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# Genetic Variation of the Green-lipped Mussel *Perna viridis* (L.) (Mytilidae: Mytiloida: Mytilicae) from the West Coast of Peninsular Malaysia

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**Chee Kong Yap, Soon Guan Tan, Ahmad Ismail and Hishamuddin Omar (2002)** Genetic variation of the green-lipped mussel *Perna viridis* (L.) (Mytilidae: Mytiloida: Mytilicae) from the west coast of Peninsular Malaysia. *Zoological Studies* **41**(4): 376-387. Horizontal starch gel electrophoresis was used to estimate the levels of genetic variation for 8 different geographical populations (Penang, Pulau Aman, Bagan Lalang, Telok Emas, Sungai Muar, Tanjung Kupang, Pantai Lido, and Kampong Pasir Puteh) of the mussel, *Perna viridis*, collected from the waters off the west coast of Peninsular Malaysia. Fourteen polymorphic loci were observed. The observed mean heterozygosity ranged from 0.108 to 0.334, while the expected mean heterozygosity ranged from 0.133 to 0.301. The highest mean value for genetic distance (0.091) was found between the populations of Penang and Telok Emas while the lowest value (0.004) was found between those of Pantai Lido and Tanjung Kupang. The populations studied could be divided into 2 groups by the UPGMA dendrogram based on Nei's (1978) genetic similarities. The groupings seemed to indicate differentiation into local populations. These results suggest that *P. viridis* has a tendency to split into a number of geographical populations regardless of larval dispersal as a potential agent of gene flow. The mean $F_{ST}$ value of 0.149 indicates that the mussel populations show a moderate degree of genetic differentiation. However, the mean genetic distance from the present study (0.048 $\pm$ 0.004) falls within the range of genetic distances between conspecific populations of mussels (0.0-0.14). Therefore, the present study supports the use of the local mussel *P. viridis* as a suitable biomonitoring agent for heavy metals. The range of genetic distance values (0.004-0.091) presented here can also serve as baseline data to which results of similar studies in the future can be compared to determine whether genetic divergence of mussel populations from the west coast of Peninsular Malaysia is taking place. http://www.sinica.edu.tw/zool/zoolstud/41.4/376.pdf

**Key words:** *Perna viridis*, Allozymes, Population.

The green-lipped mussel, *Perna viridis* (L.) (Class Bivalvia: Family Mytilidae: Order Mytiloida: Suborder Mytilicae), is widely distributed along the western coast of Peninsular Malaysia (Ismail et al. 2000). The species' wide geographical distribution and sedentary lifestyle have prompted its use as a bioindicator of heavy metal contamination in the coastal waters of Hong Kong (Phillips 1985, Wong et al. 2000), Thailand (Sukasem and Tabucanon 1993, Boonchalermkit et al. 1998), Indonesia (Hutagalung 1989), India (Senthilnathan et al. 1998), and Malaysia (Ismail et al. 2000). However, information about the population genetic structure of this species has not been documented. As mussels are widely cultured and have become an important seafood delicacy in this region, knowledge of their population genetic structure is important for proper mussel farming management. The present paper aimed to investigate the genetic variation of mussel populations collected from the west coast of Peninsular Malaysia by enzyme electrophoresis.

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*Perna viridis* is an oviparous, dioecious species whose life cycle exhibits a long larval dispersal period and a sedentary adulthood (Rosell 1991). Sexual reproduction occurs when gametes are released into the water column where fertilization takes place. The pelagic larval stages last for about 3 wk, providing ample opportunities for larval dispersal and thereby promoting gene flow (Yamanaka and Fujio 1984). After the spat begin settling, they spend the rest of their lives as sedentary organisms. The life cycle, distribution, and environmental inputs to coastal waters make *P. viridis* a marine bivalve whose population genetic structure would be interesting to study.

Wild mussels have been harvested by commercial fishermen for decades because mussels can provide cheap protein. In Malaysia, mussel farming is considered one of the potential shellfish mariculture activities in Malaysian coastal waters in addition to cockles and oysters (Mazuki 1998). However, a critical step in mussel cultivation depends heavily on natural spatfall. The origin of these spats is unknown and the supply is highly variable. Apart from the problem of supply of spats for seeding, genetic problems can arise from the use of wild stock of unknown origin. Therefore, selection and improvement of cultured mussels may be hindered by the limited amount of genetic information available. For biomonitoring purposes, good clear taxonomic as well as genetic information on this species is required because *P. viridis* has been suggested for use as a potential biomonitoring agent for heavy metals (Ismail et al. 2000). Different species may accumulate metals at different rates. For example, Lobel et al. (1990) reported that individuals of *Mytilus trossulus* accumulated about 50% more metals than did *M. edulis* from the same location. Therefore, the study of the population structure of *P. viridis* should receive more-careful consideration for the design and interpretation of biomonitoring studies.

To date, only 3 species of *Perna* have been listed, viz., *P. viridis*, *P. perna*, and *P. canaliculus* (Siddall 1980, Vakily 1989). Based on the shape and color of the shell surface, *P. viridis* and *P. canaliculus* are usually called green-lipped mussels, while *P. perna* is known as the brown mussel. The possibility that green-lipped mussels collected from the west coast of Peninsular Malaysia are a mixture of *P. viridis* and *P. canaliculus* is probably negligible. This is because the green-lipped mussel *P. canaliculus* is restricted to the waters of New Zealand. However, it is still necessary to study the genetic structure of local populations of mussels collected from different locations along the west coast of Peninsular Malaysia to ascertain the variation present within, as well as between, geographical populations.

