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RESEARCH ARTICLE

Local variation of within-host clonal diversity coupled with genetic homogeneity in a marine trematode

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Abstract Despite their ubiquity and importance to intertidal ecosystems, information is currently lacking regarding the genetic diversity of trematode parasites within coastal organisms and the distribution of their genetic variation among intertidal habitats. In this study, we quantified the clonal diversity of the coastal marine trematode *Maritrema novaezealandensis* within *Zeacumantus subcarinatus* snail hosts from three coastal bays in Otago Harbour, New Zealand, using five microsatellite loci to determine if differences exist in the frequency of occurrence of multi-clone infections. In addition, we examined gene flow among *M. novaezealandensis* collected from the three bays. The frequency of mixed-clone infections varied fourfold among bays and no genetic differentiation was detected among intertidal bays. Across the coastal bays studied, *M. novaezealandensis* comprises a single population that is potentially infecting multiple *Z. subcarinatus* populations with varying life history traits.

Introduction

Trematodes are the most common metazoan parasites of animals within intertidal ecosystems (Mouritsen and Poulin 2002) and their abundance can have major impacts on intertidal communities. Trematodes can influence the mortality, behavior, morphology, abundance, and life history trait evolution of their hosts (Sousa 1983; Sorensen and Minchella 2001; Mouritsen and Poulin 2002; Fredensborg and Poulin 2006; Miura et al. 2006) as well as the distribution and abundance of non-host organisms (Poulin 1999; Mouritsen and Poulin 2005), including macroalgae (Wood et al. 2007). Their ubiquity, abundance, and importance within these systems suggest that trematodes may be a key factor determining the overall structure and biodiversity of intertidal communities (Thomas et al. 1997; Poulin 1999; Mouritsen and Poulin 2002; Miura et al. 2006; Wood et al. 2007). Despite their importance, very little information currently exists regarding the genetic diversity and connectivity of trematodes within intertidal ecosystems.

The vast majority of digenetic trematodes utilize molluscs, typically gastropods, as first intermediate hosts (Gibson and Bray 1994; Esch et al. 2001). Within these hosts, individual trematode miracidia multiply asexually to form numerous rediae or sporocysts in which thousands of infective cercariae are produced. In many snail–trematode systems, the hosts are castrated and larval trematodes completely replace host gonadal tissue (Baudoin 1975; Kuris and Lafferty 1994; Probst and Kube 1999; Sorensen and Minchella 2001), making each snail a limiting resource for which competition can develop (Kuris 1990; Sousa 1992; Lafferty et al. 1994). Although few host species have been examined, the available data suggest that multiple genetic clones commonly exist within snail first intermediate hosts across a variety of ecosystems (e.g. Minchella et al. 1995; Davies et al. 1999; Jarne and Theron 2001; Eppert et al. 2002; Rauch et al. 2005; Keeney et al. 2007). The factors influencing the within-host genetic diversity of trematodes remain unknown.

The marine trematode *Maritrema novaezealandensis* is found throughout coastal areas on the South Island of New Zealand and it is one of the most common trematode parasites within Otago Harbour. This trematode uses the snail

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Zeacumantus subcarinatus as a first intermediate host, several crustaceans as second intermediate hosts, and shorebirds as definitive hosts (Martorelli et al. 2004). Sexual reproduction occurs within the bird's gastrointestinal tract and fertilized eggs are passed into the environment with the bird's feces. *M. novaezealandensis* eggs are accidentally consumed by snails while they are grazing on algae and sediment.

The extent of genetic structure among parasite populations is a key factor behind evolutionary processes such as local adaptation and speciation (Criscione et al. 2005; Huyse et al. 2005; Thompson 2005). However, estimates of gene flow among coastal trematode populations within intermediate hosts are currently lacking, with only one study examining the genetic structure of marine trematodes within fish definitive hosts (Vilas et al. 2004). Vilas et al. (2004) detected only moderate genetic structure among *Lecithochirium fusiforme* sampled from conger eels (*Conger conger*) collected from Portugal to France despite the relatively low mobility of all hosts utilized in *L. fusiforme*'s life cycle. They proposed that gene flow via the mobility of paratenic hosts (small fish) or possible natural selection on the allozyme loci utilized may have influenced population structure estimates. Within Otago Harbour, it is likely that the vagility of bird definitive hosts will produce high levels of gene flow among *M. novaezealandensis* from different coastal bays, but this remains to be tested.

