BIOCHEMICAL GENETIC STUDIES ON FEW SPECIES OF *CHANNA*

By

QUENDARISA KHARBULI

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Dedicated to my father,

(L) Treslington Khyriem.

"Every good gift and every perfect gift is from above, and comes down from the Father of lights, with whom there is no variation or shadow of turning." (Bible — James 1:17)

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SHILLONG—793 022

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I, Ms. Quendarisa Kharbuli, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University / Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Zoology.

Kharbuli

(Candidate)

B. B. P. Gupta

(Head)

Head
Department of Zoology
North Eastern Hill University
Shillong - 793022

K. Chakraborty

(Supervisor)

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Kharbuli

(Quendarisa Kharbuli)

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INTRODUCTION

Though two-third of our planet is covered by water, most of it supports fish life that comprises almost as many species as all the other vertebrates put together. The fish, one of the first forms of evolutionarily higher life to appear in water, is among the earliest vertebrates. They are cold-blooded, they breathe by means of pharyngeal gills, propelling and balancing themselves by means of fins. About 20,000 species of fish are known to inhabit water bodies of various descriptions. Fishes exhibit a remarkable wide range of biological adaptations to diverse habitats both spatial and temporal. Besides the conventional habitats of lakes, ponds, rivers, rock pools, and the open sea, they are able to sustain life in the deserts, the deep sea, the cold Antarctica, caves and in the warm waters of high alkalinity or of low oxygen. Fish occupy the aquatic environment, but in exceptional cases, they show occasional short-term capabilities for terrestrial and aerial life. Along with these adaptations, evolved, the most impressive specializations of morphology, physiology and behaviour, hence attracting the interest of many, to make a study on various aspects of their lives. Exemplary of such adaptive variations span from the tiny parasite candiru, *Vandellia cirrhosa*, which normally occupies the gill cavity of larger catfish of the Amazon basin, but can

make quite unpleasant alternative choices if human swimmers are available, to monstrous but benign travellers of oceans like the basking shark, *Cetorhinus maximus* (Purdom, 1993).

In developing South Asian countries such as India, Bangladesh, Myanmar, Nepal and Pakistan, fish constitutes one of the main food-item of sustenance for many people. The vast inland areas of these countries depend mainly on freshwater fishes for feeding the populace. Fish provides a staple diet and protein supplement, and the abundant water resources support a good harvest. Over two thousand species of fishes have been recorded in India, of which over nine hundred species are freshwater inhabitants (Jhingran, 1999).

Though Aristotle (384-327 B.C.) is said to be the founder of Ichthyology, knowledge of the occurrence of fish in India dates three millennium B.C (Hora 1920-1955). The first modern writer on Indian fishes, according to Day (1875-1878), was Bloch whose splendid work *Auslandiche Fische* was published in 1785. This work along with his *Ichthyologie*, and its further extension by Schneider in 1801, contain many Indian marine forms. However, studies with a more scientific, more accurate and fulfilling the needs of modern taxonomy on the Indian freshwater fish fauna started only from the nineteenth century.

Beginning with Hamilton-Buchanan's (1822) account of the fishes of the Ganges, followed by McClelland (1839), Sykes (1841), Jerdon (1849) and Blyth (1860), the vast array of fish found in this area came to light.

Day brought out for the first time, the monumental treatise "The Fishes of India" (1875-1878) embodying his own extensive observations and the results of the earlier workers. He included in his work 1418 species found within the boundaries of the present day India, Pakistan, Bangladesh, Myanmar and Sri Lanka. Though Day's work had its own limitations, his monograph is irreplaceable even today. It remains an important reference manual for the Ichthyology of this region and will continue so for many more years to come.

In the last century, valuable contributions to Indian Ichthyology have been made by the indomitable researches of Hora (1920-1955) providing the very first source of reference and further basic information. His works were further extended by many zealous workers, notably Misra (1947-1953), Menon (1951-1974) and Jayaram (1981).

During the late Devonian, the atmospheric oxygen was about one-fifth of its present level, and drying of lagoons, swamps and fresh water bodies was very prevalent. During this period, the lung that

develops as pouches of the pharynx seems to have evolved in the Dipnoi, Crossopterygii and Amphibia. Fange (1976) recorded that during the Cenozoic era, the lungs of the teleostean or bony fishes, which became masters of both fresh as well as seawater, became transformed into a specialized hydrostatic organ the swim bladder. The tertiary and quaternary periods marked a considerable drop in the level of atmospheric oxygen (0.1% PAL) that was found to affect the oxygen content of the water. Under such conditions the gills were unable to sustain the oxygen requirements of these fishes especially in rivers and swamps. Lungs were no longer available for aerial respiration as they had become modified into specialized swim bladder. Some teleostean fishes, represented by the modern forms of *Anabas, Colisa (= Trichogaster), Channa (= Ophiocephalus), Clarias, Heteropneustes, Monopterus (= Amphipnous),* etc., survived because they developed other types of accessory respiratory organs. Studies by Munshi (1980, 1985) showed that many of these air-breathing organs are essentially modifications of the gills. The swim bladder is either absent or very much reduced in these fishes.

Modern air-breathing teleosts have been found to inhabit both fresh water and intertidal environments. Thompson (1969) and

Packard (1974) found that salinity does not seem to have been an important factor, limiting the evolution of air-breathing vertebrates. The aquatic hypoxia in these waters is probably the more important selective force in the evolution of air-breathing vertebrates. A wide variety of teleostean fishes belonging to different orders and families have developed the air-breathing habit. Such adaptations are found among a wide range of groups, distributed mainly in the tropical and sub-tropical regions of South America (Carter and Beadle 1931), Africa and Asia. Few species are also found in Europe and North America.

The commercially important air-breathing fishes of India are *Heteropneustes* (Singhi), *Anabas* (Koi), *Clarius* (Magur) and *Channa* (murrel or snakeheads). They are held in some esteem as food fishes and are generally found in the swamps, marshes and wetlands etc., of Bihar, Bengal and Assam and some parts of South India. These fishes are endowed with remarkable powers of respiration that enable them to lead an amphibious life and to survive the adverse conditions in swampy areas. The extensive swamps, subject to the ravages of the seasons, permit the survival of only a limited number of species. Reclamation of the swamps for carp culture would entail considerable

expenditure, yet what a waste of valuable food sources if these vast areas are not better utilized for the culture of air-breathing fishes. The importance of the problem of culturing and propagating these fishes in swamps need to be recognized and subsequently, techniques developed for the proper utilization of swamps for these purposes. Dehadrai (1978), and Dehadrai and Thakur (1980) suggested that realization of these objectives would have considerable significance in the augmentation of fish production especially in the rural areas.

The murrels are distributed throughout the world except West Europe, North America, South America and Australia. In India, they are available throughout, in ponds as well as streams and rivers, from tropical region to high mountain range. All the representatives are very much alike in colour and shape and therefore, are often difficult to distinguish when they are of the same size.

Though snakeheads are sometimes considered as weed fishes due to their predatory habit on other fish, they certainly provide a second line of production in terms of protein. They have commercial value and some species attain very large size; in addition, they also solve the problem of utilizing swampy areas with least management. However, many a times, they are wrongly identified due to their

morphological similarities. Unless there is a definite key for identification, there will always remain a problem in studying this group of fishes from different biological angles. One such aspect studied with great interest by many biologists is the genetic variation in a population. For nearly fifty years, the workhorse method for revealing genetic variation has been electrophoresis. Protein electrophoresis is among the most cost-effective methods of investigating genetic phenomena at the molecular level. Proteins are composed of amino acids joined by covalent peptide bonds to form polypeptides. These sequences or 'primary structures' are genetically determined. Each of the twenty amino acids has a unique side chain characterized by shape, size and charge. The amino acid sequences of proteins are changed by mutations in the encoding DNA locus. Such mutations alter shape and net charge, as well as catalytic efficiency and stability (Shaw, 1965). Protein electrophoresis aims to reveal as many of these changes as possible. It entails the study of the mobility of soluble proteins primarily enzyme molecules across an electric potential. The basic methodology employs a matrix of gel, such as starch, agar or acrylamide, which may be buffered to a specific pH and to which is applied an electric potential. The material to be analyzed is

loaded over the gel, cast on gel rods, and the specific enzymes are subsequently stained to reveal the position to which they have migrated between the two electrodes.

The technique thus reveals small differences in the rate of migration in an electrophoretic field, between nearly identical macromolecules. If the enzyme present in a sample has an amino acid replacement that results in a difference in the overall ionic change of the molecule, then the enzyme will have a somewhat altered electrophoretic mobility and move at a different rate. The electrophoretic mobility changes because enzymes of the same size and shape move at a rate determined largely by the ratio of the number of positively charged amino acids to the number of negatively charged ones. Electrophoresis can therefore be used to detect a mutation that results in a difference in electrophoretic mobility of the enzyme it encodes.

Since the origin of starch gel electrophoresis (Smithies, 1955) and the histochemical visualization of enzymes on gels (Hunter and Markert, 1957) and the classic studies of Harris (1966) and Hubby and Lewontin (1966) a major revolution in understanding micro- and macro-evolutionary processes have occurred. Using enzymatic and

non-enzymatic proteins, numerous investigations have focussed on enzyme efficiency, estimating and understanding genetic variability in natural populations, gene flow, hybridization, recognition of species boundaries and phylogenetic relationships among other problems. The frequency of such investigations has not waned in recent years but rather has increased as refinements and new methods have been developed.

Two general forms of protein data can be gathered simultaneously using electrophoretic methods. One is derived from isozymes, which are all functionally similar forms of enzymes, including all polymers of subunits produced by different gene loci or by different alleles at the same locus; the other set consists of allozymes, a subset of isozymes, which are variants of polypeptides representing different allelic alternatives of the same gene locus (Markert and Möller, 1959).

The correct application of isozyme data requires that banding patterns observed on gels be correctly interpreted. The most basic assumption that biologists make in using isozyme data is that changes in the mobility of enzymes in an electric field reflect changes in the encoding DNA sequence. Thus if the banding patterns of two

individuals differ it is assumed that these differences are genetically based and heritable (Matson, 1984). Also it is assumed that enzyme expression is codominant, i.e., all alleles at a locus are expressed. To interpret these banding patterns, one must know something about the number of subunits in the enzymes. In addition to biochemical components of gel interpretation, one must be aware of compartmentalization of enzymes, or enzyme activity, in particular organs or organelles. For example, livers may have different enzymes than hearts or brains (Murphy and Matson, 1986). In plants and animals some enzymes are restricted to the cytosol whereas others are found only in the mitochondria and or chloroplast. Cell fractionation studies can demonstrate whether the enzymes are housed in the cytoplasm or one of several separate organelles (Weeden, 1983, Weeden and Wendell, 1989).

For most enzymes the genetic controls are well enough known to allow genetic inferences to be made from gel isozyme patterns. The distribution of isozymes per cell or tissue can be reliably predicted, homozygous or heterozygous individuals can be identified and conclusions about genetic polymorphism, the breeding system of individuals and population structuring can be drawn.

Population geneticists have developed statistical models for interpreting genetic population structure. The most relevant of these to gel interpretation is the Hardy-Weinberg equilibrium principle. Oversimplified, this states that in the absence of selection, drift and migration, the frequencies of alleles in a randomly mating population will maintain a stable equilibrium with genotype frequencies of $AA = p^2$, $Aa = 2pq$, and $aa = q^2$, where p is the frequency of allele A, and q is the frequency of the alternative allele a. Nonconformity to the prediction of Hardy-Weinberg equilibrium indicates that the phenotypic variation has a non-genetic basis or that one or more of the Hardy-Weinberg assumptions is not met in the population. Thus, for example, the individuals may not be randomly mating, or some natural selective force may be acting on the species, or genes from neighbouring populations may be migrating into the study site. If these principles of biochemistry, genetics, and gel interpretation are followed, electrophoresis can yield many valuable insights for the evolutionary biologists. Enzymes that differ in electrophoretic mobility as a result of allelic differences in a single gene are called allozymes. Hence, allozymes variation in a population is an indication of simple Mendelian genetic variation, which is widespread in almost all natural populations.

The earliest account on protein electrophoresis in fish is a comparative serological study by Nuttal (1904) in certain species. For almost fifty years after the first publication, no documentary work was reported in this field, until Connell (1953), demonstrated the pattern of skeletal muscle proteins of codling. Chandrasekhar (1959) initiated such work in India, by investigating the blood protein of five Indian carps belonging to the family *Cyprinidae*. Das (1961) reviewed the blood chemistry of three Indian carps. A similar study by Lillevik and Schleomer (1961) has helped to understand species differentiation in fish.

The revelation by Booke (1964) with regards to variation in fish serum protein has shed light in relation to species specificity, phylogeny and environmental conditions. Further examination by Nyman (1965, 1966) on species-specific proteins in fishes along with intra- and inter-specific variations; strongly suggest their utility as tools in biochemical systematics. Markert and Faulhaber (1965) undertook a comparative study of lactate dehydrogenase isozyme patterns in thirty fish species and showed that all these have one major isozyme system with two minor ones (in eye and gonad) and on this basis they grouped these fishes into four categories. Shaw's (1965) observation on different enzyme systems through electrophoretic

technique, hinted on their significance in biological sciences and research. Nyman (1967) extended his investigation on protein variation in salmonids and likewise, catalogued the species specificity in them. The survey made by Tsuyuki *et al.*, (1968) on protein electrophoresis has greatly contributed to rockfish systematics.

The popularity of isozymes as genetic markers in population studies became obvious in subsequent years, as well as a confinement of electrophoretic investigations to the species level. For example, Chen and Tsuyuki (1970) made a comparative study on the protein electropherograms of *Tilapia mossambica* and *T. melanopleura.* Clayton and Franzin (1970) performed experiment on muscle lactate dehydrogenase isoenzymes of lake whitefish (*Coregonus clupeaformis*), which provided evidence for the probable tetraploid nature of salmonid fishes. The electrophoretic investigation by Dando (1970) on megrim populations from English Channel and approaches revealed polymorphisms of lactate dehydrogenase and glycerol 3-phosphate dehydrogenase isoenzymes. Whitt and Booth (1970) reported the presence of LDH-E_4 isoenzyme in addition to the usual A_4 and B_4 isoenzymes in the eye tissue of *Xiphophorus helleri* that suggested its

importance in the biochemistry of vision. Bailey *et al.*, (1970) recorded multiple forms of supernatant malate dehydrogenase in salmonid fishes. Dando (1971) has detected species-specific isoenzymes in heart and muscle extracts while studying lactate dehydrogenase polymorphism in flat fish (*Heterostomata*), with individual variations in certain species. Stegeman and Goldberg (1971) investigated the distribution and characterization of hexose 6-phosphate dehydrogenase in trout and in the subsequent year revealed its polymorphism in brook trout. The presence of mono- and poly-morphic protein loci in the population of tetraploid salmon species *Onchorhynchus keta* was reported by Altukhov *et al.*, (1972). The biochemical genetics of the Atlantic salmon *Salmo salar* have been investigated with significance to population identifications. Kirpichnikov (1973) reported the biochemical polymorphism and microevolution in fishes. Yamauchi and Goldberg (1973) performed an isozymic and immunological study of glucose 6-phosphate dehydrogenase in brook trout and splake trout, and in the following year they revealed the asynchronous expression of glucose 6-phosphate dehydrogenase in splake trout embryos. A comparative

analysis of the tissue proteins in some catfishes was reported by Hussain and Siddique (1974). Utter *et al.*, (1974) conducted biochemical genetic studies with reference to fish potentialities and limitations. Basasibwaki (1975) made a comparative study of the electrophoretic patterns of lactate dehydrogenase and malate dehydrogenase isoenzymes in five Lake Victoria cichilid species.

Cederbaum and Yoshida (1976) studied the activity of glucose 6-phosphate dehydrogenase in rainbow trout. Krishnaja and Rege (1979) conducted electrophoretic studies of two species of Indian carps and their fertile hybrids. Siddique (1977) undertook a comparative study of plasma proteins of four air-breathing freshwater fishes. The detection of isozyme loci in brown trout *(Salmo trutta)* through interpretation from population data was done by Allendorf *et al.*, (1977). Frankel (1978) studied the gene activity of alcohol dehydrogenase in danio hybrids. Busack *et al.*, (1979) recorded electrophoretic variation and differentiation in four strains of domesticated rainbow trout *Salmo giardneri.* Cross *et al.*, (1979) reported a duplicate loci and allelic variation for mitochondrial malic enzymes in the Atlantic salmon *Salmo salar.*

Winans (1980) examined the geographic variation in the milkfish *Chanos chanos* using biochemical genetic parameters. Utter *et al.*, (1980) investigated the population structure of indigenous salmonid species of Pacific Northwest based on genetic variation of protein. Ramanujam and Ratha (1980) examined glucose 6-phosphate dehydrogenase and lactate dehydrogenase activities in two air-breathing and in two gill-breathing species of fish. Basu *et al.*, (1981) analyzed the egg protein in *Notopterus notopterus* and *Mystus vittatus.* The electrophoretic analysis of tissue proteins by Ferguson (1981) indicated the systematics of Irish charr. Whitt (1981) analyzed the differential gene expression of enzymes to throw light on developmental genetics of fishes.

Ståhl and Ryman (1982) have studied the simple Mendelian inheritance of a locus coding for α–glycerophosphate dehydrogenase in brown trout *Salmo trutta.* Dhar and Chatterjee (1982) reported protein variations in two species of *Channa* through electrophoretic investigations. Buth (1983) studied the duplicate isozyme loci in fishes, and their origins, distribution, phyletic consequences and locus nomenclature. McAndrew and Majumdar (1983) applied

electrophoretic markers for stock identification of *Tilapia.* Whitt (1984) investigated the lactate dehydrogenase isozyme system in understanding developmental genetics and evolutionary aspects. Dhar and Chatterjee (1984) examined two species of *Channa* electrophoretically and reported protein variation in *Channa punctatus* and *Channa striatus.*

Frankel (1985) has studied the asynchronous expression of alcohol and supernatant malate dehydrogenase loci during the development of hybrid *Barbus.* Triveni and Rao (1986) recorded the activity of lactate dehydrogenase isozyme in two Cyprinids. Baldwin and Lake (1987) showed that the lactate dehydrogenase homopolymer of hagfish heart and the single lactate dehydrogenase of lampreys display greater immunochemical similarity to LDH-C_4 than LDH-B_4 of teleost. Whitt (1987) reported species differences in isozyme tissue-pattern hence confirming their utility for systematic and evolutionary analysis.

Chatterjee *et al.,* (1988) observed a specific locus of lactate dehydrogenase in the kidney of *Clarius batrachus.* Tripathi and Shukla (1988) made a comparative study on the skeletal muscle- and

liver-lactate dehydrogenase of *Clarius batrachus.* The expression of isozyme in bighead carp, silver carp, and their reciprocal hybrids was observed by Brummett *et al.,* (1988). Padhi and Khuda-Bukhsh (1989) investigated the pattern of lactate dehydrogenase isozyme in four species of *Mugil.* Ropson and Powers (1989) examined the physical characteristics and kinetic properties of the allelic isozymes of hexose 6-phosphate dehydrogenase of the teleost *Fundulus heteroclitus.* Basaglia (1989) studied on some aspects of the isozymes of lactate dehydrogenase, malate dehydrogenase and glucose phosphate isomerase in fish. Chatterjee (1989) reported the cytotaxonomic and electrophoretic investigations on Indian air-breathing fishes. The investigation on the electrophoretic pattern of xanthine dehydrogenase in twenty species of teleostean fishes by Padhi and Khuda-Bukhsh (1990) revealed the tissue distribution and their possible taxonomic significance. Coppes (1990) reported the divergence of duplicate gene in three species of *Scianid.* Coppes *et al.,* (1990) revealed the multilocus isozymes in fishes. Buth *et al.,* (1991) performed molecular and cytological investigations in Cyprinid fishes. The genetic characterization of the Atlantic salmon

Salmo salar lines, farmed in Ireland was undertaken by Cross and Challanain (1991). Coppes (1992) studied the lactate dehydrogenase in teleost and suggested the role of LDH-C_4 isozymes in them.

Lee *et al.*, (1993) biochemically identified two closely related gobies *Yongeichthys caninus* and *Y. nebulosus* from Taiwan. Engelbrecht and Van Der Bank (1994) analyzed the isozyme and allozyme differences in four shortfin barb *Barbus brevipinnis.* Grobler and Van Der Bank (1994) showed the allozyme polymorphism and phenotypic variation in the African catfish *Clarius gariepinus.* Karakousis *et al.*, (1995) suggested the phylogenetic relationship of *Barbus peloponnesius* from Greece and other species of *Barbus* as revealed by allozyme electrophoresis. The systematics and biogeography of snubnose darters *Etheostoma* from the Black Warrior River system was reported by Clabaugh *et al.*, (1996). Palanichamy (1996) conducted cytogenetic and genetic studies on the chocolate mahseer *Acrossocheilus hexagonolepis.*

Faundez *et al.*, (1997) showed allozyme variability in the Chilean brown trout *Salmo trutta.* Sengupta and Chatterjee (1998) studied the biochemical genetic aspect of chocolate mahseer

Neolissochilus hexagonolepis. Ramirez *et al.*, (1998) experimented the karyological, biochemical and physiological aspects of *Callophysus macropterus* from the Solimoes and Negro Rivers located in the Central Amazon. Basaglia (2000) showed the distribution of ten isozymes and their loci in South American lungfish *Lepidosiren paradoxa.* The biochemical polymorphism observed in the yellow catfish *Mystus nemurus* from Thailand was reported by Leesa-Nga *et al.*, (2000). Kharbuli and Chatterjee (2001) reported the genetic expression of glucose 6-phosphate dehydrogenase in *Channa* species. Papasotiropoulos *et al.*, (2001) used the allozyme data to show the genetic divergence and phylogenetic relationship in grey mullets. Gharrett *et al.*, (2001) used a genetic marker to examine the genetic interaction among the subpopulation of pink salmon *Oncorhynchus gorbuscha.* Lin *et al.*, (2002) examined the phylogenetic relationships, biochemical properties of the duplicated cytosolic, and mitochondrial isoforms of malate dehydrogenase from a teleost fish, *Sphyraena idiastes.* A comparison of liver enzymes in osmerid fishes by Treberg *et al.*, (2002), revealed key differences between two species, the rainbow smelt (*Osmerus mordax*) which accumulate glycerol and

capellin (*Mallotus villosus*) that does not accumulate glycerol. A study on the duplication and divergence in actinopterygian fish by Merritt and Quattro (2003) provided an understanding on the evolution of the vertebrate cytosolic malate dehydrogenase gene family.

Though a number of publications has been recorded above, compilation of all the literature will always remain inadequate. The frequency of such investigations has not waned in recent years, but rather has increased as refinements and new methods have been developed. Keeping this in mind we have undertaken the genetic study of eleven enzymes in three species of *Channa* viz., *C. orientalis* Bloch and Schneider (1801), *C. punctatus* (Bloch, 1793) and *C. striatus* (Bloch, 1793).

MATERIALS AND METHOD

2.1 Material:

Three species belonging to the genus *Channa* viz. *Channa orientalis* (**Fig. 2.1a**), *C. punctatus* (**Fig. 2.1b**), and *C. striatus* (**Fig. 2.1c**) were chosen for the present study.

2.2 Systematic Position:

Kingdom	-	Animalia
Sub-kingdom	-	Metazoa
Phylum	-	Chordata
Group	-	Vertebrata / Craniata
Sub-phylum	-	Gnathostomata
Series	-	Pisces
Class	-	Teleostomii
Sub-class	-	Actinopterygii
Order	-	Channiformes
Family	-	Channidae
Genus	-	*Channa*
Species	-	*C. orientalis* Bloch & Schneider, 1801
		C. punctatus (Bloch, 1793)
		C. striatus (Bloch,1793)

Fig. 2.1(a) *Channa orientalis*

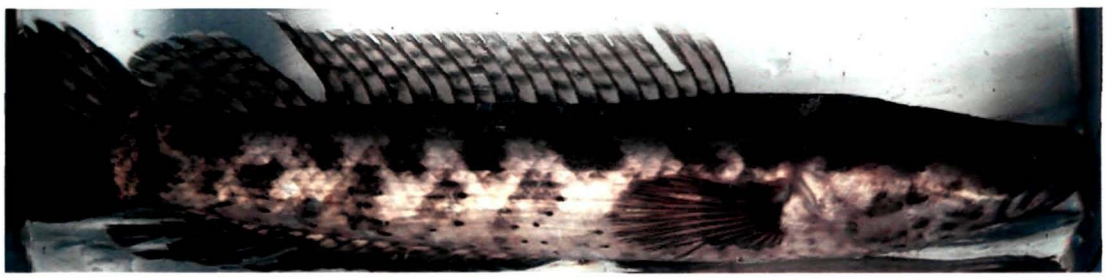


Fig. 2.1(b) *Channa punctatus*

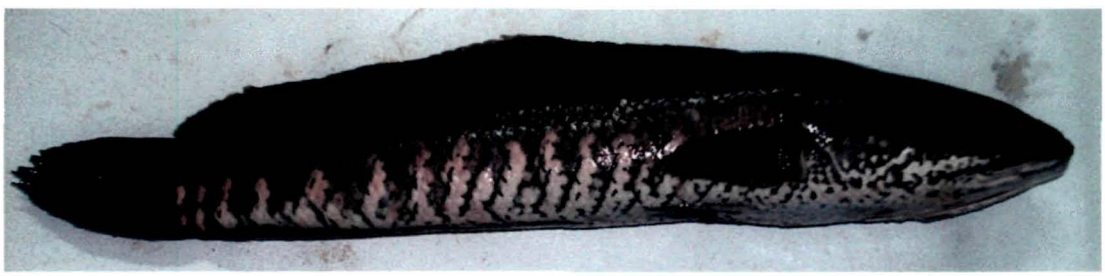


Fig. 2.1(c) *Channa striatus*

The specimens, collected live from the bheels of Assam and ponds around Shillong, were transported to the laboratory. Some were kept alive while in the rest, their tissues were dissected out and stored at -40°C in the ultra freezer (Scien Temp 2000, USA) until use. To avoid ontogenic problems only adult specimens were used for tissue extractions.

