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Contrasting mtDNA diversity and population structure in a direct-developing marine gastropod and its trematode parasites

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Abstract

The comparative genetic structure of hosts and their parasites has important implications for their coevolution, but has been investigated in relatively few systems. In this study, we analysed the genetic structure and diversity of the New Zealand intertidal snail *Zeacumantus subcarinatus* (n = 330) and two of its trematode parasites, *Maritrema novaezealandensis* (n = 269) and *Philophthalmus* sp. (n = 246), using cytochrome *c* oxidase subunit I gene (*COI*) sequences. Snails and trematodes were examined from 11 collection sites representing three regions on the South Island of New Zealand. *Zeacumantus subcarinatus* displayed low genetic diversity per geographic locality, strong genetic structure following an isolation by distance pattern, and low migration rates at the scale of the study. In contrast, *M. novaezealandensis* possessed high genetic diversity, genetic homogeneity among collection sites and high migration rates. Genetic diversity and migration rates were typically lower for *Philophthalmus* sp. compared to *M. novaezealandensis* and it displayed weak to moderate genetic structure. The observed patterns likely result from the limited dispersal ability of the direct developing snail and the utilization of bird definitive hosts by the trematodes. In addition, snails may occasionally experience long-distance dispersal. Discrepancies between trematode species may result from differences in their effective population sizes and/or life history traits.

Keywords: cytochrome *c* oxidase subunit I, genetic diversity, host–parasite, *Maritrema novaezealandensis*, *Philophthalmus*, population structure, trematode, *Zeacumantus subcarinatus*

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Introduction

The comparative genetic structure of hosts and their parasites is of crucial importance for the evolution of local host adaptations to parasites and the spread of parasites among host populations, both key elements of the coevolutionary arms race between hosts and parasites (Dybdahl & Lively 1996; Jarne & Théron 2001; Prugnolle *et al.* 2005). The outcome of host–parasite interactions is influenced by the selection pressures the different species experience and impose on one another (Lively 1999), and by how they respond to these pressures. The extent of genetic structure among populations and genetic diversity within populations are key factors dictating the ability of both hosts and their parasites to respond to these different selection pressures (Gandon *et al.* 1996; Lively 1999; Gandon & Michalakis 2002; Prugnolle *et al.* 2005), as well as maintaining their integrity as a species.

Two factors should be associated *a priori* with a high probability of observing genetic structure among populations of any organism: (i) infrequent movement of individuals among populations, due to a lack of dispersal or migration; and (ii) the potential for rapid evolutionary change, mediated by short generation times and high fecundity that allow for populations to diverge rapidly when gene flow is reduced. Most

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parasitic organisms possess these characteristics, maturing rapidly to produce large numbers of eggs and larvae with very limited innate dispersal capabilities. However, the life cycles of parasites are intimately linked with those of their hosts, and as passengers on or within the hosts, parasites may be dispersed over larger geographic areas. Host movements may therefore be the most influential avenue of gene flow for many parasite species (Nascetti *et al.* 1993; Blouin *et al.* 1995; Nadler 1995; McCoy *et al.* 2003; Criscione & Blouin 2004, 2007). The vast majority of parasitic worms (flatworms or roundworms) must pass through multiple host species in a prescribed sequence in order to complete their life cycle. The intermediate hosts of the parasite's larval stages are generally invertebrates with very limited dispersal abilities. In contrast, the definitive, or final, host species is often a vertebrate with relatively wider geographic dispersal capabilities. The genetic structure of the intermediate hosts is therefore likely to differ from that of the parasites if parasite gene flow is ultimately dictated by the most dispersive host (Jarne & Théron 2001; Prugnolle *et al.* 2005).

Our study system consists of the intertidal snail *Zeacumantus subcarinatus* (Batillariidae) and two of the common species of trematode parasites that use it as first intermediate host across the South Island of New Zealand. The snail is an abundant grazer of microalgae in many intertidal ecosystems throughout New Zealand. It typically flourishes in soft-sediment intertidal bays and is also common in many protected hard shore-lines (Morton & Miller 1968). It is often absent from long stretches of exposed sandy coastline and exposed rocky shores that experience heavy wave pressure, creating a widespread, but disjunct distribution. *Zeacumantus subcarinatus* does not possess a planktonic larval stage and its eggs hatch into crawl-away larvae, i.e. miniature copies of the adult that are believed to remain within their site of origin (Fredensborg & Poulin 2006). The two trematode species use only these snails as their first intermediate host, within which they reproduce asexually, producing free-swimming larvae that subsequently leave the snail to encyst in or on second intermediate hosts. Larvae of the trematode species *Maritrema novaezealandensis* (Microphallidae) encyst in crabs and other small crustaceans (Martorelli *et al.* 2004), whereas larvae of the species *Philophthalmus* sp. (Philophthalmidae) encyst on the outer surfaces of molluscs, crustaceans or other hard substrates once leaving the snails (Martorelli *et al.* 2008). Both trematode species are ultimately acquired by shorebirds, the definitive hosts, via ingestion of second intermediate hosts. *Maritrema novaezealandensis* parasitizes the gastrointestinal tract of birds whereas *Philophthalmus* sp. parasitizes the eyes. Infection by either of these trematodes causes complete castration of the snail, and the parasites thus combine to cause substantial reductions in the density and biomass of snail populations (Fredensborg *et al.* 2005). There is also evidence of local adaptation in snail populations, with snails from areas of high parasite prevalence displaying higher growth rates and lower age at maturity than snails from areas where the parasites are rare, as adaptive compensation against a high probability of castration (Fredensborg & Poulin 2006).

In this study, we compare the genetic structure of the intertidal snail *Z. subcarinatus* with the structure of two of its trematode parasites using cytochrome *c* oxidase subunit I gene (*COI*) sequences. As the snail does not possess a planktonic larval stage, gene flow is likely to be limited even among relatively proximate snail populations. Marine gastropods with direct development often display relatively strong genetic population structure in comparison to species with planktonic larval stages (Janson 1987; Hoskin 1997; Wilke & Davis 2000; Collin 2001; Kojima *et al.* 2004). However, the dispersal potential of larvae alone does not always explain the genetic structure observed in marine gastropods (Kyle & Boulding 2000) and factors such as ability to tolerate environmental stresses (Wilke & Davis 2000) and generation time (Rolán-Alvarez *et al.* 1995) can also be important. Both trematodes, after undergoing larval development in snails, eventually complete their life cycles in shorebirds such as gulls and oystercatchers. These birds likely provide the parasites with a dispersal route as they visit several coastal feeding sites within a broader feeding territory, allowing for gene flow among widespread trematode populations. The comparative nature of this study allows us to test several specific hypotheses. We predict that (i) genetic differentiation will occur among most snail populations, even over relatively small geographic scales; (ii) genetic divergence among snail populations will follow an isolation by distance pattern as the potential for even limited gene flow among snail populations will decrease with distance; (iii) trematode species will not display genetic differentiation over small geographic scales; and (iv) trematode species will display moderate genetic differentiation over larger geographic scales as this scale may extend beyond the bird hosts' local feeding territories, although movements of birds will maintain some gene flow.

