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Evaluation of genetic diversity of Pearly mussel, *Parreysia corrugata* by randomly amplified polymorphic DNA (RAPD)

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ABSTRACT

Bivalve *Parreysia corrugata* is an important component of freshwater ecosystem of Indian sub-continent. In the present study an attempt was made to evaluate the genetic diversity of *P. corrugata* populations using RAPD markers.

Key words: Genetic diversity, *Parreysia corrugata*, RAPD-PCR, Freshwater bivalve.

INTRODUCTION

Freshwater bivalve *Parreysia corrugata* is reported to be widely distributed in the states like Punjab, Bihar, Madhya Pradesh, Maharashtra, Orissa, and Karnataka[1]. Recently it has been found to be a potential candidate species for freshwater pearl production[2]. Further *Parreysia* spp. is known to have medicinal importance [3].

Evaluation of the genetic diversity of populations of *P. corrugata* is important to assess the health of this species. If low genetic diversity is found, measures can be put into place to protect and potentially help these endangered populations recover their genetic diversity. This will increase the long term chances of survival for this important species.

RAPD analysis based on the PCR amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence has been used to evaluate genetic diversity for species, subspecies and stock identification in a number of aquatic organisms. In spite of importance of *P. corrugata*, there are absolutely no reports available on the use of these markers for studying genetic variation in *P. corrugata*. Therefore, an attempt has been made to evaluate the use of RAPD markers to analyze genetic diversity of *P. corrugata* collected from five different locations within the state of Maharashtra.

MATERIAL AND METHODS

P. corrugata were hand collected from five distantly located districts in Maharashtra *viz.* Thane, Ahmednagar, Ratnagiri, Aurangabad and Nanded, brought alive to Aquaculture laboratory, Department of Life Sciences and acclimatized for 24 hrs in separate glass aquaria. After acclimatization, 10-15 live mussels were sacrificed and different body parts were carefully dissected and separated for isolation of genomic DNA.

Isolation of Genomic DNA

Genomic DNA was isolated from fresh mantle tissue (approximately 100 mg) by grounding in 2 ml of extraction buffer and after repeated centrifugation and treatment with suspension buffer, SDS, proteinase K, ammonium acetate, chloroform - isoamyl alcohol (24:1) and absolute ethanol. The

pellet was dissolved in 200 µl elution buffer and store at 4°C. The agarose gel electrophoresis (0.8%) was carried out to check the presence of DNA. Purity and concentration of DNA was estimated by calculating the ratio of the optical density measured at 260 and 280 nm with a spectrophotometer.

RAPD analysis

The isolated genomic DNA was subjected to PCR amplification with 12 random 10-mer primers (Operon Technologies, California). Amplification of genomic DNA was carried out in 50 µl reaction mixture containing 1µl genomic DNA as template, 25µl PCR master mix, 2 µl primer and 22 µl of nuclease free water. DNA amplification was performed in a DNA thermal cycler (Biometra, Germany) with standardized PCR conditions. Amplified PCR products were analyzed using electrophoresis in 1.4% agarose gel. DNA markers (Fermentas) of different sizes were also run along side the PCR products. The gel was stained in solution containing 5 µl of ethidium bromide and observed under UV transilluminator for DNA bands. Photographs of the gel were taken.

Statistical Analysis of RAPD profile

The RAPD markers were scored visually on the basis of their presence (1) or absence (0), separately for each individual and primer. The scores obtained from all the seven primers were then pooled together for constructing a single data matrix. The data obtained for all the five populations of *P. corrugata* was statistically analyzed using POPGENE version 1.32 software [4] under the assumption of Hardy-Weinberg equilibrium.

RESULTS AND DISCUSSION

Molecular genetic information has been increasingly used to detect the population genetic structure and genetic diversity among morphologically similar populations of a same species. Of the many molecular approaches available today, RAPD-PCR was chosen for this study as it is simple and rapid method for determination of genetic variability in various organisms without prior knowledge of the genome under study.

