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Population genetic structures of two New Zealand stream insects: *Archichauliodes diversus* (Megaloptera) and *Coloburiscus humeralis* (Ephemeroptera)

IAN D. HOGG
PIA WILLMANN-HUERNER
MARK I. STEVENS
Centre for Biodiversity and Ecology Research
Department of Biological Sciences
University of Waikato
Private Bag 3105
Hamilton, New Zealand
email: hogg@waikato.ac.nz

Abstract We examined the population genetic structure (allozyme variation) of two New Zealand aquatic insects: *Archichauliodes diversus* (Walker) (Megaloptera) and *Coloburiscus humeralis* (Walker) (Ephemeroptera); and tested the hypothesis that species with limited adult dispersal abilities would show high levels of genetic differentiation among hydrologically isolated habitats. Both species were characterised by low levels of within-population genetic variability (e.g., H_{exp} = 0.06 and 0.04 for *A. diversus* and *C. humeralis*, respectively). However, genetic differentiation among sites was considerably greater for *A. diversus* relative to *C. humeralis* (F_{ST} = 0.57 and 0.03, respectively). *A. diversus* consisted of two genetically distinct groups in the North Island (I = 0.96), and a third group from the South Island (I = 0.67). By contrast, allelic differences among *C. humeralis* were minimal (I > 0.99, in all cases), and appeared unrelated to geographic proximity or habitat type. We suggest that a combination of historic range changes and reproductive mechanisms, as well as contemporary dispersal capabilities, may be responsible for the observed population genetic structures of *A. diversus* and *C. humeralis*. Accordingly, generalisations based on present-day dispersal abilities alone may be misleading particularly for island habitats such as New Zealand where gene pools may already be restricted.

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INTRODUCTION

Identifying patterns of genetic variability within species may be critical from a conservation perspective as it is widely contended that maintaining such diversity will facilitate both population and ecosystem-level responses to environmental change. However, concern is often targeted towards overall levels of variability, while relatively little consideration is given to how the variability may be partitioned among populations (i.e., genetic differentiation). For freshwater systems, which often consist of naturally fragmented and isolated habitats, this may be particularly relevant (Hogg et al. 1998a). Specifically, interhabitat dispersal (and hence gene flow) may be restricted for some species resulting in limited within-population variability—an attribute potentially compounded by the increasing anthropogenic fragmentation of aquatic habitats.

The extent to which populations of a species become genetically differentiated will be determined in part by: (1) the biological characteristics of the species (e.g., dispersal abilities, geographic distribution); (2) selective pressures that have been imposed on local populations (Slatkin 1985); and (3) random genetic drift (Jackson & Resh 1992). However, present-day patterns of population structure are also likely to have been influenced by the history of a particular species (e.g., genetic bottlenecks, breeding systems). Accordingly, genetic assessments also need to consider the species' past (e.g., Bossart & Prowell 1998; Driscoll 1998). Unfortunately, it is often difficult to separate the relative importance of present-day events relative to the past history of a species.

New Zealand may provide an ideal opportunity to test hypotheses related to patterns of species and genetic diversity. Specifically, it has a small land mass (270 000 km^2), wide latitudinal range (c. 12°),

