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Genetic Diversity Between Two Populations of *Heteropneustes fossilis* (Bloch) Using RAPD Profile

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Abstract: The present study was aimed to elucidate its genetic diversity based on RAPD markers from 6 individuals belonging to two ecological habitats i.e., upper lake and lower lake of Bhopal. Ten random 10-mer primers were scored in each of the individuals from two locations out of which three primers, which gave polymorphism, were selected for Polymerase Chain Reaction (PCR) and used in the final RAPD analysis. The complementary approach of RAPD was used to evaluate the genetic diversity among all the accessions using 3 highly polymorphic primers. Using these primers, 128 scorable DNA fragments were found, of which 24 (18.75%) were polymorphic. By comparing RAPD banding patterns, small variations were found between and within the populations. The present study yielded data elucidating the usefulness of complementary approaches to make diversity analysis more explanatory and purposeful for optimum genetic amelioration and effective conservation of its genotypic variability.

Key words: Status of fishes in M.P., genetic diversity, RAPD, polymorphism

INTRODUCTION

The freshwater resources of India are currently experiencing an alarming decline in fish biodiversity due to several factors and a result, a sizeable portion of freshwater fishes have been categorized as threatened. This emphasizes an immediate need for initiating research and actions for alternative management techniques to protect these aquatic systems. Several species of fish have declined and some have become endangered. Therefore, in order to prioritize fish species for taking conservation action, there is an urgent need to assess the present status of freshwater fish of the Central India. Out of about 700 freshwater fishes reported from Indian inland waters, 320 were assessed in a workshop in 1998 jointly organized by National Bureau of Fish Genetic Resources, Lucknow and Zoo Outreach Organization, Coimbatore, India (CAMP, 1998). Of these 320 fish species assessed, about 166 were from Central Region and among these 21 were categorized as endangered, 01 critically endangered, 27 vulnerable, 50 at lower risk or near threatened, 8 at lower risk of least concern and 59 species were not evaluated (CAMP, 1998). The base list prepared by National Bureau of Fish Genetic Resources was assessed in a workshop held in 2006 for

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Central India, where 138 species were from Madhya Pradesh (CAFF, 2006). The overall assessment of this workshop indicates that 01 species were under critically Endangered Category (CE), 14 species under Endangered Category (EN), 26 under vulnerable (VU), 53 under Data Deficient (DD), 37 under lower risk of near threatened (LR-nt) and 08 under lower risk of least concern (LR-lc) category. Total of 41 (56.58%), 29 (34.8%) and 18 (11.16%) species were categorized as threatened in Madhya Pradesh, Chattishgarh and Rajasthan, respectively (CAFF, 2006). This observation clearly indicates that, fishes are declining at a faster rate in Madhya Pradesh than the other two states of Central India.

Genetic markers are important instruments for the study of fish populations and fish lots. The development of molecular methods has permitted genomic analysis and made it possible to analyze existing variations, both in the regions which encode genes, as well as regions with unknown functions (Jayme *et al*., 2008). Genetic monitoring is ideal for use in a reproduction program with the aim of genetic conservation (i.e., stocking). Molecular markers are realistic and useful tools for the investigation and monitoring of genetic conditions both in native populations and in captive lots (Alam and Islam, 2005). The RAPD (Random Amplified Polymorphic DNA) and microsatellite markers are among the molecular markers used to analyze genetic diversity of fish. Both of these markers may be analyzed by PCR (Polymerase Chain Reaction).

Catfish is a commercially important warm water fish species, which is distributed all over the world. Consequent to the rapid increase in hatchery-reared catfish production, there is a need to understand the genetic composition of natural catfish populations in order to evaluate the range and extent of latent genetic effects induced by hatchery operations. Little information is available on the phylogenetic relationship among the populations of catfish of India. Rapid amplified polymorphism NDA technique is the one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms (Bartish *et al*., 2000) and has provided important applications in catfish. In the present study germplasm collection of *Heteropneustes fossilis* (Bloch), a catfish freshwater fish with edible fish, was carried out from two locations of Bhopal i.e., upper lake and lower lake and find out intra-specific genetic variability.

