# A MOLECULAR APPROACH TO DETECT THE GENETIC VARIATION OF INDUCED BRED INTRASPECIFIC HYBRIDS OF *CHANNA STRIATUS* BY USING RAPD MARKERS

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## ABSTRACT

Genetic diversity is the raw material permitting species to adjust to a changing world. The hybrids were normally viable and their survival was strongly influenced by the natural parent. Intraspecific hybridization of different genetic types is an alternative to conventional selective breeding of fishes to yield qualitative or quantitative changes in commercial traits and it would show improvement over the parental species including faster growth to marketable size, greater uniformity in growth and disease resistance. RAPD comparative analysis was carried out to observe the degree of genetic similarity among the two populations of C. *striatus* and its hybrid. In the present investigation, among the 7 primers screened 3 primers (OPA04, OPA06, OPA07) generated reproducible profiles whereas 4 primers didn't amplify or produce highly inconsistent amplification products from the same individual and hence they were excluded from further analysis. The genetic distance and the genetic identity ranged from 0.0189 to 0.9163 and 0.4000 to 1.0000 respectively.

**Keywords**: Intraspecific hybrids, *Channa striatus*, RAPD

***No:of Figures: 2*** ***No:of Tables : 3*** ***No:of References: 19***

## INTRODUCTION

Murrels commonly called snakeheads occupy the top rank among commercially important freshwater fish species due to their taste, fewer intra muscular spines and medicinal qualities (Haniffa *et al.,* 2006). About 28 to 30 channa species have been reported in the global scenario of which 8 to 10 species occur in India. Murrels are distributed naturally in rivers, canals, lakes, swamps, marshes and earthen ponds. They are the important food fish species in these areas, particularly in India. *Channa* species and its categories are reported to be threat in Indian rivers and reservoirs (IUCN 1994, CAMP 1998). Hybridization, that remained the pursuit of mainly non-geneticist. However, the extension of this empirical practice may genetically improve culture fishes through heterosis (hybrid vigor), introgression i.e., the transfer of a small number of genes from one genetic group to another for improving specific traits, establishment of wild de nova gene pool as a broad way for their selection, combination of defined quality of parent services in the offsprings and production of sterile and monosex hybrids (Dehadrai *et al.,* 1993). Cross breeding of different strains of the same species is known to result in improved growth rate, viability and uniformity of F1 generation (Moav, 1979).Intraspecific hybridization of different genetic types is an alternative to conventional selective breeding of fishes to yield qualitative or quantitative changes in commercial traits. Fish hybridization is relatively easy and hybrids have potential value in aquaculture on account of their faster growth, survival, colour, fin shape and structure (Hickling, 1960; Kirpichikov, 1981; Purdom, 1993). DNA fingerprinting offers great potential in aquaculture and fisheries as a tool for identification of individuals (Heist and Gold, 1999) and population genetics (Mamuris *et al.,* 1998). In recent years RAPD is used to characterize and trace the phylogeny of diverse plant and animal species. The RAPD analysis is also employed in differentiating sex chromosome (Iturra *et al*., 1988), genetic inheritance (Elo *et al*., 1997), gene mapping (Liu *et al*., 1999) and fish conservation (Fritzch and Rieseberg, 1996; Dioh *et al*., 1977). Hence in the present study brooders of C. *striatus* collected from Tamilnadu and Kerala were cross hybridized and the feasibility of intraspecific hybridization between two populations were evaluated and genetic phylogeny of the parents with its hybrid was evaluated using RAPD fingerprinting pattern.

## MATERIALS AND METHODS

### Sample preparation:

Brood stock of C. *striatus* with average length 28-34 cm and weight 650-800 g collected from Thamirabarani river, Tamilnadu ($8.44^0$ N, $77.44^0$ E) and vembanad lake, Kerala (9.35´N; 76.25´E) were transported and reared in the live gene bank at Centre for Aquaculture Research and Extension (CARE) Aquafarm. The sexually matured brood fishes were

selected, based on external morphological feature. After identification of sex, Human Chorionic Gonadotropin hormone (HCG) was injected at (2000 IU/kg body weight), to one male and (2000 IU/kg body weight), one female. Induced breeding experiments were made with Tamilnadu Male (TM) and Kerala Female (KF); Tamilnadu Female (TF) and Kerala Male (KM). The injected fishes were separately introduced into fiber tanks (3 x 3 x 4 m). Water quality parameter during experiments was reported to have temperature 28°C ± 0.5; DO - 5.5 to 6.5 mg/l; pH - 7.5 to 8.5. Fertilized eggs were carefully collected using a beaker and observed under microscope. After the induced breeding tissue samples were taken for RAPD analysis from the parent and hybrids of *C. striatus viz;* Tamilnadu male and female, Kerala male and female, Hybrid I (Kerala male + Tamilnadu female) and Hybrid II (Tamilnadu male + Kerala female).

