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Brief Study on the Genetic Variations at Isozyme Loci in Native Catfish (*Clarias batrachus*), African Catfish (*Clarias gariepinus*) and their Hybrid (*Clarias gariepinus x Clarias batrachus*)

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Abstract

Genetic variations in native (*Clarias batrachus* Linnaeus, 1758), African (*Clarias gariepinus* Burchell, 1822) and their hybrid catfish (Magur) (*Clarias gariepinus* ♀ x *Clarias batrachus* ♂) were investigated in six isozyme systems. The isozymes were Isocitrate dehydrogenase (IDH), Malate dehydrogenase (MDH), Malic enzyme (ME), Phosphogluco mutase (PGM), Glucosephosphate isomerase (GPI) and Lactate dehydrogenase (LDH). The isozymes were studied in muscle, liver and heart tissues by using starch gel electrophoretic technique to examine the degree of genetic closeness among them. It was found that genotypes of the different catfishes varied depending on the type of isozymes and also on the type of biopised tissues. A total of three individual fish from each category of catfish was used. Isozyme, GPI in two liver tissues of native and African catfish showed heterogygosity and the other tissues showed homogygosity. However, the hybrid catfish showed heterogygosity in three samples of liver tissues. From the findings of the present electrophoretic study it may be said that Hybrid catfish received genetic traits from both the father and mother.

Key Words: Genetic variation, Electrophoresis, Isozyme, Loci.

Introduction

During the last decade, lavish data on genotype and allele frequency have been obtained from a number of fish species primarily through the means of protein electrophoresis (Allendorf, *et. al.* 1987). This has enabled the complex population structure of many species to be identified (Ryman, 1983). Systematic and population genetics are currently being investigated using the techniques of electrophoresis on starch and acrylamide gels (Degani and Veith, 1990). These methods allow accurate separation of enzymes and other proteins in biological fluids. The use of specific histochemical staining permits the study of the activity of individual enzymes on the gels. The genetic variation and natural population among the different species may be investigated by enzymatic analysis of allelic variations at specific loci of species and at desired individual.

Systematic problems have been minimized in fishes by comparative analyses of the electrophoretic patterns of lateral muscle (muscle myogens), blood serum proteins and of the eye lens (Degani and Veith, 1990). The objective of the present study was to find out the genetic variations among the three types of catfish by electrophoretic techniques to detect the genetic differences using several isozymes of different tissues in three catfish.

Materials and Method

In the present study indigenous (*Clarias batrachus*), African (*Clarias gariepinus*) and their hybrid catfish (*Clarias gariepinus* ♀ x *Clarias batrachus* ♂) having length group of 28 to 32 cm were used. A total of three individual fish from each category of catfish was used. The live fishes were collected from a stock of the Fisheries Research Institute (FRI), Mymensingh. It may be mentioned that all the fishes used in the experiments were reared in an identical condition and fed with pelleted feed.

Preparation of samples

Small pieces of skeletal muscle, the entire liver and heart samples from freshly killed fishes were taken by a scalpel and forceps for research purpose. These tissues were then preserved at -20^{O}C until completion of analysis. For elec-

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trophoresis, tissues were taken from cold storage, thawed for a few minutes and then placed in 0.5 cm. diameter wells sunk into perspex block. The samples were moistened with 25 µl of distilled water and homogenized the tissue with an equal volume of "homogenizing" buffer in a polycarbonate centrifuge tube for approximately 15-30 seconds at 3000 round per minute (RPM). (James and Clive, 1986).

Analytical Technique

Horizontal starch gel electrophoresis (McAndrew and Majumder, 1983, Hussain, 1992) was used as a technique to verify the genotype of isozyme in muscle, liver and heart samples from native, African and hybrid catfish.

Preparation of Starch-Gel

About 26g of starch (Sigma Ltd.) was mixed with 220 ml of diluted TC-l (22 ml buffer plus 198 ml of distilled water) or TM (8.8 ml buffer plus 110.2 ml of distilled water) buffer respectively in a Buchner flask. The mixture was heated with constant rotation of the flask to an almost translucent jelly state and then quickly degassed by using a vacuum water pump and then poured into a 6 mm thick gel former. The gel was covered with a glass plate and allowed to set for overnight.

