ALLOZYME COMPARISON OF BLACK-SCAR OYSTER POPULA TIONS OF INDIA, THAILAND AND MALAYSIA

A. Murugan*, M. Niklasson**, S. Bussarawit***, C. Aungtonya***, and F. Boneka*****
*Suganthi Devadason Marine Research Institute, Tuticorin - 628 001, India.
**Institute of Biological Sciences, Aarhus University, Aarhus, Denmark.
***Phuket Marine Biological Centre, Phuket, Thailand.
****Sam Ratulangi University, Manado, Indonesia.

ABSTRACT

The edible black-scar oyster *Crassostrea madrasensis* from Vellar estuary and Pitchavaram mangrove were studied for genetic variability along with black-scar oyster populations from Narathiwat and Nathap in Thailand and from Penang in Malaysia using protein electrophoresis. The Indian black-scar oysters from Pitchavaram and Vellar estuary belong to same population. The populations from Penang and Nathap are closely related. The population from Narathiwat is also related to the populations from Penang and Nathap. However, the Indian populations and that of Thailand and Malaysia differ in genetic distance at the clustering level of 0.11533. The mean genetic similarity of 0.892 was observed between Indian and Malaysian, Thailand populations. The geographical distance could be attributed to the variation among the populations.

INTRODUCTION

The black-scar edible oyster, *Crassostrea madrasensis* Preston is distributed in estuaries and back waters all along the east coast and up to Karwar on the west coast of India. The black-scar edible oysters (*C. iredalei* in Malaysia and *C. lugubris* in Thailand) are found distributed along the coasts of Malaysia and Thailand. Durve (1986) stated that *C. virginica*, the American oyster could be ancestral to *C. madrasensis*, the Indian oyster and *C. gigas*, the Japanese oyster. There is a possibility that the black-scar oysters in India, Malaysia and Thailand are closely related. The populations may be allopatric-geographically isolated. Shell morphology may be influenced by the local environmental conditions. Hence, allozyme electrophoresis has been employed to compare the black-scar oyster populations from India, Malaysia and Thailand.

MATERIALS AND METHODS

Crassostrea madrasensis, the Indian black-scar oyster was collected from two locations, Pitchavaram mangrove (L1) and Vellar estuary(L2), southeast coast of India. The black-scar oysters from Malaysia (Penang (L3)) and Thailand (Narathiwat (L4), Nathap (L5)) were used for analysis and comparison (Fig. 1). Adductor muscles were removed and deep frozen. The adductor muscles were homogenised in equal volume with 0.04% Mercaptoethanol and centrifuged at 15,000 rpm for 15 minutes at 4 °C. Whatman 3 mm chromatography paper was cut into 9x4 mm paper wicks and was soaked with the supernatant. The soaked paper wicks were inserted in a 10.6% starch gel.

Figure 1 Map showing study areas India: Pitchavaram (L_1), Vellar (L_2); Malaysia: Penang (L_3); Thailand: Narathiwat (L_4) and Nathap (L_5)

The enzymes were tested on
1. Electrode buffer: 0.223 M Tris, 0.086 M citric acid, pH 6.3. gel buffer: 0.008 M Tris, 0.003 M citric acid, pH 6.7. Running conditions: 170 V for three hours.
2. Electrode buffer: 0.30 M sodium borate, pH 8.2. gel buffer: 0.076 M Tris, 0.005 M citric acid, pH 8.7. Running conditions: 250 V for four hours.

The electrophoresis was carried out at Phuket Marine Biological Center (PMBC), Department of Fisheries, Phuket, Thailand. Three enzymes coding for four loci (Table 1) were used for analysis. The enzymes showed good activity, distinct bands and were consistently readable.

Data analysis were carried out using the computer program GENEPOP Version 2 (Raymond and Rousset, 1995). BIOSYS (Swofford and Selander, 1981) package was used to estimate Nei's genetic distance and for cluster analysis using unweighted pair group method.

RESULTS

Three enzymes coding for four loci were selected for the study (Table 1). Allele frequencies obtained for the five populations are given in Table 2. The genetic variability and distance are given in Table 3 and 4.

The two populations in India, Pitchavaram mangrove (L1) and Vellar estuary (L2) are located nearly 10 km apart. Out of the four loci studied, three (PGM-1, PGI and MDH-2) were polymorphic. The L1 and L2 populations also shared the most common allele in all the four loci. [A locus was considered polymorphic if the frequency of the most common allele was not greater than 0.95 (Hara *et al.* 1996)]. The genetic distance between L1 and L2 is zero. The dendrogram, for the five populations studied is given in Fig. 2. The data were produced from estimates of genetic distances (Nei, 1978) over four loci by unweighted pair group method. The two Indian black-scar oyster populations L1 and L2 were clustering together and the two populations could be considered identical.