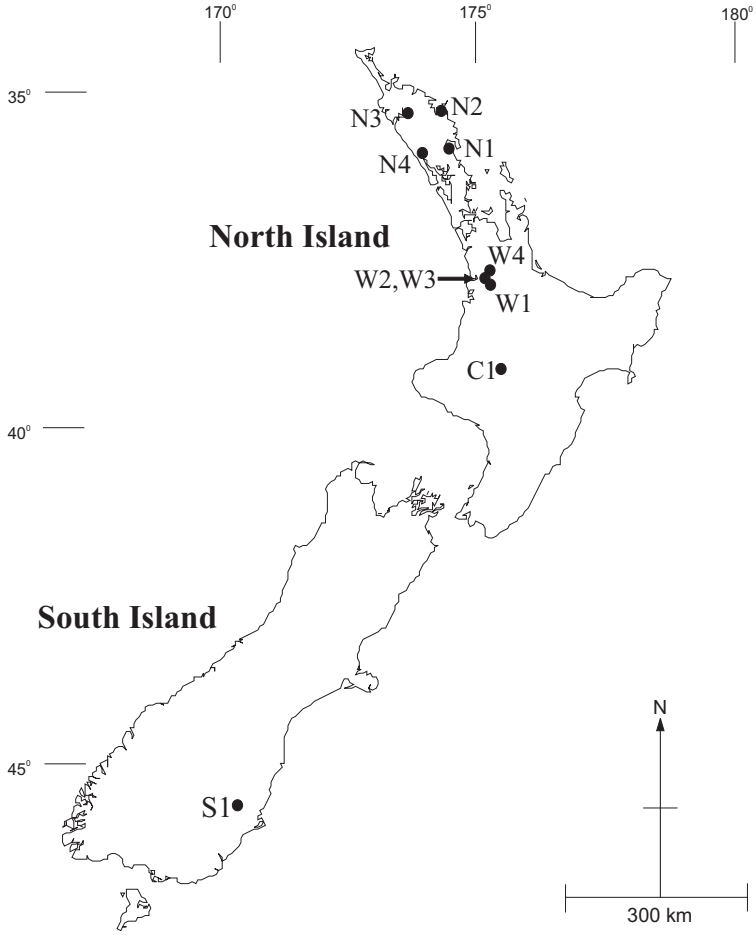

Fig. 1 Study area, showing locations of sampling sites from Northland (N1–4), Waikato (W1–4), central North Island (C1) and South Island (S1), New Zealand.

diverse topology, and well documented geologic history (Suggate et al. 1978). Furthermore, New Zealand has been isolated in the south Pacific Ocean since separation from Australia some 70 million years ago (Gibbs 1989). Accordingly, stream insects with potentially limited dispersal abilities inhabiting New Zealand's highly fragmented landscape may serve as an ideal model for comparative studies assessing the relative contributions of present and past events.

Before beginning this study, little was known of New Zealand's stream insects, although a single study has since been published (Smith & Collier 2001). Comparatively more is known of the Australian fauna (e.g., Schmidt et al. 1995; Bunn & Hughes 1997; Hughes et al. 1998, 1999, 2000). These studies have generally suggested limited levels of genetic differentiation among populations, although taxonomic coverage still remains somewhat restricted (e.g., Ephemeroptera, Trichoptera, Hemiptera). By contrast, studies of North American taxa have suggested considerably greater levels of differentiation even at smaller spatial scales (e.g., Jackson & Resh 1992; Hughes et al. 1999).

To enhance our knowledge of the New Zealand fauna, we evaluated the population genetic structures of two widespread and endemic stream insects, *Archichauliodes diversus* (Walker) (Megaloptera) and *Coloburiscus humeralis* (Walker) (Ephemeroptera). By selecting species with limited dispersal capabilities from discrete habitats (i.e., different streams and catchments), we reasoned that present day gene flow should be minimal, and that populations should show significant genetic differentiation among locations. As we show, results for *C. humeralis* are contrary to these expectations and require an alternative explanation.

METHODS

Study species

Archichauliodes diversus, the only New Zealand megalopteran, has a predacious and almost entirely aquatic (larval) existence (Devonport & Winterbourn 1976; Harding & Winterbourn 1993). Prolonged larval development (c. 18 months–3 years), long and asynchronous emergence periods (Hamilton 1940; Devonport & Winterbourn 1976), and obligate diapause of late-laid eggs (Edwards 1986) have been suggested. Adults are non-feeding and live from 6–10 days (Hamilton 1940). Their flight is slow and clumsy (Edwards 1986) and individuals manage only short distances over streams at very low height because of their heavy bodies and large, awkward wings (5–8 cm wing span).

The mayfly, *Coloburiscus humeralis* (Oligoneuriidae) exhibits a filter-feeding, aquatic larval stage (Tan 1961), with life history ranging from univoltine in the warmer, northern warmer regions, to 18–27 months in lake outlet streams and cool, forested streams (Tan 1961; Norrie 1969). Although no specific information is available for adult *C. humeralis*, mayflies are known to be short-lived as adults (e.g., 10–30 min in the burrowing mayfly *Dolania americana*) (Sweeney & Funk 1991). However, some ovoviviparous species have been reported to live up to 14 days (Brittain 1982).

Study sites

Ten sampling sites were selected to examine the genetic structure of *A. diversus* and *C. humeralis* (Fig. 1). Four sites within the Waikato region (W1–W4) were in relatively close proximity to each other, separated by 1.5–18 km. The Northland sites (N1–N4) were separated by larger distances (c. 50–75 km) and were c. 200–300 km away from the Waikato sites. The maximum distance occurred between the uppermost Northland site (N3) and the South Island site (S1) that were separated by more than 1200 km.

