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Heavy Metal Contamination and Physical Barrier are Main Causal Agents for the Genetic Differentiation of *Perna viridis* Populations in Peninsular Malaysia

(Pencemaran Logam Berat dan Penghalang Fizikal Merupakan Penyebab Utama bagi Perbezaan Genetik Populasi *Perna viridis* di Semenanjung Malaysia)

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ABSTRACT

A total of 19 polymorphic microsatellite loci were used to analyze the levels of genetic variations for six geographical populations of green-lipped Perna viridis *collected from the coastal waters of Peninsular Malaysia. In addition, the total soft tissues of all mussel populations were determined for heavy metals (Cd, Cu, Pb and Zn). F_{ST} values revealed that all the six populations of* P. viridis *in Peninsular Malaysia were categorized as showing 'moderate genetic differentiation' according to the classification of Wright (1978). Cluster analysis revealed that three populations which were located in the western part of the Johor Causeway were clustered differently from the other three populations located in the eastern part. Hierarchical* F*-statistics and cluster analysis indicated that the Johor Causeway which blocked the free flow of the pelagic larvae swimmers of* P. viridis *and a distinct effect of heavy metal contamination on the Kg. Pasir Puteh population, were the two main causal agents for the genetic differentiation of the* P. viridis *populations investigated in this study.*

Keywords: Heavy metal contamination; microsatellite markers; Perna viridis

ABSTRAK

Sejumlah 19 lokus mikrosatelit yang berpolimorfik telah digunakan untuk menganalisis tahap variasi genetik bagi enam populasi geografi kupang Perna viridis *yang disampel dari persisiran pantai Semenanjung Malaysia. Keseluruhan tisu lembut daripada kesemua populasi juga ditentukan untuk logam berat (Cd, Cu, Pb dan Zn). Nilai F_{ST} menunjukkan kesemua enam populasi* P. viridis *di Semenanjung Malaysia dikategorikan sebagai 'perbezaan genetik yang sederhana' mengikut klasifikasi Wright (1978). Analisis kluster menunjukkan bahawa tiga populasi yang berada di bahagian barat Tambak Johor telah diklusterkan berasingan daripada tiga lagi populasi yang berada di bahagian timur.* F*-statistik berhierarki dan analisis kluster menunjukkan bahawa tambak Johor yang menghalang aliran bebas bagi perenang pelagik larva* P. viridis *dan suatu kesan yang ketara hasil daripada pencemaran logam berat di populasi Kg. Pasir Puteh, merupakan dua penyebab utama bagi perbezaan genetik populasi* P. viridis *dalam kajian ini.*

Kata kunci: Penanda mikrosatelit; pencemaran logam berat; Perna viridis

INTRODUCTION

From the literature, over the past decades, the genotoxic effects of heavy metals had been extensively studied in both plants and animals. For instances, Ni was reported to promote mutations of simple sequence repeats (SSRs) in human cell lines (Zienolddiny et al. 2000). Various studies suggested that the genotoxicity of Cd may be directly, through the binding of Cd^{2+} to DNA (Hossain & Huq 2002) or indirectly through the inhibition of DNA mismatch repair (Jin et al. 2003).

Genetic variability is one of the important factors determining the success and survival of a species in stressful environments (Simonsen et al. 2004). These harsh environments include metal-polluted ecosystems. In such an environment, the chance of at least some individuals to withstand and survive would increase by possessing high genetic differentiation within a population (Simonsen et al. 2004). Anthropogenic impacts can also induce genetic changes in natural populations by causing a reduction in their genetic diversity because of their fragmentation and loss of numbers (bottleneck effect) and the effect of selection pressure (Belfiore & Anderson 2001). Fratini et al. (2008) reported that heavy metal stress is related to a loss of genetic diversity at the population level of the intertidal crab *Pachygrapsus marmoratus*. On the other hand, toxicant-induced mutagenesis can lead to an increase in genetic variability in populations. Natural and anthropogenic environmental changes acting together resulted in alterations of genetic diversity, both within and among populations. Hence, measuring genetic variability can provide insights into the consequences of environmental alterations (Fratini et al. 2008).

Previously, the genetic differentiation in the mussel populations collected from Peninsular Malaysia was evaluated by using protein allozyme (Yap et al. 2002) and DNA microsatellites (Ong et al. 2008, 2009). Findings by Ong

et al. (2005, 2008, 2009) did not evidently show the reasons of genetic variations in the green-lipped mussel *Perna viridis* populations collected from Peninsular Malaysia. Only Yap et al. (2004) explained the allozyme polymorphisms in relation to heavy metal pollution, based on protein allozymes. However, further studies are needed to confirm whether the genetic variation of mussel populations collected from different geographical sites, based on DNA microsatellites, could be attributable to heavy metal pollution. Therefore, the objective of this study was to determine the relationships of heavy metal levels with genetic variations by using DNA microsatellite markers on the *P. viridis* collected from six geographical populations (three on the eastern and three on the western part of the Johor Causeway) in the Straits of Johor, Peninsular Malaysia.

