CYTOGENETIC AND GENETIC STUDIES ON THE CHOCOLATE MAHSEER, *ACROSSOCHEILUS HEXAGONOLEPIS*

BY

M. PALANICHAMY

SUBMITTED

IN

FULFILMENT OF THE REQUIREMENT OF

THE DEGREE OF DOCTOR OF PHILOSOPHY IN

ZOOLOGY

OF

NORTH-EASTERN HILL UNIVERSITY

SHILLONG - 793 022

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CYTOGENETIC AND GENETIC STUDIES ON THE CHOCOLATE MAHSEER, *ACROSSOCHEILUS HEXAGONOLEPIS*

ABSTRACTS

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The use of aquatic resource has been a challenge to man throughout his existence on earth because approximately two-third of our planet is covered by water and most of it supports fish life. Fish contributes about 1% of the total food supply of the world. The part played by fish in the diet varies greately from country to country. In a country like India, since the intake of meat and milk is low fish has special importance as a supplement to ill-balanced diets and are obviously subjected to maximum and often ruthless exploitation. In addition, other stresses like habitat degradation due to aforestation, pollution etc. have resulted in a rapid decline of the fish stocks especially mahseer species. Therefore, is an urgent need for the conservation of fish genetic resources. There are various possible approach towards conservation of fish genetic resources, *i.e.*, genetic stock identification of fish population, stock improvement, monitoring genetic changes in their habitat etc. Biochemical genetic technique offers accurate methods for identifying discrete breeding populations, estimating contribution to stock mixtures, indicating problem in fish culture, recognizing and quantifying hybrid populations and providng insights into conservation problems.

Mahseers are important game fishes of India. They grow to a considerably large size and are generally found in mountainous streams and rivers. The commonly found mahseers in India are *Tor khudree, T. mosal, T. mossulah, T. neilli, T. progeneius, T. putitora, T. tor* and *Acrossocheilus haxagonolepis.*

There is a paucity of information on the cytogenetic and genetic structure of the indigenous game fish, *A. hexagonolepis* in the North-Eastern region of India. Such a knowledge, is of utmost importance not only from the academic view point, but also in its utility in increasing technological efficiencies of the fishery evolving judicious management measures. Keeping in view, the basic necessity to have a knowledge on the cytogenetic and genetic background of the chocolate mahseer fish and hence, on a virtual absence of such informations, a study has been undertaken to elucidate on the cytogenetic and genetic structure of chocolate mahseer species, *A. hexagonolepis* from the North-Eastern region of India.

The study pertains to the specimens collected from two different sites *viz.*, (1) The Pagladia river (Subankhata, Assam; 91° 28' Latitude and 27° 40' Longitude). (2) Umiam reservoir (Barapani, Meghalaya; 91° 56' Latitude and 24° 34' Longitude). The collections were made during May 1993 through May 1995 from Umiam reservoir, while from the Pagladia river during the month of March 1993, 1994 and May 1995. The results obtained during the present study has been presented in the thesis entitled, "Cytogenetic and genetic studies on the chocolate mahseer, *Acrossocheilus hexagonolepis*". The thesis containing 114 pages, with 20 Tables and 34 Figures, has been divided into two chapters. The first chapter deals with the cytogenetic aspects of chocolate mahseer. The second chapter contain the genetic structure of the chocolate mahseer *A. hexagonolepis*.

The following are the important results obtained during the present investigations and embodied in the thesis.

Chapter I. Cytogenetic study: We have reported the diploid chromosome number and karyotype of *A. hexagonolepis* and have also suggested that *A. hexagonolepis* evolved from the ancestral group containing 48 chromosome number by the process of polyploidization event. The karyotype 2n = 96; chromosome formula n = 7m + 15sm + 9st + 17t, was subsequently attained through Robertsonian alteration coupled withh pericentric inversion.

Chapter II. Genetic study: We examined 1237 spcimens sampled from the two sites and we have selected twelve enzymes *viz.*, Adenylate kinase (AK), Alcohol dehydrogenase (ADH), Glucose 6-phosphate dehydrogenase(G6PDH), Glutamate dehydrogenase(GDH), Glycerol 3-phosphate dehydrogenase(G3PDH), Hexokinase (HK), Hexose 6-phosphate dehydrogenase (H6PDH), Malate dehydrogenase (MDH), Malic enzyme (ME), Phosphoglucomutase (PGM) and Xanthine dehydrogenase (XDH) for our present work.

ADH, ME and GDH enzyme showed tissue specific activity, the former being expressed in liver and latter one in muscle tissue. While the expression of other enzyme has been observed in different tissues.

We have observed that G6PDH, LDH-1, LDH-2, ME and PGM-2 (Umiam reservoir) were all represented as a single invariant band on the gels, each of these enzymes is coded by single locus.

The enzymes ADH, G3PDH-2, HK, H6PDH, LDH-3, MDH and PGM-1 showed double banded pattern on the gels, each of these enzyme are coded by single locus with an allelic variant.

GDH appeared as two isozymes of different electrophoretic mobility. No variation in banding pattern was observed. Two loci were assumed responsible for the genetic control of this enzyme.

The dimer enzymes G3PDH and XDH in chocolate mahseer was represented by four banded and three banded pattern respectively. Two loci were responsible for the control of these enzymes.

We have observed the maximum number of homozygous individuals in the Umiam reservoir and its reflected in the low level of average heterozygosity (0.909) and alleles per locus (1.412) in the population comparable to the values observed in the Pagladia riverine population. MDH locus which was polymorphic in Umiam reservoir population were observed to be monomorphic in the Pagladia river population.

The maximum number of homozygous individuals were found in Umiam reservoir. This may perhaps if correlated with ecological parameter (*i.e.*, temperature Vs heterozygosity). Overall water surface temperature also shows a positive correlation and is statistically significant ($Y = 23.141 + 0.468x$; $r = 0.631$). We also observed but statistically insignificant positive correlation between the mean heterozygosity and body weight of fish ($Y = 76.058 = 0.029x$; $r = 0.0497$).

The phenetic relationship between Umiam reservoir and the Pagladia river population were estimated through comparison of genetic similarity ($I = 0.918$)/distance ($D = 0.121$) indicating low level of genetic distance. A 29% of gene duplication was noticed thus supporting the tetraploid nature of our fish *A. hexagonolepis.*

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December 1996

This is to certify that the thesis entitled "Cytogenetic and Genetic Studies on the Chocolate Mahseer, *Acrossocheilus hexagonolepis*", submitted by Mr. M. Palanichamy for the degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong (India) embodies the record of original investigations carried out under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of Ph. D. degree. This work has not been submitted for any degree of any other University

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I, Mr. M. Palanichamy, hereby declare that the subject matter of 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 any-body else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

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M. Palanichamy

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Palanichamy, M., Bose, S. and Chatterjee, K. (1995): Chromosomes of a hill stream fish, *Acrossocheilus hexagonolepis*. In: Perspectives in Cytology and Genetics (Eds. Manna, G.K. and Roy, S.C). *Vol. 8*. pp. 691 - 696

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FOREWORD

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The use of aquatic resources has been a challenge to man throughout his existence on earth because approximately two-third of our planet is covered by water and most of it supports fish life. The first men who lived on the shore of a sea, rivers or lake found food in animals, fibres in the plants, and ornaments in the shells. They gradually learned to catch fish in traps, to make hooks from bone and twist fibres into lines and nets. In the early 20th century after world war II when the weary poeple of the world began to rebuild their countries and economies, they looked first to their supply for food. Those who had been fishermen went back to sea if they could find a ship. This is enough to drive man to the study of fish or any aspect of their lives, but in addition , fish serve man as an unending source of recreational or aesthetic pleasure.

Fisheries contribute about 1% of the total food supply of the world. The part played by fish in the diet varies greately from country to country. From nutritional standpoint, the animal protein contribution of fish to the diet is especially important. In most of the countries *i.e.*, Africa, Japan and Portugal, about one-half and in some instances even more of the total amount of animal protein consumed comes from the fishery origin.

In a country like India, since the intake of meat and milk is low fish has special importance as a supplement to ill-balanced diets. By the end of the 20th century, the domestic demand for fish in India is likely to be anywhere between 60 to 125 lakh tons per year. The marine catches can meet only less than half of this demand. The balance will have to come from inland waters. Aquaculture or fish farming has the potential to increase inland fish production considerably thus augmenting the income of the rural population. At present inland water fishery contributes 19.01 lakh tons of fish production (Srivastava *et al.*, 1993).

Extensive researches on freshwater fishes in India is mainly concerned around the biology of only those species which are being used to stock dams and lakes rather than on natural fish communities. In India, the important culturable fishes are the Indian major carps, such as, *Catla catla, Labeo rohita*, and *Cirrhinus mrigala* and the exotic

carps, *viz.*, *Cyprinus carpio, Ctenopharyngodon idellus* and *Hypophthalmichthys molitrix*. Apart from these, there exists a wide variety of game fishes gaining fillip in the country as far as culture is concerned. Game fishes generally have qualities like rapid growth and capacity to fight to the last when caught by the anglers. Trout is well known among the Europeans to have all the sport qualities and during the British rule in India, the British Civil Servants introduced trout in Kashmir and Ootakamund, the summer resorts of the English men. They were also attracted by mahseers, our indigenous culturable sport fishes.

Mahseers belong to the family Cyprinidae. They grow to a considerably large size and are usually found in mountainous streams and rivers (Hora, 1939). According to Thomas (1897) and McDonald (1948) the mahseers show more sport and fighting nature among other sport fishes. Various opinions have been expressed about the etymology of the term "Mahseer". According to Lacy and Cretin (1905) this English popular name was derived from Hindustani "Maha" meaning "great" or "big" and "seer" meaning "head". It may be an attempt to import a meaning to the name!

The commonly found mahseers in India are *Tor khudree, T. mosal, T. mossulah, T. progeneius, T. putitora, T. tor* and *Acrossocheilus hexagonolepis* (Menon, 1974). Of the eight recorded species of mahseers found in India, seven species therefore, belong to a genus *Tor* but the chocolate mahseer belongs to the different genus altogether. It may be interesting to know that the chocolate mahseer has also been included with other species under the genus *Acrossocheilus*. Suzuki and Taki (1981) reported a tropical Asian Cyprinid *viz.*, *A. sumatranius*. Recently Talwar and Jhingran (1991) put the chocolate mahseer under the genus *Neolissocheilus*.

It has been stressed in many recent reports (Jhingran, 1988; Das, 1992) that a large numbers of Indian fish species are threatened or endangered and there is an urgent need for conserving the fish genetic resources. Many freshwater fish especially mahseer species are presently undergoing drastic fluctuation in size distribution and genetic makeup as result of man-made changes in the riverine habitat due to large scale water obstruction in the catchment areas and pollution from different sources (Jhingran, 1988).

Conservation of fish genetic resources is the basic premise upon which our study has been based. UNEP (1980) has recommended various possible approach towards conservation of fish genetic resources, *i.e.*, preparation of catalogue of the fish genetic materials, promotion of research directed at the level of genetics of fish which would assist in a more applicable definition of conservation and monitoring of changes in the genetic diversity. We have undertaken in our laboratory a detailed cytogenetic and genetic study of the chocolate mahseers from natural population.

The knowledge of fish cytogenetics is immensely important and help us to improve the fish stock and management. Study of the karyotype is generally accepted as a valuable method for identification of species, hybrid or more rarely population and may be of particular importance in the application of polyploidy and gynogenesis in aquaculture. Since mahseer fishes live in most diversified environmental conditions, it is naturally expected that there would be wide range of cytogenetical variation. Khuda-Bukhsh (1980 & 1982); Khuda-Bukhsh and Nayak (1982) suspected that the polyploid origin of three species of *Tor* and some other hill stream fishes was due to the ecological thrust of cold shock. Review of literature indicates a total absence of information on the chromosomes of the chocolate mahseer. We have attempted to study the diploid number and chromosome morphology of the chocolate mahseer and the result has been presented in the first chapter of the thesis.