MATERIALS AND METHODS

Fish Sampling Sites and Morphometric Measurements of Fishes

The present study was carried out from July, 2008 to December, 2008. A total of 06 specimens of *Heteropneustes fossilis* were collected from both the locations (upper lake and lower lake, Bhopal) with the help of castnet by local fishermen for morphometric measurements and estimated genetic variations. All the fish specimens were kept in the iceboxes and brought to the MPCST laboratory for further study. For the morphometric measurements, total 14 parameters i.e., total length, standard length, body weight, body width, body depth/height, head length excluding snout, width of head, snout length, eye diameter, length of caudal peduncle, length of anal fin, length of pelvic fin, length of pectoral fin, height of caudal peduncle were taken. Fish specimens were morphologically identified with taxonomic keys (Shrivastava, 2000; Jayaram, 1999). The muscle, liver and brain tissues were isolated from freshly caught fishes and preserved at -20°C for further use.

Molecular Study

For the isolation of total genomic DNA, a short procedure was applied according to a modified protocol of Wu *et al*. (1995). Tissues (100 mg) were placed in a

1.5 mL micro-centrifuge tube (Eppendorf, A.G., Humberg, Germany) and homogenized by using Eppendorf micro-pestle. In the homogenized tissue, 0.5 mL of lysis buffer (4 mM NaCl, 0.5 mM EDTA, 0.1% SDS and 0.02 NP 40) and 0.01% proteinase-K were added, gently mixed and incubated at 55°C on dry bath (Genei, model-SLM-DB-120) for 45-60 min for complete lysis of cells. After incubation, 500 µL phenol: chloroform: isoamyl alcohol (25:24:1) was added, mixed gently and centrifuged at 10,000 rpm at room temperature (High Speed Brushless Centrifuge, MPW-350R, Poland) for 5 min. The supernatant was then transferred to a new micro-centrifuge tube and half volume of 7.5 M ammonium acetate and two volume of 100% chilled ethanol was added, mixed well and centrifuged at 10,000 rpm for 5 min at room temperature. One microliter of 70% ethanol was added to the tube for washing. Again centrifuged the sample for 10 min at 10,000 rpm at 4°C. The DNA pellet was then washed with 70% ethanol, dried and dissolved in a Tris-EDTA buffer (10 mM Tris Hcl, 1mM EDTA, pH 7.6). UV-VIS Spectrophotometer (Nano-Drop ND-1000, USA) was used to check quality as well as quantity of isolated DNA. The concentration of extracted DNA was adjusted to 50 ng μL^{-1} for PCR amplification.

In this study, 10 commercially available RAPD primers of Bangalore Genei, India make were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplifications. After initial screening with all the ten primers, only 3 were used in the final study. They are RAn-1, RAn-5 and RAn-6 with accession numbers AM765825 AM750052 and AM765829, respectively.

The reaction mixture for PCR (30 µL) was composed of 3 µL of 10X Taq polymerase buffer, 1.2 µL of 10 mM dNTP, 1.2 µL of RAPD primer, 0.6 µL Taq DNA polymerase (3 U μL^{-1}), 23.4 µL sterile distilled water and 0.6 µL template DNA. A negative control, without template DNA was included in each round of reactions. After pre-heating for 5 min at 94°C, PCR was run for 45 cycles. It consisted of a 94°C denaturation step (0.45 min), 37°C annealing step (1 min) and 72°C elongation step (1.5 min) in a thermal cycler (Corbet Research, Australia). At the end of the run, a final extension period was appended (72°C, 7 min) and then stored at 4°C until the PCR products were analyzed.

The amplified DNA fragments were separated on 1.2% agarose gel and stained with ethidium bromide. A low range DNA marker was run with each gel (100, 200, 300, 600, 1000, 1500, 2000, 2500 and 3000 bp DNA ladder from Bangalore Genei, Bangalore, India). The amplified pattern was visualized and photographed by Gel Documentation System (Alpha-Innotech, USA).