Materials and methods

Sample collection

Approximately 200–500 snails were haphazardly collected from each site during low tides in an attempt to obtain 25–30 *Maritrema novaezealandensis* and *Philophthalmus* sp. individuals per site. Snails and parasites were

collected from McCormacks Bay, Christchurch and Greenpoint Domain, Bluff Harbour in December 2006 and from the Otago region from March to August 2007 (Fig. 1). Due to low prevalence at some sites, we were not able to collect the desired number of each parasite species from all sites (Table 1). Snails were dissected to screen for trematodes using a stereomicroscope and individual larval parasites were isolated with dissecting pins, rinsed in 60-mm Petri dishes containing 0.22-μm-filtered fresh water, and placed into 1.5-mL microfuge tubes for subsequent DNA extraction.

DNA extraction, PCR amplification and DNA sequencing

To extract DNA from individual *Zeacumantus subcarinatus*, ~4 mm^3 of tissue was removed from the foot and placed into 800 μL of 5% chelex containing 0.1 mg/mL proteinase K, incubated at 60 °C overnight and then heated to 100 °C for 8 min. An ~1100 bp region of the *COI* gene was amplified using the primers LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′ (Folmer *et al.* 1994) and H7005: 5′-CCGGATCCACNACRTAR-TANGTRTCRTG-3′ (Hafner *et al.* 1994). PCR reactions (25 μL) contained 1 μL of DNA extraction, 80 μM each dNTP, 1.5 mM $MgCl_2$, 0.5 μM each primer, 1× *Taq* buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl, 0.01% Tween-20] and 0.625 units BIOTAQ DNA polymerase (Bioline). PCR amplification was performed on an Eppendorf

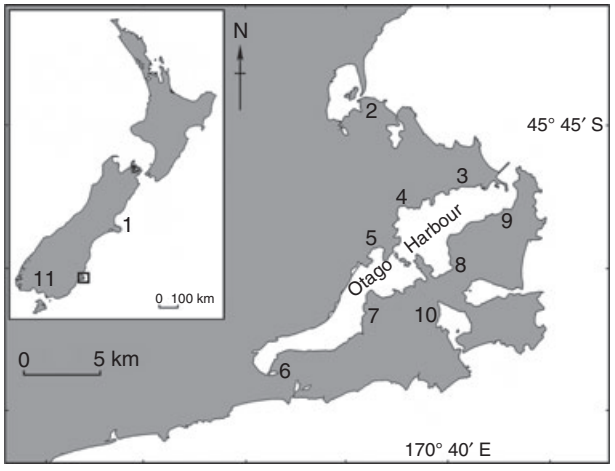

Fig. 1 Collection site locations for snails and trematodes. Abbreviations used for each site are in parentheses. Collection site numbers refer to the following sites: 1 = McCormacks Bay, Christchurch (Cch), 2 = Blueskin Bay (Blue Bay), 3 = Aramoana (Ara), 4 = Deborah Bay (Deb Bay), 5 = Sawyers Bay (Saw Bay), 6 = Andersons Bay Inlet (ABI), 7 = Company Bay (Comp Bay), 8 = Lower Portobello Bay (LPB), 9 = Otakau, 10 = Papanui Inlet (Papa Inlet), and 11 = Greenpoint Domain, Bluff Harbour (Bluff).

Table 1 Host and parasite summary statistics

Site	*Zeacumantus subcarinatus*					*Maritrema novaezealandensis*					*Philophthalmus* sp.				
	n	*Nh*	*Np*	*h*	π	*n*	*Nh*	*Np*	*h*	π	*n*	*Nh*	*Np*	*h*	π
Christchurch (1)	30	4	3	0.251 ± 0.102	0.0005 ± 0.0005	30	21	33	0.966 ± 0.021	0.0057 ± 0.0033	5	4	4	0.900 ± 0.161	0.0028 ± 0.0022
Blueskin Bay (2)	30	2	8	0.370 ± 0.084	0.0033 ± 0.0020	30	22	30	0.968 ± 0.019	0.0050 ± 0.0029	2	2	2	1.000 ± 0.500	0.0028 ± 0.0035
Aramoana (3)	30	3	16	0.131 ± 0.082	0.0013 ± 0.0009	10	9	20	0.978 ± 0.054	0.0067 ± 0.0041	3	3	4	1.000 ± 0.272	0.0038 ± 0.0034
Deborah Bay (4)	30	4	17	0.356 ± 0.106	0.0024 ± 0.0015	30	20	29	0.959 ± 0.020	0.0054 ± 0.0031	30	8	7	0.830 ± 0.035	0.0026 ± 0.0017
Sawyers Bay (5)	30	3	10	0.191 ± 0.093	0.0010 ± 0.0008	9	9	13	1.000 ± 0.052	0.0051 ± 0.0032	30	8	8	0.729 ± 0.077	0.0021 ± 0.0014
Andersons Bay (6)	30	1	0	0.000 ± 0.000	0.0000 ± 0.0000	29	21	34	0.966 ± 0.022	0.0064 ± 0.0036	30	8	8	0.775 ± 0.053	0.0024 ± 0.0016
Company Bay (7)	30	1	0	0.000 ± 0.000	0.0000 ± 0.0000	30	19	25	0.959 ± 0.020	0.0045 ± 0.0027	27	9	9	0.835 ± 0.043	0.0030 ± 0.0019
LPB (8)	30	6	11	0.460 ± 0.109	0.0037 ± 0.0022	30	26	41	0.989 ± 0.013	0.0060 ± 0.0034	30	11	13	0.782 ± 0.059	0.0029 ± 0.0019
Otakau (9)	30	3	10	0.536 ± 0.048	0.0022 ± 0.0014	30	25	37	0.986 ± 0.013	0.0060 ± 0.0034	30	9	8	0.844 ± 0.038	0.0031 ± 0.0019
Papanui Inlet (10)	30	1	0	0.000 ± 0.000	0.0000 ± 0.0000	30	24	35	0.984 ± 0.013	0.0055 ± 0.0032	29	10	11	0.860 ± 0.037	0.0031 ± 0.0020
Bluff Harbour (11)	30	4	3	0.481 ± 0.094	0.0006 ± 0.0006	11	9	15	0.964 ± 0.051	0.0054 ± 0.0033	30	6	6	0.726 ± 0.057	0.0024 ± 0.0016
Total	330	13	23	0.462 ± 0.033	0.0019 ± 0.0012	269	141	108	0.976 ± 0.004	0.0056 ± 0.0031	246	23	22	0.813 ± 0.014	0.0028 ± 0.0017

Collection site abbreviations and numbers refer to Fig. 1. Parameters are abbreviated as follows: *n* = number of individuals analysed, *Nh* = number of different haplotypes observed, *Np* = number of polymorphic sites observed, *h* = haplotype diversity ± SD, and π = nucleotide diversity ± SD.

