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Genotypic Analysis of Etroplus Maculatus in Western Ghat Rivers of India using Random Amplification of Polymorphic DNA (RAPD) Analysis

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ABSTRACT

Genotypic Analysis is used to profile the genetic variation among different populations of fishes. The freshwater fish, Etroplus maculatus was used in this study to analyze the genetic variation in different population. The Etroplus maculatus fishes were collected from four different locations of southern Western Ghats, India and the fishes from different location were designated as different population. Random Amplification of Polymorphic DNA (RAPD) analysis was used to study the genetic variation among different populations with the help of universal primers. The results displayed that the morphometric characters of population from Eraumanthurai showed little variation when compared with other population. The genomic size of the different population of E. maculatus found between 3.45 x10-6 to 3.10 x10-6 pg/mg. The result of RAPD analysis showed that the population from Eraumanthurai had eight distinct fragments. The study inferred that the population from Eraumanthurai appeared to be unique among the other population of E. maculatus. This study will give an evidence of evolutionary relationship between different populations of E. maculatus

Keywords: *Etroplus maculatus, genetic diversity, RAPD, Western Ghats, Genotypic analysis.*

INTRODUCTION

Life originated in an almost endless variety of enthralling and charming forms, from microscopically small unicellular species to giant whales and elephants. In fact, species were formed from different kinds of individuals and these by different types of organs, tissues, cells and genes. These may the main causes of genetic variation. So the number of individuals of a species varies extremely both in time and from species to species (Solbring et al., 1992).

Any differences in the nucleotides, genes, chromosomes or whole genomes of organisms are referred to as Genetic diversity. It is a state of biodiversity that refers to the entire number of genetic characteristics in the genetic makeup of a species. It is commonly considered to be vital for the existence of natural populations, because it permits them to keep high levels of fitness and acclimate to altering environmental conditions (Frankel and Soule, 1981).

The Western Ghats of India, one of the well-known biodiversity hotspots of the world, ports 289 species of fresh water fish, of which 119 are endemic (Kottelat, 1993). Among the aquatic organisms, fishes are the best acknowledged group that exist at or near the top of the food chain and that can be used as an indicator of a balanced ecosystem (Karr et al., 1986). Among the freshwater fishes, *Etroplus* is one of the lowest genus in the Cichlidae family and their dissemination ranges from South India to Sri Lanka. There are three valid species under the genus. They are *E. Suratensis, E. maculatus* and *E. canarensis*. Among the species *E. maculatus* is unveiling great difference in morphometric characters, body colour and banding patterns.

Fishing and aquaculture activities posed potential threats to genetic diversity of wild fish

populations in different way (Selandar *et al*., 1973). The conservation of aquatic genetic diversity has yet to receive the attention it deserves (Najiah and Gade, 2003). Conservation programs helped the fish population to be more sustainable while at the same time sustaining diversity. Diversity decreases the disease problems and encourages the retrieval from disruption (Najiah and Gade, 2003).

The information on biodiversity and conservation of genetic resources has strengthened during the last few decades. In the advent of modern molecular techniques like DNA fingerprinting, Restriction Fragment Length Polymorphism and Random Amplified Polymorphic DNA, which provided the means for measurable screening of genetic variation (DeLong *et al*., 1989; Giovannoni and Cary, 1993). Investigation of genetic variation by electrophoresis of the primary gene products (proteins) provided a powerful tool for the population discrimination and identification (Ferguson, 1980; Shaklee and Bentzen, 1998). Random Amplified Polymorphic DNA (RAPD) polymorphisms (Welsh and McClelland 1990; Williams *et al*., 1990) are relatively easy to generate and are increasingly used for population genetic studies in marine fishes (Dahle *et al*., 1997; Bielawski and Pumo, 1997; Mamuris *et al*., 1998).

India is one of the `mega-diversity' countries with richest storehouse for genetic resources from all organisms. The information on genetic resource of Indian fish fauna is very limited. To develop an effective protocol for the conservation and rational utilization of genetic resources the quantification of organism at different taxonomic as well as genetic level is essential. Current nomenclature and status of many species is solely based on meristic and morphometric characters often that lead to taxonomic uncertainties at various taxonomic levels (Daniels, 1997). Hence, the present study aims to document the genetic variation of *Etroplus maculatus* found in the Western Ghats drainage systems between different parts of Kanyakumari district in Tamil Nadu, India

MATERIALS AND METHODS

Fish samples were collected from four different locations such as Eraumanthurai, Attoor, Suseendram and Pachiparai. The Eraumanthurai and Attoor were present in east flowing river of Tamiraparani. The Suseendram and Pachiparai were present in Pazhayar river basin of southern Western Ghats. The sampling was performed by using mono filamentous gillnet and multi filamentous cast net. A portion of gill and muscle tissues were fixed in isopropyl alcohol and they were kept in the ice cubes for further laboratory analysis. Few individuals were also fixed in formaldehyde for further morphometric analysis. The morphometric measurements were done by following the method of Hubbes and Laggler (1958).