MATERIALS AND METHODS

Mussels and surface sediments from six different geographical locations (Figure 1; Table 1) in Peninsular Malaysia were collected. In the laboratory, the adductor muscle was excised from the mussel and kept at -80ºC prior to DNA extraction and analysis. For metal analysis, mussel and sediments were stored at -10ºC until analysis. About 15-20 individual mussels were selected for metal analysis. The shell lengths for all populations are given in Table 2. The total soft tissues of mussel and sediments were dried at 60ºC until constant dry weights. Three replicates of sediments from each site were analyzed for heavy metals. For Cd, Cu, Pb and Zn analyses, the sieved sediment samples were digested in concentrated nitric acid

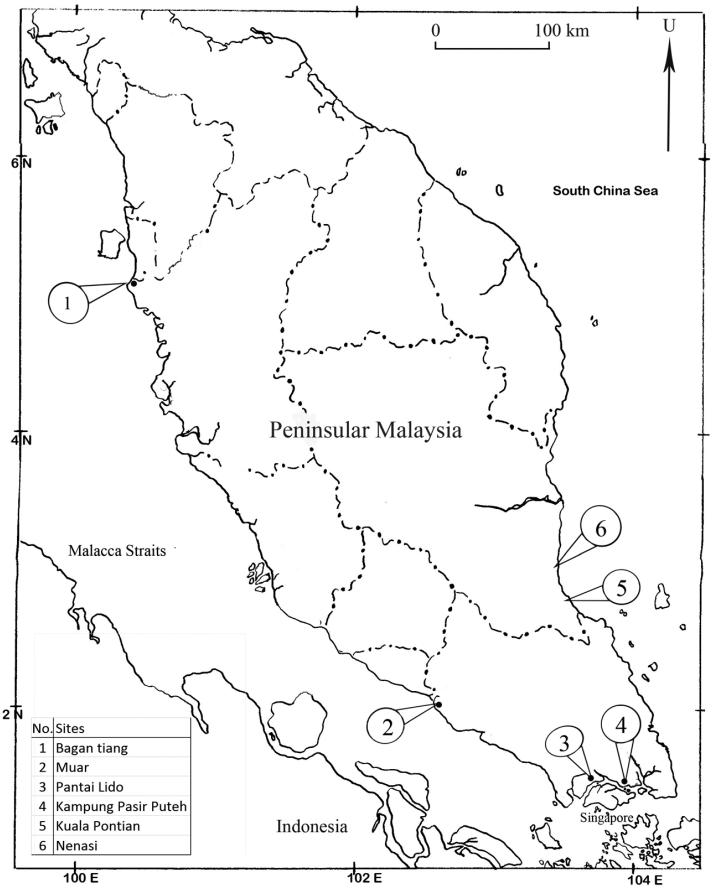

FIGURE 1. Sampling map of six *Perna viridis* populations from Peninsular Malaysia

TABLE 1. Sampling dates, GPS (longitude and latitude) of the sampling sites and site descriptions. $n = 25$

No	Sampling sites	Sampling dates	N	E	Site description
1.	Bagan Tiang	11 Apr 2002	5°07′	100°25′	Aquacultural area
2.	Muar	17 Feb 2002	2°02′	102°34′	Agricultural area
3.	Pantai Lido	17 Apr 2002	1°27′	103°41′	Urban and agricultural areas
4.	Kg. P. Puteh	17 Apr 2002	1°26′	103°55′	Industrial, shipping and urban
5.	Kuala Pontian	8 Apr 2004	2°46′	103°32′	Mussel aquacultural site; clean site
6.	Nenasi	8 Apr 2004	3°08′	103°27′	A light house; pristine

TABLE 2. Shell lengths (mm) and concentrations (mean ± SE, mg/g dry weight) of Cd, Cu, Pb and Zn in the total soft tissues of *Perna viridis* populations collected from Peninsular Malaysia

No.	Sampling sites	Shell length	Cd	Cu	Pb	Zn
1.	Bagan Tiang	135.0 ± 10.2	0.32 ± 0.09	14.1 ± 1.77	10.3 ± 3.73	65.8 ± 4.88
2.	Muar	84.40 ± 2.00	0.83 ± 0.05	7.93 ± 1.00	3.05 ± 0.02	78.9 ± 3.05
3.	Pantai Lido	89.08 ± 2.60	0.43 ± 0.14	14.3 ± 1.58	6.09 ± 1.71	105.5 ± 23.2
4.	Kg. P. Puteh	92.84 ± 3.21	5.55 ± 1.05	31.1 ± 2.48	11.9 ± 1.24	69.9 ± 5.07
5.	Kuala Pontian	67.7 ± 3.04	1.89 ± 0.05	10.3 ± 1.10	7.95 ± 0.07	119.6 ± 5.10
6.	Nenasi	76.6 ± 1.67	2.13 ± 0.07	3.84 ± 0.04	8.84 ± 0.08	93.1 ± 60.01

(BDH, 69%) and perchloric acid (BDH, 60%) in the ratio of 4:1, while the mussels' soft tissue samples were digested in concentrated nitric acid (BDH, 69%). The samples were put in a hot-block digester first at low temperature (40°C) for 1 h and then were fully digested at 140°C for at least 3 h. The prepared samples were analysed for Cd, Cu, Pb and Zn by using an Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The accuracy of the analytical procedures was checked with certified reference materials (CRM) for dogfish and soil (IAEA). These metal recoveries were acceptable between 80-110%.