Genetic differentiation has been reported among mussel populations in the New Zealand green-lipped mussel *Perna canaliculus* (Smith 1988, Sin et al. 1990) and extensively documented in the blue mussel, *Mytilus edulis* (Yamanaka and Fujio 1981, Hilbish and Koehn 1985, Gosling and McGrath 1990, Koehn 1991). All of these studies suggested environmental factors as the main causes for the observed genetic structuring.

As the green-lipped mussel *P. viridis* can be potentially cultured as a cheap protein source and has been identified as a potential biomonitoring agent for heavy-metal pollution, genetic information is required about the population structure. Moreover, the taxonomy of Malaysian *P. viridis* is confused, and the status of *P. viridis* remains under discussion. In this study, horizontal starch gel electrophoresis was used to (1) estimate the levels of genetic differentiation among geographical populations, and (2) determine the genetic distances/similarities among sampled mussel populations.

## MATERIALS AND METHODS

### Sampling

Sampling locations are shown in figure 1. Sampling was conducted in Nov. 1999 for mussels from Penang and Pulau (P.) Aman. Mussels from Telok (T.) Emas, Sungai (Sg.) Muar, Tanjung (Tg.) Kupang, Pantai (P.) Lido, and Kampong (Kg.) Pasir Puteh were collected in Jan. 2000. Bagan (B.) Lalang mussels were sampled in Apr. 2000. All mussels were sampled from the wild except for the T. Emas and Sg. Muar mussels which were bought from market stalls. In order to keep them alive, mussels were put into wet (with sea water) gunny bags. These bags were brought back to the laboratory for further analysis.

In the laboratory, only mussels of similar size from each population were chosen for analysis. The overall mean shell lengths of mussels used for analysis are given in figure 2. Small differences in shell lengths were observed among populations. These may have been due to age or environmental factors (Jordaens et al. 1998). Live samples were dissected for their posterior adductor muscles. The adductor muscle was chosen for this

study because this muscle of mussels is a readily available source of extractable proteins (Smith and Mix 1987).

### Electrophoresis

Samples were kept at -70°C until used for electrophoresis. When needed for analysis, frozen muscle tissue was added to a few drops of homogenization buffer (0.2 M Tris-HCl and glycerol). The manually homogenized fluid sample was imbibed onto Whatman no. 1. filter-paper inserts. The 10 enzyme stains and buffer systems used in this study are summarized in table 1.

Three buffer systems were used for this study. The CA-7 buffer system is described by Steiner and Joslyn (1979) while the 0.2 M phosphate and the phosphate-citrate 7 buffer systems are described by Shaw and Prasad (1970).

Horizontal starch gel electrophoresis (STAGE) was conducted at 3-5°C with 230 V/gel slab. The starch gel was prepared using 12% starch (Sigma, St. Louis, MO). Inserts were removed after running for 30 min, and the run was resumed until the tracker dye, bromophenol blue, reached the anodal end of the gel. After electrophoresis, the gels were sliced, and the enzyme staining recipes used were adapted from Shaw and Prasad (1970) and Harris and Hopkinson (1976) with slight modi-

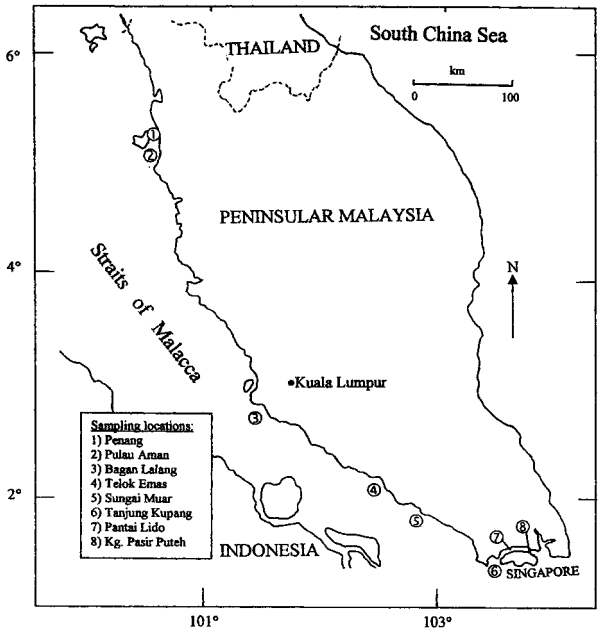


**Fig. 1.** Map showing sampling sites.

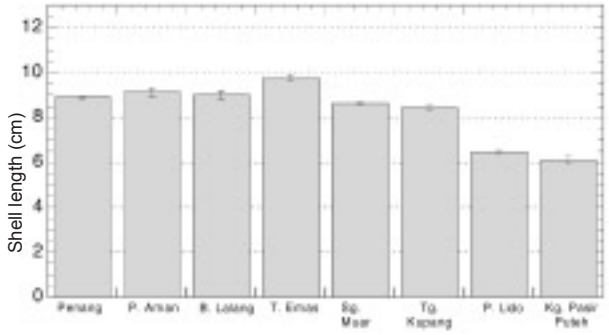


**Fig. 2.** Mean shell length of mussels (cm ± standard error) from 8 sampling locations (All samples were collected from the wild except those from T. Emas, and Sg. Muar).

