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Allozyme diversity and population genetic structure of the caddisfly *Orthopsyche fimbriata* and the mayfly *Acanthophlebia cruentata* in New Zealand streams

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SUMMARY

1. Allozymes were used to measure genetic variation within and among regional populations of the caddisfly *Orthopsyche fimbriata* and the mayfly *Acanthophlebia cruentata* in North Island New Zealand streams.
2. High levels of genetic differentiation were recorded in populations of *O. fimbriata* within and among catchments separated by more than 100 km, but little or no differentiation in populations separated by around 10 km. The Auckland isthmus appears to be a major barrier to north–south gene flow, with nearly fixed allelic differences at one locus. Genotype frequencies conformed to Hardy–Weinberg equilibrium.
3. *Acanthophlebia cruentata* had low levels of genetic variation; the results are unexpected given that *O. fimbriata* apparently has greater potential for dispersal. The limited genetic data for *A. cruentata* provided evidence for genetic differentiation among populations separated by around 100 km, or more, within catchments and some differentiation between catchment populations separated by only 25 km.

Keywords: allozymes, genetic structure, caddisfly, mayfly

Introduction

An understanding of the spatial structure of populations is a key facet of conservation biology (Hanski & Simberloff, 1997). Aquatic invertebrates exhibit a wide range of dispersal capabilities, but many species are difficult to study with mark and recapture techniques, therefore genetic measures are increasingly being used to estimate within and between stream movement (Hughes, Bunn Kingston & Hurwood, 1995, Hughes, Bunn, Hurwood, Choy & Pearson, 1996, Hughes, Bunn, Hurwood & Cleary, 1998, Hughes, Mather, Sheldon & Allendorf, 1999). In general the greater the dispersal capability the less genetic differentiation is expected between catchments. Stream species with limited potential for adult dispersal, such as some Crustacea, exhibited significant genetic differentiation between rainforest streams (Hughes *et al*., 1995, 1996). In contrast, aquatic insects with winged adult stages revealed little or no genetic differentiation among neighbouring catchments (Bunn & Hughes, 1997; Gibbs, Gibbs, Siebenmann & Collins, 1998; Hughes *et al*., 1998, 1999), but populations separated by mountain barriers showed significant genetic differentiation (Jackson & Resh, 1992). Some aquatic insects reveal significant genetic differentiation within streams, which may be the result of stochastic recruitment events (Schmidt, Hughes & Bunn, 1995; Hughes *et al*., 1998; Plague & McArthur, 1998).

In New Zealand, several studies have been undertaken into the genetic structure of stream invertebrate populations, primarily to resolve taxonomic and autecological issues. Phillips & Lambert (1989, 1990) studied the genetic structure among populations of the hydrobiid snail *Potampoyrgus antipodarum* and

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identified two major groupings and evidence for sexual and parthenogenetic modes of reproduction. More recently, Schnabel, Hogg & Chapman (1999) used allozyme analyses to distinguish eastern and western groupings of the freshwater and estuarine amphipod *Chaetocorophium lucasi*, and considered that prevailing ocean currents and overland dispersal routes best explained these patterns. Finally, Willmann-Huerner (1999) found that populations of the dobsonfly *Archichauliodes diversus* collected in the central and northern North Island were strongly differentiated from a South Island population, whereas the mayfly *Coloburiscus humeralis* showed low levels of differentiation among populations, suggesting higher levels of gene flow or conservative genetic differentiation (see also Collier, Fowles & Hogg, 2000).

The North Island of New Zealand is a relatively narrow mountainous island with many short unconnected drainage systems. Prevailing winds flow west to east across the north–south lying island and much of the environment has been modified extensively for farming over the past 200 years so that areas of native forest are now highly fragmented. These factors potentially create a series of isolated habitats in remnant forest patches for stream invertebrates that are dependent on forest cover. Here we use the technique of allozyme electrophoresis to measure genetic variation and test within and between catchment dispersal in two insect species, the caddisfly *Orthopsyche fimbriata* (McLachlan) (Hydropsychidae) and the mayfly *Acanthophlebia cruentata* (Hudson) (Leptophlebiidae), with apparently different dispersal potentials.