An efficient use of biological resources needs a thorough knowledge of identifying genetic resource, the amount and distribution of genetic variability within the species considered. Therefore, the second part of the present work, has been devoted to find out genetic background of the chocolate mahseer from the isoenzymatic investigation, which has been accomplished in four phases. In phase I, we tried to identify the genetic locus / loci coding for selected isozymes / enzymes. In phase II, the qualitative genetic variation was estimated by using the allele frequency. In phase III, relationship if any between genetic variation and water temperature and body weight were studied and lastly in phase IV, an attempt was made to find out the genetic diversity between the populations by using the heterozygosity of polymorphic loci.

CHAPTER ONE

CYTOGENETIC STUDY

INTRODUCTION

INTRODUCTION

Interest on the study of fish chromosome has been noticed since ninteen thirties by referring to the work of Iriki (1932), Prokofieva (1934), Friedman and Gordan (1934), Makino (1937 & 1939), Svardson and Wickbom (1939) etc., but the study of fish chromosome has not been as successful or widespread as in mammals. The standard karyotypes (chromosome and chromosome number) have been reported for less than 10% of the more than 20,000 extant species of fishes (Gold *et al.*, 1990). This is due to the presence of small size and large number of chromosomes. Moreover, the methodologies used for fish chromosome study has been minimal (Gold, 1979; Hartley and Horne, 1985).

Only few reports are available on teleostean fish chromosome study during the years 1941 - 1960 (Makino, 1941; Svardson and Wickbom, 1942; Wickbom, 1943; Svardson, 1945; Nogusa, 1954 & 1955; Prakken *et al.*, 1955; Bothroyd, 1959). The chromosome morphology and ploidy level have been found in fish and other vertebrates and made a significant contribution in understanding the evolution from fish to higher groups (Ohno *et al.*,1967 & 1968). Apart from this a diversified implication of fish chromosome studies could be well understood in the field of fish biology. Sick (1962) has given a comparative account of hemoglobin pattern and chromosome number of American, European and Japanese eels, *Anguilla*. Lieder (1963) has investigated the presumptive sex chromosome in *Perca, Aecrina* and *Anguilla*. The cytological aspects of speciation in two north American teleosts, *Salmo gairdneri* and *S. clarki* have been described by Simon and Doller (1963). Ojima *et al.* (1963) have described the chromosome of a number of species belonging to the family Salmonidae from the point of cytotaxonomy. Roberts (1964) has investigated the chromosome complement of twenty species of the family Centrachidae. Becak *et al.* (1966) have reported the presence of chromosomal polymorphism in green sunfish *Lepomis cyanelisus* as an evidence of genetic segregation. Chen and Ebelling (1966 & 1968) and Chen (1969) have found heterozygosity in certain fish species which, according to them is due to the chromosome number variation in these fishes.

Glydenholm and Scheel (1971) have published a check list of 200 species of fish chromosome which shows a rapid spectacular progress in the fish chromosome studies. This has been largely due to the innovated techniques like the air drying technique for chromosome preparation which provides comparatively better resolution of individual chromosome within a complement. According to Denton (1973), 83 species under 47 genera of the family Cyprinidae have so for been cytologically investigated. The realization that karyotype comparisons are crucial in assessing phylogenetic relationships within various taxa, has also been equally significant. Nikoviskij and Vasil'ev (1973) have provided a comparative account of chromosome morphology of a large number of fish species. Uyeno and Ojima (1977) studied the diploid-tetraploid complex in the genus *Cobitis* and described the chromosome complements of two species of the genus *Coreoperca* with reference to a karyotypic differentiation and evolution. Vasil'ev (1977) has studied the polyploidization and evolution of karyotypic evolution in charrs of the genus *Salvelinus*. Gold *et al.* (1978 & 1979) have reported the gross karyotypic changes and evolution in the north American fishes. Johnson *et al.* (1981) have described the karyotype of seven species of the genus *Galaxias* from Tasmania and interpreted the evolutionary relationships. Kirpichnikov (1981) has compiled the chromosome data from the fishes which included 215 species from the Cyprinidae family. Sabti (1985) found chromosomal variation in the three species of Salmonids fishes. Falcao and Bertollo (1985) have investigated the karyotype of six species of *Acestrorhynchnae* and detected karyotypic relationships among the different species. Formacion and Uwa (1985) have studied the Philippine medaka, *Oryzias luzonensis* and reported the origin and species differentiation of the fish. Rivlin *et al.* (1985), Chourrout and Happe (1986), Reddy and John (1986), Fan and Fox (1990) have described simple methods for the preparation of fish chromosome which has broad application in the fish karyotypic research. Jenkin *et al.*(1992) have described the apparent chromosomal conservation in western north American Cyprinids as reminiscent of the "canalization" hypothesis where the karyotype is strongly affected by natural selection and older lineage presumably evolved by "optimum" karyotype. Gold and Li (1994) have reported chromosome, NOR, karyotypic and genome size variation among the squaw fishes of

the genus *Ptylocheilus* (Cyprinidae). Miyaquwa and Galetti (1994) have studied the *Characidium* species and found a quite stable karyotype structure in the population.

In India, study of fish chromosome have started from the year 1960. Sharma *et al.* (1960) pioneered the chromosome study in fish and determined the chromosome number of three species of Indian fishes. Srivastava and Kaur (1964) observed the structure and behaviour of chromosomes of some freshwater teleosts. Nayar (1962, 1964, 1965 & 1966) worked out the karyotype of about twenty three species. Verma (1968) studied the structure and behaviour of chromosomes of two Indian air breathing fishes. Natarajan and Subrahmanyan (1968) have made a preliminary study on the chromosome of *Tilapia mossamibica*. Srivastava and Das (1968 & 1969) have investigated the chromosome number in *Clarias batrachus* and certain other teleostean fishes. Subrahmanyan (1969) studied the karyotype of estuarine fish with calcium treatment. Prasad and Manna (1970) studied the somatic chromosomes of a major carp, *Cirrhina mrigala*. Manna and Prasad (1971) have put forward a hypothesis on the mechanism of fish chromosome evolution. Prasad (1971), Prasad and Manna (1971) investigated the somatic and germinal chromosomes of about sixteen freshwater fishes. Chatterjee and Majhi (1973) studied karyotype of the teleostean species *Mugil persia, Cirrhina reba*. Khuda-Bukhsh and Manna (1973 & 1974) investigated karyotype of nine species of fishes including two major Indian carps, *Catla catla* and *Labeo bata*. Manna and Prasad (1973), Rishi (1973), Dhar and Chatterjee (1984 & 1986) have studied the chromosome number variation and evolutionary aspects of Indian murrels. Vasudevan *et al.* (1973), Prasad and Manna (1974) demonstrated the chromosome complements of *Heteropneustes fossilis*. Manna and Prasad (1974) investigated the chromosome of two hybrid fishes and fishes from the family Gobiidae respectively. Natarajan and Subrahmanyan (1974) studied the chromosomes of some teleostean fishes from Portonovo in south India. Khuda-Bukhsh (1975) studied the somatic chromosome of an exotic fish, *Puntius japonicus*. Rishi (1976) investigated the chromosome number of four species of fishes. Manna and Khuda-Bukhsh (1977) studied the chromosome morphology and cytological evolution of the cyprinid fishes and provided a check list of chromosomal number in Cyprinid fishes. The chromosomes

of two hill stream fishes, *Barilius bendalius* and *Rasbora daniconius* have been studied by Khuda-Bukhsh (1979). Khuda-Bukhsh (1980) and Khuda-Bukhsh *et al.* (1986) have further investigated three species of hill stream fishes with a report of high chromosome number in the genus *Tor*. Khuda-Bukhsh (1984) has also studied two species of hill stream fishes, *Labeo diplostomus* and *Garra gotyla gotyla* cytologically. Singh and Banerjee (1993) have published a bibliography of fish cytogenetics in India.

With regard to the cytogenetics of mahseer practically no work has been done so far, except the reports by Khuda-Bukhsh (1980 & 1982) on high number of chromosome in the hill stream cyprinid *Tor khudree, T. putitora, and T. tor*. Khuda-Bukhsh *et al.* (1986) have mentioned karyomorphology and evolution in some Indian hill stream fishes with particular reference to polyploidy in *T. mosal mahanadicus.*

The chocolate mahseer inhabits the hillstream, rivers and other freshwater bodies in North-Eastern India. The present investigation incorporates a detailed account of the diploid number and chromosome morphology of the chocolate mahseer, *Acrossocheilus hexagonolepis* (*Neolissocheilus hexagonolepis*).

MATERIALS AND METHODS

2.1 *The fish :*

The specimens of chocolate mahseer *Acrossocheilus hexagonolepis* (*Neolissocheilus hexagonolepis* Talwar and Jhingran, 1991) for the present study were collected from two sites, *viz.*, (1) Umiam reservoir (Barapani) about 15 km from Shillong, Meghalaya (25° 34' N; 91° 56' E) (2) Pagladia river, which flows from Uttarkuchi along Subankhata in Assam (250 km away from Shillong, Meghalaya; 27° 40' N; 91° 28' E) (Fig.1 & 2).

2.2 *Taxonomic Status :*

Phylum	-	Vertebrata
Sub-Phylum	-	Craniata
Super-Class	-	Gnathostomata
Series	-	Pisces
Class	-	Teleostomi
Sub-Class	-	Actinopterygii
Order	-	Cypriniformes
Sub-Order	-	Cyprinoidei
Family	-	Cyprinidae
Genus	-	*Acrossocheilus* (*Neolissocheilus*)
Species	-	*Acrossocheilus hexagonolepis* (McClelland, 1834)
		Neolissocheilus hexagonolepis (Talwar and Jhingran, 1991)

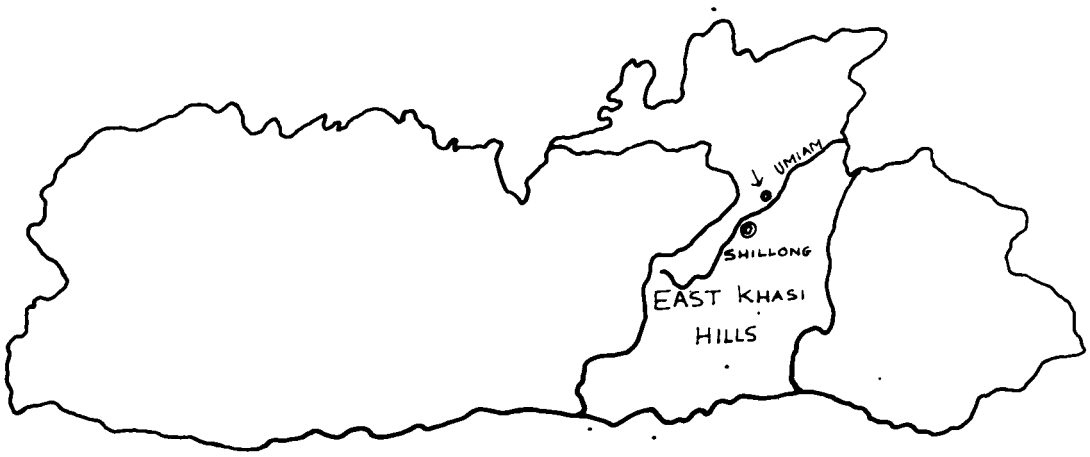
2.3 *Chromosome preparation :*

Ten fish from each sites have been brought to the laboratory and injected intraperitonially with 0.02% colchicine solution at a rate of 1 ml/100 g body weight and kept in a well aerated aquarium. The fishes have been sacrificed at interval of 2 to 3 hours after colchicine treatment. This procedure eliminates the possibility of over-action