**Table 1.** Enzyme names and abbreviations, enzyme codes (E.C.), number of loci, enzyme structures, and electrophoretic buffer systems

| Enzyme | E.C. number | Number of loci | Enzyme structure | Buffer system used |
|---|---|---|---|---|
| Alpha-esterase (α-*EST*) | 3.1.1.1 | 4 | monomeric /dimeric | CA-7 |
| Alpha-glycerophosphate dehydrogenase (α-*GPD*) | 1.1.1.8 | 3 | dimeric | phosphate 0.2 M |
| Glutamate oxaloacetate transaminase (*GOT*) | 2.6.1.1 | 2 | dimeric | CA-7 |
| Glucosephosphate isomerase (*GPI*) | 5.3.1.9 | 1 | dimeric | phosphate 0.2 M |
| Isocitrate dehydrogenase (*IDH*) | 1.1.1.42 | 2 | dimeric | CA-7 |
| Leucine aminopeptidase (*LAP*) | 3.4.11.1 | 1 | monomeric | CA-7 |
| Malate dehydrogenase (*MDH*) | 1.1.1.37 | 1 | dimeric | phosphate-citrate 7 |
| Malic enzyme (*ME*) | 1.1.1.40 | 1 | tetrameric | CA-7 |
| Peptidase B (*PEP-B*) | 3.4.11 | 1 | monomeric | CA-7 |
| Phosphoglucomutase (*PGM*) | 2.7.5.1 | 1 | monomeric | CA-7 |

fication.

For any locus, the most common allele in the control population from Penang was designated allele 100. All other alleles were labeled according to their band mobilities, more or less anodally in millimeters from the allele 100 band.

**Data analysis**

The BIOSYS-1 computer package of Swofford and Selander (1989) was used to calculate allelic frequencies, proportion of polymorphic loci (P) based on the 0.95 criterion, mean heterozygosity (*H*), and genetic distance (D), as well as the identity (I) values of Nei (1978) and *F*-statistics (Nei 1977, Wright 1978). The dendrogram based on Nei's I was drawn using the unweighted-pair group method with arithmetic averaging (UPGMA) of Sneath and Sokal (1973).

## RESULTS

**Phenotypes of loci**

Fourteen loci which could be reliably and consistently typed were *α-EST-1*, *α-EST-4*, *PGI-2*, *PGM-1*, *PGM-2*, *α-GPDH-3*, *MDH*, *ME*, *PEP-B*, *LAP*, *IDH-1*, *IDH-2*, *GOT*, and *CGOT*. All enzymes migrated anodally except *CGOT*. *GPI* and *α-GPD* showed 2 and 3 zones of activity, respectively. But only *GPI-2* and *α-GPD-3* could be consistently and reliably scored. Similarly, *α-EST* showed 4 zones of activities, but only *α-EST-1* and *α-EST-4* could be consistently and reliably scored.

*α-CEST* migrated cathodally and could not be realiably scored in some populations. Similarly, *DIA*, *SOD*, *AK*, and *6PGD* migrated anodally and could not consistently be scored in all populations. Therefore, *CEST*, *DIA*, *SOD*, *AK*, and *6PGD* were not used in the genetic data analysis.

*α-EST* was detected with α-naphthyl acetate as the substrate although a similar result could be achieved with β-naphthyl acetate as the substrate. *α-EST-1* was polymorphic in all populations. Four phenoptypes were observed for *α-EST-1*: 95, 100, 100/90, and 100/95. In the P. Aman population, polymorphic alleles were observed in *α-EST-1* and *α-EST-4*. *α-EST-1* was found to be heterozygous with 2 bands, while *α-EST-4* was observed to be heterozygous with 3 bands.

*α-GPD-3* showed 3 phenotypes: 100, 100/110, and 95/110. Populations from B. Lalang, P. Lido, and Tg. Kupang were monomorphic with phenotype 100, while all individuals of the populations from P. Aman and Penang had phenotype 100/110. Phenotypes 100 and 100/110 were found in the Sg. Muar and T. Emas populations, while the phenotypes 95/110 and 100/110 were found in the Kg. Pasir Puteh population.

*IDH* activity appeared in 2 zones which were apparently encoded by 3 genes. Both loci exhibited polymorphisms in all mussel populations. Three phenotypes of 100, 102, and 100/102 were observed for both loci. The heterozygous phenotype 100/102 was presumed to have 3 unseparated bands.

*GOT* was monomorphic in 4 populations while phenotypes 100 and 100/95 were observed in populations from P. Aman, P. Lido, Kg. Pasir Puteh, and T. Emas. *CGOT* showed a single zone of activity. The *CGOT* locus was found to have phenotypes 100 and 100/102 in all populations except B. Lalang which had only the 100 phenotype. The heterozygous phenotype 100/102 at the *CGOT* locus was presumed to have 3 unseparated bands for this dimeric enzyme.

*GPI-2* appeared to be polymorphic for 2 alleles. Homozygotes showed single banded patterns while heterozygotes showed 3 banded patterns which were indicative of a dimeric enzyme. This enzyme also showed shadow bands in that 4 bands instead of 3 could sometimes be seen for the heterozygotes of this dimeric enzyme.

The *LAP* zymogram showed a single zone of activity in all populations. *LAP* was monomorphic with phenotype 100 in 5 populations, while polymorphisms were observed in the Tg. Kupang, Kg. Pasir Puteh, and Sg. Muar populations.

