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Full Length Research Paper

RAPD analysis for genetic diversity of two populations of *Mystus vittatus* (Bloch) of Madhya Pradesh, India

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The random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was applied to analyze the genetic variation of the 2 populations of *Mystus vittatus* (Bloch) of Madhya Pradesh, India. 10 random 10-mer primers were primarily scored in 3 individuals from each of the 2 locations. Five primers, which gave polymorphism, were selected for polymerase chain reaction (PCR) and used in the final RAPD analysis of 20 individuals collected from 2 sites, namely Bhadwada reservoir (Bhopal) and Mohinisagar reservoir (Gwalior). These primers produced 388 scorable DNA fragments were found, of which 252 (64.98%) were polymorphic, 182 (46.90%) were monomorphic and 14 (3.61%) were unique. RAPD banding patterns, showed variations between and within the populations, while, the morphological variations were negligible.

Key words: Catfish, genetic diversity, RAPD, polymorphism.

INTRODUCTION

Biological diversity is the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystem and the ecological complexes. This includes diversity within species, between species and of ecosystems (Kushwaha and Kumar, 1999). In this way, biodiversity includes variety of all forms along with their genetic make up and their all possible assemblages. Species diversity is a property at the population level while the functional diversity concept is more strongly related to ecosystem stability and stressses, physical and chemical factors for determining population dynamics in the lentic ecosystem. Also, the various organisms including the plankton play a significant role in the dynamics of an ecosystem (Kar and Barbhuiya, 2004). India is one of the mega diversity hot spots contributing to the world's biological resources from greater Himalayan range on the northern plain and long stretches of east as well as Western Ghats in the west. The central India including the 3 states of Madhya Pradesh, Chattisgarh and Rajasthan have enormous potential in terms of diverse water resources in the form of streams, rivers, reservoirs, subterranean aquatic systems, traditional lakes and domestic ponds as well as harbors a wide variety of freshwater fishes. Catfishes are commercially important warm water fish species, which are distributed all over the world. As in warm water fish species, the catfish, population/density is decreased significantly owing mainly to imprudent development and reckless fishing during last 2 decades.

RAPD technique is the one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms and has provided important applications in catfish (Bartish et al., 2000). Although DNA fingerprinting offers a great potential in aquaculture and in fisheries as a tool for identification of individuals (Dinesh et al., 1993; Bardakci and Skibinski, 1994; Takagi and Taniguchi, 1995; Dinesh et al., 1996; Meruane et al., 1997; Nei and Li, 1979; Heist and Gold, 1999; Jong-Man, 2001) and population genetic (Hallerman and Beckmann, 1988; D'Amato and Corach, 1996; Bielawski and Pumo, 1997; Smith et al., 1997;

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Mamuris et al., 1998).

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). 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). The genetic diversity of catfishes of Madhya Pradesh is very much limited, so in the present study, this technique was applied to analyze the genetic relationship among *Mystus vittatus* populations. The objectives of this study are focused on morphometric identification and detection of RAPD pattern for determination of the genetic variation among *M. vittatus* populations.

MATERIALS AND METHODS

Fish sampling sites and morphometric measurements of fishes

Geographically, populations of *M. vittatus* were caught from 2 freshwater bodies of Madhya Pradesh about 300 km away from each other, that is, Bhadwada reservoir at Bhopal and Mohinisagar reservoir at Gwalior in the month of December, 2008. A total of 20 fish specimens were collected from both the locations with the help of castnet and local fishermen for morphometric measurements and estimating genetic variations. All the fish specimens were kept in the iceboxes and brought to the MPCST laboratory at Bhopal for further study. For the morphometric measurements, total 15 parameters that is, total length, standard length, body weight, body width, body depth/height, body length, 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 mor-phologically 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.

