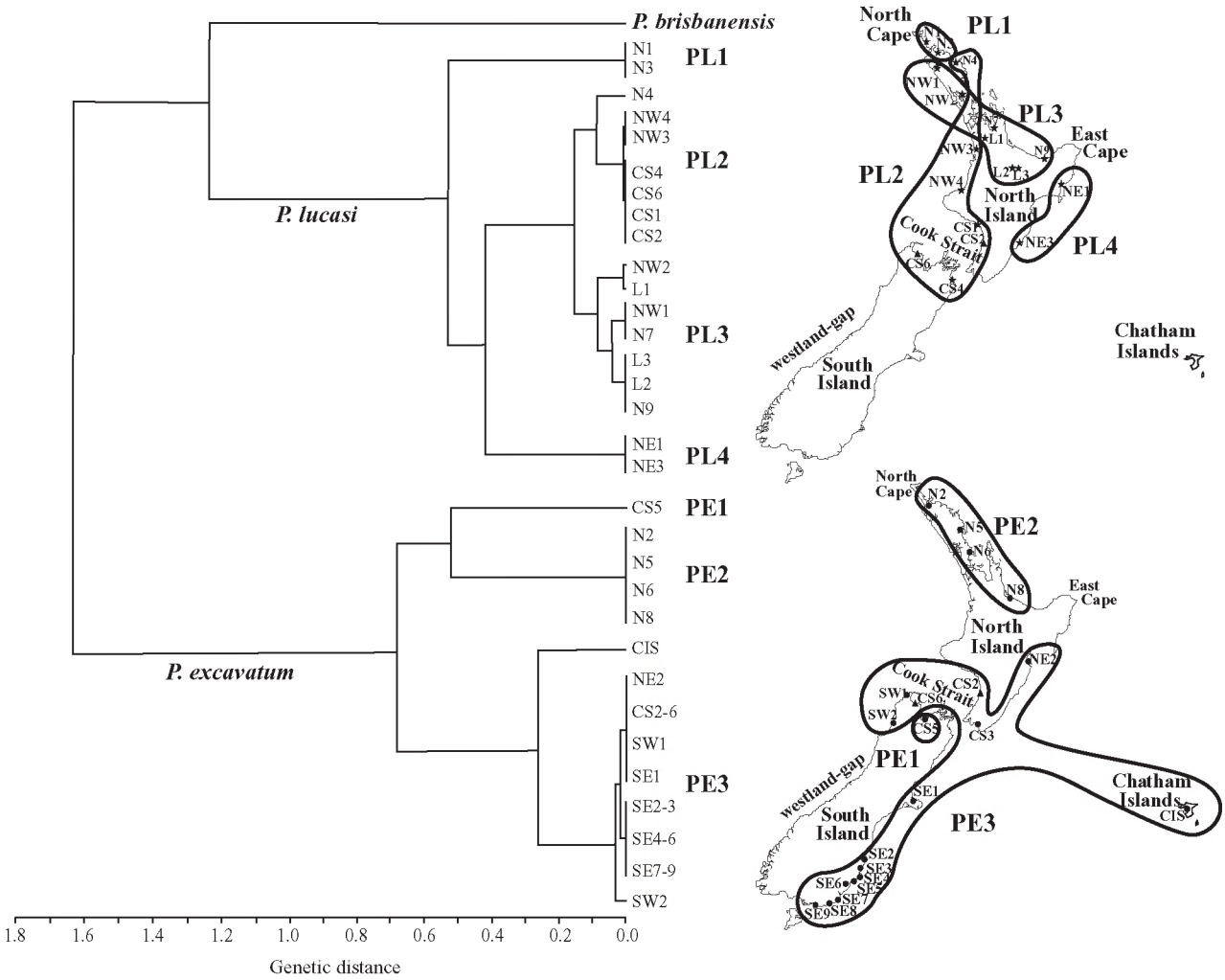

Figure 3. Phenetic analyses (UPGMA dendrogram) of genetic distance (Nei, 1978) between *Paracorophium brisbanensis* and the population clusters for *P. lucasi* (PL1–PL4) and *P. excavatum* (PE1–PE3). Clusters for each species are indicated on the adjacent maps. Pooled populations correspond to Table 1.