Fig. 1. Geographical map of Meghalaya showing site of collection

Fig. 2. Geographical map of Assam showing site of collection

→ BARA PANI



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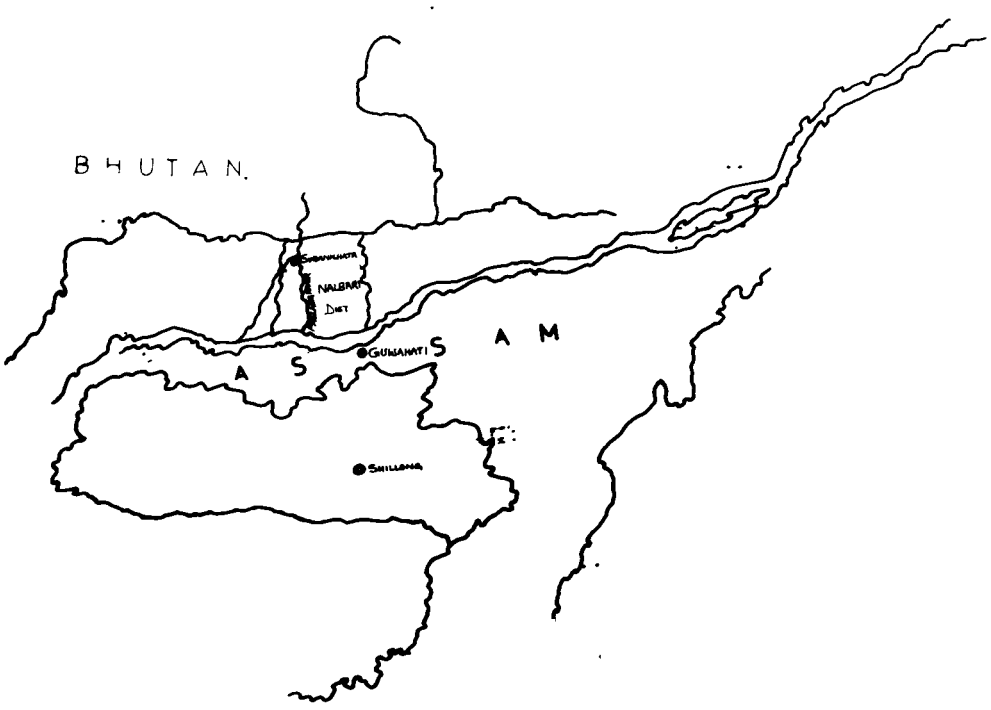

or under-action of colchicine. The kidney and gill epithelium were removed from dissected fish. These tissue were minced and transferred into separate centrifuge tube containing hypotonic solution (0.80% tri Sodium citrate and 0.56% Potassium chloride in the ratio of 1 : 1). A cell suspension was prepared by repeated flushing through a narrow pipette. The suspension was incubated in the hypotonic solution for 45 to 60 minutes at room temperature and then centrifuged at 1200-1500 rpm. for 5-10 min. The supernatant was removed and the materials of each centrifuge tubes were fixed with freshly prepared methanol-acetic acid solution (Methanol and glacial acetic acid 3:1 by volume) for atleast one hour. The fixed material was then centrifuged for 5-10 min. at 1200-1500 rpm. The supernatant was again removed and fresh fixative was added to the material. After half an hour the fixed material was dropped on the alcohol immersed, pre-chilled slides which were then ignited on a flame.

The slides were stained with 10% Giemsa's stain in phosphate buffer solution (0.908% Potassium dihydrogen phosphate and 0.946% di Sodium hydrogen phosphate; p^H 6.8) for 30-45 minutes and then observed under microscope. Photomicrographs of well spread metaphase chromosome have been taken with 18 ASA ORWO panchromatic film mounted in a Ortho Variable Leitz photocompatible unit microscope at 100X magnification with 10X occular under oil immersion.

2.4 *Chromosome analysis :*

The following parameters were considered while studying the chromosome morphology.

(i) length of the long arm of the chromosome

(ii) length of the short arm of the chromosome

(iii) arm-ratio or 'r' = $\frac{\text{length of the long arm}}{\text{length of the short arm}}$

(iv) centromeric indices = $\frac{\text{length of the short arm}}{\text{length of the long arm}}$ X 100

(v) total length of chromosome

(vi)relative length of the chromosome (RL)

$$RL = \frac{\text{total length of a chromosome}}{\text{total length of the whole chromosome set}}$$

(vii) number of arms or'NF' value : total number of arms in a complete chromosome set

(viii) centromeric position and chromosome type

The position of the centromere was determined by arm ratio 'r'. If 'r' is 1.0 to 1.7 μm the centromere has been considered median and the chromosome have been designated 'm' types. An 'r' of 1.7 to 3.0 μm reflect a submedian centromere and chromosome have been designated 'sm' types. An 'r' of 3.0 to 7.0 μm indicates subterminal centromere and the chromosomes have been designated 'st' types and 'r' of 7.0 to ∝ μm indicates terminal centromeres and the chromosome have been designated 't' types. This system of nomenclature has been suggested by Levan *et al.* (1964) at the Chicago Conference.

Based on the centromeric position and chromosome type the karyotype and idiogram were prepared.

OBSERVATIONS

The diploid number of chromosome of *A. hexagonolepis* has been determined from kidney tissue as well as gill epithelium from 20 specimens (Table 1). The kidney tissue has produced more favourable result than the gill epithelium tissue. The somatic karyotypes, therefore, are from this tissue only. The mode indicates 96 as the diploid number for this species since out of a total seventy five nuclei studied sixty five showed this number while , six nuclei were with 98 chromosomes, three with 94 and one with 100 chromosomes (Table 1; Fig. 3).

The two largest pairs (%TCL > 3.0) of chromosomes were subtelocentric and were readily identifiable in all the nuclei and could, therefore, serve as marker chromosomes. The complement could be tentatively divided into three broad groups consisting of (i) 9 pairs of large (%TCL > 2.5) chromosomes that included one pair of metacentric, three pairs of submetacentric and five pairs of subtelocentric (ii) 26 pairs of medium-sized (2.5> %TCI > 1.75) chromosomes that included three pairs of metacentric, ten pairs of submetacentric, two pairs of subtelocentric and eleven pairs of telocentric chromosomes, and (iii) thirteen pairs of small (1.75 > %TCL) chromosomes that included three pairs of metacentric, two pairs of submetacentric, two pairs of subtelocentric and six pairs of telocentric chromosomes (Table 2; Fig. 4 & 5). The two populations could not be differentiated at the chromosomal level. The length of the chromosomes ranged from 8.67 μm to 2.17 μm and the size differentiation was sometimes not at all appreciable. For example, chromosome pair number 15 and 16, 28 and 29 were almost of the same length. The chromosomes were further classified according to their arm ratio and centromeric indices (Levan *et al.*, 1964) into 7 pairs belonging to an 'm' type (Nos. 8, 11, 17, 19, 36, 41, and 44), 15 pairs belonging to 'sm' type (Nos. 3, 4, 6, 13, 14, 18, 21, 22, 26, 27, 30, 31, 42, and 47), 9 pairs belonging to 'st' type (Nos. 1, 2, 5, 7, 9, 23, 25, 38, and 45) and 17 pairs belonging to 't' type (Nos. 10, 12, 15, 16, 24, 28, 29, 32, 39, 40, 43, 46, and 48). Therefore the chromosomal formula of this species seems to be n = 7m + 15sm + 9st + 17t and the fundamental number (NF value) is 158.

Table 1. Summary of karyotype analysis of *Acrossocheilus hexagonolepis*.

Total No. of specimens	Tissue	No. of cells scored	Frequency of Chromosome counts				
			92	94	96	98	100
20	Kidney	75	0	3	65	6	1
	Gill	25	2	2	17	4	0

We did not observe any heteromorphic pair of chromosomes indicating the absence of morphologically identifiable sex chromosomes in this species.

Table 2. Morphometric data of the chromosome of *A. hexagonolepis*

Chromosome Number(n)	Absolute length in μm	Relative length (% of TCL)	Centromeric index	Chromosome designation
1	8.67±0.03	3.95±0.02	23.08±0.29	st
2	7.33±0.04	3.34±0.02	18.18±0.21	st
3	6.17±0.03	2.81±0.02	39.89±0.24	sm
4	6.00±0.04	2.73±0.02	31.58±0.23	sm
5	5.90±0.02	2.69±0.03	25.81±0.37	st
6	5.83±0.02	2.66±0.03	38.89±0.34	sm
7	5.67±0.04	2.58±0.04	26.67±0.21	st
8	5.57±0.03	2.54±0.03	50.00±0.41	m
9	5.50±0.03	2.52±0.02	28.57±0.28	st
10	5.40±0.04	2.46±0.03		t
11	5.33±0.02	2.43±0.03	44.00±0.41	m
12	5.27±0.04	2.40±0.03		t
13	5.17±0.03	2.36±0.02	33.33±0.27	sm
14	5.13±0.03	2.34±0.02	38.46±0.24	sm
15	5.07±0.02	2.31±0.03		t
16	5.03±0.03	2.29±0.03		t
17	4.93±0.05	2.25±0.03	45.45±0.41	m
18	4.83±0.04	2.20±0.03	30.43±0.27	sm
19	4.77±0.03	2.17±0.02	41.61±0.47	m
20	4.73±0.07	2.16±0.01		t
21	4.63±0.05	2.11±0.03	38.46±0.29	sm
22	4.57±0.03	2.08±0.04	33.33±0.19	sm
23	4.50±0.04	2.05±0.03	25.00±0.21	st
24	4.43±0.04	2.02±0.03		t
25	4.33±0.04	1.97±0.02	20.00±0.02	st
26	4.30±0.03	1.96±0.02	33.33±0.27	sm
27	4.23±0.03	1.93±0.03	30.77±0.24	sm
28	4.20±0.04	1.91±0.02		t
29	4.10±0.05	1.87±0.04		t
30	4.07±0.07	1.85±0.04	30.77±0.21	sm
31	4.03±0.08	1.84±0.03	30.00±0.28	sm
32	4.00±0.05	1.82±0.04		t
33	3.93±0.03	1.79±0.03	31.82±0.32	sm
34	3.87±0.02	1.76±0.02		t
35	3.83±0.04	1.75±0.02		t
36	3.80±0.04	1.73±0.04	41.67±0.31	m
37	3.77±0.04	1.72±0.04		t
38	3.73±0.04	1.70±0.02	16.00±0.17	st
39	3.67±0.04	1.67±0.03		t
40	3.63±0.04	1.65±0.02		t
41	3.53±0.03	1.60±0.02	42.11±0.29	m
42	3.47±0.02	1.58±0.02	33.33±0.27	sm
43	3.43±0.02	1.56±0.02		t
44	3.40±0.01	1.55±0.02	45.83±0.40	m
45	3.33±0.02	1.52±0.01	27.78±0.27	st
46	3.23±0.03	1.47±0.02		t
47	3.00±0.03	1.37±0.03	30.00±0.41	sm
48	2.17±0.02	0.99±0.03		t

DISCUSSION

Karyological variation in different populations of many species, paricularly in the family Cyprinidae, have been recorded by several researchers working in different countries or places (Ojima, 1985).