Methods

Study animals

The caddisfly *O. fimbriata* and the mayfly *A. cruentata* are restricted to the North Island of New Zealand where they can be abundant in small, stony streams (Winterbourn & Gregson, 1989). These species were chosen for this study because large specimens are readily identified in field collections, and because they have contrasting expected dispersal abilities (indicated by adult longevity and flight characteristics) and habitat breadth. *Orthopsyche fimbriata* occurs in streams draining forested and pasture catchments where larvae occupy fixed retreats on stable benthic substrates. *Orthopsyche fimbriata* larvae have a low propensity to drift compared with *A. cruentata* nymphs (J. Quinn, unpublished data) which are free-living among hyporheic and benthic gravels in forested streams. Adult *A. cruentata* are believed to live for only a few days and appear to stay close to the stream (McLean, 1967), whereas *O. fimbriata* adults are thought to live longer. Observations of flight distance of other hydropsychid species in North America have shown that individuals may travel considerable distances from their larval habitat (Coutant, 1982; Kovats, Ciborowski & Corkum, 1996). These species traits suggest that, compared with *O. fimbriata*, *A. cruentata* should have less ability to disperse among distant forested catchments isolated by surrounding pastoral land use.

Samples

Samples of *A. cruentata* nymphs and *O. fimbriata* larvae were collected from 12 small gravel-bed streams in native forest catchments (Fig. 1; Table 1) ranging in width from 2 to 8 m between November 1998 and December 1999. Streams were selected at different spatial scales within and between catchments aligned from north to south. Northland streams (samples labelled N) are in separate catchments, whereas Waikato River tributaries (samples labelled W) are within a common catchment covering a similar north–south spatial scale to the Northland samples. Separate catchments (A1, B1, and C1) were sampled west–east across the central Waikato region over a similar spatial scale (Fig. 1). Site W3 was sampled in consecutive years to assess temporal variation in the genetic structure of this population.

Invertebrates were collected by disturbing the stream bed and collecting dislodged insects in a net held downstream. All insects were washed into a tray, and individuals of the target species were removed and frozen in liquid nitrogen in separate tubes. Field identifications were possible for these species because *A. cruentata* has a distinctive orange colour and late instar *O. fimbriata* larvae have a distinctive notch on the anterior margin of the fronto-clypeus (Winterbourn & Gregson, 1989). Specimens were stored at −70 °C prior to analysis.

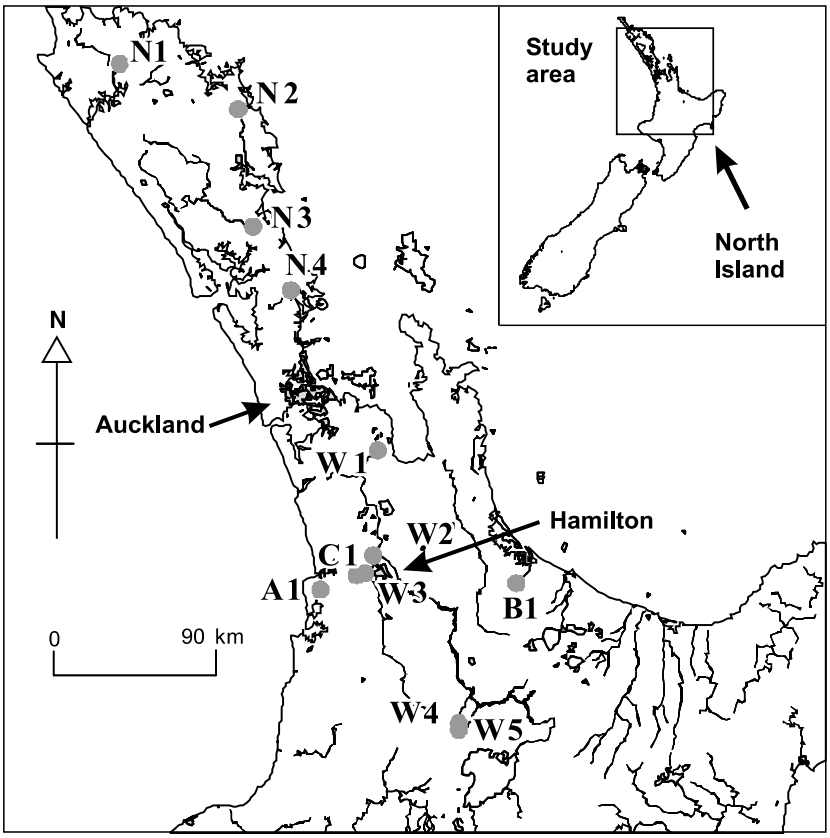

Fig. 1 Collection sites for *Orthopsyche fimbriata* and *Acanthophlebia cruentata* in the North Island of New Zealand.