2.3 Preparation of Tissue homogenate:

Each individual was dissected and tissues such as brain, eye, heart, kidney, liver and muscle were excised, blotted dry and weighed accurately. Each tissue was placed in glass homogenizing tubes, containing measured volume of ice-cold 0.25 (M) sucrose solution, and homogenized in an electric homogenizer. For mitochondrial MDH and ME we used 0.1(M) phosphate buffer pH 7.4. The homogenates were immediately transferred into polypropylene centrifuge tubes (15 ml).

2.4 Extraction of samples:

The tissue extracts were obtained by centrifuging the homogenates at approximately 14,000 X g for 20 minutes in the cooling centrifuge (Beckman J2-HS-Centrifuge USA). This removes most of the cellular debris and unhomogenized tissue fragments. The resultant clear supernatant was decanted in clean centrifuge tube

and subjected to electrophoresis. For mitochondrial extraction, the homogenates were first centrifuged at 600 X g for 10 minutes. To the clear supernatant, equal volume of triton X was added and mixed thoroughly. It was then left for 30 minutes after which each extract was sonicated for 30 seconds. They were then subjected to centrifugation at 14,000 X g for 30 minutes. The resultant clear supernatant was decanted in clean centrifuge tube and subjected to electrophoresis.

All the above procedures were carried at 4°C in ice to prevent denaturing of the enzymes.

2.5 Electrophoresis:

When a particle of effective electrical charge Q is forced to migrate in a viscous medium (liquid or gel) by action of an electrical field (Potential gradients) E this process is generally defined as electrophoresis. The driving force, which acts on the particle migrating with constant velocity, is equal to the frictional f, which the particle must overcome in the medium:

$$QE = f$$

The electrophoretic mobility m of a particle is defined by:

$$m = \frac{d}{tE} = \frac{v}{E} = \frac{Q}{f} \quad \left[\frac{cm^2}{volt.cm}\right]$$

where, d represents the migration distance of the particle in time t.

The disc electrophoresis developed by Davis (1964) deals with a discontinuous separation system with regards to pH value, buffer composition and gel pore size to create discontinuous voltage and pH gradients in which polyacrylamide gel serves as the matrix.

Prior to separation, these discontinuities produce very thin, i.e. highly concentrated, starting zones, which determine the sharpness of the separations. Three physical effects are responsible for the high resolving power of the disc electrophoresis. The concentrating effects according to Kohlrausch's (1897) regulating function, by which the sample components are concentrated in large–pore spacer (stacking) gels to form sharply defined zones prior to their separation. The molecular sieving effects observed by Smithies (1962) in which individual molecules are electrophoretically separated on the basis of their size (molecular weight) and shape or tertiary structure and the

electrocharge effect by which the sample molecules are fractionated according to their net electric charge.

Polyacrylamide gel is the polymerization and cross-linking product of the monomer acrylamide, CH_2=CH-CO-NH_2, and a cross-linking co-monomer, usually N, N'-methylene-bis acrylamide (Bis), CH_2=CH-CO-NH-CH_2-NH-CO-CH=CH_2 (Raymond, 1959; Bloemendal *et al.*, 1962; and Ott, 1963). The three-dimensional network of the gel is formed by cross-linking of polyacrylamide chains growing side-by-side by the mechanism of vinyl polymerization (co-polymerization in solution). This leads to the development of numerous, random polymer gel coils in which the polyacrylamide chains assume a state of maximum entropy, i.e. the most irregular shape. The growing coils move together and are crossed-linked by main valencies, where bifunctional compounds, such as N, N'-methylene-bis-acrylamide, are build into the polymer chains as cross-linking agents and can react with free functional groups at terminals of other chains. Ott (1963) and Ornstein (1964) found that the concentration of monomer and co-monomer in the gelating solution and the degree of polymerization (chain length) and cross-linking (i.e. the quantity of built-in cross-linker) determine the density, viscosity, elasticity and mechanical strength of the gel.

Usually certain catalyst-redox systems, which furnish free radicals, are used for the polymerization of polyacrylamide gels for electrophoresis (Logemann, 1961). For example: Ammonium-persulphate-N, N, N, N'-tetramethylethylene-diamine (TEMED) polymerizes the gel chemically, while Riboflavin-TEMED brings about photochemical polymerization.

Gels are distinguished from liquid media by high viscosities and high frictional resistances. These supporting media not only prevent convection and minimize diffusion, but also actively participate in the separation process by interacting with the migrating particles. This interaction depends on the particle size. Consequently, in gels the particles are separated according to both charge and size. The property of gels to distinguish molecular species of different sizes is ascribed to their size sieving capacity. Therefore this phenomenon has been termed "molecular sieving effect".

Polyacrylamide gel being a synthetic polymer, under constant conditions and with chemically defined components of relatively high purity, can always be prepared in a reproducible manner.

Polyacrylamide gel electrophoresis (PAGE) is a rapid and accurate method to determine the relative size and molecular weights

of macromolecular ions such as proteins and nucleic acids. The method offers several advantages over classical procedures of Mol.Wt. determination. However, according to Kingsbury *et al.*, (1970), careful standardization of the technique is essential and not only should the conditions of electrophoresis be standardized, but also those of gel formation.

2.5a Equipments:

Gel tubes preferably made from low-alkali glass and having a length of 65-70 mm and an inside diameter of 5 mm (o. d. about 7 mm) are used. The apparatus consists primarily of an upper and a lower buffer reservoir and a lid with platinum electrodes mounted therein. The upper buffer reservoir is punched and silicone-rubber grommets are inserted into the holes to hold the gel tubes. The additional equipments comprises of electrode cables, a suitable power supply (Systronic 610), racks upon which the gel tubes are mounted for loading, a polymerizing lamp, and various accessories for handling the solutions and gels. The latter include micropipettes (1000 µl) equipped with thin flexible tubing and a stainless steel injection needle (about 8 cm long) with the tip blunted and filed smooth for gel removal. Equipment for staining and destaining and photographing the stained bands complete the required equipments.

2.5b Reagents:

1. Reagent A (pH 8.9)

(i). 1(N) HCl (8.734 in 100 ml distilled water)	24.0 ml
(ii). Tris (hydroxymethyl) methylamine	36.600 g
(iii). N, N, N,' N'- Tetramethylethylenediamine (TEMED)	0.230 µl

2. Reagent B (pH 6.7)

(i). 1(N) HCl (8.734 in 100 ml distilled water)	48.0 ml
(ii). Tris (hydroxymethyl) methylamine	5.980 g
(iii). N, N, N,' N'-Tetramethylethylenediamine (TEMED)	0.460 µl

3. Reagent C

(i). Acrylamide	30.000 g
(ii). Bis (N, N'-methylene-bis-acrylamide)	0.800 g

4. Reagent D

(i). Acrylamide	10.0 g
(ii). Bis (N, N'-methylene-bis-acrylamide)	2.5 g

5. Reagent E

(i). Riboflavin	0.004 g

6. Reagent F

(i). Sucrose	40.0 g

7. Reagent G

(i). Ammonium persulphate 0.140 g

The volume of the above reagents was made to 100 ml with distilled water.

Stock buffer solution 1(M) Tris Glycine (pH 8.3).

(i). Tris (hydroxymethyl)methylamine 6.000 g

(ii). Glycine 28.800 g

For electrophoretic reservoir 10% of the stock buffer solution was used.

Destaining solution (7% Acetic acid).

(i). Acetic acid 70.0 ml

The above two solutions were diluted with distilled water to 1000 ml.

2.5c Composition of the gel system:

Separation gel was made just before casting by mixing the reagents in the following proportion (mixing ratio v/v).

Reagent A	1 part
Reagent C	2 parts
Reagent G	4 parts
Distilled water	1 part

Spacer gel system was prepared by mixing reagents in the following proportion (v/v).

Reagent B	1 part
Reagent D	2 parts
Reagent E	1 part
Reagent F	4 parts

Disc electrophoresis was carried out with small columns of polyacrylamide gel consisting of three parts:

(i). A large-pore sample and anticonvection gel containing the sample solution;

(ii). A large-pore spacer or stacking gel in which the sample constituents are concentrated;

(iii). A small-pore separation or running gel in which the sample constituents are separated.

2.5d Making the Gel:

The gel tubes were sealed with parafilm at one end and fixed in a gel rack. About 1-1.5 ml (up to 7 cm in length of the gel tubes) of the separation gel solution was poured in them. Care was taken to prevent formation of air bubble in the gel tube column. The gel solutions were layered with water to prevent adhesion forces between

the gel solution and glass, which lead to the formation of a meniscus, thus producing the smoothest possible gel surfaces. The gels were polymerized using a polymerizing lamp, which is completed in about 45 minutes to 1 hour. The water was carefully discarded and to the polymerized separation gels are pipetted about 200µl of the spacer gel and carefully layered with water. This was left to polymerize for 15-20 minutes in sunlight, after which the water was discarded.

2.5e Loading and running the samples:

The gel tubes were carefully removed from the rack and fixed vertically into the rubber grommet of the upper electrode reservoir. They must fit firmly into the rubber grommets of the electrode reservoir without leakage. Precooled (4°C) electrode buffer solution (1M Tris-glycine, pH 8.3) was slowly poured into the lower electrode reservoir. Special care was taken to prevent formation of air bubbles at the electrode and tube rims. The upper chamber was slowly lowered until the tubes were immersed to a depth of about 4 cm in the buffer solution of the lower chamber. Required concentration of the samples were pipetted over the spacer gel and the remaining space layered with buffer. The upper chamber was then filled with buffer and a drop of

1% Bromophenol blue was added, which serves as an indicator of the migrating boundary during electrophoresis. The apparatus was then placed in the refrigerator at 4°C, the electrodes were connected to the power supply. The current was initially adjusted to 1.5 mA/gel tube for 15 minutes then increased to 3 mA/gel tube. After the requisite time of running, the power supply was disconnected and the gel tubes removed from the upper chamber. The gels were carefully removed from the tubes with the help of a long needle and water forced from a syringe. These were immediately subjected to specific staining solution to obtain different isozyme banding patterns.

2.5f Enzyme staining recipe:

The gels were incubated with specific stains at 37°C in the incubator till blue bands appear. They were washed with distilled water and then preserved in 7% acetic acid that also acts as a destaining solution. The staining procedure as described by Shaw and Prasad (1970) and Pasteur *et al.*, (1988) was followed with slight modification.

1. Alcohol dehydrogenase (ADH, EC No. 1.1.1.1)

NAD^+ (Nicotinamide adenine dinucleotide)	0.080 g
PMS (Phenazine methosulphate)	0.001 g
NBT (Nitroblue tetrazolium)	0.0008 g
0.5 (M) Tris-HCl buffer pH 7.1	2.5 ml
Ethanol	1.0 ml

2. Glucose dehydrogenase (GD, EC No. 1.1.1.47)

NAD^+	0.020 g
NBT	0.015 g
PMS	0.0008 g
D-Glucose	0.721 g
0.01(M) Tris-HCl pH 8.0	40.0 ml

3. Glucose 6-Phosphate dehydrogenase (G6PD, EC No. 1.1.1.49)

$NADP^+$	0.020 g
NBT	0.015 g
PMS	0.0008 g
Glucose 6-phosphate	0.040 g
0.01(M) Tris-HCl pH 8.0	40.0 ml

4. Glutamate dehydrogenase (GDH, EC No. 1.4.1.2)

NAD^+	0.024 g
NBT	0.012 g
PMS	0.0008 g
Sodium glutamate	0.338 g
0.1 (M) phosphate buffer pH 7.5	10.0 ml

5. Glycerol 3-phosphate dehydrogenase (αGPD, EC No. 1.1.1.8)

NAD^+	0.020 g
NBT	0.012 g
PMS	0.0003 g
α–Glycerophosphate	0.200 g
0.5 (M) Tris-HCl buffer pH 7.1	0.5 ml

6. Hexose 6-phosphate dehydrogenase (H6PD, EC No. 1.1.1.47)

$NADP^+$	0.020 g
NBT	0.015 g
PMS	0.0008 g
Galactose 6-phosphate	0.040 g
0.01(M) Tris-HCl pH 8.0	40.0 ml

7. Lactate dehydrogenase (LDH, EC No. 1.1.1.27)

NAD^+	0.080 g
NBT	0.008 g
PMS	0.001 g
0.5 (M) Tris-HCl buffer pH 7.1	2.5 ml
1 (N) Lithium lactate solution	0.5 ml

8. Malate dehydrogenase (MDH, EC No. 1.1.1.37)

NAD^+	0.080 g
NBT	0.008 g
PMS	0.001 g
0.5 (M) Tris-HCl buffer pH 7.1	2.5 ml
1(N) Malic acid solution	0.5 ml

9. Malic enzyme (ME, EC No. 2.7.5.1)

$NADP^+$	0.080 g
PMS	0.001 g
NBT	0.008 g
0.5 (M) Tris-HCl buffer pH 7.1	2.5 ml
1(N) Malic acid solution	0.5 ml

10. Sorbitol dehydrogenase (SDH, EC No. 1.1.1.14)

NAD^+	0.040 g
NBT	0.005 g
PMS	0.0008 g
Sorbitol	0.200 g
0.05(M) Tris-HCl pH 8.0	40.0 ml

11. Xanthine dehydrogenase (XDH, EC No. 1.1.1.204)

NAD^+	0.024 g
NBT	0.012 g
PMS	0.001 g
1(M) hypoxanthine	1.2 ml
0.5 (M) Tris-HCl buffer pH 7.1	8.0 ml

2.6 Documentation of results:

At the completion of staining, the isozyme patterns should be documented by photography or zymogram i.e., by drawing observed patterns on paper. The nomenclature used for the enzymes follows the proposal of Shaklee *et al.*, (1990), according to which, the loci are designated by capital letters in italics followed by a number and an asterisk (for lactate dehydrognase and malate dehydrogenase the loci are designated by capital letters followed by another capital letter). The locus and the allele that codes for the less anodic isozyme were designated by *1* and *a*, respectively.

OBSERVATIONS

3.1 *Channa orientalis*

A summary of the electrophoretic expression of the eleven enzymes analysed is given in **Table 3-1**.

3.1.1 Alcohol dehydrogenase enzyme showed high activity in liver extracts of all individuals examined. The tissue exhibited two phenotypes in the natural population, a single-banded (**Fig. 3.1a**) and three-banded (**Fig. 3.1b**) pattern . Brain, eye, heart, kidney and muscle tissues, on the other hand, recorded low activity of ADH. The bands for ADH appeared at the cathodal region of the gels.

3.1.2 All the individuals examined for glucose dehydrogenase enzyme exhibited a single darkly stained band in liver tissue. This band showed a cathodal migration. A faint band was also observed in the rest of the tissues at the same position as liver (**Fig. 3.1c**).

3.1.3 One band showing a cathodal mobility was common to all the six tissues investigated for glucose 6-phosphate dehydrogenase. The band was found to be stable in brain, eye, heart, kidney and liver while in muscle its frequency of occurrence was low. A second band with high anodal mobility was also observed, in heart, kidney and liver extracts in all individuals studied. Liver exhibited an additional band

with a slightly lower mobility than the second band. This band was also detected in kidney and heart of few individuals. All the bands scored for G6PD in the six tissues displayed a high staining intensity (**Fig. 3.1d**).

3.1.4 Glutamate dehydrogenase showed two intensely stained bands in liver extracts of all the egg bearing individuals examined (**Fig. 3.1e**). Brain, eye, heart, kidney and muscle tissues, on the other hand, exhibited no activity of GDH. The males seemed to lack the activity of this enzyme in all the six tissues (**Fig. 3.1f**).

3.1.5 A single intensely stained band was observed at the cathodal region for α-Glycero 3-phosphate dehydrogenase enzyme in liver tissue only (**Fig. 3.1g**). No activity of αGPD was detected in brain, eye, heart, kidney and muscle tissues.

3.1.6 Hexose 6-phosphate dehydrogenase enzyme revealed a single band with a cathodal mobility in brain, eye, heart and kidney but in liver three bands were observed. The first band in liver was stained intensely and had the same mobility as that of brain, eye, heart and kidney. The two additional bands showed a high anodal mobility, and are moderately stained as that of brain, eye, heart and kidney. Muscle

tissue recorded the weakest activity for H6PD (**Fig. 3.1h**).

3.1.7 Two zones of activity were observed for lactate dehydrogenase. The bands at the most cathodal region of the first zone showed variation in the staining intensity and appeared diffused in brain, eye, heart, kidney and liver, while in white skeletal muscle it appeared as a faint band. The bands at the less cathodal region, on the other hand, appeared distinct in all the six tissues, with the highest intensity seen in white skeletal muscle. The second zone of LDH activity was found to be restricted to the eye tissue only. It appeared as a single moderately stained band with high anodal mobility. Additional faint bands were also observed intermediate between these two zones in this tissue (**Fig. 3.1i**)

3.1.8 For malate dehydrogenase two zones of activity was noted. The more cathodal zone showed activity only in liver with moderately stained band. At the more anodal zone two deeply stained bands were observed in all six tissues. White skeletal muscle, on the other hand, exhibited an additional band with a more anodal mobility than the above two mentioned bands and stained moderately (**Fig. 3.1j**).

3.1.9 Two zones of activity were noted for malic enzyme. The first zone consists of a single faint band with a cathodal migration. It was observed in heart and muscle tissues of all individuals but in brain, eye, kidney and liver its appearance was variable. The second zone was found to be variable in the number of bands as well as staining intensity in the different tissues. Eye, heart and muscle tissues showed four, while brain, kidney and liver showed three asymmetrical bands (**Fig. 3.1k**).

3.1.10 Four bands with a cathodal mobility were prominent in liver tissue for sorbitol dehydrogenase. Faint bands were also observed in brain, eye, heart, kidney and muscle tissues (**Fig. 3.1l**).

3.1.11 Xanthine dehydrogenase enzyme showed a single anodally migrating band of medium intensity in liver tissue. Low activity of XDH was also observed in brain, eye, heart, kidney and muscle tissues. All individuals examined showed the same staining intensity and mobility for this enzyme (**Fig. 3.1m**).

Expression of ADH in
C. orientalis

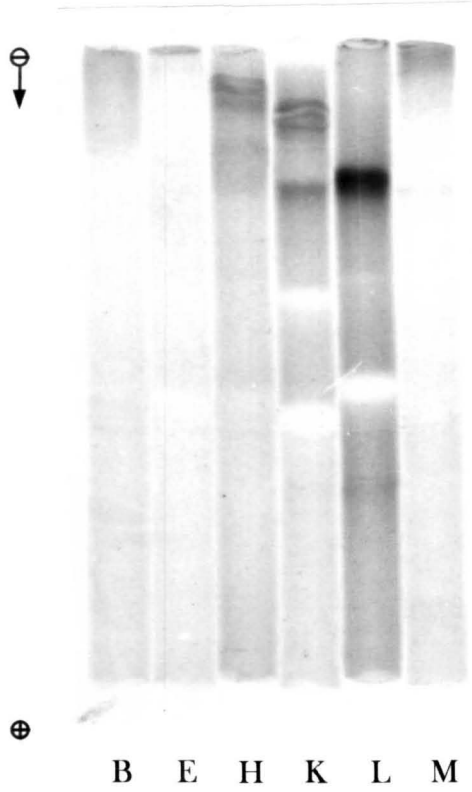


Fig. 3.1(a)
(Single-banded pattern)

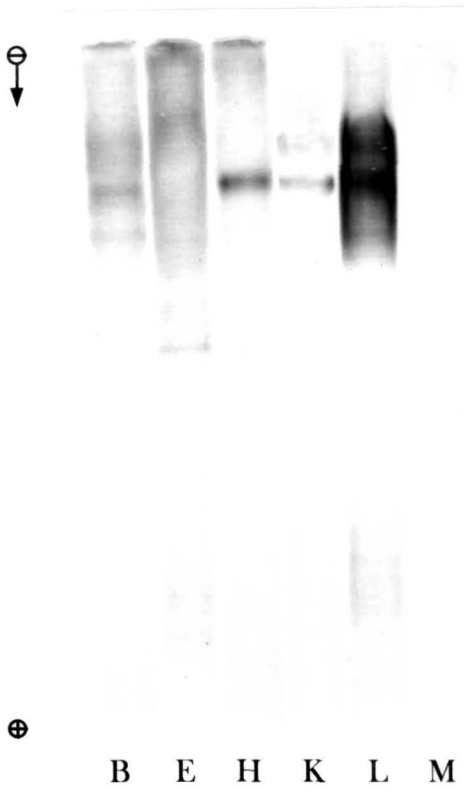
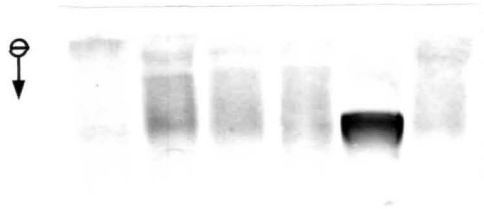


Fig. 3.1(b)
(Three-banded pattern)

Expression of GD in
C. orientalis

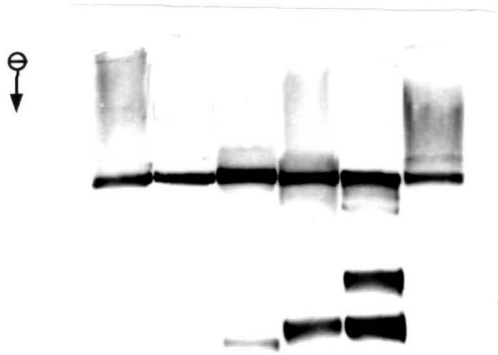


⊕

B E H K L M

Fig. 3.1(c)

Expression of G6PD in
C. orientalis



⊕

B E H K L M

Fig. 3.1(d)

Expression of GDH in *C. orientalis*

(Female)

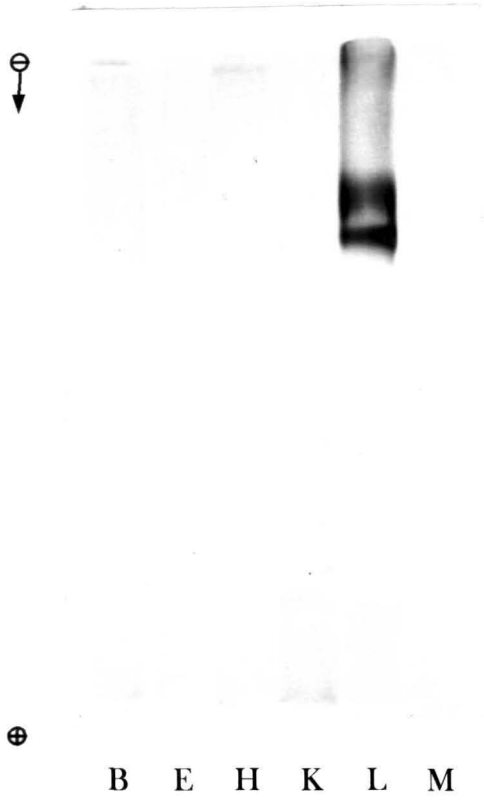


Fig. 3.1(e)

(Male)

Fig. 3.1(f)

Expression of αGPD in
C. orientalis

Fig. 3.1(g)

Expression of H6PD in
C. orientalis

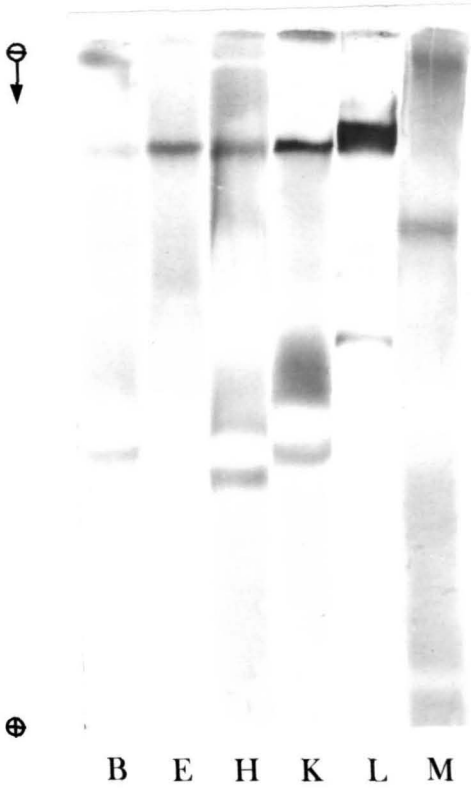


Fig. 3.1(h)