Chocolate mahseer, *A. hexagonolepis* belongs to the family Cyprinidae. Review of literature reveals that the chromosomes of chocolate mahseer have not been previously studied and this seems to be the first report. The chromosomal study has revealed that the diploid number of chromosome is 2n=96 containing 7 pairs of metacentric, 15 pairs of submetacentric, 9 pairs of subtelocentric and 17 pairs of telocentric chromosomes and the chromosome formula is n = 7m + 15sm + 9st + 17t. It has been found that the diploid number is somewhat higher than in other teleostean fishes (Makino, 1956; Nogusa, 1960; Post, 1965; Roberts, 1967; Manna and Prasad, 1971; Glydenholm and Scheel, 1971; Denton, 1973; Nikoviskij and Vasil'ev, 1973; Chatterjee and Majhi, 1973; Sabti, 1985; Jenkin *et al.*, 1992; Miyaquwa and Galetti, 1994). The members of Cyprinidae usually have a diploid number between 48 and 52 (Chiarelli and Capana, 1973; Manna, 1983 & 1984). Roberts (1964) and Ohno *et al.* (1967) reported the presence of 48 telocentric chromosomes in old world cyprinid as the presumable ancestral group. Besides these, a number of reports are available on 48 as the diploid chromosome number in cyprinid species, *viz.*, *Hemigramocypris rasborella* (Nogusa, 1960), *Barbus* species, *Barbus conconius, B. sehuberti, B. titteya, B. viviparus, B. tetrazona, Branchydanio elbolineatus, B. frankei, B. revio* and *Gobio gobio* (Post, 1965), *Scardinius erythrophthalamus* (Chiarelli *et al.*, 1969; Fontana *et al.*, 1970; Manna, 1984), *Esox* species (Davisson, 1972), *Zacco termminckii, Z. platypus, Ischikauia steenackeri* (Ojima *et al.*, 1972), *Acheilognatus lanceolata, A. limbata, Rhodeus ocellatus ocellatus, Tanakia tangao* (Ojima *et al.*, 1973), *Puntius conchonius* (Das and Srivastava, 1973; Sharma and Agarwal, 1981), *Hypophthalamiicthys molitrix* and *Ctenopharyngodon idellus* (Khuda-Bukhsh and Manna, 1974), *Tinca tinca* (Cataudella *et al.*, 1977), *Vimba vimba* (Kirpichnikov, 1981). Apart from these Manna and Khuda-Bukhsh (1977), Manna (1983 & 1984) studied

nearly 300 species belonging to 106 genera in Cyprinidae and have shown that seventy percent of the species studied have 50 as the diploid chromosome number. They confirmed the peak of 50 as the modal number in this family. Obviously this would not be true for all the species of Cyprinidae. A higher diploid number of chromosome has been reported in some members of the Cyprinidae family, e.g. *Hesperoleucus symmetricus, Opsaiichthys uncirostris* (Ojima *et al.*, 1972; Ojima, 1981), *Schizothorax niger* (Rishi and Haobam, 1984) have a diploid number of 75, 78 and 98 respectively. A diploid number of 100 (Table 3) is reported in *Carassius auratus, Cyprinus carpio* (Ojima and Hitotsumachi, 1967), *Barbus barbus plebejus, B. meridionalis* (Cataudella *et al.*, 1977), *Tor tor, T. putitora, T. khudree, T. mosal mahanadius* (Khuda-Bukhsh, 1980 & 1982; Khuda-Bukhsh *et al.*, 1986), *Autopyge hugeli, Barbus meridinalis petenyi, Carassius auratus auratus, C. a. buergeri, C. a. cuvieri, C. a. gibelio, C. a. grandoculis, C.a. langsdorfii* and *Carassius carassius* (Ojima, 1981). *Barbus aenus* and *B. kimberleyensis* posses a diploid number of 148 while 150 is the diploid number in *B. capensis, B. natalentis* and *B. polylepis* (Oellerman and Skelton, 1990).

Comparison of the karyotype of *A. hexagonolepis* with other members of Cyprinidae leads to interesting findings. In almost all the karyotypes there are large chromosomes which can be used as markers. In case of our fish, as already stated, there are two pairs of large subtelocentrics. It may be pointed out that a large acrocentric pair is present in many of the 50-chromosome Cyprinidae. It may also be pointed out that a subtelocentric may be derived from an acrocentric by pericentric inversion. In fact in few 50-chromosome Cyprinids the acrocentric marker pair has been replaced by subtelocentric pair (Cataudella *et al.*, 1977). According to Cataudella *et al.* (1977), the Cyprinid *Tinca tinca* (2n = 48) seems to have an ancestral karyotype of the Cypriniformes because of its similarities with the representatives of other families of this order, *e.g.*, the Cobitidae and the Characidae. The number of single armed chromosomes in most of the 50-chromosome karyotype of Cyprinidae is not very high. Many of the 48-chromosome Cyprinids, including *Tinca tinca*, however, possess a fairly high number of single armed chromosomes (Table 4). It may again be pointed out that

Table 3. List of Cyprinid fishes showing the karyotypic indication of polyploid origin

Species	Chromosome formula (n) 2n	m	sm	st	t	FN	Ref.
Acrossocheilus hexagonolepis	96	7	15	9	17	158	Present investigation
Barbus barbus plebejus	100	13	9	9	19	162	Cataudella *et al.*, 1977
B. meridionalis	100	11	10	6	23	154	"
Carassius auratus	100	6	18	-	26	148	Ojimaand Hitotsumachi, 1967
Cyprinus carpio	100	6	18	-	26	148	"
C. carpio var *comminis*	100	12	12	-	26	148	Khuda-Bukhsh and Barat, 1987
Tor mosal mahanadicus	100+2	22	7	20 to 22		?	Khuda-Bukhsh *et al.*, 1986
T. putitora	100	5	12	7	26	148	Khuda-Bukhsh, 1980
Barbus kimberlayensis	148	?	?	?	?	204	Cataudella *et al.*, 1977
B. aeneus	148	?	?	?	?	196	"
B. capensis	150	?	?	?	?	208	"
B. polylepis	150	?	?	?	?	206	"
B. natalensis	150	?	?	?	?	200	"

Table 4. Karyotype formula of few 48 - and 50 - chromosome Cyprinid

Species	Chromosome formula (n) 2n	m	sm	st	t	FN	Ref.
Acheilognathus lanceolata	48	4	10	-	10	76	Ojima *et al.*, 1972.
Aspidoparia morar	48	4	3	-	17	62	Khuda-Bukhsh and Barat, 1987.
Puntius stigma	48	2	1	-	21	54	"
Rhodeus ocellatus ocellatus	48	4	10	-	10	76	Ojima *et al.*, 1972
Tinca tinca	48	4	6	5	9	78	Cataudella *et al.*, 1977
Danio acqipinnatus	50	7	16	-	2	96	Khuda-Bukhsh *et al.*, 1986
D. devario	50	6	12	-	2	86	"
Gara lamta	50	6	12	1	6	86	"
Psylorhynchus bolitora	50	12	8	-	5	90	"
Puntius melanampyx	50	6	6	7	6	74	"

A. hexagonolepis possesses 34 short armed chromosomes. Three primary mechanisms *viz.*, Robertsonian alterations, polyploidy and aneuploidy may be held responsible for changes in chromosome number to be established during the course of evolution (Gold, 1979). The basic inference from Robertsonian principles is change in diploid number without accompanying change in NF (Booke, 1974; Legrande, 1981). Karyotypic evolution has been convincingly explained through Robertsonian alteration in a large member of fish family (Legrande, 1980; Dhar and Chatterjee, 1984 etc.). However, the diploid number of 96, which is exactly twice of 48 - the diploid number of a large number of Cyprinids, entices one to suggest polyploidy as the favoured pathway of chromosomal evolution in our fish. Similar circumstances have been instrumental in leading certain researchers to assume that the karyotypes with 100 chromosomes are tetraploid (Ohno and Atkin, 1966; Ohno *et al.*, 1967; Muramoto *et al.*, 1968; Wolf *et al.*, 1969; Schmidthe *et al.*, 1975; Cataudella *et al.*, 1977; Mayr *et al.*, 1986). According to Ohno *et al.* (1967), the Cyprinid family consists of species with both diploid and tetraploid origins. Incomplete meiosis II during the development of the gametes may subsequently give rise to tetraploidy by union of diploid spermatozoon and ovum. In fact, artificial production of tetraploid fish has been reported (Don and Avtalion, 1988). In many of the 96-104 - chromosome fish species the DNA contents of the cells show almost a two fold increase (Ohno *et al.*, 1968) suggesting tetraploid origin. Further evidence has been furnished by the presence of tetravalents in meiotic nuclei is some of these fishes (Ohno *et al.*, 1968). Considering the available data, we are inclined to presume that while the 50 - chromosome Cyprinids evolved from the ancestral karyotype of 48 by Robertsonian alteration coupled with pericentric inversion, our fish probably evolved from a 48-chromosome Cyprinid by what Mayr *et al.* (1986) has termed as polyploidic event which doubled the chromosome number to the present 96. The term 'polyploidic event' is used to include all possible ways in which polyploidy may have been induced *i.e.*, environmental changes and hybridization (Oellerman and Skelton, 1990). The present karyotype was subsequently attained through Robertsonian alterations coupled with pericentric inversion. Extended research with the measurement of DNA content of the cells and study of isozyme gene loci may throw more light on this subject.

A few reports are available on the presence of morphologically distinguishable sex chromosomes in fishes (Leider, 1963; Chen, 1969; Ebeling and Chen, 1970; Rishi and Gaur, 1976; Forster and Anders, 1977; Thorgaard, 1977 etc.). We did not observe any heteromorphic pair of chromosomes in both the sexes of our fish which is in accordance with the current concept that the sex chromosomes in fishes are in a very low level of differentiation.

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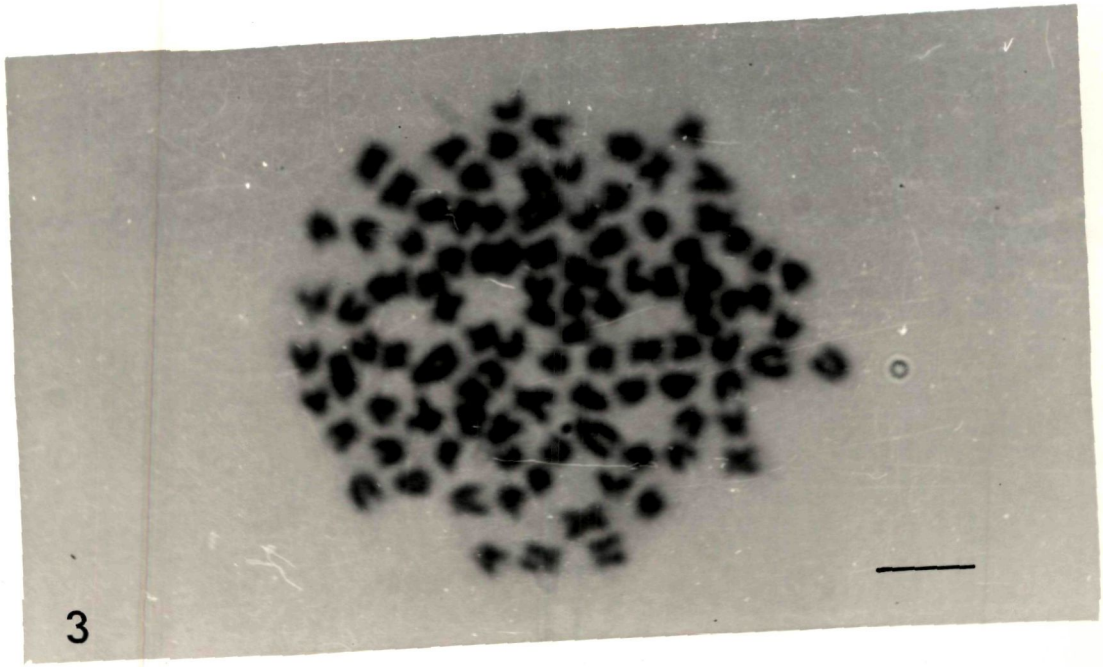
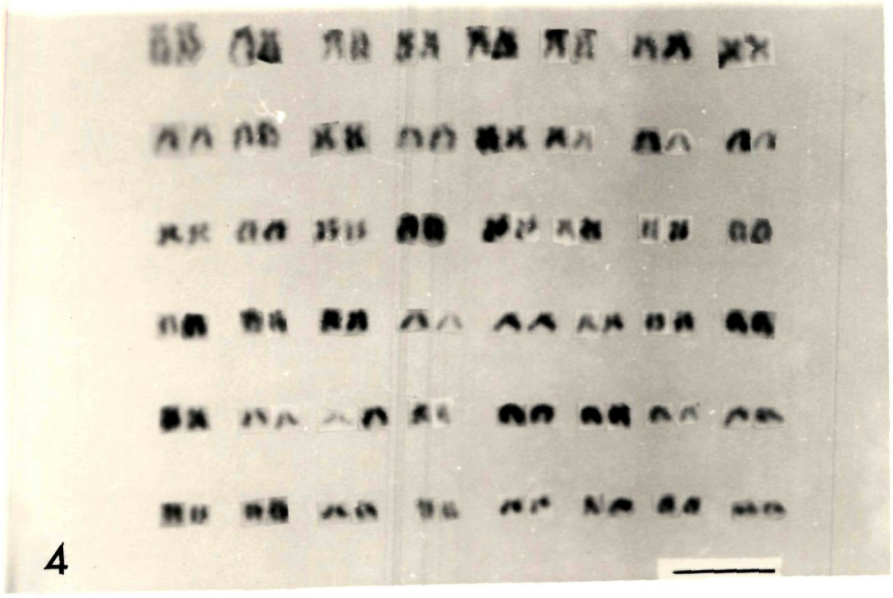
Fig. 3. Metaphase chromosome of *Acrossocheilus hexagonolepis* from kidney cell; the bar represents 10 μm.