**Isolation of genomic DNA**

DNA was isolated by High salt method. A small amount of tissue was taken and it was chopped with a sterile scalpel blade. The sample was transferred to 1.5 ml micro centrifuge tube, 600 µl of TNES buffer and 35µl of Proteinase k were added. The samples were incubated over night (or 5-24 hrs) at 50°C. If possible the samples were mixed occasionally by inverting the tubes. After incubation, 166.7µl of 6M Nacl was added to it. The samples were centrifuged at full speed (12000-14000 rpm) for 5 -10 minutes at room temperature. The supernatant was pippetted out and equal volume (~800 µl) of 100% ice cold ethanol was added till white DNA precipitate was seen.The samples were centrifuged at full speed (12000-14000 rpm) for 10-20 minutes at 4°C. The supernatant was poured or pipetted out. It was taken care that DNA was not dislodged. DNA pellet was washed with 70% ethanol.

The pellet was air dried completely and dissolved with 100-200µl of sterile distilled water or Tris-EDTA buffer.

**Amplification of DNA:**

21.9µl of the reaction mixture was added to PCR tube, which was already loaded with 3.1µl of template DNA making the final volume to 25µl (Williams et al., 1990).An eppendorf Gene Amp PCR system programmed for 47 cycles of 10 min denaturation at 94°C, 1 min low stringency annealing at 35 °C and 2 min primer extension at 72 °C. At the end, a final extension for 5 min was performed at 72 °C to amplify the DNA.The resulting PCR products were resolved (10µl product mixed with 2µl bromophenol blue dye) on 1.5% agarose gels by submarine gel electrophoresis for 1 hr in 1 X TBE (pH 8.0) buffer (Mohindra, 2002). Subsequently, gels were stained with ethidium bromide (5µg /ml) and visualized on a UV transilluminator and photographed using gel documentation system. The bands were designated according to the PCR product size in relation to standard molecular marker.

**Statistical Analysis:**

Amplification profiles of parent and hybrid fishes were compared with each other. Genetic similarity matrix among populations of samples was calculated using the standard coefficient method (Nei and Li, 1979). Nei's (1978) unbiased genetic identity and genetic distance values between parents and hybrids were calculated using the data generated from the RAPD profiles using POP GENE 32 software. Genetic distance values were utilized to construct a dendrogram through clustering analysis (UPGMA) to determine the relationship among the populations.

**RESULTS**

The breeding behavior of the two populations of *C. striatus* was observed. After spawning, fishes released eggs in the water surface. Fertilized eggs are free floating, spherical, non-adhesive and transparent, the diameter of fertilized eggs ranged from 0.68-1.10 mm. The number of eggs obtained was higher in KM+TF hybridization (4500), when compared to TM+KF hybridization (2700). Significantly higher fertilization rate (76 %) and hatching rate (60 %) were observed in KM+TF hybrid after the injection of HCG. In the case of TM+KF fertilization rate (54%) and hatching rate (45%) were not significant (Table 2). RAPD comparative analysis was carried out to observe the degree of genetic similarity among the two populations of *C. striatus* and its hybrid. In the present investigation, among the 7 primers screened 3 primers (OPA04, OPA06, OPA07) generated reproducible profiles where as 4 primers didn't amplify or produce highly inconsistent amplification products from the same individual and hence they were excluded from further analysis. Reproducible 3 primers were selected for further analysis on the basis of the number, spacing and intensity of bands amplified and degree of polymorphism revealed. An average of 3 bands for Tamilnadu male & Kerala female, 7 bands for Tamilnadu female and 9 bands for Kerala male was amplified. In the case of hybrids Tamilnadu male & Kerala female produced 6 bands and Kerala male & Tamilnadu female produced 3 bands. The 3 selected primers yielded a total of 10 loci.

The genetic distance and the genetic identity ranged from 0.0189 to 0.9163 and 0.4000 to 1.0000 respectively, which is given in Table 3. The highest genetic identity was found between the Kerala female and the hybrid of Kerala male and Tamilnadu female. Based on the genetic distance, a dendrogram (Fig.1) was constructed to depict the genetic relationships among the two populations of *C. striatus* and its hybrids. From the dendrogram it is clearly understood that the hybrid II (Tamilnadu male &Kerala female) was closer to Tamilnadu male, which subdivides from a single clade. Similarly the Kerala female was closer to Tamilnadu female and the hybrid I (Kerala male &Tamilnadu female) separating from the same clade.