The RAPD products were scored for the presence and absence of fragments on the gel photographs and RAPD fragments were compared among the *H. fossilis* populations. The RAPD banding patterns were recorded on spreadsheets, which were used to determine gene diversity, gene flow, number of polymorphic loci and genetic distance through a construct by an un-weighted pair group method of arithmetic mean of UPGMA (Nei, 1978) using past (1. 91) software. The Similarity Index (SI) values between the RAPD profiles of any two individuals on the same gel were calculated using the following formula:

$$\text{Similarity Index (SI)} = \frac{2\,N_{AB}}{N_A + N_B}$$

where, N_{AB} is the total number of RAPD bands shared by individuals A and B and N_A is the number of bands scored for each individual, respectively (Lynch, 1990).

RESULTS AND DISCUSSION

In the present study twelve classical morphometric characters were studied in the two populations of *H. fossilis* which have been shown in Table 1. The morphometric characteristics did not vary much among the populations from upper lake and lower lake of Bhopal.

DNA from all the 06 samples was extracted using phenol: chloroform:lsoamyl method (Wu *et al*., 1995). The extracted DNA of all the samples was diluted as per the requirements of PCR amplifications. Out of these, three primers (RAn-1, RAn-5 and RAn-6) generated higher number of bands (Table 2). The RAPD profile of the bands obtained in the two populations with primer RAn-05 is shown in the Fig. 1 as a representative photograph. These three primers generated a total of 128 bands in all the 06 individuals out of which 18.75% were polymorphic (Table 2, 3). The UPGMA dendrogram was prepared based on genetic distance indicating the segregation of the *H. fossilis* populations collected from the two reservoirs of Bhopal. The un-weighted dendrogram divided all the 06 individuals in two groups, as observed in the study (Fig. 2).

In capture fishery, excessive exploitation, combined with poor fishery management results in the depletion of the fishery stocks. Such depletions can result in the loss of total gene pool (Nelson and Soule, 1987; Smith *et al*., 1991). In the present study, most of

Table 1: Morphometric measurement of fishes collected from upper and lower lake, Bhopal

	Individual						
Parameters	01 (UL-1)	02 (UL-2)	03 (UL-3)	04 (LL-I)	05 (L-II)	06 (L-III)	Mean±SD
Total length (cm)	12.50	15.50	18.00	19.00	11.50	12.00	14.75±3.2360
Standard length (cm)	11.40	14.00	16.50	16.00	10.50	11.00	13.23±2.6350
Length of head (cm)	3.00	2.00	5.00	7.00	2.50	3.70	3.86±1.8560
Height (cm)	2.00	1.80	2.50	3.50	1.30	2.20	2.21±0.7460
Length of caudal peduncle (cm)	1.50	1.50	3.00	2.50	1.00	1.50	1.83±0.7520
Height of caudal peduncle (cm)	1.70	1.00	2.60	2.50	1.00	1.50	1.71±0.7020
Length of head excluding snout (cm)	3.00	2.70	4.30	5.50	4.40	2.00	3.65±1.3000
Diameter of eyes (cm)	0.30	0.50	0.30	0.50	0.20	0.30	0.35±0.1220
Snout (cm)	0.50	1.00	0.60	0.30	0.40	0.80	0.60±0.2600
Pre-dorsal length (cm)	3.50	3.50	5.30	6.60	4.00	2.50	4.23±1.4740
Width of head (cm)	2.00	2.00	3.00	2.50	2.40	2.30	2.36±0.3720
Weight (g)	11.00	19.15	25.25	50.00	7.39	9.99	20.46±15.919

UL-1, UL-2 and UL-3 are samples from upper lake; LL-I, LL-II and LL-III are samples from lower lake

Table 2: Primer wise pattern of polymorphism between six individuals

Primer	Total No. of bands	Total No. of polymorphic bands	Total No. of monomorphic bands	Total No. of unique bands	Polymorphism (%)	Monomorphism (%)	Uniqueness (%)
RAn-1	35	8	2	1	22.85	5.71	2.85
RAn-5	59	11	3	4	18.64	5.08	6.77
RAn-6	34	5	3	11	14.70	8.82	32.35