Table 1 Locations, names, sampling dates and number of individuals collected from the 12 North Island sites

Site code	Map ref. (NZMS 260)	Stream/ river name	Catchment	Date sampled	No. of *Acanthophlebia*	No. of *Orthopsyche*
N1	O05 561669	Mangamuka R.	Mangamuka R.	26/11/98	25	23
N2	Q05 224409	Mokotuna Stm.	Wairau R.	27/11/98	50	0
N3	Q08 301742	Findlayson Brook	Waipu R.	25/5/99	50	35
N4	R09 515385	Waitaraire Stm.	Hoteo R.	25/5/99	18	4
				20/12/99	32	50
W1	S12 079506	Mangatangi Stm.	Waikato R.	15/9/99	50	49
W2	S14 975888	Firewood Ck. trib.	Waikato R.	13/11/98	50	50
W3	S14 927785	Whakakai Stm.	Waikato R.	17/11/98	50	50
				16/9/99	50	50
W4	T17 458897	Kakaho Stm.	Waikato R	21/9/99	14	50
				23/12/99	8	
W5	T17 455935	Waimonoa Stm. trib.	Waikato R.	21/9/99	0	50
C1	R14 888778	Mangaokuhu Stm.	Waitetuna R.	19/11/98	50	50
A1	R15 685689	Te Rekereke Stm.	Te Rekereke Stm.	23/9/99	50	50
B1	U14 783725	McLaren Falls	Wairou R.	20/9/99	0	50

Laboratory methods

Cellulose acetate and starch gel electrophoresis were used to screen enzymes using the methods of Benson & Smith (1989). Whole larvae were homogenized in 20 μL buffer, held at 4 °C for 30 min and centrifuged at 14 900 *g* for 10 min at 4 °C. The clear supernatants were applied to Helena cellulose acetate and starch gels. Three cellulose acetate gel systems were used: C1, tris-barbital pH 8.8 (Helena Products 5805, Helena

Laboratories, TX, U.S.A.); C2, tris-glycine pH 8.5 (Hebert & Beaton, 1989); and C3, tris EDTA boric pH 8.0 (Helena Products 5802 Helena Laboratories, TX, U.S.A.); and one starch system: S1, phosphate-citrate pH 7.0 (Selander *et al*., 1971). The enzymes tested, abbreviation (and Enzyme Commission number and buffer system) were: acid phosphatase ACP (2.6.1.1, buffer C2), aconitate hydratase ACO (4.2.1.3, buffer C2), alkaline phosphatase ALP (3.1.3.1, buffer C2), creatine kinase CK (2.7.3.2, buffer C2), esterase EST (3.1.1.-, buffer C2), glucose-6-phosphate dehydrogenase GPPDH (1.1.1.49, buffer C2), glucose-6-phosphate isomerase GPI (5.3.1.9, buffer C2), glycerol-3-phosphate dehydrogenase GPDH (1.1.1.8, buffer S1), isocitrate dehydrogenase IDH (1.1.1.42, buffer S1), lactate dehydrogenase LDH (1.1.1.27, buffer C2), leucine aminopeptidase LAP (3.1.11.-, buffer C2), malate dehydrogenase MDH (1.1.1.37, buffer C3), malic enzyme MEP (1.1.1.40, buffer C2), mannose-6-phosphate isomerase MPI (5.3.1.8, buffer C2), peptidase PEP (3.4.-.-, buffer C2), phosphoglucomutase PGM (5.4.2.2, buffer C2), phosphogluconate dehydrogenase PGDH (1.1.1.44, buffer S1). Following initial electrophoretic screening, those enzymes that produced clear banding patterns were selected for population analyses; nine enzymes were tested in *O. fimbriata* and eight enzymes in *A. cruentata*.

Gel interpretation and statistical analyses

Enzyme loci were considered polymorphic if more than one electromorph was found in the initial survey of 24 animals (of each species) from three sites (eight animals × three sites). Electromorphs were given an alphabetic code with *a* for the fastest migrating electromorph. Hardy–Weinberg tests were not carried out on loci where the common electromorph was at a frequency > 0.95. Genotype frequencies were tested for conformance to Hardy–Weinberg expectations, using the χ^2 pseudo-probability programme CHIHW (Zaykin & Pudovkin, 1993), which uses a randomization procedure to estimate the significance of the χ^2 test when there are many cells with fewer than five observations. One thousand randomizations were used and the probability was estimated from the number of randomizations that were equal to or greater than the observed χ^2. Probability levels were modified by the Bonferroni procedure for multiple tests, after Rice (1989).

Allele frequencies were tested for heterogeneity among samples within species with contingency χ^2 tests using the BIOSYS-1 programme (Swofford & Selander, 1981). Significance levels were adjusted by the Bonferroni technique for multiple tests in which the significance level is divided by the number of tests (Rice, 1989). Average heterozygosity was calculated in each species by direct count of heterozygous individuals; area sample heterozygosity estimates were compared with the Mann–Whitney rank test. Genetic distances were calculated from allele frequencies after Nei (1978) and a dendrogram generated with the unweighted pair-group method using arithmetic averages with the BIOSYS programme (Swofford & Selander, 1981).