Cook Strait (CS) populations (Fig. 3). By contrast, *P. excavatum* populations in PE3 were genetically similar throughout this geographical region. A similar pattern was observed for the two most northern *P. lucasi* populations (PL1: N1, N3) which also showed moderate divergence relative to nearby populations, but this pattern was not observed for PE2 from the same region (Fig. 3).

For both *P. lucasi* and *P. excavatum* isolation-by-distance and high F_{ST} values suggest that populations are not in equilibrium (i.e. between gene flow and genetic drift, *sensu* Slatkin, 1993). This pattern of geographical differentiation would be conducive to allopatric or parapatric speciation processes (Templeton, 1980). For *P. lucasi* an island model (Slatkin, 1993) can be used to describe the populations that were connected by ocean currents (PL1, PL2 without N4 and PL4). However, when populations were on separate coastlines (PL2, PL3) they were best described by a stepping stone model (Slatkin, 1993). One possible explanation is that restricted dispersal opportunities via ocean currents are limiting gene flow among these populations. For *P. excavatum*, populations within all clusters corresponded to oceanic routes (Fig. 3). We found that a stepping stone model described PE3, but if Chatham Islands (CIS) were not included, the relationship was better described using Wright's Island model which was also found to best describe PE2 (Table 2; Fig. 3). Overall the genetic divergence and isolation-by-distance analyses show that *P. lucasi* is more divergent than *P. excavatum* over similar geographical distances.

Colonization via oceanic surface currents is possible, and this mode of transport has been implicated for the occurrence of a number of Chatham Islands taxa with affinities to both North and South Island (Knox, 1954; Craw, 1988; Emberson, 1995; Trewick, 2000b). For *P. lucasi*, it may be possible to explain the relationship of N4 to PL2, and for NW1 and NW2 to PL3 by overland dispersal (Fig. 3). Such overland transport may be possible by waterfowl which have been implicated as vectors for many small aquatic organisms (e.g. Rosine, 1956; Maguire, 1959).

Long-term extrinsic barriers to gene flow have been described for marine taxa along the Californian and Oregonian coastal regions (Dawson, 2001; Edmands, 2001; Wares, Gaines, & Cunningham, 2001). Similarly, in New Zealand Apte & Gardner (2002) have revealed that ocean currents and eddies are sufficient isolating barriers among greenshell mussel (*Perna canaliculus*) populations. Schnabel *et al.* (2000) also suggested that the patterns of ocean circulation around New Zealand were the most likely barriers to gene flow for *Paracorophium*. Because ocean currents are generally described as a mean (averaged over time and space) they indicate the dominant surface circulation. However, surface and coastal currents are highly variable and often affected by prevailing wind patterns (Roemmich & Sutton, 1998; Chiswell, 2000). Limited data on *Paracorphium* (Ford, Thrush & Probert, 1999; Stevens *et al.*, 2002) have shown that juveniles are abundant in the water column and are prone to being flushed out of bays and estuaries during tidal flows. Accordingly, dispersal among neighbouring populations may only be successful during times of rare or periodical climatic events, such as an ENSO (El Niño and southern oscillation), which results in intense rainfall and increased sea surface temperatures (Tomczak & Godfrey, 1994). Our data suggest that dispersal at greater distances is less likely where significant geographical barriers exist (e.g. North Cape and East Cape).

Speciation through allopatric isolation

We found no evidence to indicate that recent hybrids can occur between *P. lucasi* and *P. excavatum* from the two sympatric occurrences in the present study (CS2, CS6) and no hybrids were found among the three *Paracorophium* species at Tauranga Harbour (N8) (Stevens *et al.*, 2002). The single Cook Strait population from PE1 (CS5) was found to have allelic similarities at some loci to all three species. In addition, a few *P. lucasi* and *P. excavatum* individuals were found within the CS5 population; their sympatric occurrence suggests the presence of three reproductively isolated species. The degree of reproductive isolation among other clusters is uncertain in the absence of sympatry. However, for both *P. lucasi* and *P. excavatum* the levels of genetic divergence within each taxon are likely to correspond to sibling species that have diverged through allopatric isolation.