In this study, we examine the genetic diversity of *M. novaezealandensis* within *Z. subcarinatus* first intermediate hosts collected from three Otago Harbour bays approximately 3–7 km apart to determine if differences exist in the frequency of occurrence of multi-clone infections. We examine host size and parasite genetic diversity within each bay to assess whether *Z. subcarinatus* accumulate *M. novaezealandensis* clonal infections over time within any of the bays. In addition, we determine if the proximity of bays and potential for gene flow in this study has produced genetic homogeneity of *M. novaezealandensis* among intertidal bays.

Materials and methods

Host and parasite collection

Approximately 200 *Z. subcarinatus* snails were collected during low tides from the following three coastal bays in Otago Harbor, South Island, New Zealand: Lower Portobello Bay (LPB), Turnbull Bay (TB), and Company Bay (CB) in November 2005 in an attempt to obtain approximately 20 snails from each site infected with *M. novaezealandensis*. After initial screening failed to produce a sufficient number of infected snails from TB, an additional 200 snails were collected from this site in January 2006. These areas were selected because a previous study had identified large differences in overall trematode prevalence within *Z. subcarinatus* from these sites (Fredensborg and Poulin 2006). The maximum shell length of each snail was measured with Vernier calipers to the nearest 0.1 mm (mean values are followed by standard deviation) and snails were dissected to screen for *M. novaezealandensis* sporocysts. Snail measurements were taken to ensure consistency of snail sizes among bays, as Keeney et al. (2007) detected the highest prevalence of multiple *M. novaezealandensis* clones within intermediate-sized snails. Only sporocysts from snails possessing *M. novaezealandensis* single-species infections were retained for this study. However, the presence and identity of other trematode species were recorded for each infected snail. Using a stereomicroscope, individual sporocysts were isolated with dissecting pins, rinsed twice in 60 mm Petri dishes containing 0.22 µm filtered fresh water, and placed into 1.5 ml tubes for DNA extraction.

DNA extraction and PCR amplification

DNA was extracted from *M. novaezealandensis* sporocysts by placing individual sporocysts in 400 µl of 5% chelex containing 0.1 mg/ml proteinase K, incubating at 60°C for 2 h and boiling at 100°C for 8 min. The genotypes at five microsatellite loci (Mno-1, Mno-28, Mno-30, Mno-45 and Mno-47) were determined for 20 individual *M. novaezealandensis* sporocysts collected from each of 21 snails in each locality as described by Keeney et al. (2006, 2007). A previous study by Keeney et al. (2007) did not detect any additional trematode genotypes within snails once 20 sporocysts had been sampled and our estimates of the clonal diversity of *M. novaezealandensis* likely reflect the true diversity. Although we cannot rule out that low-copy parasite genotypes within snails are occasionally missed, this error should be consistent among bays.

Trematode clone identification

Identical multilocus genotypes were identified with the program GENALEX 6 (Peakall and Smouse 2006). The probabilities of observing at least as many identical trematode genotypes by chance via sexual reproduction (P_{sex}-value) were calculated using the program MLGsim (Stenberg et al. 2003). A corresponding critical P_{sex}-value was generated with 1×10^6 simulations and trematode sporocysts possessing identical genotypes with P_{sex}-values below this were considered true clones.

Statistical analyses

The following statistical analyses were performed with SPSS 13.0 for Windows. Snail total lengths from each of