The proportion of allozyme variation as a result of differentiation among samples was estimated with Nei's gene-diversity statistic, G_{ST} (Nei, 1978), which is a multiallele estimator of Wright's F_{ST} statistic (Wright, 1951). Sampling error will produce differences in allele frequencies, even when samples are drawn from the same population; therefore, a randomization test was used to test for differences as a result of sampling error (Elliott & Ward, 1992). One thousand randomizations were used and the probability was estimated from the number of randomisations that were equal to or greater than the observed F_{ST}.

Results

Orthopsyche fimbriata

Nine enzymes produced clear scoreable results in the initial testing of 24 animals and were tested in all samples. Several enzymes, (ADH, CK, G6PDH, LDH, MPI, PGDH, XDH) produced weak or no activity in the initial screening and were not tested in further samples. The enzyme AKP produced the same gel phenotypes as locus *EST-2** and was excluded from statistical analyses.

Ten loci were resolved in the 12 populations; most loci showed no significant departure from Hardy–Weinberg equilibrium. Eighty two tests were carried out on 10 loci across 12 samples and only three locus-samples had an excess of homozygotes (*EST-1** $P = 0.007$ and *MEP** $P = 0.004$, sample N1; and *IDH** $P = 0.007$, sample W6); a result no greater than expected by chance. The mean number of alleles ranged from 1.8 to 2.5 per site and observed

heterozygosities between 0.071 and 0.171 (Table 2). Northland sites had higher heterozygosities than all other sites (Mann–Whitney test $T = 6$, $P_{3,9}$ 0.001).

Heterogeneity was tested for all loci with contingency χ^2 tests. Significant heterogeneity was found among all samples at eight of the ten loci (Table 3). Allelic heterogeneity in the total data set was confirmed by gene diversity analysis (Table 3). Applying a Bonferroni-modified P, eight out of ten loci showed a significant F_{ST}. Over all 10 loci, F_{ST} was 0.129 indicating that around 13% of the observed genetic variation was as a result of differences among populations (Table 4). There was considerable variation in F_{ST} estimates among loci ranging from 0.02 for *LAP** to 0.826 for *EST-2** (Table 3). The *EST-2** locus was nearly fixed for different alleles at some Northland and central sites. Two other loci, *IDH** and *MEP**, also exhibited high F_{ST} values (Table 3).

The genetic distance analysis (Fig. 2a) revealed a major genetic break between the Northland populations and those from the central North Island. Among the three Northland populations genetic differentiation was greater than among populations from the central North Island (F_{ST} values, Table 4). A plot of genetic diversity (F_{ST}) against geographical distance (Fig. 3) shows two clusters representing (a) the Northland–Central site comparisons with high F_{ST} values and (b) the Central–Central and Northland–

Table 2 Genetic variation measures (mean ± SE) in population samples of *Orthopsyche fimbriata* and *Acanthophlebia cruentata* from 13 sites in the North Island of New Zealand. (Sites as in Fig. 1)

	Orthopsyche fimbriata			*Acanthophlebia cruentata*		
Site	No. of alleles	Het_{obs}	Het_{exp}	No. of alleles	Het_{obs}	Het_{exp}
N1	2.5 ± 0.4	0.171 ± 0.06	0.277 ± 0.08	2.0 ± 0.3	0.087 ± 0.03	0.095 ± 0.03
N2	–	–	–	1.9 ± 0.2	0.043 ± 0.02	0.045 ± 0.02
N3	2.2 ± 0.3	0.150 ± 0.07	0.169 ± 0.08	1.5 ± 0.2	0.023 ± 0.01	0.022 ± 0.01
N4	2.1 ± 0.3	0.146 ± 0.07	0.141 ± 0.07	1.6 ± 0.2	0.029 ± 0.01	0.028 ± 0.01
A1	2.1 ± 0.3	0.094 ± 0.04	0.108 ± 0.04	1.7 ± 0.2	0.035 ± 0.01	0.039 ± 0.01
B1	2.0 ± 0.3	0.115 ± 0.05	0.118 ± 0.05	–	–	–
C1	2.8 ± 0.4	0.121 ± 0.05	0.139 ± 0.05	1.6 ± 0.2	0.016 ± 0.01	0.020 ± 0.01
W1	2.0 ± 0.4	0.102 ± 0.04	0.125 ± 0.04	1.5 ± 0.2	0.025 ± 0.01	0.024 ± 0.01
W2	2.4 ± 0.4	0.077 ± 0.04	0.089 ± 0.04	1.5 ± 0.2	0.033 ± 0.01	0.032 ± 0.01
W3a	1.8 ± 0.3	0.071 ± 0.04	0.085 ± 0.04	1.5 ± 0.2	0.019 ± 0.01	0.028 ± 0.01
W3b	1.8 ± 0.4	0.078 ± 0.04	0.080 ± 0.04	1.6 ± 0.2	0.030 ± 0.01	0.032 ± 0.01
W4	2.1 ± 0.3	0.114 ± 0.04	0.122 ± 0.04	–	–	–
W5	1.8 ± 0.2	0.079 ± 0.03	0.110 ± 0.03	1.2 ± 0.1	0.053 ± 0.04	0.046 ± 0.03

