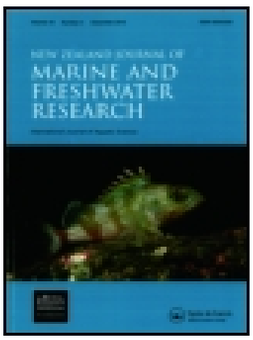

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Biochemical-genetic variation in the green-lipped mussel *Perna canaliculus* around New Zealand and possible implications for mussel farming

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Abstract Genetic variation was surveyed at 10 polymorphic enzyme loci in six samples of green-lipped mussels, *Perna canaliculus*, from around New Zealand. There is a significant heterogeneity at five loci and at four of these the heterogeneity is produced by significant differences between northern and southern samples. The differences may be explained by limited genetic exchange owing to current movements coupled with local selection. Four loci were tested in spring and autumn seed mussels from Marlborough Sounds and Ninety Mile Beach. There is a significant difference between spring and autumn seed at one locus. Seed mussels show a significant excess of homozygotes at 6 out of 16 tests whereas for adult mussels at the same loci only 4 out of 24 tests are significant. It is suggested that the differences between spring and autumn seed and the greater homozygosity in seed mussels may be produced by partial assortative mating. For farming operations there may be genetic disadvantages in transferring seed from one water mass to another for ongrowing.

Keywords green-lipped mussel; *Perna canaliculus*; electrophoresis; genetic variation; mussel farming

INTRODUCTION

The green-lipped mussel *Perna canaliculus* is a common bivalve on the lower intertidal and sublittoral rocky coasts around New Zealand. It has become the major farmed species of shellfish with exports valued at NZ$ 15 million in 1987. Most mussel farms are located in the Marlborough Sounds but some have been established in sheltered waters off the north-east coast of the North Island. A limiting factor to the extensive farming operations in the Marlborough Sounds has been an inadequate supply of seed mussels. This has been overcome by transplanting seed from other areas. Seed can be collected on strand weed in spring and autumn on Ninety Mile Beach off the north-east coast of the North Island (Hickman 1975). Casual observations suggest that mussel seed collected from northern areas grow at different rates and mature at different periods to local seed when ongrown in the Marlborough Sounds (Hickman pers. comm.).

The expansion of mussel farming activities has focused attention on seed transplantation; experiments have been set up to compare the growth and maturity characteristics of mussel stocks to test if there is a genetic component to regional differences (Hickman & Illingworth 1980; Hickman unpubl. data). An alternative, and fairly rapid, method to assess genetic similarities and differences between populations is to use gel electrophoresis. Biochemical-genetic studies with other bivalves have shown moderate to high levels of genetic variation and regional differences in electromorph frequencies, e.g., the scallop *Chlamys opercularis* (Beaumont 1982) and the blue mussel *Mytilus edulis* (Koehn et al. 1984). One feature of electrophoretic studies on marine bivalves has been an excess of homozygotes at several loci (Singh & Green 1984). In general this excess is greater in juveniles than in adults, implying that selection favours heterozygotes over the juvenile to adult growth phase (Singh & Green 1984).

The purpose of this paper is to examine regional variation in electromorph frequencies in wild stocks of *P. canaliculus* and to test for genetic differences between spring and autumn seed collected from the Marlborough Sounds and at Ninety Mile Beach.

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MATERIALS AND METHODS

Sample collection

Regional samples of wild adult green-lipped mussels were collected at six locations around New Zealand (Fig. 1). Approximately 100 mussels were removed from a small area of a large bed of mussels at low tide. The mussels were transported alive to the laboratory where they were dissected and the adductor muscle and hepatopancreas individually frozen in plastic tubes at −70 °C.

Seed mussels, 1–16 mm, were collected on drift weed at Ninety Mile Beach (Fig. 1) in December 1983 and June 1984. The weed was transported to Wellington where it was held in sea water tanks for several days. A sample was taken by removing all the visible mussels off the weed. These mussels were electrophoresed on the same day. Two samples of seed mussels were taken from spat collectors in the Marlborough Sounds (Fig. 1) in January 1984 and May 1984. These samples were "spatted" onto ropes before transport to Wellington where they were kept in sea water tanks for several days before electrophoresis.