Running, Slicing and Staining the Gel

The gel was taken out from the starch and cut parallel at 2 and 3 cm distances from the edge of the prepared gel. The small pieces of filter paper were soaked in homogenised sample and were placed along the edge of the gel. Then the gel was placed in an electrophoretic bath with the appropriate buffer. A gauze wick soaked in the buffer was applied to either end of the gel to connect the gel and buffer. The gel was then covered with a polythene sheet to reduce evaporation. The bath tray covered with appropriate transparent lid was placed in a refrigerator at $4^{O}C$, the power pack connected and the gel was allowed to run for 3-4 hours at 150-200V current.

At the end of the process, the gel was removed from the bath and sample filtres were removed from the gel which was then sliced horizontally so that each of which could be stained for a different enzyme system (Table I). The appropriate stains (Table I) for the enzyme system to be examined were weighted and mixed with stain buffer solution with 2% agar (approx. $50\text{-}60^{O}C$). This was poured over the slice and allowed to set and then incubated at $37^{O}C$ until the banding patterns became visible. The electrophorograms were then analyzed and scored for the respective genotypes. The stained starch gels were photographed with uniform lighting from above.

Determination of Relative Mobilities

The initial interpretaion of the banding patterns resulting from electrophoresis simply involves a determination of the relative mobility of each band in the unknown sample (s) compared to that of the band(s) in the reference samples(s) on the same gel (James and Clive, 1986).

Table I. Enzyme system observed in native catfish (*C. batrachus*), African catfish (*C. gariepinus*) and hybrid catfish (*C. gariepinus x C. batrachus*)

Enzyme	Abbreviation	E.C. No	Buffer	Loci No
Isocitrate dehydrogenase	IDH	1.1.1.42	TC-1 TM	3
Malate dehydrogenase	MDH	1.1.1.37	TC-1 TM	3
Malic enzyme	ME	1.1.1.40	TC-1 TM	2
Phosphogluco mutase	PGM	5.4.2.2	TM	1^{C}
Glucosephosphate isomerase	GPI	5.3.1.9	TC-1	2
Lactate dehydrogenase	LDH	1.1.1.27	TC-1	3

TC = Tris Citrate, pH 7.0
TM= Tris Maleate, pH 7.4

Results and Discussion

In the present study, the starch gel electrophoresis of biopsied tissue was used to determine the genotypes of native catfish (*Clarias batrachus*), African catfish (*Clarias gariepinus*) and their hybrid (*Clarias gariepinus* ♀ x *Clarias batrachus* ♂). Table II presents information on the genotypes of the two species of catfish and their hybrid. The numeric numbers used in the Table II represent the tissue samples of the fish used in the study. Sample nos. 1. 2, 3 represent the tissue samples from native catfish, sample nos. 4, 5, 6 that from African catfish while sample nos. 7, 8, 9 from hybrid catfish (*Clarias gariepinus* ♀ x *Clarias batrachus* ♂). Fig. I (A, B, C, D, E, F, G, H, I, J, K) illustrate the type of electrophoretic patterns obtained in analyzing different enzymes

Table II. Showing the pattern of genotype from isozyme identification in native catfish (*C. batrachus*), African catfish (*C. gariepinus*) and hybrid catfish (*C. gariepinus* x *C. batrachus*)

Isozyme	Species Examined			Tissue Samples
	Native Catfish *(C. batrachus)*	African Catfish *(C. gariepinus)*	Hybrid Catfish *(C. gariepinus x C. batrachus)*	
IDH	1) 11	4) 11	7) 22	Muscle
(Fig.A)	2) 11	5) 22	8) -	
	3) 11	6) 22	9) -	
MDH	1) 11	4) 31	7) 22	Muscle
(Fig.B)	2) 11	5)22	8) 44	
	3) 11	6)22	9) -	
ME	1) 11	4)11	7) 22	Muscle
(Fig.C)	2) 11	5)11	8)22	
(Slow zone)	3) 11	6)22	9)22	
IDH	1) 12	4)22	7)22	Liver
(Fig.D)	2) 12	5)22	8)12	
	3) 12	6)22	9)12	
MDH	1) 11	4)11	7)13	Liver
(Fig.E)	2) 11	5)33	8)11	
	3) 12	6)13	9)13	
ME (Fig.A)	1) 23	4)11	7)11	Liver
(Fast zone)	2) 11	5)11	8)11	
	3) 12	6)11	9)11	
PGM	1) 11	4)11	7)11	Liver
(Fig.G)	2) 11	5)11	8)11	
	3) 11	6)11	9)32	
GPI	1) 12	4)23	7)13	Liver
(Fig.H)	2) 22	5)33	8)12	
	3) 12	6)13	9)12	
MDH	1) 12	4)12	7)12	Heart
(Fig.I)	2) 12	5)12	8)12	
	3) 12	6)12	9)12	
LDH-2	1) 11	4)11	7)11	Heart
(Fig.J)	2) 11	5)11	8)11	
	3) 11	6)11	9)11	
LDH-3	1) 11	4)11	7)11	Heart
(Fig.K)	2) 11	5)11	8)11	
	3) 11	6)11	9)11	

systems in native, African and hybrid catfish. The results of the present study are detailed as follows.