**DISCUSSION**

Intraspecific hybridization of different genetic types is an alternative to

conventional selective breeding of fishes to yield qualitative or quantitative changes in commercial traits and it would show improvement over the parental species including faster growth to marketable size, greater uniformity in growth and disease resistance.The breeding behavior of *C. striatus* collected from Kerala and Tamilnadu was observed 6 hours after administration of the hormone and more number of eggs was produced by Kerala male and Tamilnadu female which showed the highest fertilization rate (76%). Such study was done in *Clarias gariepinus* by Salami *et al.* (1994) which showed 90% success, similarly 81% was obtained in *C. batrachus* by Zairin *et al.* (1992); 50-62 % in *C. macrocephalus* by Carreon *et al.* (1976); 81 % in *C. macrouphalus* by Nagmvongchon *et al.* (1988); 67 - 89 % in *Myatus montanus* and 80-90 % in *Ompok malabaricus* by Haniffa *et al.* (2001); 75 % in *Heterobranchus longifillus* by Legender *et al.* (1986). The hybrids were normally viable and their survival was strongly influenced by their natural parent. The low survival rate of the hybrids during larval rearing may be related to poor quality of eggs but mortality of older fish was probably due to other factors. Glamuzine *et al.* (2001) reported that the embryonic development of the hybrid (*Epinephelus costae X E. morginatus)* was slightly faster throughout embryogenesis. From this study it is assumed that two groups of the strain preserve morphometric and genetic variation because of the homing tendency of adults to the natural area. Further verification of the variation of group is needed for the genetic analysis of each spawning set. Genetic diversity is the raw material permitting species to adjust to a changing world. The level of similarity in the genetic makeup of populations of same species indicates to what extent genetic material can be exchanged between populations and still maintained a species specific gene pool. The presence of variability in intraspecific with different population is essential for the survey to successfully respond to environmental changes (Ryman *et al.,* 1995). In this study 7 different decamer primers were tested in *C. striatus* with 2 populations and its hybrid. Out of 7 primers, 3 primers generated reproducible bands. Similarly Crossland *et al.* (1993) successfully used RAPD marker to analyse the interspecific nuclear gene flow between *Lris fulva* and *L. hexagona* and to study the presumed hybrid origin of *L. nelsonii.*

## CONCLUSION

It would therefore be advisable to integrate hybridization studies into more general genetic improvement programmes. It also concludes that hybrid of *C. striatus* could improve the species diversity including faster growth to marketable size, greater uniformity in growth and disease resistance.

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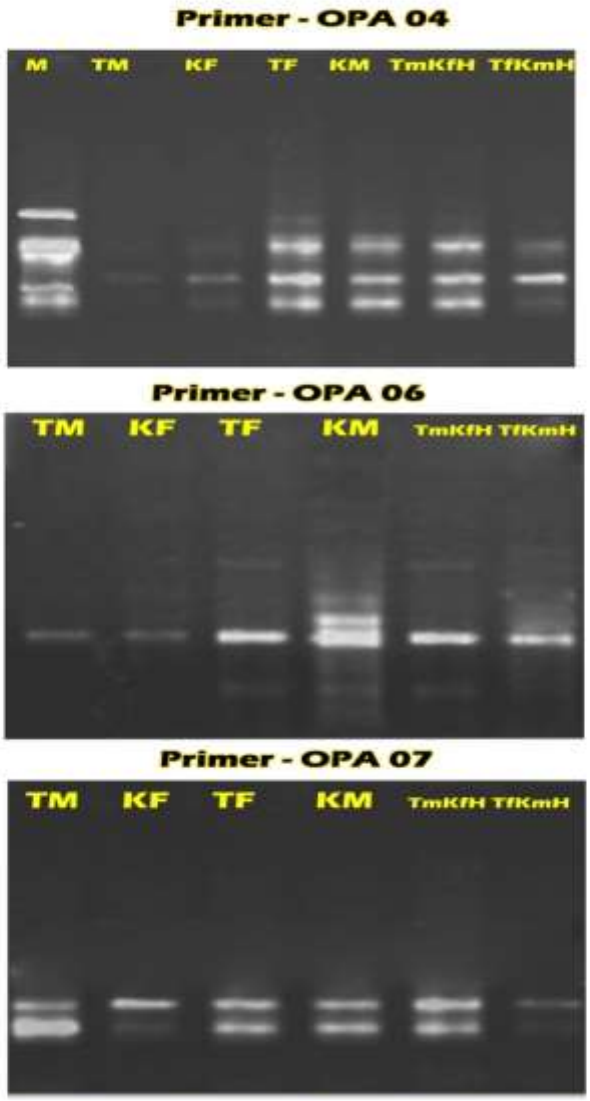
*Plate 1: Two Populations of Channa Striatus and hybrids*




M- Marker; TM – Tamilnadu Male; KF – Kerala Female; TF – Tamilnadu Female;
KM - Kerala Male; TmKfH - Tamilnadu Male Keralafemale Hyb.;
TfKmH - Tamilnadu Female Kerala Male Hyb.

