A Study of Esterase Polymorphism in Some Carps Viz. Labeo Rohita, Cirrhina Mrigala and Cyprinus Carpio

Dr. Anil Jindal[1], Dr. Mridula Sharma[2], Dr. S. Rishi[3]
[1](corresponding author) Department of Zoology, R.K.S.D. (PG) College, Kaithal-136027, Haryana
[2] (co-author) Education Department, Govt. of Haryana
[3]Research Supervisor, R.K.S.D. (PG) College, Kaithal-136027, Haryana

***Abstract*—Esterase polymorphism was studied in three carps viz. *Labeo rohita, Cirrhina mrigala* and *Cyprinus carpio*. Five tissues namely Liver, heart, kidney, eye and muscles were utilized for polyacrylamide gel electrophoretic analysis. All the tissues showed higher rate of esterase activity in *C. carpio* as compared to *L. rohita* and *C. mrigala* as is evident by the number and intensity of staining of bands. Esterase activity was highest in liver as compared to other tissues. The present study indicates that the ploidy level (diploid to tetraploid) can be confirmed on the basis of altered isozyme profiles.**

***Index Terms*—Esterase; polymorphism; *L. rohita; C. carpio; C. mrigala* and isozyme.**

I. INTRODUCTION

In fishes, genetic polymorphism seems to be widespread. In recent years electrophoretic techniques have been increasingly used to demonstrate allelic variation of proteins in conspecific animal populations. Some scientists have been stressing at the conservation of endangered fish species with the help of their isozyme profiles. Doadrio et al. (1996) investigated allozyme variations of the endangered killifish (*Aphanius iberus*) and suggested its application for the conservation of the fish. Many workers have discovered polymorphism with respect to esterase genes in the representatives of clupeids, salmonids, osmerids, cyprinids, catostomids, eels, gadoids, tunas, flatfishes etc. by the frequency and range of variability. The population analysis showed that differences between populations could be easily defined by the allelic concentration of esterase genes. Grant (1986) has studied biochemical genetic divergence between Atlantic *Clupea harengus* and Pacific *C. pallasi* using esterase enzyme system. Sarangi and Mandal (1996) reported that the heatshock induced tetraploid Indian major carp; *L. rohita* can be identified from diploid stock by polymorphism at Est-1 locus in kidney. So, Est-1 act as a reliable marker in identifying tetraploid stocks from diploid. Keeping in view the above factors, esterase polymorphism has been studied in *L. rohita, C. mrigala* and *C. carpio* during the present investigation.

II. MATERIALS AND METHODS

The live specimens of approximately similar weight (150-200 gm.) of two Indian major carps *L. rohita, C. mrigala* and one exotic carp *C. carpio* belonging to family Cyprinidae and order Cypriniformes were collected from the 'National Fish Seed Farm', Jyotisar, as well as from canals near Kurukshetra to analyze the various forms of esterase enzyme in the liver. The fishes were brought alive to the laboratory and acclimatized in well-aerated aquaria. Liver, heart, kidney, eye and muscle tissues were utilized for extract preparation. Each extract was prepared in 0.125 M tris-HCl buffer (ph 6.8) and stored at –5°C. 7% polyacrylamide gels were prepared and electrophoresis (PAGE) was carried out in anionic system at 7mA constant current, in a refrigerator to avoid heating of the gels, Davies (1964) and Ornstein (1964). The gels were stained for esterase enzymes according to the method as described by Shaw and Prasad (1970). Gels were photographed and zymograms were also prepared.

III. RESULTS

The liver, heart, kidney, eye and muscle tissues were investigated in all the three carps. The representative PAGE-esterase patterns of different tissues of *L.*

rohita and *C. mrigala* have been shown in fig.1 and that of *C. carpio* have been shown in fig.2.