Electrophoresis

Seed mussels smaller than c. 20 mm were removed from the shell and the tissue ground up in a well in a cold perspex block. To mussels >5–6 mm, about half their volume of cold distilled water was added before homogenisation; for the smaller mussels there was sufficient sea water with the tissue to act as a homogenising buffer.

Adult mussels were dissected and the adductor muscle and hepatopancreas gland homogenised separately in about half their volume of cold distilled water.

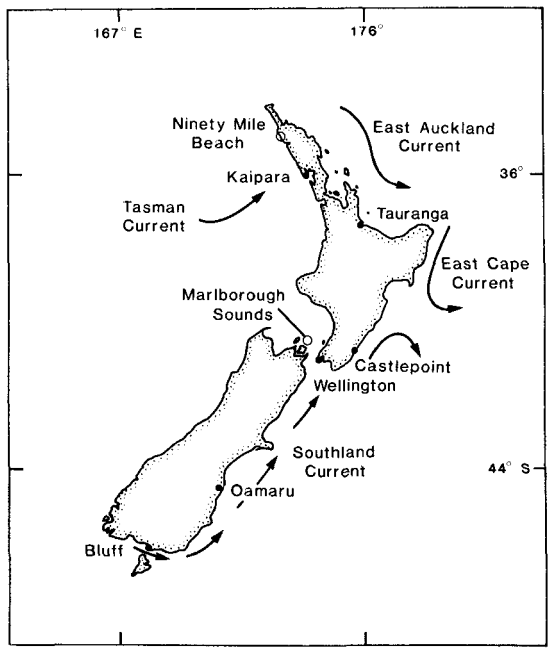

Fig. 1 Location of sampling sites of green-lipped mussel *Perna canaliculus* around New Zealand and water currents referred to in the text. • adult sample; o seed sample.

After homogenising, samples were loaded into starch or cellulose acetate gels using standard techniques (Gauldie & Smith 1978; Smith et al. 1978). Electrophoretic conditions are summarised in Table 1. Enzyme staining procedures followed those described by Harris & Hopkinson (1976). Enzymes were chosen from a preliminary survey of genetic variation in *P. canaliculus* that identified polymorphic loci (Fujio et al. 1983).

Table 1 Enzymes, loci, tissues, and electrophoretic conditions used in a survey of genetic variation in green-lipped mussels, *Perna canaliculus*. Tissue: h, hepatopancreas gland; m, adductor muscle. Gel medium: c, cellulose acetate; s, starch. Buffer: PC, phosphate-citrate; TB, tris-barbital; TC, tris-citrate; TL, tris-citrate, lithium hydroxide-boric acid.

Enzyme	Enzyme commission no.	Loci	Tissue	Gel	Buffer
Aspartate aminotransferase	2.6.1.1	*Aat*-1	m	s	TL
		Aat-2	m	s	TL
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*Gpdh*	h	s	PC
Glucosephosphate isomerase	5.3.1.9	*Gpi*	m	c	TB
Isocitrate dehydrogenase	1.4.1.42	*Idh*	h	s	PC
Leucine aminopeptidase	3.1.11 -	*Lap*	m	s	TL
Malate dehydrogenase	1.1.1.37	*Mdh*	m	s	TC
Peroxidase	1.11.1.7	*Per*	m	s	TL
6-Phosphogluconate dehydrogenase	1.1.1.44	*Pgdh*	h	s	TC
Phosphoglucomutase	2.7.5.1	*Pgm*	m	c	TB

RESULTS

Regional variation

The results of the electrophoretic survey of the *P. canaliculus* samples are summarised in Table 2. The frequency of the common electromorph is shown for each locus. At nine loci one electromorph is in high frequency (>0.60) in all samples (Table 2). Seven electromorphs were observed at *Lap* and these were pooled into three synthetic alleles (six synthetic genotypes) for statistical analyses. Allele frequencies and goodness-of-fit tests were performed using the computer program of Swofford & Selander (1981).

