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Absence of population genetic differentiation in the New Zealand greenshell mussel *Perna canaliculus* (Gmelin 1791) as assessed by allozyme variation

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Abstract

Genetic variation in the endemic New Zealand greenshell mussel, *Perna canaliculus* (Gmelin 1791), was examined using starch–gel electrophoresis at seven protein-coding loci (*Idh; Acon-1; Acon-2; Gpd; Pgi; Pgm; Pgd*) in 35 populations ($N = 1038$ mussels). For all loci and all populations, Fisher's exact tests indicated highly significant departures from Hardy–Weinberg equilibrium (HWE), but this overall result was caused by significant heterozygote deficiencies at only two loci (Pgm and Pgd), and in only three northern populations (Kuaotunu, Te Haumi and Days Bay). Allelic and genotypic differentiation between population pairs at individual loci and across all loci were nonsignificant, and genotypic disequilibrium at each locus pair was also nonsignificant for all populations. Genetic variation in all populations was high (mean heterozygosity, 0.210 ± 0.113), while Nei's D among populations was very low (0.002 ± 0.002). Low population subdivision ($\theta = -0.001 \pm 0.002$) and high levels of gene flow ($Nm_p = 10.18$; $Nm_\theta =$ infinity) also indicated that the single panmictic unit model best explains population genetic homogeneity in *P. canaliculus* over a north–south distance > 2000 km. Lack of genetic subdivision in this species is discussed in light of two previous allozyme studies, with differing results: one suggested that a north–south division exists between greenshell mussel stocks, and the other suggested that population structure in this species can be explained through isolation by distance model modified by local hydrology.

Keywords: Allozyme variation; Gene flow; Greenshell mussel; Hydrography; New Zealand; *Perna canaliculus*; Population genetic structuring

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1. Introduction

The endemic greenshell mussel, *Perna canaliculus*, is abundant and widespread throughout New Zealand's main three islands (North, South and Stewart Islands), but has not been reported from the Chatham Islands, ~850 km to the east (Finlay, 1928; Knox, 1954; Fleming, 1959; Powell, 1976). Three factors suggest that *P. canaliculus* will not exhibit extensive population subdivision in New Zealand. Firstly, *P. canaliculus* is a broadcast-spawner. Species that release gametes or weak-swimming larvae into the water column, such as *P. canaliculus*, often show less population differentiation than species with strong-swimming pelagic larvae (e.g. Burton and Feldman, 1982) since the former are more prone to passive dispersal via currents. Secondly, *P. canaliculus* possesses a pelagic larval stage of 3–4 weeks (Hayden, 1994), and pediveligers remain planktonic for several weeks if suitable substrate for settlement is not encountered (Buchanan, 1994). As a rule of thumb, species with long-lived planktotrophic larvae have broader geographic ranges (Scheltema and Williams, 1983) and display biochemical genetic homogeneity over greater distances than species with nonplanktonic and/or lecithotrophic (nonfeeding) larvae (e.g. Allock et al., 1997; but see Burton, 1983; Grant and da Silva-Tatley, 1997). Thus, high levels of gene flow accompanied by low levels of population genetic structuring have been reported in numerous marine invertebrate species (e.g. Hunt and Ayre, 1989; Creasey et al., 1996; Murray-Jones and Ayre, 1997). Thirdly, aquaculture of this commercially important species is based on, at 80%, Kaitaia spat, which are collected in northern New Zealand and transferred to mussel farms in mainly four areas: Houhora, around Coromandel Peninsula, the Marlborough Sounds and Stewart Island (Fig. 1). Human-mediated mass transfer of mussel spat from Kaitaia to aquaculture sites throughout New Zealand has occurred for almost three decades (Hickman, 1983) and may indirectly have added to the population genetic homogeneity of this species.

The first allozyme survey of greenshell mussels in New Zealand (Smith, 1988) reported significant heterogeneity between two northern and four southern samples from 4 out of 10 polymorphic loci. Smith (1988) suggested that local hydrography, as well as genetic–physiological adaptation to different thermal environments, might partially isolate mussel populations, which could result in a warm water-adapted northern group and a cold water-adapted southern group, between which there only was limited gene flow. Gardner et al. (1996a) extended Smith's (1988) study by examining 7 polymorphic loci in 10 populations. These authors found no evidence of a north–south genetic split and explained the population genetic structuring that they observed through isolation by distance model and regional hydrographic conditions. Thus, despite both biological and human-mediated transfer arguments in favour of a model of panmixia for this species, the two previous studies of this species have observed differing patterns (north–south population model and isolation by distance model) of population genetic structuring.

Neither of the previous studies of *P. canaliculus* had sampled at a fine geographic scale nor covered the entire species' range. In the present study, seven nuclear protein-encoding allozyme loci were used to examine biochemical genetic variation in 30 wild (naturally occurring) and 5 cultured mussel populations ($N = 1038$ individuals) throughout New Zealand. Five of the seven loci are shared with Smith's (1988) study

