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HIGH LEVELS OF GENETIC VARIATION REVEALED IN WILD POPULATIONS OF THE STRIPPED DWARF CATFISH *MYSTUS VITTATUS* (BLOCH) (BAGRIDAE: SILURIFORMES) IN BANGLADESH BY RANDOM AMPLIFIED POLYMORPHIC DNA TECHNIQUES

Faozia Mehbuba Tamanna, Jonaira Rashid, and Md. Samsul Alam
Department of Fisheries Biology and Genetics
Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ABSTRACT
Random amplified polymorphic DNA (RAPD) markers were used to assess genetic variation in three wild populations of the striped dwarf catfish, *Mystus vittatus* (Bloch) namely Chalan *beel* (Natore), Mohanganj *haor* (Netrakona) and Kangsha river (Netrakona) in Bangladesh. Four primers used in the study yielded a total of 44 reproducible and consistently scorable bands all of which (100%) were found to be polymorphic (P_{95}). The proportions of polymorphic loci were more or less similar in the three studied populations. The intra-population similarity index, gene diversity and Shannon's Information Index were found to be the highest in the Chalan *beel* population followed by those of the Kangsha river and Mohanganj *haor* population. The inter-population similarity index was highest between Chalan *beel* and Kangsha river population and the gene flow was highest between Mohanganj *haor* and Kangsha river population. The population differentiation values (PhiPT) were found to be insignificant indicating no significant differentiation among the three populations. No population-specific bands were detected. The RAPD analysis revealed a high level of genetic variation in the three wild populations of the dwarf catfish *M. vittatus*.

KEYWORDS: Genetic variation, *Mystus vittatus*, RAPD, Polymorphic Loci

INTRODUCTION

The striped dwarf catfish, *Mystus vittatus* (Bloch) belonging to the family *Bagridae* of the order Siluriformes is naturally distributed in Bangladesh, India, Pakistan, Sri Lanka, Nepal, Myanmar and Thailand (Talwar and Jhingran, 1991; Roberts, 1992). The fish is demersal and inhabits mostly freshwater and occasionally brackish water. In Bangladesh *M. vittatus* is usually found among marginal vegetation in lakes and swamps with a mud substrate (Rahman, 2005). The species is rich in food value with a protein, fat, moisture and ash content of 15.62±0.32%, 7.53±1.10%, 73.99±3.13%, and 6.50±0.63% respectively (Kamal, *et al*., 2007). Recently, this species has been used as an aquaculture species to a very limited scale by some fish farmers and for that purpose the seeds are being produced in a few hatcheries. As a country of wetlands, Bangladesh is very rich in fish diversity. Unfortunately, however, combination of reasons including overexploitation, indiscriminate use of pesticide and aquatic pollution, habitat modification due to industrialization, river-valley projects, excessive water abstraction and siltation, there is a declining trend in the availability of *M. vittatus* along with many species in natural water bodies of the country that might have an impact on the allelic diversity of the population. In small populations, the individuals are likely to be genetically, anatomically, and physiologically more homogeneous than in larger populations and less able to adapt to variable environmental conditions. Genetic diversity is a characteristic of ecosystems and gene pools that describes an attribute which is commonly held to be advantageous for adaptation and survival, therefore, a key component for conservation efforts associated with population management (Andayani, *et al*., 2001).

Molecular markers combined with new statistical developments have revolutionized the analytical power, necessary to explore the genetic diversity. Various DNA markers such as RAPD, AFLP, microsatellites or SNP are now being used in fisheries and aquaculture. RAPD analysis is a technique for rapidly detecting genomic polymorphisms, utilizing a single short oligonucleotide primer (usually 10 bases in length of arbitrary sequence (Welsh and McClelland, 1990) in a polymerase chain reaction (PCR). RAPD assay is simple, fast, and comparatively low-cost and it has quickly become the method of choice for genotype identification, gene mapping, detection of strain diversity, population and pedigree analysis, phylogenic studies and the demonstration of phylogenetic and taxonomic relationships (Avise, 1994). Furthermore, RAPD analysis can be carried out on organisms for which there is little or no information concerning genomic sequences or organization, thus making it possible to analyze polymorphisms for virtually any organism from which relatively pure genomic DNA can be isolated. The objective of the present study was to determine the intra- and inter-population genetic variation among three *M. vittatus* populations in Bangladesh.