Genomic DNA extraction

For the isolation of total genomic DNA, a short procedure was applied according to a modified protocol than reported (Wu et al., 1995). Tissues (200 - 500 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, mixed gently and incubated at 55°C on dry bath (Genei, model-SLM-DB-120) for 45 - 60 min for complete lysis of cells. After incubation, chloroform (250 µl) and phenol (250 µl) were 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 2 volume of 100 % chilled ethanol was added, mixed well and centrifuged at 10,000 rpm for 5 min at room temperature. 1 ml of 70 % ethanol was added to the tube for washing. Again, centrifuged 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, 1 mM 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 for PCR amplification.

PCR primers

In this work, 10 commercially available RAPD primers (10 to 20 base long) make Bangalore Genei, India 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 10 primers, only 5 were used in final study. They are RAn-3, RAn-4, RAn-5, RAn-6 and RAn-8 with accession numbers AM765834, AM 750059, AM 7500 52, AM765829 and AM 765833, respectively.

PCR amplification

The reaction mixture (30 µl) for PCR was composed of 3 µl of 10 X 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), 23.4 µl sterile distilled water and 0.6 µl template DNA. A negative control, without template DNA was also 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.

Agarose gel electrophoresis

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 on an UV transilluminator and photographed by gel documentation system (Alpha-Innotech, USA).

Statistical analysis

The RAPD fragments were scored for the presence and absence of fragments on the gel photographs and RAPD fragments were compared among the *M. vittatus* populations. 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). The similarity index (SI) values between the RAPD profiles of any 2 individuals on the same gel were calculated using following formula:

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

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

RESULTS

In the present study 15 classical morphometric characteristics were studied in the 2 populations of *M. vittatus* (Table 1). The morphometric characteristics did not vary much among the populations from Bhadwada reservoir (Bhopal) and Mohinisagar reservoir (Gwalior). However, all values of morphometric characteristics of fishes of Mohinisagar reservoir, Gwalior were found higher than

Table 1. Range of variation, mean and standard deviation of morphometric measurements of *M. vittatus.*

S/N	Measurements	Bhopal (n = 10)			Gwalior (n = 10)		
		Minimum	Maximum	Mean ± SD	Minimum	Maximum	Mean ± SD
1.	Total length	15.30	18.80	16.59 ± 1.064	13	21	18.11 ± 1.999
2.	Standard length	12.10	14.50	13 ± 0.874	11.5	17	14.92 ± 1.477
3.	Body weight, gm	28.02	49.39	37.46 ± 7.350	17.85	75.1	55.74 ± 16.629
4.	Body width	2.00	2.80	2.42 ± 0.214	2	3.5	2.77 ± 0.441
5.	Body depth/height	2.80	3.80	3.38 ± 0.345	2.3	5	3.88 ± 0.715
6.	Head length	2.50	3.00	2.81 ± 0.191	2.5	3.6	3.15 ± 0.287
7.	Head length excluding snout	1.60	2.20	1.88 ± 0.154	1.6	2.4	2.05 ± 0.227
8.	Width of head	1.90	2.50	2.16 ± 0.206	1.5	3	2.47 ± 0.392
9.	Snout length	0.90	1.40	1.11 ± 0.137	0.9	1.3	1.10 ± 0.128
10.	Eye diameter	0.60	0.90	0.72 ± 0.078	0.6	0.9	0.77 ± 0.099
11.	Length of caudal Peduncle	2.00	2.80	2.53 ± 0.330	1.9	2.8	2.40 ± 0.259
12.	Length of anal fin	1.50	2.40	2 ± 0.298	1.5	2.6	2.12 ± 0.295
13.	Length of pelvic fin	2.00	2.40	2.19 ± 0.159	1.8	2.8	2.36 ± 0.280
14.	Length of pectoral fin	1.80	2.50	2.15 ± 0.271	2	2.8	2.30 ± 0.270
15.	Height of caudal peduncle	1.10	1.90	1.34 ± 0.302	1	2.5	2.01 ± 0.320

Note: All values in cm otherwise stated.