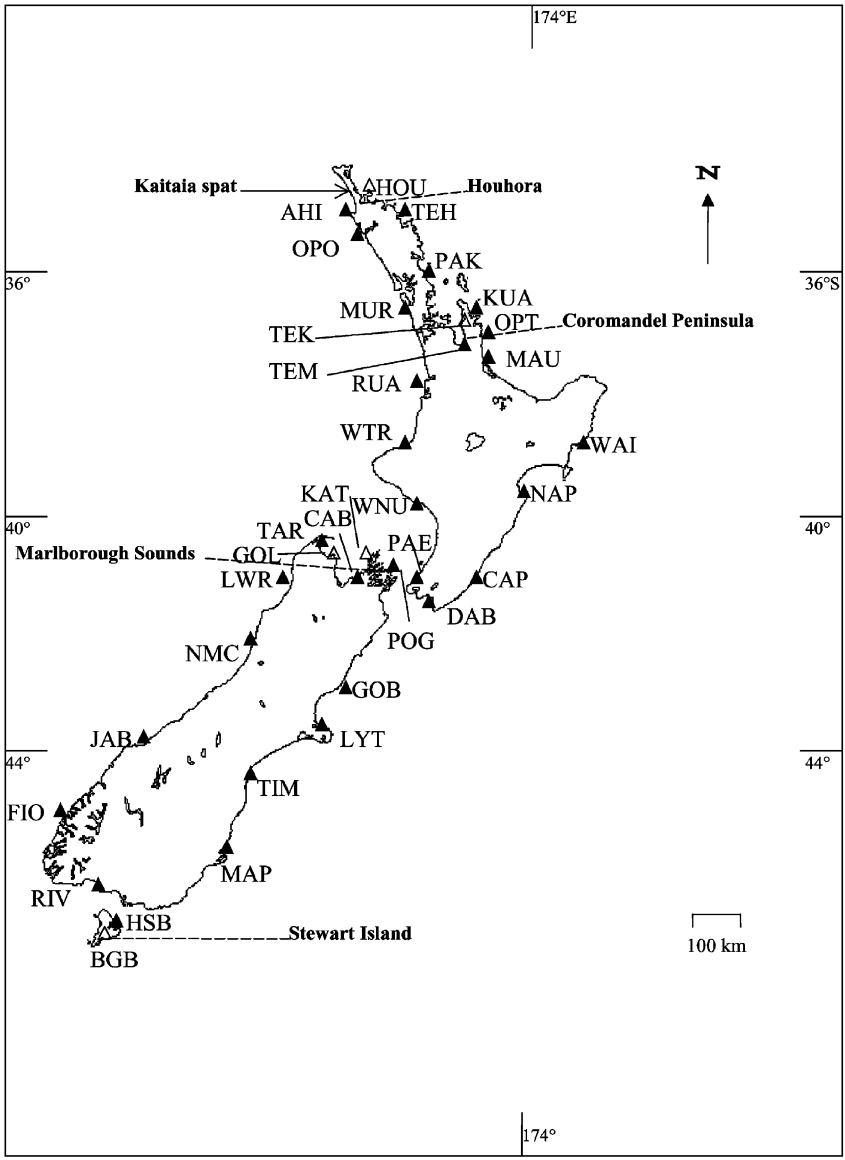

Fig. 1. *P. canaliculus*. Map of New Zealand showing Kaitaia spat collection site (arrow) and destinations (dashed lines), and sample collection sites: Houhora (HOU); Ahipara (AHI); Te Haumi (TEH); Opononi (OPO); Pakiri (PAK); Muriwai (MUR); Kuaotunu (KUA); Te Kouma (TEK); Opoutere (OPT); Te Mata (TEM); Maunganui (MAU); Ruapuke (RUA); Waikanae (WAI); Waitara (WTR); Napier (NAP); Waiinu (WNU); Castlepoint (CAP); Paekakariki (PAE); Days Bay (DAB); Tarakohe (TAR); Golden Bay (GOL); Kaitaia (KAT); Port Gore (POG); Little Wanganui River (LWR); Cable Bay (CAB); Nine Mile Creek (NMC); Gore Bay (GOB); Lyttleton (LYT); Jacksons Bay (JAB); Timaru (TIM); Fiordland (FIO); Mapoutahi (MAP); Riverton (RIV); Horseshoe Bay (HSB); Big Glory Bay (BGB). Solid triangles (▲), wild samples; unfilled triangles (△), individuals obtained from mussel farms.

and two are shared with Gardner et al.'s (1996a) study. Our large-scale allozyme data set was used to test for the existence of Smith's (1988) north–south population model and Gardner et al.'s (1996a) isolation by distance model. The findings indicate that neither model is appropriate to our data set, and that a model of panmixia best explains the observed genetic variation among the 35 assayed populations.

2. Materials and methods

2.1. Collection of specimens

An average of 28.3 mussels/population was collected from the intertidal zone or from depths of 1–20 m at 35 sites from North, South and Stewart Island, from 1996 to 1998 (Fig. 1). Mussels were dissected in the field. Tissue was snap-frozen in liquid nitrogen, transported to Victoria University of Wellington, and subsequently transferred to −70°C for storage.

2.2. Electrophoresis

Horizontal starch–gel electrophoresis was carried out according to Richardson et al. (1986). Digestive gland was prepared by homogenising 0.1 g of tissue in 50-μl distilled water. The resulting homogenate was centrifuged at 5000 rpm for 3 min, and the supernatant applied to the gel on 2 × 10-mm strips of filter paper. Gels were kept cool with ice and were run at room temperature for 3–5 h at 150 V. Enzymes (Table 1) were stained by means of agar overlays, following standard procedures (Allendorf et al., 1977; Harris and Hopkinson, 1978; Richardson et al., 1986). Genotypes were scored

Table 1
Resolved enzymes, Enzyme Commission number, number of polymorphic loci, and used buffer systems (*P. canaliculus*)

Enzyme	Enzyme Commission number	Number of polymorphic loci	Buffer system
Aconitase (Acon)	4.2.1.3	2	TEC
Glycerol-3-phosphate dehydrogenase (Gpd)	1.1.1.8	1	TEC
Isocitrate dehydrogenase (Idh)	1.1.1.42	1	TEC
Phosphoglucoisomerase (Pgi)	5.3.1.9	1	TEC
Phosphoglucomutase (Pgm)	2.7.5.1	1	TM
6-Phospholuconate dehydrogenase (Pgd)	1.1.1.44	1	AC

TEC: electrode (0.14 M Tris, 0.04 M citric acid, 0.0013 M EDTA, pH 7.4), gel (0.009 M Tris, 0.0029 M citric acid, 0.0013 M EDTA, pH 7.2); TM: electrode (0.1 M Tris, 0.1 M maleic acid, 0.01 M EDTA, 0.01 M $MgCl_2$, pH 7.4), gel (1:10 dilution of electrode buffer); AC: electrode (0.04 M citric acid adjusted with *N*-[3-aminopropyl]morpholine, pH 6.1), gel (1:20 dilution of electrode buffer).

immediately after staining by assigning the letter A or the number 1 to the most cathodal allele or locus, respectively.

2.3. Data analysis

Data analyses were carried out using the software programmes GENEPOP version 3.2 (Raymond and Rousset, 2000), FSTAT 2.9.1 (Goudet, 1995, 2000), POPGENE version 1.32 (Yeh et al., 1999) and PAUP beta 4a (Swofford, 2000). For all analyses, the data set consists of 35 populations. For analyses of genetic diversity, conformation to Hardy–Weinberg Equilibrium (HWE), genotypic disequilibrium, gene flow and randomisation, the populations were also grouped into a northern and a southern class in order to test for the north–south split that Smith (1988) observed. The northern class (21 populations) included all of North Island and the four cultured populations on-grown from Kaitaia spat (HOU, TEK, KAT, BGB). The southern class (14 populations) consists of all South Island populations, including the Golden Bay population originating from locally caught spat in Golden Bay and the Horseshoe Bay population from Stewart Island. For the analyses of conformation to HWE and genotypic disequilibrium, all 35 populations were also pooled.

2.3.1. Conformance of populations to HWE

Genotypic frequencies observed in each of the 35 populations at the seven loci were tested for conformation to HWE using an exact test (Rousset and Raymond, 1995), where the null hypothesis tested was a random union of gametes and the alternative hypothesis was heterozygote-deficit. Fisher's exact tests were performed to test for conformation to HWE across populations ("All pops") and across loci ("All loci"). A Markov chain method (Guo and Thompson, 1992) was used to estimate the exact *P*-values. Whenever possible, standard errors associated with the *P*-values were kept < 0.01 by adjusting the Markov chain parameters (dememorisation number, number of batches, and iterations per batch; Raymond and Rousset, 2000). *P*-values were corrected for multiple testing with the sequential Bonferroni technique (Holm, 1979; Rice, 1989). Fisher's exact tests were also performed on pooled data sets of northern and southern populations and on the pooled data set from all 35 populations.

2.3.2. Genotypic disequilibrium

We tested the null hypothesis that genotypes at one locus were random in occurrence with respect to those at another locus within each population, i.e. that there was no evidence for genotypic disequilibrium. Genotypic disequilibrium was tested for by performing exact tests using a Markov chain method and correcting for multiple testing, as outlined above. *P*-values were also obtained for each locus pair across all 35 populations, between pooled northern and pooled southern populations, and for the pooled data set.

2.3.3. Allelic and genotypic differentiation

Population differentiation was analysed by testing for allelic and genotypic heterogeneity at each locus between all possible pairs of populations and across all popula-

tions. Unbiased estimates of *P*-values of log-likelihood (G)-based exact tests (Goudet et al., 1996) were estimated using a Markov chain method and corrected for multiple testing, as described above.