Genomic DNA from *P. viridis* adductor muscle was isolated by using a CTAB-based protocol described by Winnepennincks et al. (1993) with minor modifications. PCR amplifications were performed in a 10 µL final reaction volume containing 25 ng of genomic DNA, 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl and 0.1% Triton® X-100), 0.25 mM each of dNTPs, 0.15 µM of each reverse and forward primers, 1-3.75 mM of $MgCl_2$ and 0.5-1.5 U of *Taq* DNA polymerase (Promega, USA). Amplifications were performed in a Peltier Thermal Cycler PTC-220 (MJ Research, USA) with an initial 3 min of predenaturation at 95°C, followed by 35-40 cycles of denaturation at 94°C for 30 s, an optimum annealing temperature (Table 2) for 30 s and extension at 68°C for 30 s. The amplifications were concluded with a 5 min final extension at 68°C.

The *P. viridis* specific primer pairs that were used in this study are loci BP2-49-1, BP2-49-2, VJ1-12-2 and VJ1-18-1 which were reported by Ong et al. (2005), loci BP2-35-2, BP9-7-1, BP9-13-2, BP9-16-2, BP9-19-2, BP9-27-1, BP14-7-1, VJ1-9-1, VJ1-15-1, VJ1-21-2 and VJ1-22-2 were reported by Ong et al. (2008) while loci BP10-5-1, BP10-16-1, BP10-17-2 and LR1-58-1 were reported by Ong et al. (2009).

For data analysis, genetic variability measures including mean number of alleles per locus and mean heterozygosity were calculated for all the populations. The *F*-statistics was calculated according to Wright (1978). This is a measure of the deficiency or excess of heterozygosity. Chi–square goodness of fit tests were used to determine whether the observed genotypic numbers were consistent with Hardy-Weinberg expectations for each population. Nei's (1978) unbiased genetic distance (D_N) was calculated to assess the genetic distances among the populations. All the genetic data were analysed by using the POPGENE (version 1.32) computer software program (Yeh & Boyle 1997). By using the multivariate analysis software BIOSYS-1 computer package of Swofford and Selander (1989), an UPGMA dendrogram was constructed based on Nei's (1978) unbiased genetic distance estimates to depict the genetic relationships among the populations of *P.* viridis based on divisions into 2 and 3 regions were also conducted by using the Analyses software BIOSYS-1 computer package.

RESULTS

The background concentrations of Cd, Cu, Pb and Zn in the total soft tissues of *P. viridis* collected from the six geographical populations are presented in Table 2. Mussel soft tissues collected from Kg. Pasir Puteh recorded the highest values in the concentrations of Cu, Cd and Pb among the six mussel populations. On the other hand, the mussels collected from Bagan Tiang possessed the lowest values in Zn and Cd concentrations when compared with the others. The Nenasi population showed the lowest Cu concentration among the six populations, whereas the mussels collected from Muar possessed the lowest Pb concentration when compared with the others.

Based on the heavy metal concentrations in sediments (Table 3), Kg. Pasir Puteh could be considered as a metal-polluted site as it possessed the highest concentrations of Cu, Pb and Zn among the six sampling sites. Hence, the high metal levels found in the soft tissues of *P. viridis* collected from Kg. Pasir Puteh were well supported by these sediment data. Sediment collected from Muar showed the highest concentration of Cd. On the other hand, Nenasi showed the lowest concentrations of Pb, Cd and Zn among the six sampling sites.

Genetic variability at 19 loci in the six mussel populations is presented in Table 4. The mean sample size per locus for all populations ranged at 18.2-19.5 while the mean no. of alleles per locus was 2.1-2.5. The percentages of polymorphic loci were also ranged between 68.4-73.7%. All the above measures of genetic variations were based on DNA microsatellite loci. The majority of the population-locus cases deviated from the Hardy–Weinberg expectations, showing heterozygote deficiency (Table 4). Deviation from Hardy–Weinberg equilibrium could have resulted from admixture of more than two independent populations, non-random mating or artificial and natural selection during mussel seed production and cultivation.