the three bays were tested for deviations from normality and equality of variances with the Kolmogorov–Smirnov test and Levene's test of homogeneity of variances, respectively. Differences in mean snail length among bays were examined using a one-way ANOVA. The mean number of *M. novaezealandensis* genotypes within infected snails was compared among the three bays using a Kruskal–Wallis test. The strength of the relationship between snail length and number of trematode genotypes was examined using Kendall's rank-order correlation (T) within each bay and over all bays. The following analyses used unique genotypes within snails only, as identical trematode clones within snails are the products of asexual reproduction. Trematode genetic diversity was estimated within individual bays and over all bays by calculating the number of alleles per locus and estimating gene diversity using Nei's (1987) unbiased estimator of H_S with FSTAT version 2.9.3 (Goudet 1995). To test for deviations from Hardy–Weinberg equilibrium (HWE), Weir and Cockerham's (1984) f estimator of F_{IS} was calculated for each locus within each bay and over all bays, and significance values determined with 1,500 randomizations using FSTAT version 2.9.3 (Goudet 1995). Tests of genotypic disequilibrium between all pairs of loci within bays (locally) and over all bays (globally) were conducted with FSTAT version 2.9.3 (Goudet 1995) using 3,000 randomizations. Alpha significance values were corrected for multiple pairwise comparisons within bays for tests of HWE deviations and genotypic disequilibrium using the sequential Bonferroni approach (Rice 1989) (initial significance determined at $P \leq 0.05/5 = 0.010$, $P \leq 0.05/4 = 0.013$, and $P \leq 0.05/12 = 0.004$ for HWE, local disequilibrium, and global disequilibrium tests, respectively).

Genetic structure among intertidal bays

Genetic differentiation of parasite populations among bays was analyzed using two approaches. First, individual parasites were clustered into populations based on their microsatellite genotypes using the Bayesian approach of STRUCTURE version 2.2 (Pritchard et al. 2000). The number of populations (K) was allowed to vary from 1 to 3 and 10 replications were run for each K. The admixture-ancestry model with α inferred and correlated allele-frequency model with $\lambda = 1$ were used for all simulations. Each simulation was run with a burn-in of 50,000 iterations and a Markov chain Monte Carlo of 100,000 iterations. Second, individual parasites were classified into three populations based on their sampling site, Weir and Cockerham's (1984) θ estimator of F_{ST} was calculated over all loci and 95% confidence intervals were obtained by bootstrapping over loci with FSTAT version 2.9.3 (Goudet 1995). Genetic differentiation among bays was examined using the G-based test (Goudet et al. 1996) in FSTAT with 10,000 permutations of genotypes among sites. Pairwise θ values were also calculated between all pairs of collection sites with FSTAT.

Results

A total of 200 (size range 10.9–17.4 mm, mean = 13.3 ± 1.1 SD), 208 (size range 10.0–19.7 mm, mean = 13.8 ± 1.5 SD), and 407 (size range 9.3–19.1 mm, mean = 13.0 ± 1.5 SD) snails were dissected from LPB, CB, and TB, respectively. Prevalences of *M. novaezealandensis* within snails at each site were 95.5 (LPB), 18.8 (CB), and 7.9% (TB). Five additional trematode species were recovered from snails and their total prevalences at each site were 24.5 (LPB), 16.3 (CB), and 27.5% (TB). The frequencies of mixed-species infections involving *M. novaezealandensis* and one of these additional trematode species were 23.0 (LPB), 7.7 (CB), and 18.8% (TB). The resulting percentages of snails uninfected by any trematode species at each site were 2.0% (LPB), 66.3% (CB), and 65.8% (TB). Twenty-one snails infected only with *M. novaezealandensis* from each site were haphazardly selected for genetic analyses. Summary statistics for snails whose trematodes were used for genetic analyses are provided in Table 1. Host sizes for genetic samples did not deviate significantly from normality for any of the three sites (Kolmogorov–Smirnov tests, $P > 0.05$) and Levene's test supported homogeneity of variances ($P = 0.684$). No significant difference was detected among mean size of snails used for genetic analyses from the three sample sites (one-way ANOVA, $F_{2,60} = 1.41$, $P = 0.252$).

The genotypes of 1,260 *M. novaezealandensis* sporocysts were determined for five loci (20 sporocysts × 21 snails × 3 sites). Summary data for microsatellites are provided in Table 2. None of the loci deviated significantly from HWE within any of the bays after Bonferroni corrections [locus Mno-45 was nominally significant for heterozygote deficiency in all bays: LPB ($P = 0.043$), CB ($P = 0.038$), and TB ($P = 0.014$)], but locus Mno-45 deviated significantly from HWE over all bays ($P < 0.001$). Genotypic disequilibrium was not detected between any loci at any level of analysis. Eighty-two *M. novaezealandensis* genotypes were identified and 95% of the distinct genotypes differed by ≥2 alleles. No identical sporocyst clones were recovered from different snails. MLGsim results indicated that sporocysts with identical genotypes were true genetic clones (P_{sex}-values < 1.94×10^{-6} critical value). The mean number of *Maritrema* clones detected within snails differed significantly among sites (Kruskal–Wallis test, $H = 6.78$, $df = 2$, $P = 0.034$) and was 1.52 ± 0.68 for LPB, 1.29 ± 0.72 for CB, and 1.10 ± 0.30 for TB.