2.3.4. Population differentiation

F-statistics based on the analysis of variance of weighted allele frequencies (Weir and Cockerham, 1984) were calculated to assess within-population (f) and among-population (θ) components of total population genetic variation (F) using the software program FSTATS 2.9.1 (Goudet, 1995, 2000). Standard errors of *F*-estimates were obtained by jackknifing over populations. Confidence intervals of overall *F*-estimates were determined by bootstrapping over loci (15,000 replicates). Significance tests of *F*-estimates were carried out as described by Waples (1987).

2.3.5. Gene flow

Gene flow (Nm_p) was calculated as the number of migrants exchanged per generation among populations in an island model using the private alleles method with correction for sample size (Slatkin, 1985; Barton and Slatkin, 1986). Nm_p was estimated among all 35 populations, among the 21 northern, among the 14 southern, between the pooled northern and the pooled southern, among the 18 east-coast, among the 17 west-coast populations, and between the pooled east-coast and pooled west-coast populations. Gene flow (Nm_f and Nm_θ) was also estimated from F_{ST} (Nei, 1987) and θ (Weir and Cockerham, 1984), respectively, using the relationship $\mathrm{Nm} = 0.25(1 - F_{ST})/F_{ST}$ (Slatkin and Barton, 1989).

2.3.6. Isolation by distance

Coastal distances between collection sites were estimated in kilometers from a New Zealand Atlas map (1:250,000; McKenzie, 1987). Since θ is independent of mutation rate, whereas D is not (Slatkin, 1993), estimates of θ rather than the genetic distance were used to detect a pattern of isolation by distance. The regression between pairwise estimates of $\theta/(1-\theta)$ and geographic distance was calculated with the ISOLDE routine (GENEPOP v 3.2) to test for isolation by distance under a one-dimensional model (Rousset, 1997). Significance of a positive or negative correlation between $\theta/(1-\theta)$ and geographic distance was tested using the Spearman rank correlation coefficient computed with two one-sided Mantel's tests (Mantel, 1967) with 1000 random permutations. The slope of the regression line was tested for significant difference from zero.

2.3.7. Randomisation testing of population genetic structure

Smith (1988) found that two northerly samples of *P. canaliculus* exhibited significant genetic heterogeneity from four southerly samples from 4 out of 10 polymorphic allozyme loci. We tested two hypotheses that might explain the difference in population differentiation between Smith's (1988) and the present study. Firstly, we predicted that we would detect less population heterogeneity among our 35 populations than Smith (1988) did among his six populations. This might be the case because the average frequency of an allele is an increasing function of the number of populations in which it

is found (Slatkin, 1981). For example, alleles occurring at very low frequency will incorrectly be scored as private alleles (alleles occurring in only one population) if the number of sampled populations is not sufficiently high, and estimators of population differentiation, such as F_{ST}, will consequently increase. We therefore tested to see if our observed F_{ST}-value (Nei, 1987) among all 35 populations ($F_{ST} = 0.0199$, nonsignificant) lay within ± 2 standard deviations of the 95% confidence interval of a frequency distribution of 100 F_{ST}-values among six populations. For each of the 100 iterations, six populations were chosen at random from the 35 populations, irrespective of their northern or southern origin.

Secondly, we hypothesised that if differing numbers of sampled populations do not cause differences in population genetic differentiation in our data set, restriction of sampling to geographic regions might. We therefore simulated Smith's (1988) sampling approach with our data by generating F_{ST}-values again among six populations chosen from our 35 populations. However, for each of these 100 iterations, two populations were chosen at random from our 21 northern populations, and four populations were chosen at random from our 14 southern populations. F_{ST}-values for the two separate randomisation tests were generated with POPGENE, and their frequencies of occurrence were plotted. Significance levels of all 200 F_{ST}-values were calculated (i.e. gene flow is $< 100\%$ if F_{ST}-values are significantly > 0; Waples, 1987). The mean values ($\pm$S.D.) of the two F_{ST} frequency distributions were tested for significant difference by *t*-test.

3. Results

3.1. Allele frequencies

Five electromorphs were observed at the Acon-1 locus, six at the Gpd locus, seven at the loci *Idh*, *Acon-2*, *Pgm* and *Pgd*, and 10 at locus *Pgi* (data not shown due to large size of allele frequency table). The frequencies of the most common allele (mean = 0.875 ± 0.06) at each of the seven allozyme loci did not exhibit a geographic cline when plotted against sampling locations (Fig. 2). *Idh*, *Pgi*, *Pgm* and *Pgd* had a second allele at high frequency (mean = 0.123 ± 0.041) in each population, and *Acon-1* had a second allele at high frequency in all populations except Golden Bay (0.149 ± 0.06). All loci either had a population-specific allele or class-specific alleles (see Section 2.3) at very low frequency (0.001–0.002). The remaining alleles at low frequency (0.016 ± 0.03) were neither population- nor class-specific.

3.2. Genetic diversity

Observed and expected heterozygosities (H_{obs} and H_{exp}) calculated over all loci for each population were high, and ranged from 0.143 ± 0.127 to 0.258 ± 0.147 and from 0.158 ± 0.113 to 0.261 ± 0.136, respectively. For each separate population, observed and expected heterozygosities were calculated, and then differences between northern and southern populations were tested for significance with the Mann–Whitney *U*-test (Sachs, 1984). The null hypothesis that northern and southern sample heterozygosities were identical could not be rejected: neither differences in observed nor in expected

