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Full Length Research Paper

Genetic structure of populations of *Mugil cephalus* using RAPD markers

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Genetic structure of four populations of *Mugil cephalus* from Gujarat, Maharashtra, Andhra Pradesh and Tamil Nadu in India was studied using randomly amplified polymorphic DNA (RAPD) markers. Five selective primers provided distinct and consistent RAPD profiles in all the four populations. The bands in the range 400 to 1200 bp were scored for consistent results. The RAPD profiles generated by all the five primers revealed varying degrees of polymorphism, ranging from 50.76 (primer E03) to 72.41% (primer E05). Nei's genetic diversity (h) among the four populations varied from 0.3717 ± 0.1460 (Gujarat population) to 0.5316 ± 0.1720 (Maharashtra population). Nie's highest genetic distance (0.8556) was observed between Tamil Nadu and Gujarat populations.

Key words: *Mugil cephalus*, randomly amplified polymorphic DNA (RAPD), genetic structure, India.

INTRODUCTION

Information on the genetic structure of fish is useful for optimizing identification of stocks, stock enhancement, breeding programs, management of sustainable yield and preservation genetic diversity (Dinesh et al., 1993; Gracia and Benzie, 1995; Tassanakajon et al., 1997, 1998). DNA polymorphisms have been extensively employed as a means of assessing genetic diversity in aquatic organisms (Ali et al., 2004). Randomly amplified polymorphic DNA (RAPD) fingerprinting offers a rapid and efficient method for generating a new series of DNA markers in fishes (Foo et al., 1995).

RAPD analysis is a technique based on the polymerase chain reaction (PCR) amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence (Welsh and McClelland, 1990; Williams et al., 1990). This method is simple and quick to perform and most importantly, no prior knowledge of the genetic make-up of the organism is required (Hadrys et al., 1992). This technique has been used extensively to detect genetic diversity in plants (Williams et al., 1993), animals (Cushwa and Medrano, 1996) and microbes (Carretto and Marone, 1995). It has also been used to evaluate genetic diversity in various fish species such as tilapia (Naish et al., 1995), striped bass (Bielawski and Pumo, 1997), grouper (Asensio et al., 2002) and murrel (Nagarajan et al., 2006). *Clarias batrachus* (Garg et al., 2010), *Eutropiichthys vacha* (Chandra et al., 2010) and *Plecropomus maculates* respectively. The striped mullet, *Mugil cephalus* is the most widely distributed and of aquaculture importance among mullets. It is euryhaline and also fairly resistant to changing temperature (Chondar, 1999). This species is one of the most popular warm water fishes being cultured in tropical and sub-tropical regions (Pillai et al., 1984). Indian aquaculture is

Abbreviations: RAPD, Random amplified polymorphic DNA; **PCR,** polymerase chain reaction; **EDTA,** ethylenediaminetetra acetate; **SDS,** sodium dodecyl sulfate.

Table 1. *Mugil cephalus* RAPD profiles obtained by five random primers.

Primer	Number of band	Band size (bp)	Total DNA band	Polymorphic DNA band	Percentage polymorphic DNA band
E02 (5'GGTGCGGGAA3')	1 - 4	512 - 930	38	22	57.89
E03 (5'CCAGATGCAC3')	3 - 6	548 - 1200	65	33	50.76
E04 (5'GTGACATGCC3')	1 - 3	416 - 822	60	39	65.00
E05 (5'TCAGGGAGGT3')	1 - 3	746 - 884	29	21	72.41
E06 (5'AAGGCCCCTC3')	1 - 4	522 - 1196	40	27	67.50

mainly restricted to carps and shrimps. To achieve higher aquaculture production, species diversification must be prioritized. *M. cephalus* is one of the candidate species for diversification in the aquaculture sector due to its euryhaline nature easy availability of seeds along the coasts. Therefore, studying genetic variation in *M. cephalus* could provide base line data for identifying stock with superior traits for breeding programs and also to formulate management strategies for sustainable utilization of the species. Despite its aquaculture importance, there is no information available on genetic structure of this species. Hence, the present study was carried out to ascertain the genetic stock structure of *M. cephalus* populations using versatile RAPD markers.