*MDH* was polymorphic for 2 alleles in the P. Aman population, while phenoypes 100 and 100/110 were found in the Penang, Kg. Pasir Puteh, and Sg. Muar populations. The remaining populations were all monomorphic for the 100 allele.

*ME* activity appeared in a single zone encoded by a single gene. The *ME-1* locus was found to have 3 phenotypes. This locus was monomorphic in populations from Tg. Kupang, Sg. Muar, and T. Emas.

The enzyme pattern for *PEP-B* was consistent with that expected for a polymorphic locus with 2 alleles. *PEP-B* was monomorphic at 100 in the P. Lido and T. Emas populations.

*PGM* appeared as 2 zones, of both which migrated anodally in all populations. *PGM-2* was found to have 2 alleles in most populations.

**Table 2.** Allele frequencies of 14 loci in 8 mussel populations

| Locus | Population | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | B. Lalang | P. Aman | Penang | P. Lido | Tg. Kupang | P. Pasir Puteh | Sg. Muar | T. Emas |
| α-*EST-1* | | | | | | | | |
| (*N*) | 26 | 19 | 21 | 29 | 34 | 20 | 20 | 31 |
| 100 | 0.769 | 0.816 | 0.333 | 0.707 | 0.765 | 0.700 | 0.825 | 0.758 |
| 95 | 0.000 | 0.184 | 0.667 | 0.293 | 0.235 | 0.300 | 0.175 | 0.242 |
| 90 | 0.231 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| α-*EST-4* | | | | | | | | |
| (*N*) | 25 | 18 | 27 | 31 | 36 | 18 | 18 | 30 |
| 100 | 1.000 | 0.638 | 0.648 | 0.984 | 1.000 | 1.000 | 0.806 | 1.000 |
| 105 | 0.000 | 0.361 | 0.352 | 0.016 | 0.000 | 0.000 | 0.194 | 0.000 |
| α-*GPD-3* | | | | | | | | |
| (*N*) | 24 | 20 | 16 | 22 | 22 | 19 | 22 | 20 |
| 100 | 1.000 | 0.500 | 0.500 | 1.000 | 1.000 | 0.474 | 0.545 | 0.575 |
| 95 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.026 | 0.000 | 0.000 |
| 110 | 0.000 | 0.500 | 0.500 | 0.000 | 0.000 | 0.500 | 0.455 | 0.425 |
| *CGOT* | | | | | | | | |
| (*N*) | 24 | 17 | 28 | 30 | 32 | 37 | 35 | 29 |
| 100 | 1.000 | 0.971 | 0.964 | 0.967 | 0.875 | 0.946 | 0.943 | 0.966 |
| 102 | 0.000 | 0.029 | 0.036 | 0.033 | 0.125 | 0.054 | 0.057 | 0.034 |
| *GOT* | | | | | | | | |
| (*N*) | 24 | 35 | 20 | 18 | 35 | 37 | 31 | 33 |
| 100 | 1.000 | 0.986 | 1.000 | 0.972 | 1.000 | 0.986 | 1.000 | 1.000 |
| 95 | 0.000 | 0.014 | 0.000 | 0.028 | 0.000 | 0.014 | 0.000 | 0.000 |
| *GPI-2* | | | | | | | | |
| (*N*) | 23 | 24 | 22 | 24 | 24 | 21 | 23 | 19 |
| 100 | 0.565 | 0.625 | 0.523 | 0.680 | 0.771 | 0.500 | 0.522 | 0.895 |
| 110 | 0.435 | 0.375 | 0.477 | 0.313 | 0.229 | 0.500 | 0.478 | 0.105 |
| *IDH-1* | | | | | | | | |
| (*N*) | 22 | 15 | 29 | 22 | 26 | 31 | 17 | 23 |
| 100 | 0.957 | 0.867 | 0.983 | 0.818 | 0.885 | 0.742 | 0.382 | 0.457 |
| 102 | 0.000 | 0.133 | 0.017 | 0.182 | 0.115 | 0.258 | 0.618 | 0.543 |
| *IDH-2* | | | | | | | | |
| (*N*) | 24 | 19 | 29 | 22 | 25 | 32 | 19 | 24 |
| 100 | 0.917 | 0.895 | 0.948 | 0.977 | 0.920 | 0.833 | 0.737 | 0.750 |
| 102 | 0.083 | 0.105 | 0.052 | 0.023 | 0.080 | 0.167 | 0.263 | 0.250 |
| *LAP* | | | | | | | | |
| (*N*) | 23 | 19 | 35 | 36 | 30 | 30 | 36 | 35 |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 0.867 | 0.783 | 0.903 | 1.000 |
| 98 | 0.000 | 0.000 | 0.000 | 0.000 | 0.133 | 0.217 | 0.097 | 0.000 |
| *MDH* | | | | | | | | |
| (*N*) | 23 | 27 | 27 | 28 | 25 | 25 | 25 | 25 |
| 100 | 1.000 | 0.685 | 0.537 | 1.000 | 1.000 | 0.728 | 0.860 | 1.000 |
| 110 | 0.000 | 0.315 | 0.463 | 0.000 | 0.000 | 0.280 | 0.140 | 0.000 |
| *ME* | | | | | | | | |
| (*N*) | 24 | 27 | 20 | 38 | 25 | 37 | 20 | 35 |
| 100 | 0.979 | 0.907 | 0.800 | 0.855 | 1.000 | 0.800 | 1.000 | 1.000 |
| 98 | 0.021 | 0.093 | 0.200 | 0.145 | 0.000 | 0.200 | 0.000 | 0.000 |
| *PEP-B* | | | | | | | | |
| (*N*) | 23 | 37 | 36 | 18 | 20 | 37 | 28 | 20 |
| 100 | 0.870 | 0.770 | 0.931 | 1.000 | 0.900 | 0.919 | 0.750 | 1.000 |
| 95 | 0.130 | 0.230 | 0.069 | 0.000 | 0.100 | 0.081 | 0.250 | 0.000 |
| *PGM-1* | | | | | | | | |
| (*N*) | 20 | 13 | 13 | 20 | 20 | 19 | 13 | 25 |
| 100 | 0.950 | 0.885 | 0.962 | 0.975 | 0.975 | 1.000 | 0.846 | 1.000 |
| 105 | 0.025 | 0.115 | 0.038 | 0.000 | 0.025 | 0.000 | 0.154 | 0.000 |
| 95 | 0.025 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 |
| *PGM-2* | | | | | | | | |
| (*N*) | 26 | 19 | 21 | 20 | 20 | 22 | 19 | 20 |
| 100 | 0.635 | 0.342 | 0.381 | 0.725 | 0.600 | 0.864 | 0.447 | 0.450 |
| 95 | 0.365 | 0.658 | 0.619 | 0.275 | 0.400 | 0.136 | 0.553 | 0.550 |
| P | 0.429 | 0.786 | 0.643 | 0.357 | 0.571 | 0.786 | 0.857 | 0.429 |
| $H_{obs}$ | 0.143 | 0.334 | 0.303 | 0.108 | 0.149 | 0.289 | 0.319 | 0.195 |
| $H_{exp}$ | 0.133 | 0.279 | 0.263 | 0.148 | 0.162 | 0.268 | 0.301 | 0.181 |