TABLE 3. Concentrations (mean ± SE, mg/g dry weight) of Cd, Cu, Pb and Zn in the surface sediments collected from the mussel habitats

No.	Sampling sites	Cd	Cu	Pb	Zn
1.	Bagan Tiang	2.24 ± 0.17	13.6 ± 0.49	38.1 ± 1.46	106 ± 3.81
2.	Muar	2.51 ± 0.66	42.2 ± 0.47	64.5 ± 1.46	162 ± 0.353
3.	Pantai Lido	1.68 ± 0.01	34.7 ± 0.30	68.8 ± 0.97	141 ± 2.18
4.	Kg. P. Puteh	1.67 ± 0.21	136.2 ± 2.77	83.3 ± 0.85	309 ± 1.56
5.	Kuala Pontian	1.54 ± 0.05	7.23 ± 0.18	34.9 ± 2.24	60.3 ± 0.21
6.	Nenasi	1.49 ± 0.01	13.4 ± 4.30	30.3 ± 0.04	60.9 ± 5.62

TABLE 4. Genetic variability at 19 loci in six *Perna viridis* populations

No.	Population	Mean sample size per locus	Mean no. of alleles per locus	% of polymorphic loci	Mean heterozygosity Observed	Expected
1.	Bagan Tiang	19.3 ± 0.2	2.4 ± 0.2	73.7	0.173 ± 0.047	0.238 ± 0.053
2.	Muar	19.5 ± 0.2	2.5 ± 0.3	68.4	0.203 ± 0.045	0.242 ± 0.057
3.	Pantai Lido	18.2 ± 0.3	2.2 ± 0.3	68.4	0.179 ± 0.044	0.258 ± 0.053
4.	Kg. P. Puteh	19.5 ± 0.2	2.4 ± 0.2	73.7	0.197 ± 0.047	0.257 ± 0.049
5.	Kuala Pontian	19.5 ± 0.2	2.1 ± 0.2	68.4	0.205 ± 0.050	0.218 ± 0.047
6.	Nenasi	18.9 ± 0.2	2.2 ± 0.2	73.7	0.187 ± 0.043	0.226 ± 0.043

Note: A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95

Values of *F* statistics for *P. viridis* are presented in Table 5. The mean F_{IS}, F_{IT}, and F_{ST} values of 0.184, 0.247 and 0.077, respectively, indicated the greater contribution of the among-population differentiation than the within-population differentiation to the total genetic differentiation.

TABLE 5. *F*-statistics values based on 19 loci of six *Perna viridis* populations

No.	Locus	F_{IS}	F_{IT}	F_{ST}
1.	CC9 O	-0.064	0.035	0.093
2.	C13 O	-0.055	-0.037	0.018
3.	C25 O	0.032	0.044	0.013
4.	C28 O	-0.441	-0.339	0.071
5.	C32 O	-0.097	-0.074	0.021
6.	C36 O	-0.026	-0.004	0.021
7.	C42 O	0.17	0.201	0.038
8.	C43 O	-0.123	-0.086	0.032
9.	C44 O	0.828	0.838	0.055
10.	C46	0.656	0.68	0.071
11.	CH24	-0.058	-0.021	0.035
12.	CH27	-0.05	-0.045	0.004
13.	CH49	0.236	0.262	0.033
14.	CH53	-0.254	-0.243	0.01
15.	CH55	-0.051	-0.023	0.027
16.	CH8 O	-0.104	-0.081	0.021
17.	H15 O	0.845	0.888	0.277
18.	H38 O	-0.161	-0.116	0.038
19.	H52	-0.081	-0.013	0.063
	Mean	0.184	0.247	0.077

Note:
F_{IT} is the inbreeding coefficient of an individual (I) relative to the total (T) population; F_{IS} is the inbreeding coefficient of an individual (I) relative to the subpopulation (S); and F_{ST} is the effect of subpopulations (S) compared to the total population (T)

Wright's (1978) hierarchical *F*-statistics (Table 6) showed that populations within the 2 and the 3 regions accounted for 42.9% and 50.7%, respectively, of the total variance, while the between and among region variance components were 57.1%, and 49.3% respectively, of the total variance depending on which hierarchy was considered.

Based on the allelic frequencies, Nei's (1978) D and I values were calculated and are presented in Table 7. The lowest mean value of D was 0.003 between the populations of Nenasi and Kuala Pontian. This result showed that the populations of Nenasi and Kuala Pontian were very closely related. The highest mean value of D was 0.053 between the Muar and Nenasi populations.

In the dendrogram drawn based upon Nei's (1978) I values for the six populations (Figure 2), the populations subdivided into two major clusters: The populations from Muar, Bagan Tiang and Pantai Lido were clustered into one major group; while the other major cluster included the Kg. Pasir Puteh, Kuala Pontian and Nenasi populations.