Mastercycler® gradient thermal cycler and consisted of 2 min at 94 °C, followed by 40 cycles of 45 s at 94 °C, 50 s at 41 °C and 1 min at 72 °C and a final extension for 8 min at 72 °C.

DNA was extracted from individual parasite sporocysts (*M. novaezealandensis*) and rediae (*Philophthalmus* sp.) similarly to that described for *Z. subcarinatus* using 400 μL of 5% chelex containing 0.1 mg/mL proteinase K. An ~800 bp region of the *COI* gene was amplified using the primers JB3: 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′ (Bowles *et al.* 1995) and CO1-R trema: 5′-CAACAAATCATGATGCAAAAGG-3′ (Miura *et al.* 2005). PCR reactions and amplifications were performed as described for *Z. subcarinatus* with the following amplification conditions: 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 53 °C and 1 min at 72 °C and a final extension for 8 min at 72 °C.

For snails and parasites, PCR products were purified using PureLink PCR Purification Kits (Invitrogen). DNA from purified PCR products was sequenced using forward primers (LCO1490 and JB3) at the Allan Wilson Centre Genome Sequencing Service at Massey University with an ABI3730 Genetic Analyzer. A subset of individual DNA samples was also sequenced using the reverse primer for snails (one to five individuals of each unique haplotype) and parasites (five individuals from each sample site for both species when available). No discrepancies were detected between sequences from the same individuals.

Statistical analyses

All sequences were edited and aligned manually using Sequencher version 4.8 (Gene Codes Corp.). A 95% statistical haplotype network was constructed using TCS 1.21 (Clement *et al.* 2000) for each of the three species. The number of unique haplotypes, number of polymorphic sites, haplotype diversity (h) and nucleotide diversity (π) were calculated for each species within each sample site and over all sample sites (all individuals treated as one sample) with Arlequin version 3.1 (Excoffier *et al.* 2005). Tajima's D test (Tajima 1989) and Fu's F_S test (Fu 1997) were used to assess the consistency of observed genetic variation with a neutral model of evolution in each sample site and over all sample sites combined for each species with 1000 permutations, using Arlequin version 3.1 (Excoffier *et al.* 2005). Significant deviations from neutrality can be caused by selection, as well as population expansions or bottlenecks. Fu's F_S test is highly sensitive to population demographic expansions, which produce large negative values. Therefore, when one species (*M. novaezealandensis*) consistently deviated from neutral expectations, Fu and Li's D^* test (Fu & Li 1993) was performed using DnaSP v5 (Librado & Rozas 2009). This additional test is more powerful for detecting background selection although the previous two tests have more power to detect population growth and genetic hitchhiking (linkage of a neutral locus to a locus under selection). Contradictory results between them can therefore provide insight into the mechanism producing the observed patterns of variation (Fu 1997).

Genetic population structure was examined for each species at multiple levels using Arlequin version 3.1. The following analyses refer to sample site numbering in Fig. 1. Genetic structure (Φ_{ST}) was examined among all sites by treating sites 1–11 as separate populations. Genetic structure (Φ_{CT}) was examined among the three relatively distant sampling regions by treating sites 1 (Christchurch) and 11 (Bluff) as separate regions and sites 2–10 (Otago region sites) as subpopulations nested within a third region. Genetic structure (Φ_{ST}) was also examined among sites 2–10 within the Otago region and among sites 3–9 within Otago Harbour. In addition, pairwise Φ_{ST} values were calculated between all collection localities. The corrected Akaike information criterion (AICc) of ModelTest version 3.7 (Posada & Crandall 1998) was used to select the most appropriate model of sequence evolution for each species. Consequently, for each species, all Φ statistics incorporated Tamura & Nei's (1993) model of sequence evolution and analyses for *M. novaezealandensis* further incorporated gamma-distributed substitution rate variation, with shape parameter $\alpha = 1.137$. Significance of genetic structure was determined via 10 000 nonparametric permutations (Excoffier *et al.* 1992).

The relationship between genetic ($\Phi_{ST}/1 - \Phi_{ST}$; Rousset 1997) and geographic surface distances among all populations was examined for each species using Mantel tests (Mantel 1967) with 1000 randomizations as implemented in the Isolation by Distance Web Service version 3.15 (Bohonak 2002; Jensen *et al.* 2005). As *Z. subcarinatus* cannot independently disperse over land while both parasites can presumably be transported by birds, an additional analysis was conducted for *Z. subcarinatus* using coastal distances between sites instead of surface (linear) distances. The results of this analysis were identical to the surface distance analysis (data not shown).

Migration rates among sample sites were estimated using the Bayesian inference method of Migrate version 3.0.3. (Beerli & Felsenstein 1999, 2001; Beerli 2006). Estimates incorporated the observed transition:transversion ratios obtained from Modeltest. Preliminary Migrate runs consisting of three replicate runs each with one long chain, uniform prior distribution, 2 000 000 visited and 100 000 recorded genealogies with a burn-in of 10 000, and an adaptive heating scheme with start

temperatures of 1.00, 1.50, 3.00 and 6.00 were used to estimate the boundaries of Θ ($2N_{ef}\mu$ for snails; N_{ef} = female effective population size, μ = mutation rate; and $2N_e\mu$ for trematodes; N_e = effective population size for haploid marker in hermaphroditic species) and M (m/μ; m = immigration rate) for exponential prior distributions in the final run for each species. Final Migrate runs for each species consisted of one long chain, an exponential prior distribution, 80 000 000 visited and 4 000 000 recorded genealogies with a burn-in of 50 000, and a static heating scheme with start temperatures of 1.00, 1.50, 3.00 and 6.00. Migration rates were then estimated as $N_{ef}m_i = 0.50 \times \Theta_i \times M_{j \to i}$, where i = receiving site and j = source site for snails and $N_em_i = 0.50 \times \Theta_i \times M_{j \to i}$ for trematodes. The 95% confidence intervals incorporated the 2.5% and 97.5% estimates of both Θ_i and $M_{j \to i}$. Due to computational limitations, a subset of two populations was selected to represent the Otago region along with Christchurch and Bluff in Bayesian analyses. Deborah Bay and Otakau were selected because they represent approximate average Otago region values for snail and trematode genetic differentiation from Christchurch and Bluff.