MATERIALS AND METHODS

Experimental animal

Specimens *M. cephalus* (n=200; 50 from each location) were collected from four different locations in India: Navsari, Gujarat (20.5800°N, 72.5500°E); Ratnagiri, Maharashtra (16.9800°N, 73.3000°E); Kakinada, Andhra Pradesh (16.9300°N, 82.2230°E) and Chennai, Tamil Nadu (13.0810°N, 80.2740°E). The muscle tissue was collected and preserved in 95% ethanol, and transported in ice to the lab. The samples were stored at -20°C until DNA was extracted.

Extraction of genomic DNA

Total genomic DNA was isolated from muscle tissue according to DNA extraction method of Williams et al. (1990). Tissue (150 to 200 mg) was cut into smaller pieces in the presence of 1 ml lysis buffer (50 mM Tris-HCl, pH 8.0, 10 mM ethylenediaminetetra acetate (EDTA), 100 mM NaCl) and transferred to a 2 ml Eppendorf tube. Then, proteinase K (300 µg/ml), sucrose (1%), and sodium dodecyl sulfate (SDS) (2%) were added to the tube. After incubation at 60°C, the lysate was extracted with phenol and chloroform/isoamyl alcohol. The DNA was precipitated with isopropanol and pellet was washed with 70% ethyl alcohol, dried, suspended in TE buffer (50 mM Tris-HCl, 10 mM EDTA). DNA quality and quantity were determined by 1.0% agarose gel electrophoresis and biophotometer (Eppendorf, Germany).

RAPD-PCR amplification and product analysis

Five random primers (E02 to E06; Operon, USA) were screened based on the presence of intense, well distinguished and reproducible bands for further analysis. PCR reactions were performed in 25 µl volume containing 200 µmol/l each dNTP, 2 mmol/l $MgCl_2$, 1 x standard *Taq* polymerase buffer, 0.2 µmol/l random primer, 40 ng genomic DNA, and 0.75 U *Taq* polymerase. PCR reactions were carried out with initial denaturation of 4 min at 94°C, followed by 35 cycles of denaturation for 30 s at 94°C, 45 s annealing at 36°C, 2 min extension at 72°C, and one 8 min cycle at 72°C for final extension. Amplified products were separated on 1.5% agarose gel stained with ethidium bromide, run in 1 X TAE buffer at a constant 80 V (Sambrook and Russell 2001). The gels were imaged using a Syngene gel documentation system (USA).

Data analysis

Only the reproducible and intense bands ranging from 400 to 1200 bp were scored to maintain the consistency across the samples of different populations. Bands observed in each lane were compared with all the other lanes of the same gel and reproducible bands were scored as present (1) or absent (0). Fragment sizes were estimated based on the 100 bp Plus DNA Ladder (Bangalore Genie, India) according to the algorithm provided in the Gene Tools Software. Data was analyzed using the POPGENE version 1.31 software (Yeh et al., 1999). It was also used to construct dendrograms based on genetic distances (Nei, 1972; Sneath and Sokal, 1973; Reynolds et al., 1983). The robustness of the dendrogram was tested using 1000 bootstraps.

RESULTS AND DISCUSSION

The RAPD profiles of different populations from Navsari (Gujarat), Ratnagiri (Maharashtra), Kakinada (Andhra Pradesh) and Chennai (Tamil Nadu) were generated for four geographically different populations of *M. cephalus*. The RAPD fingerprints of a total of 200 individuals of *M. cephalus* were carried out using optimized RAPD-PCR conditions for five selected primers. The polymorphism pattern obtained for four populations is shown in Table 1.

All the selected five primers produced distinct and consistent RAPD profiles for *M. cephalus* from all the four populations (Figures 1 and 2). The primers generated bands in the range of 200 to 2,200 bp. However, only the repeatable major bands ranging from 400 to 1200 bp were scored for consistency. A total of 142 reproducible bands were obtained in the three populations for the five primers (Table 1). Generally, the number and size of the bands generated strictly depend upon the nucleotide sequence of the primer used and the source of the template DNA; resulting in the genome-specific finger-prints of random DNA bands (Welsh et al., 1991).