*N*, sample size; P, proportion of polymorphic loci (0.95 criterion); $H_{obs}$, observed mean heterozygosity; $H_{exp}$, expected mean heterozygosity.

### Genetic variability within populations

Allelic frequency data for the 14 loci that could be scored in *Perna viridis* are presented in table 2. The proportion of polymorphic loci, P, ranged from 0.36 in the P. Lido population to 0.86 in the Sg. Muar population. Observed mean heterozygosities were higher than those expected in 6 mussel populations examined, indicating excesses of heterozygosity. The observed mean heterozygosity ($H_{obs}$) ranged from 0.108 in the P. Lido population to 0.334 in the P. Aman population, while the expected mean heterozygosity ($H_{obs}$) ranged from 0.133 in the B. Lalang population to 0.301 in the Sg. Muar population.

### Conformation with Hardy-Weinberg equilibrium

Excesses or deficiencies of heterozygotes can be analyzed in more detail by testing the significance of deviations of the observed genotypic frequencies from those expected under Hardy-Weinberg equilibrium. These tests, for each locus in each population, are summarised in table 3. Three loci (*PGM-1*, *PGM-2*, and *IDH-1*) showed consistent observed deficiencies of heterozygotes. *PGI-2*, *ME*, *LAP*, and *IDH-2* showed observed deficiencies of heterozygotes to a lesser extent, while *EST-4*, *α-GPD-3*, *MDH*, and *PEP-B* showed consistent observed excesses of heterozygotes. The observation of deficiencies of heterozygotes is in

**Table 3.** Summary of chi-square tests for deviation from Hardy-Weinberg equilibrium

| Locus | B. Lalang | P. Aman | Penang | P. Lido | Tg. Kupang | P. Pasir Puteh | Sg. Muar | T. Emas |
|---|---|---|---|---|---|---|---|---|
| α-*EST-1* | ns | ns | - | + | (+) | (+) | ns | (+) |
| α-*EST-4* | homo | + | (+) | ns | homo | homo | ns | homo |
| α-*GPD-3* | homo | + | + | homo | homo | + | + | + |
| *CGOT* | homo | ns | ns | ns | ns | ns | ns | ns |
| *GOT* | homo | homo | ns | ns | homo | ns | homo | homo |
| *GPI-2* | (+) | - | ns | (-) | - | ns | ns | ns |
| *IDH-1* | - | ns | ns | - | ns | - | ns | - |
| *IDH-2* | ns | ns | ns | ns | ns | ns | - | ns |
| *LAP* | homo | homo | homo | homo | ns | - | ns | homo |
| *MDH* | homo | + | + | homo | homo | (+) | ns | homo |
| *ME* | ns | - | ns | ns | homo | ns | homo | homo |
| *PEP-B* | ns | (+) | ns | homo | ns | ns | (+) | homo |
| *PGM-1* | ns | - | ns | ns | ns | homo | ns | - |
| *PGM-2* | - | ns | - | - | ns | ns | ns | ns |

ns, not significant; +, significant observed excess of heterozygotes, $p < 0.05$; (+), $0.05 < p < 0.10$; -, significant observed deficiency of heterozygotes, $p < 0.05$; (-), $0.05 < p < 0.10$; homo, homozygous locus.

