Biochemical population genetics of the black mussel *Choromytilus meridionalis*

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Gene products of nine loci were examined by horizontal starch-gel electrophoresis in five samples of black mussels, *Choromytilus meridionalis*, from the south-western Cape coast. Allelic frequency variation for four polymorphic proteins suggests no racial differences between west and south coast populations. Mean heterozygosity ($\bar{H}$) showed a non-significant increase from south to west coast populations. The duration of the pelagic larval stage and currents may both contribute to long-distance larval recruitment, although gene flow between neighbouring populations may be more important.

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Geenprodukte van nege loci in vyf monsters van swart mossels, *Choromytilus meridionalis*, van die Suiwes-Kaapse kus is bestudeer deur middel van styseljel-elektroforese. Alleliese frekwensievariasie vir vier polimorfiese proteïene dui daarop dat geen rasverskille tussen wes- en suidkusbevolkings voorkom nie. Gemiddelde heterosigositeit ($\bar{H}$) toon 'n onbeduidende toename van suid- na weskusbevolkings. Die duur van die pelagiese larwale stadium, sowel as seestrome, mag tot die langafstand-werwing van larwes bydra, alhoewel genevloei tussen naburige bevolkings van groter belang mag wees.

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The black mussel, *Choromytilus meridionalis* (Kr.), is endemic to southern Africa and is distributed from Walvis Bay on the west coast to Port Alfred on the east coast (Day 1974). However, Barnard (1964) noted that it is common on the west coast and is rare east of Hermanus in the Cape Province. Over this range patchy mussel beds are distributed on gently sloping and slow draining rocky intertidal surfaces having moderate to large amounts of wave exposure (Griffiths 1981). Spawning takes place intermittently during spring and summer with peaks in the proportion of spawners that vary annually (Griffiths 1977). Pelagic larvae spend 5 to 7 weeks in the water and settle sporadically throughout most of the year (du Plessis 1977). However, successful large-scale settlement may occur only every 4 to 6 years at a given location (Griffiths 1981).

Recent studies have shown that there are metabolic differences between populations of black mussels on the west and south coasts. Seiderer, Davis, Robb & Newell (1984) found regional differences in bacteriolytic activity and speculated that lower temperatures from west-coast upwelling may induce lytic activity. However, lytic activity was not induced by experimentally lowering temperatures of south-coast mussels. Another study showed that the specific activities of carbohydrases, such as laminarinase, alpha- and beta-amylase, of west-coast mussels were about three times greater than those of south-coast mussels (B.C. Clarke, University of Cape Town, unpublished data). This may be due to the induction of carbohydrase by greater concentrations of dissolved kelp carbohydrates, such as laminarin, on the west coast or by some other kind of geographic adaptation. Both of these studies suggest that there may be genetic differences between south- and west-coast mussels.

Studies of other species of mussels have shown that, in spite of their large potential for unimpeded gene flow along a coast, mussel populations may be genetically subdivided. For example, populations of the northern hemisphere mussel, *Mytilus edulis*, are subdivided into three genetic races along the eastern coast of North America (Koehn, Hall, Innes & Zera 1984). Along the west coast of Europe, this same species also shows some degree of population subdivision (Murdock, Ferguson & Seed 1975; Gosling & Wilkins (1981); Skibinski, Beardmore & Cross 1983).

The purpose of this study was to examine the genetic population structure of black mussels located around the Cape Peninsula using the geographic distributions of electrically detectable protein variants. Strong temperature gradients in this area may affect the survival of drifting larvae and may act as a barrier to gene flow between the Cape south and west coasts. If these mussel populations are reproductively isolated

from one another by divergent water currents or by environmental gradients, then allelic frequency differences may be apparent.