Similar levels of divergence have been found among morphologically distinct species of the talidrid amphipod *Orchestia* ($D = 0.51–0.59$) (Conceição, Bishop & Thorpe, 1998; De Matthaeis *et al.*, 2000) and are similar to reported divergence among congeneric species of other Crustacea (Hedgecock, Tracey & Nelson, 1982; Stewart, 1993; Thorpe & Solé-Cava, 1994). Such patterns may also explain, to a lesser extent, the genetic similarity of *P. excavatum* on CIS to southern populations from PE3 (Fig. 3). However, common alleles with the northern cluster PE2 may reveal common ancestry. The level of divergence between CIS and PE3 (Fig. 3) is consistent with Campbell's (1998) hypothesis that the Chatham Islands were totally submerged less than 4 Mya. The divergence of one Cook Strait population (CS5) may suggest an older coalescence in the Miocene. One possibility is that this population was isolated in a lake prior to the separation of North and South Island, but now has access to the coast due to the intrusion of seawater in the strait (Fig. 1). The degree of divergence among the sibling species for *Paracorophium* suggests that these groups were isolated well before the Pleistocene glaciations, and are more likely to have origins in the Pliocene or Miocene.

An increased rate of speciation followed by a high rate of extinction has been hypothesized for New Zealand terrestrial biota during the Oligocene (Cooper & Cooper, 1995). For aquatic taxa, we suggest that an increase in land surface during the Miocene isolated east and west coastal regions, leading to many populations becoming extinct or locally restricted. Subsequent range expansion/overlap may have occurred during the Pliocene when more frequent east–west exchange among populations would have been possible (Fig. 1). During the Pliocene, tectonic changes altered New Zealand from a collection of islands to the two main islands present today (Fleming, 1979; Craw, 1988; Cooper & Cooper, 1995; Stevens *et al.*, 1995; Campbell, 1998). It is apparent that the Pleistocene glaciations, sea-level changes and Pliocene landmass alterations were agents for divergence in several New Zealand terrestrial taxa (Craw, 1988; Trewick & Wallis, 2001 and references therein). For *Paracorophium*, we suggest that the presence of ephemeral islands throughout the Pliocene for the New Zealand landmass may have allowed for more frequent aquatic dispersal. Substantial barriers to aquatic east–west dispersal may have arisen towards the beginning of the Pleistocene (*c.* 2 Mya) once the present-day New Zealand landmass formed. However, some some east–west dispersal may have been possible during interglacial

periods due to an increase in sea-level (Fleming, 1979; Stevens *et al.*, 1995).

The advance and retreat of glaciers and fluctuating sea-level has also had a considerable influence on the distribution, abundance and diversity of taxa in New Zealand (e.g. Fleming, 1979; Craw, 1988; Wardle, 1991). Much of the west coast was covered by sea-ice during the last glacial maximum (*c.* 17 Kya) (Stevens *et al.*, 1995) (Fig. 1), and the inability of poorly dispersing organisms to recolonize such denuded regions is especially evident (Main, 1989; McDowall, 1997). The westland-gap constitutes an area of the west coast of South Island that lacks beech forest between 42°30′ and 43°30′S (Wardle, 1991). *Paracorophium* was absent from the westland-gap (see Fig. 2) and may provide support for the effects of Pleistocene climate conditions. Re-colonization via oceanic dispersal to the south appears unlikely during the present interglacial with the prevailing ocean current flowing northward along this coastline (Fig. 1). However, we found no evidence to suggest that divergence was promoted over the last 2 Myr as a consequence of the Pleistocene climatic fluctuations.

CONCLUSION

We suggest that *P. lucasi* and *P. excavatum* are species complexes consisting of genetically distinct sibling species. Such genetic patterns are likely to reflect geological isolation that has occurred since the Miocene. In addition, climatic shifts throughout the Pleistocene may have restricted the distribution of *Paracorophium*, particularly in the westland-gap. With no larval stage, dispersal opportunities may be greatly reduced where geographical barriers are concerned. In particular, geological formations and the patterns of present-day ocean currents may be sufficient to isolate populations. With limited gene flow among biogeographical regions throughout New Zealand the apparent fragmentation of an ancestral gene pool suggests that allopatric speciation has played an important role in the origin of taxon diversity for New Zealand corophiid amphipods.