Results

A 900-bp fragment of the *COI* gene was analysed from a total of 330 *Zeacumantus subcarinatus* individuals, resulting in 23 polymorphic sites and 13 different haplotypes (GenBank accession numbers GQ868066–GQ868078). Summary statistics are presented in Table 1. Each of the three broad geographic regions (Otago, Bluff and Christchurch) possessed a different numerically dominant haplotype. These three haplotypes were separated by few mutations. Within the Otago region, the dominant haplotype was separated from two groups of less common haplotypes by seven and eight mutations. There was one reticulation in the haplotype network (Fig. 2). Overall, *Z. subcarinatus* was characterized by few haplotypes, and low haplotype and nucleotide diversity within collection sites, and several sites within the Otago region possessed only the most common haplotype (Table 1).

A 706-bp *COI* fragment was analysed from 269 *Maritrema novaezealandensis* individuals, resulting in 108 polymorphic sites and 141 different haplotypes (GenBank accession numbers GQ868102–GQ868242). There were no dominant, common haplotypes or geographic associations of haplotypes and all haplotypes were connected to another by 3 or fewer mutations. There were many alternative connections among haplotypes within the network (Fig. 2). *Maritrema novaezealandensis* sample sites typically possessed a large number of haplotypes and high haplotype and nucleotide diversities (Table 1).

The homologous 706-bp COI fragment used for *M. novaezealandensis* was analysed for 246 *Philophthalmus* sp. individuals, resulting in 22 polymorphic sites and 23 different haplotypes (GenBank accession numbers GQ868079–GQ868101). Four haplotypes were commonly observed (frequency ≥32, all other haplotypes <7), all haplotypes could be connected to another by three or fewer mutations, and the same haplotypes were often recovered from multiple geographic regions. There were no reticulations in the haplotype network (Fig. 2). *Philophthalmus* sp. collection sites possessed fewer haplotypes and lower haplotype and nucleotide diversities than *M. novaezealandensis* (Table 1).

The only species for which neutrality was consistently rejected by both Tajima's D and Fu's F_S tests was *M. novaezealandensis* (Table 2). Although comparatively fewer *M. novaezealandensis* collection sites were significant for Fu & Li's D^* test (6 out of 11, none with $P < 0.001$) than for Fu's F_S test (10 out of 11, 9 with $P < 0.001$) (Table 2), many tests were significant, suggesting that demographic and selective forces may be influencing the *COI* variation observed in *M. novaezealandensis*. Results from the combined location analyses for *Z. subcarinatus* and *Philophthalmus* sp. may have been influenced by the presence of genetic structure among their populations.

Significant genetic differentiation ($P < 0.0001$) was detected among samples collected over all geographic scales for *Z. subcarinatus* (Table 3). Forty-one out of 55 pairwise comparisons were significant ($P < 0.05$), including all comparisons involving Christchurch and Bluff (Table 4). Genetic differentiation was not detected among *M. novaezealandensis* samples over any geographic scale and only 3 out of 55 pairwise comparisons were significant ($P < 0.05$; Tables 3 and 4). For *Philophthalmus* sp., weak to moderate genetic differentiation was detected over all populations ($\Phi_{ST} = 0.026$, $P = 0.0372$) and to a lesser extent within the Otago region ($\Phi_{ST} = 0.023$, $P \sim 0.0500$), but not among the three distant regions ($\Phi_{CT} = 0.009$, $P = 0.2796$) or within Otago Harbour ($\Phi_{ST} = 0.006$, $P = 0.2708$) (Table 3). Seven out of 55 pairwise comparisons were significant ($P < 0.05$), four of which involved Papanui Inlet (Table 4).

A significant correlation between genetic and geographic distances was detected with the Mantel test for *Z. subcarinatus* ($r = 0.649$, $P < 0.001$), but not for *M. novaezealandensis* ($r = 0.189$, $P = 0.179$) or *Philophthalmus* sp. ($r = -0.132$, $P = 0.719$) (Fig. 3).

Mean migration estimates were broadly consistent with results obtained from AMOVA analyses. *Zeacumantus subcarinatus* had a low number of effective female migrants in all comparisons (Table 5) with the highest immigration between the two sites in Otago Harbour.