Table 3 Heterogeneity χ^2 tests and genetic diversity (F_{ST}) at nine allozyme loci in regional population samples of the caddisfly *Orthopsyche fimbriata*

	Location of sites (number of population samples)									
Locus	Total (12) χ^2	Total (12) F_{ST}	North (3) χ^2	North (3) F_{ST}	Central (6) χ^2	Central (6) F_{ST}	Waikat (6) χ^2	Waikat (6) F_{ST}	Waikat (3) χ^2	Waikat (3) F_{ST}
ACP	59.5*	0.034*	7.2	0.028	–	0.030	–	0.009	–	0.007
EST-1*	183*	0.071*	53.6*	0.086*	55.6*	0.032*	31.6	0.014	5.2	0.003
EST-2*	876*	0.826*	26.2*	0.147*	–	0.009	–	0.004	–	0.005
GPDH*	53.5*	0.034*	15.3*	0.050*	7.3	0.007	28.0*	0.022	–	0.010
GPI*	44.0*	0.024*	2.4	0.013	12.5	0.009	30.3*	0.036*	3.4	0.010
IDH*	238*	0.125*	15.8	0.025	52.8*	0.051*	23.9*	0.032*	5.9	0.013
LAP*	34.8	0.020	3.9	0.004	26.5*	0.033*	15.3	0.018	–	0.008
MDH*	61.4*	0.027*	7.2	0.028	–	0.010	–	0.017	–	0.014
MEP*	121*	0.104*	16.4*	0.052*	14.3	0.018	23.0	0.032*	–	0.010

*Significant at the 5% level with a Bonferroni-modified P for multiple tests; – loci excluded where allele frequencies in all samples >0.98).

Table 4 Genetic differentiation, geographical distance and number of catchments for population samples of the caddisfly *Orthopsyche fimbriata*

Region	Distance (km) & direction	No. of catchments	Sites	χ^2	F_{ST}
All	450 N–S	7	All	1698***	0.129***
Northland	170 N–S	3	N1 N3 N4	151.6***	0.045***
Waikato	180 N–S	1	W1 – W5	171.7***	0.020***
Central	120 W–E	4	A1 B1 C1 W2 W3a W3b	225.4***	0.020***
Waikato local	20 N–S	1	W2 W3a W3b	34.6NS	0.009NS
Waikato south	2	1, 2 streams	W4 W5	28.09*	0.028*
Central west	25 W–E	2	A1 C1	31.6*	0.007NS
Central local	2	2	C1 W3a W3b	59.06*	0.013**

NS = non-significant; *P = 0.05–0.01, **P < 0.01, >0.001, ***P < 0.001.