ACKNOWLEDGEMENTS

We thank C. E. C. Gemmill, J. D. S. Witt, S. A. Trewick and an anonymous reviewer for comments on the manuscript, and M. A. Chapman, N. Loussert, P. Willmann-Huerner, K. E. Schnabel and J. L. Laboyrie for assistance in the field. This study was funded in part by a grant from the New Zealand Lotteries Board to I. D. H. and a University of Waikato doctoral scholarship to M. I. S.

REFERENCES

Apte S, Gardner JPA. 2002. Population genetic subdivision in the New Zealand greenshell mussel (*Perna canaliculus*) inferred from single-strand conformation polymorphism analysis of mitochondrial DNA. *Molecular Ecology* **11:** 1617–1628.

Avise JC. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation. *Oikos* **63:** 62–76.

Campbell HJ. 1998. Fauna and flora of the Chatham Islands: less than 4m.y. old? Geology and Genes. *Geological Society of New Zealand, Misc. Publ.* **97:** 15–16.

Chapman MA. 2002. Australasian species of *Paracorophium* (Crustacea, Amphipoda): the separate identities of *P. excavatum* (Thomson, 1884) and *P. brisbanensis* sp.nov. *Journal of the Royal Society of New Zealand* **32:** 203–228.

Chapman MA, Hogg ID, Schnabel KE, Stevens MI. 2002. Synonymy of the New Zealand corophiid amphipod genus, *Chaetocorophium* Karaman, 1979, with *Paracorophium* Stebbing, 1899: morphological and genetic evidence. *Journal of the Royal Society of New Zealand* **32:** 229–241.

Chiswell SM. 2000. The Wairarapa coastal current. *New Zealand Journal of Marine and Freshwater Research* **34:** 303–315.

Conceição MB, Bishop JDD, Thorpe JP. 1998. Genetic relationships between ecologically divergent species of talitrid amphipod (Crustacea). *Marine Ecology Progress Series* **165:** 225–233.

Cooper A, Cooper RA. 1995. The Oligocene bottleneck and New Zealand biota: genetic record of a past environmental crisis. *Proceedings of the Royal Society of London B* **261:** 293–302.

Cooper RA, Millener PR. 1993. The New Zealand biota: historical background and new research. *Trends in Ecology and Evolution* **8:** 429–433.

Craw R. 1988. Continuing the synthesis between panbiogeography, phylogenetic systematics and geology as illustrated by empirical studies on the biogeography of New Zealand and the Chatham Islands. *Systematic Zoology* **37:** 291–310.

Dawson MN. 2001. Phylogeography in coastal marine animals: a solution from California? *Journal of Biogeography* **28:** 723–736.

De Matthaeis E, Davolos D, Cobolli M, Ketmailer V. 2000. Isolation by distance in equilibrium and nonequilibrium populations of four Talidrid species in the Mediterranean Sea. *Evolution* **54:** 1606–1613.

Edmands S. 2001. Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. *Molecular Ecology* **10:** 1743–1750.

Emberson RM. 1995. The Chathams Islands beetle fauna and the age of separation of the Chatham Islands from New Zealand. *New Zealand Entomology* **18:** 1–7.

Felsenstein J. 1976. The theoretical population genetics of variable selection and migration. *Annual Review of Genetics* **10:** 253–280.

Fleming CA. 1979. *The geological history of New Zealand and its life.* Auckland: Auckland University Press.

Ford RB, Thrush SF, Probert PK. 1999. Macrobenthic colonisation of disturbances on an intertidal sandflat: the influence of season and buried algae. *Marine Ecology Progress Series* **120:** 163–174.

Hebert PDN, Beaton MJ. 1993. *Methodologies for allozyme analysis using cellulose acetate electrophoresis.* Beaumont, Texas: Helena Laboratories.