The genomic DNA was isolated by phenol-chloroform method based on Sambrook et al. (1989). Amount of DNA present in each sample were determined by using UV-spectrophotometer. Isolated DNA samples were subjected to RAPD using two universal primers (forward CTC CCC AGA C reverse AGA ACC GAG G, forward CAC TTC CGC T reverse GTG ACG TAG G). The PCR was done by using PCR kit (Cat No: 616104400011730) The PCR reaction was carried out in the thermal cycler (PC-320), programmed for an initial denaturation of one minute at 94^{o}C followed by 40 cycles each consisting of 30s at 94^{o}C (denaturation), one minute at 36^{o}C (annealing) and 120s at 72^{o}C (extension). A final extension was carried out at 72^{o}C for two minutes. The PCR products were separated in 0.8% agarose gels containing 1x TBE buffer at 100V for about 3 h. After electrophoresis gel was placed in the gel document unit and bands were visualized and they were photographed using NIKON digital camera. The separated DNA fragment in each lane was viewed and the presence/ absence matrix of DNA fragments in each lane corresponding to each study site/sample was analyzed.

Table 1. *Morphometric Characteristics of Etroplus maculatus from different locations*

Characters	Attoor (n=10)		Eraumanthurai(n=10)		Pachiparai (n=9)		Suseendrum (n=9)	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
% of Standard Length								
Body width	6.02-7.14	06.73±0.395	05.81-06.89	6.36±0.38	6.16-7.02	6.57±0.29	6.25-6.97	6.59±0.23
Body Depth	20.23-21.08	20.55±0.298	20.35-21.55	20.91±0.42	17.12-19.29	18.27±0.90	19.12-20.54	19.87±0.63
Head Length	12.65-13.09	12.94±0.146	13.79-14.53	14.01±0.29	13.16-13.50	13.35±0.13	12.74-13.01	12.89±0.10
Length of Caudal peduncle	5.37-7.38	06.45±0.841	5.17-7.56	6.60±0.84	5.26-6.16	5.60±0.29	5.36-6.37	5.95±0.44
Predorsal length	18.07-19.05	18.69±0.450	17.24-19.19	18.67±0.72	17.37-18.30	17.78±0.32	18.13-19.12	18.62±0.42
Length of pelvic fin	3.01-3.57	03.33±0.162	2.72-3.74	3.26±0.43	3.42-3.51	3.45±0.05	2.68-3.18	2.91±0.20
Length of anal fin	21.08-22.18	21.65±0.508	22.09-24.14	23.59±0.68	21.05-24.66	22.90±1.26	23.21-23.57	23.48±0.12
Length of Pectoral fin	9.04-10.71	09.74±0.648	8.72-9.48	9.12±0.23	8.22-8.77	8.57±0.19	9.55-9.82	9.67±0.09
% of Head Length								
Eye diameter	18.75-20.00	19.36±0.424	15.79-18.75	17.25±1.19	15.53-17.39	16.33±0.68	17.64-18.52	18.17±0.32
Snout length	25.00-30.00	27.89±1.596	28.13-31.58	29.81±1.08	26.09-26.32	26.16±0.07	23.53-25.93	24.87±0.88
Inter orbit width	50.00-56.25	54.029±2.096	52.63-53.13	52.91±0.17	56.52-58.16	57.35±0.68	55.56-58.82	57.27±1.26

RESULTS

In the present study eleven classical morphometric characters were studied in different population of *E. maculatus* (Table 1). The morphometric characters did not vary much among the population from Pazhayar River basin to Tamiraparani river basin; however, the population from Eraumanthurai had significant difference in some morphometric characters. It distinguished from Pachiparai and Suseendrum population in body depth (20.91 vs. 18.27 and 19.87 in % of standard length), head length (14.01 vs. 13.35 and 12.89 in % of standard length), pectoral fin length (9.12 vs. 8.57 and 9.67 in % of standard length), Predorsal length (18.67 vs. 17.78 and 18.62), length of caudal peduncle (6.6 vs. 5.6 and 5.95) and pelvic fin (3.26 vs. 3.45 and 2.91 in % of standard length). It also differed from Attor population in Body width (6.36 vs. 6.73 in % of standard length), Head length (14.01 vs. 12.94 in % of standard length), Length of pectoral fin (9.12 vs. 9.74) and length of anal fin (23.59 vs. 21.65 in % of standard length).