Expression of LDH in
C. orientalis

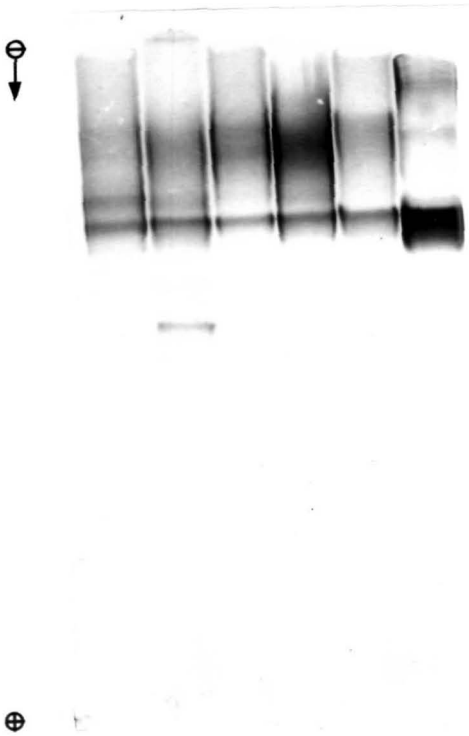


Fig. 3.1(i)

Expression of MDH in
C. orientalis

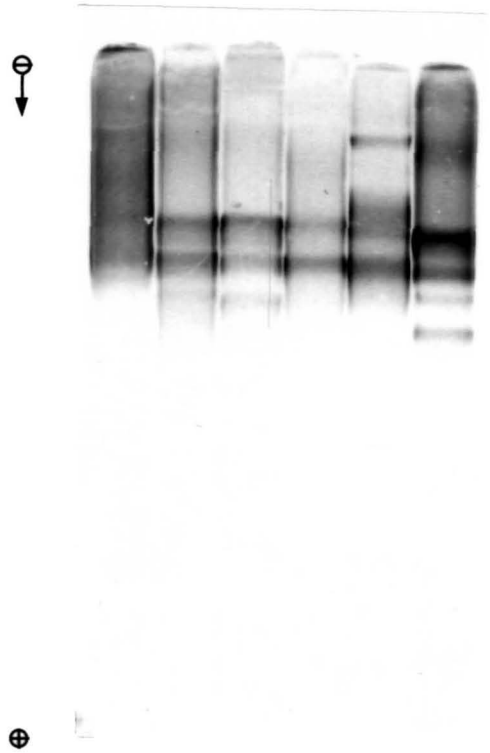


Fig. 3.1(j)

Expression of ME in
C. orientalis

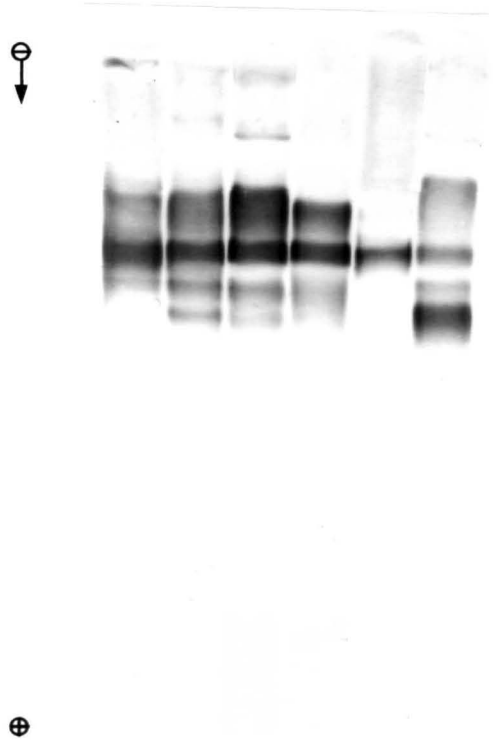
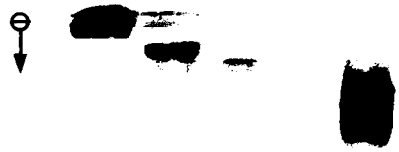


Fig. 3.1(k)

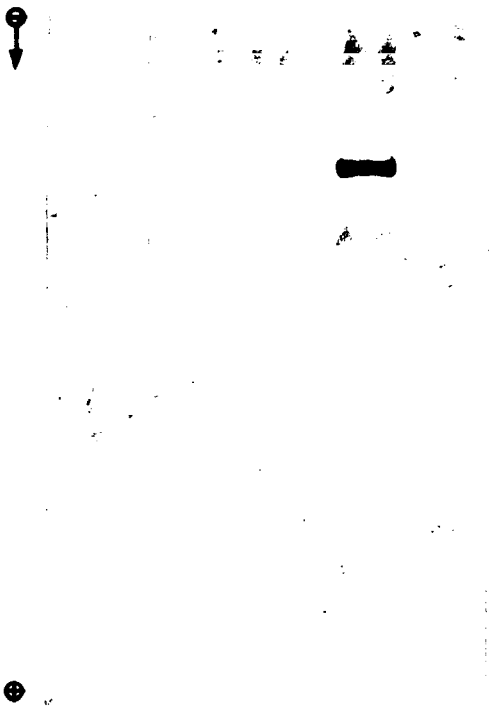
Expression of SDH in
C. orientalis



B E H K L M

Fig. 3.1(l)

Expression of XDH in
C. orientalis



B E H K L M

Fig. 3.1(m)

TABLE 3-1

Summary of electrophoretic expression of the eleven enzymes analyzed in *Channa orientalis* :

Sl. No	Protein	EC No.	Protein Structure	Loci	Activity					
					B	E	H	K	L	M
1	Alcohol dehydrogenase (ADH)	1.1.1.1	Dimer	*ADH-A*(P)*	+	+	+	++	+++++	+
2	Glucose dehydrogenase (GD)	1.1.1.47		*GD*	+	+	+	+	+++++	+
3	Glucose 6-phosphate dehydrogenase (G6PD)	1.1.1.49	Dimer	*G_6PD-1**	+++	+++	+++	+++	+++	+++
				G_6PD-2(P)*	+	+	+++	+++	+++++	+
4	Glutamate dehydrogenase (GDH)	1.4.1.2	Monomer	**Female**						
				*GDH-1**	-	-	-	-	+++++	-
				*GDH-2**	-	-	-	-	+++++	-
				Male	-	-	-	-	-	-
5	Glycerol 3-phosphate dehydrogenase (αGPD)	1.1.1.8	Dimer	*αGPD*	-	-	-	-	+++++	-
6	Hexose 6- phosphate dehydrogenase (H6PD)	1.1.1.47	Dimer	*H_6PD-1**	+++	+++	+++	+++	+++++	+
				H_6PD-2(P)*	-	-	-	-	+++	-
7	Lactate dehydrogenase (LDH)	1.1.1.27	Tetramer	*LDH-A**	+++	+++	+++	+++	+++	+++++
				*LDH-B**	+++	+++	+++++	+++	+++	+
				*LDH-C**	-	+++++	-	-	-	-
8	Malate dehydrogenase (MDH)	1.1.1.37	Dimer	*$_c$MDH-A**	+++++	+++++	+++++	+++++	+++++	+++++
				*$_c$MDH-B**	-	-	-	-	-	+++
				*$_m$MDH**	-	-	-	-	+++	-
9	Malic enzyme (ME)	2.7.5.1	Tetramer	*$_c$ME-1**	+++++	+++++	+++++	+++++	+++	+++++
				*$_c$ME-2**	+++	+++++	+++++	+++	+	+++
				*$_m$ME**	-	-	+	-	-	+
10	Sorbitol dehydrogenase (SDH)	1.1.1.14	Tetramer	*SDH-1**	+	+	+	+	+++++	+
				*SDH-2**	+	+	+	+	+++	+
11	Xanthine dehydrogenase (XDH)	1.1.1.204	Dimer	*XDH*	+	+	+	+	+++++	+

(+) – weak; (+++) – moderate; (+++++) – strong; (-) – no activity.
B – brain; E – eye; H – heart; K – kidney; L – liver; M – muscle; P– polymorphic.

3.2 Channa punctatus

A summary of the electrophoretic expression of the eleven enzymes analysed is given in **Table 3-2**.

3.2.1 Alcohol dehydrogenase activity was observed in the six tissues under investigation. Five equally spaced bands of moderate staining intensity were resolved in brain, eye, heart and kidney tissues. In the latter two tissues, only the first two bands were prominent while the rest three were weakly stained. Liver exhibited two phenotypes for ADH, a common single-banded (**Fig. *3.2a***) and a less frequent three-banded (**Fig. *3.2b***) pattern . The weakest activity was observed in the muscle tissue. The bands for ADH exhibited a cathodal mobility.

3.2.2 Glucose dehydrogenase enzyme seemed to lack activity in all six tissues investigated in all individuals (**Fig. *3.2c***).

3.2.3 The six tissues examined for glucose 6-phosphate dehydrogenase showed a single zone of activity at the cathodal region of the gels. Besides the general pattern, a majority of the individuals exhibited an additional band located more cathodally to the common band. This band was found to be fixed in kidney but unstable in brain,

eye, heart and liver tissues. Muscle showed the weakest activity of G6PD. The staining intensity for G6PD in all these tissues was found to be high (**Fig. 3.2d**).

3.2.4 In all the individuals investigated for glutamate dehydrogenase enzyme (**Fig. 3.2e**), the egg bearing females seemed to have a high intensity of GDH activity. Deeply stained bands were observed in brain, eye, heart, kidney, liver and muscle tissues. Additional bands were also observed in the eye and heart but their frequency of occurrence are high in the former. The males, on the other hand, seemed to lack activity of GDH in all the tissues studied (**Fig. 3.2f**).

3.2.5 Faint bands were scored for α-Glycero 3-phosphate dehydrogenase enzyme. Brain, eye, heart and kidney tissues showed five bands of low staining intensity, while liver showed a single band with a slightly higher staining intensity than the rest of the tissues (**Fig. 3.2g**). No activity of αGPD was recorded in white skeletal muscle.

3.2.6 Brain, eye, heart, kidney and liver tissues showed a single invariant region of activity for hexose 6-phosphate dehydrogenase

enzyme at the cathodal region of the gels. The highest activity was recorded in eye and liver and the weakest in muscle tissue (**Fig. 3.2h**).

3.2.7 Lactate dehydrogenase enzyme revealed a five-banded pattern in brain, eye, heart, kidney and liver tissues that were equally spaced, while in white skeletal muscle only two bands were resolved on the gel (**Fig. 3.2i**). The most cathodal and anodal bands were ubiquitous in all the six tissues studied, the former being predominant in heart (red muscle) while the latter in white skeletal muscle. The intermediate bands, which appeared in brain, eye, heart, kidney and liver, were found to be missing in white skeletal muscle. All the five bands showed equal staining intensity in brain, eye and liver, while heart and kidney tissues showed a decrease in the staining intensity from the anodal to the cathodal region.

3.2.8 Two distinct zones of activity were displayed for malate dehydrogenase enzyme. The more cathodal bands of the first zone were deeply stained and present in all six tissues. The more anodal bands, on the other hand, were observed in heart and muscle and are weakly stained, but in brain, eye, kidney and liver, their occurrence was highly unpredictable. White skeletal muscle, on the other hand, showed an additional band more anodal to the above two bands. The second zone consisted of two equally spaced but weakly stained bands. The bands

appeared in all the tissues and showed a high anodal migration (**Fig. 3.2j**).

3.2.9 Malic enzyme showed two zones of activity (**Fig. 3.2k**). A zone of deeply stained bands was observed at the cathodal region in all six tissues. The other zone was seen to have a high anodal mobility with a number of variations as well. Muscle tissue exhibited three to five asymmetrical bands in this zone and in addition two bands appeared intermediate between the two major zones. The rest of the tissues showed in general a single band, but occasionally an additional band was also noted, but their appearance and disappearance in these tissues was highly unpredictable.

3.2.10 Sorbitol dehydrogenase displayed five bands in brain, eye, heart and kidney (**Fig. 3.2l**), and a band with high intensity was recorded in liver tissue. Faint bands were also detected in white skeletal muscle.

3.2.11 A single cathodal band was observed in liver extracts of all individuals examined for xanthine dehydrogenase enzyme (**Fig. 3.2m**). Five bands of low intensity were also seen in brain, eye and heart and kidney tissues. In white skeletal muscle no XDH activity was detected. In all individuals studied, the relative mobility of the bands were found to be the same.

Expression of ADH in
C. punctatus

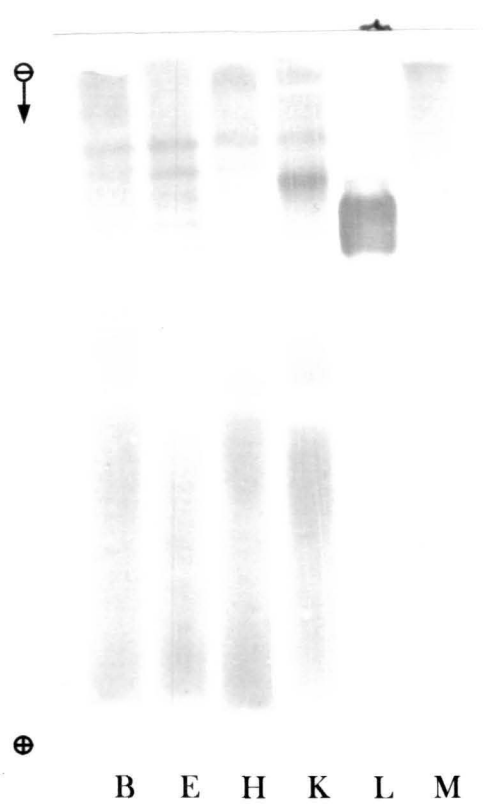


Fig. 3.2(a)
(Single-banded pattern)

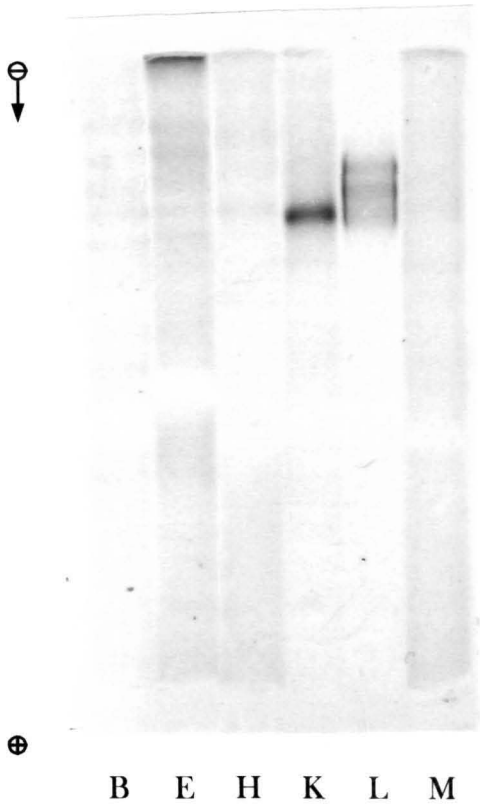


Fig. 3.2(b)
(Three-banded pattern)

Expression of GD in
C. punctatus

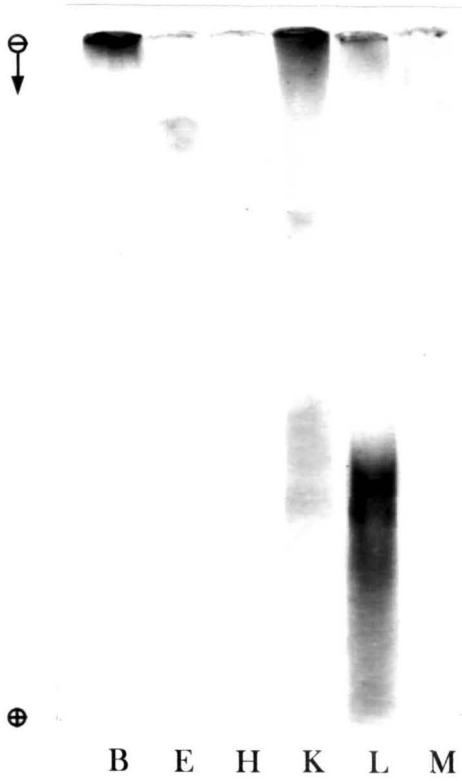


Fig. 3.2(c)

Expression of G6PD in
C. punctatus

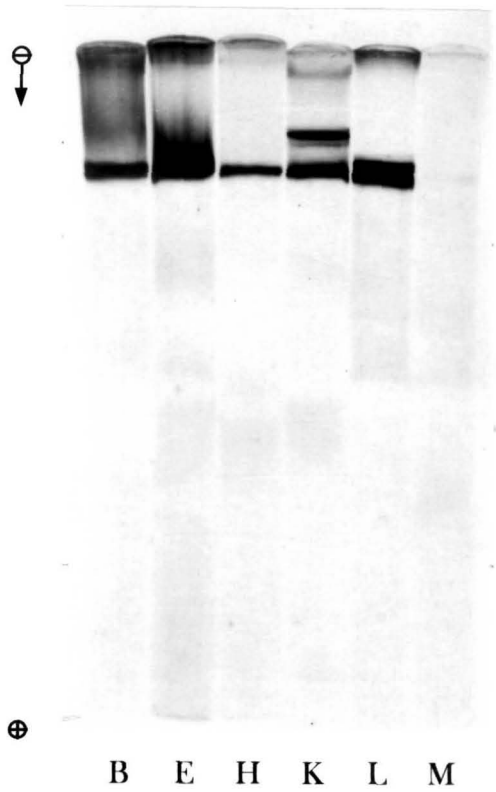


Fig. 3.2(d)

Expression of GDH in
C. punctatus

(Female)

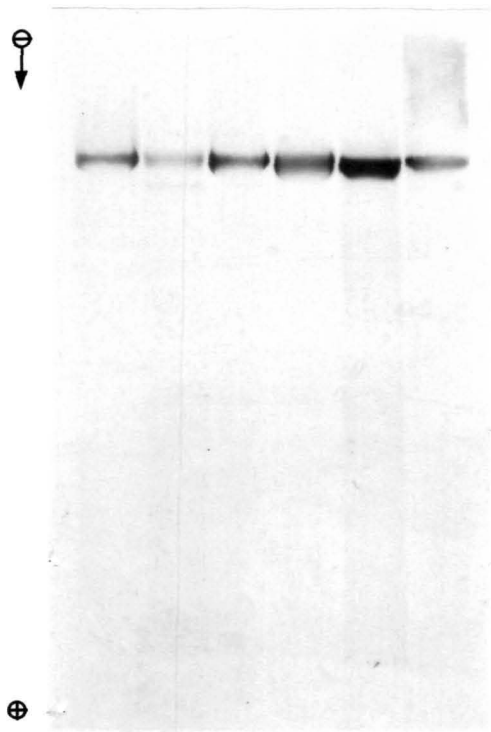


Fig. 3.2(e)

(Male)

Fig. 3.2(f)

Expression of αGPD in
C. punctatus

B E H K L M

Fig. 3.2(g)

Expression of H6PD in
C. punctatus

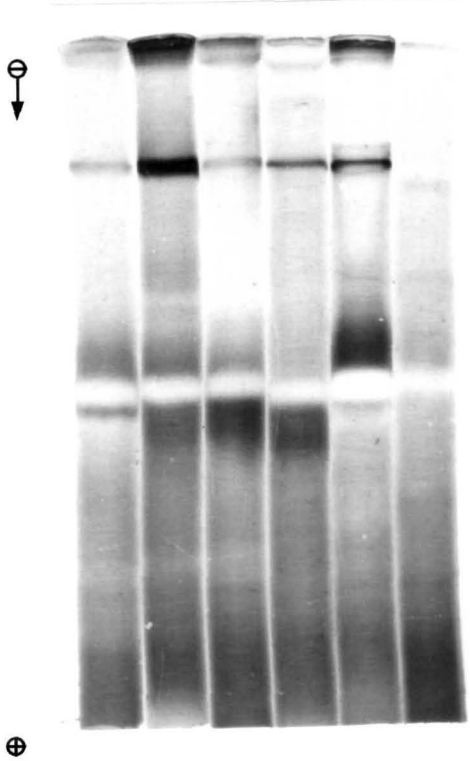


Fig. 3.2(h)

Expression of LDH in
C. punctatus

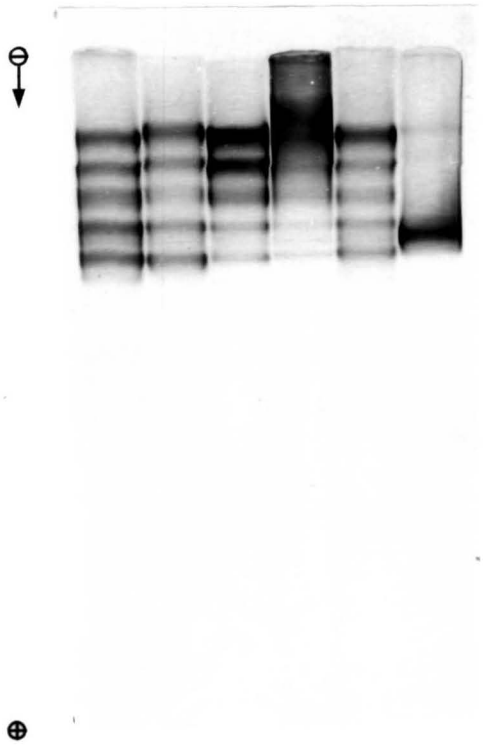


Fig. 3.2(i)

Expression of MDH in
C. punctatus

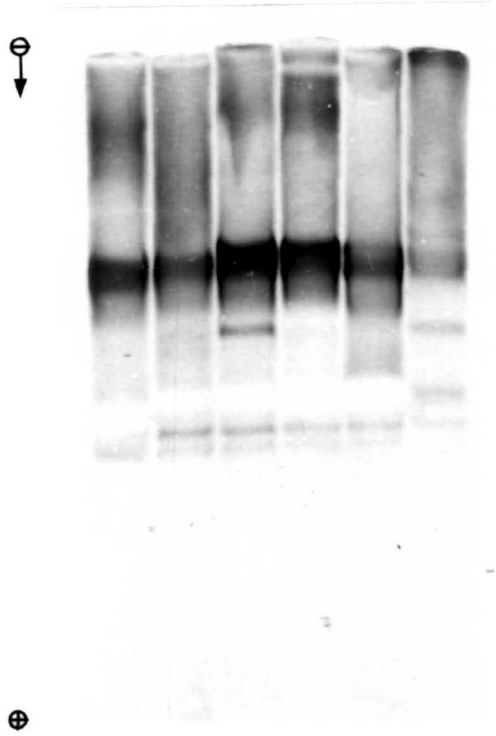


Fig. 3.2(j)

Expression of ME in
C. punctatus

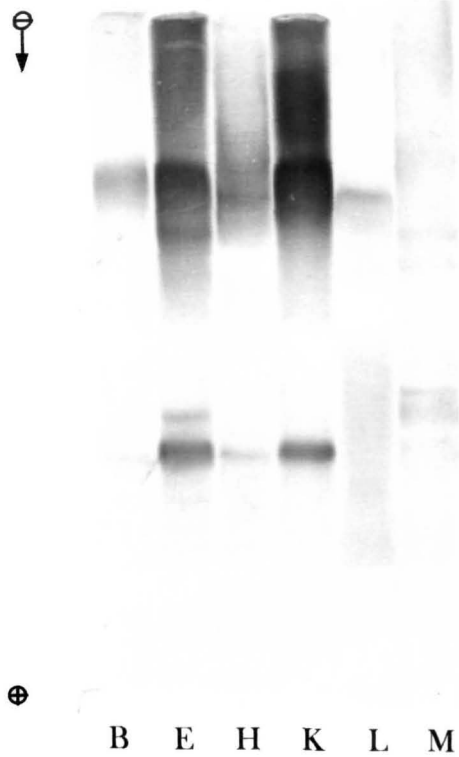


Fig. 3.2(k)

Expression of SDH in
C. punctatus

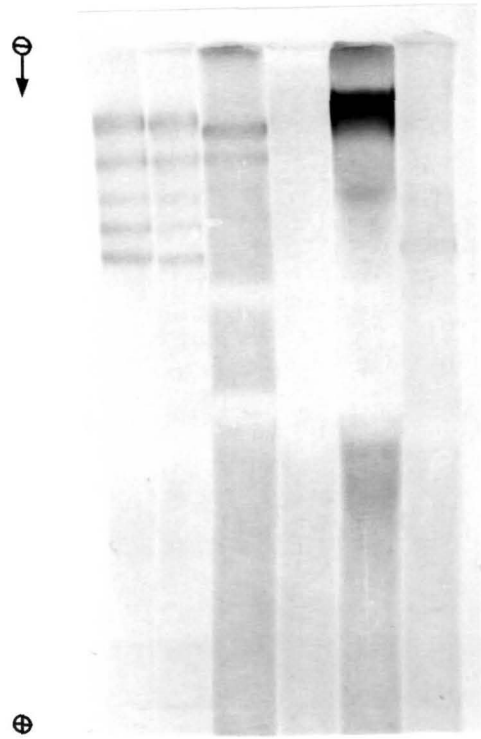


Fig. 3.2(l)

Expression of XDH in
C. punctatus

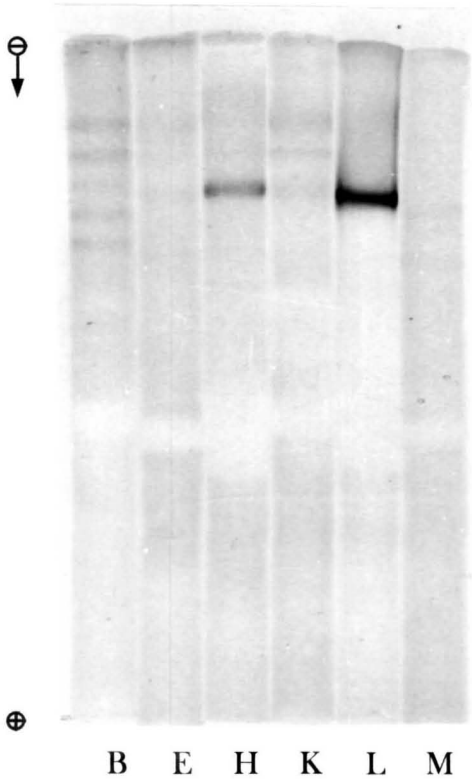


Fig. 3.2(m)

TABLE 3-2

Summary of electrophoretic expression of the eleven enzymes analyzed in *Channa punctatus* :

Sl. No	Protein	EC No.	Protein Structure	Loci	Activity					
					B	E	H	K	L	M
1	Alcohol dehydrogenase (ADH)	1.1.1.1	Dimer	*ADH-A*(P)*	+++	+++	+	+	+++++	+
2	Glucose dehydrogenase (GD)	1.1.1.47		*GD*	-	-	-	-	-	-
3	Glucose 6-phosphate dehydrogenase (G6PD)	1.1.1.49	Dimer	*$G_6PD(P)$*	+++	+++	+++	+++	+++++	+
4	Glutamate dehydrogenase (GDH)	1.4.1.2	Monomer	**Female**						
				GDH(P)	+++++	+++++	+++++	+++++	+++++	+++++
				Male	-	-	-	-	-	-
5	Glycerol 3-phosphate dehydrogenase (αGPD)	1.1.1.8	Dimer	*αGPD*	+++	+++	+++	+++	+++++	-
6	Hexose 6- phosphate dehydrogenase (H6PD)	1.1.1.47	Dimer	*H_6PD*	+++	+++++	+++	+++	+++++	+
7	Lactate dehydrogenase (LDH)	1.1.1.27	Tetramer	*LDH-A**	+++	+++	+++	+++	+++	+++++
				*LDH-B**	+++	+++	+++++	+++	+++	+
8	Malate dehydrogenase (MDH)	1.1.1.37	Dimer	*$_c$MDH-A**	+++++	+++++	+++++	+++++	+++++	+++++
				*$_c$MDH-B**	-	-	-	-	-	+++
				$_m$MDH(P)*	+++	+++	+++	+++	+++	+++
9	Malic enzyme (ME)	2.7.5.1	Tetramer	*$_c$ME-1**	+++	+++	+++	+++	+++	+++++
				*$_c$ME-2**	-	-	-	-	-	+++
				*$_m$ME**	+++	+++	+++	+++	+++	+++
10	Sorbitol dehydrogenase (SDH)	1.1.1.14	Tetramer	*SDH-1**	+++	+++	+++	+++	+++++	+
				*SDH-2**	+++	+++	+++	+++	+++++	+
11	Xanthine dehydrogenase (XDH)	1.1.1.204	Dimer	*XDH*	+++	+++	+++	+++	+++++	-

(+) – weak; (+++) – moderate; (+++++) – strong; (-) – no activity.