The habitats of both study organisms consisted of low order (2nd and 3rd order) freshwater streams. Six of the 10 study streams flowed through native podocarp-hardwood forest. For the remaining sites, one site (W1) was within exotic *Pinus radiata* forest, Site C1 was within mixed (exotic and secondary native forest) and Sites W2 and S1 occurred within agricultural land vegetated with clover and pasture grasses. Substrates of all streams were predominantly gravels and cobbles, although some silt and clay were also present at the exotic forest and pasture sites.

Collection of samples

Mature (late instar) larvae of *A. diversus* and *C. humeralis* were collected between February and October 1998. Larvae were caught with dipnets (250 µm) by kick sampling and by turning and washing large stones to dislodge individual animals. The contents of the nets were then placed into sorting trays where the study organisms were removed and counted. Individuals were placed in plastic sampling jars containing stream water and transported back to the laboratory on ice. When fieldwork extended over several days, individual animals were frozen immediately in liquid nitrogen in the field. In the laboratory, the samples were stored at –75°C in individual 1.5 ml microcentrifuge tubes until needed for electrophoretic analysis.

Genetic analysis

All study organisms were morphologically confirmed using Winterbourn & Gregson (1989). To evaluate genetic differences within and among populations, we used cellulose acetate electrophoresis following techniques described by Hebert & Beaton (1993) and Larose & Hogg (1998).

Initially, enzyme systems showed very poor resolution for *A. diversus* with smudged bands complicating reliable identification of allelic variation. Since it was suspected that the gut contents of this predatory organism could be confounding band resolution, trial runs were conducted using different body parts (e.g., head, thorax, abdomen). Although banding patterns were the same, best results were obtained when only the heads were used. Accordingly, individual heads were homogenised in 20–30 µl of a grinding buffer containing distilled water (100 ml), NADP (10 mg), β-mercaptoethanol (100 µl), and detergent Tween 80 (100 µl) (Richardson et al. 1986). Samples were then centrifuged at 10 000 rpm for 5 s and 10 µl of each sample was immediately transferred into the sample wells for electrophoresis.

For *C. humeralis* the entire body was used for analyses as trial runs involving different body parts showed no differences in band resolution or allozyme variation. Individual larvae were homogenised in 30 µl of the grinding solution and centrifuged at 10 000 rpm for 15 s before extracting the supernatant.

After preliminary screening, the following enzymes were found to have scorable activity for both species: aldehyde oxidase (AO: EC 1.2.3.1), isocitrate dehydrogenase (IDH: EC 1.1.1.42), malate

dehydrogenase (MDH: EC 1.1.1.37), phosphoglucomutase (PGM: EC 2.7.5.1), and peptidase (PEP: EC 3.4.11/13). Additional enzymes were also run for each species; lactate dehydrogenase (LDH: EC 1.1.1.27), and phosphogluconate dehydrogenase (PGDH: EC 1.1.1.44) for *A. diversus*, and arginine phosphokinase (APK: EC 2.7.3.3), malic enzyme (ME: EC 1.1.1.40), and phosphoglucose isomerase (PGI: EC 5.3.1.9), for *C. humeralis*. As several enzymes were scorable for more than one locus, a total of 15 presumptive gene loci were analysed for *A. diversus* and 13 for *C. humeralis*.

The fastest alleles (i.e., those migrating closest to the anode) were designated as "A", and sequentially slower migrating alleles were designated "B", "C", "D", etc. Enzymes encoded by more than one locus were assigned numbers in order of increasing electrophoretic mobility (i.e., *IDH–2* was closer to the anode than *IDH–1*). To minimise potential scoring errors at least one individual from each run was analysed on subsequent runs to verify allelic designations. We further verified presumed allelic homologies by running individuals from each of the locations on the same gel.

Data analysis

For both species, we recorded genotype frequencies for each locus at each site. From these data we calculated: (1) allele frequencies; (2) mean number of alleles per locus; (3) percentage of polymorphic loci (loci were considered monomorphic if the frequency of the most common allele was >95%); (4) expected and observed heterozygosity (H_{exp} and H_{obs}); (5) Wright's (1978) F_{IS} and F_{ST}; and (6) Nei's (1972) genetic identity (I). Deviations of observed genotype frequencies from predictions of Hardy-Weinberg equilibrium for each locus were tested for all sites using Chi-square goodness-of-fit tests. A dendrogram was constructed using an unweighted pair-group method of analysis (UPGMA) to illustrate genetic differences among populations. F statistics for each locus were tested for significance using the formulas given by Waples (1987):

$\chi^2 = F_{IS}{}^2 N\,(k-1)$, d.f. $= k(k-1)/2$ and $\chi^2 = 2NF_{ST}\,(k-1)$, d.f. $= (k-1)(s-1)$

where N = total number of individuals, k = number of alleles at the locus, and s is the number of populations. All other calculations were performed using BIOSYS-1 (Swofford & Selander 1981).