MATERIALS AND METHODS

Collection of fish samples and isolation of genomic DNA

Fish samples were collected from three wild populations: Chalan *beel* (Natore district), Mohanganj *haor* (Netrakona district) and the river Kangsha (Netrakona district) during September'09-January'10. In order to perform RAPD analysis for genetic diversity study, a total number of 20 fish samples were taken randomly from each population.

Tissue samples were clipped from the caudal fin of each fish and immediately preserved in individual microfuge tube containing 95% ethanol and stored at -20°C. Genomic DNA was isolated from fin tissues according to the method described by Islam and Alam (2004)**.** In brief, approximately 40 mg of fin tissues was cut into small pieces and taken into 1.5 ml microfuge tube. The fin tissue was digested with proteinase-K in extraction buffer (100 mM Tris, 10 mM EDTA and 250 mM NaCl, pH= 8.0 and 1% Sodium Dodecyl Sulfate) overnight at 37ºC. DNA was purified once with equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and once with chloroform: isoamyl alcohol (24:1) and precipitated using 0.6 volumes of isopropanol. The DNA samples were tested qualitatively on 1% agarose gel and quantified by using a spectrophotometer (Biophotometer plus, Eppendorf, Germany).

PCR amplification and electrophoretic separation of amplified products

Thirty three decamer primers of random sequences from three kits (20 from kit A, 8 from kit B and 5 from kit C) of random sequence (Operon Technologies, Inc., Alameda, CA, USA) were initially screened in this study. Four primers exhibiting good quality banding patterns and sufficient variability for population analysis were then selected for analysis of the entire samples.

The amplification conditions were based on Williams *et al*. (1990) with some modifications. PCR reactions were performed on each DNA sample in a 10 µl reaction mix containing 1 µl of 10 × *Taq* polymerase buffer, 2.0 µM of primer, 0.25 mM of each dNTPs, 1 unit of *Taq* DNA polymerase (GENEI, Bangalore, India) and 150 ng of genomic DNA. DNA amplification was performed in a gradient thermal cycler (Master Cycler Gradient, Eppendorf, Germany). The reaction mix was preheated at 94ºC for 3 min followed by 40 cycles consisting of 1 min denaturation at 94ºC, 1 min annealing at 38ºC and 2 min extension at 72ºC. After the last cycle, a final step of 7 min at 72ºC (final extension) was added to allow complete extension of all amplified fragments followed by holding at 4ºC.

Total volume of the amplified product (10µl) of each sample was subjected to electrophoresis on 1.4% agarose gel (GENEI, India) containing ethidium bromide in 1xTBE buffer at 100V for 1 hr. Two molecular weight DNA markers (Lambda DNA-EcoRI/HindIII digest and 100bp ladder) were electrophoresed alongside the RAPD-PCR products. DNA bands were observed on GelDoc system and image was saved in a computer.

Analysis of RAPD data

RAPD patterns were visually scored from the photographs and the bands were compared among the *M. vittatus* populations. All distinct bands or fragments (RAPD markers) were given identification numbers according to size and the RAPD markers were determined by recording the presence (1) or absence (0) of these bands for each fish and each primer separately. The sizes of the RAPD markers were estimated by using the software AlphaEaseFC (Version4.0).

The scores obtained using all primers in the RAPD analysis were pooled for constructing a single data matrix. This was used to estimate allele frequency, proportion of polymorphic loci, Nei's gene diversity **(**Nei, 1973), observed number of alleles, effective number of alleles, mean expected heterozygosity (*h*), Shannon's Information Index (I) gene flow (N_m), genetic distance (*D*) using the software POPGENE (Version 1.31) **(**Yeh *et al*., 1999**).** Analysis of Molecular Variance (AMOVA) was performed using the software GenAlEx (version 6.4) **(**Peakall and Smouse, 2006**).** The similarity index values (SI) between the RAPD profiles of any two individuals on the same gel were calculated from RAPD markers of the same molecular weight on the data matrix according to the following formula given by Nei and Li **(**Nei and Li, 1979):

Similarity index (SI) = $2N_{AB}/ (N_A + N_B)$

Where, N_{AB} is the total number of RAPD bands shared by individuals A and B, and N_A and N_B are the total number of bands produced by individual A and B, respectively, with regard to all assay units **(**Lynch, 1990).