Table 2. Pattern of polymorphism and uniqueness (primer wise) in 10 individuals of *M. vittatus.*

S/N	Polymorphism	Bhopal				
		RAn-3	RAn-4	RAn-5	RAn-6	RAn-8
1.	Total No. of bands	30	68	41	51	58
2.	Total No. of polymorphic bands	18	47	12	31	58
3.	Total No. of monomorphic bands	12	20	27	18	24
4.	Total No. of unique bands	0	1	2	2	2
5.	Polymorphism, %	60	69	29.2	60.78	55.17
6.	Monomorphism, %	40	29	65.85	35.29	41.37
7.	Uniqueness, %	0	1.47	4.87	3.92	3.44

the fishes of Bhadwada reservoir, Bhopal except snout length.

DNA from all the 20 samples was extracted using phenol: chloroform: isoamyl method. 10 primers of Bangalore Genei, India (RAn-1 to RAn-10) were employed to initially perform the amplification reaction on the DNA isolated from 3 fishes. Out of these, 5 primers (RAn-3, RAn-4, RAn-5, RAn-6 and RAn-8) generated higher number of bands (Table 2). The RAPD profile of bands obtained in the 2 populations with primer RAn-04, RAn-6 and RAn-8 are shown in the Figures 1 to 3 as representative photographs. These 5 primers generated a total of 388 bands in all the 20 individuals out of which 64.98 % were polymorphic (Table 3 and 4 and Figures 1-3). The UPGMA dendrogram was prepared based on genetic distance indicating the segregation of the *M. vittatus* populations collected from the 2 sites of Madhya Pradesh. The unweighted dendrogram divided all the individuals (except one individual of Bhopal, code no. B-09) in 2 clusters as samples collected from 2 locations

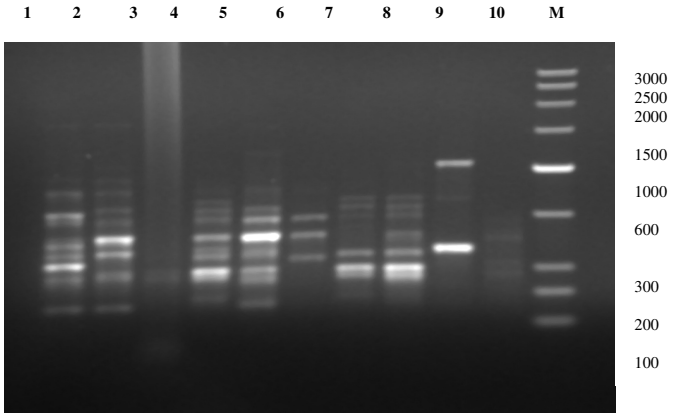

Figure 1. Random amplified polymorphic DNA fragment patterns generated using RAn-6. 1-10 are samples from Bhadwada reservoir Bhopal. M is the molecular marker (bp) of low range DNA ladder.

(Figure 4). The intra-specific genetic similarity between fishes of Gwalior was higher than the fishes of Bhopal.

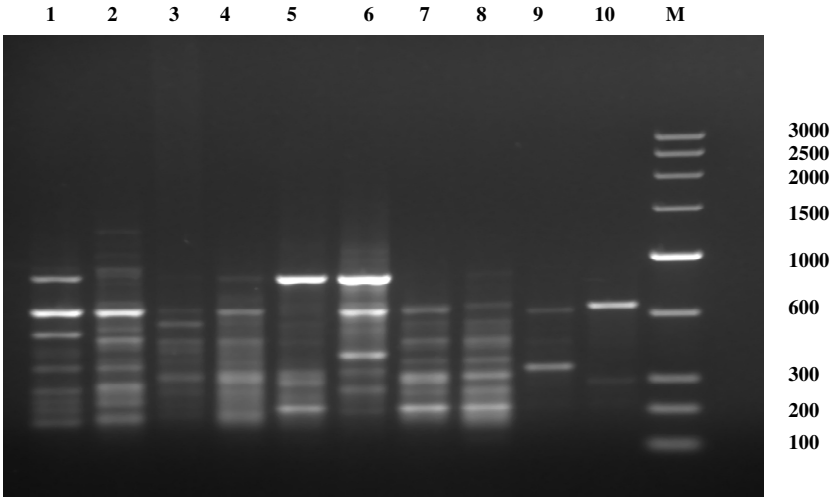

Figure 2. Random amplified polymorphic DNA fragment patterns generated using RAn-4. 1-10 are samples from Bhadwada reservoir, Bhopal. M is the molecular marker (bp) of low range DNA ladder.