In the liver tissue of *L. rohita*, the zymogram (Fig.3) shows five different zones. Est-4 and Est-5 are darkly stained. An exceedingly high activity of esterases has been detected in liver. Est-3 stains very faintly. Rm values are- .21, .24, .30, .44, .46, .47, .49, .52, .53, .54, .60, .64, .67 and .71 respectively. In case of heart, kidney, eye and muscle the zymogram shows two darkly stained bands and a faintly stained band at Est-4 locus. Extremely low activity was detected at Est-1 locus. Rm values are- .24, .47, .49 and .53 respectively.

The zymogram (Fig.3) shows three zones of activity in the liver of ***C. mrigala***. The bands representing Est-1 and Est-3 loci indicate extremely low activity in this fish. The intensity of staining was moderate. Rm values are- .21, .34, .49, .52, .53, .66, .67 and .71 respectively. Heart, kidney, eye and muscle tissues have esterase activity mainly in two zones, Est-4 and Est-2. Both of these zones have two darkly stained bands of esterase activity. Rm values are- .34, .38, .49 and .53 respectively.

In the zymogram (Fig.4) of ***C. carpio***, there has been observed an exceedingly high rate of activity of esterases in the liver. The staining intensity of all the bands was found to be much more as compared to other tissues and that of the two Indian major carps. All the five zones have increased number of bands with diverse Rm values viz. .36, .41, .45, .52, .55, .65, .69, .77 and .96 respectively. In case of muscle, heart, kidney and eye, the tissues have been observed to possess five major bands with dark to moderate staining. Kidney and muscle tissues are comparatively less stained than other tissues. The Rm values are .36, .41, .42, .45, .55 and .65 respectively.

IV. DISCUSSION

During the present investigations on three carps, *C. carpio* reported an increase in the number and intensity of staining of bands as compared to the other two carps. In a similar study on heat shock induced tetraploid fish *L. rohita*, it was observed that number of zones and degree of intensity of staining was very high (Sarangi & Mandal, 1996). Earlier, confirmation of tetraploid status of fish has largely been based on chromosome counting and DNA content. The present study indicates that the ploidy level (diploid to tetraploid) can be confirmed on the basis of altered isozyme profiles. Thus, there is a great possibility of employing such altered profiles as marker for identification of tetraploid or polyploid fishes. There are marked differences in the zymograms of *L. rohita* and *C. mrigala*. The adult *L. rohita* has been found to possess a remarkable number of esterase isozymes. Fourteen separable forms of activity are observed. In *C. mrigala* 8 to 9 separable forms of esterases are present. Est-3 and Est-1 has little or no activity in the liver of *C. mrigala* while *L. rohita* has very little Est-2 activity. The differences can be due to species-specific presence of the isozyme patterns. Enzymes are mostly the primary products of transcriptionally active genes and it is assumed that a specific isozyme profile is the reflection of the genetic makeup of a given species and may be used as a fingerprint in identifying different species, considering all other variables as constant. In the case of esterases, more intensely stained bands in different loci of tetraploid *C. carpio* perhaps indicate increased gene dose due to gene duplication and the absence of some less active region might be an epigenetic modification or post-translational modification (Pasteur et al., 1988). Several workers in different fishes have reported the increase in gene duplication due to tetraploidy, eg. Salmonids (Allendorf & Utter, 1977), herring (Pasteur et al., 1988), induced tetraploid *Labeo rohita* (Sarangi & Mandal, 1996). Gene duplication must have enhanced enzyme expression in *C. carpio*, which probably could have failed to stain due to negligible amount present in the diploid condition. This enhanced enzyme expression could have led to the appearance of additional bands in tetraploids (Sarangi & Mandal, 1996). In many fish species that have undergone extensive gene duplication, hybrid polymorphs have been reported (Richardson et al., 1986). Formation of these hybrid heteropolymorphs due to hybridization of protein products of separate loci might be another plausible explanation for development of new bands with entirely different Rm values. Nevertheless, the esterase band patterns can form dependable criteria for discrimination between fish species and for determining their evolutionary status via polyploidy.

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LEGENDS

Fig. 1 Esterases separated from the extracts of muscle, eye, kidney, heart and liver tissues of two Indian major carps viz. *Cirrhina mrigala* and *Labeo rohita* showing tissue-specific and species-specific pattern.