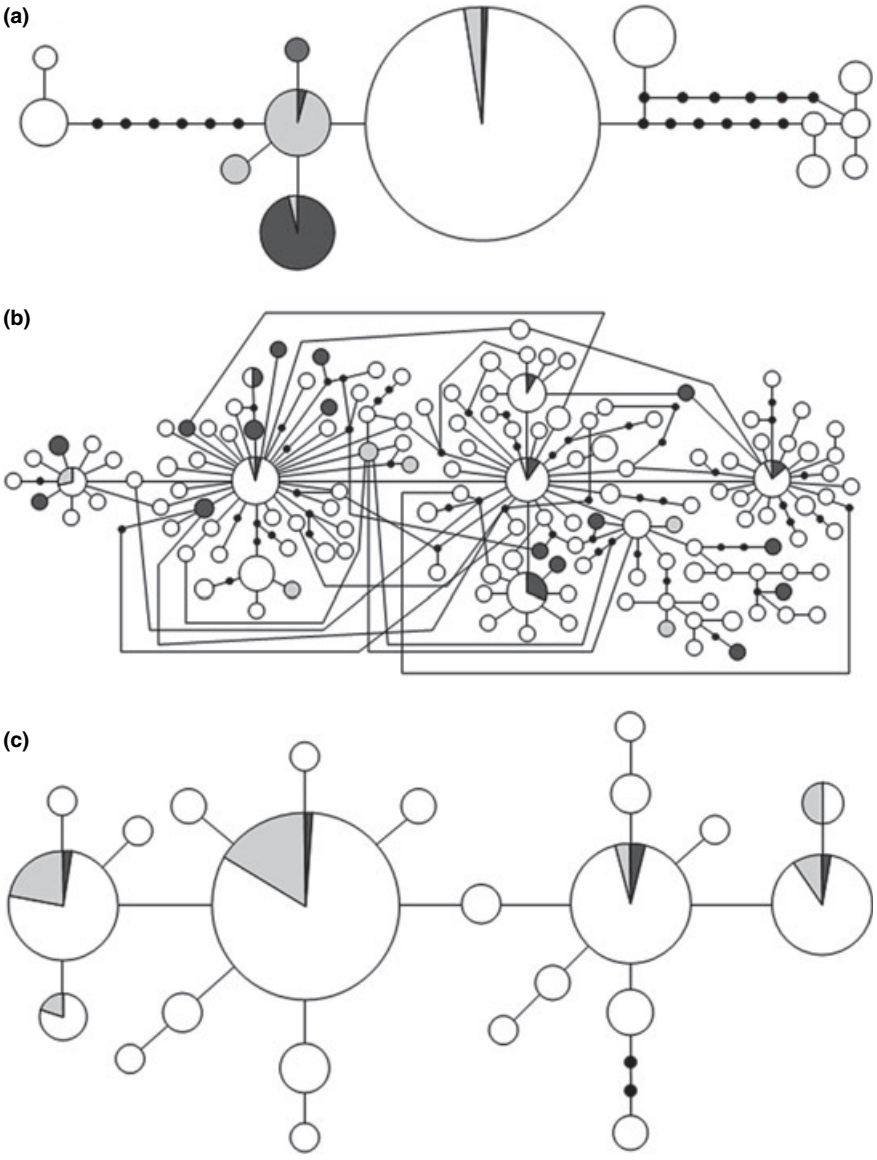

Fig. 2 Statistical parsimony haplotype networks. Haplotype frequencies are proportional to the area of the associated circle. Small black circles represent inferred intermediate haplotypes not observed in the data. All connections between haplotypes represent one mutation. The proportion of each haplotype recovered from each of the three geographical regions is represented by shading: Christchurch = dark grey, Otago = white, Bluff = light grey. (a) *Zeacumantus subcarinatus* (n = 330); (b) *Maritrema novaezealandensis* (n = 269); (c) *Philophthalmus* sp. (n = 246).

There was no strong evidence of skewed, unidirectional gene flow between any sites. In contrast, *M. novaezealandensis* was characterized by high migration among all sites. There was again no strong evidence of unidirectional gene flow between any sites, although estimates of migration into Bluff tended to be lower than out of Bluff. Migration estimates for *Philophthalmus* sp. were typically intermediate between *Z. subcarinatus* and *M. novaezealandensis*, with estimates into Bluff lower than estimates out of Bluff.

Discussion

Populations of the snail *Zeacumantus subcarinatus* displayed strong genetic differentiation and low levels of gene flow over all geographic scales incorporated in this study, consistent with expectations based on their direct development. The pattern of genetic differentiation and distribution of genetic diversity among *Z. subcarinatus* populations reveal that multiple factors have influenced their distribution and genetic makeup. The isolation by distance pattern indicates that dispersal is typically to proximate areas of suitable habitat, again consistent with the developmental strategy of the species as dispersal may often be dictated by the active movements of adults and/or juveniles given that there is no planktonic larval stage. Snail dispersal is rare even among proximate localities, as genetic differentiation was detected among bays separated by only a few kilometres within Otago Harbour. In addition, snails from

Table 2 Neutrality tests

Species & location	Tajima's D	Fu's F_S	Fu & Li's D^*
Zeacumantus subcarinatus			
Christchurch (1)	−1.003	−1.530	−0.263
Blueskin Bay (2)	1.416	8.422	1.324
Aramoana (3)	−2.447*	1.949	−4.049**
Deborah Bay (4)	−1.728*	2.668	−0.912
Sawyers Bay (5)	−2.025*	1.378	−2.363
Andersons Bay Inlet (6)	—	—	—
Company Bay (7)	—	—	—
Lower Portobello Bay (8)	0.600	2.113	0.938
Otakau (9)	−0.698	3.866	−2.363
Papanui Inlet (10)	—	—	—
Bluff Harbour (11)	−0.717	−1.093	−0.263
All locations combined	−1.367	−1.815	0.313
Maritrema novaezealandensis			
Christchurch (1)	−1.870*	−13.819**	−2.597*
Blueskin Bay (2)	−1.927*	−17.715**	−2.770*
Aramoana (3)	−1.553*	−3.775	−1.614
Deborah Bay (4)	−1.740*	−12.659**	−2.600*
Sawyers Bay (5)	−1.169	−6.127**	−1.300
Andersons Bay Inlet (6)	−1.748*	−13.052**	−2.429
Company Bay (7)	−1.774*	−12.782**	−1.931
Lower Portobello Bay (8)	−2.167*	−24.775**	−2.897*
Otakau (9)	−2.017*	−22.611**	−2.704*
Papanui Inlet (10)	−2.043*	−21.172**	−2.674*
Bluff Harbour (11)	−1.160	−3.749*	−1.441
All locations combined	−2.352**	−25.466**	−4.485*
Philophthalmus sp.			
Christchurch (1)	0.273	−1.012	0.273
Blueskin Bay (2)	0.000	0.693	+
Aramoana (3)	0.000	−0.341	+
Deborah Bay (4)	0.155	−1.540	−0.120
Sawyers Bay (5)	−0.818	−2.380	−1.211
Andersons Bay Inlet (6)	−0.517	−1.910	−1.211
Company Bay (7)	−0.340	−2.268	−1.455
Lower Portobello Bay (8)	−1.216	−4.069*	−1.589
Otakau (9)	0.213	−1.877	−0.577
Papanui Inlet (10)	−0.733	−2.892	−1.537
Bluff Harbour (11)	0.317	−0.120	0.439
All locations combined	−1.228	−10.284*	−1.674

Collection site numbers refer to Fig. 1. Populations possessing only a single haplotype are indicated with —. Samples sizes too small ($n < 4$) for Fu & Li's D^* test are indicated with +.
*Significant P-values [<0.05 for Tajima's D and Fu & Li's D^*, and <0.02 for Fu's F_S (see Fu 1997)].
**P-values <0.001.

two sites within the harbour and Papanui Inlet possessed only the most common haplotype, revealing a lack of influx of additional haplotypes following either colonization by and/or drift to fixation of the common haplotype. Genetic drift and/or founder effects may partially explain the lack of diversity within several Otago sites, although the dominance of the most common haplotype within the region also increases the likelihood of colonizing snails possessing it, as well as the pattern of dominance by specific haplotypes within each region. In addition, lowering of sea levels and associated changes in coastal habitat during glacial periods can decrease the genetic diversity of coastal gastropods (Kojima *et al*. 2004) and may partially explain the low haplotype diversity within each area, as the South Island of New Zealand experienced reduced sea levels during the last glacial maximum *c*. 20 000 years ago (Brown *et al*. 1968; Fleming 1979). The lack of genetic differentiation detected in pairwise comparisons between sites possessing only the dominant haplotype within the Otago region (Table 4) likely reflects the colonization of the common haplotype, and not high levels of gene flow.