Thus, Genetic-similarity reflects the proportion of bands shared between two individuals and ranges from 0 (no common bands) to 1 (all bands identical). Within population similarity index (S_i) was calculated as the average of SI across all possible comparisons between individuals within a population. Between population similarity index (S_{ij}) was calculated as the average similarities between randomly paired individuals from population i and j **(**Lynch, 1991**).**

RESULTS

RAPD polymorphisms

Among the 33 primers initially tested, four primers: OPA02, OPA04, OPA05 and OPA12 were selected after several round of screening that yielded relatively large number of good quality bands. All the primers produced different RAPD patterns, and the number of fragments amplified per primer varied. The four primers yielded a total of 44 reproducible and consistently scorable RAPD bands all of which (100%) were found to be polymorphic (P_{95}). The number of bands per primer ranged from 6 to 14 (Table 1). Among the primers, OPA04 gave DNA profile with highest number of bands while OPA05 gave the least (Table 1) (Fig. 1). The number of private bands in the Chalan beel, Mohongonj and Kangsha population were 2, 1 and 3 respectively (Table 2). No population specific bands were found.

Genetic variability parameters

The proportion of polymorphic loci was higher in the Kangsha river population (90.91%) than that of the other two populations (Table 2). The gene diversity and Shannon's Information Index values of the Chalan *beel* population were found to be the highest followed by those of the Kangsha river and Mohanganj *haor* population (Table 2) respectively. The observed numbers of alleles were more or less similar in all three populations however the effective number of alleles was higher in Chalan *beel* population.

TABLE 1. Random Amplified Polymorphic DNA primers, corresponding bands scored with their size ranges and polymorphic bands amplified by PCR from the samples of the three populations of *M. vittatus*

Primers	Sequence (5′-3′)	No. of scorable bands	Size range (bp)	Polymorphic bands	Polymorphism (%)
OPA02	TGCCGAGCTG	13	331-1415	13	100.0
OPA04	AATCGGGCTG	14	200-1565	14	100.0
OPA05	AGGGGTCTTG	6	557-1512	6	100.0
OPA12	TCGGCGATAG	11	421-1630	11	100.0
Total		44		44	100.0