DISCUSSION

The DNA microsatellite markers used in this study for measuring genetic variations are based on both non-expressed and expressed genes (Heckenberger et al. 2003). Previously, Yap et al. (2002) found a wider range of percentages of polymorphic loci (42.9-85.7%) in eight mussel populations and a higher percentage of polymorphic loci in the metal-polluted mussel population at Kg. Pasir Puteh than in the relatively unpolluted populations based on 14 polymorphic allozyme loci. These results were most likely due to the fact that the allozyme work was based on expressed genes only. For our study, in general, the

TABLE 6. Wright's (1978) hierarchical *F*-statistics of genetic differentiation for six *Perna viridis* populations grouped into two (western and eastern) and three (western, southern and eastern) regions

No.	Contrast	Variance component	(%)	F_{xy}
1.	Populations in 2 regions	0.114	42.9	0.024
2.	Populations in 3 regions	0.142	50.7	0.030
3.	Between 2 regions	0.152	57.1	0.032
4.	Among 3 regions	0.138	49.3	0.029
5.	Among all populations	0.266 (0.280)	100.0	0.056

Note: The two regions were western (Bagan Tiang; Muar, Pantai Lido) and eastern southern (Kampung Pasir Puteh; Kuala Pontian and Nenasi); the three regions were western (Bagan Tiang and Muar), southern (Pantai Lido and Kampung Pasir Puteh) and eastern (Kuala Pontian and Nenasi)

TABLE 7. Nei's (1978) genetic identity (below diagonal) and genetic distance (above diagonal) for *Perna viridis* populations from Peninsular Malaysia

Population	Muar	Bagan Tiang	Pantai Lido	Kg. P. Puteh	Kuala Pontian	Nenasi
Muar	*****	0.008	0.017	0.032	0.051	0.053
Bagan Tiang	0.992	*****	0.01	0.023	0.026	0.031
Pantai Lido	0.983	0.99	*****	0.012	0.03	0.028
Kg. P. Puteh	0.969	0.977	0.988	*****	0.013	0.007
Kuala Pontian	0.95	0.975	0.971	0.987	*****	0.003
Nenasi	0.948	0.97	0.972	0.993	0.997	*****

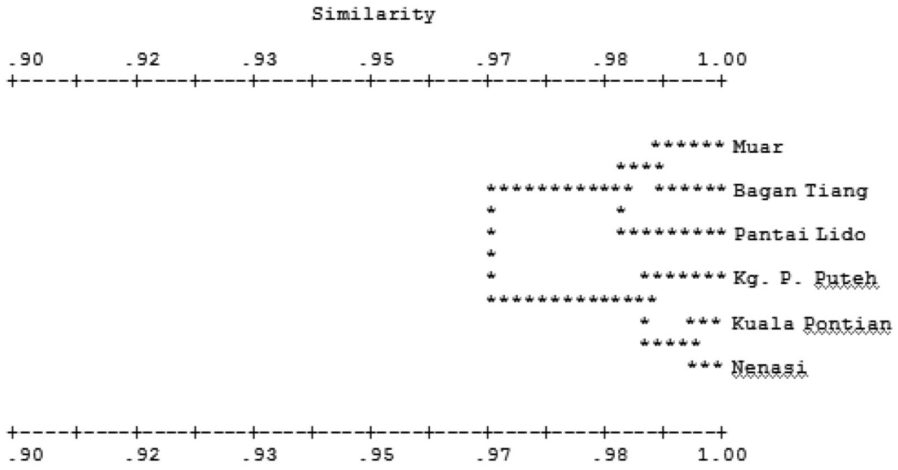

FIGURE 2. UPGMA dendrogram of genetic relationships among six *Perna viridis* populations based on Nei's (1978) genetic similarity

expected mean heterozygosity (He) values (0.218-0.258) were all higher than the observed mean heterozygosity (Ho) values (0.173-0.205). Our expected and observed mean heterozygosities were lower than those of five cultured populations of Pacific oysters (*Crassostrea gigas*) examined at seven polymorphic microsatellite loci (expected: 0.916-0.949; observed: 0.474-0.616) by Li et al. (2006).

The genetic variability obtained by using DNA microsatellite markers of *P. viridis* in the present study was much higher than that based on allozymes in the eight *P. viridis* populations collected from the west coast of Peninsular Malaysia (Yap et al. 2002). This phenomenon was in agreement with that reported for the Pacific oyster populations by Li et al. (2006) by microsatellite.

Compared with those of other wild molluscs species, the variation levels at the microsatellite loci of the six mussel populations were similar to those of the Pacific abalone, pearl oyster, geoduck clam and the zebra mussel (Naish & Boulding 2001; Smith et al. 2003) and somewhat higher than those of the Eastern oyster and blacklip abalone (Evans et al. 2004; Reece et al. 2004).

In this study, the occurrence of high F_{is} values (0.184) for all loci suggested that non-random mating might have

occurred. Since heterozygote deficit has always been reported in the literature for marine mussels, a similar finding for this work was not unexpected. The heterozygote deficit observed from this study using DNA microsatellites was in agreement with those observed using allozymes in *Mytilus* (Koehn 1991; Sanjuan et al. 1990) and in other marine bivalves (Myrand et al. 2002; Ríos et al. 2002). Heterozygote deficiency has also been documented with respect to allozyme and microsatellite loci in natural populations of the Pacific oyster (Ozaki & Fujio 1985; Sekino et al. 2003) and other marine mollusks such as *C. virginica* (Brown et al. 2000; Gaffney & Scott 1984) and *Haliotis discus hannai* (Li et al. 2002).

