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GENETIC DIVERSITY DETERMINATION IN AQUARIUM BRED FRESH WATER ORNAMENTAL FISH VARIETY (*POECILIA* SPECIES) USING RAPD MOLECULAR MARKERS

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Abstract: Successful conservation of fish such as *Poecilia species* depend on analysis of genetic signature and relations among fish species and subspecies. Knowledge about the genetic signature and structure of *Poecilia species* needs to increase several times to enable us to conserve these economic fish in the future. Many factors have contributed to the destruction of fish habitats. Hydroelectric dams, water pollution and other environmental changes have resulted in the eradication of natural stocks. In the present study ten species of Mollies were collected from aquariums in Bangalore, the genomic DNA was extracted from fin tissues using standard protocol by phenol – chloroform method and using ten decamer oligo-nucleotides as single primers in Polymerase Chain Reaction (PCR), DNA fingerprinting, dendrogram analysis and genetic similarity matrix were estimated, revealing variations between selected ten species of *Poecilia*. From the analyzed species three groups that were found to be 100% similar are first group of sample 1 and 2, second group of sample 3, 4, and 6 and third group of sample 7 and 8. In past few decades, molecular markers have evolved themselves and have totally changed our view of nature. In last ten years, advance technologies has supported greatly in determination of population diversity. The described approach holds great promise for further analyses and gives support to biodiversity maintenance as well as for conservation of genetic resources.

Key words: Molly, *Poecilia,* Ornamental fish, Genetic diversity

INTRODUCTION

Mollies (*Poecilia*) are one of the most common aquarium fishes of freshwater origin. Because of their growth and prolific breeding nature, they are also considered one of the most important ornamental fish. *Poecilia* is native to brackish waters of North America and widely distributed and capable of tolerating a wide range of water conditions (Al-Akel, 2010). Though they prefer a little salt with their water, they can live in fresh water or salt water aquariums. *Poecilia sphenops* is a species of fish, of the genus *Poecilia*, known under the common name molly. Molly fish can be found normally where fresh water meets salt water in various regions of the world. The black molly, *Poecilia sphenops* is a common aquarium fish native to Mexico and Central America which often interbreeds with the sailfin molly (Shipp 1986). The guppy *Poecilia reticulala* and neon tetra *Paracheirodon innesi* are the most popular ornamental fish (Frank, 2007). The report shows that the ornamental fish trade is growing from past two decades and still expanding with the global import value of ornamental fishes goes up to million dollars (Dawes, 2001).

Although *Poecilia* has been widely introduced, there is no information on the reproductive biology of the introduced populations (e.g., sex ratio, size at maturity, fecundity) and little is known even about native populations. This information is important because poeciliids usually exhibit great phenotypic plasticity and the environment has a big influence on its phenotype (Reznick and Miles, 1989; Trexler, 1989). According to molecular studies, *P. latipinna* and *P. mexicana* are the parental species involved in the hybridization event that gave rise to *P. formosa (*Avise, 1991; Schart, 1995). It has been introduced to many countries around the world via the aquarium fish trade and also as a biological control agent for insects, especially mosquitoes (Courtenay and Meffe, 1989; Lever, 1996). In past few decades, molecular markers have evolved and advance

technologies has supported greatly in determination of population diversity. As the random amplified polymorphic DNA (RAPD) technique uses universal sets of primers, and no preliminary work such as nucleotide sequencing, filter preparation or probe isolation is necessary, it has an advantage over other systems of genetic documentation (Bahy, 2004). In this study, in order to detect the genetic variability of *Poecilia* fish species, some genetic markers were analyzed, through the use of RAPD. The results were extremely useful, not only to characterize these fish species but also to give support to the biodiversity maintenance of this species.

MATERIALS AND METHODS

Sample Collection

Total ten species of Molly fish (*Poecilia*) family Poeciliidae were collected from local pet aquarium shops, Bangalore.

DNA Isolation

Genomic DNA was extracted from fin tissues as described previously. In brief, approximately 50 mg of fin tissues was cut into small pieces and taken into 2 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 chilled isopropanol.

PCR

The polymerase chain reaction was carried out in final volume of 25 ìl containing 100 ng DNA, 2 U of Taq DNA polymerase (Chromous Biotech, Bangalore, 2.5 mM $MgCl_2$ (Chromous Biotech, Bangalore), 2.5 mM each dNTPs (Chromous Biotech, Bangalore) and 100 p mol of primers (Eurofins Genomics, Bangalore). The DNA amplification was performed in the Corbett RG 6000 thermo cycler using the following conditions: complete denaturation (94°C for 5 min), 10 cycles of amplification (94 °C for 45 sec, 35 °C for 1 min and 72 °C for 1.5 min) followed by 30 cycles of amplification (94 °C for 45 sec, 38 °C for 1 min and 72 °C for 1 min) and the final elongation step (72°C for 5 min).