Hedgecock D, Tracey ML, Nelson K. 1982. Evolutionary divergence and speciation. In: Bliss DE, ed. *The biology of Crustacea*, Vol. 2. New York: Academic Press, 339–347.

Hellberg ME. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution* **50:** 1167–1175.

Kimura M. 1953. 'Stepping Stone' model of population. *Annual Report National Institute of Genetics (Japan)* **3:** 62–63.

Kimura M, Weiss GH. 1964. The stepping stone model of population structures and the decrease of genetic correlation with distance. *Genetics* **49:** 561–576.

Knowlton N, Weigt LA, Solórzano LA, Mills DK, Bermingham E. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* **260:** 1629–1632.

Knox GA. 1954. The intertidal flora and fauna of the Chatham Islands. *Nature* **174:** 871–873.

Maguire B. 1959. Passive overland transport of small aquatic organisms. *Ecology* **40:** 312.

Main MR. 1989. Distribution and post-glacial dispersal of freshwater fishes in South Westland, New Zealand. *Journal of the Royal Society of New Zealand* **19:** 161–169.

Mayr E. 1954. Change of genetic environment and evolution. In: Huxley J, Ford EB, eds. *Evolution as a process*. London: Allen & Unwin, 157–180.

McDowall RM. 1997. Indigenous vegetation type and the distribution of shortjawed kokopu, *Galaxias postvectis* (Teleostei, Galaxiidae), in New Zealand. *New Zealand Journal of Zoology* **24:** 243–255.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 583–590.

Parker PG, Snow A, Schug MD, Booton GC, Fuerst PA. 1998. What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* **79:** 361–382.

Pole M. 1989. Early Miocene floras from Central Otago, New Zealand. *Journal of the Royal Society of New Zealand* **19:** 121–125.

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* **43:** 223–225.

Richardson BJ, Baverstock PR, Adams M. 1986. *Allozyme electrophoresis: a handbook of animal systematics and population studies*. New York: Academic Press.

Roemmich D, Sutton P. 1998. The mean and variability of ocean circulation past northern New Zealand: determining the representativeness of hydrographic climatologies. *Journal of Geophysical Research* **103:** 13041–13054.

Rosine W. 1956. On the transport of the common amphipod, *Hyalella azteca*, in South Dakota by the mallard duck. *Proceedings of the South Dakota Academy of Sciences* **35:** 203.

Schnabel KE, Hogg ID, Chapman MA. 2000. Population genetic structure of two New Zealand corophiid amphipods and the presence of morphologically cryptic species: implications for the conservation of diversity. *New Zealand Journal of Marine and Freshwater Research* **34:** 637–644.

Slatkin M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47:** 264–279.

Sneath PHA, Sokal RR. 1973. *Numerical taxonomy*. San Francisco: W. H. Freeman.

Stevens MI, Hogg ID, Chapman MA. 2002. Corophiid amphipods in Tauranga Harbour, New Zealand: evidence of an Australian crustacean invader. *Hydrobiologia* **474:** 147–154.

Stevens G, McGlone M, McCulloch B. 1995. *Prehistoric New Zealand*. Auckland: Reed Publishing.

Stewart BA. 1993. The use of protein electrophoresis for determining species boundaries in amphipods. *Crustaceana* **65:** 265–277.

Swofford DL, Selander RB. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72:** 281–283.

Taylor DJ, Finston TL, Hebert PDN. 1998. Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. *Evolution* **52:** 1648–1670.

Templeton AR. 1980. Modes of speciation and inferences based on genetic distances. *Evolution* **34:** 719–729.

Thorpe JP, Solé-Cava AM. 1994. The use of allozyme electrophoresis in invertebrate systematics. *Zoologica Scripta* **23:** 3–18.

Tomczak M, Godfrey JS. 1994. *Regional oceanography: an introduction*. London: Elsevier.

Trewick SA. 2000a. Mitochondrial DNA sequences support allozyme evidence for cryptic radiation of New Zealand Peripatoides (Onychophora). *Molecular Ecology* **9:** 269–281.

Trewick SA. 2000b. Molecular evidence for dispersal rather than vicariance as the origin of flightless insect species on the Chatham Islands, New Zealand. *Journal of Biogeography* **27:** 1189–1200.