Table 1. Enzymes Screened

Enzyme	E. C. No.	Locus
Phoshoglucom-utase (PGM)	5.4.2.2	PGM-1 PGM-2
Phoshoglucosei-somerase (PGI)	5.3.1.9	PGI
Malatedehydro-genase (MDH)	1.1.1.37	MDH-2

Though the black-scar oysters of Thailand and Malaysia resemble the Indian black-scar, different species names have been ascribed to these oysters (*C. lugubris* in Thailand and *C. iredalei* in Malaysia). The three populations from Malaysia (Penang, L3); Thailand (Narathiwat (L4) and Nathap (L5) were compared with the Indian black scar oyster.

As far as the L3, L4 and L5 populations are concerned they share the most common allele in three loci (PGM - 1, PGM - 2 and MDH - 2). Three loci (PGM - 1, PGM - 2 and PGI) were polymorphic and MDH - 1 was monomorphic (using 0.95 criterion). The proportion of polymorphic loci were same (0.75).

Considered together, the five populations, L1, L2, L3, L4 and L5 share the most common allele in two loci (PGM - 1 and MDH - 2). Three loci (PGM - 1, PGM - 2 and PGI) were polymorphic in all the five populations. The dendrogram for the five populations showed two distinct groups in accordance with geographical distance. The two Indian populations L1 and L2 cluster together and were considered identical. The populations L3 and L5 cluster together indicating that they were closely related. The population L4 cluster with L3 and L5. The Indian populations L1, L2 and the Malaysian and Thailand populations L3, L4 and L5 cluster at a distance level of 0.115. These clustering level could be attributed to the geographical

Table 2. Allele frequencies in five populations of black-scar oyster: Pitchavaram (L_1), Vellar (L_2), Penang (L_3), Narathiwat (L_4) and Nathap (L_5) (N - Number)

Locus		Populations				
		L1	L2	L3	L4	L5
PGM-1	N	23	18	37	20	17
	1	0	0	0.176	0.325	0.176
	2	0	0.028	0.095	0.025	0
	3	0.087	0.167	0.081	0	0.029
	4	0.304	0.167	0.595	0.475	0.618
	5	0.435	0.444	0.054	0.15	0.118
	6	0.13	0.196	0	0.025	0.059
	7	0.043	0	0	0	0
PGM-2	N	23	18	36	20	17
	1	0	0	0	0.025	0
	2	0	0.194	0.056	0.175	0.265
	3	0.978	0.806	0.944	0.775	0.735
	4	0.022	0	0	0.025	0
PGI	N	23	19	37	20	17
	1	0.304	0.237	0.257	0.375	0.441
	2	0.304	0.316	0.149	0.025	0.059
	3	0.196	0.211	0.203	0.325	0.147
	4	0.174	0.237	0.108	0.2	0.029
	5	0.022	0	0.284	0.075	0.324
MDH-2	N	16	19	37	20	17
	1	0.156	0.158	0.014	0	0
	2	0.813	0.789	0.986	1	1
	3	0.031	0	0	0	0
	4	0	0.053	0	0	0

Table 3. Genetic variability in five populations of black-scar oyster: Pitchavaram (L_1), Vellar (L_2), Penang (L_3), Narathiwat (L_4) and Nathap (L_5)

Population	Proportion of polymorphic Loci	Heterozygosity		Ho/He	allele/locus
		Observed Ho	Expected He		
Pitchavaram	0.75	0.398	0.449	0.886	3.75
Vellar	1.00	0.512	0.529	0.968	3.50
Penang	0.75	0.359	0.377	0.952	3.50
Narathiwat	0.75	0.475	0.430	1.105	3.75
Nathap	0.75	0.368	0.409	0.900	3.25
Average	0.8	0.422	0.439	0.962	3.55

Table 4. Genetic distance in five populations of black-scar oyster: Pitchavaram (L_1), Vellar (L_2), Penang (L_3), Narathiwat (L_4) and Nathap (L_5)
(N - Number)

Population	Pitchavaram	Vellar	Penang	Narathiwat	Nathap
Pitchavaram	—	—	—	—	—
Vellar	0.000	—	—	—	—
Penang	0.083	0.129	—	—	—
Narathiwat	0.092	0.109	0.028	—	—
Nathap	0.126	0.153	0.019	0.020	—

Table 5. Genetic relationships of five populations of black-scar oyster. (Nei's 1978) genetic distance - UPGM cluster analysis).