Table 3: Pattern of polymorphism and uniqueness (4 primers) between 6 individuals

Pattern of polymorphism	No. of bands
Analysis	RAPD
Total No. of bands	128.00
Total No.of polymorphic bands	24.00
Total No. of monomorphic bands	8.00
Total No. of unique bands	16.00
Polymorphism (%)	18.75
Monomorphism (%)	6.25
Uniqueness (%)	12.50

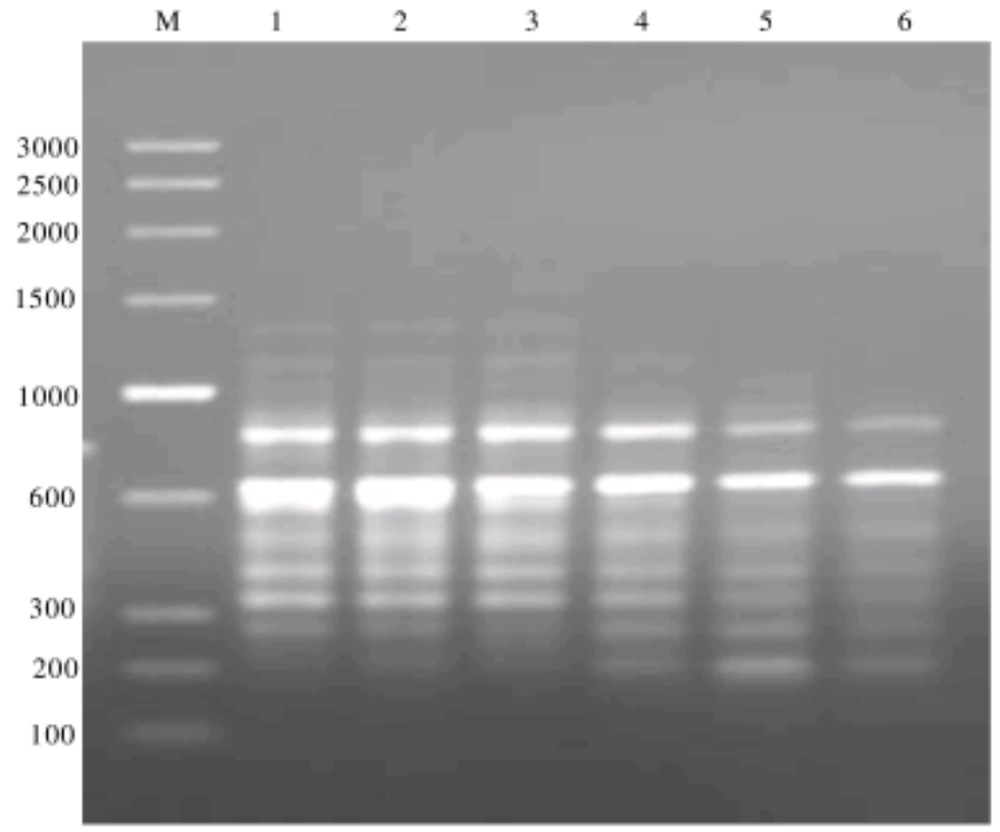

Fig. 1: Random amplified polymorphic DNA fragment patterns generated using primer 5. 1-3: Fishes of upper lake, 4-6: Fishes of lower lake. M is the molecular marker of low range DNA ladder