Hardy-Weinberg (HW) tests were carried out on the 10 loci analysed in 6 populations. Ten tests show a significant departure from HW equilibrium (Table 2), all with an excess of homozygotes. Four of these departures occur at *Lap*, 2 at *Aat*-1, and 1 at *Aat*-2, *Gpi*, *Idh*, and *Pgm* (Table 2). Ten departures is greater than expected by chance (1 in 20) and is typical of electrophoretic data on marine molluscs.

Testing for heterogeneity in the six samples at 10 loci shows a significant heterogeneity at 5 loci (Table 2). This heterogeneity is produced by a change in frequency of the common electromorphs (Table 2) and is not caused by the occurrence of regional or rare electromorphs, in some samples. There is no evidence for clinal variation in the six samples (Table 2), however the two northerly samples, Kaipara and Tauranga, are significantly different from the four southerly samples at *Aat*-2 ($\chi^2_1 = 53.97$, $P < 0.001$), *Idh* ($\chi^2_1 = 5.90$, $P < 0.02$), *Lap* ($\chi^2_1 = 10.78$, $P < 0.01$), and *Per* ($\chi^2_1 = 21.66$, $P < 0.001$).

Seed mussels

Results of the electrophoretic analyses of the seed mussels are summarised in Table 3. Only four loci could be scored routinely in seed < 5 mm, these were *Aat*-1, *Aat*-2, *Gpi*, and *Pgm*. Four other loci, *Idh*, *Lap*, *Per*, and *Pgdh*, stained weakly or not at all in seed < 5 mm and two loci, *Gpdh* and *Mdh*,

Table 2 Number of *Perna canaliculus* tested, common allele frequencies, and agreement with Hardy-Weinberg equilibrium in six samples of adult mussels tested for ten loci. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; – not significant.

		Location						Heterogeneity
Locus		Kaipara	Tauranga	Castle Point	Wellington	Omaru	Bluff	χ^2
Aat-1	*N*	94	94	96	81	93	93	
	allele	0.718	0.707	0.813	0.827	0.672	0.720	**
	HW	–	–	–	**	–	*	
Aat-2	*N*	96	95	94	95	94	94	
	allele	0.828	0.905	0.617	0.753	0.617	0.660	***
	HW	–	–	–	–	*	–	
Gpdh	*N*	96	99	96	94	92	93	
	allele	1.000	0.975	0.990	0.989	0.995	0.989	–
	HW	–	–	–	–	–	–	
Gpi	*N*	96	94	95	96	94	94	
	allele	0.828	0.867	0.795	0.870	0.793	0.824	–
	HW	–	–	–	–	–	*	
Idh	*N*	96	99	96	94	92	91	
	allele	0.932	0.924	0.891	0.878	0.935	0.841	–
	HW	–	–	*	–	–	–	
Lap	*N*	96	104	77	101	97	68	
	allele	0.458	0.447	0.682	0.465	0.464	0.551	***
	HW	***	**	–	**	*	–	
Mdh	*N*	96	96	96	99	92	96	
	allele	1.000	0.979	1.000	0.960	0.989	0.958	–
	HW	–	–	–	–	–	–	
Per	*N*	94	95	96	94	94	94	
	allele	0.931	0.895	0.833	0.750	0.803	0.835	***
	HW	–	–	–	–	–	–	
Pgdh	*N*	96	96	96	91	96	96	
	allele	0.938	0.958	0.938	0.951	0.979	0.990	*
	HW	–	–	–	–	–	–	
Pgm	*N*	97	95	90	96	95	92	
	allele	0.716	0.737	0.739	0.661	0.684	0.620	–
	HW	–	–	*	–	–	–	

were not tested as they had low levels of polymorphism in adult samples (Table 2). HW tests on *Aat*-1, *Aat*-2, *Gpi*, and *Pgm* showed 7 out of 16 tests with a significant departure from genetic equilibrium (Table 3). Six of these had a significant excess of homozygotes and one, *Gpi* in the Marlborough Sounds (May), had a significant excess of heterozygotes.