Table 1 Summary of characteristics for *Zeacumantus subcarinatus* hosts whose *Maritrema novaezealandensis* were used in genetic analyses

	LPB	CB	TB	Overall
Prevalence (%)	95.5	18.8	7.9	32.1
Sample size	21	21	21	63
Length range	12.0–16.4	11.9–15.0	11.1–16.7	11.1–16.7
Length mean ± SD	13.5 ± 1.1	13.4 ± 1.1	13.0 ± 1.2	13.3 ± 1.1
Total # clones	32	27	23	82
Mean # clones ± SD	1.52 ± 0.68	1.29 ± 0.72	1.10 ± 0.30	1.30 ± 0.61
Mixed-clones (%)	42.9	19.0	9.5	23.8

"Clones" refers to the number of distinct *M. novaezealandensis* clones within hosts. The mean number of clones per snail (mean # clones) has been calculated using infected snails only. *SD* standard deviation, *LPB* Lower Portobello Bay, *CB* Company Bay, and *TB* Turnbull Bay

Table 2 Microsatellite data for each locus and collection site

Site	Parameter	Mno-1	Mno-28	Mno-30	Mno-45	Mno-47	All loci
LP	*A*	16	6	6	3	6	37
	H_S	0.731	0.731	0.645	0.491	0.622	0.644
	f	0.102	-0.026	0.128	0.300	0.096	0.107
CB	*A*	22	8	5	4	7	46
	H_S	0.899	0.769	0.675	0.503	0.618	0.693
	f	0.052	−0.108	0.013	0.337	0.041	0.048
TB	*A*	19	6	6	4	6	41
	H_S	0.706	0.810	0.705	0.419	0.529	0.634
	f	−0.109	−0.073	−0.049	0.481	−0.069	−0.002

A number of alleles, H_S gene diversity (all loci = average across loci), *f* Weir and Cockerham's (1984) estimator of F_{IS}, *LPB* Lower Portobello Bay, *CB* Company Bay, and *TB* Turnbull Bay

The percentages of infected snails with more than one *M. novaezealandensis* genotype were 42.9, 19.0, and 9.5 for LPB, CB, and TB, respectively. Mixed-clone infections were found within snails 12.0–14.3 mm long, including one snail from CB that was 14.3 mm long and possessed four genotypes (Fig. 1). This was the only snail longer than 14 mm ($n = 16$; four from LP, eight from CB, and four from TB) that harbored more than one *M. novaezealandensis* genotype and the eight largest snails (three from LPB, four from CB, and one from TB) all possessed a single *M. novaezealandensis* genotype. No significant correlation was detected between snail length and number of trematode genotypes overall ($T = -0.031$, $P = 0.767$) or within any individual bay ($T = -0.146$ to -0.025, $P = 0.426$–0.893). Although the data include only three sites and a formal correlation test is not appropriate, a clear association among sites could still be seen between the prevalence of *M. novaezealandensis* and the percentage of infected snails harboring multi-cone infections (Fig. 2).

The probabilities for each *K* (number of populations) in Bayesian clustering analyses were similar for each replication, with $K = 1$ having the highest probability (values are mean ± standard deviation): Pr $(K = 1) = 0.955 \pm 0.076$