Muscle Tissue

The results of isozyme IDH, MDH & ME are shown in Fig. 1 (A, B, C). All the muscle samples from native catfish were observed found to be homogygous for the isozyme IDH, MDH & ME. Further the allelic distance among the triplicate samples were found equal (Table II). Similarly homogygosity was also observed in two samples of African catfish while the other one sample showed heterogygosity (Table II). Unlike the African and naive catfish, homogygosity was observed among six samples of hybrid catfish while three samples showed no mobility (Table II). In case of native,

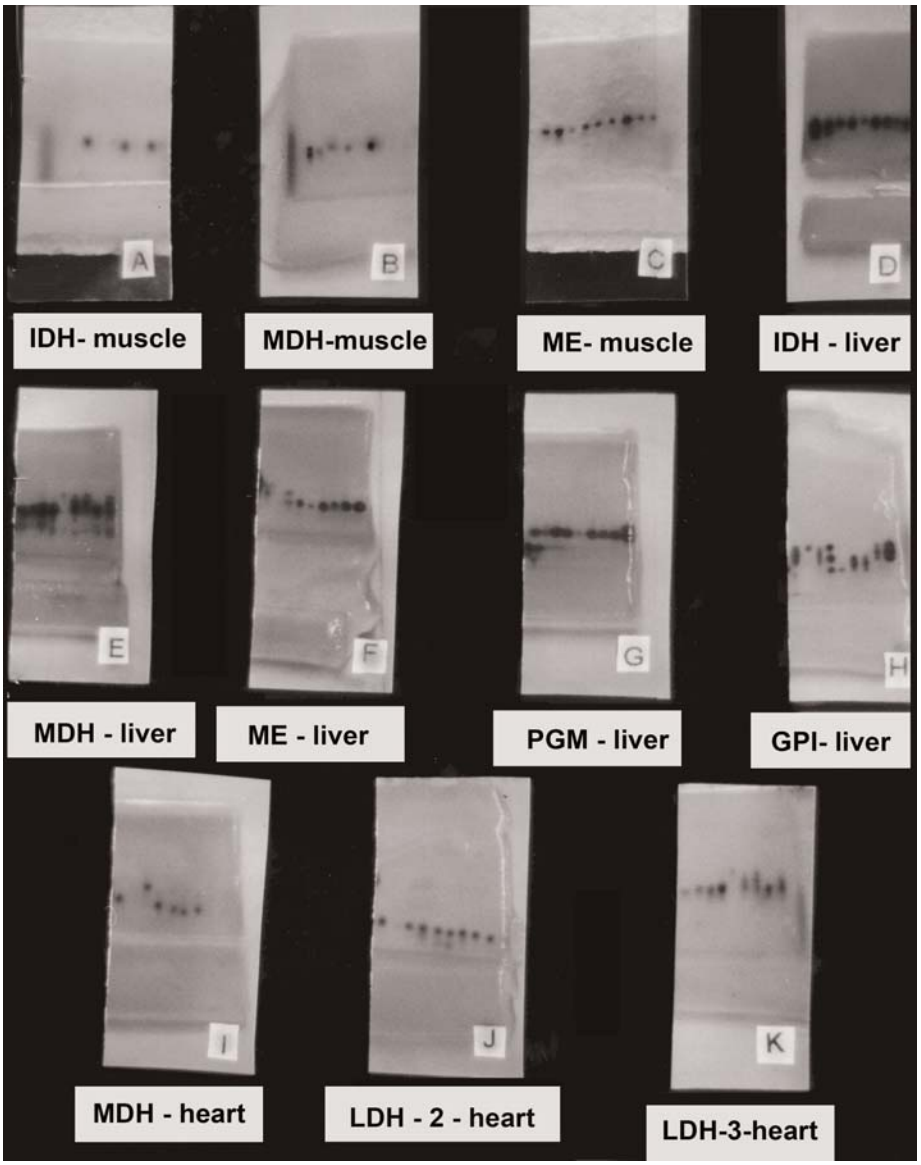

Fig. 1. A-K. Photographs showing the starch gel isozyme patterns in muscle, liver and heart tissue of native (*C. batrachus*), African (*C gariepinus*) and hybrid catfish (*C gariepinus x C.batrachus*):