Table 1 Allele frequencies at nine polymorphic allozyme loci for individuals of *Archichauliodes diversus* collected from 10 locations on the North Island and South Island of New Zealand. Site designations refer to Fig. 1.

Locus		Site									
		N1	N2	N3	N4	W1	W2	W3	W4	C1	S1
N	Allele	27	45	36	35	31	50	41	30	44	6
AO–1	A	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00
	B		0.01							0.01	
IDH–1	A										0.08
	B			0.01							0.92
	C	1.00	1.00	0.99	1.00	0.97	0.98	0.99	1.00	1.00	
	D					0.03	0.02	0.01			
IDH–2	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	B										1.00
LDH	A	0.94	0.79	0.78	0.77	0.89	0.93	0.92	0.82	0.77	0.08
	B	0.06	0.21	0.22	0.23	0.11	0.07	0.08	0.18	0.23	0.92
MDH	A	0.34	0.42	0.36	0.36	0.05		0.11	0.10	0.10	0.42
	B	0.06		0.02		0.92	0.97	0.85	0.80	0.80	0.58
	C	0.58	0.56	0.61	0.51	0.03	0.03	0.04	0.10	0.10	
	D	0.02	0.02	0.01	0.13						
PEP–1	A	1.00	0.99	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00
	B		0.01						0.08		
PEP–2	A			0.02		0.04	0.01				1.00
	B	0.95	0.97	0.96	1.00	0.96	0.98	1.00	0.94	0.95	
	C	0.05	0.03	0.02			0.01		0.06	0.05	
PGM	A	0.04	0.01								
	B	0.02	0.01		0.10	0.05	0.12	0.02	0.05	0.03	
	C	0.83	0.92	0.97	0.87	0.93	0.88	0.97	0.91	0.97	1.00
	D	0.11	0.06	0.03	0.03	0.02			0.03		
PGDH	A										1.00
	B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	

RESULTS

Genetic variability within populations

Of the 15 presumed loci examined for *A. diversus*, six showed no allelic variation and a further locus (*AO*), was considered monomorphic using the 95% criterion (frequency of the most common allele >95%). Two other loci (*IDH–2* and *PGDH*) showed fixed differences (i.e., non-shared alleles) between the North and South Islands. Thus, the remaining six loci (*IDH–1*, *LDH*, *MDH*, *PEP–1*, *PEP–2*, *PGM*) were polymorphic in at least one population of *A. diversus* (Table 1). Several geographic discontinuities in allele frequencies were found, separating the Northland (N1–N4) from the Waikato (W1–W4) and central North Island (C1) populations. For example, Allele D of *IDH–1* was present only in Waikato populations and Allele D of *MDH* only in Northland populations.

Similarly, *C. humeralis* showed no allelic variation at seven of the 13 loci examined, and one additional locus (*PEP–1*) was monomorphic using the 95% criterion. Five loci were polymorphic (*AO–1*, *AO–2*, *PEP–2*, *PGI*, and *PGM*) in at least one population of *C. humeralis* (Table 2). The southernmost site (S1) showed no allelic variation at 11 loci and was the lowest of all sites. One of the most northerly sites (N2), also showed limited allelic variation and was considered monomorphic (95% criterion) at all 13 loci. A discontinuity of Alleles A and C was detected at *PEP–2* which were present only at the four Waikato sites (W1–W4) and this locus showed no allelic variation for all other sites north and south of the Waikato (Table 2). For *C. humeralis*, alternative alleles were generally found at low frequencies (i.e., 0.10) suggesting a tendency towards fixation of alleles.

The average number of alleles per locus was low for both species, ranging from 1.2 to 1.5 for *A. diversus* (Table 3) and 1.2–1.6 for *C. humeralis* (Table 4) with an overall mean of 1.4 (±0.2), and 1.5 (±0.2), for *A. diversus* and *C. humeralis*, respectively. The percentage of polymorphic loci ranged from 13.3 to 33.3% (mean = 18.7%) for *A. diversus* and from 0 to 23.1% (mean = 15.4%) for *C. humeralis*.