The hierarchical *F*-statistics suggested that a greater amount of the genetic variation was due to differentiation between regions (western and eastern) only. However, among the three (western, southern, and eastern) regions, the hierarchical *F*-statistics suggested that almost a similar amount of the genetic variation was due to genetic differentiation. This phenomenon was most likely due to two reasons: The Johor Causeway that blocked the free flow of pelagic larvae swimmers of *P. viridis* and a distinct heavy metal contamination in the Kg. Pasir Puteh population as evidenced in the metal data based on the soft tissues of *P. viridis* and sediment samples. Previously, Yap et al. (2006) reported higher heavy metal bioavailabilities in different soft tissues of *P. viridis* in the eastern part of the Johor Causeway than in the western part. Based on the hierarchical *F*-statistics in Table 6, there was a higher variation between two region divisions than three region divisions. This indicated that the Johor Causeway has more effect on the genetic differentiation rather than that due to geographical distance. The Johor Causeway might have blocked the free flow of larval exchange between the two sides of the Causeway.

The component of variation within regions was larger than that between regions, indicating that no genetic structuring exists between mussel populations from different regions. However, a possibility exists that the genetic variation within regions is unevenly distributed due to the environmental heterogeneity reported between the waters of the eastern and western parts of the Johor Causeway and between the two parts of the Causeway (due to water circulation patterns and topographic features of each bank) (Yap et al. 2006).

In this study, the populations of Nenasi and Kuala Pontian were very closely related as indicated by the lowest mean value of D (D=0.003). Owing to the close geographical distance between these two populations, both populations recorded low levels of heavy metal pollution. On the other hand, the highest mean D value was 0.053 between the populations of Muar and Nenasi. Interestingly, these two populations are geographically farther apart, Muar is located in western of Johor while Nenasi is located in eastern of Johor. In addition to that, these two populations differed in terms of levels of heavy metal pollution.

In term of genetic similarity, the populations can be divided into two major clusters: Populations from Muar, Bagan Tiang and Pantai Lido were clustered into one major group; while the other major cluster included the Kg. Pasir Puteh, Kuala Pontian and Nenasi populations.

TABLE 8. 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)

No.	Population	Technique	F_{ST}	Comment	Reference
1.	Mussel *Perna viridis*	14 polymorphic loci by allozyme	0.149	Moderate	Yap et al. (2002)
2.	Brown mussel *Perna perna* from Penha and Canto Grande, Brazil	1 polymorphic loci by DNA microsatellite markers	0.098 to 0.589	Moderate to very great	Noel et al. (2004)
3.	Five cultured populations of *Crassostrea gigas* from China	7 polymorphic loci by DNA microsatellite markers	0.0138 to 0.0348	Low	Li et al. (2006)
4.	Three hatchery populations of *P. yessoensis* in China and two wild Japanese populations	6 polymorphic loci by DNA microsatellite markers	0.0931 to 0.1549	Moderate	Li et al. (2007)
5.	Five hatchery populations of the Pacific oyster (*Crassostrea gigas*) from China and two wild populations from Japan.	7 polymorphic loci by DNA microsatellite markers	0.0105 to 0.0348	Low	Yu & Li (2007)
6.	*Mytilus galloprovincialis* from Galician Rias	6 polymorphic loci by DNA microsatellite markers	0.0122	Low	Diz et al. (2008)
7.	Freshwater pearl mussel (*Hyriopsis cumingii)*	8 polymorphic loci by DNA microsatellite markers	0.000 to 0.063	Low to moderate	Li et al. (2008)
8.	Mussel *Perna viridis*	19 polymorphic loci by DNA microsatellite markers	0.077	Moderate	This study

The populations of Kuala Pontian and Nenasi were further sub-grouped into a minor cluster and the populations of Muar and Bagan Tiang were also sub-grouped together into another minor cluster. These populations were similar genetically. This may be due to the fact that they are geographically close to one and another. However, geographical distance alone is insufficient to explain the dendrogram of genetic similarity. The populations of Kg. Pasir Puteh and Pantai Lido are geographically close each other and yet they were clustered in different groups. Thus, we speculated that heavy metal concentrations played an important role in affecting the genetic structures of the mussel populations. Kg. Pasir Puteh population was in highly metal-polluted waters when compared with the Pantai Lido population. In terms of the heavy metal concentrations analyzed from the surface sediments (Table 3), Bagan Tiang, Muar and Pantai Lido populations were considered moderate metal-polluted; whereas Kuala Pontian and Nenasi recorded low level of metal-pollution. This could explain the clustering patterns observed in the UPGMA dendrogram.

F_{ST} values can be used to determine the degree of genetic differentiation among populations of *P. viridis*. According to Wright (1978), there are four 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 (0.077) falls in the range for moderate genetic differentiation, thus supporting the report of Yap et al. (2002) based on allozyme studies. Therefore, the use of *P. viridis* as a biomonitor which is fundamentally based on the use of a single species for biomonitoring purposes, is still well supported although there is moderate genetic differentiation among the mussel populations. However, this occurrence is considered an adaptation to the ever-changing environment in the coastal area and a process of microevolution.