A. Isozyme, IDH in Muscle Tissue ; B. Isozyme, MDH in Muscle Tissue; C. Isozyme, ME in Muscle Tissue ; D. Isozyme, IDH in Liver Tissue ; E. Isozyme, MDH in Liver Tissue; F. Isozyme, ME in Liver Tissue ; G. Isozyrne, PGM in Liver Tissue ; H. Isozyme, GPI in Liver Tissue ; I. Isozyme, MDH in Heart Tissue ; J. Isozyme, LDH-2 in Heart Tissue ; K. Isozyme, LDH-3 in Heart Tissue.

African and hybrid catfish, IDM, MDH & ME in muscle samples were monomorphic, dimorphic & dimorophic loci respectively.

Liver tissue

The results of isozyme IDH, MDH, ME, PGM & GPI are illustrated in Fig. 1 (D, E, F, G, H) collected from liver sample. Homogygosity was observed in seven samples of native catfish and other eight samples showed heterogygosity (Table II). Similarly, three liver samples of African catfish showed heterogygosity in the isozymes and other twelve samples showed homogygosity (Table II). On the other hand, eight liver samples of hybrid catfish showed hcterogygosity while homogygosity was observed in other seven samples of hybrid catfish (Table II). All the samples of native, African & hybrid catfish showed both monomorphic & dimorphic loci.

Heart tissue

The results of isozyme MDH, LDH-2 & LDH-3 of heart samples are shown in Fig. 1 (I, J, H). Heterogygosity was observed in the isozyme and MDH in all three heart samples of native, African & hybrid catfish and the allelic distances were the same. All the three heart smaples from native, African & hybrid catfish showed homogygosity (Table II) in the isozyme, LDH-2 & LDH-3. The isozyme from all heart samples from native, Afrian & hybrid catfish had monomorphic loci.

According to Campton (1987) biochemical analysis by electrophoresis are very effective to detect species specific protein and to demonstrate hybrid specimens which otherwise are hardly distinguishable from their parental species at the level of morphological or meristic characters. In the present study, all the loci of six enzymes have been determined to be suitable for the study of genetic variation. The adaptation of buffer to tissue facilitates the detection of enzyme loci, thus permitting the study of the genetic distance among two species and their hybrid as well as the genetic variation among population of different types.

Degani and Veith (1990) stated that the same genus (*Clarias batrachus* and *Clarias gariepinus*) having at least one of the various alleles in the gene was common to all the tested species, which is in good agreement with the present study. This situation was detected in IDH, MDH, ME, PGM, GPI, LDH-2, LDH-3. The loci may be suitable for studying the genetic variations between the species belonging to the genus *Clarias.* In agreement with the study of Degani and Veith (1990), the present study supports the idea that African, native and their hybrid catfish are different species from the genetic point of view. Sezaki *et. al.* (1994) observed that hybrid specimens generally show an allozygous phenotype in electrophoretic patterns as a result of specific gene expression in the parental species. The present electrophoretic analysis of proteins also clearly demonstrated allozygous gene expression for IDH, MDH & GPI in liver, suggesting hybrid catfish originated from hybridization between African and native catfish. Introgressive hybridization like the present results have been found in the taxa of many fishes (Hubbs, 1955, Campton, 1987, Verspoor and Hammer, 1991).

In liver, GPI showed that the two samples are heterogygous and one sample is homogygous in native and African catfish where all the samples of hybrid catfish showed heterogygosity. From the above findings of ME, PGM, GPI in the liver, it is evident that the individuals have only two allele but in a population they may have more than two alleles. From the investigation of GPI in liver, it can be said that hybrid catfish or progeny of African and native catfish showed the contribution of both the father and the mother's gene.

The present electrophoretic study leads to the conclusion that in a number of species the presence of high polymorphism in some enzymes can be used as a genetic markening in these species. It may be proved as an aid to the new field of aquaculture dealing with these systematic or taxonomic groups.

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