Data Analysis

Total volume of the amplified product (25µl) of each sample was subjected to electrophoresis on 1.5 % agarose gel containing ethidium bromide in 1xTAE buffer at 100V for 1 hr. Finally, the DNA bands were observed on a Gel Doc system and the photographs were captured. The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The genetic distance was calculated by the coefficient of Frequency similarity. The matrix of genetic distance was used for grouping the fish samples based on the dendrogram constructed by UPGMA (Unweighed Pair Group Method with Arithmetic averages).

RESULTS AND DISCUSSION

Genomic DNA was extracted from all ten samples (Fig. 1) and tested qualitatively on 0.8% agarose gel and quantified by using a Nanodrop spectrophotometer (Thermo Scientific). The quality was observed in Agarose gel, bands were found to be intact and without RNA contamination. In quantitative analysis, ratio of 260:280 was found between 1.81 to 1.97 for all samples. A set of 6 decamer oligonucleotides was used in this study as single primers in the PCR (Table 1). The RAPD patterns of genomic DNA of fish species were analyzed for polymorphism, two primers gave clear distinctive band pattern as shown (Figure 2 and 3). Total 38 bands were generated by OPU 10 and 56 bands by OPU15 primer respectively. For primer OPU10 approximately 300bp band was observed in all samples except sample no 9. OPU 15 produced three monomorphic bands in all samples except sample no 2.

Table 1. List of primers

Primer	Sequence (5'→3')
U-10	ACC TCG GCA C
U-11	AGA CCC AGA G
U-12	TCA CCA GCC A
U-13	GGC TGG TTC C
U-14	TGG GTC CCT C
U-15	ACG GGC CAG T

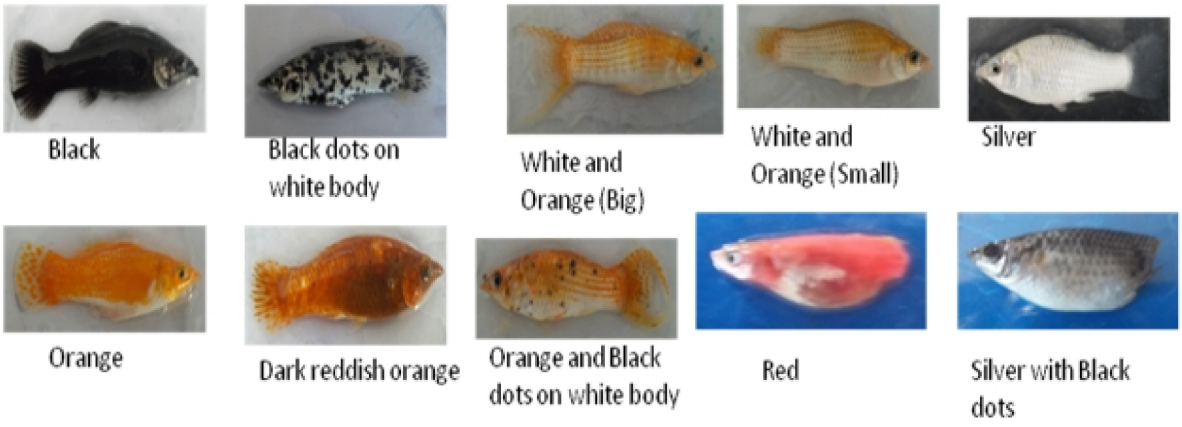

Fig. 1 . Fish species

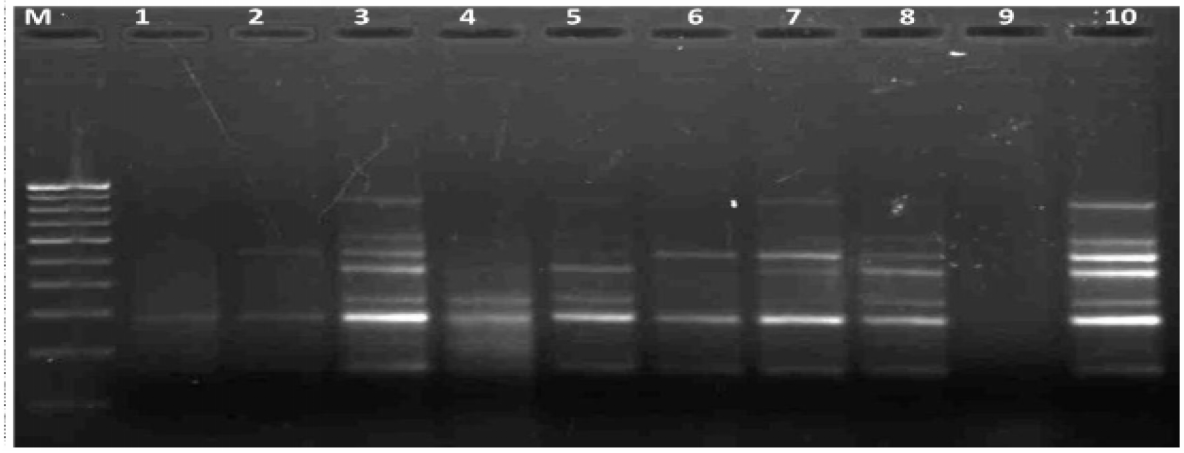

Fig. 2. RAPD patterns from Fish species using OPU 10.