B – brain; E – eye; H – heart; K – kidney; L – liver; M – muscle; P– polymorphic.

3.3 *Channa striatus*

A summary of the electrophoretic expression of the eleven enzymes analysed is given in **Table 3-3**.

3.3.1 All six tissues exhibited a single zone of alcohol dehydrogenase activity at the cathodal region with varying staining intensities, but the highest being in liver. Worthy of note is the occurrence of a large number of individuals that showed a symmetrical, two-banded (**Fig. 3.3a**) and very few individuals with three-banded (**Fig. 3.3b**) pattern in liver tissue. These bands also showed equal staining intensity. The mobility of the single band seen in brain, eye, heart, kidney and muscle tissues when compared with liver, appeared along with the most anodal band of liver. The bands of ADH were observed at the cathodal region of the gels.

3.3.2 In all individuals investigated for glucose dehydrogenase, a single invariant band with an anodal migration was observed only in liver tissue. The rest of the tissues showed no activity of GD (**Fig. 3.3c**).

3.3.3 A single invariant band was detected for glucose 6-phosphate dehydrogenase (**Fig. 3.3d**) in brain, eye and liver tissues.

In brain and eye the band appeared at the cathodal region, while liver showed an anodal migration. White skeletal muscle in most individuals lacked the activity of G6PD, but in very few individuals it was detected as a faint band. Heart and kidney tissues, on the other hand, displayed two phenotypes with regard to the relative mobility for this enzyme. In some a cathodal migration was observed along with brain and eye while in others they showed an anodal migration as in liver. The staining intensity was variable for all tissues, with the highest activity seen in eye and liver tissues.

3.3.4 The activity levels of glutamate dehydrogenase enzyme seemed to have a pronounced relationship with the egg bearing females. In them the level of GDH activity was found to be very high and appeared as a single band which migrated cathodally in brain, eye, heart, kidney and liver tissues (**Fig. 3.3e**). Males seemed to record no activity for GDH in any of the six tissues under investigation (**Fig. 3.3f**).

3.3.5 α-Glycero 3-phosphate dehydrogenase activity was observed in brain, eye, heart, kidney and liver tissues as a single cathodal band. The band showed the same intensity of staining and

electrophoretic mobility. Very weak αGPD activity was detected in white skeletal muscle tissue (**Fig. 3.3g**).

3.3.6 Hexose 6-phosphate dehydrogenase was observed as a single band in brain, eye, heart, kidney and liver tissues. White skeletal muscle showed no activity of H6PD. Brain and kidney tissues exhibited low levels of activity, while heart displayed a slightly higher activity. The highest activity was recorded in eye and liver, which in some individuals appeared as two bands in the eye. H6PD showed a cathodal mobility in brain, eye, heart and kidney tissues. In liver two phenotypes were observed with regards to the relative mobility of the band. Some individuals showed an anodal migration along with the rest of the tissues while in some a cathodal migration was noted (**Fig. 3.3h & i**).

3.3.7 The gels stained for lactate dehydrogenase showed an invariant appearance of the enzyme in all individuals subjected to electrophoresis (**Fig. 3.3j**). Three equally spaced bands were observed in brain, eye, heart, kidney and liver with equal staining intensity. In white skeletal muscle, the intermediate band was missing hence only two bands are displayed in which the more anodal band was stained

intensely and the less anodal band appeared faintly in comparison to the other tissues.

3.3.8 Three major zones of activity were resolved (**Fig. *3.3k***) for malate dehydrogenase enzyme. The bands present in the cathodal region are weakly stained and are exhibited in brain, eye, heart, kidney and liver tissues while skeletal muscle showed very faint bands for this zone. Three bands were resolved at the anodal region of the gels, at the least anodal zone, bands with varying staining intensity were observed in all the six tissues but the highest being in kidney and liver. The most anodal zone showed darkly stained bands of almost equal intensity in all the six tissues. An intermediate band between the two was also scored in brain, eye, heart, kidney and liver tissues.

3.3.9 Two zones of activity were resolved for malic enzyme that migrated anodally on the gels (**Fig. *3.3l***). The most anodal zone was found to be most active in liver, while brain, eye, heart, and kidney showed lesser activity, but the least activity was observed in white skeletal muscle tissue. The slower migrating zone was found to be most active in skeletal muscle that was represented by three bands. Liver, on the other hand, exhibited a single band and the rest of the tissues viz., brain, eye, heart and kidney showed faint bands in this region.

3.3.10 Sorbitol dehydrogenase was observed as a single band with cathodal migration in brain, eye, heart and kidney while in liver two bands were scored with variable electrophoretic mobility. Muscle tissue, on the other hand, showed no activity of SDH (**Fig. 3.3m**). The bands scored for this enzyme showed very low activity.

3.3.11 Xanthine dehydrogenase appeared as a single band, which migrated anodally and was predominantly expressed in liver tissue. Brain, eye, heart, kidney and muscle tissues also showed the XDH band but with very low intensity. Variations in width and relative position of the bands in all the six tissues were noted (**Fig. 3.3n & o**).

Expression of ADH in
C. striatus

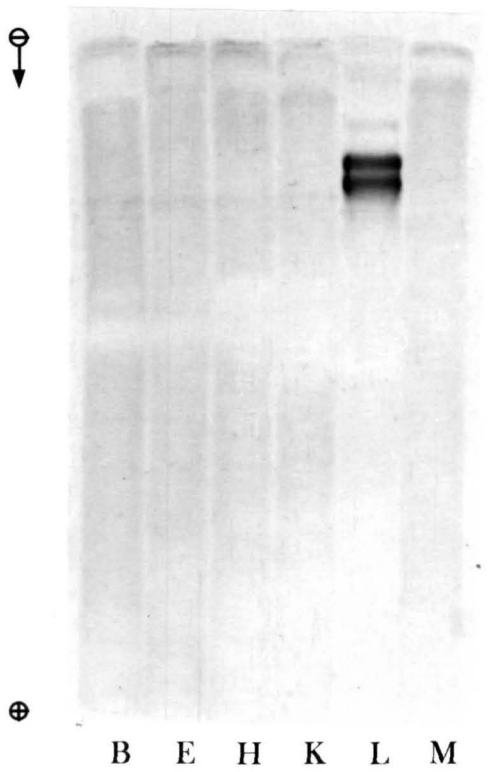


Fig. 3.3(a)
(Two-banded pattern)

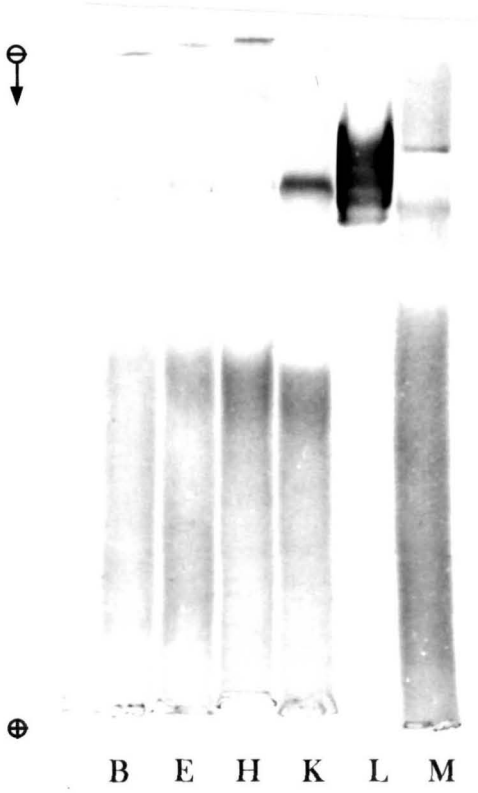


Fig. 3.3(b)
(Three-banded pattern)

Expression of GD in
C. striatus

Fig. 3.3(c)

Expression of G6PD in
C. striatus

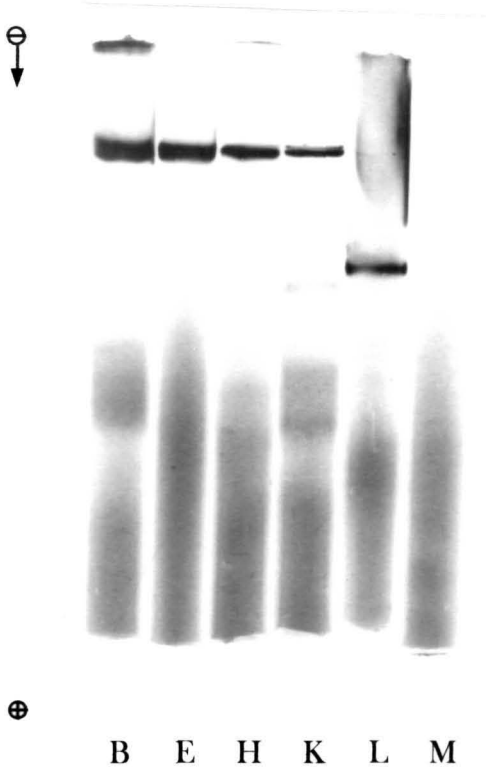


Fig. 3.3(d)

Expression of GDH in
C. striatus

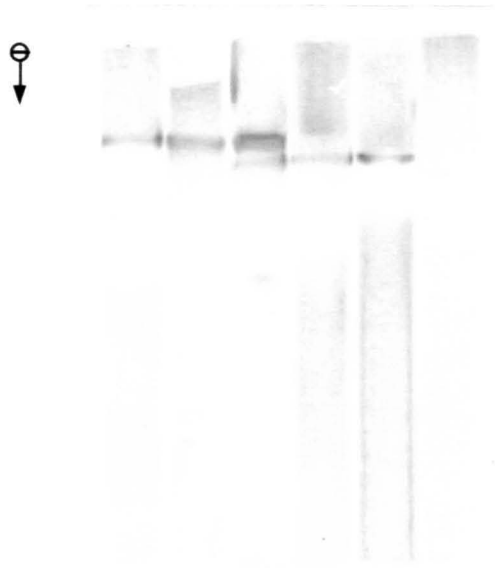
(Female)

Fig. 3.3(e)

(Male)

Fig. 3.3(f)

Expression of αGPD in
C. striatus



B E H K L M

Fig. 3.3(g)

Expression of H6PD in
C. striatus

Fig. 3.3(h)

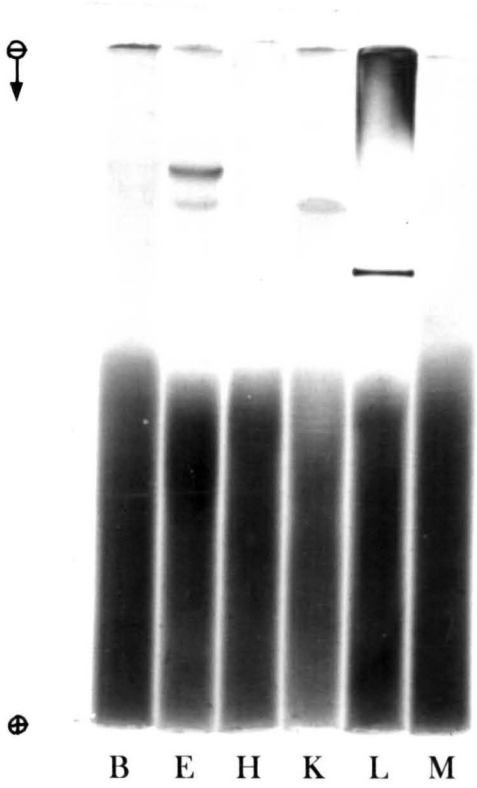


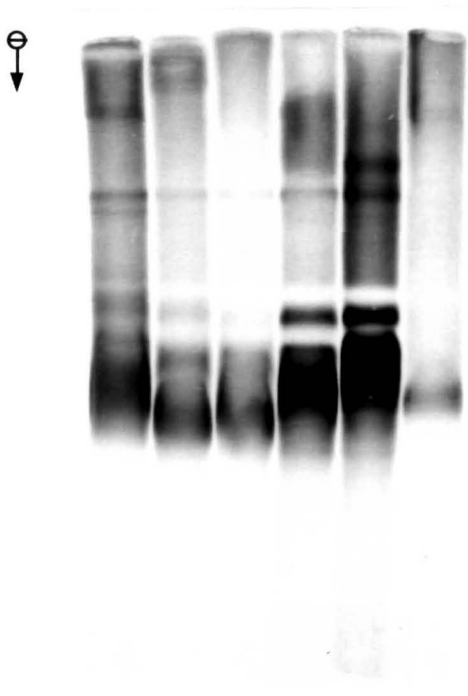
Fig. 3.3(i)

Expression of LDH in
C. striatus

B E H K L M

Fig. 3.3(j)

Expression of MDH in
C. striatus



B E H K L M

Fig. 3.3(k)

Expression of ME in
C. striatus

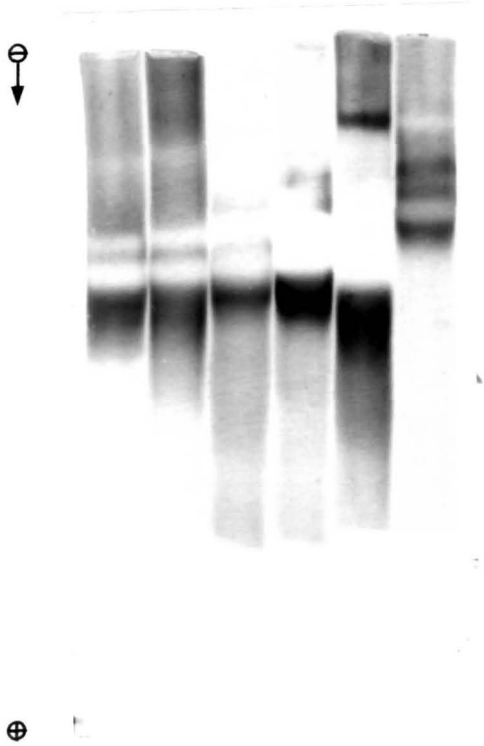


Fig. 3.3(l)

Expression of SDH in
C. striatus

Fig. 3.3(m)

Expression of XDH in
C. striatus

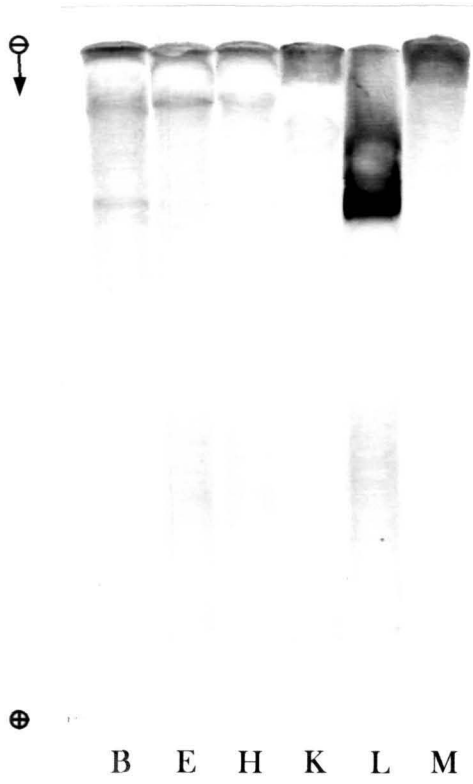


Fig. 3.3(n)

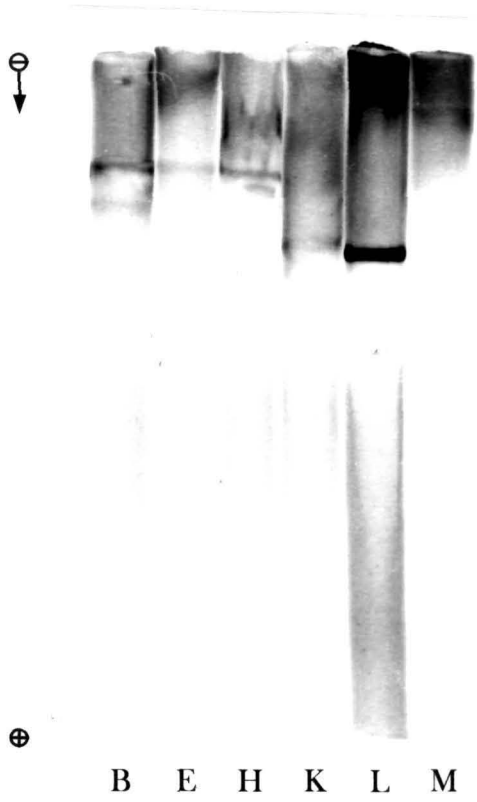


Fig. 3.3(o)

TABLE 3-3

Summary of electrophoretic expression of the eleven enzymes analyzed in *Channa striatus*:

Sl. No	Protein	EC No.	Protein Structure	Loci	Activity					
					B	E	H	K	L	M
1	Alcohol dehydrogenase (ADH)	1.1.1.1	Dimer	*ADH-A*(P)*	+	+	+	+	+++++	+
2	Glucose dehydrogenase (GD)	1.1.1.47		*GD*	-	-	-	-	+++++	-
3	Glucose 6-phosphate dehydrogenase (G6PD)	1.1.1.49	Dimer	*G_6PD-1**	+++++	+++++	+++++	+++++	-	+
				*G_6PD-2**	-	-	+++	+++	+++++	-
4	Glutamate dehydrogenase (GDH)	1.4.1.2	Monomer	**Female**						
				GDH	+++++	+++++	+++++	+++++	+++++	+
				Male	-	-	-	-	-	-
5	Glycerol 3-phosphate dehydrogenase (αGPD)	1.1.1.8	Dimer	*αGPD*	+++	+++	+++	+++	+++	+
6	Hexose 6- phosphate dehydrogenase (H6PD)	1.1.1.47	Dimer	*H_6PD-1*(P)*	+	+++++	+++	+	+++	-
				*H_6PD-2**	-	-	-	-	+++++	-
7	Lactate dehydrogenase (LDH)	1.1.1.27	Tetramer	*LDH-A**	+++	+++	+++	+++	+++	+++++
				*LDH-B**	+++	+++	+++++	+++	+++	+
8	Malate dehydrogenase (MDH)	1.1.1.37	Dimer	*$_c$MDH-A**	+++	+	+	+++++	+++++	+
				*$_c$MDH-B**	+++++	+++++	+++++	+++++	+++++	+++
				*$_m$MDH**	+++	+++	+++	+++	+++	+
9	Malic enzyme (ME)	2.7.5.1	Tetramer	*$_c$ME-1**	+++	+++	+++	+++	+++++	+
				*$_m$ME**	+	+	+	+	+++	+++++
10	Sorbitol dehydrogenase (SDH)	1.1.1.14	Tetramer	*SDH-1**	+	+	+	+	+++++	+
				*SDH-2**	-	-	-	-	+++	-
11	Xanthine dehydrogenase (XDH)	1.1.1.204	Dimer	*XDH-1**	+	+	+	+	+++++	+
				*XDH-2**	-	-	-	+	+++++	-

(+) – weak; (+++) – moderate; (+++++) – strong; (-) – no activity.
B – brain; E – eye; H – heart; K – kidney; L – liver; M – muscle; P– polymorphic.

DISCUSSION

Murrels, also called snakeheads, belong to the family Channidae and are represented by a single Genus *Channa (= Ophiocephalus).* In 1777, Scopoli erected the Genus *Channa,* although no particular species was mentioned. Some sixteen years later, Bloch described a similar fish and gave it the name *Ophiocephalus.* He named them so, probably because of the similarities of structure and appearance of the head of these fishes with that of a snakehead (ophidian – snake, cephalus – head). This fish unlike *Channa,* possessed pelvic fins and the name embraced both Asian and African species.

Regarding the classification of this group, some differences were observed between the classification of Day (1875-1878) and the recent classification of Greenwood *et al.,* (1966). Day grouped the murrels under the family Ophiocephalidae and brought it under the order Acanthopterygii, characterized by spiny rays. He also observed the differences in this regard of this family with other members of the order and probably that is why he grouped all the snake-headed fishes under a separate sub-division — Channiformes (the 13th group under the order Acanthopterygii). He classified this group under the genus *Ophiocephalus* and one species under the genus *Channa.* Regan (1929) included the murrels under the family Ophiocephalidae under the

sub-order Ophiocephaloidea. The latter, along with other three sub-orders, viz., Percoidea, Gobiodea and Anabantoidea, was listed under the order Percomorphi. Myers and Shapovalov (1931) in a significant taxonomical reclassification discussed in detail the differences between *Ophiocephalus* and *Channa* and rejected the former as a generic synonym. This was based on a comparison of *Ophiocephalus gachua* (with pelvic fins) and *Channa orientalis* (without pelvic fins). The genus *Ophiocephalus* and *Channa* had been previously separated by the single character of the pyloric or caecal appendages being present or absent (where the protrusion for the insertion of pelvic fins is situated). Hora (1921) and Deraniyagala (1929) found that the pyloric caeca were present in both *O. gachua* and *C. orientalis.* Deraniyagala (1929) stated that the Sri Lankan species *O. gachua* (later to become *Channa gachua*) and *C. orientalis* were identical in the important character of head shield (scales) patterns on top of the head. He also gave a detailed description of the two species and found no significant differences (apart from lack of pelvic fins). The bold pectoral barring and overall colouration is similar. *O. gachua* and *C. orientalis* can be found in the same biotope. Quoting Day (1878-1888); "It is not uncommon in India to find specimens of

Ophiocephalus gachua having a ventral fin deficient, but I have not observed both wanting". A specimen of *O. gachua* lacking both pelvic fins was taken on the Island of Formosa by Leo Shapovalov. Following the strict rules of Zoological Nomenclature, Myers and Shapovalov (1931) united the two genera including the African species and boldly affirmed that *Ophiocephalus* be merged into the single genus *Channa*. Concluding that *C. orientalis* may be regarded as a series of anomalous specimens to the similar *O. gachua* they strangely, after an excellent discussion on the basis of the species merging, however, were hesitant to synonymize, as did Deraniyagala (1929), listing the fish as *C. orientalis* and *C. gachua*. Herse and Myers (1937) and McAllister (1968) expressed their opinion in favour of *Channa* to *Ophiocephalus* and subsequently most of the taxonomists are using this name. Greenwood *et al.*, (1966) have grouped all the murrels under a single genus *Channa* and a new order Channiformes, to accommodate only the snake-headed fishes.