Materials and Methods

Samples of black mussels were collected at five locations on the west and south coasts of the Cape Province during low tide in April and May 1984 (Figure 1). The mussels were

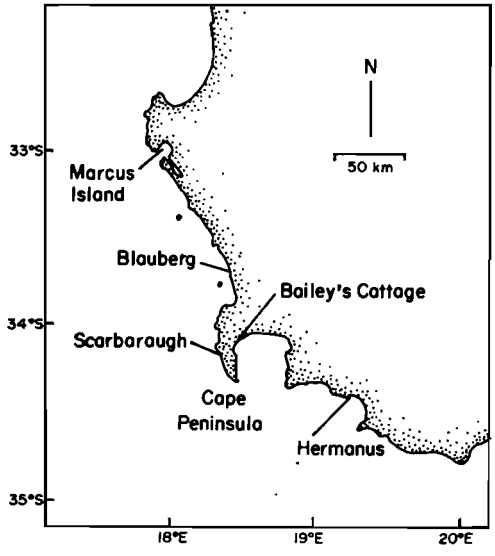

Figure 1 Map of south-west coast of Cape Province showing locations of samples of black mussels.

divided into two age-size classes for electrophoresis where the smallest class consisted of mussels 30 to 55 mm in shell length and the largest class consisted of mussels 70 to 115 mm in shell length. Sizes were chosen to represent two different cohorts at each site. This was achieved for Hermanus, Bailey's Cottage and Marcus Island (Figure 1). It was not possible to separate age classes in the samples from Scarborough and Blouberg (Figure 1), according to the population age structure of *C. meridionalis* determined by Griffiths (1981). The allelic frequencies of the different cohorts were compared in order to determine the possibility of the cohorts having different geographical origins. Soluble proteins were extracted from about 2,0 g of mantle and posterior adductor muscle tissues with distilled water. After centrifugation for 5 min at 1000 g, clear supernatants were used for starch-gel electrophoresis following May, Wright & Stoneking (1979). Histochemical methods followed Harris & Hopkinson (1976). The enzymes examined, their abbreviations and buffer systems giving the best results are presented in Table 1. Loci were designated numerically beginning from the cathodic end of a gel and alleles were designated by their mobilities relative to the most common allele, which was designated 100.

Deviations from Hardy-Weinberg proportions for each locus were detected using the chi-square test for goodness of fit (Sokal & Rohlf 1969). Degrees of freedom were equal to $(x^2 - x)/2$ where x was the number of alleles for a locus. Expected heterozygosity (h) for a polymorphic locus was calculated by

$$h = 1 - \Sigma p_i^2$$

where p_i is the relative frequency of the ith allele. Average heterozygosity ($\bar{H}$) was calculated by averaging h over all loci.

Table 1 Enzymes, locus abbreviations and buffers used in this study

Enzyme	Locus	Buffer*
Glucosephosphate isomerase	*Gpi*	1
Isocitrate dehydrogenase	*Idh* – 2	2
Malate dehydrogenase	*Mdh* – 1	2
	Mdh – 2	2
Peptidase		
substrate: glycyl-leucine	*Gl*	3
substrate: phenylalanyl-proline	*Php*	3
Phosphogluconate dehydrogenase	*Pgd*	2
Phosphoglucomutase	*Pgm*	1

*1 = gel (pH 8,5) tris 0,03 mol dm^{-3}; citric acid 0,3 mol dm^{-3}; lithium hydroxide 0,006 mol dm^{-3}; boric acid 0,03 mol dm^{-3}; electrode (pH 8,1) lithium hydroxide 0,06 mol dm^{-3}; boric acid 0,3 mol dm^{-3}. 2 = gel (pH 6,9) 1:15 dilution of electrode buffer; electrode (pH 6,9) tris 0,15 mol dm^{-3}; citric acid 0,05 mol dm^{-3}. 3 = gel (pH 8,7) tris 0,18 mol dm^{-3}; boric acid 0,1 mol dm^{-3}; NaEDTA 0,004 mol dm^{-3}.

Significant differences in allelic frequencies were detected using a nested contingency-table analysis and the chi-square test statistic (Sokal & Rohlf 1969). For this analysis, samples were divided into comparisons between size classes at each location, among locations on each coast, and between south and west coasts. Only loci having common-allele frequencies less than 0,95 were used to avoid low expected values in the chi-square statistic. The probability level of the rejection statistic was modified to account for the increase in type I error that ensues when multiple tests of the same hypothesis are made (Cooper 1968). Hence, tests were considered significant if the rejection criterion exceeded the value in a chi-square table associated with a probability of 0,05/4 = 0,0125 where 4 was the number of tests (polymorphic loci) of each hypothesis.