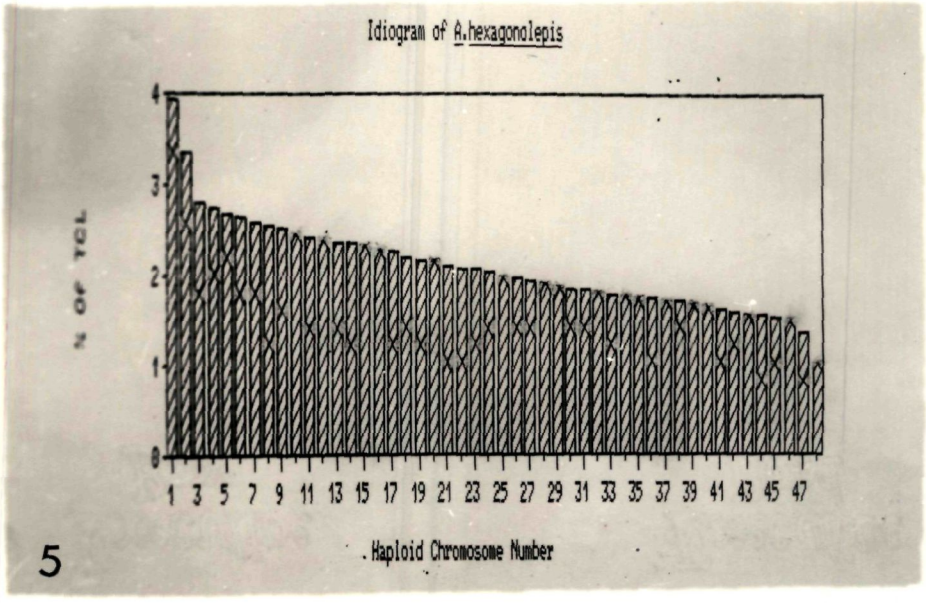
Fig. 4. & 5. Karyotype and Idiogram of chromosome in *A. hexagonolepis*; the bar represents 10 μm



3



4


Idiogram of A.hexagonolepis
N OF TCL
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Haploid Chromosome Number

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CHAPTER TWO

GENETIC STUDY

INTRODUCTION

INTRODUCTION

The year 1950 roughly marks the beginning of the trend of a continuing process of integrating biochemical genetics into fishery management. The change of focus is reflected by the proportion of studies that used genetic data out of the total number of studies dealing with intraspecific genetic variation in papers published between 1950 and 1995 in the reputed journals of worldwide circulation. There were no such genetic studies until the 1950s. At that time a single blood protein study (Drilhon, 1953) was followed by a surge of primarily biochemical genetic studies that has persisted since then. The absence of published genetic studies on fish species before 1950 masks both an awarness of the need and an active search for genetic information on population structuring at this time. Most of the genetic studies focussed on immunological procedures. Gradually, new understanding and technologies arose that were independent of these activities as clarification of the structure of the DNA molecule by Watson and Crik (1953) led to an understanding of the direct relationship between genes and genetic variation. Starch gel eletrophoresis (Smithies, 1955), enhanced by the application of histochemical staining methods (Hunter and Markert, 1957), added simplicity and sensitivity to the study of protein variation. Mendelian variation detected in serum protein by electrophoresis in humans during this time (*e.g.*, Smithies and Walker, 1955; Smithies, 1957) anticipated expanded application in fish *e.g.*, cat fish (Cushing and Durall, 1957) and Cyprinids (Chandrasekhar, 1959). All of these studies were concerned on blood-group protein differences that were potentially useful for identifying genetically discrete breeding groups within species.

Living beings adapt to the environment through genetic variation. Therefore, genetic diversity is of fundamental importance to the continued productivity of natural populations. On the other hand genetic diversity is also the source of varied traits important to artificial propagation. Successful planning and implementation of any management programme of fish populations require detailed information with regard to the genetic organization of the population (Smith *et al.*, 1975; Allendorf and Utter, 1979).

Identifying a genetic resource is therefore, a starting point for the efficient use and conservation of these species and such information constitutes a requisite for an optimal utilization of existing variability (Smith and Chesser, 1981). Further, for a correct understanding of the genetic basis of evolutionary changes, the patterns of genetic diversity present in many species must be examined.

Genetic variability should be confirmed by suitable mating experiment or by pedigree data. But these experiments are often difficult or impossible in natural population and are thus usually restricted to organisms easily bred in the laboratory.

Biochemical genetic technique offer accurate methods for identifying discrete breeding populations, estimating contribution to stock mixtures, indicating problem in fish culture, recognizing and quantifying hybrid populations and providing insights into conservation problems (Ryman and Utter, 1987). The analysis of genetic variablity in the organism can be detected relatively easily by biochemical means particularly protein phenotype with the aid of electrophoresis by using 'isozymes' as indicators. The terms "isozyme" and "isoenzyme" are synonymous and occuring in the same species (Markert and Moller, 1959). It has been found that at least half of all enzymes exist in nature in multiple molecular forms. Each enzyme has a precise metabolic role and is related to the functional divergence of the tissue. It is well known that cells of different tissues within the same organism do not exhibit the same enzyme (or protein) pattern, while all the cells presumbably contain the same genome. Therefore, it is very important to examine several different tissues of an organism for an insight of the genomic structure (Fisher *et al.*, 1980). Some enzymes, particularly those involved in vital biological processes (such as glycolysis, the pentose cycle, and Kreb's cycle, etc.) are constitutional. The genes which code for these enzymes are usually active under all environmental condition, as long as compatible with the organism. A few enzymes have activities that may vary with the development of the organisms. Similarly, variations can be correlated to season and environment as well. Thus, many studies have shown that enzyme and isozyme systems are good instruments to answer the question concerning genetics, developmental adaptation, evolutionary process in these fish.

Therefore, the present work has emphasis on protein *i.e.*, isozyme, more than nucleic acid, because it is now well established that the genetic variability may be expressed as altered enzyme activity and it has been appreciated since Garrod (1908) and Beadle and Tatum (1941) showed that a gene codes for a single enzyme.

Currently the only technique of electrophoresis helps us to study simultaneously several genes in the same individual, rapidly and relatively cheaply and allows between 20 and 30 individuals to be tested at the same time. Indeed, it is doubtful, whether the recent advances made in this field could have been achieved without electrophoresis (Rider and Taylor, 1980). The electrophoretic separation of unpurified raw extract contains hundreds of different proteins, and only few coloured bands appear on the gel by using the specific enzyme staining solution. Each band corresponds to a different protein, the synthesis of which is controlled by genes. The enzymatic phenotypes observed on the gel can, therefore, be interpreted in terms of genotypes that is, in term of genes and their alleles.

Detailed analysis of population structure and quantitative estimation of genetic variation require the examination of a large number of loci (Nei and Roychoudhury, 1974; Nei, 1975; Allendorf and Utter, 1979). We have selected twelve enzymes *viz.*, Adenylate kinase, Alcohol dehydrogenase, Glucose 6-phosphate dehydrogenase, Glutamate dehydrogenase, Glycerol 3-phosphate dehydrogenase, Hexokinase, Hexose 6-phosphate dehydrogenase, Malate dehydrogenase, Malic enzyme, Phospho-glucomutase and Xanthine dehydrogenase for our present work.

Adenylate kinase (AK., 2.7.4.3) converts ATP to ADP to release energy. Allendorf *et al.* (1977) has reported in brown trout that AK is coded by three loci and is monomeric in structure. Harris and Hopkinson (1978) have found that AK is coded by four loci in *Tilapia zilli*. Philipp *et al.*(1979) have examined large mouthbass and small mouthbass and found that AK is coded by two loci *AK-A** and *AK-B**, *AK-A** being present in white skeletal muscle and *AK-B** in all the tissues. Winans (1980) has reported in milk fish *Chanos chanos* that AK is coded by single locus, two alleles producing two bands. Ferguson (1981) has found in Arctic charr liver the existence of two loci. Cruz *et al.*'s (1982) investigation on *Tilapia zilli* revealed that AK is monomeric coded by two loci. Andersson *et al.* (1983) has reported that AK in Arctic charr is coded by three loci

designated as *AK-1**, *AK-2** and *AK-3** and assumed these to code for the three anodal zones. According to them, all the three loci are expressed in eye and muscle whereas only *AK-3** is expressed in liver in contrast to Ferguson's (1981) report. McAndrew and Majumdar (1983) have reported a single isozyme band in muscle tissue in *Sarotherodon* and *Tilapia* species and inferred that AK is coded by a single locus.

Alcohol dehydrogenase (ADH., EC. 1.1.1.1) catalyzes the conversion of alcohol to aldehyde or ketones. It is known to be dimeric in nature being encoded in the majority of vertebrate at a single gene locus (Hitzeroth *et al.*, 1968; Shaklee *et al.*, 1974; Frankel, 1978, 1980, 1981a & 1983). ADH has been examined in a wide variety of teleostean species and is usually found in highest concentration in liver tissue though it may be present to a lesser extent in both stomach and kidney tissues (Hitzeroth *et al.*, 1968; Shaklee *et al.*, 1977). Investigation on the tissue specificity of ADH in *Brachydanio*, however, has revealed that its expression is restricted to liver extracts (Frankel, 1978 & 1980) only. Allendorf *et al.* (1975) have reported that ADH is encoded in single locus in rainbow trout and it is dimeric in nature. Buth (1977) has reported that ADH is under the apparent control of a single gene with two alleles designated as *ADH-A**$_a$ and *ADH-A**$_b$ in the Cypriniformes *Hyperentelium nigricans*. ADH is encoded in single locus with dimeric nature in Quebec brook trout (McGlade and McCrimmon, 1979), small and large mouthbass (Philipp *et al.*, 1979), brook trout (May *et al.*, 1979), *Petromyzon marinus* (Kruger, 1980), eagle lake trout *Salmo gairdneri* (Busack *et al.*, 1980), *Atherina boyeri* (Berrebi and Davidian, 1980), Irish Arctic charr (Ferguson, 1981; Andersson *et al.*, 1983), *Tilapia zilli* (Cruz *et al.*, 1982; McAnderew and Majumdar, 1983), black sea bream (Taniguchi *et al.*, 1983), vendae *Coregonus albula* (Vuorinen, 1984), Barbus hybrid (Frankel, 1985) and big head silver carp (Brummet *et al.*, 1988).