Expected heterozygosity (H_{exp}) ranged between 0.03 and 0.07 (mean = 0.06) for the *A. diversus* populations, and 0.01 and 0.06 (mean = 0.04) for *C. humeralis* (Tables 3 and 4, respectively). Observed values (H_{obs}) were similar to expected values for both species for most sites and loci (Tables 3 and 4).

Allele frequencies deviated from Hardy-Weinberg equilibrium in only one instance for *A. diversus* (*MDH* at W1), and in two cases for *C. humeralis* (*PEP–2* at W4 and *PGI* at N3), and in all cases were the result of heterozygote deficiencies.

Table 2 Allele frequencies at six polymorphic allozyme loci for individuals of *Coloburiscus humeralis* collected from 10 locations on the North Island and South Island of New Zealand. Site designations refer to Fig. 1.

Locus		Site									
		N1	N2	N3	N4	W1	W2	W3	W4	C1	S1
N	Allele	49	43	40	41	48	19	60	70	45	21
AO–1	A	0.03	0.04	0.02	0.10	0.10	0.18	0.02	0.17	0.10	0.19
	B	0.97	0.96	0.98	0.90	0.90	0.82	0.98	0.83	0.89	0.81
	C									0.01	
AO–2	A	0.06	0.01	0.04	0.03		0.03		0.01	0.13	
	B	0.93	0.97	0.92	0.90	0.96	0.97	0.95	0.96	0.85	0.88
	C	0.01	0.02	0.04	0.07	0.04		0.05	0.03	0.02	0.12
PEP–1	A	0.02	0.01			0.04				0.03	
	B	0.98	0.99	1.00	1.00	0.96	1.00	0.98	1.00	0.96	1.00
	C							0.02		0.01	
PEP–2	A					0.05	0.08	0.03	0.11		
	B	1.00	1.00	1.00	1.00	0.93	0.92	0.96	0.88	1.00	1.00
	C					0.02		0.01	0.01		
PGI	A	0.01		0.02	0.02	0.01		0.03	0.03		
	B	0.92	1.00	0.94	0.93	0.99	0.97	0.97	0.96	0.96	1.00
	C	0.07		0.04	0.05		0.03		0.01	0.04	
PGM	A	0.01			0.01						
	B	0.95	0.99	0.96	0.99	1.00	0.97	0.94	0.96	0.98	1.00
	C	0.04	0.01	0.04			0.03	0.06	0.04	0.02	

Table 3 Measures of genetic variability (+SE) for *Archichauliodes diversus* collected from 10 locations on the North Island and South Island of New Zealand. Site designations refer to Fig. 1.

Site	Location	Mean no. of alleles/locus	% polymorphic loci	Observed heterozygosity	Expected heterozygosity
N1	Finlayson Brook	1.5 (0.2)	20.0	0.07 (0.03)	0.07 (0.03)
N2	Mokotuna River	1.5 (0.2)	20.0	0.07 (0.03)	0.07 (0.03)
N3	Manganuka River	1.5 (0.2)	13.3	0.06 (0.03)	0.07 (0.04)
N4	Waipoua River	1.3 (0.2)	20.0	0.06 (0.03)	0.07 (0.04)
W1	Te Puroa	1.5 (0.2)	20.0	0.03 (0.01)	0.04 (0.01)
W2	Whatawhata pasture	1.4 (0.2)	13.3	0.03 (0.01)	0.03 (0.01)
W3	Whatawhata forest	1.3 (0.2)	13.3	0.03 (0.02)	0.03 (0.03)
W4	Hakarimata	1.5 (0.2)	33.3	0.07 (0.03)	0.07 (0.03)
C1	Pureora	1.4 (0.2)	13.3	0.06 (0.03)	0.06 (0.03)
S1	Stony Creek	1.2 (0.1)	20.0	0.06 (0.03)	0.06 (0.04)

Table 4 Measures of genetic variability (+SE) for *Coloburiscus humeralis* collected from 10 locations on the North Island and South Island of New Zealand. Site designations refer to Fig. 1.