The type II error statistic was calculated using methods described by Sokal & Rohlf (1969) for detecting 'true' differences between two percentages. According to the mussel literature, differences of approximately 20% in allelic frequencies of *Pgm*, *Gpi* and *Pgd* can be expected in natural populations (Koehn & Gaffney 1984; Koehn, Milkman & Mitton 1976; Levinton & Koehn 1976; Gartner-Kepkay & Zouros 1983). We thus calculated the sample size needed to determine a 'true' difference between the percentages 0,60 and 0,80, as the frequencies of the most common alleles in this study fell mostly within this range. With a sample size of $2N$ (where N was the number of mussels sampled) the type II error (β) committed in this study was found to be only 10%.

Results

Electrophoretic variation

We identified gene products of 10 protein encoding loci (Table 1). Gels stained for *Idh* showed two zones of banding. An anodic zone, *Idh* – 1, consisted of single-banded and triple-banded phenotypes, but banding was too weak to reliably determine genotypes. A more anodic zone consisted of a single band and was designated *Idh* – 2. Products of two loci were also observed for *Mdh* where the least anodic zone, *Mdh* – 1, consisted of a single weak band and a more anodic zone, *Mdh* – 2, consisted of single- and triple-banded phenotypes typical of a dimeric enzyme. Products of two peptidase loci were detected using the dipeptides, glycyl-leucine and phenylalanyl-proline, as substrates. Rare triple- and broad-banded phenotypes were observed, in addition to narrow single-banded phenotypes, and were interpreted to represent hetero-

zygous genotypes. None of these loci had allelic frequencies greater than 0,05 and were not used for population analysis.

Four polymorphic loci were also observed. The products of a single *Gpi* locus were observed which had several different mobility variants producing single-banded homozygotes and triple-banded heterozygotes (Figure 2). A single zone of banding was observed for *Pgd* near the origin. The zone had several triple- and single-banded phenotypes typical for a dimeric enzyme. Finally, the products of two loci, *Pgm* – 1 and *Pgm* – 2, were detected for *Pgm* which had double- and single-banded phenotypes typical for a monomeric enzyme. No significant departures from Hardy-Weinberg proportions were detected for any of these four polymorphic loci.

Geographic variation

Allelic frequencies for *Gpi*, *Pgm* – 1, *Pgm* – 2 and *Pgd* are presented in Table 2. No significant allele-frequency differences were detected between the two size class samples at any of the locations. Nor were any significant differences detected among samples collected on the west coast or on the south coast. However, significant differences between pooled west-coast samples and pooled south-coast samples were detected for *Pgm* – 2 ($0,05 > P > 0,01$). Paired comparisons between successive populations around the coast, however,

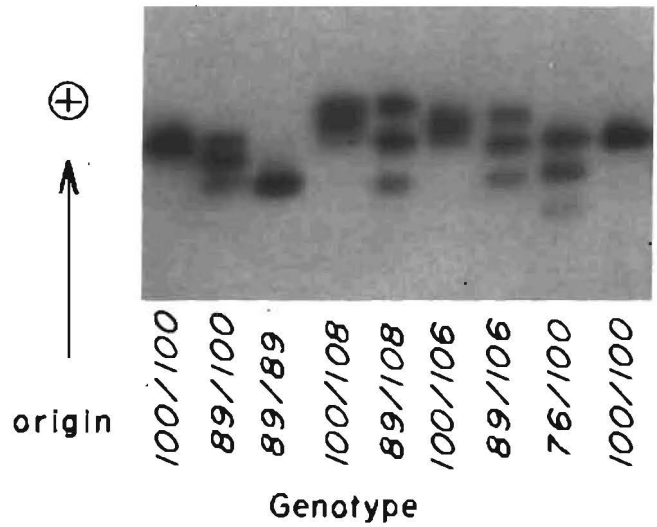

Figure 2 Genotypes of phosphoglucose isomerase in black mussels.