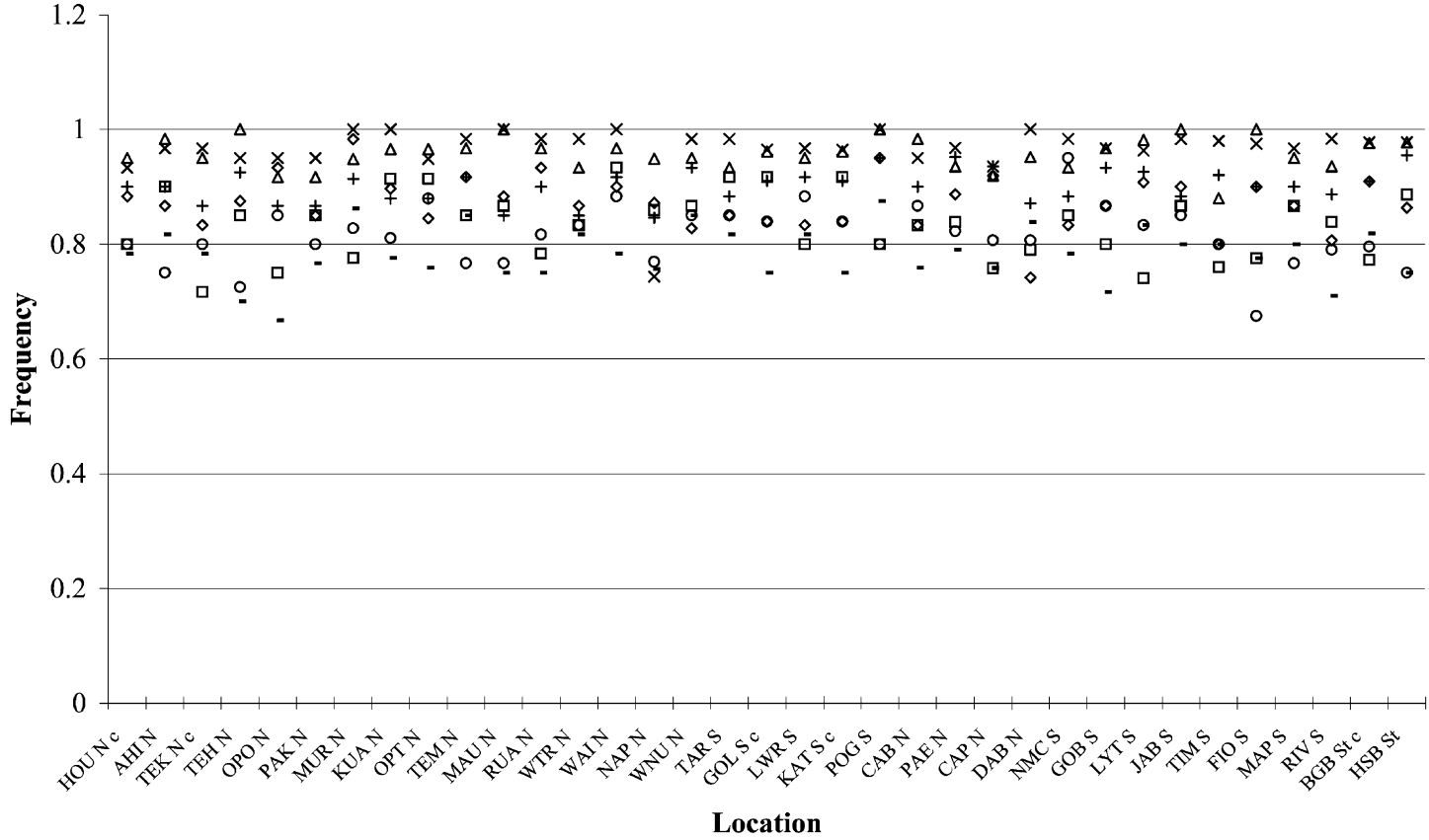

Fig. 2. *P. canaliculus*. Frequencies of the most common allele at seven allozyme loci (*Idh* ◇, *Acon-1* □, *Acon-2* △, *Gpd* ×, *Pgi* -, *Pgm* ○, *Pgd* +) plotted for each of the 35 mussel populations (see legend of Fig. 1 for full population names). Populations are arranged from sampling locations in the north (HOU N c) to the south (HSB St) of New Zealand. North Island (N); South Island (S); Stewart Island (St); cultured mussels (c).

heterozygosities were significant at the 5% level (two-sided test, $Z_{H\text{obs}} = 0.4547$, $P \gg 0.05$; $Z_{H\text{exp}} = 0.7577$, $P \gg 0.05$). Genetic diversity was high across all loci with mean $H_{\text{ave}} = 0.210 \pm 0.113$ (Table 2).

3.3. Conformance of populations to HWE

All deviations from HWE were caused by significant heterozygote deficiencies observed from five out of the seven loci, in one or more populations (Table 3, *Idh*: Little Wanganui River and Days Bay; *Acon-1*: Muriwai; *Pgi*: Te Haumi; *Pgm*: Kuaotunu, Little Wanganui River, Fiordland and Days Bay; *Pgd*: Te Mata and Waitara and Big Glory Bay). However, after table-wide sequential Bonferroni correction of *P*-values for multiple testing ($\alpha = 0.05$, $k = 7 \times 35$), all deviations from HWE were nonsignificant. When the *P*-values from the Fisher's exact tests across all populations ("All pops") and across all loci ("All loci") were included in the table-wide correction procedure ($\alpha = 0.05$, $k = 8 \times 36$), deviations from HWE at the *Pgm* and *Pgd* loci and the overall result ("All pops" × "All loci") were significant ($P < 0.0001$, S.E. < 0.0001). Bonferroni correction on a population by population basis ($\alpha = 0.05$, $k = 7$ and $\alpha = 0.05$, $k = 8$ when "All loci" values were included in the correction procedure) revealed that genotype frequencies of only three populations (Kuaotunu, Te Haumi, and Days Bay) did not conform to HWE. Fisher's exact tests performed on pooled data sets of northern and southern populations revealed that none of the seven loci exhibited deviations from HWE after correction for multiple testing. However, the pooled northern populations were not in HWE ($P < 0.0001$, S.E. < 0.0002), whereas the pooled southern populations were ($P = 0.1147$, S.E. $= 0.0116$). A Fisher's exact test indicated a highly significant ($P < 0.0001$, S.E. < 0.0001) departure from HWE for the pooled data set from all 35 populations.

3.4. Genotypic disequilibrium

Tests for genotypic disequilibrium between pairs of loci within each population produced only 22 out of 735 (3%) significant disequilibria, in 16 out of 35 (45.7%)

Table 2
Heterozygosities for seven loci across 35 populations as a measure of genetic diversity (*P. canaliculus*)

Locus	Sample size	H_{obs}	H_{exp}	H_{ave}
Idh	1986	0.2256	0.2261	0.2204
Acon-1	1958	0.2666	0.2825	0.2734
Acon-2	1982	0.0868	0.0844	0.0798
Gpd	1988	0.0523	0.0514	0.0500
Pgi	1986	0.3545	0.3543	0.3494
Pgm	1988	0.2797	0.3132	0.3093
Pgd	1986	0.1732	0.1881	0.1833
Mean ± standard deviation	1982	0.2055 ± 0.1083	0.2143 ± 0.1142	0.2094 ± 0.1131

Sample size given as the number of genes. Observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}) and average heterozygosity (H_{ave}) were computed according to Nei (1978).

Table 3
Probabilities (P) that genotype frequencies observed in 35 populations at seven loci conform to Hardy–Weinberg expectations (H_0: random union of gametes; H_1: heterozygote deficit) (*P. canaliculus*)