**Table 4.** *F*-statistics values for the 14 loci of 8 populations of *Perna viridis*

| Locus | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|
| α-*EST-1* | -0.126 | 0.029 | 0.138 |
| α-*EST-4* | -0.463 | -0.131 | 0.227 |
| α-*GPD-3* | -0.896 | -0.408 | 0.258 |
| *CGOT* | -0.077 | -0.048 | 0.026 |
| *GOT* | -0.024 | -0.005 | 0.018 |
| *GPI-2* | 0.170 | 0.232 | 0.074 |
| *IDH-1* | 0.287 | 0.472 | 0.260 |
| *IDH-2* | 0.045 | 0.105 | 0.063 |
| *LAP* | 0.248 | 0.336 | 0.116 |
| *MDH* | -0.523 | -0.176 | 0.228 |
| *ME* | 0.128 | 0.208 | 0.092 |
| *PEP-B* | -0.217 | -0.121 | 0.080 |
| *PGM-1* | -0.114 | -0.048 | 0.059 |
| *PGM-2* | 0.253 | 0.341 | 0.117 |
| Mean | -0.084 | 0.078 | 0.149 |

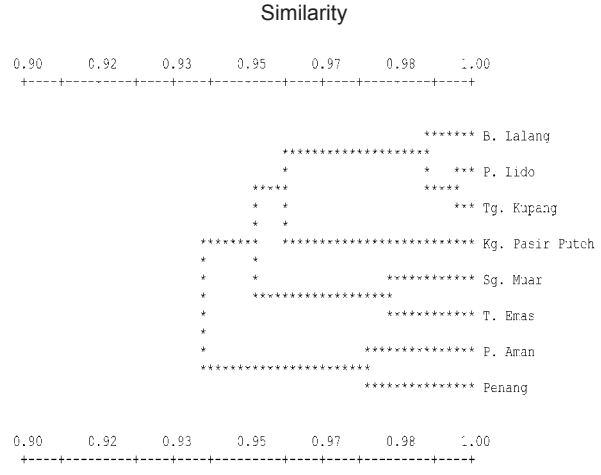


**Fig. 3.** UPGMA dendrogram of genetic relationships among 8 mussel populations based on Nei's (1978) genetic similarity.

agreement with observations conducted in other marine bivalves (Gosling 1989, English et al. 2000).

### Genetic differentiation

Values of *F* statistics for *Perna viridis* are presented in table 4. The mean $F_{IS}$, $F_{IT}$, and $F_{ST}$ values of -0.084, 0.078, and 0.149, respectively, indicate the greater contribution of among-population differentiation than within-population differentiation to the total genetic differentiation.

Wright's (1978) hierarchical *F*-statistics (Table 5) show that populations within the 2 and 3 regions accounted for 12.1% and 13.1%, respectively, of the total variance, while the between and among region variance components were 87.9% and 86.9% respectively, of the total depending on which hierarchy was considered. Therefore, the hierarchical *F*-statistics suggest that a substantial amount of the genetic variation is due to differentiation between (northern, and southern) or among (northern, central, and southern) regions.

**Table 5.** Wright's (1978) hierarchical *F*-statistics of genetic differentiation for 8 *Perna viridis* populations grouped into 2 (northern, and southern) and 3 (northern, central, and southern) regions

| Contrast | Variance component | (%) | $F_{xy}$ |
|---|---|---|---|
| Populations in 2 regions | 0.0552 | 12.1 | 0.016 |
| Populations in 3 regions | 0.0598 | 13.1 | 0.017 |
| Between 2 regions | 0.3997 | 87.9 | 0.116 |
| Among 3 regions | 0.3951 | 86.9 | 0.115 |
| Among all populations | 0.4549 | 100 | 0.130 |

Note: The 2 regions were northern (P. Aman, and Penang), and southern (B. Lalang, T. Emas, Sg. Muar, Tg. Kupang, P. Lido, and Kg. Pasir Puteh); the 3 regions were northern (P. Aman, and Penang), central (B. Lalang, T. Emas, and Sg. Muar), and southern (Tg. Kupang, P. Lido, and Kg. Pasir Puteh).

### Genetic similarity/distance

Based on the allelic frequencies presented in table 2, Nei's (1978) D and I values were calculated and are presented in table 6. The lowest mean value of D was 0.004 between the populations of P. Lido and Tg. Kupang. This result shows that the populations of P. Lido and Tg. Kupang are very closely related and that genetic similarity over a relatively large geographical distance (about 30 km) is probably the result of dispersal distance of the pelagic mussel larvae. In the dendrogram drawn based upon Nei's (1978) I values for the 8 *Perna viridis* populations (Fig. 3), the populations collected from the northern part of Peninsular Malaysia (Penang and P. Aman) clustered together reflecting their close geographical proximity while populations collected from central and southern parts of Peninsular Malaysia clustered together in the dendrogram to form another major group. The highest mean value of D was 0.091 between the populations of Penang and T. Emas.