Many studies have been done by various researchers which revealed the F_{ST} values of bivalves based on microsatellite markers (Table 8). Some researchers found that the populations studied had low levels of genetic differentiation (Diz & Presa 2008; Li et al. 2006; Yu & Li 2007). But as in our current study, several researchers found that regional bivalve populations had undergone moderate genetic differentiations (Li et al. 2007, 2008; Noel et al. 2004; Yap et al. 2002).

CONCLUSION

Based on the hierarchical *F*-statistics from the present study, it is found that a greater amount of the genetic variation was due to differentiation between regions (western and eastern) only and there was almost a similar amount of the genetic variation among the three (western, southern and eastern) regions due to differentiation. We speculated that this phenomenon is most likely due to the Johor Causeway which blocked the free flow of the pelagic larvae swimmers of *P. viridis*. In addition to that, there was a distinct heavy metal contamination in the Kg. Pasir Puteh population as evidenced in the metal data based on the soft tissues of *P. viridis* and sediment samples. Therefore, the physical barrier (Johor Causeway) and heavy metal contamination could be plausibly two major causal agents for the genetic diferentiations of the *P. viridis* populations in this study.

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REFERENCES

Belfiore, N.M. & Anderson, S.L. 2001. Effects of contaminants on genetic patterns in aquatic organisms: A review. *Mutation Research* 489(2-3): 97-122.

Brown, B., Franklin, D.E., Gaffney, P.M., Hong, M., Dendanto, D. & Kornfield, I. 2000. Characterization of microsatellite loci in the eastern oyster, *Crassostrea virginica*. *Molecular Ecology* 9: 2216-2218.

Diz, A.P. & Presa, P. 2008. Regional patterns of microsatellite variation in *Mytilus galloprovincialis* from the Iberian Peninsula. *Marine Biology* 154: 277-286.

Evans, B., Bartlett, J., Sweijd, N., Cook, P. & Elliott, N.G. 2004. Loss of genetic variation at microsatellite loci in hatchery produced abalone in Australia (*Haliotis rubra*) and South Africa (*Haliotis midae*). *Aquaculture* 233: 109-127.

Fratini, S., Zane, L., Ragionieri, L., Vannini, M. & Cannicci, S. 2008. Relationship between heavy metal accumulation and genetic variability decrease in the intertidal crab *Pachygrapsus marmoratus* (Decapoda; Grapsidae). *Estuarine and Coastal Shelf Science* 79(4): 679-686.

Gaffney, P.M. & Scott, T.M. 1984. Genetic heterozygosity and traits in natural and hatchery populations of bivalves. *Aquaculture* 42: 289-302.

Heckenberger, M., van der Voort, J.R., Melchinger, A.E., Peleman, J. & Bohn, M. 2003. Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties: II. Genetic and technical sources of variation in AFLP data and comparison with SSR data. *Molecular Breeding* 12: 97-106.

Hossain, Z. & Huq, F. 2002. Studies on the interaction between Ag+ and DNA. *Journal of Inorganic Biochemistry* 91(2): 398-404.

Jin, Y.H., Clark, A.B., Slebos, R.J.C., Al-Refai, H., Taylor, J.A., Kunkel, T.A., Resnick, M.A. & Gordenin, D.A. 2003. Cadmium is a mutagen that acts by inhibiting mismatch repair. *Nature Genetics* 34: 326-329.

Koehn, R.K. 1991. The genetics and taxonomy of species in the genus *Mytilus*. *Aquaculture* 64: 125-147.

Li, J., Wang, G. & Bai, Z. 2008. Genetic variability in four wild and two farmed stocks of the Chinese freshwater pearl mussel (*Hyriopsis cumingii*) estimated by microsatellite DNA markers. *Aquaculture* 287: 286-291.

Li, Q., Park, C. & Kijima, A. 2002. Isolation and characterization ofmicrosatellite loci in the Pacific abalone, *Haliotis discus hannai*. *Journal of Shellfish Research* 21: 811-815.

Li, Q., Xu, K. & Yu, R. 2007. Genetic variation in Chinese hatchery populations of the Japanese scallop (*Patinopecten yessoensis*) inferred from microsatellite data. *Aquaculture* 269(1-4): 211-219.

Li, Q., Yu, H. & Yu, R. 2006. Genetic variability assessed by microsatellites in cultured populations of the Pacific oyster (*Crassostrea gigas*) in China. *Aquaculture* 259: 95-102.

Myrand, B., Tremblay, R. & Sévigny, J.M. 2002. Selection against blue mussels (*Mytilus edulis* L.) homozygotes under various stressful conditions. *Journal of Hereditary* 93: 238-248.

Naish, K.A. & Boulding, E.G. 2001. Trinucleotide microsatellite loci for the zebra mussel *Dreissena polymorpha*, an invasive species in Europe and North America. *Molecular Ecology Notes* 1: 286-288.

Nei, M. 1978. Estimation of heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 538-590.