Phenotypically it exhibited variation in body colour patterns (sample from Pazhayar river basin i.e Pachiparai and Suseendrum are dark orange to light orange in colour whereas sample from Tamiraparani river basin i.e Attor and Eraumanthurai are dark yellow to pale yellow in colour). Moreover, it had variation in shape and size of body blotch. Among the sample from Tamiraparani river basin a black oval solid blotch seen in Attor whereas more than three black oval solid blotch seen in Eraumanthurai. Among the samples from Pazhayar river basin, more than four black oval solid blotch seen in Pachiparai whereas more than two black oval solid blotch seen in Suseendrum (Figure 1).

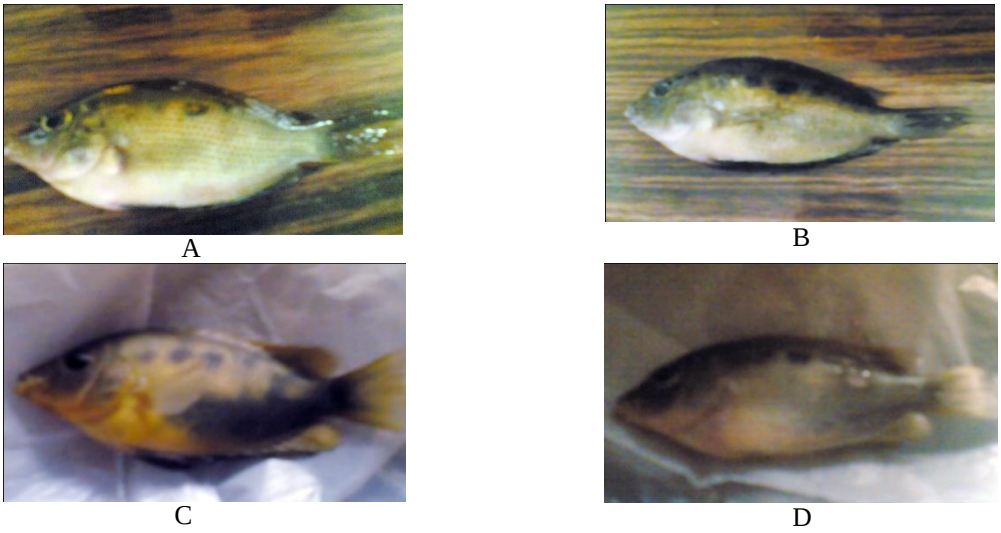

Figure1. *Etroplus maculatus from different locations A: Attor, B: Eraumanthurai, C: Pachiparai, D: Suseendrum*

The DNA content of each population and the corresponding OD values were given in Table 2. The genomic size of *Etroplus maculatus* ranged from 4.30 $x10^{-6}$ to 3.10 $x10^{-6}$ pg/mg. The genomic size of the Eraumanthurai population showed higher rate (4.30 $x10^{-6}$ pg/mg). The result of RAPD analysis (Figure 2) showed that there was a unique DNA banding patterns in different population and the fragment migration were ranged from 2176 to 154 bp. The number of fragments of electrophorogram of *E. maculatus* was given in table 3. The Eraumanthurai population has eight bands and that population is unique among the other populations.

Table2. *Genomic size of Etroplus maculatus from different locations*

Population	OD Value	DNA content (pg/mg)
Attor	0.062	3.10 $x10^{-6}$
Eramanthurai	0.069	4.30 $x10^{-6}$
Pachiparai	0.086	3.45 $x10^{-6}$
Suseendrum	0.070	3.50 $x10^{-6}$

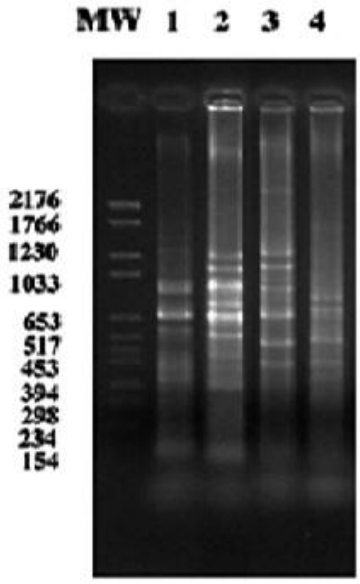

Figure2. *RAPD Pattern of E. maculatus collected from different regionsLane M: Marker, Lane 1: Attor, Lane 2: Eraumanthurai, Lane 3: Pachiparai, Lane 4: Suseendrum*

Table3. *Number of fragments in the electrophorogram of E. maculatus*

Population	Number of Fragments
Attor	4
Eraumanthurai	8
Pachiparai	6
Suseendrum	5

DISCUSSION

Genetic diversity is the least studied component of biodiversity and its assessment is partial because, the availability of techniques is ineffective to measure it. Little is known about the pattern of genetic variability in most species and it is known only in a few selected examples and in a small number of domesticated species (Okumu and Çiftci, 2003). In the recent years, interest in the conservation of aquatic organisms has increased due to the degradation of aquatic environments. The present study evaluated the morphometric characters and patterns of genetic variation in four populations of *E. maculatus*.