Studies made during the 'All India Co-ordinated Research Project On Air-Breathing Fish Culture' (ICAR Final Report, 1971-1985) revealed that of the ten species of murrels described by

Day (1889), only the following eight are valid. *Channa marulius* (Hamilton-Buchanan, 1822), *C. striatus* (Bloch, 1793), *C. punctatus* (Bloch, 1793), *C. orientalis* Bloch and Schneider, 1801, *C. stewartii* (Playfair, 1867), *C. micropeltes* (Cuvier, 1831), *C. barca* (Hamilton-Buchanan, 1822) and *C. gachua* Hamilton-Buchanan, 1822.

Presently, the taxonomy of the genus *Channa* is complicated particularly by the morphological characteristic of the presence (18 species) or absence (3 species) of pelvic fins, listed as follows:

With Pelvic Fins:

Channa argus, C. bankanensis, C. barca, C. bistriata, C. gachua, C. leucopunctatus, C. lucius, C. maculatus, C. marulioides, C. marulius, C. melanopterus, C. siamensis, C. melanosoma, C. micropeltes, C. pleuropthalmus, C. punctatus, C striatus, C. stewartii.

Without Pelvic Fins:

Channa asiatica, C. burmanica, C. orientalis.

Researchers and aquarists have recently observed important behavioural characters in *Channa gachua* and *C. orientalis* are alike. *Channa gachua* does not build a compact nest at the surface of the water (like other Channidae). It scoops out a small hole in the mud not

far from the edge of a pond or river bank in shallow water and normally it lays between 1500-2000 eggs (Mookerjee *et. al.*, 1950)

Channa orientalis orally incubates its eggs. A large area of substrate is removed and after spawning, the male's throat is distended with 80-100 fry (approximately 3mm long) which emerge from the mouth 10 days later (Roth, 1985).

It is widely accepted that the differences in the breeding behaviour of Betta (Belontidae) mouthbrooder and bubblenesting species are a direct result of the change of environmental conditions and in all cases mouthbrooders can be found in flowing water biotopes. The habitat of *Channa orientalis* in Sri Lanka is small streams, in many cases next to the ponds inhabited by *Channa gachua* and it can be assumed that the former is undergoing an evolutionary change similar to that of the mouthbrooding Betta species. It may be mentioned here that Deraniyagala (1929) have merged *C. gachua* with *C. orientalis.* We are therefore, inclined to accept the name *Channa orientalis* for this fish.

Macromolecules such as proteins and nucleic acids are the most important components of the cell that regulate all biological processes. Since the genetic information transferred by nucleic acids reveal many

interesting aspects of evolution and heredity, it will also help to rationalize the chemotaxonomic approach to systematize the animal kingdom.

In general sex, spawning, food, age, hibernation, disease, osmotic pressure, temperature, light, oxygen depletion and other seasonal factors have some role on the total protein species of a fish (Booke, 1964). According to Kirpichnikov (1992), biochemical polymorphism is highly related to the type of environment (including fool availability, temperature gradient, and duration of the seasons of the year) and population size. To minimize the influences of such factors, adult fishes were obtained during the same season and almost from the same environmental conditions taking sex and age as constant. It may therefore, be presumed that the patterns and distribution of the electrophoretic bands in different tissues could not be affected by factors other than genetic, so that species specificity could be understood only from the genetic level.

4.1 Alcohol dehydrogenase (ADH, EC No. 1.1.1.1) is the major catalyst of ethanol oxidation in the body (Havre *et al.*, 1977; Plapp *et al.*, 1984). ADH is also capable of catalysing the reversible interconversion of a wide variety of alcohols and their corresponding aldehydes and ketones, including sterols, w-hydroxy fatty acids and food flavour alcohols (Pietruszko, 1979), and may function as a major detoxification mechanism for biological aldehydes. Recent studies have suggested that ADH is involved in reductive metabolism of a range of such aldehydes, including the toxic peroxidic aldehyde, 4-hydroxynonemal (Esterbauer *et al.*, 1985) and biogenic aldehydes derived from serotonin, dopamine, and norepinephrine metabolism (Mardh *et al.*, 1985; Mardh and Vallee, 1986; Consalvi *et al.*, 1986).

Alcohol dehydrogenase has been examined in a wide variety of teleostean species (Hitzeroth *et al.*, 1968; Shaklee *et al.*, 1974, 1977; Frankel, 1981; Krueger, 1980; Andersson *et al.*, 1983; Basiao and Taniguchi, 1984; Vuorinen, 1984; Menezes *et al.*, 1992; Engelbrecht and Van Der Bank, 1994; Ramirez *et al.*, 1998; Leesa-Nga *et al.*, 2000; Peres *et al.*, 2002). These studies, along with those on tetrapods, have demonstrated that ADH is being encoded in the majority of

vertebrates at a single gene locus presumed to be controlled by two alleles. Homozygous individuals show a single-banded phenotype and heterozygotes a three-banded phenotype indicating a probable dimeric structure of the enzyme. The ADH locus was designated as *ADH-A** and its corresponding allele as *A'*.

While the enzyme is usually found at its highest concentrations in the liver tissue as seen in *Petromyzon marinus* (Krueger, 1980), Atlantic cod *Gadus morhua* (Mork *et al.*, 1982), *Barbus species* (Frankel, 1985), Bighead Carp, Silver Carp and their reciprocal hybrids (Brummett *et al.*, 1988), Grey mullets (Menezes *et al.*, 1992), *Barbus brevipinnis* (Engelbrecht and Van Der Bank, 1994), it may be present to a lesser extent in both stomach and kidney (Hitzeroth *et al.*, 1968; Shaklee *et al.*, 1974; Leesa-Nga *et al.*, 2000). Investigation into the tissue specificity of ADH in *Brachydanio,* however, has revealed that its expression is restricted to liver extracts (Frankel, 1976, 1978, 1980,1981), as well as in *Salvelinus alpinus* (Andersson *et al.*, 1983), Trout strains (Thompson, 1985), *Hoplias malabaricus* (Peres *et al.*, 2002).

The expression of ADH was presumed to be encoded by a single locus in liver, two loci in most tissues, and three in eye in *Tilapia zillii* . This high activity of alcohol dehydrogenase is not usually present in fish tissues. It may be an adaptation to survival in very low oxygen concentrations where anaerobic respiration is likely to be of great use (Cruz *et al.*, 1982).

The liver extracts of all the three species of *Channa* showed both the single (**Fig. 4.1a**) and three banded phenotypes (**Fig. 4.1b-d**) as in a majority of fishes. Investigation into the tissue specificity of ADH, however, have revealed that its expression is highest in the liver with decreased activity in the other tissues viz., brain, eye, heart, kidney and muscle. The five bands as observed in brain and eye tissues in *C. punctatus* were also reported in other fishes (**Fig. 4.1c**).

We, therefore, consider that ADH is encoded in a single locus in the presently studied fishes and both homozygous and heterozygous individuals are prevalent in natural populations. Analysis of the zymograms clearly reveals the occurrence of a single type of homozygous individuals and thus suggests recent origin of the mutant allele. The additional bands observed in *C. punctatus* are indicative of the presence of three loci in these tissues.

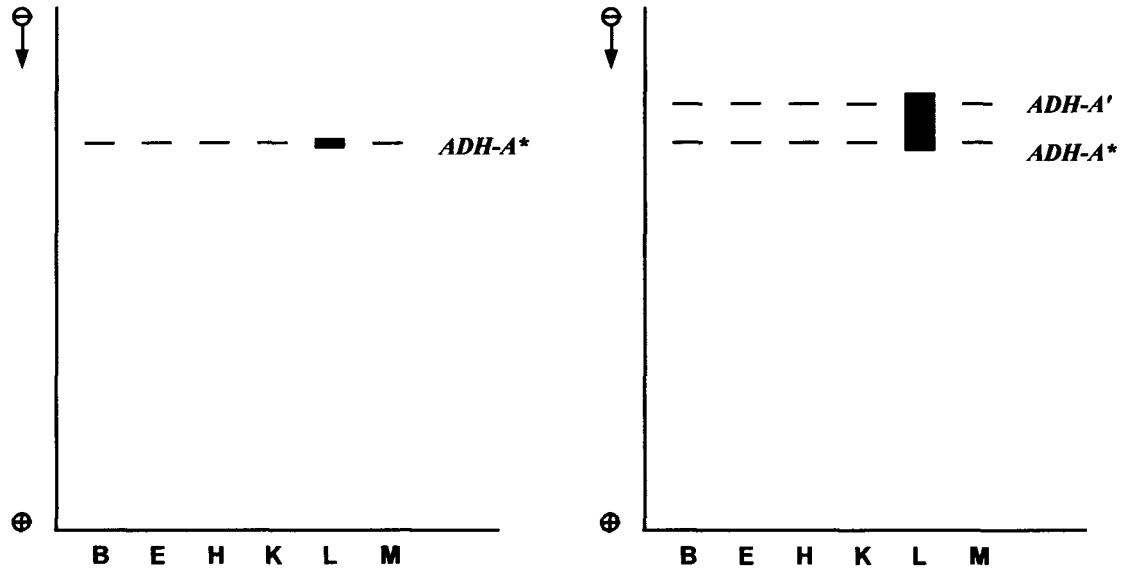


Fig. a Fig. b

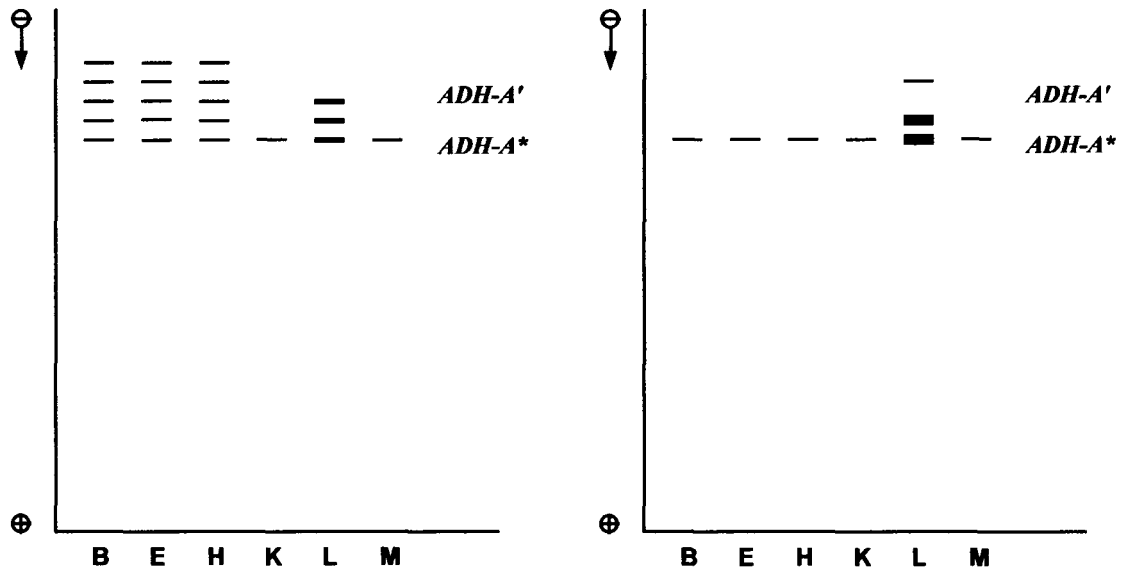


Fig. c Fig. d

Fig. 4.1 Zymograms illustrating the activity of ADH —
(a) Homozygous phenotype in the three species of *Channa*;
heterozygous phenotypes in (b) *Channa orientalis* (c) C. *punctatus*
and (d) C. *striatus*.
(B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.2 Review of literature shows limited data on biochemical genetic studies of glucose dehydrogenase (GD, EC No. 1.1.1.47) enzyme systems. Lavery and Fielder (1993) reported the expression of a single locus coding for glucose dehydrogenase in various tissues of coconut crab, *Birgus latro.* In *Hoplias malabaricus,* Peres *et al.,* (2002) also reported a single locus coding for GD whose expression is predominant in liver. Sengupta (2002) reported two loci in *Acrossocheilus hexagonolepis* that were predominant in liver.

GD activity was resolved in *C. orientalis* (**Fig. 4.2a**) and *C. striatus* (**Fig. 4.2b**), as a single invariant band which was slow migrating in the former and fast migrating in the latter. In both species it was found to be liver specific. No GD activity was resolved in *C. punctatus.* In conclusion, GD can be presumed to be encoded by a single locus in *C. orientalis* and *C. striatus.*

Glucose dehydrogenase acts on β-D-glucose and produces D-glucono-1, 5-lactone. Based on properties, other than substrate specificity reported for GD, Strecker and Korkes (1952), Metzger *et al.,* (1965), and Beutler and Morrison (1967) suggested that GD is in fact identical to hexose 6-phosphate dehydrogenase (H6PD). In addition,

Stegeman and Goldberg (1971) found a commercially available GD (Sigma) that exhibited catalytic activity with the various substrates and coenzymes acted upon by H6PD. Therefore it is, likely that the vertebrate enzyme glucose dehydrogenase can be considered to be homologous to H6PD. However, we have observed substantial difference in the expression of these two enzymes in the tissues studied in our fishes and we are confident that these two enzymes are encoded in different genes.

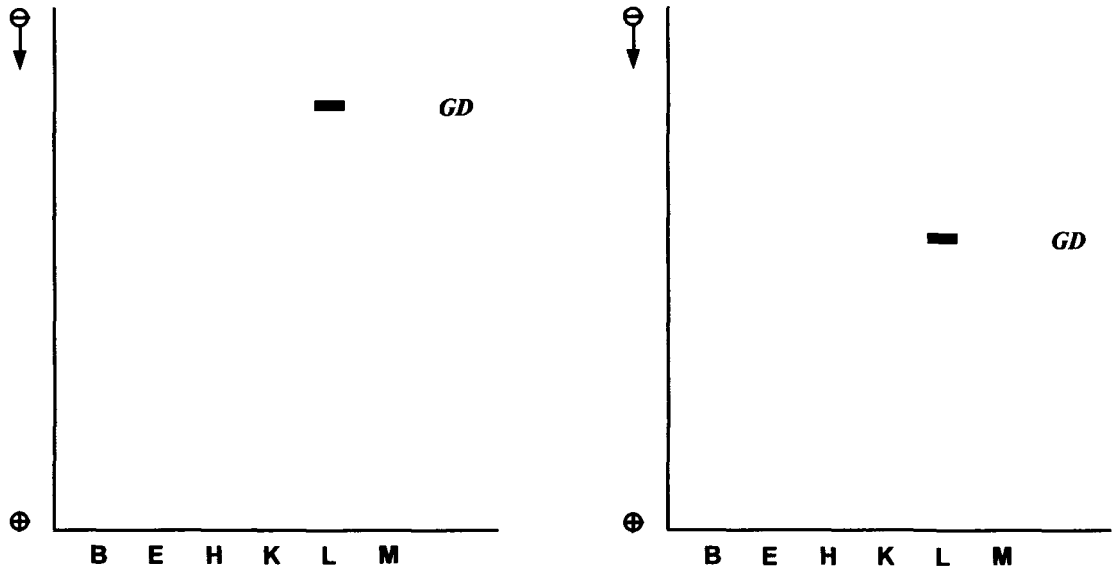


Fig. a

Fig. b

Fig. 4.2 Zymograms illustrating the activity of GD —
(a) *Channa orientalis;* (b) C. *striatus.*
(B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.3 Glucose is the primary substrate for synthesis of fat (Maynard *et al.*, 1979). It is converted to pyruvate via the glycolytic pathway and further to acetyl-CoA, which is the precursor for fatty acid synthesis. Synthesis of fatty acid also requires reducing power for the formation of double bonds. This power is supplied by NADPH, which is produced in the reactions mentioned below.

The pentose-phosphate pathway is an alternative route to the glycolytic pathway for the oxidation of glucose. In contrast to the glycolytic pathway, the pentose-phosphate pathway does not produce ATP. Instead, it generates NADPH for reductive synthesis, such as fatty acid biosynthesis, and provides ribose sugars for nucleotide and nucleic acid production.The pentose-phosphate pathway has two phases (Mayes, 1988): (1) The oxidative phase in which glucose 6-P, the substrate common to all the biochemical pathways of glucose, undergoes dehydrogenation and decarboxylation to give pentose sugar; this reaction is catalysed by glucose-6 phosphate dehydrogenase (G6PD) requiring $NADP^+$ as a hydrogen acceptor and the dehydrogenation and decarboxylation reactions of glucose 6-P generate NADPH and produces CO_2; and (2) The non-oxidative phase, in which the ribose sugar is converted back to glucose 6-P.

It is significant that tissues, which possess an active lipogenesis, i.e., liver and adipose tissue, also possess an active pentose-phosphate pathway. It seems that liver is a more important site for fatty acid synthesis in fish than is adipose tissue, as reported by Lin *et al.*, (1977) in coho salmon and Likimani and Wilson (1982) in channel catfish.

There seems to be limited data on this enzyme in fish. G6PD was found to be encoded in a single locus in *Chanos chanos* (Winans, 1980), *Petromyzon marinus* (Krueger, 1980), *Theragra chalcogramma* (Grant and Utter, 1980), *Salvelinus alpinus* (Andersson, 1983), *Ctenopharyngodon idella* and *Hypophthalmichthys nobilis* (Beck *et al.*, 1983), *Gadus morhua* (Mork *et al.*, 1982), and *Hoplias malabaricus* (Peres *et al.*, 2002).

Cederbaum and Yoshida (1976) suggested that the banding pattern in rainbow trout might be determined by two different alleles at two loci. The earlier findings of varying patterns in some fish could not be explained by the suggested hypothesis and a single locus-single allele system in which the polypeptide chain could assume multiple stable electrophoretic forms was more appealing (Cederbaum and Yoshida, 1976). *Tilapia zillii* presented a banding pattern, which also suggests the expression of two *G6PD* loci (Cruz *et al.*, 1982).

The observed pattern for G6PD suggests the expression of a single locus in *Channa punctatus* and two loci for *C. orientalis* and *C. striatus.*

In *C. orientalis* the cathodal band that expressed in all the six tissues of all the individuals examined, can be assumed to be the activity of *G_6PD-1** locus and the additional band (with a high anodic rate) predominant in heart, kidney and liver of *G_6PD-2**. An allele was detected in liver for the *G_6PD-2** locus, which appeared to be stable. The frequency of occurrence of the product of wild type allele of *G_6PD-2** locus was high in heart and kidney while the mutant allele product was unstable. Both the allelic products are found to be unstable in expression in brain, eye and muscle tissues (**Fig. 4.3a**).

Channa punctatus (**Fig. 4.3b**) exhibited two codominant alleles at a single locus, the wild type was found to be expressed in all the six tissues, while the mutant allele was highly unstable. It appeared to be fixed in kidney but unstable in brain, eye, heart, liver and muscle tissues. The rare occurrence of heterozygotes with a three-banded phenotype suggests the dimeric nature of this enzyme.

G6PD in *C. striatus* appeared to be encoded by two loci like in *C. orientalis*. The expression of *G6PD-1** locus was predictable in brain and eye and *G6PD-2** in liver. Heart and kidney showed a flexible expression for both loci, in which some individuals exhibited the expression of *G6PD-1** locus (**Fig. 4.3c**) and some showed *G6PD-2** (**Fig. 4.3d**) activity but not both at the same time in an individual. The pattern of gene expression of this species strongly revealed that both loci are never required for the synthesis of G6PD enzyme, either one is sufficient to produce a functional G6PD enzyme.

From these results observed in all the three species of *Channa*, it is tempting to conclude that the second locus of G6PD has arisen as a result of gene duplication.

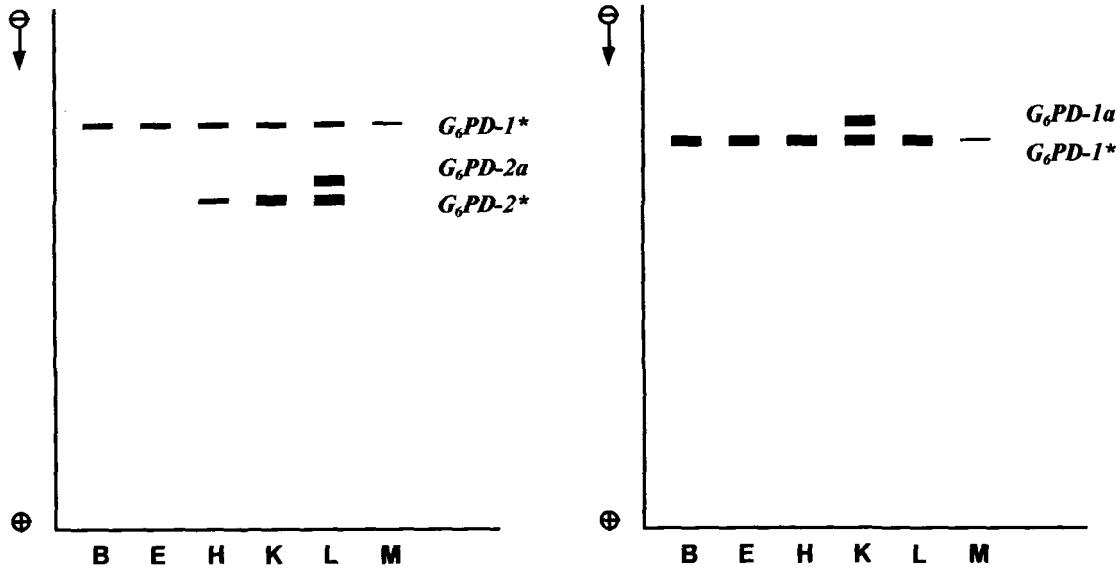


Fig. a

Fig. b

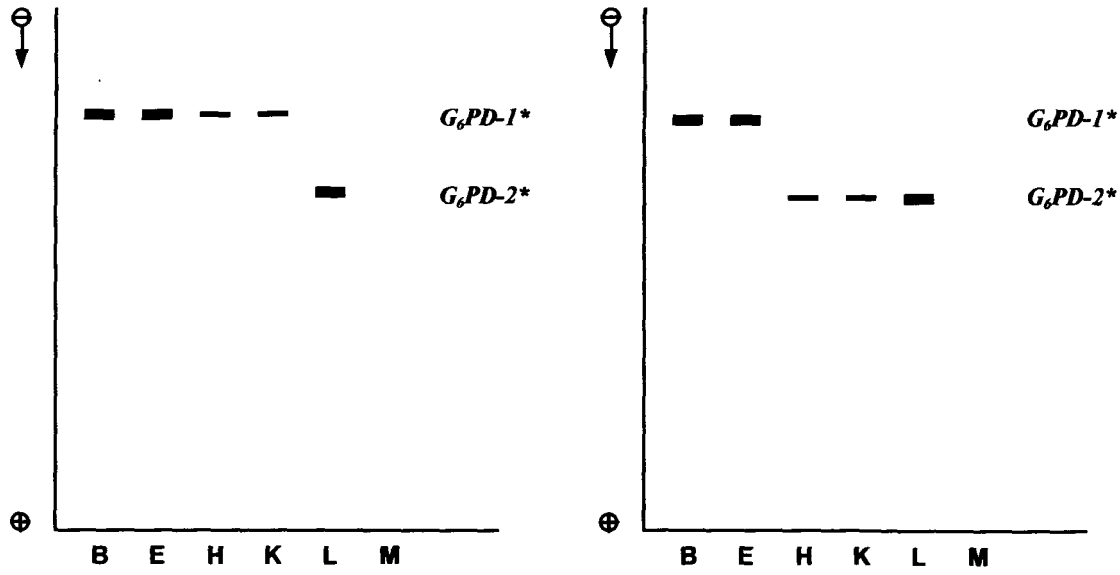


Fig. c

Fig. d

Fig. 4.3 Zymograms illustrating the activity of G6PD — (a) *Channa orientalis;* (b) C. *punctatus* (c) & (d) C. *striatus.* (B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.4 Glucose 6-phosphate dehydrogenase (G6PD, ICE No. 1.1.1.49) is among the most thoroughly studied of the enzymes in mammals. It has been of particular interest to geneticists because it is controlled in man by a gene located on the X-chromosome. Studies of the G6PD locus have generally confirmed this hypothesis particularly the demonstration by Davidson *et al.*, (1963) that clones of cells cultured from skin of Negro females heterozygous for a G6PD variant carried either one or the other form of the enzyme, but not both.

G6PD, more recently has been shown to be X-linked also in equine species (Mathai *et al.*, 1966) and in *Drosophila* (Young *et al.*, 1964), findings that have led to the speculation that there may be some general advantage in having this enzyme controlled by the X-chromosome. As stated earlier, G6PD is encoded in a sex-linked gene. Thus, it was of particular interest when a G6PD was found in the deer mouse (*Peromyscus maniculatus*), which is autosomally controlled (Shaw and Barto, 1965). This enzyme is probably not homologous with the X-linked enzyme of human erythrocytes.