Table 2 Allelic frequencies of four polymorphic enzymes in *Choromytilus meridionalis*

		Location				
Locus	Allele	Hermanus	Bailey's Cottage	Scarborough	Blouberg Strand	Marcus Island
Pgi	76	0,005	–	0,01	–	–
	85	–	0,010	–	0,005	–
	89	0,075	0,040	0,055	0,085	0,095
	92	–	–	0,010	–	0,015
	100	0,885	0,910	0,895	0,890	0,865
	102	–	–	–	–	0,005
	106	0,005	0,010	0,015	0,010	0,005
	108	0,030	0,030	0,015	0,010	0,015
N		100	99	100	100	100
Pgm – 1	70	0,010	0,010	–	–	–
	85	0,010	0,025	–	0,030	0,010
	90	–	–	0,005	–	–
	97	–	–	–	0,005	–
	100	0,890	0,860	0,910	0,830	0,850
	102	–	–	–	0,005	–
	108	0,005	0,015	0,020	0,025	0,030
	116	0,025	0,060	0,055	0,060	0,070
	120	–	0,010	–	–	–
	125	0,060	0,010	0,010	0,040	0,010
	138	–	0,010	–	0,005	0,030
N		99	100	100	100	99
Pgm – 2	89	0,055	0,035	0,070	0,070	0,105
	92	0,010	0,015	0,005	–	–
	94	0,230	0,330	0,310	0,350	0,310
	97	0,005	–	0,030	0,005	0,025
	100	0,690	0,595	0,555	0,530	0,530
	104	0,010	0,025	0,020	0,045	0,015
	107	–	–	0,010	–	0,015
N		99	100	97	100	100
Pgd	5	–	–	0,010	0,005	–
	– 100	–	–	–	–	0,010
	30	0,030	0,005	0,030	0,055	0,040
	70	0,010	0,015	0,010	0,075	0,030
	85	0,010	0,035	0,010	–	0,020
	100	0,780	0,795	0,750	0,760	0,710
	250	–	–	–	–	0,010
	300	0,170	0,150	0,190	0,105	0,180
N		98	100	95	100	99

resulted in no significant differences for *Pgm* – 2. Average heterozygosity based on the four polymorphic loci varied clinally from Hermanus to Marcus Island but the trend was not statistically significant.

Discussion

Our results suggest that populations of black mussels are not genetically subdivided into races around the Cape Peninsula from Hermanus to Saldanha Bay. We can be 90% certain that no type II error (β) has been committed as β was calculated to be 10%. Although a significant difference in allele frequencies for *Pgm* – 2 was found between south and west coasts, paired comparisons of successive populations around the coast showed no significant differences in allele frequencies for *Pgm* – 2. We thus regard differences at the *Pgm* – 2 locus as insufficient evidence of genetic subdivision between south and west coast mussel populations, considering that no other locus studied was found to differ significantly between the coasts.

There is a large potential for gene flow between populations during the pelagic larval stage. Developing larvae may spend 5 to 7 weeks drifting in near-shore currents before metamorphosis and settlement on to low intertidal filamentous substrates. During this time larvae may be carried considerable distances by currents. There is a northerly jet current off Cape Point associated with an off-shore frontal system between cool inshore upwelled water and warm off-shore water (Bang & Andrew 1974). Seasonal velocities of this current may average as much as 0,312 m s^{-1} (Harris 1978). However, inshore surface currents, which are likely to have a greater influence on planktonic larvae, are dominated by seasonal northwesterly and south-easterly winds, and south-easterly movement of larvae is possible. Drift cards released at Scarborough on the west coast and Bailey's Cottage in False Bay were recovered near Hermanus on the south coast (G. Nelson, Sea Fisheries Research Institute, personal communication). Thus, current direction and velocity, and a lengthy pelagic larval stage may account for apparent gene flow between south and west coast black mussel populations.