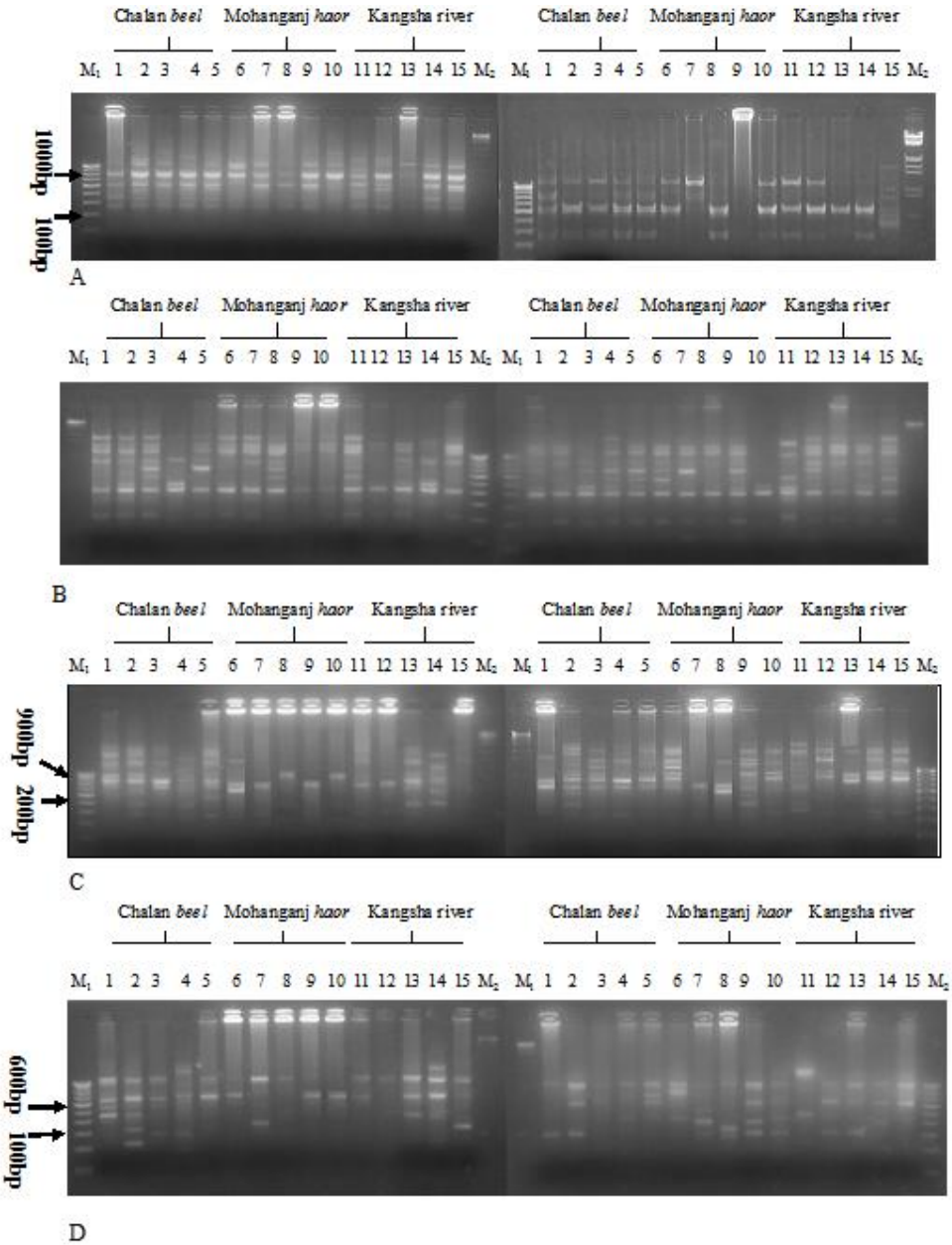

FIGURE 1. Electrophoretic pattern of *M. vittatus* generated by RAPD primer OPA02 (A), OPA04 (B), OPA05 (C), OPA12 (D); M_1: Molecular weight marker (100bp DNA ladder); M_2: Molecular weight marker (Lambda DNA/EcoRI/HindIII digest).

Intra- and inter-population similarity indices

The value of intra-population similarity or within population similarity index (S_i) was the highest (28.99%) in the Chalan *beel* population and the lowest (21.8%) in the Mohanganj *haor* population (Table 2). The inter-population similarity or between populations similarity index (S_{ij}) for Chalan *beel* vs Kangsha river populations was found to be higher (27.70%) than other two population-pairs (Table 3).

TABLE 2. Estimates of genetic variation**:** number and proportion of polymorphic loci, gene diversity, no. of alleles (n_a), effective no. of alleles (n_e) and intra- and inter-population similarity indices for the studied *M. vittatus* populations

Parameters	Population		
	Chalan *beel*	Mohanganj *haor*	Kangsha river
No. of polymorphic loci	39	37	40
Percentage of polymorphic loci	88.64	84.09	90.91
Gene diversity (Mean±SD)	0.259±0.163	0.198±0.136	0.216±0.138
Observed no. of alleles (n_a)	1.773	1.682	1.818
Effective no. of alleles (n_e)	1.419	1.288	1.320
No. of private bands	2	1	3
Shannon's Information Index (I)	0.403±0.03	0.327±0.03	0.354±0.02
Intra-population similarity index (S_i)	28.99	21.80	25.10

TABLE 3. Inter-population similarity indices, population differentiation (Phi-PT), gene flow (N_m) and genetic distance (D) values between the population pairs

Population pairs	Inter-population similarity indices	Phi-PT	Gene flow (N_m)	Genetic distance (*D*)
Chalan *beel* - Mohanganj *haor*	20.96	0.088^{NS}	8.132	0.036
Chalan *beel* - Kangsha river	27.70	0.022^{NS}	12.865	0.024
Mohanganj *haor* - Kangsha river	18.65	0.00^{NS}	17.329	0.015

NS: Statistically not significant (P>0.05)

Gene flow, genetic distance and population differentiation

The gene flow between Mohongonj *haor*-Kangsha river population was the highest while that between Chalan *beel*-Mohongong haor population was the lowest. The highest (0.036) and lowest (0.015) genetic distances were found between the Chalan *beel* and Mohanganj *haor* populations and between the Mohanganj *haor* and Kangsha river populations respectively (Table 3). The population differentiation (Phi-PT) values all the population-pairs were found to be insignificant indicating that there was no significant differentiation among the three populations of *M. vittatus*. Partitioning of RAPD variance within and among populations was performed using the AMOVA (Analysis of Molecular Variance) procedure. The percentages of molecular variance i.e. genetic variation among and within populations were 3% and 97%, respectively (Table 4).

TABLE 4. Analysis of molecular variance (AMOVA) for the three populations of *M. vittatus*. Significance levels are based on 999 permutations.