Glucose 6-phosphate dehydrogenase (G6PDH., EC. 1.1.1.49) is involved in the pentose phosphate shunt and converts glucose 6-phosphate into 6-phosphoglucose lactone. It is encoded in single locus in *Xiphophorus xiphidium, X. helleri* and the carps *Ctenopharyngodon idella, Hypophthlmichthys mobilis* (Scholl and Anders, 1973; Beck *et al.*, 1983). Vuorinen (1984) found that G6PDH is expressed as single band in liver extracts of vendae, *Coregonus albula*. However, some populations have shown

multiple bands. Cederbaum and Yoshida (1976) observed a complex banding pattern of G6PDH in rainbow trout due to the post translational modification of the enzyme coded by a single locus.

Ohno *et al.* (1966) have studied G6PDH in rainbow trout and observed multiple electrophoretic banding pattern, which they suggested might be determined by two different alleles at two loci. In splake trout nine forms of G6PDH were resolved by Yamauchi and Goldberg (1973). They proposed that G6PDH isozyme is tetrameric and the product of two codominant autosomal gene loci. Yamauchi and Goldberg (1975) and Allendorf *et al.* (1977) have reported that G6PDH is encoded in two loci in brown and lake trout. Ferguson (1981) and Andersson *et al.* (1983) have reported that G6PDH is encoded in two loci in Arctic charr and designated as *G6PDH-1** and *G6PDH-2**. The less mobile band is *G6PDH-1** expressed only in liver and the more mobile band *G6PDH-2** is expressed in all the tissues. On the contrary, three loci have been reported in some salmonids species (Lynch and Vyse, 1979; Dehring *et al.*, 1981).

Glutamate dehydrogenase (GDH., EC. 1.4.1.2) is a regulatory enzyme and plays a major role in channeling of carbon into Kreb's cycle (Hochachka and Somero. 1973) and converts glutamate to oxyglutarate and ammonia ions. In rainbow trout GDH is encoded in a single locus represented by a single invariable band and is predominantly or only expressed in liver tissue (Allendorf *et al.*, 1977). A single locus inheritance has also been reported in dermersal fish (Utter et al., 1980). However, GDH is encoded in two loci in the milk fish *Chanos chanos* (Winans, 1980). Cruz *et al.* (1982) screened white muscle, liver, heart and brain tissue of *Tilapia zilli* and could not find any apparent variation. The two banded pattern observed in white muscle is readily explained by the monomeric nature of GDH and its being encoded in two loci as in other vertebrates (Harris and Hopkinson, 1978).

Glycerol 3-phosphate dehydrogenase (G3PDH., EC. 1.1.1.8) mediates the conversion of glycerophosphate into dihydroxy acetone phosphate in Emden-Mayerhof pathway. Dando (1970) has described five phenotypes in megrim (*Lepidorhombus whiff iagonis*) white skeletal muscle which he explained assuming the presence of four codominant alleles (*GpA*, GpB*, GpC* and GpZ**). The five phenotypes may be

designated as AA, AB, BB, AC and AZ respectively. Johnson *et al.* (1970), Johnson and Beardsley (1975) have described the presence of two alleles - Fast (F) and Slow (S) in *Sebastus alutus*. G3PDH is dimeric and is mainly expressed in liver tissue coded by single locus in Sockeye salmon (Utter *et al.*, 1973), loach *Cobitis delicata* (Kimura, 1978), Atlantic salmon (Cross and Ward, 1980), Atlantic herring (Andersson *et al.*, 1981), sea bream (Taniguchi *et al.*, 1983). Buth and Burr (1978) reported the presence of three alleles in Cyprinid genus *Campstoma*. Stahl and Ryman (1982) have reported that G3PDH is predominantly expressed in skeletal muscle in brown trout with a pair of codominant alleles as originally described by Engle *et al.* (1971).

In the Cypriniformes species *Moxostoma rhothoecuny*, G3PDH is found in duplicated form controlled by two loci and each locus has two alleles (Buth, 1977). In large and small mouthbass (Philipp *et al.*, 1979) and in *Sarotherodon* and *Tilapia* species (McAndrew and Majumdar, 1983) G3PDH is encoded in two loci designated as *G3PDH-1** and *G3PDH-2**. Both are expressed in muscle and liver tissue although *G3PDH-1** has greater activity in muscle. Andersson *et al.* (1983) examined Arctic charr and revealed that G3PDH activity is represented by two invariant zones with different tissue predominance due to the expression of two loci. The cathodal zone is muscle specific whereas the anodal zone is liver specific. Similar observation has been made in the brown trout (Allendorf *et al.*, 1977). May *et al.* (1979) have resolved two zones of activity of G3PDH in muscle extracts of Salmonids, brook and lake trout, each zone being encoded in a single locus.

Clayton *et al.* (1973) showed that three loci coded for G3PDH in *Coregonus clupeaformis*, in white muscle the most anodal *G3PDH-3** shows at best, weak activity and in liver both *G3PDH-1** and *G3PDH-3** are active but *G3PDH-2** shows no activity. In *Tilapia zilli*, three bands are observed for this enzyme in all individuals (Cruz *et al.*, 1982). It is usually dimeric (Ward, 1978), but the spacing of the bands suggests the presence of three loci rather than two. G3PDH is encoded in three loci in muscle tissue of *Salvenlineus* species (Clayton and Ihssen, 1980).

Hexokinase (HK., EC. 2.7.1.1) converts glucose into glucose 6-phosphate in the Emden-Mayerhof pathway. Allendorf *et al.* (1977) have reported that HK is encoded in

two loci in brown trout *Salmo trutta*. But Andersson *et al.* (1983) have reported that HK is encoded in a single locus in Arctic charr with the expression of single invariable anodal liver specific band.

Hexose 6-phosphate dehydrogenase (H6PDH., EC. 1.1.1.47) has the ability to catalyze the oxidation of glucose 6-phosphate as well as galactose 6-phosphate, 2-deoxyglucose 6-phosphate and glucose. Ohno *et al.* (1966) have found a second form of G6PDH in human tissue which they designated as Hexose 6-phosphate dehydrogenase. Though H6PDH shows about equal activity toward galactose 6-phosphate, it is slightly larger than G6PDH molecules. H6PDH otherwise is known as glucose dehydrogenase, and has broad substrate specificity compared to narrower specificity of G6PDH. G6PDH occurs in a wide variety of animals, while H6PDH is found in echinoderm and vertebrates (Onishi and Horri 1977; Horri *et al.*, 1977) and is encoded in two loci in rainbow trout, brook trout and lake trout (Stegemen and Goldberg, 1971 & 1972; Shaw and Koen, 1968; Ruddle *et al.*, 1968).

Lactate dehydrogenase (LDH., EC. 1.1.1.27) has been the most extensively studied isozyme system since Markert and Moller (1959) first discovered and described the existence of isozymes. It is a very suitable system for studying genetic aspect of an organism (Shaklee *et al.*, 1973; Place and Powers, 1979; Powers *et al.*, 1986; Coppes *et al.*, 1987; Coppes, 1990). LDH catalyzes the inter-conversion of lactate to pyruvate; during anaerobic periods when the Kreb's cycle is inoperative, pyruvate is converted to lactate by LDH.

In most fishes LDH is coded by three independent loci, *LDH-A*, LDH-B** and *LDH-C**. The enzyme is a tetrameric protein. Random combination of these loci products (Whitt, 1970; Shaklee *et al.*, 1973; Markert *et al.*, 1975) result in the formation of the different LDH isozymes. For example, the products of the two loci *LDH-A** & *LDH-B** may give rise to five isozymes *viz.*, A_4, A_3B_1, A_2B_2, A_1B_3 and B_4. Isozyme A_4 is expressed mainly in skeletal white muscle (primarily anaerobic tissue) but may be present in almost all the tissues. Isozyme B_4 is expressed mainly in heart muscle (primarily aerobic tissue) and it is present in several other tissues. The aerobic tissues predominately contain a high proportion of B subunits which are effectively lactate oxidase, and typical anaereobic

tissues may contain higher proportion of A subunits which are pyruvate reductase (Wilson, 1964; Everse and Kaplan, 1973).

In Petromyzontiformes, LDH is encoded in a single *LDH-A** gene locus. In Myxiniformes, however, two loci, *LDH-A** and *LDH-B** are already present forming the two homotetramers A_4 and B_4 (Whitt *et al.*, 1975; Callegarini, 1978; Fisher *et al.*, 1980; Whitt, 1984 & 1987; Baldwin *et al.*, 1987; Baldwin and Lake, 1987). In fish, the lowest number of LDH isozymes phenotypes have been observed in flat fishes, showing only the A_4 and B_4 isozymes (Cahn *et al.*, 1962; Markert and Faulhaber, 1965).

A third locus *LDH-C** was first observed in chondrostei and is the result of a duplication of a *LDH-B** locus (Whitt, 1969; Morizot and Siciliano, 1983). It is a characteristic of the bony fish, Actinopterygi (Markert *et al.*, 1975; Fisher *et al.*, 1980; Whitt, 1984). In primitive taxa of Osteichthyes 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). In advanced teleost the structure and expression of this third locus is specialized. LDH-C_4 is expressed in brain and retina tissue and it has been reported in many species of various order (Massaro and Markert, 1968; Callegarini and Vendamiati, 1975). In order Gadiformes and Cypriniformes, the C_4 isozyme has a cathodal migration and is predominantly expressed in the liver tissue (Sensabaugh and Kaplan, 1972; Shaklee *et al.*. 1973 & 1974; Shaklee and Whitt, 1981; Morizot adn Siciliano, 1983; Frankel, 1985). Many authors have reported the association between B and C subunits in the retina and neural tissue (Callegairini and Vendemiati, 1975; Philipp and Whitt, 1977; Philipp *et al.*, 1979; Salvatorelli *et al.*, 1987; Coppes *et al.*, 1987). Shaklee *et al.* (1973) and Garlick and Terwilliger (1978) have described some species of the fish in which an association between C and A subunits was observed.