Testing for heterogeneity in the four seed samples at four loci shows a significant heterogeneity only at *Aat*-1 ($\chi^2_3 = 27.25$, $P < 0.001$). This heterogenity is produced by significant differences between autumn and spring seed at both localities (Ninety Mile Beach $\chi^2_1 = 4.94$, $P < 0.05$; Marlborough Sounds $\chi^2_1 = 7.55$, $P < 0.01$) although the electromorph frequency changes have moved in opposite directions at the two localities (Table 3).

The seed samples were compared with adults from the nearest geographical locality, Ninety Mile Beach seed with Kaipara adults and Marlborough Sound seed with Wellington adults. There is a general decrease in frequency of the common electromorph accompanied by a general increase in heterozygosity from seed to adult (Table 3). Observed heterozygosities in the four seed samples (0.257–0.271) are less than the observed heterozygosities in the six adult samples (0.287–0.394). There are significant differences between Kaipara adults and Ninety Mile Beach seed at *Aat*-1 (spring seed $\chi^2_1 = 5.5$, $P < 0.5$; autumn seed $\chi^2_1 = 14.69$, $P < 0.001$) and *Aat*-2 (spring seed $\chi^2_1 = 8.90$, $P < 0.01$; autumn seed $\chi^2_1 = 5.84$, $P < 0.05$) and between Wellington adults and Marlborough Sound seed at *Aat*-1 (autumn seed $\chi^2_1 = 8.5$, $P < 0.01$) and *Aat*-2 (spring seed $\chi^2_1 = 14.84$, $P < 0.001$; autumn seed $\chi^2_1 = 15.44$, $P < 0.001$). In six of these seven differences between seed and adult electromorph frequencies the change is a reduction in frequency of the common allele in the adult samples, i.e., an increase in heterozygosity.

DISCUSSION

There is a significant heterogeneity at five out of ten loci in the green-lipped mussel samples from around New Zealand, indicating that they cannot have been taken from a single genetic unit. The genetic differences are due to changes in frequency of the common electromorphs and there are no unique regional alleles. Part of the heterogeneity is produced by regional differences in electromorph frequencies. The two northerly samples, from Kaipara on the west coast of the North Island and from Tauranga on the north-east coast of the North Island, are significantly different from the other four

Table 3 Number of *Perna canaliculus* tested, common allele frequencies, and agreement with Hardy-Weinberg equilibrium, and heterozygosities in four samples of seed mussels and two samples of adult mussels tested for four loci. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; – not significant.

		Seed				Adult	
		Ninety Mile Beach		Marlborough Sounds		Kaipara	Wellington
Locus		Dec	Jun	Jan	May		
Aat-1	*N*	210	288	90	210	94	81
	allele	0.786	0.844	0.817	0.710	0.718	0.827
	HW	–	***	*	***	–	**
Aat-2	*N*	204	288	89	210	96	95
	allele	0.912	0.894	0.904	0.879	0.828	0.753
	HW	–	–	–	–	–	–
Gpi	*N*	219	288	87	212	96	96
	allele	0.815	0.786	0.816	0.807	0.828	0.870
	HW	–	–	–	*	–	–
Pgm	*N*	219	288	92	211	97	96
	allele	0.715	0.724	0.707	0.730	0.716	0.661
	HW	***	**	**	–	–	–
Mean observed heterozygosity (±s.e.)		0.271(±0.04)	0.263(±0.04)	0.257(±0.04)	0.269(±0.06)	0.340(±0.05)	0.287(±0.05)
Mean expected heterozygosity (±s.e)		0.302(±0.05)	0.298(±0.05)	0.298(±0.05)	0.334(±0.05)	0.347(±0.04)	0.335(±0.05)

more southerly samples at four of the five loci which show a significant heterogeneity in the total data. These northerly samples occur in warmer water masses than the other samples. Tauranga receives subtropical water from the north in the East Auckland current whilst the Kaipara receives water from the Tasman current (Fig. 1). The samples on the east coast are influenced by cooler water of the Southland Current (Fig. 1).