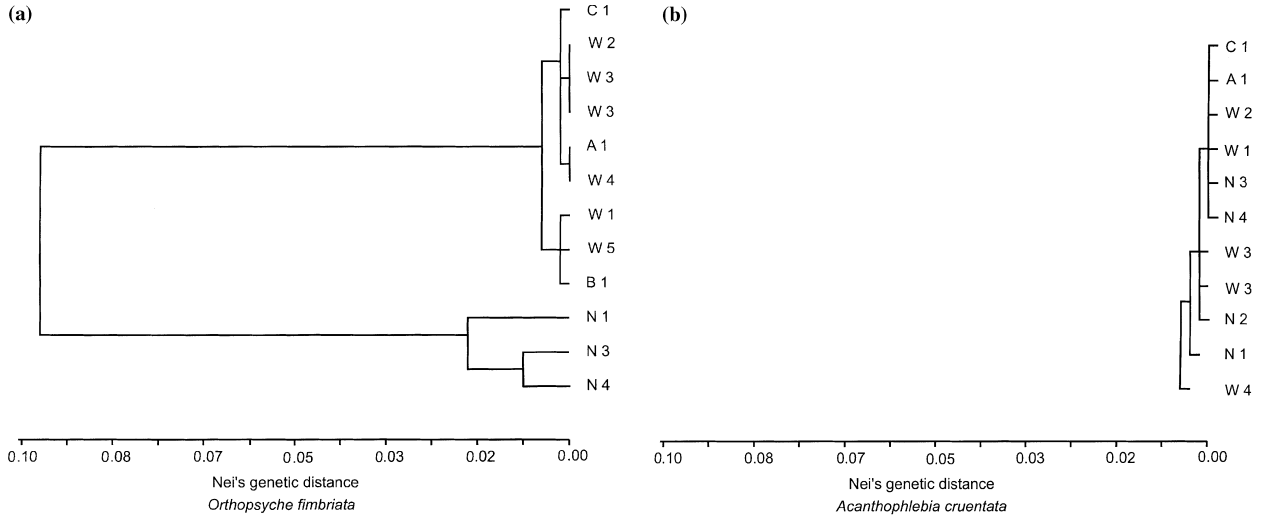

Fig. 2 Genetic distances (Nei, 1978) for population samples of (a) *Orthopsyche fimbriata* and (b) *Acanthophlebia cruentata*.

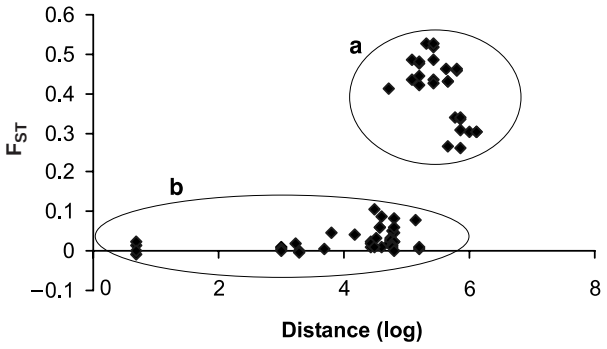

Fig. 3 Genetic diversity (F_{ST}) plotted against geographical distance for populations of *Orthopsyche fimbriata*. The two clusters show (a) the Northland–Central and (b) the Northland–Northland and the Central–Central population comparisons.

Northland site comparisons with low F_{ST} values. A linear regression of F_{ST} against square root of geographical distance gave an R^2 value of 0.49.

To further test for geographical structure, the data were subdivided into geographical regions and catchments (Table 4). Significant differentiation was found among the Northland samples taken from three catchments over a distance of 170 km. Likewise, significant differentiation was found among 6 Waikato samples taken from one catchment over a distance of 180 km and among the six central samples taken from four catchments over a distance of 120 km from west to east.

Differentiation over smaller scales within and between catchments was tested among the central sites. In the south Waikato, samples from neighbouring streams (W4 and W5) had different allele frequencies at one locus, *IDH**, a difference that resulted in an overall significant F_{ST} (Table 4). Within streams, including two temporal samples from one site in the

central Waikato (W2, W3a and W3b), showed no significant differentiation (Table 4). However, streams separated by only 2 km but in different catchments (sites C1, W3a, and W3b) showed significant differentiation (Table 4). These streams were separated by hills and flowed in opposite directions.

Acanthophlebia cruentata

Eight enzymes produced clear scoreable results in the initial testing of 24 animals and were tested in all samples (Table 5). Several enzymes (AKP, CK, G6PDH, LDH, MPI, PGDH, XDH) produced weak or no activity in the initial screening and were not tested in further samples. The enzyme ACP produced the same gel phenotypes as locus *EST-4** and was excluded from statistical analyses.

Eleven loci were resolved in the 11 populations; most loci were monomorphic or weakly polymorphic in all samples and Hardy–Weinberg tests were not undertaken. The mean number of alleles ranged from 1.2 to 2.0 per site and observed heterozygosities varied between 0.016 and 0.087 (Table 2). No evidence for regional differentiation in heterozygosity levels was found (Northland versus Central; Mann–Whitney test $T = 20$, $P_{4,7} > 0.10$).

The genetic distance analysis (Fig. 2b) showed a very shallow division among the populations with no obvious break between the Northland and central populations.

However, significant heterogeneity was demonstrated with contingency χ^2 tests among all samples at six of eight loci (Table 5). The allelic heterogeneity in the total data set was confirmed by the gene diversity analysis for four loci; applying a Bonferroni-modified P, four of eight loci show a significant F_{ST} (Table 5). Over all loci, F_{ST} was 0.043 (Table 5), indicating that around 4% of the observed genetic variation was the result of differences among populations. At some loci the heterogeneity χ^2 tests gave a significant result when the F_{ST} was non-significant (e.g. *EST-2** and *EST-4** Central, Table 5); rare alleles can inflate the heterogeneity χ^2 result because of the large number of cells with few observations.