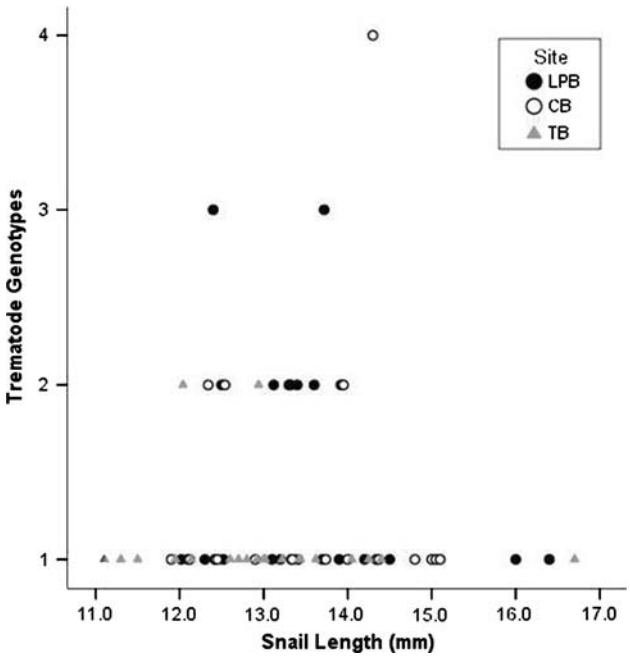

Fig. 1 *Zeacumantus subcarinatus* shell size versus number of *Maritrema novaezealandensis* clones for each sample site. *LPB* Lower Portobello Bay, *CB* Company Bay, and *TB* Turnbull Bay

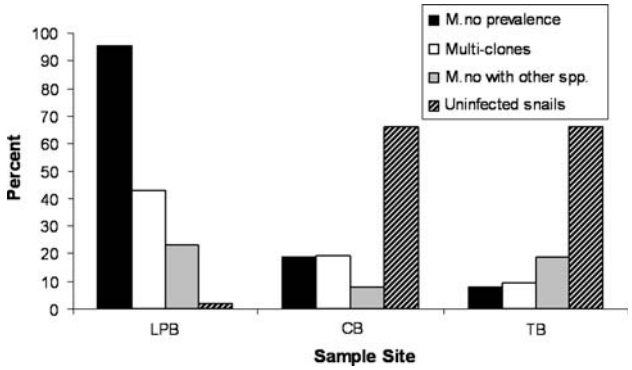

Fig. 2 Prevalence of *Maritrema novaezealandensis* infections in *Zeacumantus subcarinatus* (M. no prevalence), the percentage of infected *Z. subcarinatus* possessing multiple *M. novaezealandensis* genetic clones (Multi-clones), the percent of *M. novaezealandensis*-infected snails infected with additional trematode species (M. no with other spp.), and the percentage of *Z. subcarinatus* not infected by any trematode species (Uninfected snails) in each sample site. *LPB* Lower Portobello Bay, *CB* Company Bay, and *TB* Turnbull Bay

versus Pr $(K = 2) = 0.004 \pm 0.006$, and Pr $(K = 3) = 0.041 \pm 0.077$. In addition, with $K = 2$, the inferred ancestry of all individuals was approximately 50% for both populations and with $K = 3$, the inferred ancestry of all individuals was approximately 33% for each population, indicating a lack of genetic population structure and an inability to assign individuals to specific populations, when $K > 1$. Significant genetic differentiation was not detected among sites with F_{ST} estimates [$\theta = 0.000$ (95% confidence interval = −0.011, 0.009), $P = 0.953$ ($P \geq 0.426$ for any individual locus)] or between any pair of collection sites: LPB versus CB, $\theta = 0.000$, $P = 0.767$; LPB versus TB, $\theta = 0.002$, $P = 0.783$; and CB versus TB, $\theta = 0.001$, $P = 0.993$. Removal of locus Mno−45 due to deviations from HWE did not alter genetic differentiation results ($\theta = 0.001$, $P = 0.921$; bootstrapping not possible with four loci).

Discussion

Trematode clonal diversity within snails

This study detected significant differences in the frequency of mixed-clone infections of the trematode *M. novaezealandensis* within the snail first intermediate host *Z. subcarinatus* among relatively proximate coastal bays in Otago Harbour. The frequency of mixed-clones was positively associated with parasite prevalence within bays. The association between prevalence and frequency of mixed-clone infections likely reflects that the factors influencing trematode prevalence (i.e. frequency of snails encountering trematode eggs and parasite successfully establishing within snails), such as shorebird abundance (Fredensborg et al. 2006), in these areas would also affect the frequency with which snails encounter multiple trematode eggs and multiple parasites initially establish within the snail hosts. Snails from the bay with the highest *M. novaezealandensis* prevalence (LPB) also had the highest mean number of genotypes per infected snail, similar to results obtained for pooled infected and uninfected *Biomphalaria glabrata* snails infected with *Schistosoma mansoni* (Eppert et al. 2002).