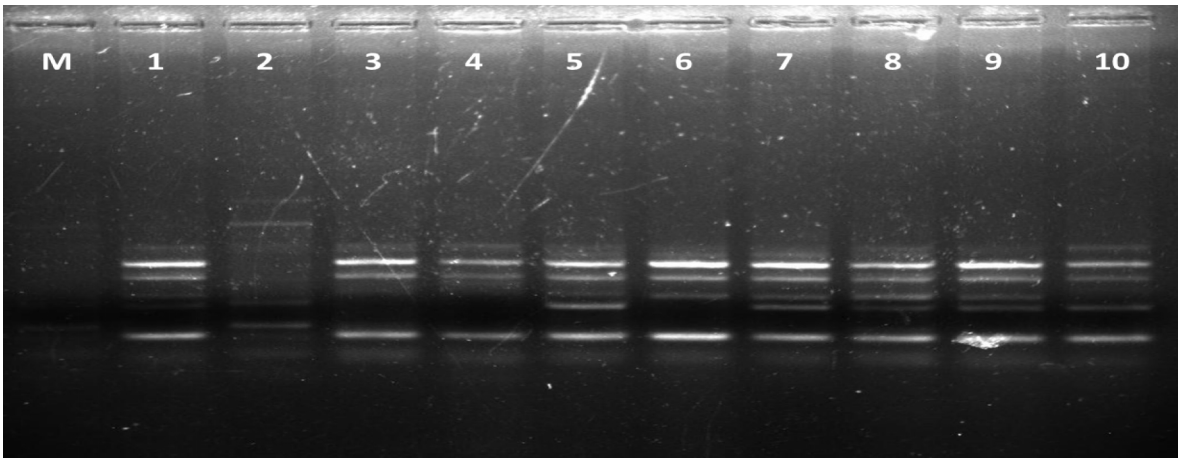

Fig. 3. RAPD patterns from Fish species using OPU 15.

Genetic similarity was calculated using Similarity frequency coefficient and dendrogram was generated to access the genetic relationship among ten selected species (Fig. 4). Dendrogram constructed by cluster analysis of RAPD markers showed three cluster formations. Cluster one contained sample 4, 5, 6, Cluster two contained sample 7, 8, 9, 10, cluster three contained sample 1, 2, 3.

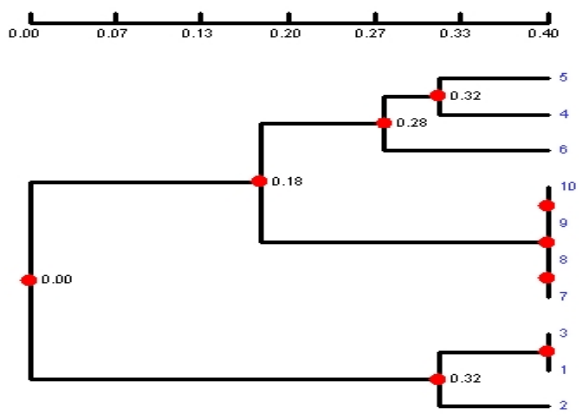

Fig. 3. Dendrogram for ten selected fish species.

Similarity matrices can be related to many population genetics methods in a two-stage approach to population genetics by first computing the pairwise similarities, and then perform clustering or other analyses on this summary of the dataset (Biswas, 2009; Gao, 2007). There are many possible interpretations of what it means for individuals to be correlated, leading to different matrices being constructed. A simple interpretation of relatedness is the average genetic distance (McVean, 2009). The frequency similarity (Table 2) shows that species 7, 8, 9 and 10 are 100% similar and grouped together also species 1 and 3 showed 100% similarity.

CONCLUSIONS

The RAPD profiles in the present study displayed a high degree of polymorphism. This confirms the suitability of RAPD markers for discrimination of fish species. The results from RAPD assays can be extended to further separate traits in a more refined way to exactly associate specific genes and genetic pathways using other molecular methodologies. There is also the opportunity and need to study sequences of specific polymorphic bands, to determine the genes detected by RAPD experiments with other molecular methodologies.

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Table 2. Frequency similarity matrix of ten fish species based on RAPD markers

Sl.No.	1	2	3	4	5	6	7	8	9	10
1	100	83.33	100	50	33.33	50	0	0	0	0
2	83.33	100	83.33	33.33	16.67	33.33	16.67	16.67	16.67	16.67
3	100	83.33	100	50	33.33	50	0	0	0	0
4	50	33.33	50	100	83.33	66.67	50	50	50	50
5	33.33	16.67	33.33	83.33	100	83.33	66.67	66.67	66.67	66.67
6	50	33.33	50	66.67	83.33	100	50	50	50	50
7	0	16.67	0	50	66.67	50	100	100	100	100
8	0	16.67	0	50	66.67	50	100	100	100	100
9	0	16.67	0	50	66.67	50	100	100	100	100
10	0	16.67	0	50	66.67	50	100	100	100	100

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