To test for geographical structure, the data were subdivided into geographical regions and catchments. Significant differentiation was found among the Northland sites and among the Waikato sites over north–south distances of 170–180 km (Table 6), and over 25 km among the central sites from three different catchments (Table 6). No differentiation was observed among two western catchments, or among different streams in neighbouring catchments at distances of 25 km and less (Table 6).

Discussion

A high level of genetic differentiation was found among *O. fimbriata* populations in the North Island. The major break between the Northland and Central North Island populations (Fig. 2a) indicated there is little north–south movement across the relatively narrow Auckland isthmus. Historically, the Northland area was separated from the central North Island by the Manukau Strait that ran across the Auckland isthmus during the Pliocene (Stephens, 1980), so it is

Table 5 Heterogeneity χ^2 tests and genetic diversity (F_{ST}) at eight allozyme loci in population samples of the mayfly *Acanthophlebia cruentata*

	Location of sites (number of population samples)							
Locus	Total (11) χ^2	Total (11) F_{ST}	Waikat (5) χ^2	Waikat (5) F_{ST}	Central (5) F_{ST}	Central (5) F_{ST}	North (4) χ^2	North (4) F_{ST}
EST-2*	54.4*	0.031	9.6	0.032	23.5*	0.021	–	0.008
EST-4*	64.7*	0.044*	13.1	0.022	14.6	0.013	25.1*	0.07*
EST-5*	78.2*	0.051*	19.8	0.038	18.9	0.022	21.9*	0.03*
GPI*	38.3*	0.039*	–	0.011	–	0.014	3.6	0.010
LAP*	119.0*	0.095*	45.0*	0.13*	12.7	0.012	0.6	0.002
MDH*	27.2	0.026	6.9	0.022	7.7	0.014	–	0.000
MEP*	59.7	0.033	–	0.013	–	0.013	21.6*	0.03*
PGM*	46.9*	0.030	14.4	0.032	11.7	0.030*	12.3	0.022

*Significant at the 5% level with a Bonferroni-modified P for multiple tests; – loci excluded where allele frequencies in all samples >0.98.

Table 6 Genetic differentiation, geographical distance and number of catchments for population samples of the mayfly *Acanthophlebia cruentata*

Region	Distance (km) & direction	No catchments	Sites	χ^2	F_{ST}
All	450 N–S	6	All	488***	0.043***
Northland	170 N–S	4	N1 N2 N3 N4	92***	0.025***
Waikato	180 N–S	1	W1 W2 W3a W3b W5	124***	0.056***
Central	25 W–E	3	A1 C1 W2 W3a W3b	115***	0.018***
Waikato local	20 N–S	1	W2 W3a W3b	31.1*	0.010*
Central west	25 W–E	2	A1 C1	13.60 NS	0.009 NS
Central local	2	2	C1 W3a W3b	33.3 NS	0.007 NS

NS = non-significant; *P = 0.05–0.01, ***P < 0.001.

possible that the observed genetic differentiation is partly a result of historical processes. Today, this isthmus is characterized by few streams and extensive urban development; the unfavourable habitat coupled with the prevailing south–westerly windflow across the North Island is hypothesized to limit gene flow within *O. fimbriata*.

Mitochondrial DNA data for the landlocked mudfish *Neochanna diversus* showed significant sequence divergence between samples from Northland and Waikato (Gleeson, Howitt & Ling, 1999). This differentiation, at a selectively neutral region of the genome, was attributed to processes that divided northern New Zealand into isolated islands during the Pliocene. However, it is not valid to use allozyme data to estimate time of divergence, because some loci are assumed to be under selection or linked with loci under selection (e.g. Verspoor, 1983; Synder & Hendricks, 1997). Experimental studies with aquatic insects have demonstrated differential survival rates among allozyme genotypes exposed to heavy metals (Benton & Guttman, 1992; Synder & Hendricks, 1997) indicating that allozyme variation may measure short-term population events rather than phylogeny.

The Northland sites were characterized by higher levels of genetic diversity than other sites at which *O. fimbriata* were sampled (Table 2). Overall, there was a general trend towards increasing genetic differentiation with increasing geographical distance, although two distinct clusters represented the within (Northland and Central) and between (Northland–Central) area comparisons (Fig. 3). It is apparent that the high F_{ST} obtained for *O. fimbriata* was produced to a large extent by one locus. Greater genetic differentiation was observed among catchments in Northland ($F_{ST} = 0.045$) than within the Waikato River catchment ($F_{ST} = 0.023$) over a similar north–south range (Table 4). East–west samples across the central region and three catchments showed similar levels of differentiation ($F_{ST} = 0.023$) to north–south sites within the Waikato catchment (Table 4). Similar levels of genetic differentiation within and among catchments over spatial scales of 100 + km suggests that movement within catchments is limited. The higher differentiation among Northland sites is probably produced by restricted dispersal among north–south catchments and reinforced by the prevailing south–west winds.