Fig. 2 Esterases from the extracts of muscle, liver, heart, eye and kidney of *Cyprinus carpio.*

Fig. 3 Zymograms for Esterases of different tissues of *Cirrhina mrigala* and *Labeo rohita* resolved on 7% polyacrylamid gel under non-dissociating conditions.

Fig. 4 Zymograms for Esterases of different tissue of *Cyprinus carpio* resolved on 7% polyacrylamid gel under non-dissociating conditions.

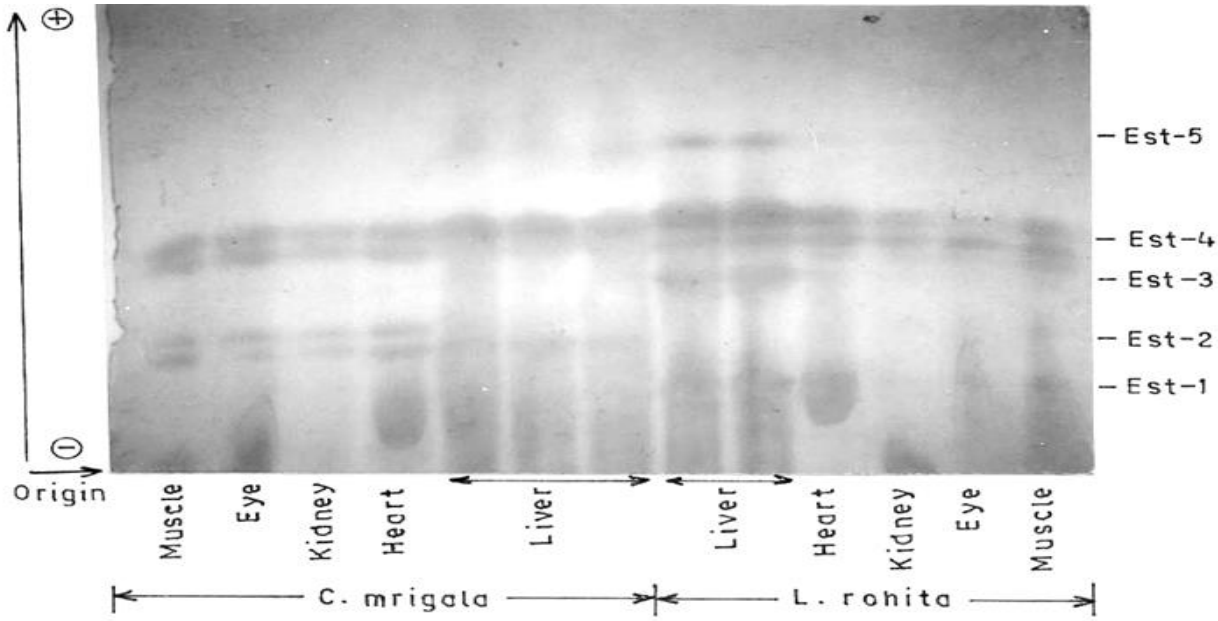
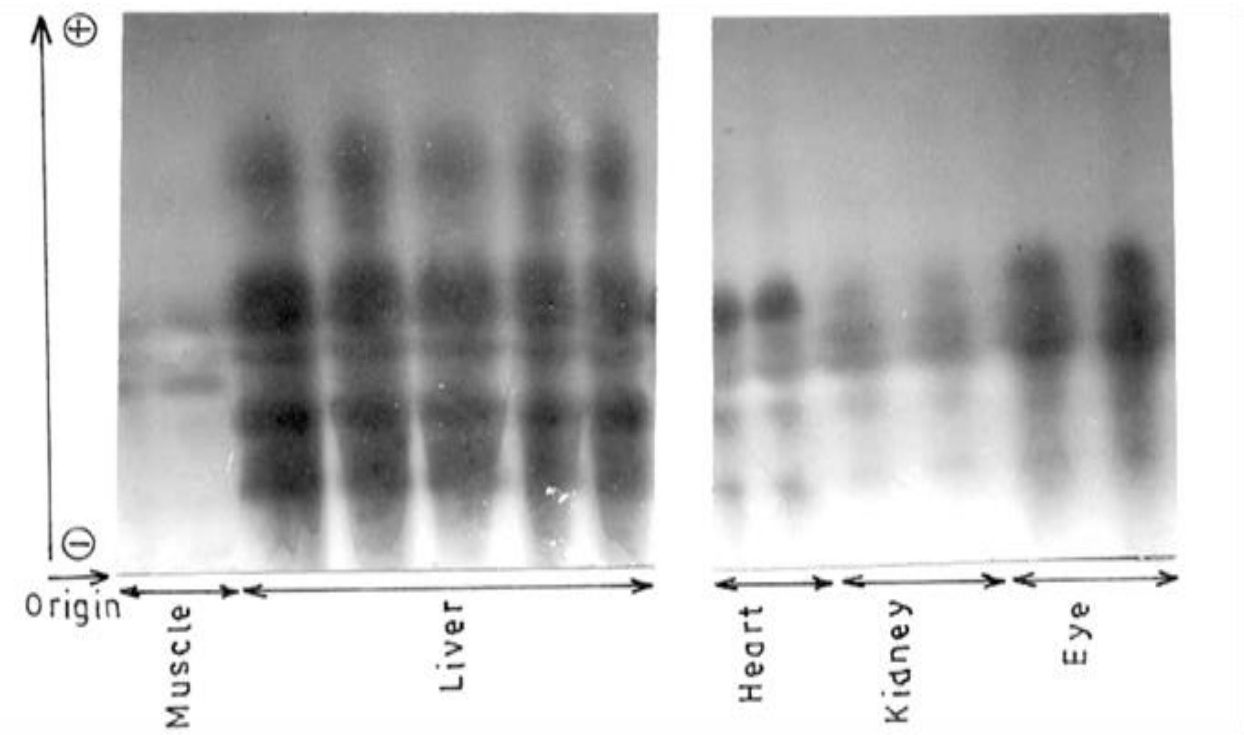