The size distribution of snails harboring multi-clone infections was not significantly associated with a linear increase in the number of trematode clones with snail size over all bays or within any of the three bays examined (Fig. 1) as would be expected if snails accumulate parasite infections throughout their life. Instead, the largest snails each possessed a single parasite clone. This is consistent with the findings of Keeney et al. (2007) for an independent sample of 25 snails from LPB. In the present study, the four largest LPB snails possessed single genotypes as in the previous study. Although CB and TB had fewer multi-clone infections and therefore, a greater probability that the large snails sampled have single parasite genotypes by chance, the four largest (and seven of the eight largest) CB snails and ten largest TB snails possessed a single *M. novaezealandensis* genotype. These data are not sufficient for a rigorous analysis of the relationship between snail size and intraspecific trematode genetic diversity, but do indicate that multi-clone infections are relatively rare within larger snails versus intermediate-sized snails in several bays of Otago Harbour. Experimental studies controlling for the timing of infection and number of trematodes that snails are exposed to would shed important light on the role of parasite competition within snails, but were not feasible with this study.

Keeney et al. (2007) proposed three nonexclusive scenarios (timing between initial and subsequent infections, resource competition, and increased host mortality/reduced host growth of snails harbouring multiple clones) to explain the relatively low frequency of mixed-clone infections in larger snails. These hypotheses invoke competition for gonadal tissue and/or space within the snail host that could create selective-pressures favouring trematode clones that are more efficient at reproducing asexually once inside the snail host. Theoretical models predict that in multi-clone infections faster replicating clones have an advantage leading to the evolution of high virulence in multi-clone infections (e.g. May and Nowak 1995; Frank 1996) and recent empirical studies have identified asymmetry in competitive success among different parasite clones (de Roode et al. 2005; Bell et al. 2006), including trematodes (Gower and Webster 2005). These studies suggest that faster replicating genetic clones may have more of a selective advantage in

populations where mixed-clone infections are common versus populations where single-clone infections dominate. Differences in the frequency of mixed-clone infections throughout a parasite species' range could therefore result in geographically variable selection pressures, setting the stage for local adaptations to evolve. Alternatively, facultative responses may evolve if parasites can detect the presence of other clones and alter their reproductive rates accordingly (Frank 1992; van Baalen and Sabelis 1995; Davies et al. 2002).

Sousa (1990) proposed that intense interspecific competition among trematode species would result in intermediate-sized snails possessing the maximum trematode species diversity. Although this prediction may not be sensitive enough to allow detection of competition in many multispecies systems (Kuris and Lafferty 1994), our data and the previous study by Keeney et al. (2007) suggest it may be representative of intraspecific competition among conspecific trematode genetic clones within snail hosts. This may reflect the fact that there is less heterogeneity within a snail population in recruitment among conspecific trematode clones versus different trematode species, which can obscure patterns of competition within snails (Kuris and Lafferty 1994; Lafferty et al. 1994).

Trematode genetic homogeneity among proximate bays

We did not detect any evidence of genetic structure among *M. novaezealandensis* collected from the three coastal bays and our data suggest that these trematodes are members of one panmictic population. The most parsimonious explanation for the observed genetic homogeneity is the ability of the mobile bird definitive hosts to create *M. novaezealandensis* gene flow among relatively proximate intertidal bays. However, given the small geographic scale of our study, additional factors could also account for the observed genetic homogeneity, including movement of trematode eggs via tidal currents.