Noel, M.B., Manzoni, G.C. & Weber, L.I. 2004. Genetic differentiation in natural and cultured mussels of *Perna perna* (Mollusca, Mytilidae) in Santa Catarina. *Notas Téc. Facimar* 8: 55-63.

Ong, C.C., Teh, C.H., Tan, S.G., Yusoff, K. & Yap, C.K. 2008. Eleven novel polymorphic microsatellite DNA markers from the green-lipped mussel *Perna viridis*. *Russian Journal of Genetics* 44: 498-500.

Ong, C.C., Yusoff, K., Yap, C.K. & Tan, S.G. 2009. Genetic characterization of *Perna viridis* L. in Peninsular Malaysia using microsatellite markers. *Journal of Genetics* 88: 153-163.

Ong, C.C., Tan, S.G., Yusof, K. & Yap, C.K. 2005. Isolation of DNA microsatellite markers in the green-lipped mussel, *Perna viridis*. *Pertanika Journal of Tropical Agricultural Science* 28: 79-85.

Ozaki, H. & Fujio, Y. 1985. Genetic differentiation in geographical populations of the Pacific oyster (*Crassostrea gigas*) around Japan. *Tohoku Journal of Agricultural Research* 36: 49-61.

Reece, K.S., Ribeiro, W.L., Gaffney, P.M., Carnegie, R.B. & Allen, S.K. Jr. 2004. Microsatellite marker development and analysis in the eastern oyster (*Crassostrea virginica*): Confirmation of null alleles and non-Mendelian segregation ratios. *Journal of Heredity* 95: 346-352.

Ríos, C., Sanz, S., Saavedra, C. & Peña, J.B. 2002. Allozyme variation in populations of scallops, *Pecten jacobaeus* (L.) and *P. maximus* (L.) (Bivalvia: Pectinidae), across the Almeria-Oran front. *Journal of Experimental Marine Biology and Ecology* 267: 223-244.

Sanjuan, A., Quesada, H., Zapata, C. & Alvarez, G. 1990. On the occurrence of *Mytilus galloprovincialis* Lmk. on the N.W. coast of the Iberian Peninsula. *Journal of Experimental Marine Biology and Ecology* 143: 1-14.

Sekino, M., Hamaguchi, M., Aranishi, F. & Okoshi, K. 2003. Development of novel microsatellite DNA markers from the Pacific oyster *Crassostrea gigas*. *Marine Biotechnology* 5: 227-233.

Simonsen, V., Holmstrup, M. & Niklasson, M. 2004. Genetic differentiation of the parthenogenetic soil collembolan *Isotoma notabilis* along a copper gradient based on random amplified polymorphic DNA. *Pedobiologia* 48(4): 297-303.

Smith, C., Benzie, J.A.H. & Wilson, K.J. 2003. Isolation and characterization of eight microsatellite loci from silver-lipped pearl oyster *Pinctada maxima*. *Molecular Ecology Notes* 3: 125-127.

Swofford, D.L. & Selander, R.B. 1989. BIOSYS-1: A computer program for the analysis of allelic variation in population genetics and biochemical systematics (Release 1.7). Champaign, IL: Illinois Natural History Survey.

Winnepennincks, B., Backeljau, T. & Watchter, R.D. 1993. Extraction of high molecular weight DNA from mollusks. *Trends in Genetics* 9: 407.

Wright, S. 1978. *Evolution and the Genetics of Populations. Variability Within and Among Natural Populations* Vol. 4. Chicago: Univ. of Chicago Press.

Yap, C.K., Ismail, A. & Tan, S.G. 2002. Genetic variation of green-lipped mussel *Perna viridis* (Linnaeus) collected from the west coast of Peninsular Malaysian. *Zoological Studies* 41(4): 376-387.

Yap, C.K., Ismail, A., Edward, F.B., Tan, S.G. & Siraj, S.S. 2006. Use of different soft tissues of *Perna viridis* as biomonitors of bioavailability and contamination by heavy metals (Cd, Cu, Fe, Pb, Ni and Zn) in a semi-enclosed intertidal water, the Johore Straits. *Toxicological and Environmental Chemistry* 88(1-4): 683-695.

Yap, C.K., Tan, S.G., Ismail, A. & Omar, H. 2004. Allozyme polymorphisms and heavy metal levels in the green-lipped mussel *Perna viridis* (Linnaeus) collected from contaminated and uncontaminated sites in Malaysia. *Environment International* 30: 39-46.

Yeh, F.C. & Boyle, T.H.B. 1997. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belgian Journal of Botany* 129: 157.

Yu, H. & Li, Q. 2007. Genetic variation of wild and hatchery populations of the Pacific Oyster *Crassostrea gigas* assessed by microsatellite markers. *Journal of Genetics and Genomics* 34(12): 1114-1122.

Zienolddiny, S., Svendsrud, D.H., Ryberg, D., Mikalsen, A.B. & Haugen, A. 2000. Nickel(II) induces microsatellite mutations in human lung cancer cell lines. *Mutation Research* 452(1): 91-100.

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