Fig.1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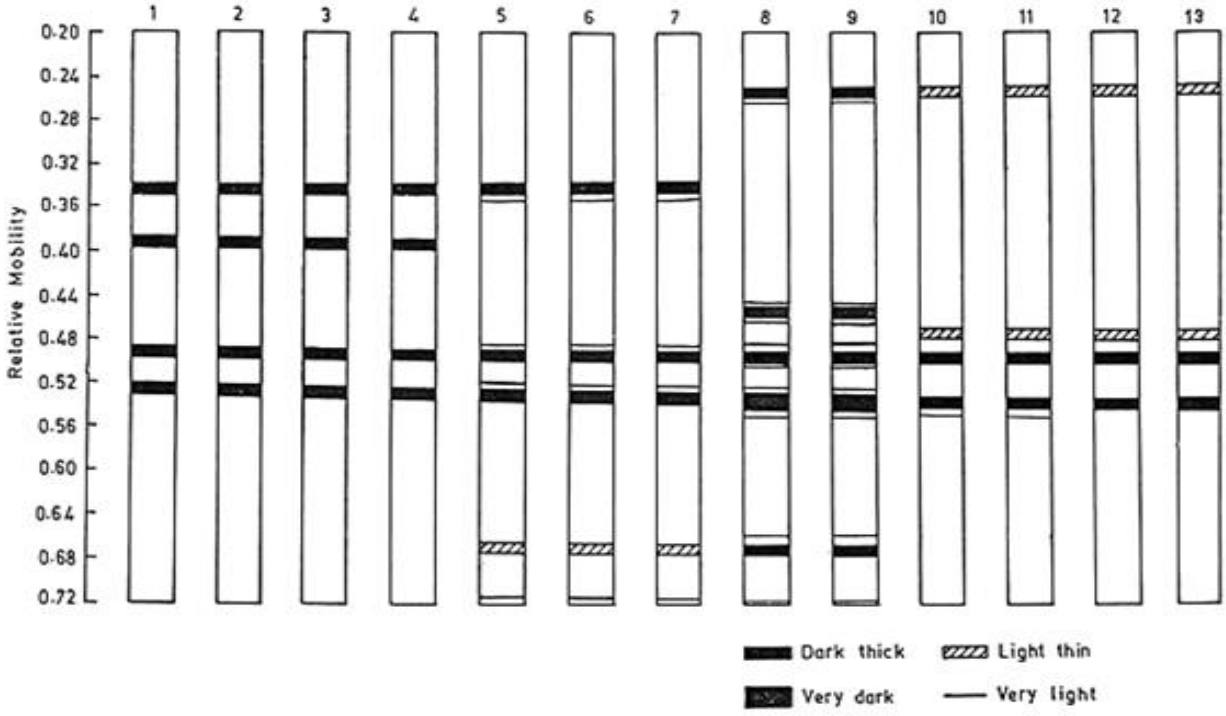
Origin
Muscle
Liver
Heart
Kidney
Eye