There are two different forms of G6PD in deer mouse tissues, which were arbitrarily, designated the A and B forms. The B form is the autosomally controlled type, and does not occur in deer mouse

erythrocytes. The A form, which was found in all tissues studied including erythrocytes, was postulated to be homologous with the G6PD of human erythrocytes. A second form of G6PD was found subsequently in human tissues (Shaw, 1966; Ohno *et al.*, 1966), and this appears to be homologous with the B enzyme of deer mouse.

The presence of multiple forms of G6PD in various mammalian systems has been the subject of several recent reports, some of which have noted with interest the nonhomology of two molecular species of this enzyme. The A form, which is generally specific for glucose 6-phosphate and $NADP^+$, is ubiquitous in its phylogenetic and tissue distribution (Noltman and Kuby, 1963). This enzyme is X-linked in diverse mammals (Kirkman and Hendrickson, 1963; Epstein, 1969) and is localized in nuclear and soluble fractions of the cell (Beutler and Morrison, 1967; Shaw and Koen, 1968).

The second form of mammalian G6PD can be distinguished electrophoretically and by its ability to catalyse the oxidation of G6P, as well as Gal6P, 2-deoxyglucose 6-phosphate and glucose, with either NAD^+ or $NADP^+$ serving as coenzyme (Beutler and Morrison, 1967; Shaw and Koen, 1968). Although these two enzymes fit the criteria of

Shaw (1969) for primary isozymes, certain investigators (Shaw, 1966; Ohno *et al.*, 1966) designated the second form of G6PD enzyme with broader substrate specificity as hexose 6-phosphate dehydrogenase (H6PD, EC No. 1.1.1.47) for convenience in distinguishing this enzyme from G6P-specific G6PD as it was found not to be specific for glucose 6-phosphate but showed about equal activity toward this broader specificity, whereas the G6PD of erythrocytes, both in man and deer mouse, showed little or no activity on galactose 6-phosphate. Because of this, and also because of possible confusion of the A and B designation of the deer mouse enzymes with the well-known A and B variants of G6PD in human erythrocytes, it has been accepted that the B form of G6PD would be designated as hexose 6-phosphate dehydrogenase (H6PD). Both the enzymes oxidize glucose 6-phosphate, and both require $NADP^+$ as a coenzyme. Aside from these two similarities, all other parameters of study disclosed significant differences between the two molecules, and these included: substrate specificities, molecular weights, intracellular localization, and tissue-specific variation in both enzyme concentration and electrophoretic pattern. Molecular weights of the two enzymes appear to differ, with H6PD being a slightly larger molecule. The evidence for

the homology of hexose 6-phosphate dehydrogenase and glucose 6-phosphate dehydrogenase was reported by Matsuoka *et al.*, (1983) by comparison of the amino acid compositions. Their findings strongly suggest the previous prediction; that the two enzymes have diverged from a common ancestral molecule.

H6PD is present in liver and kidney of several vertebrates (Ohno *et al.*, 1966 Shaw and Koen, 1968), and also in rat testes, ovary, spleen, and lung (Shrivastava *et al.*, 1968). The enzyme is autosomally controlled (Shaw and Barto, 1965; Ruddle *et al.*, 1968) and is localized in the microsomal fraction of the cell (Beutler and Morrison, 1967).

Analysis of hepatic extracts from a large number of individual lake trout has shown only one H6PD phenotype which was expressed as a single electrophoretic band. Apparently, the gene coding for H6PD in lake trout was invariant, controlling production of a single type of subunit and therefore a single electrophoretic species. Brook trout and rainbow trout, however, exhibited polymorphism of H6PD with as many as six phenotypes resolved electrophoretically (Stegeman and Goldberg, 1971). Ropson and Powers (1989) reported the allelic isozymes of hexose 6-phosphate dehydrogenase from *Fundulus heteroclitus.*

The banding patterns observed in *Channa orientalis* revealed the presence of two loci of H6PD designated as *H6PD-1** and *H6PD-2** (**Fig. 4.4a**). The bands located at the cathodal region are presumed to be the expression of *H6PD-1** whose activity was found to be the highest in liver. The two anodal bands observed only in liver arises as a result of the expression of two codominant alleles encoded by *H6PD-2**.

In *C. puncatus* the presence of a single locus is explicit and its expression is highest in eye and liver (**Fig. 4.4b**).

The appearance of two zones of activity in *C. striatus* suggests the presence of two loci for H6PD as in *C. orientalis*. The expression of *H6PD-1** was observed in brain, eye, heart and kidney and the occurrence of two bands in eye (**Fig. 4.4d**) and heart suggest two alleles for this locus. In some individuals, the liver H6PD appeared to be encoded by *H6PD-1** (**Fig. 4.4c**) while in others by *H6PD-2** locus (**Fig. 4.4d**).

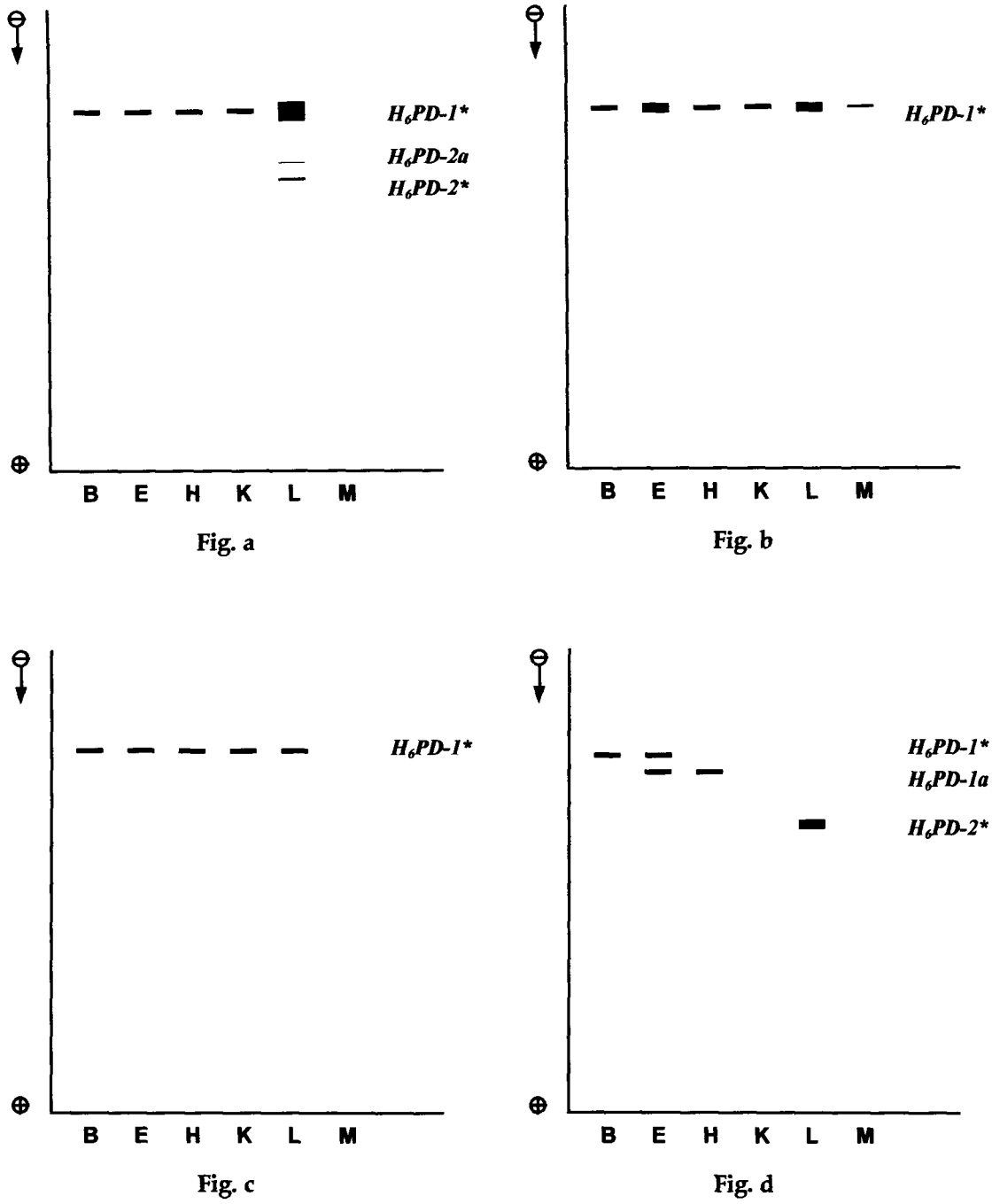


Fig. 4.4 Zymograms illustrating the activity of H6PD — (a) *Channa orientalis;* (b) C. *punctatus* (c) & (d) C. *striatus.* (B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.5 Glutamate dehydrogenase (GDH, EC No. 1.4.1.2) is a regulatory enzyme and plays a major role in the channelling of carbon into Krebs cycle (Hochachka & Somero, 1973). Thus, any fluctuation in GDH activity levels in liver could be indicative of increased or decreased energy demands during various developmental phases. Besides, GDH also collects the NH^{+4} ion and produces glutamate. Thus an increase in its level could also result in high levels of glutamate production.

Korsgaard (1982) studied the enzyme activity levels of glutamate dehydrogenase in *Zoarces viviparus* through vitellogenesis, ovulation, pregnancy and parturition. He observed changes in the levels of GDH during all these reproductive phases, and significantly, during mid-term pregnancy. A constant but low level of GDH was observed during vitellogenesis, which showed a marked increase during mid-term pregnancy. Similar observation was made in northern pike, *Esox lucius*, by Medford and Makay (1978).

A review of literature shows scarce data on biochemical study on glutamate dehydrogenase isozyme in fish. Glutamate dehydrogenase enzyme is encoded by two loci in *Chanos chanos* (Winans, 1980), and *Tilapia zillii* (Cruz *et al.*, 1982), and a single locus in

Theragra chalcogramma (Grant and Utter, 1980), *Gadus morhua* (Mork *et al.*, 1982) and *Salmo salar* (Ståhl, 1981). In *Tilapia zillii* and *Theragra chalcogramma*, the activity was found to be high in muscle tissue. The banding pattern observed in *Tilapia zillii* by Cruz (1982) is readily explained by assuming that glutamate dehydrogenase has a monomeric structure, as in other vertebrates (Harris and Hopkinson, 1976). *Petromyzon marinus* showed insufficient staining activity (Krueger, 1980), while no activity was reported in Arctic Charr (Andersson *et al.*, 1983).

From our observations in all three species of *Channa*, we can conclude that there is some correlation between the activity level of GDH and the reproductive phases as observed in *Zoarces viviparus*. The banding pattern resolved in all three species is indicative of a monomeric structure for this enzyme. In *Channa orientalis* it appeared to be encoded in two loci designated as *GDH-1** and *GDH-2** (**Fig. 4.5a**), while in *C. punctatus* (**Fig. 4.5b**) and *C. striatus* (**Fig. 4.5c**), a single locus codes for this enzyme. The absence of GDH activity in all the males of the three species investigated tempts us to suggest the non-expression of the gene for GDH at this particular season of the year, or maybe due to very low expression we were unable to detect its activity on the gel.

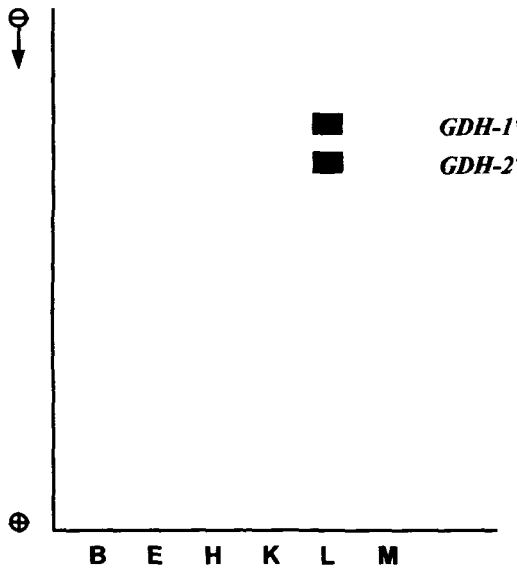


Fig. a

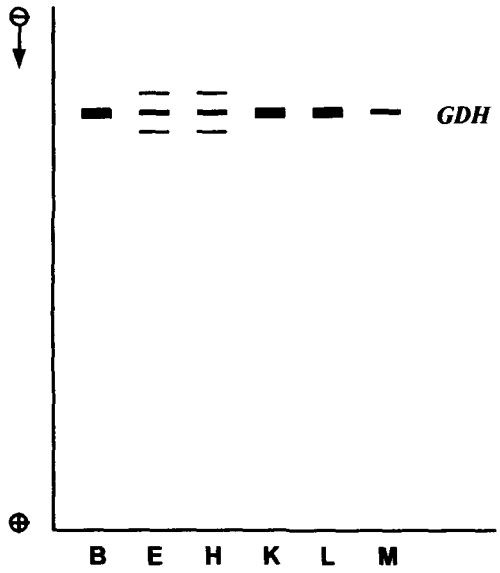


Fig. b

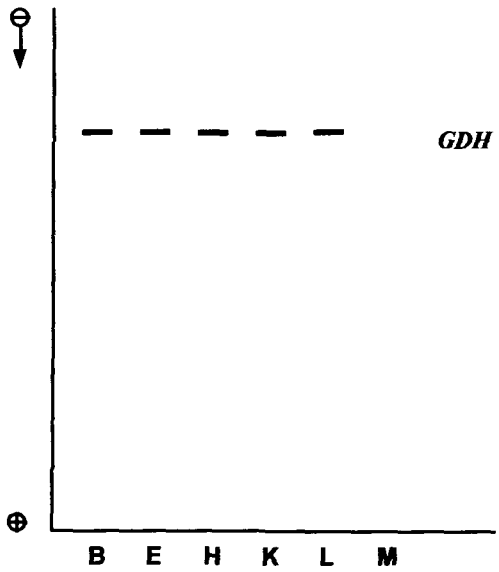


Fig. c

Fig. 4.5 Zymograms illustrating the activity of GDH — (a) *Channa orientalis;* (b) C. *punctatus* (c) C. *striatus.* (B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.6 The trivial name of the glycerol 3-phosphate dehydrogenase is α-Glycero 3-phosphate dehydrogenase (αGPD, EC No. 1.1.1.8). It mediates the conversion of glycerophosphate into dihydroxy acetone phosphate in Emden-Mayerhof pathway. It is a dimeric enzyme. There is a controversy in tissue-specific activity of αGPD in fish species, i.e., one group found αGPD activity mainly in liver tissue (Utter *et al.*, 1974; Kimura, 1978; Cross and Ward, 1980; Andersson *et al.*, 1983; Taniguchi *et al.*, 1983) and the other group found that αGPD is predominantly expressed in skeletal muscle tissue (Dando, 1970; Engel *et al.*, 1971; Ståhl and Ryman, 1982).

αGPD is expressed solely in muscle in *Hypentelium* (Buth, 1980), *Oncorhynchus nerka* (Grant *et al.*, 1980), *Salvelinus alpinus* (Andersson *et al.*, 1983), and Bighead carp, silver carp and their hybrids (Brummett *et al.*, 1988) but in grey mullets from Spain, liver is the tissue of choice (Papasotiropoulos *et al.*, 2001). Japanese stock of tilapia *Oreochromis niloticus* and *Tilapia zillii* (Basiao and Taniguchi, 1984), *Platycephalidae* (Keenan, 1991), and grey mullets from Goa showed expression in both liver and muscle (Menezes *et al.*, 1992) while,

Theragra chalcogramma in eye and muscle (Grant and Utter, 1980) and *Mystus nemurus* in kidney, liver and muscle (Leesa-Nga *et al.*, 2000).

A single locus with two codominant alleles has been reported in *Salmo trutta* (Engel *et al.*, 1971), *Petromyzon marinus* (Krueger, 1980), *Hypentelium* (Buth, 1980), *Salmo giardneri* aquilarum (Guyomard, 1981), *Gadus morhua* (Mork *et al.*, 1982), *Salvelinus alpinus* (Andersson *et al.*, 1983), Bighead carp, silver carp and their hybrids (Brummett *et al.*, 1988), *Barbus brevipinnis* (Engelbrecht and Van Der Bank, 1994), *Mystus nemurus* (Leesa-Nga *et al.*, 2000).

Two loci have been found to code for αGPD, *αGPD-1** and *αGPD-2** in *Oncorhynchus nerka* (Grant *et al.*, 1980), *Theragra chalcogramma* (Grant and Utter, 1980), Japanese stock of tilapia *Oreochromis niloticus* and *Tilapia zillii* (Basiao and Taniguchi, 1984), *Platycephalidae* (Keenan, 1991), grey mullets from Goa (Menezes *et al.*, 1992), and grey mullets from Spain (Papasotiropoulos *et al.*, 2001)

Three loci were reported for *Tilapia zillii* (Cruz *et al.*, 1982), and *Coregonus albula* (Vuorinen *et al.*, 1986).

In all the three species of *Channa* the banding pattern for αGPD observed are presumed to be encoded in a single locus. The locus seemed to be liver-specific in *C. orientalis* (**Fig. 4.6a**) but in *C. punctatus and C. striatus* it showed a broader tissue distribution (**Fig. 4.6b&c**). The presence of five bands in brain, eye, heart and kidney of *C. punctatus* (**Fig. 4.6b**) is perhaps due to multiple loci arising out of gene duplication.

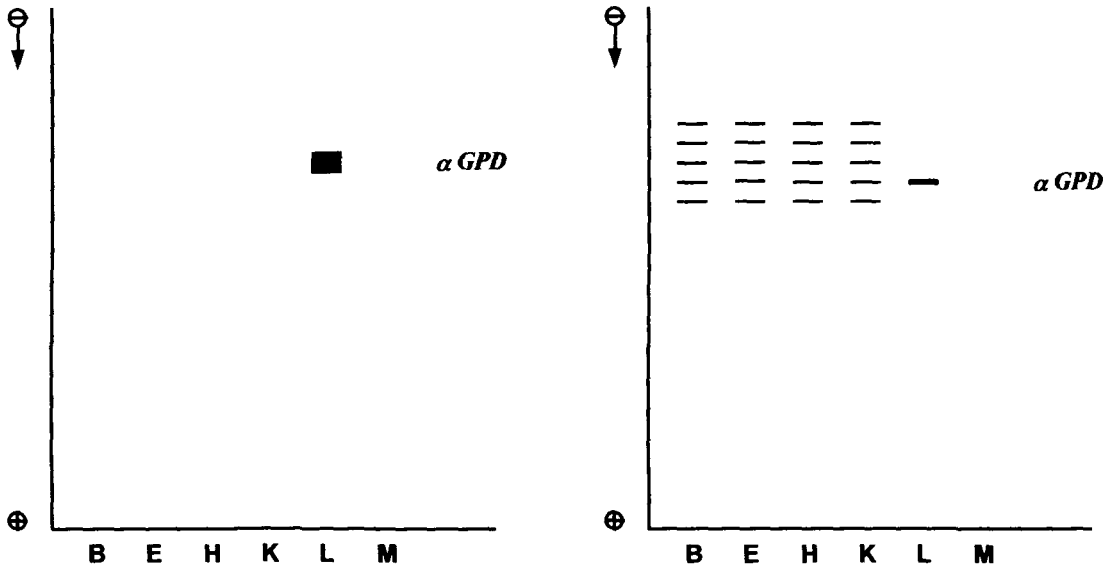


Fig. a

Fig. b

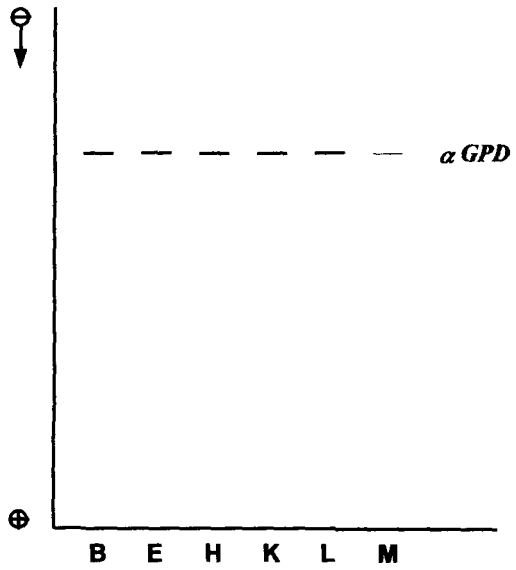


Fig. c

Fig. 4.6 Zymograms illustrating the activity of αGPD — (a) *Channa orientalis;* (b) *C. punctatus* (c) *C. striatus.* (B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.7 Lactate dehydrogenase (lactate: NAD-oxidoreductase, LDH, EC No. 1.1.1.27) is the most studied enzyme in vertebrates and in all it is a tetrameric protein with a mol.wt. of 140 kDa. It has four subunits of mol.wt. 35 kDa each (Appella and Markert, 1961; Jaenicke and Knof, 1968; Adams *et al.*, 1970, 1973; Darnall and Klotz, 1975; Shaklee, 1975; Vallee, 1975). LDH catalyzes the reversible dehydrogenation of lactate converted into pyruvate in the presence of NAD^+ as a hydrogen acceptor.

Lactate dehydrogenase (LDH) enzyme constitutes a multigene family whose members are developmentally regulated and differentially expressed (Fine *et al.*, 1963; Lindsay, 1963). In all vertebrates there are two major LDH isozymes, A and B encoded by two gene loci *LDH-A** and *LDH-B**. The A isozyme is found predominantly in tissues such as skeletal muscle which may undergo anaerobic glycolysis (Nadal-Ginard and Markert, 1975) whereas the B isozyme is the major form of LDH in tissues with an aerobic metabolism (example; heart) (Wilson *et al.*, 1963; Everse and Kaplan, 1975).

The two subunits, A and B, are associated in cytoplasm to produce five different tetramers: the two homotetramers A_4 and B_4, and the three heterotetramers A_3B_1, A_2B_2, and A_1B_3, with different distribution and different kinetic and physico-chemical properties (Kaplan *et al.*, 1956; Markert and Apella, 1961; Markert, 1963). Distribution of the isozymes formed by the subunits A and B is not binomial in many species. This absence of heteropolymers is presumably due to the genetically specified intrinsic properties of the subunits (Markert and Faulhaber, 1965; Whitt, 1970a) or instability of the heteropolymers (Shaklee, 1975).

The migration of the A_4 isozymes, which normally have a low anodic speed, and B_4, with a greater negative charge, is inverted in one third of teleosts studied, the homotetramer A_4 is the most anodic band and the B_4 homotetramer is the less anodic (Markert and Faulhaber, 1965; Whitt, 1969; Callergarini and Ricci, 1973; Markert *et al.*, 1975; Odense and Leung, 1975; Philipp and Whitt, 1977; Philipp *et al.*, 1979, 1983; Ladewig De Panepucci *et al.*, 1984; Chatterjee and Dhar, 1985; Coppes *et al.*, 1987). Noteworthy of mention is the relative mobility of the A_4 (with low anodic rate) and B_4 (with average anodic rate)

homotetramers, which appeared to be reversed in the genus *Channa*. This phenomenon has been reported in 30% of the fish species. The C_4 homotetramer (with high anodic rate) prevalent in most teleosts was expressed in *C. orientalis* only. Its function was restricted to the neural tissue, specifically eye tissue. Heteropolymers between B_4 and A_4 and between A_4 and C_4 homotetramers were also observed.

Some vertebrates also possess a third type of LDH. In mammals and birds this isozyme has been called LDH-X or the C isozyme and it is only expressed in primary spermatocytes (Zinkham *et al.*, 1969; Goldberg, 1972). Many fish have a third form of LDH, which Markert *et al.*, (1975) have also referred to as the C isozyme encoded by the *LDH-C* locus. This was first observed in chondrostei (Whitt, 1969, 1970a; Morizot and Siciliano, 1983). Unlike the C isozyme of warm-blooded vertebrates, the C isozymes of fish with high anodic speed are not restricted to primary spermatocytes. In the primitive taxa of osteichthyes, which have the *LDH-C** locus, the C_4 isozyme is present in a variety of tissues, parallel to the *LDH-B** locus (Whitt *et al.*, 1975; Fisher *et al.*, 1980). Most modern teleosts limit the expression of this form of LDH to neural tissues such as the

eye and the brain. This has led to the terms E_4, eye-band and retinal isozyme I being used (Lush *et al.*, 1969; Whitt, 1970a).

Considering the most primitive fishes, the Agnatha, they appear to have a more primitive isozyme repertory. The lampreys (Petromyzontiformes) have only one LDH isozyme, the LDH-A_4 (Wilson *et al.*, 1964; Markert *et al.*, 1975; Whitt *et al.*, 1975). This observation suggests that an LDH-A-like locus was the ancestral LDH gene (Whitt, 1981). The hagfishes (Myxomizontiformes) have *LDH-A** and *LDH-B** genes like the other fishes and higher vertebrates, but are less functionally divergent (Sidell and Beland, 1980). Upon the scale of fishes the cartilaginous fishes show, generally a four-banded pattern of LDH isozymes (Markert *et al.*, 1975; Whitt *et al.*, 1975), only the *LDH-A** and *LDH-B** genes are found in these fishes. Bony fishes constitute a group of critical importance with regard to vertebrate evolution, since one branch of them leads to the advanced fishes and another leads to higher vertebrates. Only lungfishes and the coelacanth represent this second branch of bony fishes. A third locus *LDH-C** characterizes all osteichthyes except the most primitive of them, the Dipnoi that lack this third LDH-C locus. It was proposed that the

ancestral form of LDH closely resembled the present A type LDHs and that the gene for this protein duplicated to give rise to the A and B forms. The C type LDHs were then produced by a succession of independent duplications of the gene, which coded for the B isozyme (Holmes, 1972).