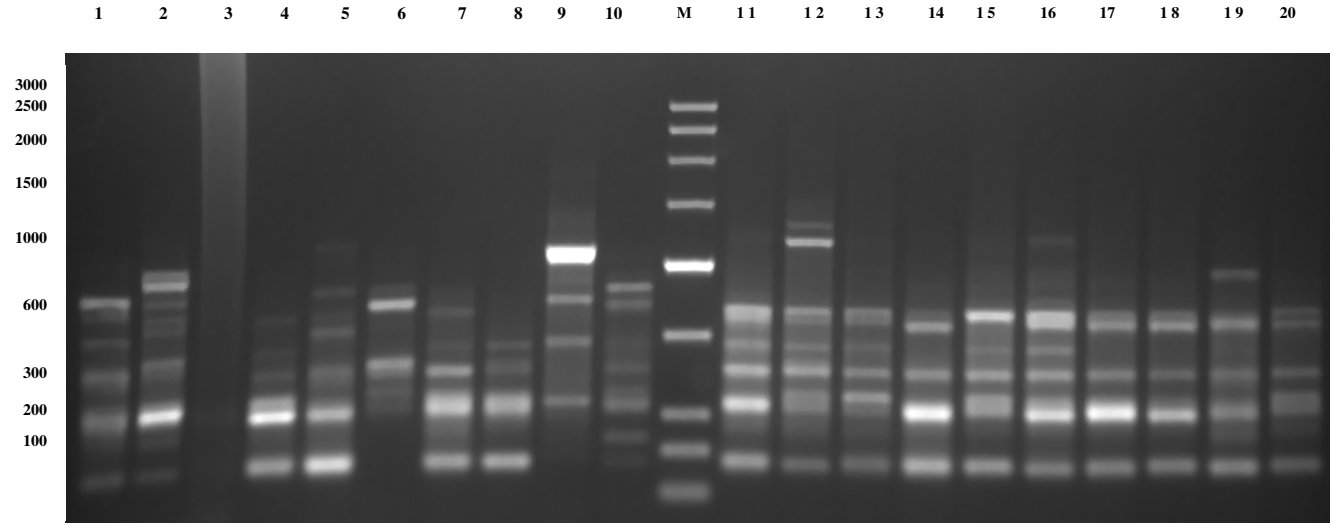

Figure 3. Random amplified polymorphic DNA fragment patterns generated using RAn-8. Lanes 1 - 20 = individuals of Mohinisagar reservoir (Gwalior), M = molecular marker (bp) of low range DNA ladder.

Table 3. Pattern of polymorphism and uniqueness (primer wise) in 10 individuals of *M. vittatus.*

S/N	Polymorphism	Gwalior				
		RAn-3	RAn-4	RAn-5	RAn-6	RAn-8
1.	Total No. of bands	17	29	20	15	59
2.	Total No. of polymorphic bands	10	19	18	13	52
3.	Total No. of monomorphic bands	6	8	2	1	4
4.	Total No. of unique bands	1	2	0	1	3
5.	Polymorphism, %	58.8	65.5	90	86.66	88.13
6.	Monomorphism, %	35.2	27.5	10	6.66	6.17
7.	Uniqueness, %	5.88	6.89	0	6.66	5.08

Table 4. Pattern of polymorphism and uniqueness (4 primers) between 20 individuals of *M. vittatus* collected from Bhopal and Gwalior.