Pop	*N*	*P*							
		Locus							
		Idh	*Acon-1*	*Acon-2*	*Gpd*	*Pgi*	*Pgm*	*Pgd*	All loci
WAI	30	1.0000	1.0000	1.0000	–	0.7468	1.0000	1.0000	0.8423
RUA	30	1.0000	0.2376	1.0000	–	0.2313	0.6735	0.2409	0.2013
MUR	29	–	0.0134	1.0000	–	1.0000	0.2937	1.0000	0.2438
OPO	30	0.1008	0.4086	1.0000	1.0000	0.7006	0.1911	1.0000	0.0746
PAK	30	1.0000	0.4144	1.0000	1.0000	0.6788	0.1445	1.0000	0.3934
TEK	30	1.0000	0.5909	1.0000	1.0000	0.6788	0.1125	0.1179	0.1134
TEM	30	1.0000	1.0000	1.0000	–	1.0000	0.6485	0.0336	0.1228
KUA	29	0.2489	1.0000	1.0000	–	0.6940	0.0004*	0.1200	0.0021*
OPT	29	1.0000	1.0000	1.0000	1.0000	0.6314	0.1707	0.2489	0.2358
MAU	30	1.0000	0.3253	–	–	0.2313	0.8673	1.0000	0.4491
AHI	30	1.0000	1.0000	–	1.0000	1.0000	0.2627	1.0000	0.7232
HOU	30	1.0000	0.2247	1.0000	1.0000	1.0000	0.1125	1.0000	0.3673
TEH	20	1.0000	0.3537	–	1.0000	0.0003*	0.1875	1.0000	0.0011*
WTR	30	1.0000	1.0000	1.0000	–	0.1547	1.0000	0.0087	0.5270
WNU	30	1.0000	0.3253	1.0000	–	1.0000	1.0000	1.0000	0.7387
LYT	27	0.1121	0.4662	–	1.0000	0.5488	1.0000	0.1121	0.0192
LWR	30	0.0156	0.3071	1.0000	1.0000	0.1853	0.0201	1.0000	0.0080
NMC	30	0.5919	0.5045	1.0000	–	0.8101	1.0000	0.2409	0.3024
JAB	30	1.0000	0.3253	–	–	0.7495	1.0000	0.2072	0.3182
MAP	30	1.0000	0.5045	1.0000	1.0000	1.0000	0.2247	1.0000	0.5170
TIM	25	0.5825	0.4110	1.0000	–	1.0000	0.1405	1.0000	0.3183
GOB	30	0.4144	0.6735	1.0000	1.0000	0.3192	1.0000	1.0000	0.2994
TAR	30	1.0000	1.0000	1.0000	–	0.1547	1.0000	0.1791	0.3751
CAB	30	1.0000	0.5919	–	1.0000	0.8747	0.3253	1.0000	0.7031
RIV	31	0.7324	1.0000	1.0000	–	0.7707	0.1831	1.0000	0.6790
BGB	22	0.1378	0.5381	–	–	1.0000	0.2815	0.0233	0.0529
HSB	22	1.0000	1.0000	–	–	0.2712	0.3631	1.0000	0.3484
POG	20	1.0000	0.5807	–	–	0.2460	0.0788	1.0000	0.1064
FIO	20	1.0000	0.5807	–	–	0.4678	0.0393	1.0000	0.0845
NAP	39	0.4826	1.0000	1.0000	1.0000	0.5782	0.6830	0.4126	0.5583
DAB	31	0.0460	0.2909	1.0000	–	1.0000	0.0015*	0.1127	0.0008*
CAP	31	1.0000	1.0000	1.0000	1.0000	0.3443	0.1078	1.0000	0.4882
PAE	31	0.3156	0.5776	1.0000	1.0000	0.3792	0.6588	1.0000	0.2396
GOL	22	1.0000	–	–	–	0.8512	1.0000	0.2587	0.5405
KAT	22	0.5332	1.0000	1.0000	1.0000	0.5433	0.1483	1.0000	0.1650
All pops		0.4039	0.0779	1.0000	1.0000	0.2937	0.0001*	0.0000*	0.0000*

Fisher's exact tests were performed to test for conformation to HWE across populations (All pops) and across loci (All loci). Pop, population abbreviations; *N*, sample size; –, locus either monomorphic or number of expected and observed heterozygotes is the same; standard errors (not shown) associated with *P*-values were kept < 0.01 whenever possible.

* Significant after Bonferroni correction for multiple testing.

populations. After population-wide sequential Bonferroni correction for multiple testing ($\alpha = 0.05,\ k = 21$), none of the 22 results was significant. All 21 possible locus pairs were in genotypic equilibrium across all 35 populations ($P > 0.05$ in all cases). Genotypic disequilibrium between each of the 21 possible locus pairs was also not observed in pooled northern or pooled southern populations ($P > 0.18$ and $P > 0.05$, respectively) or within the pooled data set ($P > 0.05$ in all cases).

3.5. Allelic and genotypic differentiation

Neither for individual loci nor across all loci did significant allelic or genotypic heterogeneity exist between any pair of populations after sequential Bonferroni adjustment for multiple testing ($\alpha = 0.05,\ k = 595$). Across all 35 populations, allelic and genotypic frequencies were not significantly heterogeneous at any of the seven loci (Fisher's exact tests, $P > 0.05$ in all cases). These results indicate homogeneity of allelic, as well as genotypic distributions, among all populations.

3.6. Population differentiation

The reduction in heterozygosity of the total population (F) was mainly due to the effects of nonrandom mating within populations (f), rather than the effects of population subdivision (θ) (Table 4). θ-values for individual loci were consistently low and highly significant for the *Idh*, *Acon-1* and *Acon-2* loci. Significant f-values and F-values were observed at the *Pgm* and *Pgd* loci. This is in agreement with the departures from HWE at these two loci (Table 3). However, overall θ, f and F-estimates were all nonsignificant.

Table 4
F-statistics for 35 populations, estimated as in Weir and Cockerham (1984) (*P. canaliculus*)

Locus	f	θ	F
Idh	0.003^{ns} (0.033)	0.001^{***} (0.005)	0.004^{ns} (0.035)
Acon-1	0.055^{ns} (0.028)	0.006^{***} (0.005)	0.060^{ns} (0.027)
Acon-2	-0.029^{ns} (0.004)	0.000^{***} (0.003)	-0.029^{ns} (0.003)
Gpd	-0.020^{ns} (0.003)	-0.003^{ns} (0.002)	-0.023^{ns} (0.002)
Pgi	0.002^{ns} (0.024)	-0.003^{ns} (0.003)	-0.001^{ns} (0.024)
Pgm	0.108^{***} (0.030)	-0.001^{ns} (0.004)	0.107^{*} (0.030)
Pgd	0.093^{***} (0.031)	-0.008^{ns} (0.002)	0.086^{*} (0.031)
Overall	0.042^{ns} (0.022)	-0.001^{ns} (0.002)	0.042^{ns} (0.022)
CI	0.006, 0.078	−0.004, 0.003	0.006, 0.077

The null hypothesis that $f = 0$ (total within-population similarity) and the null hypothesis that $\theta = 0$ (100% gene flow) were tested with equations taken from Waples (1987). Significance levels for F as a measure of deviation from HWE in the compound population were obtained by pooling all subdivisions, after Skibinski et al. (1983). Values in brackets are standard errors associated with jackknifed estimates over populations and, for the overall result, over loci. CI, 95% confidence interval of overall estimates, was determined by bootstrapping over loci (15,000 replicates).

* $P \leq 0.05$.

*** $P \leq 0.001$.

3.7. Gene flow

For a deme size of 25, based on the mean frequency of private alleles (Slatkin, 1985), the number of migrants per generation (Nm_p) was 11.53. Correction for sample size by multiplying the uncorrected value (11.53) with the ratio of assumed deme size (25) to the actual average deme size of this study (28.32) (Barton and Slatkin, 1986) produced an Nm_p of 10.18. Nm_p-values among populations within various regions around New Zealand were very similar to this overall value, whereas gene flow between pooled northern and pooled southern locations, as well as between pooled eastern and pooled western locations, was apparently approximately three times higher (Table 5). Using Slatkin and Barton's (1989) equation, the estimate of gene flow from F_{ST} (0.0199) was $Nm_f = 12.31$. Using the same equation and the bootstrap confidence interval for $\theta(-0.004,\ 0.003)$, this corresponds to gene flow values of $Nm_\theta = 83$ to effectively infinite.