In contrast to the isolation by distance pattern, long-distance dispersal may occasionally occur with *Z. subcarinatus* as several haplotypes were shared among distant regions (Fig. 2), representing either very low levels of contemporary gene flow or historic long-distance dispersal coupled with incomplete lineage sorting. In either scenario, dispersal has occurred over relatively large geographic scales, unless extirpation of intermediate populations has occurred. Evidence of relatively long-distance dispersal exists in other direct developing marine gastropods that otherwise display genetic differentiation over smaller geographic scales, and has been attributed to shipping (Miura *et al*. 2006) and possibly migratory birds (Wilke & Davis 2000).

Within the Otago region, three phylogenetically distinct groups of haplotypes were recovered, one of which was found in Christchurch and Bluff (Fig. 2). One explanation for this pattern is secondary admixture in Otago Harbour of haplotypes that evolved allopatrically. Haplotypes in one of the groups (group of five haplotypes on the right of Fig. 2) are phylogenetically more similar to a *Z. subcarinatus* haplotype recovered from Sydney, New South Wales, Australia (GenBank accession number AY296834; Colgan *et al*. 2000, 2003) than to other haplotypes in Otago Harbour, differing by only 1–2 out of 864 nucleotides. *Zeacumantus subcarinatus* is believed to have established itself in New South Wales, Australia from New Zealand via shipping (Powell 1979). Whether the present individuals arrived in Otago Harbour from Australia, from elsewhere in New Zealand or are related to the source individuals that colonized Australia cannot be determined as we do not know the genetic diversity of *Z. subcarinatus* in Australia or the remainder of New Zealand. The proposed shipping hypothesis does suggest that shipping may also contribute to *Z. subcarinatus* dispersal throughout New Zealand. Other dispersal mechanisms have been proposed for marine gastropods, including floating (Anderson 1971; Adachi & Wada 1999), drifiting (Martel & Chia 1991), rafting (Helmuth *et al*. 1994; Donald *et al*.

Table 3 Analysis of molecular variance for each species

Species and geographic analysis	Source of variation	d.f.	Percent of variation	Fixation indices	*P*-value
Zeacumantus subcarinatus					
Overall	Among sites	10	31.18	Φ_{ST} = 0.312	<0.0001
Sites 1–11	Within sites	319	68.82		
Among 3 regions	Among regions	2	39.42	Φ_{CT} = 0.394	<0.0001
Sites 1–11	Among sites within regions	8	9.52		
	Within sites	319	51.06		
Within Otago region	Among sites	8	13.82	Φ_{ST} = 0.138	<0.0001
Sites 2–10	Within sites	261	86.18		
Within Otago Harbour	Among sites	6	12.05	Φ_{ST} = 0.121	<0.0001
Sites 3–9	Within sites	203	87.95		
Maritrema novaezealandensis					
Overall	Among sites	10	0.14	Φ_{ST} = 0.001	0.3783
Sites 1–11	Within sites	258	99.86		
Among 3 regions	Among regions	2	0.48	Φ_{CT} = 0.005	0.2063
Sites 1–11	Among sites within regions	8	0.00		
	Within sites	258	99.52		
Within Otago region	Among sites	8	0.01	Φ_{ST} = 0.000	0.4546
Sites 2–10	Within sites	219	99.99		
Within Otago Harbour	Among sites	6	0.13	Φ_{ST} = 0.001	0.3988
Sites 3–9	Within sites	161	99.87		
Philophthalmus sp.					
Overall	Among sites	10	2.63	Φ_{ST} = 0.026	0.0372
Sites 1–11	Within sites	235	97.37		
Among 3 regions	Among regions	2	0.93	Φ_{CT} = 0.009	0.2796
Sites 1–11	Among sites within regions	8	2.35		
	Within sites	235	96.72		
Within Otago region	Among sites	8	2.27	Φ_{ST} = 0.023	0.0500*
Sites 2–10	Within sites	202	97.73		
Within Otago Harbour	Among sites	6	0.58	Φ_{ST} = 0.006	0.2708
Sites 3–9	Within sites	173	99.42		

Collection site numbers refer to Fig. 1.
**P*-value ranged from 0.045 to 0.055 in multiple runs with 10 000 permutations.

2005) and dispersal by migrating birds and fish (Drake & Arias 1995; Aarnio & Bonsdorff 1997), but we currently have no evidence for these mechanisms operating with *Z. subcarinatus*. In addition, genetic bottlenecks may have removed intermediate haplotypes within the Otago region, and representatives of additional *COI* clades may exist in the Christchurch and Bluff areas as their sample sizes were relatively small compared to the Otago region.

The overall lack of or low genetic structure and high levels of gene flow in parasite populations compared to their snail hosts is likely due to dispersal via migratory bird hosts (Dybdahl & Lively 1996; Miura *et al.* 2006; Keeney *et al.* 2008). Depending on the trematode species, birds can create parasite gene flow by passing parasite eggs with faeces, tears, etc. during movements among proximate locations while feeding or over larger geographic areas during migrations. Despite the movements of bird hosts, *Philophthalmus* sp. did display some genetic structure, although this was driven largely by Papanui Inlet. This species did not display an isolation by distance pattern, suggesting that dispersal is not consistently limited by geographic distance, a pattern consistent with dispersal by birds. This raises the question of why one trematode that utilizes birds displays genetic structure while another does not. If the adults of one species are shorter-lived or utilize a narrower range of second intermediate hosts, they may display more genetic differentiation within snails (Miura *et al.* 2006). In the present study, the geographic scale of sampling would not likely require long survival periods and both species utilize a range of second intermediate hosts.