The most precise method for determining the evolutionary relationships among related proteins is to compare their amino acid sequences (Wilson *et al.*, 1977). This is now possible for three A and two B type LDH isozymes (Eventoff *et al.*, 1977) and for the C isozymes from rat and mouse (Pan *et al.*, 1983). Li *et al.*, (1983) have made use of these amino acid sequences to re-evaluate the evolutionary pathway leading to the A, B and C isozymes of mammals. Their results indicate that a C type LDH was the ancestral form. This is radically different from the acknowledged view of LDH isozyme evolution (Holmes 1972; Markert *et al.*, 1975; Holbrook *et al.*, 1975).

The LDH isozymes are like other systems in which there had been a gene duplication event followed by divergent evolution (e. g. globins, Wilson *et al.*, 1977; lysozyme-lactalbumin, White *et al.*, 1977; pancreatic ribonuclease, Lenstra and Beintema 1979; Beintema

1983), in that the rates of point mutation fixation have been constant in the duplicated genes. Thus, there has not been an increase in the evolutionary rate in the rodent C type LDHs. This analysis refutes part of the Holmes model of LDH evolution (Holmes 1972; Holbrook *et al.*, 1975) and brings into question the validity of the rest of it. In particular, the relationship of the fish C-type LDHs to the other LDH isozymes need to be determined.

Rehse and Davidson (1986) in their comparison of cod liver C-type LDH to other LDH isozymes concluded that the cod and rodent C type LDHs are orthologous proteins, i.e., they correspond to the same genetic locus and their different structures are the result of divergence due to speciation events rather than gene duplications. If this interpretation were incorrect, and the rodent C type LDH is as ancient as it appears, then one would expect to find more than three genetic loci for LDH in some vertebrates. To date, this has not been observed except in the case of some animals known to have undergone entire genome duplication (i.e., a stable tetraploidisation, Markert *et al.*, 1975).

In other fish species belonging to the orders gadiformes and cypriniformes, C_4 isozyme having cathodic migration, liver is the tissue

of choice. Investigations employing immunochemical, genetic, physical and phylogenetic approaches have demonstrated that the eye-band LDH seen in many groups of teleosts and the liver-band LDH seen in other groups are encoded in the same basic locus, even though the isozymic products have somewhat different properties in these different groups. Thus, among teleosts the general trend in evolution is clearly for specialization of the C gene and restriction of its function to neural tissues or, for a few fish, to the liver (Markert *et al.*, 1975; Whitt *et al.*, 1975).

In these fish the liver-specific form has been called the D_4, or F_4 LDH (Sensabough and Kaplan, 1972; Shaklee *et al.*, 1973, Shaklee and Whitt, 1981; Morizot and Siciliano, 1983; Frankel, 1987). Recently a co-expression of the *LDH-C* locus with a C_4 isozyme, having a high anodic speed, has been observed in some cichlid fish species (Perciformes, Cichlidae), in the eye, with an L_4 isozyme having cathodic speed, in the liver. This fact might support the hypothesis of the presence of a fourth LDH locus in cichlids (Holt and Liebel, 1987).

In keeping with the nomenclature advocated by Markert *et al.*, (1975), we shall refer to all vertebrate LDH isozymes which are not of the A or B form as C-type.

Contrary to what Ladewig De Panepucci *et al.*, (1984) reported regarding the absence of heteropolymers formed by the C subunits with those of A or B, many authors have found an association between B and C subunits in the retina and neural tissues (Callergarini and Vendemiati, 1975; Philipp and Whitt, 1977; Philipp *et al.*, 1979; Salvatorelli *et al.*, 1987, 1989; Coppes *et al.*, 1987; Basaglia *et al.*, 1989). Shaklee *et al*, (1973) and Garlick and Terwilliger (1978) have described some species of fish in which an association between C and A subunit was observed.

The electrophoretic expression of this tetrameric enzyme has been previously described for this genus (Chatterjee and Dhar, 1985). The LDH isozymes of the three species under investigation viz., *Channa orientalis* (**Fig. 4.7a**), *C. punctatus* **Fig. 4.7b**) and *C. striatus* (**Fig. 4.7c**) have its own pattern. The A_4 and B_4 homotetramers typical of this enzyme in the more highly evolved teleosts are found to be monomorphic and ubiquitous in expression in all the three species of *Channa.* As was observed in a majority of vertebrates, A_4 homotetramer was found to be predominant in white skeletal muscle and B_4 in red muscle (heart).

The results confirmed the presence of two active gene loci for LDH isozyme designated as *LDH-A** and *LDH-B** in all the three species studied and an additional locus in *C. orientalis* designated as *LDH-C** (**Fig. 4.7a**). None of the three species exhibited the fourth or liver-specific locus as seen in some species.

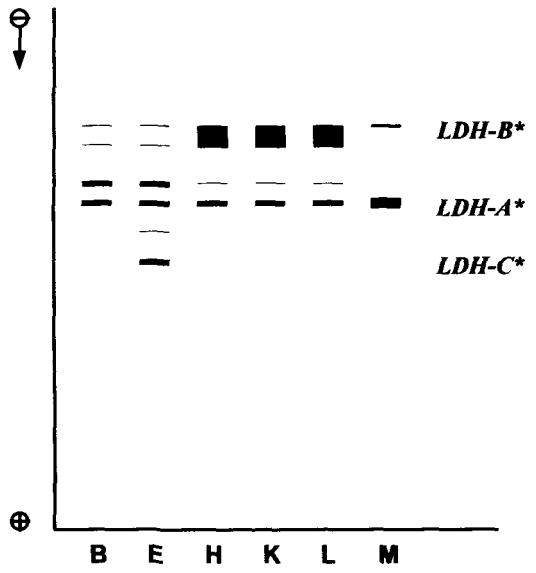


Fig. a

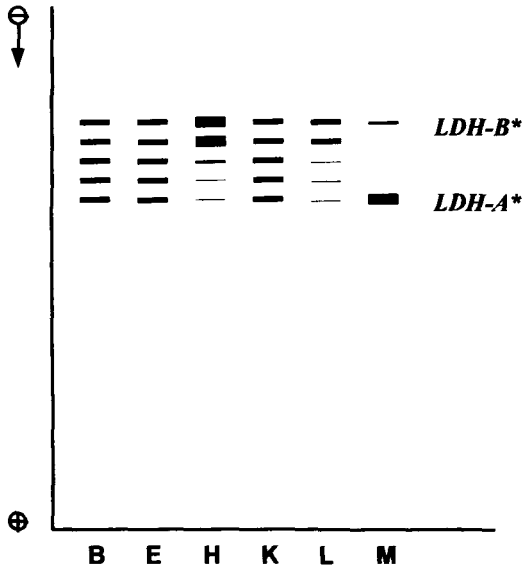


Fig. b

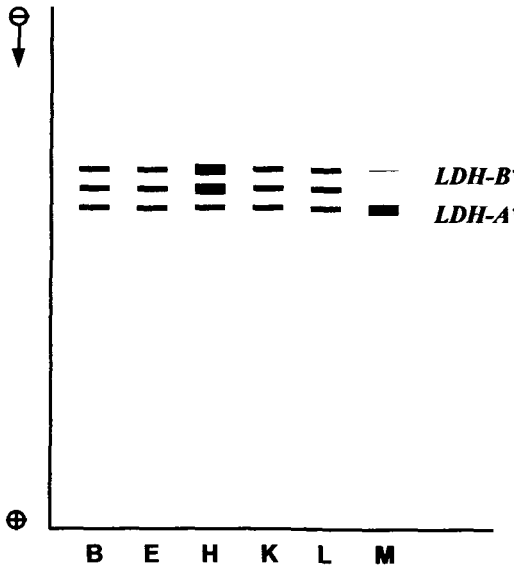


Fig. c

Fig. 4.7 Zymograms illustrating the activity of LDH — (a) *Channa orientalis;* (b) C. *punctatus* (c) C. *striatus.* (B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.8 Malate dehydrogenase (L-malate: NAD^+-oxidoreductase, MDH, EC 1.1.1.37) is a dimer of mol.wt 60-80 kDa. It catalyzes the interconversion of malate and oxalocetate (Banaszak and Bradshaw, 1975; Darnall and Klotz, (1975) in the krebs cycle. The MDH isozymatic system consists in invertebrates and vertebrates of two main forms: (1) supernatant/cytosolic ($_c$MDH) in extramitochondrial cytoplasm, and (2) mitochondrial malate dehydrogenase ($_m$MDH) in the mitochondrial matrix. These two forms have the same mol. wt. but because of their different subcellular location they differ in electrophoretic mobility, kinetic behaviour, amino acid composition and antigenic properties; and they are controlled by separate gene loci (Whitt, 1970b, 1971). The two forms, cytosolic and mitochondrial do not combine with each other to form heterodimers (Mankwell and Baker, 1970); a characteristic that distinguishes between the two different forms beyond any doubt (Fisher *et al.*, 1980; Buth, 1983).

In the majority of fish species duplicated cytosolic MDH loci are found, *MDH-A** and *MDH-B**, encoding polypeptides with molecular weights of 30,000-35,000 respectively. Random association of these polypeptides results in the formation of dimeric isozymes A_2, AB and B_2. In primitive fish the two cytoplasmic subunits are

expressed equally in all tissues. In advanced teleosts the A_2 homodimer exhibits the highest activity and is present in most tissues (Fisher *et al.*, 1980; Pasdar *et al.*, 1984; Salvatorelli *et al.*, 1987; Basaglia and Callergarini, 1988; Coppes *et al.*, 1987; Basaglia, 1989). The B_2 homodimer is usually of low activity with restricted tissue expression, predominating in skeletal muscle extracts (Bailey *et al.*, 1970; Wheat and Whitt, 1971; Wheat *et al.*, 1972, 1973; Whitt *et al.*, 1973; Rainboth and Whitt, 1974; De Luca *et al.*, 1983; Buth, 1983; Philipp *et al.*, 1979, 1983; Coppes *et al.*, 1987; Salvatorelli *et al.*, 1987, 1989; Papasotiropoulos *et al.*, 2001; Yang *et al.*, 2001). These findings suggest that the A and B subunits are the products of duplicate genes, which have undergone limited evolutionary divergence.

The mitochondrial MDH ($_m$MDH) is not very active in any tissue. In fishes it has been suggested that a single gene is involved in the production of $_m$MDH, which usually occurs as a single cathodal band. In *Fundulus heteroclitus* the mitochondrial MDH isozymes migrated more anodally during electrophoresis than the cytosolic MDH isozymes (Whitt, 1970b). The relative electrophoretic mobility of *Fundulus* $_c$MDH and $_m$MDH isozymes is reversed compared to that

for most vertebrate. This reversal has also been observed in sea urchins and tuna, but does not occur in all fish.

Three loci were scored in *C. orientalis* (**Fig. 4.8a**) for malate dehydrogenase enzyme. Two loci are assumed to code for the two subunits A and B of cytosolic malate dehydrogenases. As has been observed in other teleosts the species show a characteristic tissue expression of $_{c}$MDH isozymes. The A_2 subunit showed a high degree of activity in all tissues. The B_2 isozyme showed a restricted tissue distribution, predominating in extracts of skeletal muscle. In addition, the relative mobilities of $_{c}$MDH isozymes conform to the typical teleostean distribution pattern for this system, with the B_2 homodimer being the most anodal. The AB heterodimer between A_2 and B_2 was also observed in all tissues. A single locus codes for the mitochondrial MDH whose expression is low and variable. The product of the gene appeared at the cathodal region of the gel.

In *C. punctatus*, we observed the expression of three gene loci for malate dehydrogenase. $_{c}$*MDH-A** and $_{c}$*MDH-B** loci code for the A_2 and B_2 subunits of cytosolic MDHs respectively and $_{m}$*MDH* codes for mitochondrial MDH. $_{c}$*MDH-A** was expressed in all the six tissues

and showed high level of activity. *$_c$MDH-B**, on the other hand showed low activity and restricted its expression to the white skeletal muscle. The heterodimer AB between the A_2 and B_2 subunits was also observed in white skeletal muscle and heart but with very low activity. The relative mobility of the supernatant and mitochondrial MDHs was reversed as exhibited in *Fundulus heteroclitus* and other few fishes. It appeared at the anodal region and showed activity in all the tissues. From the banding pattern, we can conclude that the $_m$MDH is encoded in a single locus (**Fig. 4.8b**).

Two forms of malate dehydrogenases were recorded in *C. striatus* as in the rest of the teleosts (**Fig. 4.8c**). The A_2 subunit was predominant in liver and to a lesser extent in kidney, while B_2 subunit was present in all the tissues and showed high activity. The A_2,B_2 heterodimer was also detected with high activity in brain, eye, heart, kidney and liver. Mitochondrial MDH appeared predictably at the cathodic region in brain, eye, heart, kidney and liver but showed weak activity. None of the individuals showed the expression of *$_m$MDH* in white skeletal muscle.

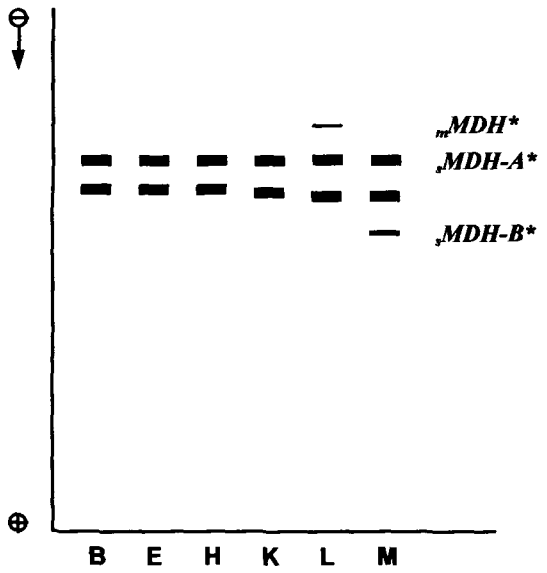


Fig. a

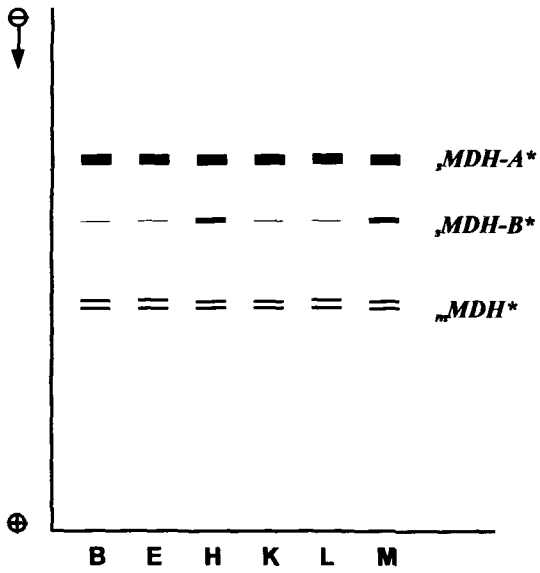


Fig. b

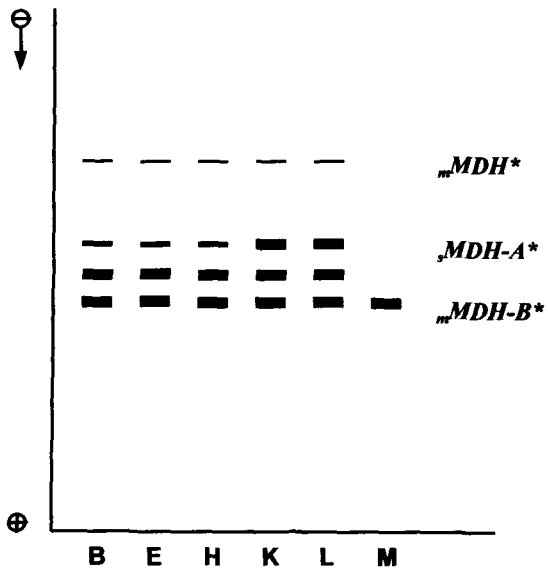


Fig. c

Fig. 4.8 Zymograms illustrating the activity of MDH — (a) *Channa orientalis;* (b) *C. punctatus* (c) *C. striatus.* (B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.9 Malic enzyme (ME, EC No. 2.7.5.1), otherwise known as $NADP^+$ MDH converts malate into pyruvate and during the reaction provides NADPH for lipogenesis. Malic enzyme, in most vertebrates is known to exist in two forms, mitochondrial (ME_m) and cytosolic (ME_c). In all fish species studied it has been found to be a tetramer.

The slowest migrating anodal zone is expressed in skeletal muscle, and corresponds to the mitochondrial ME. This zone was in general expressed as a single band, but three rare phenotypes with five asymmetrical stained bands were also found. These phenotypes are most probably produced by a single copy of a variant allele, which would correspond to the staining patterns of a tetrameric enzyme postulated by the duplicate model. The duplicate status of mitochondrial ME has been reported for many salmonids (Allendorf *et al.*, 1977; Cross *et al.*, 1979; Stoneking *et al.*, 1979). May (1980) however, stated that only one locus, codes for ME_m in three North American coregonids. In Arctic charr the less anodal zone obtained in muscle extracts was represented by three bands assumed to represent the expression of a duplicate pair of loci (*ME-1** and *ME-2**) fixed for different alleles (Anderson *et al.*, 1983). Similar deviations from the expected five-banded pattern reported for brook trout was assumed to be caused by reduced

expression of *ME-1** (Stoneking *et al.*, 1979). When the duplication of a locus coding for a mitochondrial enzyme is connected with a polyploidization event, this can be taken as evidence that the loci are located in the chromosomes and not in the mitochondrial DNA.

The faster moving zone represents the cytosolic form of the enzyme, which predominates in liver. In vendace this zone showed a relatively high amount of variation, it consisted usually of three phenotypes, a single-banded type with additional asymmetrical and symmetrical five-banded patterns towards the anode. The most plausible explanation for this variation involves a duplicate pair of loci with one fixed and one variable locus. Both loci share common alleles coding for electrophoretically identical proteins. These enzymes also interact forming heterotetramers between each other (Vuorinen, 1984). In vendace the ME phenotypes indicate clearly duplicate loci in ME_c and ME_m. Both show one fixed and one variable locus. The five-banded banding patterns confirm also the tetrameric quaternary structure of ME, as found in all vertebrate species examined (Nevaldine *et al.*, 1974).

Malic enzyme has been reported to be encoded in two loci in trout, *Salmo trutta* (Allendorf *et al.*, 1977; Stoneking *et al.*, 1979; Thompson, 1985; Loudenslager *et al.*, 1986; Cross and Challanain,

1991), *Theragra chalcogramma* (Grant and Utter, 1980), Italian freshwater *Gobies* (Miller *et al.*, 1994), Japanese stock of tilapia *Oreochromis niloticus* and *Tilapia zillii* (Basiao and Taniguchi, 1984), *Coregonus albula* (Vuorinen, 1984), *Gyrinocheilus aymonieri* (Rainboth *et al.*, 1986), *Gobius* (McKay and Miller, 1991), Grey mullets of Spain (Papasotiropoulos *et al.*, 2001), *Hoplias malabaricus* (Peres *et al.*, 2002)

In contrast, only one locus has been found in some *Coregonus* species (May, 1980), *Petromyzon marinus* (Krueger and Spangler, 1981), *Salmo giardneri aquilarum* (Guyomard, 1981), *Gadus morhua* (Mork *et al.*, 1982), *Procambarus clarkii* and *P. acutus* (Busack, 1988) Grey mullets from Goa (Menezes *et al.*, 1992), shortfin barb *Barbus brevipinnis* (Engelbrecht and Van Der Bank, 1994), yellow catfish *Mystus nemurus* from Thailand (Leesa-Nga *et al.*, 2000).

In *C. orientalis* the slowest migrating band was expressed in heart and skeletal muscle, and corresponds to the mitochondrial ME. The anodal zone representing the cytosolic ME showed a relatively high amount of variation and usually consisted of five bands. The most plausible explanation for this variation involves two loci, with one fixed and one variable locus. We therefore, conclude the presence

of three loci for malic enzyme, one for ME_m and two for Me_c, designated as *ME-1** and *ME-2** (**Fig. 4.9a**).

The two zones of activity for ME in *C. punctatus* corresponds to the mitochondrial and cytosolic ME (**Fig. 4.9b**). The banding pattern suggests the present of a single locus for ME_m and two loci for ME_c, with one fixed and one variable locus.

In *C. striatus* malic enzyme showed two zones of activity (**Fig. 4.9c**). The zone closest to the cathode is the mitochondrial ME and is mainly expressed in muscle, while the faster moving zone represents the cytosolic form of the enzyme, which predominated in liver. The ME_m obtained in muscle extracts was represented by three bands, which can be explained as the expression of two loci *$_m$ME-1** and *$_m$ME-2** fixed for different alleles. A similar deviation from the expected five-banded pattern reported for *C. orientalis* and *C. punctatus* was assumed to be caused by reduced expression of *$_m$ME-1**. The cytosolic ME on the other hand appeared to be encoded by a single locus.

The five-banded pattern also confirms the tetrameric quaternary structure of ME as found in all vertebrates.

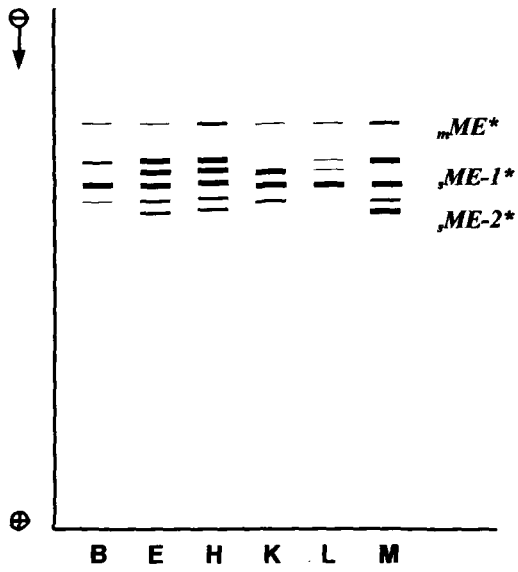


Fig. a

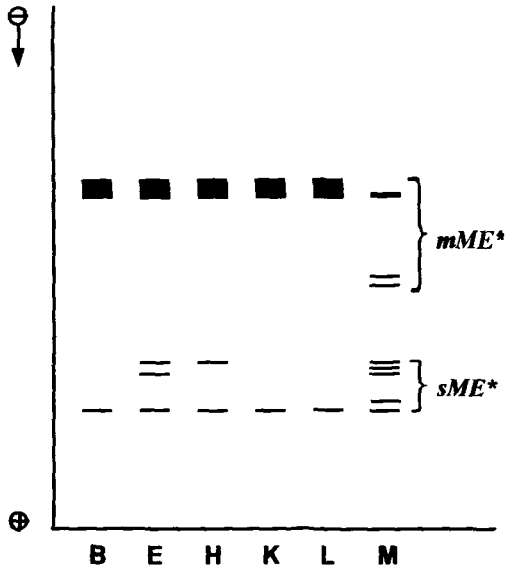


Fig. b

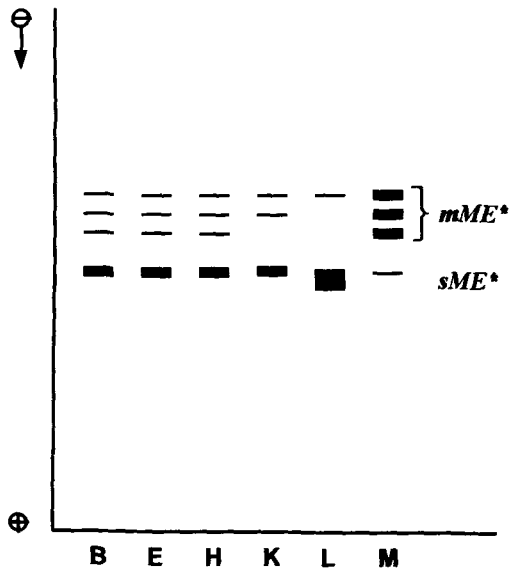


Fig. c

Fig. 4.9 Zymograms illustrating the activity of ME — (a) *Channa orientalis;* (b) C. *punctatus* (c) C. *striatus.* (B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.10 Sorbitol dehydrogenase (SDH, EC No. 1.1.1.14) is a tetrameric enzyme (Ward, 1978) that constitutes the polyol pathway, an alternative route of glucose metabolism. It converts L-sorbitol to fructose using NAD^+ as a cofactor. Amano *et al.*, (2003) have found that SDH isozyme activity might make a greater contribution to the etiology of diabetic retinopathy.

SDH was observed as a single invariant band in brook and lake trout (May *et al.*, 1979), sea lamprey (Krueger, 1980), *Hypentelium* (Buth, 1980), Arctic charr (Andersson *et al.*, 1983), Australian species of flatheads, Platycephalidae (Keenan, 1991), and shortfin barb *Barbus brevipinnis* (Engelbrecht and Van Der Bank, 1994). In all the above fishes, SDH was found to be liver-specific.