3.8. Isolation by distance

Pairwise $\theta/(1-\theta)$ estimates between each population were plotted as a function of geographic distances between each pair of populations ($y = -1.05 \times 10^{-6} x + 0.0009$, Fig. 3). There was no significant correlation between pairwise $\theta/(1-\theta)$ estimates and geographic distance ($r^2 = 0.0036,\ df = 593,\ P > 0.05$). The expected correlation generated from 1000 Mantel permutations was not significantly larger ($P > 0.713$) or smaller ($P > 0.287$) than the observed correlation. The slope of the plot was not significantly different from zero ($t = -1.564,\ df = 593,\ P > 0.05$). These results are evidence for an absence of genetic isolation by distance.

3.9. Randomisation testing of population genetic structure

The mean of the frequency distribution of 100 F_{ST}-values generated from combinations of six populations chosen at random from northern and southern class populations (0.017 ± 0.004) was significantly larger ($t = 2.925,\ df = 198,\ P < 0.01$) than the mean

Table 5
Average number of migrants per generation (Nm_p) estimated among all populations, and among northern, southern, eastern, and western populations, as well as between pooled northern and pooled southern, and between pooled eastern and pooled western populations (*P. canaliculus*)

Region	Number of populations	Nm_p
Among all populations	35	10.18
Among northern populations	21	10.77
Among southern populations	14	11.34
Among eastern populations	18	9.60
Among western populations	17	10.51
Between northern and southern populations	2	38.96
Between eastern and western populations	2	35.49

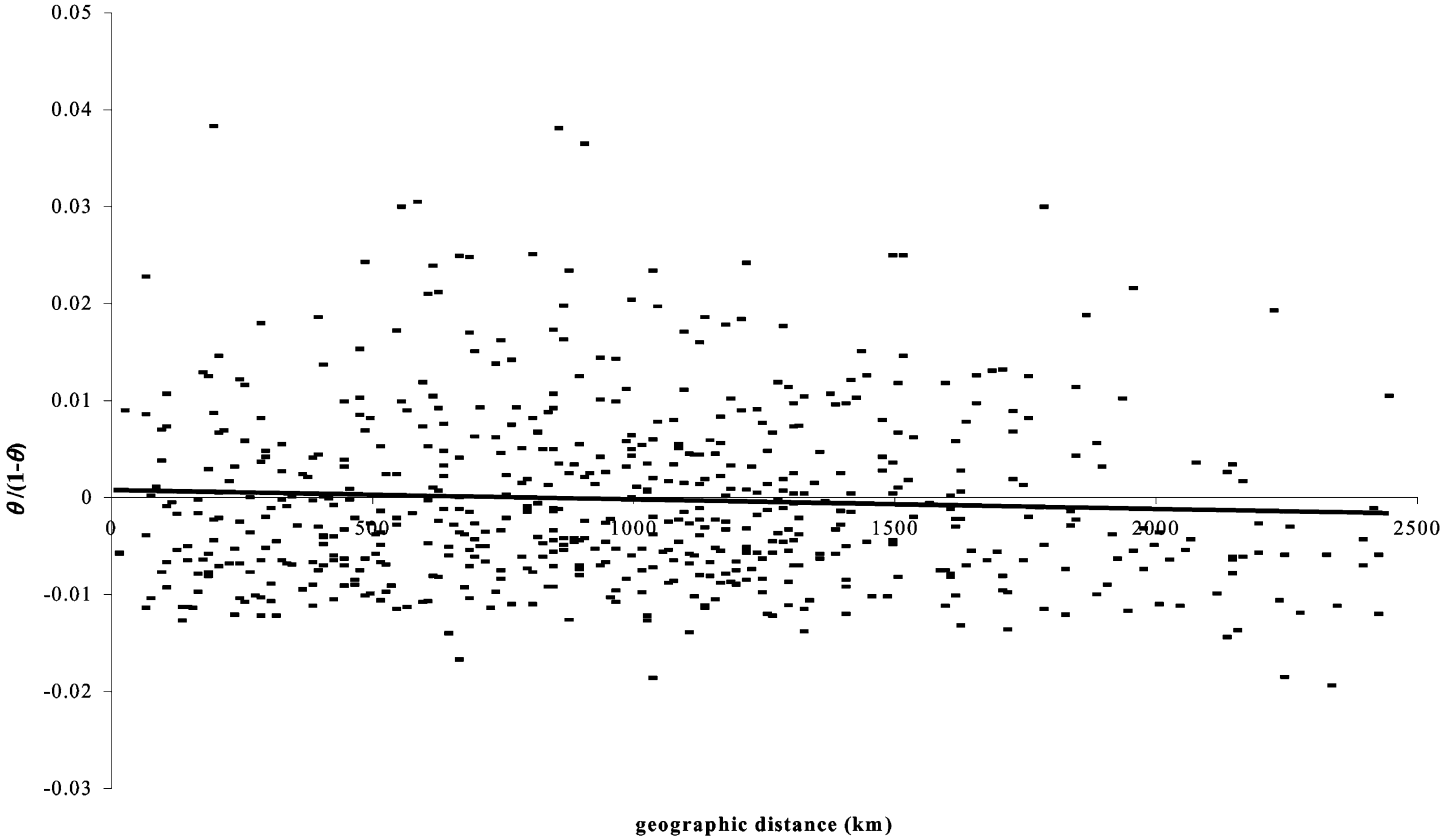

Fig. 3. *P. canaliculus*. $\theta/(1-\theta)$ estimates between each pair of populations plotted as a function of geographic distance between each population pair; $y = -1.05\times10^{-6}x + 0.0009$, $r^2 = 0.0036$.

of the frequency distribution of 100 F_{ST}-values generated from combinations of six populations chosen at random from 35 populations (0.016 ± 0.003). Ten values of the F_{ST} frequency distribution for combinations of six populations chosen at random from northern and southern class populations were significantly greater than zero (i.e. gene flow was $< 100\%$; $\chi^2 > 43.86$, $df = 30$, $P < 0.05$). Less than 5% of the values of F_{ST} frequency distribution for combinations of six populations chosen at random from 35 populations were significantly greater than zero. The F_{ST}-value (0.0199) obtained across 35 populations fell within the 95% interval of both frequency distributions of the F_{ST}-values.

4. Discussion

4.1. Genetic diversity and HWE

The seven loci surveyed are suitable markers to detect population genetic structure on a large geographic scale because allelic variation at each locus is high (5–10 alleles/locus), and because genetic diversity (0.210 ± 0.113, mean of seven loci, Table 2) is at the upper range of most protein-electrophoretic surveys (0.0–0.2, Avise, 1994). High levels of genetic variation may reflect broad environmental adaptation in species, which are, geographically, widely distributed due to the dispersal capacity of their long-lived larvae (Scheltema and Williams, 1983). For example, the starfish *Linkia laevigata* exhibited an average heterozygosity of 0.384 in populations sampled over distances > 1000 km throughout the Great Barrier Reef (Williams and Benzie, 1993), and the sea urchin *Evechinus chloroticus* (Echinoidea, Echinodermata) exhibited mean heterozygosities of 0.35–0.42 from six widespread populations in New Zealand (Mladenov et al., 1997).