Trewick SA, Wallis GP. 2001. Bridging the 'beech-gap': New Zealand invertebrate phylogeography implicates Pleistocene glaciation and Pliocene isolation. *Evolution* **55:** 2170–2180.

Väinölä R. 1995. Origin and recent endemic divergence of a Caspian *Mysis* species flock with affinities to the 'Glacial Relict' crustaceans in boreal lakes. *Evolution* **49:** 1215–1223.

Wallis GP, Bland J, Judge KF, Waters JM, Berra TM. 2001. Genetic diversity in New Zealand *Galaxias vulgaris* sensu lato (Teleostei: Osmeriformes: Galaxiidae): a test of a biogeographic hypothesis. *Journal of Biogeography* **28:** 59–68.

Wardle P. 1991. *Vegetation of New Zealand*. Cambridge: Cambridge University Press..

Wares JP, Gaines SD, Cunningham CW. 2001. A comparative study of asymmetric migration events across a marine biogeographic boundary. *Evolution* **55:** 295–306.

Wright S. 1943. Isolation by distance. *Genetics* **28:** 139–156.

Wright S. 1978. *Evolution and the genetics of populations*, Vol. 4. Variability within and among natural populations. Chicago: University of Chicago Press.

APPENDIX

Allele frequencies at 12 loci for all populations of *Paracorophium lucasi*, *P. excavatum* (* = pooled populations corresponding to Fig. 3) and *P. brisbanensis* (PB). Locations refer to those used in the text, figures, and tables. Alleles are labelled according to their relative mobility (see text).

	Paracorophium lucasi																	
	N1	N3	N4	N7	N9	NW1	NW2	NW3	NW4	L1	L2	L3	CS1	CS2	CS4	CS6	NE1	NE2
n	26	29	26	26	27	23	17	41	27	37	31	26	23	11	26	24	28	18
AO																		
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
B																	1.00	1.00
C																		
D																		
E																		
F																		
PEP																		
A	1.00	1.00									0.13					0.04	0.07	
B																		
C			1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.87	1.00	1.00	1.00	1.00	0.96	0.93	1.00
D																		
E																		
F																		
IDH-1																		
A																		
B					0.02													
C	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
D																		
IDH-2																		
A																		
B								0.04	0.02						0.06	0.02		
C	1.00	1.00	1.00	0.98	1.00	1.00	1.00	0.92	0.96	1.00	1.00	1.00	0.94	0.96	0.87	0.94	0.70	0.64
D				0.02				0.04	0.02				0.04		0.08	0.04	0.30	0.36
E													0.02	0.05				
LDH-1																		
A																		
B	1.00	1.00	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C					0.04													
D																		
E																		
F																		
LDH-2																		
A				1.00	1.00	1.00	1.00	0.01		1.00	1.00	1.00						
B	1.00	1.00	1.00					0.98	1.00				1.00	1.00	1.00	1.00	1.00	1.00
C								0.01										
G3PDH																		
A																		
B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C																		
D																		
6PGDH																		
A				0.02														
B	1.00	1.00	1.00	0.98	0.87	1.00	1.00	1.00	0.94	1.00	1.00	1.00	0.91	0.91	1.00	0.98		
C																0.02	0.21	0.50
D					0.13				0.06				0.09	0.09			0.79	0.50
E																		