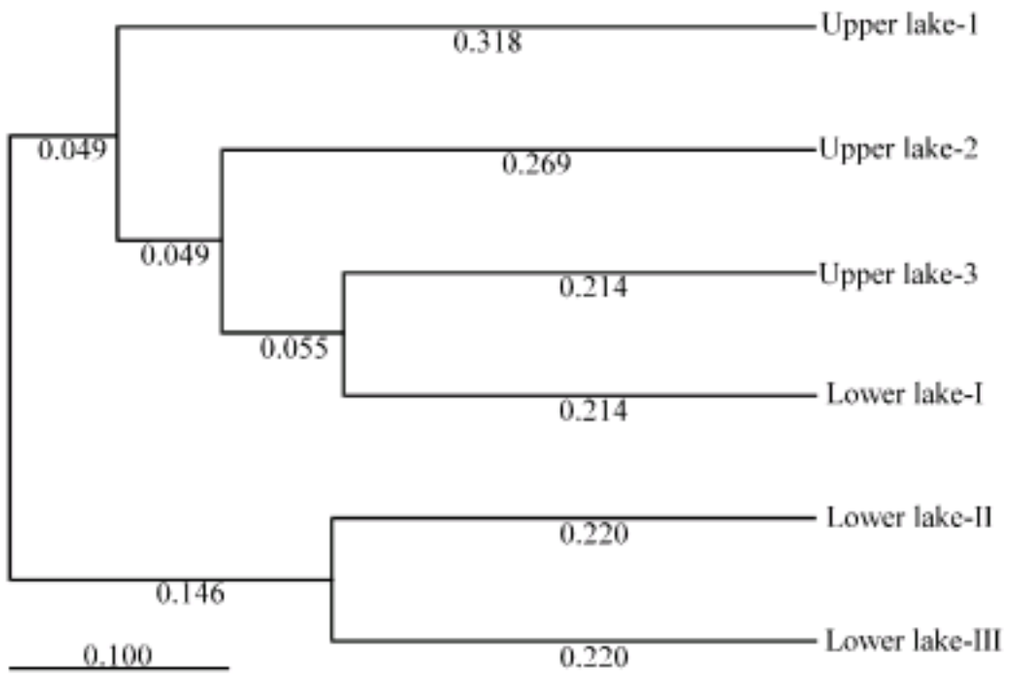

Fig. 2: Phylogenetic tree constructed by distance matrix (Jaccard's and UPGMA)

the morphometric characteristics of fishes were similar and often overlapped with population (Table 1). These morphometric data are not enough to support the established genetic structure of the population that often leads to taxonomic uncertainty (Daniel, 1997; Ponniah and Gopalakrishnan, 2000). Allozymes and morphometric analyses were used to discriminate Hilsa populations which were collected from 9 different sites within Bangladesh (Salini *et al*., 2004). They had observed significant differences in allele frequencies and morphological variations in Hilsa, which may be due to the local environment.

The RAPD markers have been found to have a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding (Bardakci and Skibinski, 1994; Ertas and Seker, 2005). The RAPD technique consists

of amplification by PCR, of random segments of genomic DNA using a single-short primer of arbitrary sequence. There is no requirement of prior knowledge of the sequence of DNA. Its cost effectiveness provides an advantage in population genetics studies. The RAPD technique has been applied to the study of phylogentic relationship in tilapiine and cichlid species (Bardakci and Skibinski, 1994). The presence of variability among populations as well as individuals within a population is essential for their ability to survive and successfully respond to environmental changes (Ryman *et al*., 1995). Intra-population genetic variation in *Tilapia* was studied using different RAPD primers (Bardakci and Skibinski, 1994). This technique is more sensitive than the mt-DNA analysis, which failed to reveal variations within the *Tilapia* populations (Capili, 1990; Seyoum and Kornfield, 1992). Genetic variation was studied between four different populations of Hilsa Shad from Ganga, Yamuna, Hoogly and Narmada Rivers of India using RAPD technique (Brahmane *et al*., 2006). Garg *et al*. (2009) studied genetic diversity of two populations of *Mystus vittatus* of Madhya Pradesh using RAPD markers and obtained high polymorphism (64.98%). Thus, RAPD has been used in population studies in fisheries and can be used efficiently for variation analysis of populations with differential degrees of geographic isolation.

The genetic stock of *H. fossilis* has not been studied in the reservoirs of Madhya Pradesh. In the present investigation, RAPD analysis has been used to discriminate between the two different populations of *H. fossilis* of Madhya Pradesh. RAPD fragments observed in the 06 individuals, showed a reasonable degree of genetic diversity within and between the populations. The average percentage of polymorphism was 18.75 as obtained using the 3 primers. The present study may serve as a reference point for future examinations of genetic variations within the populations of fishes which are not only commercially important but also play a significant role in food chain in lentic as well as lotic habitats.

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