Factors that may be important in our system are discrepancies in the effective population sizes of the parasites, the resulting differences in genetic diversity and the amount of host migration required to create genetic homogeneity. Several hundred to 1000 *Maritrema novaezealandensis* can be found within a single crab second intermediate host (Martorelli *et al.* 2004) and few of these are genetically identical clones (Keeney *et al.*

Table 4 Pairwise analysis of molecular variance estimations

	Cch (1)	Blue Bay (2)	Ara (3)	Deb Bay (4)	Saw Bay (5)	ABI (6)	Comp Bay (7)	LPB (8)	Otakau (9)	Papa Inlet (10)	Bluff (11)
Cch (1)		0.000/0.425	−0.006/0.490	0.003/0.337	−0.024/0.802	0.003/0.329	0.007/0.200	−0.003/0.577	0.010/0.158	−0.001/0.450	0.004/0.351
	—	0.168/0.322	−0.146/0.824	−0.055/0.681	0.065/0.211	−0.051/0.660	−0.084/0.846	−0.094/0.961	−0.111/0.999	−0.116/0.999	0.051/0.231
Blue Bay (2)	0.479/<0.001*		−0.002/0.433	−0.010/0.794	−0.005/0.496	0.031/0.011*	−0.009/0.743	−0.013/0.949	−0.006/0.674	0.006/0.280	0.036/0.083
		—	−0.199/0.806	0.053/0.290	0.056/0.225	0.126/0.157	0.072/0.250	0.156/0.150	0.091/0.340	0.248/0.073	−0.018/0.431
Ara (3)	0.664/<0.001*	0.108/0.051		−0.020/0.748	−0.003/0.521	−0.022/0.799	−0.022/0.773	−0.022/0.883	−0.013/0.719	−0.025/0.916	−0.011/0.569
			—	−0.08	−0.044/0.469	−0.045/0.537	−0.133/0.820	−0.062/0.557	−0.115/0.753	−0.030/0.533	−0.026/0.458
Deb Bay (4)	0.549/<0.001*	0.055/0.074	−0.004/0.471		0.002/0.378	0.029/0.021*	−0.017/0.951	−0.010/0.829	−0.002/0.482	0.009/0.187	0.015/0.204
				—	0.011/0.259	−0.012/0.532	−0.008/0.440	0.010/0.226	−0.001/0.374	0.071/0.031*	0.000/0.371
Saw Bay (5)	0.707/<0.001*	0.154/0.006*	−0.017/0.745	−0.001/0.409		−0.008/0545	0.012/0.279	−0.002/0.464	0.004/0.348	−0.011/0.658	0.014/0.286
					—	0.012/0.209	0.020/0.170	0.060/0.024*	0.062/0.033*	0.157/0.002*	−0.005/0.453
ABI (6)	0.882/<0.001*	0.207/0.015*	0.004/0.501	0.063/0.015*	0.024/0.216		0.026/0.026*	0.013/0.124	0.020/0.062	−0.014/0.877	−0.012/0.627
						—	−0.003/0.390	−0.015/0.691	0.011/0.236	0.058/0.034*	0.034/0.124
Comp Bay (7)	0.882/<0.001*	0.207/0.007*	0.004/0.499	0.063/0.022*	0.024/0.239	0.000/0.999		−0.009/0.822	−0.001/0.455	0.005/0.247	0.043/0.067
							—	0.003/0.316	−0.0274725	0.031/0.114	0.020/0.183
LPB (8)	0.518/<0.001*	0.173/<0.001*	0.102/0.023*	0.071/0.030*	0.095/0.044*	0.190/0.003*	0.190/0.004*		−0.0076104	−0.007728	0.003/0.376
								—	0.001/0.352	0.010/0.229	0.080/0.015*
Otakau (9)	0.630/<0.001*	0.185/<0.001*	0.198/<0.001*	0.073/0.048*	0.172/0.003*	0.329/<0.001*	0.329/<0.001*	0.153/<0.001*		0.002/0.371	0.022/0.129
									—	0.003/0.303	0.052/0.064
Papa Inlet (10)	0.882/<0.001*	0.207/0.011*	0.004/0.495	0.063/0.020*	0.024/0.246	0.000/0.999	0.000/0.999	0.190/0.002*	0.329/<0.001*		0.009/0.286
										—	0.156/<0.001*
Bluff (11)	0.594/<0.001*	0.270/<0.001*	0.413/<0.001*	0.311/<0.001*	0.474/<0.001*	0.708/<0.001*	0.708/<0.001*	0.349/<0.001*	0.461/<0.001*	0.708/<0.001*	
											—

Collection site abbreviations and numbers refer to Fig. 1. Values are Φ_{ST} estimates/*P*-values. For each comparison between localities, values in the lower matrix are for *Zeacumantus subcarinatus* and values in the upper matrix are for the trematodes (*Maritrema novaezealandensis* is listed above *Philophthalmus* sp. in each comparison).
**P*-values less than 0.05.

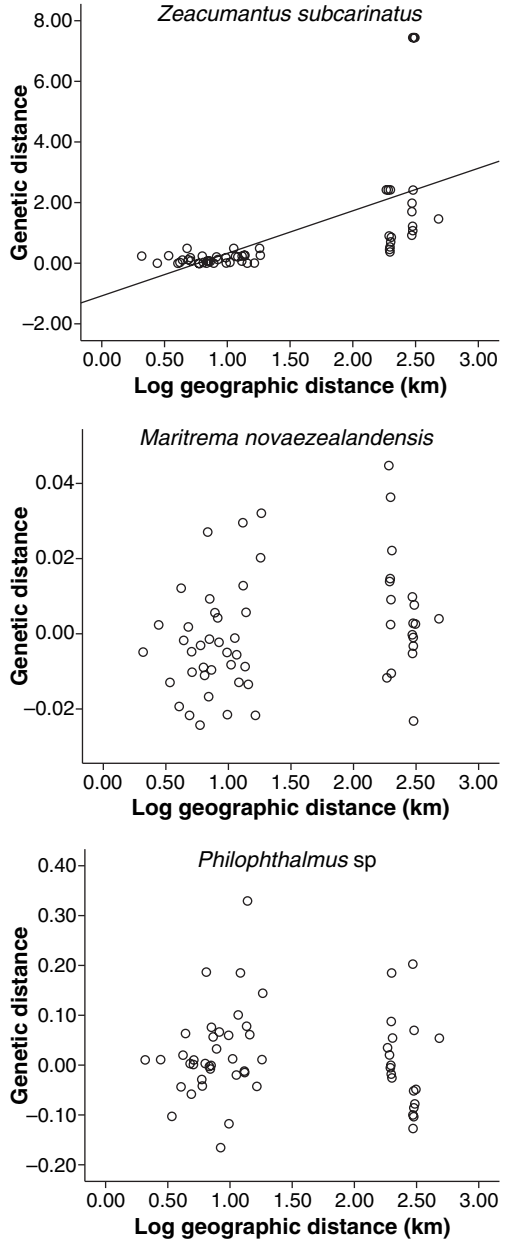

Fig. 3 Relationships between pairwise geographical distances and genetic distances ($\Phi_{ST}/1 - \Phi_{ST}$).