Paracorophium excavatum

N2 26	N5 24	N6 26	N8 32	NE2 30	CS* 57	CS5 25	SW1 28	SW2 27	SE1 27	SE2-3* 34	SE4-6* 35	SE7-9* 51	CIS 80	PB 38
				1.00	1.00		1.00	1.00	1.00	1.00	1.00	1.00		
1.00	1.00	1.00	1.00										1.00	
														1.00
						1.00								
														1.00
						0.02								
						0.98								
1.00	1.00	1.00	1.00										1.00	
				1.00	1.00		0.98	1.00	1.00	0.98	1.00	1.00		
							0.02			0.02				
						0.35							0.04	
1.00	1.00	1.00	1.00	1.00	1.00	0.52	1.00	1.00	1.00	1.00	1.00	1.00	0.96	
						0.13								
														1.00
					0.01	0.08	0.07	0.61	0.02			0.01	0.01	
		0.04		1.00	0.98	0.92	0.93	0.39	0.98	1.00	1.00	0.98	0.99	
0.98	0.94	0.96	0.98		0.01							0.01		
0.02	0.06		0.02											1.00
			0.00											
1.00	1.00	1.00	1.00						0.07	0.05	0.03	0.05	0.07	
					0.01	1.00							0.02	1.00
									0.05	0.03				
				0.75	0.92		0.75	0.59	0.52	0.68	0.77	0.89	0.91	
				0.23	0.07		0.25	0.41	0.36	0.23	0.21	0.06		
				0.02										
1.00	1.00	1.00	1.00			1.00			0.02				0.03	
													0.04	1.00
				1.00	1.00		1.00	1.00	0.98	1.00	1.00	1.00	0.93	
														1.00
0.98	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	0.04													
0.02														
					0.06	0.05							0.04	
1.00	1.00	1.00	1.00	0.88	0.82	0.95	0.98	0.98	1.00	1.00	0.97	0.89	0.90	
				0.12	0.12		0.02	0.02			0.03	0.11	0.03	1.00
					0.01								0.03	

APPENDIX *Continued*

	Paracorophium lucasi																	
	N1	N3	N4	N7	N9	NW1	NW2	NW3	NW4	L1	L2	L3	CS1	CS2	CS4	CS6	NE1	NE2
ARK																		
A																		
B					0.19								0.05					
C	1.00	1.00	1.00	1.00	0.81	1.00	0.97	1.00	1.00	1.00	1.00	1.00	0.96	1.00	1.00	0.98	0.98	1.00
D							0.03									0.02	0.02	
E																		
MPI																		
A																		
B			0.07		0.05			0.03					0.08	0.05	0.02	0.04	1.00	1.00
C				0.02												0.02		
D	0.21	0.15	0.76	0.65	0.86	0.42	0.50	0.87	0.70	0.42	0.95	0.96	0.69	0.96	0.91	0.72		
E	0.74	0.85	0.17	0.33	0.10	0.54	0.50	0.10	0.30	0.58	0.05	0.04	0.22		0.02	0.20		
F	0.05					0.04									0.05	0.02		
PGM																		
A				0.02	0.52			0.24	0.02		0.47	0.56	0.11	0.09	0.04	0.09		
B															0.02			
C	0.25	0.10	0.08	0.98	0.48	1.00	1.00	0.73	0.98	1.00	0.53	0.44	0.89	0.86	0.94	0.91	1.00	1.00
D																		
E	0.67	0.69	0.88					0.03						0.05				
F	0.04	0.17	0.04															
G	0.04	0.04																
ME																		
A			1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
B																		
C	1.00	1.00																

Paracorophium excavatum

N2	N5	N6	N8	NE2	CS*	CS5	SW1	SW2	SE1	SE2-3*	SE4-6*	SE7-9*	CIS	PB
			0.05											
1.00	1.00	1.00	0.75	1.00	1.00		1.00	0.98	0.83	0.56	0.39	0.77	1.00	
			0.21			1.00		0.02	0.17	0.41	0.61	0.24		1.00
										0.03				
	0.04													
	0.04					0.07					0.03	0.01		0.04
0.30		0.26		0.87	0.86	0.10	0.98	0.71	0.85	0.32	0.67	0.39	0.44	0.96
0.63	0.82	0.74	0.98	0.13	0.14	0.77	0.02	0.29	0.15	0.66	0.30	0.59	0.48	
0.07	0.11		0.02			0.07				0.02		0.01	0.09	
											0.02			
0.02	0.02	0.02	0.13	0.08	0.07		0.02		0.04	0.02	0.02	0.02		
			0.02											
0.98	0.96	0.98	0.86	0.92	0.92	1.00	0.71	0.93	0.96	0.94	0.95	0.97	0.97	
	0.02				0.01		0.25	0.07		0.02	0.02	0.01	0.03	1.00
							0.02			0.03				
														1.00
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	