Although a highly significant heterozygote deficit across all loci and all populations was detected for *P. canaliculus* ("All pops" × "All loci", Table 3), this significant deviation from HWE resulted from only two loci (*Pgm* and *Pgd*) and three northern populations. The highly significant result of the exact tests performed on the 35 pooled populations, and on the 21 pooled northern and 14 pooled southern populations, confirmed this because neither the 35 pooled populations nor the 21 pooled northern populations were in HWE, whereas the southern populations were. Significant heterozygote deficiency has commonly been reported in marine invertebrate species and has been assigned to a number of different causes, including the mis-scoring of gels, null alleles, selection against heterozygotes and the Wahlund effect (e.g. Zouros and Foltz, 1984; Gardner, 1992). Whilst our findings are generally consistent with a large body of published data, we cannot determine the cause(s) of this heterozygote deficiency.

4.2. Population genetic structuring and gene flow

Despite a high level of heterozygosity, populations of *P. canaliculus* from sites separated by > 2000 km displayed surprisingly high genetic homogeneity. A genetic distance matrix (Nei's, 1978 genetic distance, *D*) for all possible pairs of the 35

populations across the seven loci was calculated with POPGENE. Nei's D among populations was very low (mean, 0.002 ± 0.002). A UPGMA dendrogram based on the genetic distance matrix was constructed with PAUP beta 4a (Swofford, 2000) and showed no grouping of populations by geographic localities. A Neighbor Joining dendrogram reflected a similar topology (data not shown).

Absence of allelic and genotypic differentiation indicated that allele and genotype distributions among all pairs of 35 populations were nonsignificantly different. The absence of population differentiation, as manifested by low D, low F_{ST} (0.0199) and consistently low θ-values (mean $= -0.001 \pm 0.002$, Table 4), as well as high levels of gene flow (Nm_p, Nm_f and Nm_θ), argues for the existence of a single panmictic population. Both the mean frequency of private alleles and F_{ST} resulted in Nm_p and Nm_f-values of the same order of magnitude (10.18 and 12.31, respectively). Gene flow (Nm_p) between northern and southern regions, as well as between eastern and western regions, was approximately three times higher than among populations within any region or among all 35 populations (Table 5). This observation is probably an artefact because the number of private alleles (on which Nm_p is based) is likely to decrease upon pooling all populations within each region. Nm_θ was 83 to effectively infinite. Unlike F_{ST}, Weir and Cockerham's (1984) θ is corrected for the number of populations and sample size. θ is therefore calculated as an unbiased estimate of F_{ST} and represents an underestimate of F_{ST}, so that the inverse relationship between F_{ST} (or θ) and Nm leads to an overestimate of Nm (Slatkin and Barton, 1989). Thus, although Nm_θ-values of 83 or infinite are probably overestimates, gene flow in *P. canaliculus* is at least > 10 migrants/generation, sufficient to promote panmixia. Particularly for F_{ST}-values < 0.1, the confidence interval for Nm can be enormous due to the sampling error caused by finite samples of individuals and alleles from a finite number of populations and from the random evolutionary history of each locus (Whitlock and McCauley, 1999).

Higher levels of population genetic differentiation in *P. canaliculus* were, however, reported by Gardner et al. (1996a), among 10 greenshell mussel populations collected from around New Zealand ($F_{ST} = 0.095$, from which we have calculated $Nm_f = 2.38$). Similarly, Gardner et al. (1996b) observed an Nm-value (corresponding to Nm_p) of 2.01 between a natural *P. canaliculus* population from the southern end of the North Island and a cultured population from the northern tip of the South Island, with a distance of ~ 120 km.

4.3. Previous studies of P. canaliculus

Smith (1988) observed that significant heterogeneity in 4 out of 10 polymorphic loci was produced by the change in frequency of the common electromorph between northern and southern samples. We did not observe such a frequency shift at any locus (Fig. 2) nor could we detect allelic and genotypic differentiation. Population genetic structuring among 35 populations ($F_{ST} = 0.0199$, $P > 0.05$) was not significantly different from either population genetic structuring among random subsets of any six populations of the total 35 populations or of six populations consisting of combinations from two northern and four southern populations. This result implies that F_{ST}-values are independent of the number of populations sampled (35 populations vs. 6 populations).

When testing for significant difference between the means of the two F_{ST} frequency distributions, we found the difference to be minimal albeit significant. Ten of the F_{ST}-values of the frequency distribution for combinations of six populations chosen at random from northern and southern populations were significantly > 0. This corresponds to five more significant values than can be expected by chance alone. By contrast, $< 5\%$ of the F_{ST}-values of the F_{ST} frequency distribution for combinations of six populations randomly chosen from the 35 populations were significantly > 0. The two randomisation procedures show that F_{ST}-values indicating population differentiation and $< 100\%$ gene flow (i.e. those that were significantly > 0) could, at least for our data set, not be generated by reducing sample size alone, but by reducing sample size and by restricting sampling to northern and southern regions. One possible explanation for the heterogeneity among two northern and four southern mussel populations observed by Smith (1988) is, therefore, an undersampling of the species' range.

The absence of isolation by distance (no relationship between $\theta/(1-\theta)$ estimates and geographic distance) in the present study indicates that the effect of gene flow is higher relative to that of genetic drift. The $\theta/(1-\theta)$-values are not only independent of geographic distance, but the narrow scatter of the values (low $\theta/(1-\theta)$ estimates, slope not significantly different from zero) implies that mussel populations are not fragmented into isolated demes, within which genetic drift can act independently without the compensating influences of gene flow. This pattern of gene flow might be observed among populations that have recently invaded an area from a homogenous source population, reflecting the genetic similarity among these populations with a shared evolutionary history, or it might be observed if gene flow remains strong relative to genetic drift, thus spreading genetic variants throughout the region (Hutchison and Templeton, 1999). *P. canaliculus* is first recorded in the fossil record in the late Oligocene to early Miocene (30–25 million years ago, Fleming, 1979), and is widespread and common throughout New Zealand (Hutton, 1880; Powell, 1979; personal observations). The sampled individuals, therefore, do not appear to originate from recently invading populations but rather seem to belong to one ancestral homogeneous population, within which levels of gene flow are high. Gardner et al. (1996a), on the other hand, found a significant positive relationship between geographic and genetic distance, and explained the observed genetic structure with the isolation by distance model in conjunction with local hydrography. Theoretically, it would be possible for the 3–5-week planktonic larval stage of *P. canaliculus* to recruit over large coastal distances, either dispersed in the water column or drifting on kelp. For an average daily current velocity of 1 kn (1.852 km/h), a particle could drift 44.45 km/day, and it would take that particle 21 days to reach a destination 926 km away. Thus, the biology and history of *P. canaliculus*, the absence of known barriers to gene flow and the hydrography of New Zealand (Harris, 1990) are all consistent with the observed model of panmixia for this species, and neither the north vs. south (Smith, 1988) nor the isolation by distance models (Gardner et al., 1996a) of population genetic structuring applies to this study.