Malate dehydrogenase (MDH., EC. 1.1.1.37) catalyzes the interconversion of malate into oxaloacetate in the Kreb's cycle (Banaszak and Bradshaw, 1975; Darnall and Klotz, 1975). The MDH isozyme system consists in invertebrate and vertebrates of two main forms (1) supernatant MDH (sMDH) in extramitochondrial cytoplasm and (2) mitochondrial MDH (mMDH) in the mitochondrial matrix. These two forms have the same molecular weight but because of their diffferent subcellular location they differ in

electrophoretic mobility, kinetic behaviour, amino acid composition, antigenic properties and are controlled by separate gene loci (Whitt, 1970).

sMDH has been studied in a diverse group of vertebrate system from Agnathas to man and such studies have revealed that MDH exists as a dimeric molecule (Davidson and Cortner, 1967; Bailey *et al.*, 1970) and is usually encoded in two loci designated as *sMDH-A** and *sMDH-B**, located on separate chromosomes (Bailey *et al.*, 1970; Wheat and Whitt, 1971; Wheat *et al.*, 1972; Whitt *et al.*, 1973; Rainboth and Whitt, 1974; De Luca *et al.*, 1983; Buth, 1983; Coppes *et al.*, 1987). However, the number of genes encoding for sMDH typically exists as a single major anodal form (Kitto and Wilson, 1966; Davidson and Cortner, 1967; Karig and Wilson, 1971), which suggests single gene control. In fish and amphibians, three equally spaced anodal bands of sMDH are commonly observed. Presumably, these bands are produced by two loci encoding for different sMDH subunits designated as A and B (Bailey *et al.*, 1970). The random association of A and B polypeptides results in the formation of three dimeric sMDH isozymes, A_2, AB and B_2. The sMDH-A_2 is present in all tissues and its often dominant in the liver (Fisher *et al.*, 1980; Pasdar *et al.*, 1984; Salvatorelli *et al.*, 1987; Basaglia and Callengarini, 1988; Coppes *et al.*, 1987). While sMDH-B_2 is ordinarily present in white skeletal muscle and to a lesser extent in other tisues as well (Philipp *et al.*, 1979 & 1983; Salvatorelli *et al.*, 1987 & 1989; Coppes *et al.*, 1987). Bailey *et al.* (1970) have demonstrated the presence of duplicate loci encoding for the sMDH-B subunit in the king salmon and sMDH-A subunit in the brown trout.

The mMDH isozyme migrates more anodaly during electrophoresis than the sMDH isozyme. But the reversed electrophoretic mobility of sMDH and mMDH has been observed in *Fundulus*, sea urchin and Tuna (Ozaki and Whiteley, 1967; Kitto and Lewis, 1967) mMDH is controlled by a single locus.

Malic enzyme (ME., EC. 1.1.1.40) is one of the oxidative NADP - enzyme that provides NADPH for lipogenesis. ME exists in vertebrates in two forms *i.e.*, mitochondrial (mME) and supernatant (sME). The slow moving zone towards the cathode represents the mitochondrial one and is mainly expressed in muscle tissue. The fast moving zone represents the supernatant form and is predominantly expressed in liver tissue,

and to some extent in muscle tissue. mME has been reported to be coded by two loci in Atlantic salmon and brown trout and brook trout (Allendorf *et al.*, 1977; Stoneking *et al.*, 1979). Vuorinen (1984) has given a detailed description of sME variation in vendae which showed a five banded asymmetric pattern indicating the activity of two loci. This agrees with the results from most other salmonids (Cross *et al.*, 1979; Stoneking *et al.*, 1979). McAndrew and Majumdar (1983) observed that sME is encoded in two loci designated as *ME-1** (liver specific) and *ME-2** (muscle specific) in *Tilapia* species. In brown trout *Salmo trutta* (Allendorf *et al.*, 1977), walleye pollock *Theragra chalcogramma* (Utter et al., 1980), Atlantic salmon *Salmo salar* (Stahl, 1981; Cross and King, 1983), sME is encoded in two loci producing five banded pattern. In contrast, sME is encoded in a single locus in *Tilapia zilli* (Cruz *et al.*, 1982), *Barbus meridonalis* (Triantaphyllidis *et al.*, 1981) and *Gadus morhua* (Mork *et al.*, 1982).

Phosphoglucomutase (PGM., EC. 2.7.5.1) is a phospho transferase enzyme which catalyzes the reaction of glucose 1-phosphate to glucose 6-phosphate. PGM is encoded in single locus in arrow squid (Smith *et al.*, 1981), milk fish *Chanos chanos* (Winans, 1980), walleye pollock *Theragra chalcogramma* (Grant and Utter, 1980), sea lamprey (Krueger, 1980), sockeye salmon (Utter *et al.*, 1973), small and large mouthbass (Philipp *et al.*, 1979), *Anoplarchus purpurrescens* (Sassaman and Yoshiyama, 1979) *Sarotherodon* and *Tilapia* species (McAndrew and Majumdar, 1983), Atlantic cod, *Gadus morhua* (Mork *et al.*, 1982), Atlantic salmon, *Salmo salar* (Stahl, 1983), and Cyprinids species (Al-Hasson and Elias, 1988). However, a number of reports states that PGM is coded by two loci designated as *PGM-1** and *PGM-2** in brown trout *Salmo trutta* (Utter *et al.*, 1973; Allendorf *et al.*, 1975 & 1977), Salmonid *Salvelinus* and rainbow trout *Salmo gairdneri* (May *et al.*, 1980; Allendorf *et al.*, 1982), Arctic charr (Kornfield *et al.*, 1981; Ferguson, 1981; Andersson *et al.*, 1983), *Tilapia zilli* (Cruz *et al.*, 1982), *Tilapia* species (Basiao and Taniguchi, 1984).

Xanthine dehydrogenase (XDH., EC. 1.1.1.204) is an enzyme having a physiological role in purine metabolism catalyzing the direct oxidation of Xanthine or hypoxanthine using NAD as the hydrogen acceptor (Johnson, 1974). According to Cruz *et al.* (1982) XDH is coded by single locus in *Tilapia zilli* and expressed in liver and

heart with reduced activitiy in skeletal muscle. XDH is coded by a single locus in a large number of fish (Allendorf *et al.*, 1977; Busack *et al.*, 1979; Andersson *et al.*, 1983; Padhi and Khuda-Bukhsh, 1990).

Review of literature reveals that there is a total lack of information on the biochemical genetics of the different mahseer species in comparison to the work done in the other fish species. Keeping this in mind, we have selected the chocolate mahseer *A. hexagonolepis* (*Neolissocheilus hexagonolepis*) for the present investigation. The aim of the present work is to study the inheritance pattern of twelve enzymes in two well contrasted populations (see Material and Methods for detail) and to determine the amount of genetic variation and genetic distance within and between the two populations to unravel their relationship. Subsequently we have also used the electrophoretic data in correlating heterozygosity/polymorphism with water temperature and body weight.

MATERIAL AND METHODS

2.1 *Sample preparation and storage*

2.1.1 *Samples*

Collection has been made from two different localities; Umiam reservoir, a closed water system near Shillong in Meghalaya and Uttarkuchi (Assam) near the India-Bhutan border (Table 1; Fig. 1, 2 & 3). The Pagladia river starts in the Himalayan mountain and flows towards Uttarkuchi along Subankhata (Fig. 1) (250 km away from Shillong). Live specimens collected from Umiam reservoir (Fig. 2 & 3) were transported to the laboratory for processing the different tissues. The specimens collected from Pagladia river were dissected and brain, eye, heart, liver, kidney and muscle tissue were immediately wrapped in aluminium foil and stored in liquid nitrogen and few live specimens were also transported to laboratory for further processing. Tissue samples were stored in a -60° C ultra freezer (ScienTemp 2000, USA).

2.1.2 *Homogenization*

Tissues were blotted and weighed accurately and homogenized in an electric homogenizer with measured volume of the ice cold 0.25 M sucrose solution. The homogenates were immediately transferred into poly-propylene centrifuge tubes (15 ml) kept in on ice bath.

2.1.3 *Homogenates*

The homogneized slurry was clarified by centrifugation at -10° C. We rountinely centrifuge at approximately 24,000 X g for 20 min. in a cooling centrifuge (REMI - C24, India) which was adequate to remove most of the cellular debris and unhomogenized tissue fragments. The resultant clear supernatant was decanted in test tubes and subjected to electrophoresis. Subsequently the remaining samples were stored at -60°C in ultra freezer.

Table 1. Collection data of chocolate mahseer, *Acrossocheilus hexagonolepis*.

Sample No	Site	Date	Total number of fish collected	Location		Depth (m)	Body weight (g)	Water temperature (°C)
				N. Long.	E. Lat.			
1.	Umiam reservoir	Mar.'93 to May '95	1047	24° 34'	91° 56'	7.5	280 - 9	28 - 16
2.	Pagladia river	Mar '93 Mar.'94 May '95	210	27° 40'	91° 28'	3.0	115 - 5	27 - 20.5

Fig. 1. Pagladia river (collection site, Subankhata, Assam)

Fig. 2 & 3. Umiam reservoir (collection site, Barapani, Shillong, Meghalaya)


1


2

2.2 *Polyacrylamide Gel Electrophoresis (PAGE)*

The following reagent solutions are stored in dark bottle in a refrigerator and their shelf life time are upto two months.

Reagent A: pH 8.9

1 N Hydrochloric acid (HCl)	48.00 ml
N,N,N,N-Tetramethyl ethylene diamine (TEMED)	00.23 ml
Tris (hydroxyl methyl) methylamine	36.60 g

Reagent B: pH 6.7

1 N HCl	48.00 ml
TEMED	00.46 ml
Tris	5.98 g

Reagent C:

Acrylamide	30.00 g
N,N- Methylene bis-acrylamide	0.80 g

Reagent D:

Acrylamide	*10.00 g*
Bis-acrylamide	2.50 g

Reagent E:

Riboflavin	0.40 g

Reagent F:

Sucrose	40.00 g

Reagent G:

Ammonium persulfate	0.14 g

The reagent A, B, C, D, E, F and G are dissolved in distilled water and the volume is made upto 100 ml.

Stock buffer solution: pH 8.3

Tris	6.00 g
Glycine	28.80 g

Destaining solution

Acetic acid	70.00 ml

The above two solutions are made upto 1000 ml of distilled water. For electrophoretic reservoir buffer 10% of this stock solution is to be used.

2.3 *Composition of the gel system*

Separation gel was prepared just before use 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

2.4 *Electrophoresis:*

2.4.1 *Procedure:*

The vertical disc elcctrophoresis system of Davis (1964) was employed. It is a discontinous separating system with regard to pH value, buffer composition on gel pore size in which polyacrylamide gel serves as the matrix. Disc electrophoresis is carried out with small columns of polyacrylamide gel consisting of three layers in a suitable container like cylindrical tubes. The three layers are : (1) a large pore spacer gel (2) a small pore separation or running gel in which the sample constituents are separated and (3) the sample gel.

2.4.2 *Making the gel:*

About 1 ml to 1.5 ml (upto 7 cm in length of the gel tubes) of the separation gel solution was poured into the gel tubes which were capped at the bottom . Care was taken not to allow any air buble in the gel tube column. A few drops of distilled water were carefully layered above the separation gel. After polymerization, the water from the top of the gel column was removed carefully and about 0.2 ml of the spacer gel solution was dropped carefully into the gel tubes. A few drops of water were again layered above this gel solution and the gel system were kept under fluorescense light for 30 - 45 min. to polymerize. The easiest method was to expose the solution to sunlight to get polymerization within a minute.

2.4.3 *Loading and running the gel samples:*

The gel tubes were than fixed vertically in the disc electrophoresis chamber as described by Davis (1964). About 0.1 ml of the sample solution was directly placed over the spacer gel after removing the water layer. The remaining part of the gel tubes were filled with the buffer used for the chambers. Then both the upper and lower chambers were filled with buffer (1 M Tris-Glycine, pH 8.3) completely. A drop of 1% Bromophenol indicator solution was mixed in the upper buffer chamber. The apparatus was then placed in a refrigerator at 4° C and the two electrodes were connected with the electrophoretic power supply. The current strength was initially upto 1.5 mA/gel tube for 15 min. then raised upto 3.0 mA/gel tube. After the requisite time of running, the current supply was stopped and the gel tubes were removed from the upper chamber. The gels were taken out carefully with the help of a long needle and water force from a syringe and immediatley subjected to specific staining solution to obtain different isozyme patterns.

2.5 *Reagent solution for enzyme staining:*

The gels were incubated with specific stains for a specific period. Gels incubated without the substrate served as controls. After the reaction the gels were washed and preserved in 7% acetic acid (v/v).