Average heterozygosity, the proportion of heterozygous loci in an average mussel, increased clinally from 0,314 at Hermanus on the south coast to 0,402 in Saldanha Bay on the west coast. The increase in average heterozygosity was non-significant, but this result cannot be ignored. A study of average heterozygosity in *Mytilus edulis* by Koehn & Gaffney (1984) in Long Island Sound, North America, showed a correlation between average heterozygosity and growth rate. Of the five loci examined by these authors [*Gpi, Pgm, Ap* (aminopeptidase), *Odh* (octopine-dehydrogenase) and *Lap* (leucine-aminopeptidase)], heterozygosities at only the *Odh* and *Lap* loci correlated statistically significantly with growth rate, whereas the remaining loci showed correlations with growth rate that were non-significant. A similar study by Beaumont, Beveridge & Budd (1983) in Europe revealed no significant correlation between average heterozygosity and growth rate. Koehn & Gaffney (1984) attributed this discrepency in results to the experimental design employed by Beaumont *et al.* (1983), where half-sib families were used. Koehn & Gaffney (1984) unsuccessfully attempted to relate heterozygosity to relative growth rate among individuals in sibships, possibly owing to the reduction in heterozygosity at both marker loci and the genetic background in half-sib families. In the present study, natural populations were sampled but only four polymorphic loci were examined, which may be insufficient to detect a statistically significant increase in heterozygosity around the coast if it does exist. This would have occurred in the work of Koehn & Gaffney (1984) had they not examined the *Odh* and *Lap* loci.

Indeed, it seems probable that recruitment in the present study occurs more often between neighbouring populations since Griffiths (1981) observed that successful recruitment of juveniles occurred only every 4 to 6 years, even though mussels spawned annually and larvae were present in the water. Thus gene flow may proceed in a 'stepping-stone' fashion along the coast by sporadic recruitment of locally spawned larvae drifting to nearby populations.

The amount of gene flow between populations of *C. meridionalis* may be great enough so that regional differences do not appear in frequencies of selectively-neutral genetic markers, such as the electrophoretic variants examined in this study. Kimura & Weiss (1964) have shown that only a small amount of gene flow is needed to prevent the differentiation of selectively-neutral genetic variation. At the same time, gene flow may be weak enough to permit local differentiation of genetic variation that is under some kind of selection. Such a model may explain the differences in digestive activity between south- and west-coast mussels reported by Seiderer *et al.* (1984). However, another possibility is that the specific activities of the enzymes or additional digestive isozymes are induced by temperature or substrate concentrations and cannot be ruled out by the data presented here.

A model of differential selection with partial gene flow may also account for allele-frequency differences between populations of blue mussels across Cape Cod for *aminopeptidase* – 1 and the absence of differences for other polymorphic proteins over the same distance (Koehn *et al.* 1976; Koehn *et al.* 1984). Free amino acids appear to be important for intracellular osmoregulation in *Mytilus*, and the aminopeptidases encoded by various alleles of *Ap* – 1 are differentially selected by habitat differences in salinity (Koehn, Bayne, Moore & Siebenaller 1980). Variation in proteins with other functions appears to be selectively neutral to salinity variation or to other environmental gradients over the same distance.

The geographic range of the samples examined in the present study included a small portion of the range of *C. meridionalis*. If gene flow is largely restricted to nearby populations, as our results suggest, then greater genetic differences may be apparent between more distantly separated populations through isolation by distance (Wright 1943).

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References

BANG, N.D. & ANDREWS, W.R.H. 1974. Direct current measurements of a shelf-edge frontal jet in the southern Benguela system. *J. Mar. Res.* 32: 405 – 417.

BARNARD, K.H. 1964. Contributions to the knowledge of South African marine Mollusca. 5. Lamellibranchiata. *Ann. S. Afr. Mus.* 47: 390 – 391.

BEAUMONT, A.R., BEVERIDGE, C.M. & BUDD, M.D. 1983. Selection and heterozygosity within single families of the mussel *Mytilus edulis* (L.). *Mar. Biol. Lett.* 4: 151 – 161.

COOPER, D.W. 1968. The significance level in multiple tests made simultaneously. *Heredity* 23: 614 – 617.