RAPD analysis clearly indicated polymorphism in the wild study populations of bivalve, *P. corrugata* collected from five different geographical regions of Maharashtra. Out of the twelve primers tried out for PCR, seven primers *viz*. OPA- 01, OPA-09, OPA-13, OPB-01, OPD-02, OPD-08 and OPD-20 showed amplification product and DNA fingerprint for DNA samples from at least a single location. Remaining five primers i.e. OPB-02, OPB-08, OPB-16, UBC456 and UBC457 however, failed to produce any amplification. Primer OPD-20 produced maximum number of polymorphic bands i.e. 13 whereas OPA-01 produced a single polymorphic band (Table 1). Primer OPA-09 and OPA-13 both gave rise to 13 polymorphic bands followed by primer OPD-02 and OPD-08 with 10 and 9 polymorphic bands respectively. Primer OPB-01 produced only couple of polymorphic bands. Thane sample showed a total of 9 polymorphic bands, whereas Aurangabad, Nanded, Ratnagiri and Ahmednagar samples produced a total of 19, 11, 4 and 14 polymorphic bands respectively. Total number of polymorphic bands produced by the 7 RAPD primers for *P. corrugata* from five different locations was 57.

Statistical analysis of RAPD profile
Polymorphism in different populations

The maximum polymorphic loci were found in Aurangabad population (100%) and minimum (28.57%) in Ratnagiri population indicating polymorphism in each of the five populations of *P. corrugata* (Table 2).

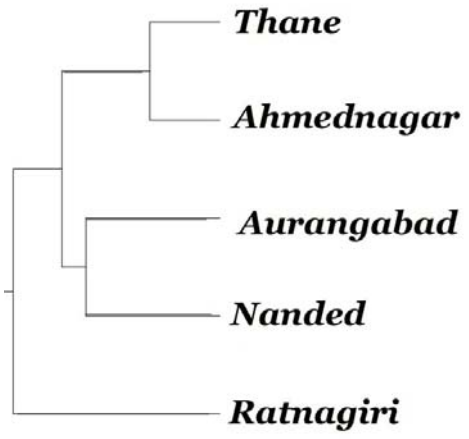

Fig. 1: UPGMA dendrogram for *P. corrugata* based on Nei's Genetic distances

Gene diversity index (h)

The overall genetic diversity ranged from 0.0208 (Ratnagiri) to 0.1025 (Aurangabad) with a mean of 0.0606 (Table 3).

Shannon's Informative index (I)

The informative index ranged from 0.0459 to 0.2077 in all the individuals of the five populations (Table 3). Average value was 0.1339. This mean index value clearly indicates diversity between and within the populations proving that the genetic diversity has been retained in *P. corrugata.* This is in agreement with results observed in freshwater mussels [5] and marine bivalves [6, 7] suggesting that

Table 1: RAPD analysis of *P. corrugata*

Primer Name	Sample	Total Scorable Bands	Molecular weight range	Total Polymorphic bands
OPA-01	Thane	0		1
	Aurangabad	1	1900	
	Nanded	0		
	Ratnagiri	0		
	Ahmednagar	0		
OPA-09	Thane	1	650	11
	Aurangabad	3	400-1700	
	Nanded	4	300-800	
	Ratnagiri	0		
	Ahmednagar	3	320-650	
OPA-13	Thane	3	550-750	11
	Aurangabad	3	800-1100	
	Nanded	1	750	
	Ratnagiri	0		
	Ahmednagar	4	350-720	
OPB-01	Thane	0		2
	Aurangabad	2	600-950	
	Nanded	0		
	Ratnagiri	0		
	Ahmednagar	0		
OPD-02	Thane	0		10
	Aurangabad	3	500-1300	
	Nanded	3	480-1000	
	Ratnagiri	2	450-550	
	Ahmednagar	2	450-550	
OPD-08	Thane	2	500-1300	9
	Aurangabad	4	500-1700	
	Nanded	1	500	
	Ratnagiri	0		
	Ahmednagar	2	500-650	
OPD-20	Thane	3	450-1000	13
	Aurangabad	3	750-1200	
	Nanded	2	200-300	
	Ratnagiri	2	450-650	
	Ahmednagar	3	450-1000	