Locus / Population	Number	Heterozygosity	
		Expected	Observed
PGM - 1			
Pitchavaram	23	70%	52%
Vellar	18	68%	58%
Penang	37	60%	57%
Narathiwat	20	65%	75%
Nathap	17	59%	53%
PGM - 2			
Pitchavaram	23	4%	4%
Vellar	18	32%	26%
Penang	37	11%	5%
Narathiwat	20	40%	30%
Nathap	17	41%	29%
PGI			
Pitchavaram	23	78%	65%
Vellar	19	79%	74%
Penang	37	78%	78%
Narathiwat	20	75%	85%
Nathap	17	71%	65%
MDH - 2			
Pitchavaram	23	22%	26%
Vellar	19	37%	42%
Penang	37	3%	3%
Narathiwat	—	—	—
Nathap	—	—	—
Average		49.61%	45.9%

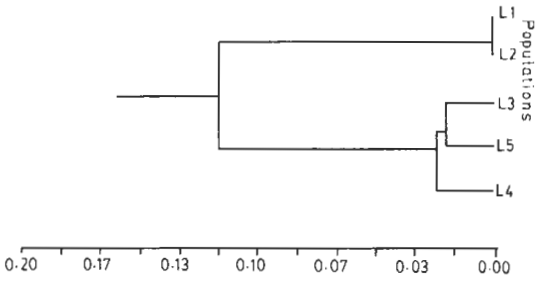

Figure 2. Genetic relationships between five populations (Nei, 1978) genetic distance - UPGM cluster analysis.

distance.

Higher genetic distance of 0.153 was obtained between populations of L2 and L5. The genetic distance ranged from 0.000 to 0.153 (mean 0.0765) for all five populations. The genetic distance were low when compared to 0.707 - 2.293 observed in 8 species of *Crassostrea* (Ostreidae) (Buroker *et al.* 1979b).

The expected heterozygosity ranged from 3% to 79% and the observed values varied between 3% and 85% with the mean expected and observed heterozygosity of 49.6% and 45.9% (Table 5).

DISCUSSION

Durve (1986) stated that *C. madrasensis* is considered morphologically very close to *C. virginica*, the American oyster. Morphological and biochemical relationships have also been established between *C. virginica*, *C. gigas* (the Japanese oyster) and *C. rhizophorae* (South American Oyster). The ancestral *C. virginica*, which probably had a wide distribution in the Pacific coast of North and South America got distributed along the Arctic circle to Japanese islands and down south (through the Java Trench) in the Cretaceous period. The steep thermal and environmental gradients during the Tertiary period lead to isolation and speciation. Thus, the ancestral *C. virginica* got distributed up to the Indian subcontinent via this pathway to become *C. gigas* in Japan and *C. madrasensis* on the east coast of India and Sri Lanka (Durve, 1986).

The distribution pathway of *C. virginica* to the Indian subcontinent through the Java Trench (Durve *op. cit.*) indicate that the black scar oysters of India, Malaysia, Thailand and Indonesia might have the same ancestor. The low genetic distance values may be attributed to this previous history of the oysters. Also, no specific diagnostic locus was detected in this study (may be due to low number of loci) and the differences could be attributed to the geographical distance. Philip Samuel and Thangavelu (1991) observed variations in protein patterns in *C. madrasensis* from different locations along the southeast coast of India and attributed the same to the physiological stresses due to complex environmental changes. However, it should be noted that only a small sample size was used in this study and only 3 enzymes coding for 4 loci were used for the analysis. Further specific studies with large sample size and comparative anatomical studies would bring more light on the specific status of these populations from India, Malaysia and Thailand.

ACKNOWLEDGEMENT

We would like to thank TMMP for supporting travel grant of A. Murugan from India and F. Boneka from Indonesia and partially sharing of chemical expenses to do this study at PMBC. Thanks to Mr. Kongkiat Kittiwattanawong for reading and comment of an early draft of manuscript, PMBC Director, Mr. Praween Limpsaichol for his support and Ms. Teunchai Srisawad for laboratory assistance.

REFERENCES

Buroker, N.E., Hershberger, W.K. & Chew, K.K., 1979b. Population Genetics of the family Osteridae. II. Interspecific studies

of the genera *Crassostrea* and *Saccostrea*. - Marine Biology. **54**: 171-184.

Durve, V.S. 1986. On the Ancestry & Distribution Pathways of Three species of Indian Oysters. - Indian Journal of Marine Sciences. **15**: 56-58.

Hara M., M. Sekino & U. Na-Nakhon, 1996. Enzyme polymorphism and population structure of snake-head fish *Channa striatus* in Thailand. Pages 61-71 *in* Motoyuki Hara and Uthairat Na-Nakorn (eds.). Development of sustainable Aquaculture Technology in southeast Asia - Focused on Genetics and Physiology. Under the co-operative research program between JIRCAS, Japan and Faculty of Fisheries in Kasetsart University, Thailand. 79 pp.

Samuel, P. & R. Thangavelu, 1991. Electrophoretic protein patterns of farm grown and wild edible oyster *Crassostrea madrasensis* from different locations. - Journal of Marine Biological Association of India **33**(1&2):346-349.

Mi R. & F. Rousset, 1995. GENEPOP (Version 1.2): A population genetics software for exact tests and ecumenicism. - Journal of Heredity. **86**: 248-249.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. - Genetics **89**: 583-590.

Swofford, D.L. & R.B. Selander, 1981. BIOSYS - 1. A Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. - Journal of Heredity. **72**: 281-283.