Site	Location	Mean no. of alleles/locus	% polymorphic loci	Observed heterozygosity	Expected heterozygosity
N1	Finlayson Brook	1.6 (0.2)	23.1	0.04 (0.01)	0.04 (0.02)
N2	Mokotuna River	1.4 (0.2)	0.0	0.01 (0.01)	0.01 (0.01)
N3	Manganuka River	1.5 (0.2)	15.4	0.02 (0.01)	0.03 (0.01)
N4	Waipoua River	1.5 (0.2)	23.1	0.04 (0.02)	0.04 (0.02)
W1	Te Puroa	1.5 (0.2)	15.4	0.04 (0.01)	0.04 (0.02)
W2	Whatawhata pasture	1.4 (0.1)	15.4	0.05 (0.03)	0.05 (0.02)
W3	Whatawhata forest	1.5 (0.2)	15.4	0.03 (0.01)	0.03 (0.01)
W4	Hakarimata	1.6 (0.2)	15.4	0.05 (0.02)	0.06 (0.03)
C1	Pureora	1.6 (0.2)	15.4	0.05 (0.02)	0.05 (0.02)
S1	Stony Creek	1.2 (0.1)	15.4	0.03 (0.02)	0.04 (0.02)

Table 5 Wright's (1978) F_{IS} and F_{ST} values for *Coloburiscus humeralis* and *Archichauliodes diversus* collected from 10 locations on the North Island and South Island of New Zealand. F_{ST} values for *Archichauliodes diversus* from the nine North Island locations are also shown.

	C. humeralis		*A. diversus*		
Locus	F_{IS}	F_{ST}	F_{IS}	F_{ST}	F_{ST} (North Island)
AO–1	0.03	0.04 $P < 0.001$	–0.01	0.01	0.01
AO–2	0.19 $P < 0.001$	0.03 $P < 0.001$			
IDH–1			–0.06	0.84 $P < 0.001$	0.02 $P < 0.05$
IDH–2				1.00 $P < 0.001$	
LDH			0.08	0.32 $P < 0.001$	0.03 $P < 0.01$
MDH			–0.02	0.44 $P < 0.001$	0.42 $P < 0.001$
PEP–1	0.13 $P < 0.01$	0.02 $P < 0.05$	–0.08	0.06 $P < 0.001$	0.06 $P < 0.001$
PEP–2	0.32 $P < 0.001$	0.05 $P < 0.001$	–0.04	0.76 $P < 0.001$	0.02
PGI	0.02	0.02 $P < 0.05$			
PGM	0.11	0.02 $P < 0.05$	–0.10	0.04 $P < 0.001$	0.03 $P < 0.001$
PGDH				1.00 $P < 0.001$	
Mean	0.12	0.03 $P < 0.05$	–0.01	0.57 $P < 0.001$	0.24 $P < 0.001$

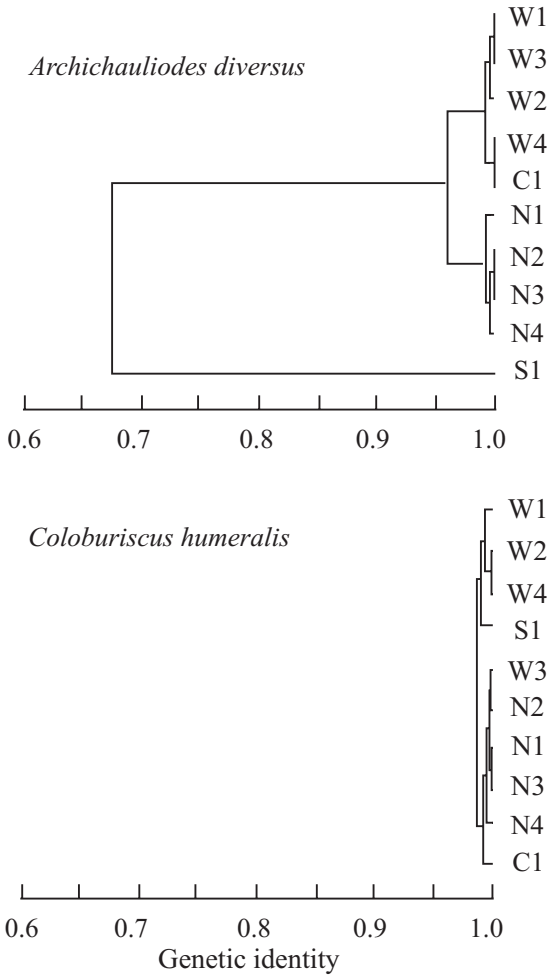

Fig. 2 Dendrogram for *Archichauliodes diversus* and *Coloburiscus humeralis* populations based on unweighted pair-group method of analysis (UPGMA) clustering of sites using Nei's (1972) genetic identity. Site designations correspond to Fig. 1.