DAY, J.H. 1974. Marine life on South African shores. A.A. Balkema, Cape Town.

DU PLESSIS, A.J. 1977. Larval development, settlement and growth of the black mussel *Choromytilus meridionalis* in the Saldanha Bay region. *Trans. Roy. Soc. S. Afr.* 42: 303 – 316.

GARTNER-KEPKAY, K.E. & ZOUROS, E. 1983. Genetic differentiation in the face of gene flow: A study of mussel populations from a single Nova Scotian embayment. *Can. J. Fish. Aquat. Sci.* 40: 443 – 451.

GOSLING, E.M. & WILKINS, M.P. 1981. Ecological genetics of the mussels *Mytilus edulis* and *M. galloprovincialis* on Irish coasts. *Mar. Ecol. Prog. Ser.* 4: 221 – 227.

GRIFFITHS, R.J. 1977. Reproductive cycles in littoral populations of *Choromytilus meridionalis* (Kr.) and *Aulacomya ater* (Molina) with a quantitative assessment of gamete production in the former. *J. Exp. Mar. Biol. Ecol.* 30: 53 – 71.

GRIFFITHS, R.J. 1981. Population dynamics and growth of the bivalve *Choromytilus meridionalis* (Kr.) at different tide levels. *Estuar. cstl. mar. Sci.* 10: 417 – 435.

HARRIS, T.F.W. 1978. Review of coastal currents in southern African waters. *S. Afr. Nat. Sci. Prog. Rep.* No. 30.

HARRIS, H. & HOPKINSON, D.A. 1976. Handbook of enzyme electrophoresis in human genetics. Am. Elsevier, New York.

KIMURA, M. & WEISS, G.H. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics* 49: 561 – 576.

KOEHN, R.K. & GAFFNEY, P.M. 1984. Genetic heterozygosity and growth rate in *Mytilus edulis*. *Mar. Biol.* 82: 1 – 7.

KOEHN, R.K., BAYNE, B.L., MOORE, M.N. & SIEBENALLER, J.F. 1980. Salinity related physiological and genetic differences between populations of *Mytilus edulis*. *Biol. J. Linn. Soc.* 14: 319 – 334.

KOEHN, R.K., HALL, J.G., INNES, D.J. & ZERA, A.J. 1984. Genetic differentiation of *Mytilus edulis* in eastern North America. *Mar. Biol.* 79: 117 – 126.

KOEHN, R.K., MILKMAN, R. & MITTON, J.B. 1976. Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel, *Mytilus edulis*. *Evolution*. 30: 2 – 32.

LEVINTON, J.S. & KOEHN, R.K. 1976. Population genetics. In: Marine Mussels: their ecology and physiology. (Ed.) Bayne, B.L. Cambridge University Press. pp. 357 – 384.

MAY, B., WRIGHT, J.E. & STONEKING, M. 1979. Joint segregation of biochemical loci in Salmonidae: results from experiments with Salvelinus and review of the literature on other species. *J. Fish. Res. Bd. Can.* 36: 1114 – 1128.

MURDOCK, E.A., FERGUSON, A. & SEED, R. 1975. Geographical variation in leucine aminopeptidase in *Mytilus edulis* L. from the Irish coasts. *J. Exp. Mar. Biol. Ecol.* 19: 33 – 41.

SEIDERER, L.J., DAVIS, C.L., ROBB, F.T. & NEWELL, R.C. 1984. Utilisation of bacteria as nitrogen resource by kelp-bed mussel *Choromytilus meridionalis*. *Mar. Ecol. Prog. Ser.* 15: 109 – 116.

SKIBINSKI, D.O.F., BEARDMORE, J.A. & CROSS, T.F. 1983. Aspects of the population genetics of *Mytilus* (Mytilidae; Mollusca) in the British Isles. *Biol. J. Linn. Soc.* 19: 137 – 183.

SOKAL, R.R. & ROHLF, J.F. 1969. Biometry. W.H. Freeman & Co., San Francisco.

WRIGHT, S. 1943. Isolation by distance. *Genetics* 28: 114 – 138.