A question of considerable interest is why did the three separate studies of this species observe different models of population genetic structuring. Several different possible explanations exist, none of which is easily testable. Reasons for the observed difference in estimates of gene flow based on sampling differences among the studies

include: (1) the assayed allozyme loci were not identical. Smith (1988) reported heterogeneity at four loci (*Aat-2*, *Idh*, *Lap*, and *Per*) among two northern (Kaipara and Tauranga) and four southern (Castle Point, Wellington, Oamaru, and Bluff) mussel populations, and suggested that local hydrography, as well as genetic–physiological adaptation to different thermal environments, might partially isolate mussel populations, which could result in a warm water-adapted northern group and a cold water-adapted southern group, between which there was only limited gene flow. Gardner et al. (1996a) also reported a significant difference in allele frequencies at loci *La-1* and *Lgg-1* (loci coding for amino peptidases) between their pooled two northern (Ahipara and Coromandel) and pooled eight southerly populations (Napier, Wellington, Cultured [Beatrix Bay], Golden Bay, Tasman Bay, Akaroa Harbour, Horseshoe Bay, and Watercress Bay). It is interesting to note that, of the 10 polymorphic loci examined by Smith (1988), only four exhibited significant heterogeneity in allele frequencies between the northern and southern populations. Only one of the five loci shared between Smith's (1988) and this study (*Idh*) showed this heterogeneity, whereas the other four shared loci between the two studies did not (*Gpdh*, *Gpi*, *Pgdh*, and *Pgm*). Furthermore, two of the three loci shared between Smith's (1988) and Gardner et al.'s (1996a) study (*Gpi* and *Pgm*), which are the only two loci shared between the present and Gardner et al.'s (1996a) study, did not exhibit significant allele frequency heterogeneity among populations. If, as is usually assumed, all allozyme loci are neutral markers of gene flow, then differences among studies of assayed loci should not result in different patterns of population genetic structuring. However, the present findings lead one to the conclusion that *Gpi* and *Pgm* are neutral allozyme loci, whereas the loci at which population heterogeneity was detected are directly subject to selective forces, such as variation in sea surface temperature or salinity, or are linked to loci that are under selection. In particular, the five amino peptidases (*La-1*, *La-2*, *Lap*, *Lgg-1*, and *Lgg-2*) used by Gardner et al. (1996a) may not be selectively neutral. There is strong evidence that variation at the *Lap* locus (coding for an amino peptidase) is associated with salinity variation (e.g. Koehn et al., 1976, 1980). Mussels (*Mytilus edulis*) carrying different *Lap* alleles can accumulate cellular free amino acids at different rates (Hilbish et al., 1982). Thus, the capacity to osmoregulate is *Lap* genotype-dependent, and *Lap* genotypes are under direct selective influence of environmental salinity (Hilbish and Koehn, 1985). Therefore, the discrepancies in results between Smith's (1988) and Gardner et al.'s (1996a) findings regarding a north–south population subdivision and the absence of such population differentiation in the present study might be based on the different allozyme loci examined.

(2) Parametric variation (mutation rates vary among loci) inherent in allele frequencies and (3) sampling variation (the observed frequency of an allele is an estimate of the true frequency of this allele in the population) (Slatkin and Arter, 1991) may each have contributed to different results among allozyme studies. Alternatively, the differences among the three studies may be real and have resulted from actual changes in population genetic structuring of this species as a consequence of interannual variability. For example, profound temporal change in allele frequencies at the *Lap* locus in a 2-year period has been reported in *M. galloprovincialis* in New Zealand (Gardner and Kathiravetpillai, 1997; Gardner and Palmer, 1998). The weak El Niño events in

1986/1987 and 1994, and the strong El Niño event in 1997/1998, roughly coincided with the sampling of mussels by Smith (1988) prior to 1988, Gardner et al. (1996a) in 1994 and the present study in 1996–1998, respectively. The pronounced changes in environmental and hydrographic conditions associated with such events (e.g. McPhaden, 1993) may have led to temporal and/or spatial variation in allele frequencies of populations and, therefore, genuine alterations of population genetic structuring.

4.4. Do other New Zealand marine species show population genetic structuring?

One might expect the influence of regional hydrography (e.g. Heath, 1985; Mercer, 1979) and a gradient in the habitat range of >2000 km, from the warm-temperate north to the cool-temperate south, to be reflected in the genetic structuring of populations, not just of *P. canaliculus*, but of other New Zealand species, too. For example, there is evidence of allele frequency clines in populations of marine species between regions characterised by different environmental regimes, e.g. salinity, water temperature differences, and hydrographic barriers (Burton and Feldman, 1982; Grant et al., 1992; Powers et al., 1993; Saavedra et al., 1993; Quesada et al., 1995; Pannacciulli et al., 1997). Several allozyme studies conducted in New Zealand suggest that water temperature and hydrographic conditions may indeed be responsible for promoting population genetic structuring in New Zealand. For example, Smith (1978) identified two stocks in the New Zealand ling *Genypterus blacodes* (Gadiformes, Teleostei), one located at the Pukaki Rise off the southeast coast of New Zealand, and the other comprised of five mainland populations. Smith et al. (1978) reported three groups of New Zealand snapper, *Pagrus auratus* (Perciformes, Teleostei), located on the west- and east-coast of North Island, and in Hawke Bay. Paua, *Haliotis iris* (Gastropoda, Mollusca; Frusin, 1982), populations were divided into a mainland and a Chatham Island group. Thirteen populations of the surf clam, *Paphies subtriangulata* (Bivalvia, Mollusca) from around New Zealand fell into two mainland groups (northern and central coasts), a Chatham Island, and possibly a Stewart Island group (Smith et al., 1989). Significant heterogeneity was exhibited among populations of pea crabs, *Pinnotheres novaezelandiae* and *Pin. atrinicola* (Decapoda, Crustacea; Stevens, 1990, 1991), commonly symbiotic with bivalve mussels, in the North Island of New Zealand. Finally, Intasuwan et al. (1993) separated 17 New Zealand populations of the red alga *Gracilaria chilensis* (Gracialiares, Rhodophyta) into a northern group (northern-half of the North Island) and a group found throughout the country. By contrast, there was no evidence for biochemical genetic differentiation among New Zealand red rock lobster populations, *Jasus edwardsii* (Decapoda, Crustacea), from Gisborne, Wellington, and Stewart Island (Smith et al., 1980), and six populations of the sea urchin *E. chloroticus* from around New Zealand exhibited low values of genetic distance (0–0.019) and low levels of population differentiation ($F_{ST} = 0.01–0.02$, Mladenov et al., 1997). These discordant results based on allozyme data suggest that the reproductive biology of a species influences its potential habitat range to a great extent (e.g. Scheltema and Williams, 1983). For example, snapper (*Pag. auratus*) form temporal spawning aggregations (Smith et al., 1978). Pea crabs (*Pinnotheres* spp.) brood their young within their molluscan hosts, larvae are strong-swimming, and have an abbreviated larval development and obligatory

requirement for association with a host (Stevens, 1990). In the case of the red alga (*G. chilensis*), vegetative reproduction cannot be excluded (Intasuwan et al., 1993). The behaviour and/or reproductive biology of these three species may contribute to genetic subdivision of their populations due to fertilisation occurring in a confined geographical space. However, both the red rock lobster *J. edwardsii* and the sea urchin *E. chloroticus* possess long pelagic larval stages of 8–15 and 1–2 months, respectively, which are conducive to high dispersal via ocean currents.

We conclude that the absence of population genetic structure in *P. canaliculus* is best explained by high levels of gene flow mediated by larvae with high dispersal capacity and lack of effective hydrographic barriers. Comparison of the allozyme results with those obtained by mitochondrial SSCP and RFLP and nuclear microsatellite analyses from the large sample set is likely to provide valuable insights into the extent of genetic variation in this endemic mussel species.

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