Genetic differentiation among populations

For *A. diversus*, Wright's (1978) F_{ST} averaged across all loci and sites was 0.57 (Table 5), indicating "very great" (*sensu* Hartl & Clark 1997) levels of genetic differentiation. Removing the South Island individuals from the analysis still resulted in a mean F_{ST} value of 0.24 (Table 5), suggesting little or no present day gene flow among at least some of the North Island sites. Nei's (1972) genetic identity (*I*) for the 10 *A. diversus* populations ranged from 0.99 to 0.67. Analysis of identity values using UPGMA (Fig. 2), revealed three clusters: (1) the four Northland sites (N1–N4); (2) the four Waikato sites (W1–W4), including the central North Island site (C1); and (3) the South Island site (S1). Genetic differences for those populations within each geographical area (cluster) were very small ($I > 0.99$ in all cases). Genetic identity (*I*) for the four Northland sites relative to the Waikato and central North Island sites was 0.96. However, South Island individuals were very different with a genetic identity of 0.67 relative to all other sites reflecting fixed allelic differences at two loci (*IDH–2* and *PGDH*).

For *C. humeralis*, Wright's (1978) F_{ST} averaged across all loci and sites was 0.03 (Table 5), indicating very low levels of genetic differentiation and potentially high gene flow among populations. By contrast, mean F_{IS} was 0.12 (Table 5), suggesting non-random mating within locations, and/or other violations of Hardy-Weinberg expectations.

Nei's (1972) genetic identity (*I*) for the 10 populations of *C. humeralis* ranged from 1.00 to 0.99. The very low levels of genetic differentiation are illustrated in Fig. 2 by the UPGMA clustering of sites. The four Northland sites (N1, N2, N3, N4) were grouped together with one of the Waikato sites (W3) and the central North Island site (C1). Two other Waikato sites (W2, W4) were joined as a separate cluster to W1 and to the South Island site (S1). However, given that the differences among the sites were so small, the clusters demonstrate the high degree of similarity found among these geographically separated populations.

DISCUSSION

Genetic differentiation among locations for *A. diversus* corresponded to geographic proximity with three genetically distinct groups (i.e., Northland; the Waikato and central North Island; and the South Island; Fig. 2). In particular, South Island *A. diversus* were genetically very different compared with North Island locations, showing fixed allelic differences (non-shared alleles) at two loci (*IDH–2*, *PGDH*; Table 2). It is uncertain whether these differences are sufficient to consider them as separate species. However, Thorpe (1982) states that identity values of >0.85 usually represent populations of the same species whereas values <0.45 are likely to represent separate species. Values between 0.45 and 0.85 constitute a "gray zone", and additional criteria for recognising species boundaries such as behavioural, ecological, and/or morphological features are

required (Thorpe 1982). The genetic identity value obtained for the South Island population ($I = 0.67$) falls within the gray zone, although the isolation of the North and South Islands and the limited dispersal of *A. diversus* would presumably facilitate speciation.

By contrast, genetic differentiation among locations for *C. humeralis* was very low ($F_{ST} = 0.03$), and showed no discernible relationship with geographic proximity (Fig. 2). Although we anticipated lower genetic differentiation among *C. humeralis* populations relative to *A. diversus* as a result of their stronger flight characteristics, we did not expect a near panmictic population structure across the entire range of study locations. A recent study of the mayfly *Acanthophlebia cruentata* in the North Island of New Zealand (Smith & Collier 2001), has also found similarly low levels of genetic differentiation.

One possible explanation is that our initial expectation of limited gene flow for New Zealand mayflies may be invalid and that dispersal abilities are better than expected—a potentially questionable explanation given the very short life spans (e.g., <48 h) for most adult Ephemeroptera. However, relatively long distance movements across land have been suggested for some North and Central American Ephemeroptera species (e.g., Edmunds et al. 1976). Furthermore, Sweeney et al. (1992) point out that the presence of some mayfly species in areas that were presumably uninhabitable during the most recent glaciation would indicate the ability of those species to traverse great distances, and to colonise new habitats. With respect to the presently widespread distribution of both *C. humeralis* and *A. diversus* in New Zealand, this certainly suggests that both species have been able to disperse widely. Whether conditions that might have once facilitated dispersal still exist today is uncertain.