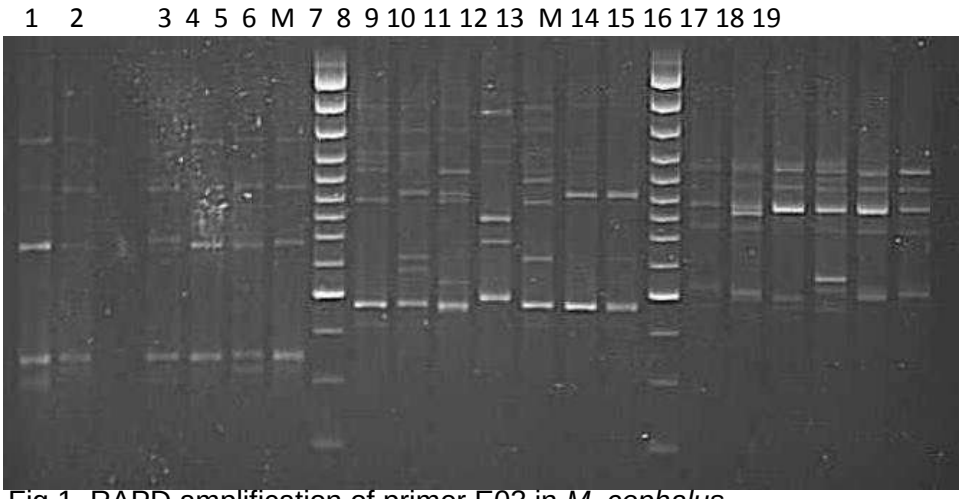

Fig.1. RAPD amplification of primer E03 in *M. cephalus*

Figure 1. RAPD amplification of primer E03 in *M. cephalus* . Lanes 1-6, Samples from Gujarat; Lane 1- 3 samples from Gujarat, 7- 13 Maharashtra and 14-19 Andhra lanes 7-13, Maharashtra; lanes 14-19, Andhra Pradesh; M, molecular maker (100 bp plus Pradesh;DNAladder)M. – Molecular maker (100 bp plus DNA ladder)

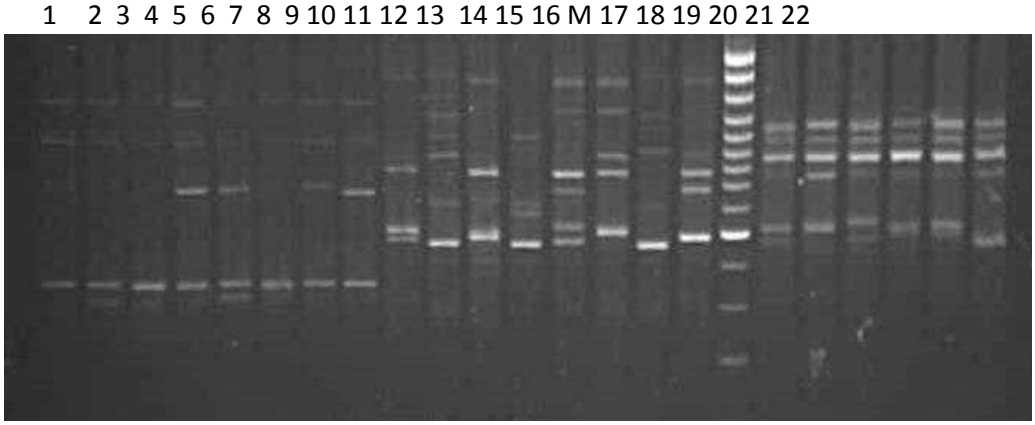

Fig.2. RAPD amplification of primer E04 in *M. cephalus*

Figure 2. RAPD amplification of primer E04 in *M. cephalus.* Lane 1-8 samples from Gujarat; lanes 9-16, Maharashtra; lanes 17-22, Tamil Nadu; M, molecular maker (100 bp plus DNA ladder). Lane 1-8 samples from Gujarat, 9- 16 Maharashtra and 17-22 Tamil Nadu M – Molecular maker (100 bp plus DNA ladder)

Table 2. Genetic diversity within four populations of *Mugil cephalus*.