Table 2: Percentage of polymorphic loci of five populations of *P. corrugata*

Population	Percentage of Polymorphic loci
Thane	57.14 %
Aurangabad	100.0 %
Nanded	71.43 %
Ratnagiri	28.57 %
Ahmednagar	71.43 %

the wild populations of aquatic invertebrates maintain the genetic diversity.

Coefficient of genetic differentiation

To assess genetic differences among the populations and the level of population differentiation, the index of inter population differentiation (Gst) was calculated. The value of Gst normally varies from 0 to 1 [8]. Gst varied from 0.0001 to 0.1886. Mean Gst between populations

Table 3: A summary of coefficient of genetic variability

Populations	Observed number of alleles n_a subscript	Effective number of alleles n_e	h = Nei's gene diversity	Shannon's information Index (I)
Thane	1.5714	1.0512	0.0464	0.0989
Aurangabad	2.0000	1.1158	0.1025	0.2077
Nanded	1.7143	1.0630	0.0566	0.1196
Ratnagiri	1.2857	1.0224	0.0208	0.0459
Ahmednagar	1.7143	1.0806	0.0719	0.1473
Mean	2.0000	1.0657	0.0606	0.1339

Table 4: Analysis of gene diversity in subdivided populations

Primer / Locus	Ht	Hs	Gst	Nm
OPA-01	0.0074	0.0073	0.0150	32.8096
OPA-09	0.0878	0.0858	0.0224	21.8227
OPA-13	0.0805	0.0789	0.0199	24.6819
OPB-01	0.0150	0.0145	0.0304	15.9315
OPD-02	0.0731	0.0722	0.0119	41.3832
OPD-08	0.0661	0.0648	0.0198	24.7805
OPD-20	0.0939	0.0938	0.0019	258.4096
Mean	0.0606	0.0596	0.0153	32.1347

Table 5: Genetic identity and Genetic distance between the five populations of *P. corrugata*

Population	Thane	Aurangabad	Nanded	Ratnagiri	Ahmednagar
Thane	*****	0.9989	0.9987	0.9991	0.9996
Aurangabad	0.0011	*****	0.9992	0.9983	0.9994
Nanded	0.0013	0.0008	*****	0.9991	0.9993
Ratnagiri	0.0009	0.0017	0.0009	*****	
Ahmednagar	0.0004	0.0006	0.0007	0.0013	*****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

was found to be 0.0153 (Table 4) which shows that genetic differentiation is moderate suggesting that all the populations under study are not completely isolated. The number of migrants among local populations (Nm), total genetic diversity (Ht) and the mean sample genetic diversity (Hs) were also computed. Hs and Ht was 0.0596 and 0.0606 respectively. Gene flow plays a critical role in differentiation among populations. When Nm is greater than 1, gene flow can resist genetic drift in the population and prevent differentiation among them [9]. Mean Nm value was 32.1347 indicating that the gene flow of these populations has resisted genetic drift.

Dendrogram based on Nei's (1972) Genetic distances[10]

The genetic distance values ranged from 0.0004 to 0.0013 among the five populations (Table 5). Low genetic distances confirmed that all the populations belong to same species. A UPGMA dendrogram based on Nei's genetic distance [10] indicated the segregation of these populations into distinct clusters according to geography (Fig. 1). The dendrogram linked Thane and Ahmednagar populations separated from Aurangabad and Nanded populations with Ratnagiri population as an outgroup.

Overall, the RAPD technique showed genetic variation in wild populations of *P. corrugata*. The present study may serve as a reference point for future examinations of the genetic variation within populations of this important freshwater pearly mussel. Furthermore, it can be used as a model for other studies relating to genetic diversity.

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