## DISCUSSION AND CONCLUSIONS

Studies of marine bivalves often report many significant deviations from Hardy-Weinberg equilibrium. Several studies have reported that frequencies of observed heterozygosity are often lower than those expected under Hardy-Weinberg equilibrium in natural populations of mussels (Koehn and Gaffney 1984, Yamanaka and Fujio 1984, Gosling and Wilkins 1985, Colgan 1987, Gentili and Beaumont 1988, Zouros et al. 1988, Gosling 1989, Gosling and McGrath 1990, Beaumont 1991, Koehn 1991, Gosling 1992). For example,

**Table 6.** Nei's (1978) genetic identity (below diagonal) and genetic distance (above diagonal) for 8 populations of mussel *Perna viridis* from Peninsular Malaysia

| Population | B. Lalang | P. Aman | Penang | P. Lido | Tg. Kupang | P. Pasir Puteh | Sg. Muar | T. Emas |
|---|---|---|---|---|---|---|---|---|
| B. Lalang | | 0.050 | 0.088 | 0.010 | 0.009 | 0.049 | 0.063 | 0.059 |
| P. Aman | 0.951 | | 0.023 | 0.056 | 0.050 | 0.046 | 0.027 | 0.047 |
| Penang | 0.916 | 0.977 | | 0.076 | 0.083 | 0.057 | 0.081 | 0.091 |
| P. Lido | 0.990 | 0.945 | 0.927 | | 0.004 | 0.036 | 0.058 | 0.040 |
| Tg. Kupang | 0.991 | 0.951 | 0.921 | 0.996 | | 0.045 | 0.054 | 0.036 |
| P. Pasir Puteh | 0.952 | 0.955 | 0.945 | 0.965 | 0.956 | | 0.041 | 0.049 |
| Sg. Muar | 0.939 | 0.974 | 0.922 | 0.943 | 0.947 | 0.960 | | 0.018 |
| T. Emas | 0.943 | 0.955 | 0.913 | 0.961 | 0.964 | 0.952 | 0.982 | |

deficiencies of heterozygosity for *LAP* were observed in several populations of *Mytilus edulis* (Beaumont 1991). Yamanaka and Fujio (1981) observed that deficiencies of heterozygosity were significant for *PGM*, *LAP*, and *GPI* in the mussel *M. edulis* collected in the coastal waters of Japan.

Several explanations have been offered such as inbreeding (particularly self-fertilization), the Wahlund effect, null alleles, and aneuploidy (Singh and Green 1984, Zouros and Foltz 1984, Zouros et al. 1988) to account for this phenomenon. Of these possible explanations, the Wahlund effect and inbreeding are the 2 most plausible agents for the observed deficiencies of heterozygosity in natural populations of mussels (Beaumont 1991).

Smith (1988) observed significant genetic differentiation at 4 polymorphic loci between northern and southern populations of the green-lipped mussel *P. canaliculus* collected from New Zealand coastal waters. In the dendrogram of genetic relationships obtained in the present study, we may assume that the 2 major clusters observed are possibly related to limited genetic exchange resulting from movements of currents and/or local selection pressure. Close proximity between localities will increase gene flow which tends to make gene frequencies uniform among populations. One of the agents of gene flow is the presence of a pelagic larval stage (Hedgecock 1986). Extensive gene flow is expected due to the long duration of the planktonic phase. However, the successful migration of individuals is heavily dependent on whether the dispersing larvae can successfully survive, settle, and then reproduce in new environments (Hedgecock 1986). Geographically, the sea is narrower in the southern end of the Straits of Malacca compared to the northern part. This may promote greater genetic exchange in the southern part.

$F_{ST}$ values can be used to determine the degree of genetic differentiation among populations of *Perna viridis*. According to Wright (1978), there are 4 qualitative guidelines for the interpretation of $F_{ST}$: 0-0.05 for little genetic differentiation, 0.05-0.15 for moderate genetic differentiation, 0.15-0.25 for large genetic differentiation and above 0.25 for very large genetic differentiation. Based on these guidelines, the mean $F_{ST}$ value from the present study falls in the range for moderate genetic differentiation. Our $F_{ST}$ value is also within the range reported by other authors for several molluscs species (Table 7).

The question as to whether *P. viridis* collected from the west coast of Peninsular Malaysia can be used as a good biomonitoring agent for heavy metals, is an interesting problem. D values between different geographical populations of *P. viridis* from the west coast of Peninsular Malaysia ranged from 0.004 to 0.091, with a mean of 0.048 $\pm$ 0.004. This mean value is within the range generally observed between conspecific populations (0.000-0.050; Ferguson 1980). In addition, the range is much lower than those between subspecies (D= 0.230; Ayala 1975). The D values from the present study are also lower than those reported for allopatric species of other mussels (Gosling 1992). As listed in table 8, the value of D observed between allopatric species of *M. edulis*

**Table 7.** $F_{ST}$ values reported for different species of molluscs and populations. Comments are based on the degree of genetic differentiation as suggested by Wright (1978)

| Population | $F_{ST}$ | Comment | Reference |
|---|---|---|---|
| Mussel *Amblema plicata* | 0.082 | moderate | Johnson et al. (1998) |
| Mussel *Plectomerus dombeyanus* | 0.121 | moderate | Johnson et al. (1998) |
| Mussel *Quadrula pustulosa* | 0.108 | moderate | Johnson et al. (1998) |
| Mussel *Q. quadrula* | 0.160 | great | Johnson et al. (1998) |
| Clam *Potamocorbula amurensis* | 0.014 | moderate | Duda (1994) |
| Snail *Semisulacospira libertina* | 0.174 | great | Oniwa and Kimura (1986) |
| Snail *S. reiniana* | 0.031 | moderate | Oniwa and Kimura (1986) |
| Snail *Potamopyrgus antipodarum* | 0.060-0.108 | moderate | Phillips and Lambert (1990) |
| Snail *Littorina saxatilis* | 0.137 | moderate | Janson and Ward (1984) |
| Snail *Biomphalaria straminea* | 0.098 | moderate | Wooddruff et al. (1985) |
| Snail *Mandarina aureola* | 0.280 | very great | Chiba (1993) |
| Snail *M. ponderosa* | 0.130 | moderate | Chiba (1993) |
| Mussel *Perna viridis* | 0.149 | moderate | this study |

(U. K.) and *M. galloprovincialis* (Italy) was 0.172. Allopatric species of *M. edulis* (North Sea) and *M. trossulus* (Baltic Sea) were observed to have a D of 0.280.