Evidence from other marine organisms shows that temperature and salinity are selective agents in maintaining some enzyme polymorphisms (Place & Powers 1979; Koehn et al. 1980; DiMichele & Powers 1982; Hilbish & Koehn 1985; Hoffmann 1985) and thus the genetic differences observed between northern and southern mussels may reflect a genetic-physiological adaptation to different thermal environments. In addition current directions may partially isolate mussel populations by limiting the movement of pelagic larvae between areas. Beaumont (1982) has suggested that partial isolation, plus local selection, maintains the different electromorph frequencies between geographically separate populations of the scallop *Chlamys opercularis* off North-West Europe. In this respect the east coast mussel samples from the South Island and southern North Island (Fig. 1) would form one unit; genetic exchange with areas north of East Cape, such as Tauranga, would be restricted by the East Auckland and East Cape currents (Fig. 1). The regional genetic differentiation of mussel populations is supported by similar breaks in the genetic composition of the teleost *Chrysophrys auratus* (Smith et al. 1978) and the surf clam *Paphies subtriangulata* (Smith unpubl. data) off the east coast of the North Island.

In the seed mussels, only one out of four loci showed a significant heterogeneity between autumn and spring samples from two localities. However, two of the loci, *Gpi* and *Pgm*, showed no regional variation in adult samples (Table 2) and so these markers may be insensitive to population events. The heterogeneity at *Aat*-1 is produced by significant changes in electromorph frequencies between spring and autumn seed at both Ninety Mile Beach and in the Marlborough Sounds. These changes are in opposite directions at the two sites and it is not clear if this is a chance observation or reflects genetic differences between northern and southern mussels.

The seed mussels show a greater genetic imbalance than the adult samples. Six out of 16 tests show a significant homozygous excess in seed mussels while at the same loci in adults only 4 out of 24 tests deviate significantly from HW equilibrium (Table 2 and 3). This observation is typical of marine molluscs in which homozygous excess is greater in juveniles than in adults (Singh & Green 1984).

The observed change in electromorph frequencies between samples of seed mussels and the greater excess of homozygotes in juveniles than in adults may be products of the same mechanism. Partial assortative mating, under which animals of similar genotype mature and spawn at the same time periods, but at different time periods to animals of dissimilar genotype, would produce cohorts of different electromorph frequencies and lead to homozygous excess in mixed samples of juveniles. Supporting evidence for partial assortative mating is seen in settling cohorts of the blue mussel, *Mytilus edulis*, in which there are significant differences between discrete settlement groups (Gosling & Wilkins 1985), and in the sand flounder *Rhombosolea plebeia* where there is a significant change in electromorph frequencies between early and late spawners (Smith 1987).

The relatively small sample of loci used in this study has revealed a significant heterogeneity between mussel populations around New Zealand. If this sample is representative of the total genome, then there may be major genetic differences between mussel populations, in particular the northern and southern groups. Electromorph frequency differences, as opposed to fixed differences, characterise two species of mussels, *Mytilus edulis* and *M. galloprovincialis,* in the north-east Atlantic Ocean (Ahmad & Beardmore 1976; Skibinski et al. 1978, 1983); changes in electromorph frequency between samples of *M. edulis* from southern and northern Canada have led Koehn et al. (1984) to raise the possibility of two distinct species in the north-west Atlantic. If some electromorphs are adaptive, and there is evidence to support this in marine organisms (Koehn et al. 1980; DiMichele & Powers 1982; Hilbish & Koehn 1985; Hoffmann 1985), then the transfer of seed between different water masses might be detrimental to farming practices; seed might not grow at the optimum rate under unsuitable conditions and the subsequent reproduction of large quantities of introduced seed would result in an increased homozygous excess in juveniles, through the Wahlund effect of population mixing, and an increased mortality when heterozygotes have higher survival values.

This preliminary genetic survey has shown significant differences between mussel populations and suggests that further studies of the genetic aspects of growth and condition are warranted. In the meantime, mussel farmers would be advised to choose seed from an area similar to the ongrowing region in preference to transporting seed between water masses.

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