SDH exhibited two loci for *Chanos chanos* (Winans, 1980), *Tilapia zillii* (Cruz *et al.*, 1982), *Salmo salar, Salmo trutta* (Vuorinen 1982, 1984), Atlantic cod, *Gadus morhua* (Mork *et al.*, 1982), Japanese stock of Tilapia; *Oreochromis niloticus* (Basaio and Taniguchi, 1984). The isozyme was found to be polymorphic and indicated a probable tetrameric structure. In vendace, *Coregonus albula* (Vuorinen, 1984), five alleles were observed all segregating at both loci. A high amount

of polymorphism was also noted in *Salmo salar* (Cross and Challanain, 1991) lines farmed in Ireland.

The presence of two loci for SDH is clearly indicated from the banding patterns exhibited by the presently studied three species. These are designated as *SDH-1** and *SDH-2**. The activity was highest in liver and in *C. orientalis* it appeared to be liver-specific (**Fig. 4.10a**). In *C. punctatus* it showed activity in all the tissues (**Fig. 4.10b**) while in *C. striatus* low activity of SDH was exhibited in brain, eye, heart and kidney tissues (**Fig. 4.10c**). Indications of the tetrameric structure of the isozyme were pronounced in *C. punctatus*, in which five equally spaced bands were observed in brain, eye, heart and kidney tissues.

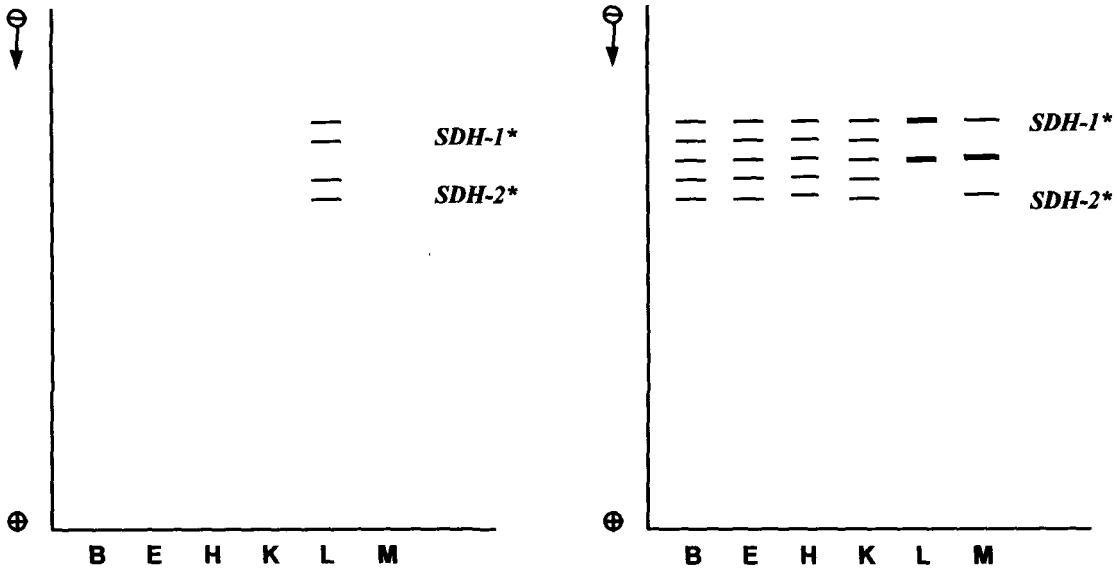


Fig. a

Fig. b

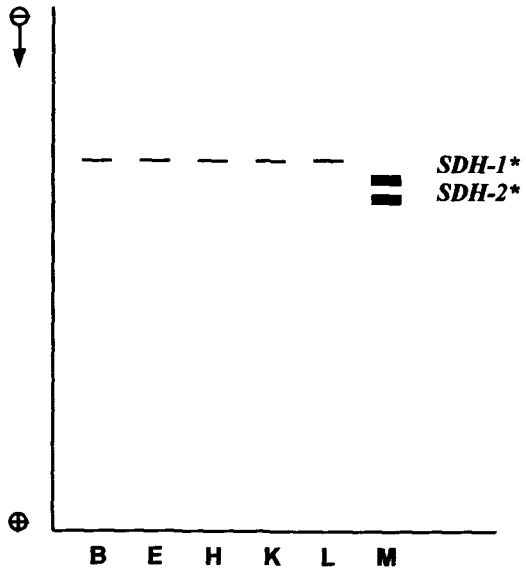


Fig. c

Fig. 4.10 Zymograms illustrating the activity of SDH — (a) *Channa orientalis;* (b) C. *punctatus* (c) C. *striatus.* (B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

4.11 Xanthine dehydrogenase enzyme (XDH, EC No. 1.1.1.204) performs a physiological role in purine metabolism as well as protein and amino acid catabolism. XDH contains a single atom of molybdenum; it catalyzes the oxidization of hypoxanthine to purine and uric acid using NAD^+ as the hydrogen acceptor (Johnson, 1974). This enzyme has also been implicated in the synthesis of pigmentation in some insects (Watt, 1967, 1972; Watt and Bowdan, 1966) and in amphibia (Levy, 1964). Smith and Jamieson (1978) proposed that xanthine dehydrogenase is an allelic isozyme having a dimeric structure. Xanthine dehydrogenase remains to be one of the least studied dehydrogenase (Utter and Folmar, 1978; Kirpichnikov, 1981; Magee and Philipp, 1982) in animal kingdom.

Xanthine dehydrogenase isozyme was found to be coded by a single locus in torrent suckers, *Thoburnia* (Buth, 1979), *Hypentelium* (Buth, 1980), *Salmo giardneri aquilarium* (Busack *et al.*, 1979), *Salvelinus alpinus* (Andersson *et al.*, 1983), *Corgonus albula* (Vuorinen, 1984), *Salmo salar*, *Salmo trutta* (Vuorinen and Piironen, 1984), Native Arizona and New Mexico Trout (Loudenslager, 1986), Australian species of flatheads, Platycephalidae (Keenan, 1991) and the activity in all of them was found to be predominant in the liver tissue.

In Atlantic cod (*Gadus morhua*) Mork *et al.*, (1982), detected activity of XDH only in skeletal muscle extracts and also noted some variation in width and relative position of bands. Padhi and Khuda-Buksh (1990) studied the activity of XDH isozyme in twenty species of teleostean fishes that revealed wide distribution in all tissues viz., brain, eye, heart, kidney, liver and muscle.

Papasotiropoulos *et al.*, (2001), detected two loci for XDH isozyme: designated *XDH-1** and *XDH-2**, both located in liver tissue of the Grey mullets of Spain.

In *Channa orientalis* (**Fig. 4.11a**) and *C. punctatus* (**Fig. 4.11b**) we observed a single invariant band in liver tissue, which revealed the monomorphic as well as tissue-specific nature of this isozyme as observed in most of the fish species studied. A single locus was assumed to code for XDH in *C. orientalis* and *C. punctatus*. In *C. striatus*, we assumed presence of two XDH loci. A wide tissue distribution as well was noted. Brain, eye and heart appeared to be encoded by *XDH-1** locus while kidney and liver in some individuals showed the expression of *XDH-1** (**Fig. 4.11c**) and in others *XDH-2** (**Fig. 4.11d**) locus. Both loci did not appear together in the same tissue in an individual.

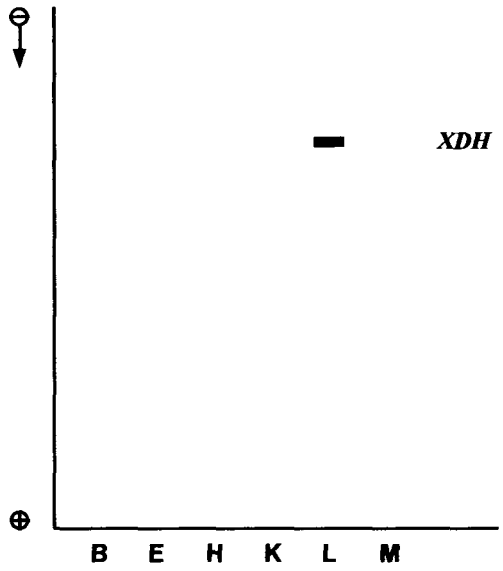


Fig. a

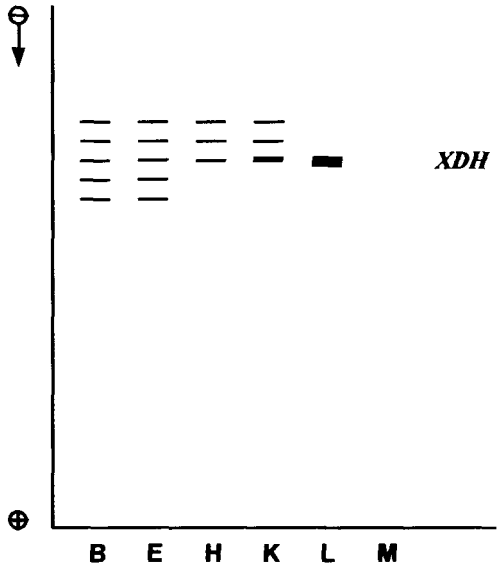


Fig. b

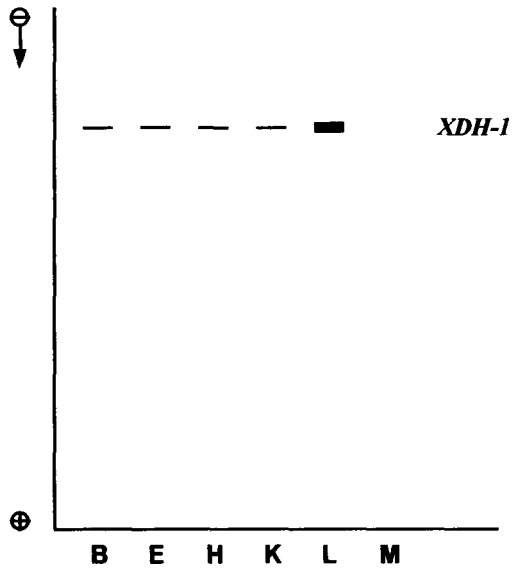


Fig. c

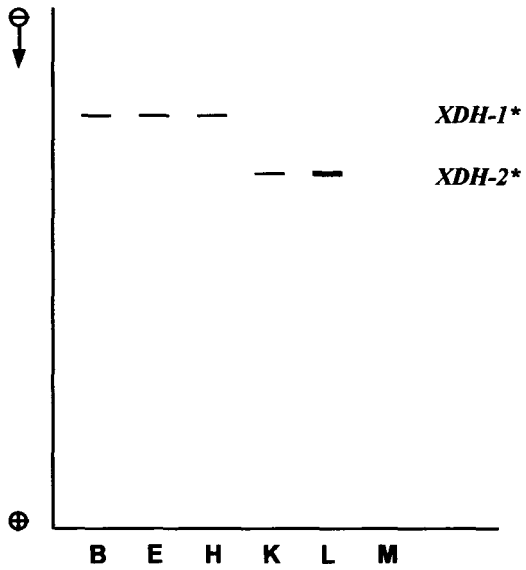


Fig. d

Fig. 4.11 Zymograms illustrating the activity of XDH — (a) *Channa orientalis;* (b) C. *punctatus* (c) & (d) C. *striatus.* (B-brain, E-eye, H-heart, K-kidney, L-liver, M-muscle)

Many enzymes exist in nature in multiple molecular forms called "isozymes" a term first introduced by Markert and Möller (1959). Its biological significance was well appreciated and is now widely found in biochemical literature. Fishes, the most primitive of vertebrates, are excellent organisms for studying the formation and evolution of isozymes loci (Whitt, 1981). The heterogeneity of proteins found in all the species of fishes, involves not only structural proteins of myogenic type but also enzymes (Kirpichnikov, 1973). It has been recognized for over a decade that multigenic enzyme systems provide one of the best systems for studying the molecular mechanisms involved in gene evolution and gene regulation (Markert *et al.*, 1975). Any interpretation of results at the nucleic acid level will depend upon a sound knowledge of the evolutionary relationships among the proteins, that the phylogenetic relationships of these isozymes had been well established (Whitt 1970a; Holmes 1972; Shaklee *et al.*, 1973; Holmes and Scopes 1974; Markert *et al.*, 1975; Whitt *et al.*, 1975). The variability of isozymic patterns in fishes is an important source of information about the epigenetic and genetic mechanisms responsible for the synthesis of isozymes. This may happen because of the primary structure of subunits, or because of

the epigenetic mechanisms that operate in order to restrict the association of subunits, or because of the evolutionary divergence of subunits, in more specialized fishes (Markert and Faulhaber, 1965; Markert, 1968). Owing to the fundamental position fishes occupy in the vertebrate scale, as well as their plasticity and the great number of species comprehended (fishes constitute approximately half of the existing vertebrate species), they are important organisms to study isozymes as tools for evolutionary purpose. When considering the evolution of vertebrates, it is evident that the genetic principle, on which the origin of mammals, and particularly of man is based, is related to the first vertebrates, the fishes (Ohno, 1970). Bony fishes (Teleostomi) represent the line from which advanced vertebrates (amphibians, reptiles, birds and mammals) evolved. Two lines are found in these fishes, one line leads to the advanced fishes, including the teleosts, and another line, poorly represented by lung fishes (Dipnoi) and the coelacanth, leads to the advanced vertebrates or land vertebrates.

A prime purpose of the present study was to identify electrophoretically detectable loci that could be used in routine population genetic surveys. We have detected a minimum of forty-six

loci (46) in the three species of *Channa* coding for the eleven (11) enzymes. We observed that the patterns and distributions of the enzymes under investigation are species-specific. The occurrence of these multiple forms of enzymes has enabled us to separate the three species of *Channa* without controversy. The unique banding pattern in *C. punctatus*, in which brain, eye, heart and kidney displayed five isozymes for most of the enzymes, has specially marked this species from the rest two. This has proved the utility of isozymes as a powerful tool for identifying morphologically indistinguishable individuals, hence making the study of genetic variation in a population interesting.

Lactate dehydrogenase is one isozyme system that owing to its multigenic nature, becomes an interesting model for evolutionary studies, and from this aspect has been widely investigated. In most investigated vertebrates the LDH enzymes are tetrameric proteins formed from the subunits polypeptides. In teleosts subunit A predominates in white muscle tissue, and subunit B generally predominates in heart muscle tissue. In tissues where both subunits occur, heteropolymers may form, but the proportions of the five LDH tetramers formed by the subunits A and B may deviate from the

expected random subunit association (Markert 1968; Markert *et al.*, 1975; Shaklee 1975; Shaklee and Whitt 1981). Subunit C has a very specialized occurrence. In the order Percomorphi the subunit is almost exclusively found in the tissues of eye and brain (Markert *et al.*, 1975). In the order gadiformes (Gadoidei, Macrouroidei and Muraenolepoidei) the subunit C predominates in liver (Markert *et al.*, 1975; Shaklee and Whitt 1981). In addition, the C_4 homopolymer in the gadiformes shows a low anodal or cathodal electrophoretic mobility, whereas the C_4 homopolymer of the eye in the Percomorphi shows a high anodal mobility relative to the electrophoretic mobilities of the A_4 and B_4 homopolymers. In the order Channiformes (Greenwood *et al.*, 1966), *Channa orientalis* exhibits eye specific C subunits with a high electrophoretic mobility of C_4 towards the anode, a pattern closer to the Percomorphi than to the Gadiformes (Shaklee and Whitt 1981). It, therefore, clearly stands out from the other two presently studied species, i.e., *C. punctatus* and *C. striatus.*

Besides lactate dehydrogenase, the other multigene enzyme systems such as glucose 6-phosphate dehydrogenase, hexose 6-phosphate dehydrogenase, malate dehydrogenase, and malic enzyme

have provided an insight on the evolutionary relationships among related proteins of the presently studied fish. The banding patterns of these enzymes indicated the evolution of these isozymes as a result of gene duplication. This was interestingly noted in the banding pattern of G6PD, H6PD and XDH in *Channa striatus*. The fact that the genus *Channa* is a diploid and not polyploid has been previously investigated by Chatterjee and Dhar (1984). This finding has solved our problem to conclude indisputably that the evolution of these isozymes is the outcome of gene duplication and not polyploidization.

The use of malate dehydrogenase as a genetic marker to examine genetic interaction among subpopulations of pink salmon (*Oncorhynchus gorbuscha*) has proved to be successful. The observations suggested that genetic isolation exists between temporally distinct spawning runs and that small temporal and spatial (or ecological) differences contribute to population structure (Gharett *et al.*, 2001). Recently Treberg *et al.*, (2002) while comparing the liver enzymes in osmerid fishes, found that the rainbow smelt (*Osmerus mordax*), which can accumulate high levels of glycerol has high levels of αGPD, than the non-glycerol accumulating capelin (*Mallotus villosus*).

We were also able to obtain information on the overall physiological function and regulation of these enzymes. The over expression of the locus for glutamate dehydrogenase in the egg-bearing females and non-expression as it appeared in the males shows the role they play during such conditions. The synthesis of glucose 6-phosphate dehydrogenase and hexose 6-phosphate in the three species appeared very peculiar. Two loci seemed to be responsible for its synthesis in *Channa orientalis*, a single locus in *C. punctatus*, while in *C. striatus* either one of the two loci is able to produce the product. This may be of some biological importance to the species (e.g. ecological, physiological etc.) and needs further investigation. In mammals, the homology between G6PD and H6PD is confirmed but in fishes no data in this regard has been obtained. Though the three species show some similarity in the banding patterns of the two enzymes, confirmation on their homology requires further in depth examinations.

The murrel have lost much of its natural habitat due to rampant destruction to its ecological niche and as a result its number is dwindling in many of its natural populations. Its conservation and propagation should be seriously considered lest it completely disappear

like many other fish species.

We suggest a detailed genetic survey to identify the natural populations as well as the genetic constitution of the snake-heads. Attempt should be made to link some of the gene loci to various environmental factors and coupled with mating experiments suitable stocks can be generated. The present study, in its own humble way may serve as a stepping stone for such a project.

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BIO-DATA

Name	:	**Quendarisa Kharbuli.**
Father's Name	:	**(L) Treslington Khyriem.**
Date of Birth	:	**27th March 1972.**
Address	:	**C/o Mrs. L. M. Kharbuli, Madan Laban, Shillong — 793 004, Meghalaya.**

***ACADEMIC QUALIFICATIONS*:**

Name of Examination	*Board/ University*	*Year*	*Division/ Class*	*Percentage of marks*
I. C. S. E.	CISCE	1988	Second	57.00%
Pre-University (Sc)	NEHU	1990	First	60.00%
B. Sc. (Hons)	NEHU	1993	First	60.50%
M. Sc.	NEHU	1995	First	64.94%

Specialization in M. Sc. : **Cytogenetics**

***SEMINAR / SYMPOSIA / WORKSHOP*:**

1. **National Symposium on Trends in Environmental Biology, NEHU, Shillong, June 23-25, 1999.**
2. **10th All India Congress of Cytology and Genetics, Kalyani, October 29-31, 1999.**
3. **Basic Computer Concepts and Applications, Bio-Informatics Centre, NEHU, Shillong, June 12-14, 2000.**

***PAPER PUBLISHED*:**

1. **Kharbuli, Q. and Chatterjee, K. (2001): Genetic expression of Glucose 6-phosphate dehydrogenase in *Channa* species. In: Perspective in Cytology and Genetics (Eds. Manna, G. K. and Roy, S. C.), 10: Pp 763-765.**

***RESEARCH EXPERIENCE*:**

1. **Two weeks training in National Bureau of Fish Genetic Resources (NBFGR), Lucknow, on mitochondrial DNA.**

***PRIZES / AWARDS*:**

1. **B. Sc. Rank Sixth.**
2. **M. Sc. Rank Fourth.**
3. **Zoology Departmental Seminar (NEHU), Second.**
4. **College Quiz, First Prize.**
5. **Khasi National Awakening Day Award.**
6. **4 x 100 m Relay Race (School), Third Prize.**

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GENETIC EXPRESSION OF GLUCOSE 6 PHOSPHATE DEHYDROGENASE IN *CHANNA* SPECIES

Q. KHARBULI and K. CHATTERJEE

Department of Zoology, North-Eastern Hill University, Shillong 793022, Meghalaya

ABSTRACT

The ever increasing environmental problems have accelerated intensive studies on ecology and thus placed heavy demands upon the existing genetic studies. Studies on the occurrence of protein polymorphisms are of importance not only in determining inter-specific relationships but also in revealing genetically controlled variants among the populations of the same species. Attempts are currently being made to assess how much and what kind of genetic variations provide the basis of adaptive evolution. In this paper, the electrophoretic expression of the gene coding for the enzyme glucose 6 phosphate dehydrogenase in two species belonging to the genus *Channa* has been described. The electrophoretic expression of G6PDH, which is a dimer, suggests the presence of two alleles segregating at one locus resulting in the formation of more than two isozymes.

INTRODUCTION

The technique of electrophoresis is useful for detecting the differences in proteins (mostly enzymes) between species. A minor difference in the molecular structure of a protein may result in it having a different net charge and mobility leading to an altered position in the gel. The proteins thus identified serve as valuable tools for comparison of the genetic constitution of related and unrelated species.

The murrels commonly known as snakeheads are represented by only one genus *Channa*. These fishes show unique environmental adaptations for direct use of atmospheric oxygen, in addition to their gill respiration. By virtue of their aerial respiratory habit, they can survive in water deficient in oxygen, such as those of swamps and marshy areas with foul water, where the usual gill breathing fish cannot thrive. The culture of these fish could certainly constitute a substantial second line of production in terms of fish protein from the inland water. While considering the development and standardization of the fishery management techniques of these fish, many gaps in information can be experienced in the biology, systematics and genetics of these fish. We have undertaken in our laboratory a detailed study of electrophoretic analysis of the murrels and this paper reports the genetic expression of glucose 6 phosphate dehydrogenase in different tissues of two species of *C hanna viz., C. punctatus and C. orientalis.*

MATERIALS and METHODS

Channa punctatus (Bloch) and *Channa orientalis* (Bloch and Schneider) were collected alive from natural populations from streams in and around Shillong and from some fresh water bodies near Gauhati. Live specimens were transported to the laboratory and acclimatised for 10-20 days. To avoid ontogenic problems only adult specimens were used for tissue extraction.

Tissues such as brain, eye, heart, kidney, liver and muscle were excised, blotted dry and weighed accurately. The tissues were then homogenised in 0.25M sucrose solution at 4°C using the Umetrex electric homogenizer. The extracts were then centrifuged at 15,000 g for 20 min at 4°C and the supernatant was collected. The samples were subjected to vertical polyacrylamide gel electrophoresis (Davis 1964) and the staining procedure as described by Shaw and Prasad (1970) and Pasteur et al. (1988) was followed.

RESULTS and DISCUSSION

The enzyme glucose 6-phosphate dehydrogenase is, in general expresed as a single band in all tissues studied i.e. brain, eye, heart, kidney, liver and muscle of *C. punctatus*. Some populations showed, however, multiple bands suggesting at least two different alleles segregating at one locus. On the other hand, in *C. orientalis* one band was common to all the tissues studied i.e. brain, eye, heart, kidney, liver and muscle but two additional zones of activity of G6PD were found in kidney and liver.

Glucose 6 phosphate dehydrogenase is among the most thoroughly studied enzymes.Hellman (1964) and Kauffman et al. (1969) have shown that the presence of G6PD is a strong evidence of the hexose monophosphate shunt which is an alternative pathway of glucose oxidation. The shunt is initiated by the conversion of glucose 6 =phosphate into 6-phosphogluconelactone, reducing NADP (Pon 1964). It has been of particular interest to geneticists because it is controlled in man by a gene located on the X chromosome (Shaw and Koen 1968). More recently, Young et al., (1964) and Mathai et al., (1966)have shown that G6PD is sex-linked also in *Drosophila* and *Equine* species respectively.

Glucose 6 phosphate is a dimer and the electrophoretic expression in *C. punctatus* can be explained by the presence of two alleles segregating at one locus. Ohno et al.,(1966) demonstrated multiple electrophoretic bands for rainbow trout G6PD and Ohno (1967) postulated the existence of two codominant gene loci each with different alleles determining these bands. It may however, be also argued that this kidney and liver specific band may be the expression, of a second gene.This same inheritance pattern was postulated in brook trout, lake trout and the hybrid between them, the so called splake trout (Yamauchi and Goldberg 1973). It may therefore, be also argued that the kidney and liver specific band in *C. orientalis* may be the expression, of a second gene. However, in both instances

(Ohno et al., 1966 and Yamauchi and Goldberg 1973), the data presented were entirely consistent with a hypothesis of a single gene locus with multiple electrophoretic bands generated by post translational modification of the enzymes. These several metastable electrophoretic forms are made of a single polypeptide chain which may assume two or more conformational states (Cederbaum and Yoshida 1976). A single locus hypothesis has thus been suggested for rainbow trout with a post translational modification responsible for the complexity of the banding patterns (Cederbaum and Yoshida 1976). We presume the relatively cathodal of the two anodally migrating bands in *C. orientalis* is due to the expression of allele and therefore taken as allozyme. The allozyme seems to be liver and kidney specific since we did not find any expression in other tissues. High activity of G6PD in these two tissues indicate that these tissues are the major sites of G6PD synthesis.

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