2007). Birds are therefore consuming hundreds of genetically different *M. novaezealandensis* when they eat an infected crab, and prevalence in three crab species collected in the Otago region was 80–100% (Martorelli *et al.* 2004). The digestive system of birds is likely to contain numerous genetically distinct *M. novaezealandensis* capable of reproducing with each other. This would facilitate the production and maintenance of genetically diverse trematode populations with large effective population sizes. To date, only 16 red-billed gulls (*Larus novaehollandiae scopulinus*) have been examined for *M. novaezealandensis*, which was found in 56% of the birds with infection intensities reaching 10 individuals (Fredensborg *et al.* 2004). However, a larger survey, including additional host species may reveal increased infection intensities. Although we do not have data on the intensity of infection of *Philophthalmus* sp. in our system, infection intensities of philophthalmids are typically lower than microphallids within shorebirds, with often only one or two philophthalmid individuals infecting an eye (Sanmartín *et al.* 2005; Álvarez *et al.* 2006). This could result in lower *Philophthalmus sp.* genetic diversity and smaller effective population sizes compared to *M. novaezealandensis*, and may even increase self-fertilization in philophthalmids, a strategy that may be a last resort in intestinal trematodes (Criscione & Blouin 2006) such as *M. novaezealandensis*. Differences in generation times for the two trematodes could also influence their effective population sizes and genetic diversity, but we do not have estimates for our species. Because our migration estimates reflect the products of effective population sizes and migration rates, the discrepancies in effective population sizes may in large part explain the differences in mean estimates of migration for the two trematodes. In addition, less gene flow may occur with *Philophthalmus* sp. than *M. novaezealandensis* per bird host due to lower prevalence, infection intensities, or differences in the dispersal effectiveness of eggs shed from the eyes or nasal passages by species of *Philophthalmus* (Alicata 1962) compared to eggs shed in faeces by *M. novaezealandensis*. However, differences in the population genetic patterns of the two trematodes were slight, and cannot be unambiguously linked with their life histories.

Because we could not reject the influence of demography or natural selection on the *COI* variation present in *M. novaezealandensis*, it is possible that the genetic homogeneity is misleading. However, we have confidence that there is a large extent of *M. novaezealandensis* gene flow among the sites included in this study as genetic homogeneity was also detected with microsatellite markers among three sites within Otago Harbour ($F_{ST} = 0.000$, $P = 0.953$; Keeney *et al.* 2008) as well as between Otago Harbour and Christchurch ($F_{ST} = 0.000$, $P = 0.882$; D. B. Keeney, unpublished). Discrepancies between the genetic structure of snails and parasites, or between the two trematode species, could also reflect the smaller influence of genetic drift on trematode populations with large effective population sizes (Table 5) and longer times to drift-migration equilibrium (Dybdahl & Lively 1996).

The differences in genetic differentiation and gene flow between the snail host and its trematodes could have important implications for their evolution. The relative rate of host and parasite gene flow influences the

Table 5 Bayesian estimates of Θ and pairwise migration rates ($N_{ef}m$ for snails and N_em for trematodes)

Sites	*Zeacumantus subcarinatus*	*Maritrema novaezealandensis*	*Philophthalmus* sp.
Cch (1) Θ	0.0002 (0.0000, 0.0018)	0.0651 (0.0000, 0.1820)	0.0154 (0.0000, 0.0520)
Deb Bay (4) Θ	0.0025 (0.0000, 0.0075)	0.0781 (0.0000, 0.2100)	0.0217 (0.0000, 0.0640)
Otakau (9) Θ	0.0011 (0.0000, 0.0035)	0.1038 (0.0000, 0.2300)	0.0178 (0.0000, 0.0560)
Bluff (11) Θ	0.0007 (0.0000, 0.0028)	0.0373 (0.0000, 0.1500)	0.0013 (0.0000, 0.0140)
Cch → Deb Bay	2.00 (0.00, 16.88)	120.58 (0.00, 918.75)	58.08 (0.00, 440.00)
Cch ← Deb Bay	0.40 (0.00, 8.31)	116.96 (0.00, 966.88)	36.36 (0.00, 341.25)
Cch → Otakau	1.00 (0.00, 8.75)	152.26 (0.00, 1078.13)	47.10 (0.00, 385.00)
Cch ← Otakau	0.36 (0.00, 7.66)	100.30 (0.00, 910.00)	37.00 (0.00, 357.50)
Cch → Bluff	1.40 (0.00, 12.72)	40.64 (0.00, 562.50)	2.20 (0.00, 74.38)
Cch ← Bluff	0.54 (0.00, 10.50)	77.48 (0.00, 739.38)	57.95 (0.00, 455.00)
Deb Bay → Otakau	2.72 (0.00, 19.69)	210.68 (0.00, 1293.75)	43.76 (0.00, 367.50)
Deb Bay ← Otakau	5.82 (0.00, 39.38)	163.62 (0.00, 1378.13)	54.02 (0.00, 420.00)
Deb Bay → Bluff	1.10 (0.00, 10.31)	50.94 (0.00, 703.13)	2.04 (0.00, 65.63)
Deb Bay ← Bluff	2.46 (0.00, 20.63)	108.16 (0.00, 918.75)	93.78 (0.00, 580.00)
Otakau → Bluff	0.96 (0.00, 9.63)	51.82 (0.00, 703.13)	2.00 (0.00, 65.63)
Otakau ← Bluff	1.18 (0.00, 10.06)	138.50 (0.00, 1078.13)	72.08 (0.00, 490.00)

Values are mean (95% confidence interval). Collection site abbreviations and numbers refer to Fig. 1. Arrows indicate direction of migration for each estimate.

evolution of local adaptations in these organisms (Dybdahl & Lively 1996; Gandon *et al.* 1996; Lively 1999; Gandon & Michalakis 2002; Prugnolle *et al.* 2005). The low level of gene flow among snail populations indicates that snails will be able to adapt locally to selective pressures that arise from parasitism by the trematodes. Indeed, local adaptations to parasitism have been detected among these snail populations over relatively small geographic distances, with populations experiencing higher trematode prevalences maturing earlier (Fredensborg & Poulin 2006). Our results therefore represent a demonstrable agreement between genetic and ecological patterns observed in nature. Extremely limited snail gene flow could decrease their ability to adapt to changes in selective pressures from specific parasite genotypes as new genetic variation is rarely introduced into snail gene pools (Gandon & Michalakis 2002), but trematode genetic homogeneity would not make this a major problem for responding to parasite loads. In contrast, genetic homogeneity suggests that the trematodes are not as likely to adapt locally, unless local selective pressures are strong enough to overcome extensive gene flow (Dybdahl & Lively 1996). The relatively small difference in genetic structure detected between trematode species makes any statements regarding the ability of *Philophthalmus* sp. to respond to local selective pressures more efficiently than *M. novaezealandensis* highly speculative.

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