At smaller spatial scales (less than 25 km) less genetic differentiation was observed in *O. fimbriata*. No significant differentiation was apparent within streams ($F_{ST} = 0.008$), but samples from neighbouring streams (sites W4 and W5), separated by 2 km land distance and stream distance of 25 km, differed at one locus ($F_{ST} = 0.028$). The observed genetic differentiation between streams within catchments implies that, at small spatial scales, movement occurs primarily along streams and rivers, rather than via land corridors, and that recolonization will occur primarily within and not between streams. In the American hydropsychid caddisfly *Hydropsyche cockerelli*, radioisotope labelling demonstrated that adults could migrate as much as 16 km upstream from the labelling site thereby counteracting the downstream drift of larvae (Coutant, 1982). Light trapping of lake-dwelling caddisflies and mayflies in North America revealed limited inland dispersal, with the majority of caddisflies caught within 100 m of the lake edge, although some individuals did travel considerably further inland (Kovats *et al*., 1996). Similarly, most caddisflies were caught within 20–30 m of streams in riparian forest in a New Zealand study (Collier & Smith, 1998).

In contrast to the results obtained for *O. fimbriata*, the North American mayfly *Siphlonisca aerodromia* showed minimal genetic differentiation (based on microsatellite DNA markers) between drainages over distances of about 100 km, suggesting that adult flight is an important mode of dispersal in this species (Gibbs *et al*., 1998). Allozyme studies of the caddisfly *Tasiagma ciliata* also indicated low levels of genetic differentiation among catchments over distances of 30–40 km (Hughes *et al*., 1998). However, the same study also indicated genetic departure from Hardy–Weinberg equilibrium as a result of stochastic effects of recruitment (Hughes *et al*., 1998); similar Hardy–Weinberg deviations were found in two other insect species inhabiting subtropical streams in Australia (Bunn & Hughes, 1997) and four species of caddisflies in North America (Plague & McArthur, 1998). Patchy deviations from Hardy–Weinberg equilibrium in *T. ciliata* and *Baetis* sp. were interpreted as the result of populations being derived from relatively few matings (Schmidt *et al*., 1995; Hughes *et al*., 1998). However, as in our study of *O. fimbriata*, work on the North American stonefly *Yoraperla brevis* found no significant departure from Hardy–Weinberg equilibrium (Hughes *et al*., 1999), suggesting that larval populations of this stonefly were not based on relatively few parents.

Unlike *O. fimbriata*, the genetic data for *A. cruentata* collected from many of the same sites showed little genetic differentiation (Fig. 2b); *A. cruentata* exhibited low genetic heterozygosity at most loci and thus the data provided a weak test of genetic differentiation in this species. Low genetic heterozygosity has been reported in the North American mayfly *Dolania americana* (Sweeney & Funk, 1991). Nevertheless, heterogeneity was apparent in the total *A. cruentata* data set, with evidence for differentiation between Northland–Central populations, among the Northland populations in separate catchments, and within the Waikato catchment at the 100+ km scale (Table 6). Greater heterogeneity between than within catchments in the central region suggests that within-catchment dispersal is greater than dispersal between catchments in *A. cruentata* (Table 6), and supports the contention that this species does not disperse between catchments as readily as *O. fimbriata*.

Our finding of low genetic differentiation in *A. cruentata* was unexpected because species traits suggested it would be a weaker disperser than *O. fimbriata* and would be expected to show greater genetic divergence over the same sampling range. It is possible that additional allozyme markers, or other regions of the genome, such as those detected with DNA-based markers, might reveal greater population structure in *A. cruentata*. The weak structure evidence using allozymes may partly reflect optimization of genetic diversity by *A. cruentata* in response to the narrow range of environmental conditions that it occupies. This species is characteristically found in cool, forested streams where it often occupies parafluvial zones that are relatively high in oxygen and efficient in retaining fine particulate organic matter (Boulton *et al*., 1997). Conservation genetics theory suggests that species with lower overall genetic variability may be restricted to a narrower range of environmental conditions than species with higher levels of genetic variability (e.g. *O. fimbriata*), and therefore may be less able to adapt to environmental changes (Hogg, Eade & de Lafontaine, 1999). Alternatively, historical extinction and recolonization processes may have reduced genetic variation in *A. cruentata* populations. Whatever may be the cause, our results indicate that factors other than dispersal ability can have an over-riding influence on the genetic structure of stream invertebrate populations, and that an understanding of past geographical events can be important for the interpretation of results from genetic studies. This is particularly so for island archipelagos, such as New Zealand, where landmass changes have occurred over relatively small spatial scales.

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