**Fig:1 Dendrogram based Nei's (1972) Genetic distance: Method = UPGMA**

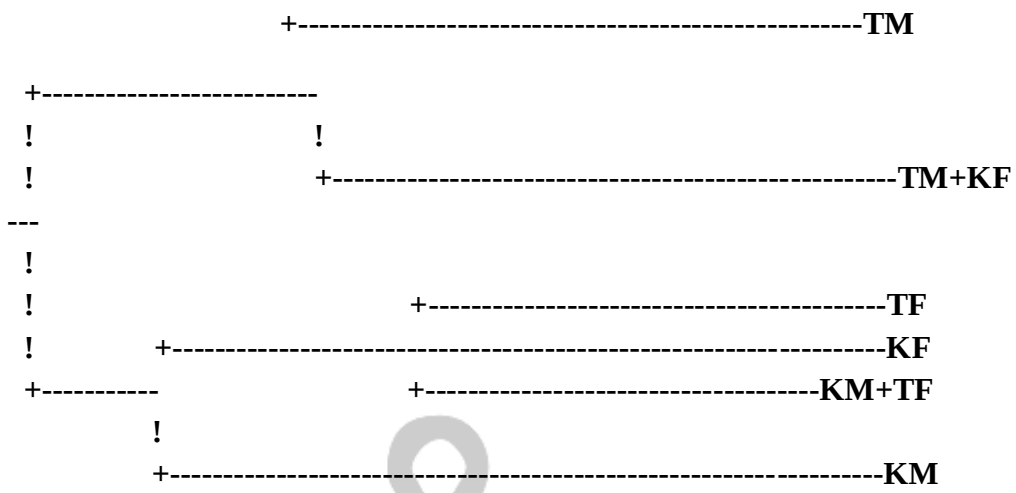



**Table 1. The sequence of primers used for RAPD analysis**

| S. No | Primer code | Primer Sequences | Molecular Weight (Da) |
|---|---|---|---|
| 1 | OPA 03 | 5' AGTCAGCCAC 3' | 2988 |
| 2 | OPA 04 | 5' AATCGGGCTG 3' | 3059 |
| 3 | OPA05 | 5' TTGCAGGCAG 3' | 3090 |
| 4 | OPA 06 | 5' GGTCCCTGAC 3' | 2995 |
| 5 | OPA 07 | 5' GAAACGGGTG 3' | 3108 |
| 6 | OPA 08 | 5' GTGACGTAGG 3' | 3099 |
| 7 | OPA 11 | 5' CAATCGCCGT 3' | 2979 |

A: Adenine T: Thymine G: Guanine C: Cytosine

**Table. 3. Pair wise value for Nei's unbiased genetic identity and genetic distance between two populations of *C. striatus* and hybrids**

| Pop id | Tamilnadu male | Kerala female | Tamilnadu female | Kerala male | Hybrid TM + KF | Hybrid TF + KM |
|---|---|---|---|---|---|---|
| **Tamilnadu male** | ****** | 0.8000 | 0.6000 | 0.4000 | 0.7000 | 0.8000 |
| **Kerala female** | 0.2231 | ****** | 0.6000 | 0.4000 | 0.7000 | 1.0000 |
| **Tamilnadu female** | 0.5108 | 0.1508 | ****** | 0.6000 | 0.9000 | 0.6000 |
| **Kerala male** | 0.9163 | 0.9163 | 0.5108 | ****** | 0.7000 | 0.4000 |
| **Hybrid TM + KF** | 0.3567 | 0.3567 | 0.1054 | 0.3567 | ****** | 0.7000 |
| **Hybrid TF + KM** | 0.2231 | 0.0189 | 0.5180 | 0.9163 | 0.3567 | ******* |

**Table 2. Induced breeding of *C. striatus* in two population using hormone HCG.**

| S. No | Male | | Female | | Concentration of hormone HCG | Type | Fertilization (%) | Incubation Hours | No of eggs | Hatchlings (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | L (cm) | W (g) | L (cm) | W (g) | | | | | | |
| 1 | 33 | 710 | 30 | 620 | 3 unit | TM + KF | 54 % | 31 hrs | 2700 | 45 % |
| 2 | 34 | 790 | 29 | 710 | 3 unit | TF + KM | 76 % | 31 hrs | 4500 | 60 % |

L – Length; W – Weight;

TM + KF = Tamilnadu male and Kerala female

TF + KM = Tamilnadu female and Kerala male

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