Source	df	SS	MS	Estimated Variance	Percentage (%)
Among populations	2	20.333	10.167	0.258	3
Within populations	57	432.573	7.589	7.589	97
Total	59	452.906		7.847	100

DISCUSSION

Genetic variation holds the key to the ability of populations and species to persist over evolutionary time through changing environments. No organism can predict the future nor can any organism be optimally adapted for all environmental conditions. Nonetheless, the current genetic composition of a species influences how well its members will adapt to future physical and biotic environments. Therefore, it is necessary to assess the amount of genetic diversity, the structure of diversity in samples and populations, rates of genetic divergence among populations and the distribution of diversity in populations found in different locations. The genetic diversity data has varied applications in research on evolution, conservation and management of natural resources and genetic improvement programmes, etc. RAPD analysis for genetic diversity study provides a basis to obtain genetic variation within and among populations. In the present study among the 33 single decamer random primers, four primers generated a total of 44 bands in the three populations, all (100 %) of which were polymorphic. The percentage of polymorphic loci was found to be

higher in the Kangsha river population (90.91%) which is higher than the average (64.98%) polymorphic loci in two populations of *Mystus vittatus* (Bloch) of Madhya Pradesh **(**Garg *et al*., 2009a)**,** and the average (44.9%) polymorphic loci in *Mystus guttatus* from Xijiang branch of Pearl river **(**Jiao *et al*., 2007). We found that 100% of the loci in our study were polymorphic as compared to the 83.87% **(**Sultana *et al*., 2010) in *Heteropneustes fossilis,* 60.48% in endangered yellow catfish, *Horabagrus brachysoma* **(**Muneer *et al*., 2009), 86.66% in *Clarias batrachus* (Garg *et al*. 2010) and 64.98% in *Mystus vittatus* (Garg *et al*., 2009a). In contrast to the findings of the present study, a very low level of polymorphism (18.75%) was obtained in two Indian populations of *H. fossilis* in RAPD loci (Garg *et al*., 2009b). The present study indicates that comparatively higher level of genetic variation exists in the studied *M. vittatus* populations in Bangladesh. Almost similar level of intra-population similarity indices showed by all the population in the present study indicates that individuals within each population were genetically closer to each other. On the other hand, inter-population similarity or between populations similarity index (S_{ij}) for Chalan *beel* vs Kangsha river populations was found to be higher (32.70%) than other two between populations similarity indices. The S_{ij} values for Chalan *beel* vs Mohanganj *haor* populations and Mohanganj *haor* vs Kangsha river populations were 25.96% and 23.65% respectively. The similar result was also observed in catfish (*Clarias batrachus*) populations from three regions of Indian riverine system **(**Khedkar, *et al*., 2010). The level of band sharing values within the catfish populations were 0.26±0.021 for Banaras, 0.60±0.033 for Bhubaneshwar and 0.377±0.058 for Hussainabad respectively. This implies that individuals within each population are genetically more similar to each other, as is expected, than to individuals from all other populations. Greater genetic identity (0.984) was found between the Mohanganj *haor* and Kangsha river population indicating that they were more genetically similar than the others. Nei's (Nei, 1972) original measures of genetic distance (D) were also used to evaluate the genetic variability and relatedness among the *M. vittatus* populations. The highest pair-wise genetic distance was found between the Chalan *beel* and Mohanganj *haor* population (0.036). The genetic distance obtained among individuals of *Mystus guttatus* from Xijiang branch of Pearl river ranged from 0.0467 to 0.2804 (Jiao *et al*., 2007). The population differentiation (PhiPT) values were found to be insignificant indicating that there were no significant differentiation among three studied populations of *Mystus vittatus.* The method of RAPD for genetic diversity analysis is important since it is relatively easy to obtain valuable data. Once the population structure is known, scientific management for optimal harvest and conservation of the catfish fishery resource can be undertaken. Therefore, the present study may serve as a reference point for future examinations of genetic variations within the populations of fishes which are commercially important but also play a significant role in food chain in lentic as well as lotic habitats. Genetic diversity serves an important role in evolution by allowing a species to adapt to a new environment. Analyses of genetic diversity can be applied to studies of the evolutionary ecology of populations. In the present study, relatively lower level of overall intra-population similarity index (25.29%) was found which indicates a relatively higher level of genetic diversity. The presence of higher proportion of polymorphic loci (100%) and higher gene diversity and Shannon's Information Index values are also an indicative of relatively higher level of genetic variation exists in the populations.

CONCLUSION

RAPD technique was found to be quite effective in determining genetic variation within and among three populations of the dwarf catfish *M. vittatus*. RAPD analysis revealed a relatively high level of genetic variation in the studied populations of *M. vittatus*. More samples however should be analyzed from across the country to have a complete picture of the population genetic structure of this important fin fish species.

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