The observed genetic homogeneity could have important implications regarding the ability of *M. novaezealandensis* to respond to potential varying selective-pressures among bays. First, *M. novaezealandensis* within Otago Harbour may experience different local selective-pressures from their snail hosts. Snails from LPB, CB, and TB exhibit differences in their life-history strategies in response to trematode parasitism with individuals from populations experiencing higher parasite prevalence maturing earlier in response to castration by trematodes (Fredensborg and Poulin 2006). This enables snails to reproduce before complete castration by infecting trematodes (Fredensborg and Poulin 2006) and the earlier host maturation is likely to favor faster replicating parasite clones that are more efficient at utilizing host resources. The development of local differences in snail host populations may be facilitated by the direct development of juveniles without a planktonic larval stage (Fredensborg and Poulin 2006), but the actual extent of *Z. subcarinatus* gene flow among bays should be quantified in the future as it is the relative rate of host and parasite gene flow that influences the evolution of local adaptation in host–parasite systems (Gandon et al. 1996; Lively 1999; Gandon and Michalakis 2002; Prugnolle et al. 2005).

Second, differences among bays in the frequency with which *M. novaezealandensis* clones encounter additional, potentially competitive trematode species or conspecific clones could create additional variation in selective-pressures. Although within-host competition among trematodes has been relatively well studied (Kuris 1990; Sousa 1992; Lafferty et al. 1994), the within-host interactions of conspecific trematode clones remain virtually unknown. Trematode reproductive rates can be higher in mixed- versus single-clone infections (Davies et al. 2002) and *M. novaezealandensis* clones that utilize host resources more efficiently, i.e., asexually reproduce earlier or faster (Bremermann and Pickering 1983), may have a selective advantage in areas where mixed-clone infections are common, such as LPB. However, because mixed-clone infections and/or faster trematode replication rates have been associated with increased host mortality and decreased host reproductive success (Davies et al. 2002; but see Davies et al. 2001) as well as decreased infectivity to subsequent hosts (Davies et al. 2001), the selective advantage of "efficient" clones may be decreased in areas where single-clone infections dominate, such as TB. Again, the ability of birds to disperse trematode clones among bays could interfere with the ability of natural selection to provide specific clones with selective advantages within bays as discussed below. For variable selective-pressures from the presence of either conspecifics or other trematode species to develop among bays, the differences in mixed-clone infections and trematode prevalence need to remain relatively stable over time. Within the bays of Otago Harbour, the prevalence of trematode infections in *Z. subcarinatus* is stable over time (Fredensborg et al. 2006). We do not have data regarding the temporal stability of mixed-clone infections within bays, but given the positive relationship between prevalence and frequency of mixed-clone infections, it is likely to also remain relatively stable within bays.

If the differences in host life-history traits and/or the frequency of the presence of conspecifics or other trematode species within hosts in individual bays create variable selective-pressures, *M. novaezealandensis* populations will not be able to respond to these differences at the scale of the individual bays. Although moderate amounts of parasite migration can facilitate their local adaptation by introducing new parasite genotypes into populations (Gandon et al. 1996; Gandon and Michalakis 2002), the

complete genetic homogeneity detected in this study suggests that *M. novaezealandensis* would not be able to adapt to any among-bay local selective-pressures. These trematodes may possess competitive traits that are "average" characters for the varying geographic selective regimes the trematode population experiences. Alternatively, facultative responses may have evolved within these trematodes that would allow the parasites to alter their strategy, i.e., virulence levels, when they encounter other conspecific clones or trematode species within a host (Frank 1992; van Baalen and Sabelis 1995; Davies et al. 2002). This may be a successful strategy for marine trematode species whose genetic lineages experience heterogeneous selective-pressures throughout their evolution. Although our statements regarding varying local selective-pressures resulting from conspecific competition among clones are speculative, given our data, this is an interesting area of research that deserves more attention.

To conclude, we have detected differences in the frequency of multi-clone trematode infections within snail first intermediate hosts among proximate coastal bays. *M. novaezealandensis* sampled from proximate intertidal bays are genetically homogeneous, likely resulting from the movement of definitive bird hosts. Our results suggest that among the coastal bays studied, *M. novaezealandensis* comprises a single population that is potentially infecting multiple host populations with varying life-history traits. This may be a common pattern for coastal trematodes utilizing intermediate hosts with direct development, such as *Z. subcarinatus*, and birds as definitive hosts. However, on a larger geographical scale, *M. novaezealandensis* may exhibit genetic structure and adaptations to Otago Harbour snails versus *M. novaezealandensis* from more distant locations. A comparative examination of host–parasite gene flow encompassing larger geographic areas and infection experiments would help to identify if local adaptations exist in *M. novaezealandensis*, and the geographical scale over which they occur.

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