Alternatively, observed patterns of population structure for *C. humeralis* may be the result of their reproductive modes. Specifically, analyses of the *F* statistics for *C. humeralis* revealed that F_{IS} was greater than F_{ST} in most cases and may indicate the effects of inbreeding or other violations (e.g., asexual reproduction) of Hardy-Weinberg expectations for some loci (Slatkin 1985; Hartl & Clarke 1997). To suggest that inbreeding is a characteristic of this taxon is uncertain because inbreeding is usually associated with small populations and *C. humeralis* appeared to be very abundant in our study streams (c. 100 m^{-2}). However, it is possible that effective population sizes may be limited due to their patchy distribution in spatially and temporally heterogeneous stream habitats (*sensu* Palmer et al. 1996, see also Bunn & Hughes 1997). Elevated F_{IS} values may also indicate that *C. humeralis* represents a facultative parthenogenetic species. This possibility is partially supported by two earlier studies (Wisely 1965; Norrie 1969), which recorded female biased sex ratios at several localities. Although mayflies are widespread and comprise a significant portion of the stream fauna, parthenogenetic populations are often overlooked because these taxa are usually collected as larvae that can be difficult to sex. However, it is now evident that parthenogenesis (both facultative and obligatory) is fairly common in both European and North American mayfly taxa (Brittain 1982; Sweeney & Vannote 1987). One facultative parthenogenetic species from North America (*Eurylophella funuralis*) also had very low within-population genetic variability, extremely low genetic distance ($D < 0.01$) and moderate differentiation ($F_{ST} = 0.07$) among the populations sampled (Sweeney & Vannote 1987). In some facultative parthenogenetic species "unisexual" (only females), bisexual populations (normal sex ratio), and female- biased bisexual populations can be found. Unisexual populations were detected mainly at the periphery of the geographic range of the species and had heterozygosity values of zero (Funk et al. 1988). In the present study, observed heterozygosity values of 0.01 and 0.02 and the percentage of polymorphic loci were lowest in the northernmost populations sampled (N2, N3) and may indicate the occurrence of (facultative) parthenogenetic populations at the periphery of the geographic range of *C. humeralis*.

Genetic variability within respective populations was low for both species. Specifically, we found evidence for low levels of average heterozygosity (i.e., 5.7% for *A. diversus*; 3.8% for *C. humeralis*) and low numbers of alleles per locus (mean = 1.4 for *A. diversus* and 1.5 for *C. humeralis* both species) compared with other aquatic invertebrates which have generally exceeded mean levels of 3–4 alleles per locus (e.g., Sweeney et al. 1987; Jackson & Resh 1992; Robinson et al. 1992; Hogg et al. 1998b; Plague & McArthur 1998)—a feature they share with other New Zealand insect (Smith & Collier 2001) and amphipod (crustacean) taxa (Schnabel et al. 2000). The possibility that low levels of genetic variability may be characteristic of the New Zealand invertebrate fauna is certainly noteworthy and may indicate the insular nature of the fauna.

Relatively recent (20 000 ya) glaciation (Suggate et al. 1978), sea level fluctuations, major volcanism, and habitat fragmentation through extensive deforestation may have led to several range contractions

and expansions which have interacted to form the present-day genetic structure and distribution of both study species. Recent range contractions may have led to isolated, possibly small populations with low genetic variability. Recolonisations by small genetically depauperate founder populations, followed by subsequent habitat fragmentation, would then explain the low allelic diversity and heterozygosity in *A. diversus* and *C. humeralis*.

Although it could be argued that the different dispersal capacities of the two species are principally responsible for the differences in population genetic structure, the lack of differentiation among *C. humeralis* populations across a large geographical area appears inconsistent with the generally short reproductive/adult phase of Ephemeroptera. Boileau et al. (1992) have proposed historical events as an alternative explanation and provided evidence that the effects of small founder populations can persist for thousands of generations. In addition, it has also been suggested that the effects of delayed population growth after colonisation can lead to both increased and decreased differentiation depending on the actual pattern of migration (Ingvarsson 1997). However, since data on patterns of colonisation and migration for *A. diversus* and *C. humeralis* are unavailable, it remains uncertain whether differential dispersal rates or different life history traits (e.g., facultative parthenogenesis) are the ultimate reasons for the lack of genetic differentiation in *C. humeralis*, and greater levels of differentiation in *A. diversus*. Thus, conclusions based on present-day dispersal alone cannot easily be generalised even if species exhibit similar life history characteristics and coexist in the same habitat.

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