Population	Polymorphic loci (%)	Average genetic diversity
Gujarat	88	0.3717 ± 0.1460
Maharashtra	76	0.5316 ± 0.1720
Andhra Pradesh	83	0.4419 ± 0. 2112
Tamil Nadu	70	0.4012 ± 0.1310

The RAPD profiles generated by all the five primers revealed varying degrees of polymorphism, ranging from 50.76% (primer E03) to 72.00% (primer E05). The range of number of bands and band size were 1 - 6 and 416 - 1196 bp, respectively.

The present study revealed a wide variation of polymorphic loci (70 - 88%) among the four populations. The highest level of polymorphism (88%) was exhibited by the Gujarat population whereas the lowest level of polymorphism (70%) was exhibited by the Tamil Nadu population. Nei's (1973) genetic diversity (h) among the four populations varied from 0.3717 ± 0.1460 (Gujarat population) to 0.5316 ± 0.1780 (Maharashtra population) (Table 2). Interestingly, two population specific bands

Table 3. Nie's genetic identity (above diagonal) and distance (below diagonal).

Population	Gujarat	Maharashtra	Andhra Pradesh	Tamil Nadu
Gujarat	***	0.9214	0.1959	0.1544
Maharashtra	0.0886	***	0.2086	0.1974
Andhra Pradesh	0.8241	0.8014	***	0.9010
Tamil Nadu	0.8556	0.8126	0.1091	***

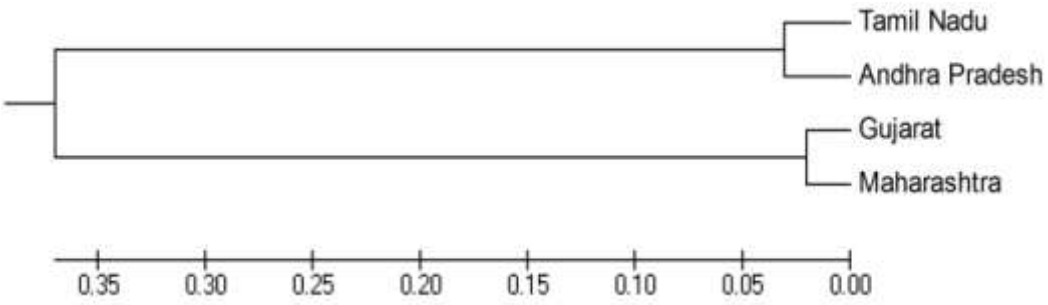

Figure 3. UPGMA dendrogram using Nei's unbiased genetic distance.

were found in the population of Andhra Pradesh (350 bp in E06 primer) and Gujarat (1000 bp in E04 primer). These population-specific unique bands can be used to detect any possible mixing of these populations, especially during selective breeding programmes (Ferguson et al., 1995). Tassanakajon et al. (1998), Mishra et al. (2009), Nagarajan et al. (2006) and Lakra et al. (2010) and Saad et al. (2012) also observed population specific bands in *Penaeus monodon Metapenaeus dobsonii Chaanna punctatus* and *Monoporeia affinis*, *Plectropomus leopardus* respectively.

Estimates of Nei's (1978) genetic distance demonstrated sufficient genetic divergence to discriminate the samples of different populations of *M. cephalus* (Table 3). The highest genetic identity (0.9214) and genetic distance (0.8556) was observed between the populations of Gujarat and Maharashtra and Tamil Nadu and Gujarat, respectively. A dendrogram based on Nei's genetic distance is shown in Figure 3. Two separate clades were identified on the dendrogram with the Maharashtra and Gujarat populations appearing one cluster, while the Tamil Nadu and Andhra Pradesh populations formed the other clade.

In conclusion, genetic stock structure of *M. cephalus* identified in this study using RAPD primers will be helpful in developing superior strain for aquaculture practices through selective breeding and formulating stock specific management measures for conservation and sustainable utilization of the species.

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