Sin et al. (1990) reported that D values between different geographical populations of *P. canaliculus* from New Zealand ranged from 0.004 to 0.166 and Masden et al. (1995) reported the range to be 0.007-0.139 in the freshwater zebra mussel *Dreissena polymorpha* from Europe. These authors did not mention the existence of species complexes in the populations they studied. Conspecific populations of 2 species of worms, the bloodworm *Glycera dibranchiata* (Bristow and Vadas 1991) and the fanworm *Sabella spallanzanii* (Andrew and Ward 1997), had ranges of Nei's D values of 0.003-0.093 and 0.000-0.086, respectively, values similar to those reported here for the geographical populations of *P. viridis* from the west coast of Peninsular Malaysia. Therefore, the present study supports the use of the local mussel *P. viridis* as a suitable biomonitoring agent for heavy metals. Further studies using DNA level markers on this species should be done to confirm our conclusion that specimens of the green-lipped mussel *P. viridis* collected from the west coast of Peninsular Malaysia are genetically similar enough so that populations across the complete natural distribution in Malaysia may be used as a biomonitoring agent for heavy metals.

From this study, both the D and $F_{ST}$ values obtained indicate that *P. viridis* is split into a number of geographical populations. Genetic differentiation may have resulted from natural selection and adaptation to local environments or from vagaries of larval settlement associated with settlement of non-random collections of genotypes in the spat. Gene flow is most likely more extensive between neighboring populations which should then be more similar genetically than more distant populations. The range of D values presented here can also serve as baseline data to which similar studies in the future can be compared so as to determine whether genetic divergence of populations is taking place.

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**Table 8.** Genetic distances reported for different invertebrate species and populations

| Population | No. of loci | Genetic distance | Comment | Reference |
|---|---|---|---|---|
| *Mytilus edulis* (UK) and *M. galloprovincalis* (Italy) | 16 | 0.172 | allopatric species | Skibinski et al. (1980) |
| *M. edulis* (Denmark) and Southern African *M. galloprovincialis* | 23 | 0.162 | allopatric species | Grant and Cherry (1985) |
| *M. edulis* (North Sea) and *M. trossulus* (Baltic Sea) | 22 | 0.28 | allopatric species | Vainola and Hvilsom (1991) |
| *M. edulis* from Hokkaido to Hiroshima, Japan | 12 | 0.0-0.042 | possibility of a species complex | Yamanaka and Fujio (1981) |
| *M. edulis* from the coast of California | 5 | 0.023-0.475 | existence of subspecies | Sarver and Loudenslager (1991) |
| Zebra mussel *Dreissena polymorpha* from North America | 15 | 0.005-0.025 | conspecific populations | Masden et al. (1995) |
| Zebra mussel *D. polymorpha* from Europe | 15 | 0.007-0.139 | conspecific populations | Masden et al. (1995) |
| Oysters *Crassostrea gigas* and *C. sikamea* | 5 | 0.440 | allopatric species | Banks et al. (1994) |
| *Perna canaliculus* from New Zealand | 14 | 0.004-0.166 | adaptation to local environments | Sin et al. (1990) |
| Bloodworm *Glycera dibranchiata* | 11 | 0.003-0.093 | conspecific populations | Bristow and Vadas (1991) |
| Fanworm *Sabella spallanzanii* | 23 | 0.000-0.086 | conspecific populations | Andrew and Ward (1997) |
| *Perna viridis* from the west coast of Peninsular Malaysia | 14 | 0.004-0.091 (0.048 $\pm$ 0.004) | conspecific populations | this study |

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# 馬來半島西岸之綠唇貽貝族群遺傳變異之研究

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本研究利用水平式澱粉膠電泳法分析來自Penang、Pulau Aman、Bagan Lalang、Telok Emas、Sungai Muar、Tanjung Kupang、Pantai Lido、Kampong Pasir Puteh等8個不同地理區貽貝*Perna viridis*族群的遺傳變異。這些貽貝均採自馬來半島西岸的水域。本實驗結果共得14個多型性基因座、歧異度觀察值介於0.108及0.334間、平均歧異度期望值則介於0.133到0.301間。最大平均遺傳距離值(0.091)出現於Penang和Telok Emas的族群間，而最小值(0.004)則出現於Pantai Lido和Tanjung Kupang的族群間。依據Nei的遺傳相似度理論建構的UPGMA表型圖將這8個地理區族群分作2群。由本結果顯示具有播遷能力的貽貝幼生雖有促成基因漂流的潛力，但不同地理區間的貽貝仍有族群分化的趨勢。平均族群分化指數(0.149)顯示貽貝族群間遺傳分化的程度應為中度，然而平均遺傳距離數值(0.048 ± 0.004)卻歸類於未分化的範圍中(0-0.14)。本研究結果支持貽貝適合作為偵測重金屬的生物監測指標。另外，本研究中所得的遺傳距離可視為底線值，並可作為與其他相似研究比較之理想材料，進而檢測馬來半島西岸之貽貝族群遺傳分化之可能趨勢。

**關鍵詞**：綠唇貽貝，同功異構酵素，族群。

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