S/N	Pattern of polymorphism	Bhopal	Gwalior	Total Number of bands
1.	Total no of bands	248	140	388
2.	Total no of polymorphic bands	140	112	252
3.	Total no of monomorphic bands	161	21	182
4.	Total no of unique bands	7	7	14
5.	Polymorphism, %	56.45	80	64.98
6.	Monomorphism, %	64.91	15	46.9
7.	Uniqueness, %	2.82	5	3.61

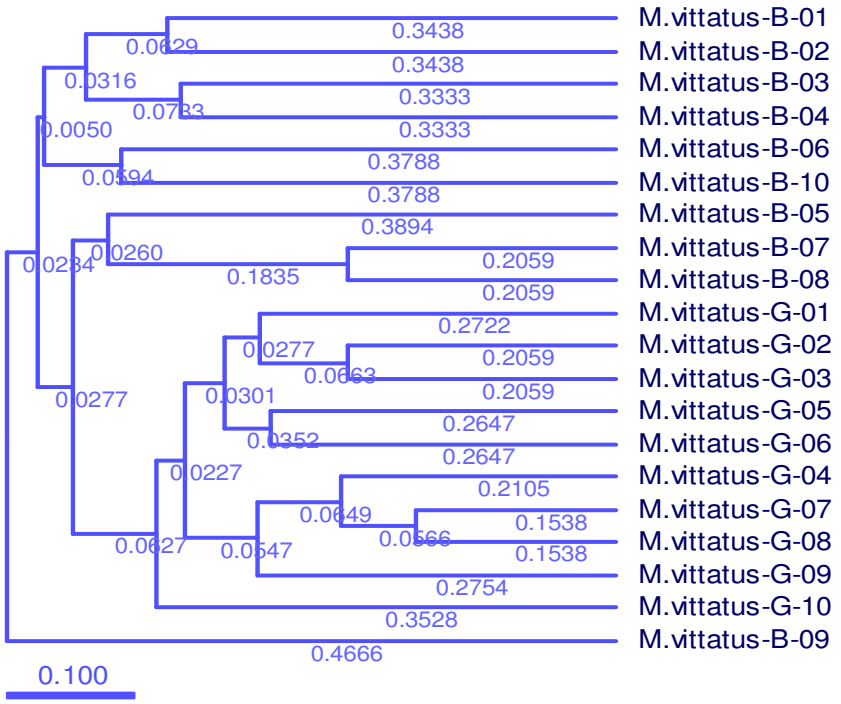

Figure 4. Phylogenetic tree constructed by similarity coefficient (Jaccard's).

DISCUSSION

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 the morphometric characteristics of fishes were similar and often over-lapped 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 mophometric 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.

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 genetic studies. RAPD technique has been applied to the study of phylogentic relationship in tilapia 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 4 different populations of Hilsa Shad from Ganga, Yamuna, Hoogly and Narmada rivers of India using RAPD technique (Brahmane et al., 2006). 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 catfishes are commercially important fishery species in central India. The genetic stock of *M. vittatus* has not been studied in the reservoirs of Madhya Pradesh. In the present investigation, RAPD analysis has been used to discriminate between the 2 different populations of *M. vittatus* of Madhya Pradesh. RAPD fragments observed in the 20 individuals, showed a reasonable degree of genetic diversity within and between the populations. The percentages of polymorphism, monomorphism and uniqueness were 64.98, 46.90 and 3.61, respectively in all the bands obtained from the 5 primers. The population specific bands could not be discerned from the fragment patterns generated.

The populations of *M. vittatus* from Mohinisagar reservoir Gwalior in one cluster while rests of the individuals of Bhadwada reservoir, Bhopal were in another cluster except an individual (*M. vittatus*-B-09). This observation clearly indicated that, both the populations of *M. vittatus* have separate gene pool. The intra-specific genetic similarity between individuals of Mohinisagar reservoir was higher than the individuals of Bhadwada which may be due to geological variations or changes in aquatic environments. All values of morphometric parameters of individuals of Mohinisagar reservoir were higher than the fishes of Bhadwada reservoir. These morphometric observations also indicated that, the aquatic environment of Mohinisagar reservoir may be more favorable than the Bhadwada reservoir of Bhopal.

Conclusion

Once the population structure is known, scientific management for optimal harvest and conservation of the catfish fishery resource can be undertaken. This method of DNA fingerprinting is important since it is relatively easy to obtain valuable data. It allows for a more introspective interpretation of diversity within a population. 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.

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