Most of the morphometric characters of fishes are similar and often overlap within the population. But the population from Eraumanthurai showed little variation in the morphometric characteristics. This morph-ometric data are not enough to support the recognized genetic structure of the population often that leads to taxonomic uncertainty (Daniel, 1997; Ponniah and Gopalakrishnan, 2000; Garg *et al*., 2009b). The investigation was further prolonged to analyze the genetic variation.

The genomic size of *E. maculatus* from different location is ranged from 4.30 $x10^{-6}$ to 3.10 $x10^{-6}$ pg/mg and that was not much varied between different populations. But the population from Eraumanthurai showed higher genomic size (4.30 $x10^{-6}$ pg/mg) than the other population. In cyprinids, the genomic size varies between 1.6 and 4.4 ng/mg (Buth *et al*., 1991; Gold *et al*., 1992). In general, intra-population genome size is very small and it is also not much in related species (Fontana, 1976). The genomic size is essential for at least three reasons. First, it provides some valuable clue regarding genome evolution. Secondly, genome size can be correlated to some quantitative characteristics such as cell volume. Thirdly, during molecular genetic study it is used in calculation of number of copies of gene present in genome of species (Dolittle and Sapienzi, 1980; Orgel and Crick, 1980). The isolation of high quality DNA is essential for many molecular biology applications using polymerase chain reaction (Chakraborty *et al*., 2008). This systematics realm of methods provides new suites of characters for analyzing the relationship among the fishes (Hillis, *et al*., 1996; Carvalho and Pitcher, 1995).

Genetic approaches offer powerful tools for examining the current status of populations, for understanding the population changes for its conservation (Belfiore and Anderson, 2001). RAPD technique is one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms (Garg *et al*., 2009a; 2009b). Using a RAPD analysis, the intra-population variation was detected with different primers in tilapia (Bardakci and Skibinski, 1994). This technique was more sensitive than the mtDNA analysis which failed to reveal the variation within the tilapia populations (Seyoum and Kornfield, 1992). The RAPD method was successfully used to detect the variation between the different species of fish (Bardakci and Skibinski, 1994) and separation of mollusc species (Crossland *et al*., 1993). Moreover, Dahle *et al*. (1997) found the genetic variation between the three different populations of *Hilsa shad* from Bangladesh waters using the RAPD technique. In the present study, a RAPD analysis discriminated between the four different populations of *E. maculatus* collected from Western Ghats of India.

Among the population, sample from Eraumanthurai River was genetically different among the other population and stands apart largely owing to high genetic diversity. Moreover, it also exhibits phenotypic characters such as body colour pattern and shape of blotch at the caudal peduncle (Figure 1). These variations in fragment length may be due to mutation. The existence of many closely related haplotypes that are only partially and geographically localized has been associated with species or subset of species with historically intermediate levels of gene flow between geographic populations (Avise *et al*., 1987). In this scenario, ancestral haplotypes may be dispersed over a wide area whereas more recent mutation is conformed to specific areas (Bermingham and Avise, 1986).

The differentiation among samples from separate region is consistent with previous findings for fish species using protein electrophoresis (Gyllelnsten, 1985; Shakleen and Kannon, 1986) and DNA fingerprinting (DeLong *et al*., 1989; Giovannoni and Cary, 1993). In this present study, it was inferred that genetic variation has marked distinction and the genetic structure of the population of *E. maculatus* from Eraumanthurai River appears to be unique among population of other region. For conserving such unique genetic makeup, the combination of our understanding of how ecology and habitat specialization relates to genetic variation should be of value in designing and management of rare germplasm. The present structure of genetic diversity is the invisible dimension of biological diversity, being the result of the evolutionary history of the species exposed to natural selection pressures in variable environmental conditions. Natural selection at the local level is an evolutionary force opposed to gene flow. The combination of the two forces creates a powerful mechanism for maintaining within-species diversity (Edward *et al.*, 2002)

CONCLUSIONS

The present investigation revealed the morphometric and genetic variation in four populations of *E. maculatus*. The results of morphological approach revealed that there is no much difference among the population from Pazhayar River basin to Tamiraparani river basin; however, the population from Eraumanthurai had significant difference in some morphometric characters. The RAPD pattern exhibits distinct variation among the four populations of *E. maculatus*. The present investigation contributes to the knowledge on morphological and genetic variation to the *Etroplus* species. However, much specific molecular biomarkers are required for understanding the taxonomical relations of many other species of this group, which are widely distributed in various fresh water basins of India.

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