Fig.2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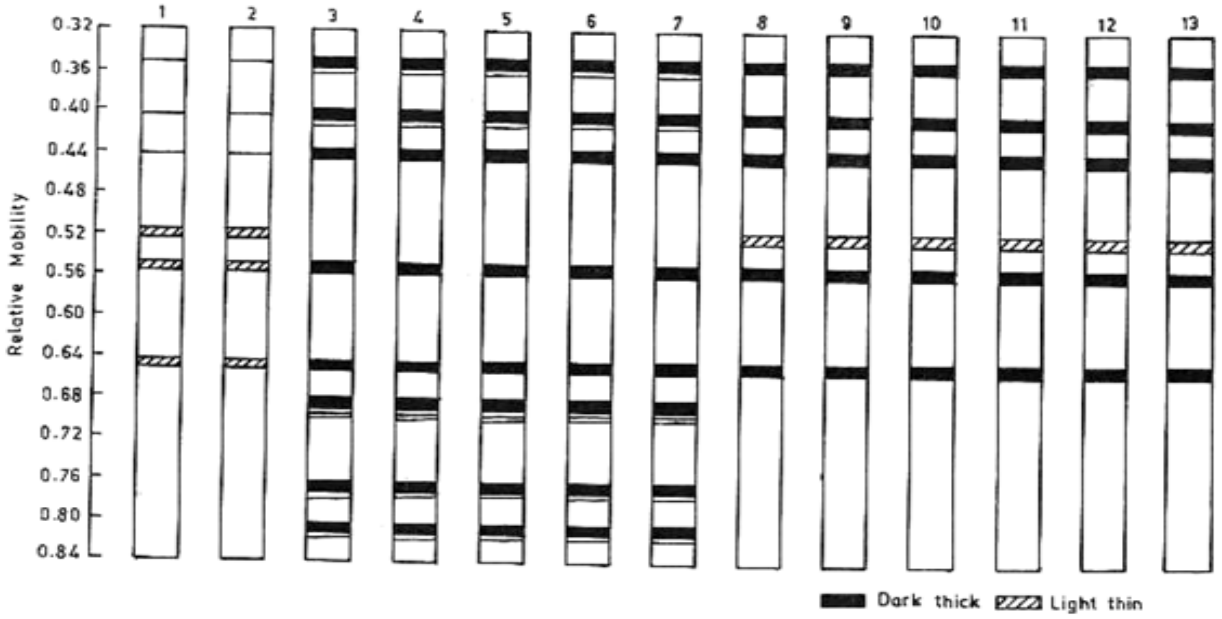
Relative Mobility
Dark thick
Light thin
Very dark
Very light

Fig.3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0.56
0.60
0.64
0.68
0.72
0.76
0.80
0.84
Relative Mobility
Dark thick
Light thin

Fig. 4