Staining buffers - Stock:

0.1 M Tris-HCl (p^H from 7 to 9)

Tris 12. 10 g

titrated to desired p^H with 1 N HCl.

0.1 M Phosphate buffer (p^H 7.5)

Pottassium dihydrogen phosphate 13.60 g

Pottassium orthophosphate added to bring the pH 7.5.

Both the buffer volume is made upto 100 ml.

2.6 *Enzyme staining Receipes:*

Adenylate kinase (AK., EC. 2.7.4.3)

Nitroblue tetrazolium (NBT)	0.008 g
Phenazine methosulfate (PMS)	0.0012 g
Nictoninamide adenine dinucleotidephosphate (NADP)	0.010 g
Magnesium chloride ($MgCl_2$)	0.0084 g
Glucose	0.036 g
Tris - HCl	4.000 ml
Hexokinase	0.003 units
Glucose 6-phosphate dehydrogenase	0.0013 units

Alcohol dehydroganse (ADH., EC. 1.1.1.1)

Nictoninamide adenine dinucleotide (NAD)	0.080 g
PMS	0.0012 g
NBT	0.008 g
Tris - HCl	2.5 ml
Ethanol	1.0 ml

Glucose 6-phosphate dehydrogenase (G6PDH., EC. 1.1.1.49)

Glucose 6-phosphate	0.040 g
NADP	0.020 g
PMS	0.006 g
NBT	0.015 g

Glutamate dehydrogenase (GDH., EC. 1.4.1.2)

NAD	0.024 g
NBT	0.012 g
PMS	0.008 g
Sodium glutamate	0.338 g
Phosphate buffer	10.0 ml

Glycerol 3-phosphate dehydrogenase (G3PDH., EC. 1.1.1.8)

NBT	0.0035 g
PMS	0.0035 g
NAD	0.0200 g
Glycero phosphate	0.0200 g
Tris - HCl	0.5 ml

Hexokinase (HK., EC. 2.7.1.1)

Glucose	0.0360 g
$MgCl_2$	0.0084 g
Adenosine-5-triphosphate (ATP)	0.0100 g
NADP	0.0100 g
PMS	0.0012 g
NBT	0.0080 g
G6PD	0.002 units
Tris - HCl	4.0 ml

Hexose 6-phosphate dehydrogenase (H6PDH., EC. 1.1.1.47)

NBT	0.0200 g
NADP	0.0200 g
PMS	0.0080 g
Tris - HCl	4.0 ml
0.01% galactose phosphate	2.0 ml

Lactate dehydrogenase (LDH., EC. 1.1.1.27)

NAD	0.0800 g
PMS	0.0012 g
NBT	0.0080 g
Tris - HCl	2.5 ml
1 N Lithium lactate solution	0.5 ml

Malate dehydrogenase (MDH., EC. 1.1.1.37)

NAD	0.0800 g
NBT	0.0080 g
PMS	0.0012 g
Tris - HCl	2.5 ml
1 N Malic acid solution	0.5 ml

Malic enzyme (ME., EC. 1.1.1.40)

NADP	0.0800 g
PMS	0.0012 g
NBT	0.0080 g
Tris - HCl	2.5 ml
1 N Malic acid solution	0.5 ml

Phosphoglucomutase (PGM., EC. 2.7.5.1)

Sodium glucose 1-phosphate	0.2400 g
$MgCl_2$	0.0800 g
NADP	0.0040 g
G6PD	0.002 units
PMS	0.0040 g
NBT	0.0080 g
Tris - HCl	4.0 ml

Xanthine dehydrogenase (XDH., EC. 1.1.1.204)

NAD	0.0240 g
NBT	0.0120 g
PMS	0.0010 g
1 M hypoxanthine	1.2 ml
Tris - HCl	8.0 ml

Each one of the enzyme staining solution was made upto 40 ml.

2.7 *Data recording and gel interpretation:*

2.7.1 *Photography*

It is important to keep a permanent recored of the result of each experiment, especiallly if future litigation is a possibility. The stained polyacrylamide gels were photographed on a translucent red background with transmitted light from below the gel.

2.7.2 *Determination of relative mobilities:*

The initial interpretation of the banding patterns resulting from electrophoresis simply involves a determination of the relative mobility of the each band. This was readily accomplished by laying a small scale on the top surface of the stained gel and measuring the distance from the sample origin to the centre of each band on the gel. The relative electrophoretic mobility of each band was then calculated as a percentage of the mobility of the most common allele, which was arbitrarily assigned the value of 100 (Please see Fig. 4).

2.7.3 *Interpretation of polymorphism:*

Genetic polymorphism are interesting characteristics of the species, and must be correctly recognised and interpreted if sample identification is to be reliable. The banding pattern of heterozygous individuals has been predicted from information concerning the molecular structure of the protein as given by Pasteur *et al.* (1988). Alleles and locus are designated according to the nomenclature described by Shaklee *et al.* (1989).

2.8 *Determination of Genetic distance:*

Average heterozygosity (H) was calculated, using the formula of Nei (1975). The Chi-square test for goodness of fit was used to test deviation from expected Hardy-Weinberg proportion for polymorphic loci. We used the correlation and regression statistics to test the relationship between the heterozygosity Vs temperature and heterozygosity Vs fish body weight. We calculated genetic distance between the population by using Nei's formulae (1978) as explained by Pasteur *et al.* (1988) and Strickberger (1990).

OBSERVATIONS

3.1 *Adenylate kinase (AK)*

The enzyme appears in the cathodal zone and is polymorphic in both populations (*i.e.*, Umiam reservoir and Pagladia river population). Two types of expression *viz.*, single banded and double banded pattern have been observed in all the tissues examined (Fig. 5). The single banded pattern has been observed in 79 specimens while the double banded appeared in 73 specimens out of 152 specimens examined. One of the two isozymes, therefore, is present in all the specimens and has been designated as AK-1 100 (Fig. 18). The second isozyme is relatively anodal and has been designated as AK-1 134 according to its relative mobility. The liver tissue shows the highest activity. The rest of the tissues exhibit weak activity (Table 2).

3.2 *Alcohol dehydrogenase (ADH)*

Six tissues were used for ADH isozyme pattern analysis *i.e.*, brain, eye, heart, liver, kidney and muscle. A zone of high activity at the cathodal end has been resolved in the liver tissue extracts. The other tissue, kidney and muscle show very faint activity of ADH. The maximum number of band resolved is two in liver tissue of 38 specimens (Fig. 6). Accordingly the prevalent isozyme has been designated a ADH-1 100 and ADH-1 125 (Fig. 19).

3.3 *Glucose 6-phosphate dehydrogenase (G6PDH)*

Six tissues including brain, eye, heart, liver, kidney, and muscle have been scored to study the G6PDH isozyme pattern in this species. Brain, eye, liver and kidney tissues exhibited high G6PDH activity. A minimum of one band was consistently observed in brain, eye and kidney tissue extracts. The brain and liver tissue, eye and kidney tissues exhibited almost equal activity as observed on the gel (Fig. 7).

3.4 *Glutamate dehydrogenase (GDH)*

The activity of GDH has been resolved only in muscle tissue extract. We did not observe GDH activity in any other tissues extract of *A. hexagonolepis*. A two banded pattern phenotype was observed in all the individuals of the population (Fig.8).

Table 2. Summary of enzymes analysed in chocolate mahseer, *A. hexagonolepis*

Protein	EC. No.	Protein Structure	No. of Loci	Activity and technical quality of the enzyme						
				Brain	Eye	Heart	Liver	Kidney	Muscle	
Adenylate kinase (AK)	2.7.4.3	Monomer	one	++	++	++	++++	++	+++	G
Alcohol dehydrogenase (ADH)	1.1.1.1	Monomer	one	-	-	-	++++	-	-	G
Glucose 6-phosphate dehydrogenase (G6PDH)	1.1.1.49	Monomer	one	++++	+++	-	++++	++++	-	G
Glutamate dehydrogenase(GDH)	1.4.1.2	Monomer	two	-	-	-	-	-	+++	V
Glycerol 3-phosphate dehydrogenase (G3PDH)	1.1.1.8	Dimer	two	-	-	-	+++	-	++	D
Hexokinase (HK)	2.7.1.1	Monomer	one	++	-	-	+++	++	++++	D
Hexose 6-phosphate dehydrogenase (H6PDH)	1.1.1.47	Monomer	one	++	++	++	+++	++	++	D
Lactate dehydrogenase (LDH)	1.1.1.27	Tetramer	three	++++	++++	++++	++++	++++	++++	G
Malate dehydrogenase (MDH)	1.1.1.37	Monomer	one	++	++	++	++++	+++	++	D
Malic enzyme (ME)	1.1.1.40	Monomer	one	-	-	-	++++	++	-	G
Phosphoglucomutase (PGM)	2.7.5.1	Monomer	two	++	++	++	++++	+++	+++	V
Xanthine dehydrogenase (XDH)	1.1.1.204	Dimer	two	-	-	-	+++	++	-	D

++ Weak; +++ Moderate; ++++ Strong; - No activity

W - Weak; D - Diffuse; V - Variable; G - Good

3.5 *Glycerol 3-phosphate dehydrogenase (G3PDH)*

The activity of G3PDH has been observed in liver tissue only. A maximum of four banded phenotype has been observed in some members of the Pagladia population, while two is the common pattern (Fig. 9, 10 & 20). The Umiam population, however, presented a uniformly two banded pattern in 27 specimens out of 97 specimens examined for this enzymes (Fig. 10).

3.6 *Hexokinase (HK)*

Brain, eye, heart, liver, kidney and muscle tissues were used for studying the HK isozyme banding pattern. The expression of HK has been observed in brain, liver, kidney and muscle tissues extracts of both the populations. While two isozymes are commonly observed, the highest activity of HK has been observed in muscle tissues extracts. We could not detect HK activity in eye and heart tissues extract (Fig.11 & 21).

3.7 *Hexose 6- phosphate dehydrogenase (H6PDH)*

The activity of H6PDH has been resolved in brain, heart, liver and kidney tissue extracts. The maximum activity of H6PDH is observed in kidney tissue extracts as compared to the other tissues in both the populations. A single banded pattern has been observed in kidney tissue and a maximum of two banded pattern has been observed in liver and heart tissues extracts (Fig. 12 & 22).

3.8 *Lactate dehydrogenase (LDH)*

A number of different isozyme patterns have been seen in this species. LDH-A_4 is found to be present in eye, liver and muscle tissues while LDH-B_4 is predominant in brain, eye, heart and kidney and is also observed in liver and muscle tissues with lesser intensity. LDH-A_2B_2 is found in brain, eye, heart, liver and kidney. LDH-A_3B_1 and LDH-A_1B_3 are not observed in any of the tissues. Another LDH isozyme LDH-C_4 is found in all the tissues, i.e., brain, eye, heart, liver, kidney and muscle tissues. However, the exact location varies from tissue to tissue. Based on the relative mobility three isozymes could be detected in this zone of activity. It may also be pointed out no tissues showed more than one band in this region (Fig. 13 & 23).

3.9 *Malate dehydrogenase (MDH)*

The activity of MDH has been resolved in brain, heart, liver and kidney tissue extracts. The highest activity of MDH is observed in liver tissue extracts of both the populations (Fig. 14). A minimum of single banded pattern of MDH in homozygous individuals and maximum of two banded pattern of MDH in heterozygous individuals has been observed in liver tissue extracts of Umiam reservoir population (Fig. 24). The Pagladia river population has show only single banded phenotype in all the individuals.

3.10 *Malic enzyme (ME)*

The activity of ME has been observed in liver, kidney and muscle tissue extracts. The highest activity of ME is observed only in liver tissue as compared to other tissues. A uniformly of single banded pattern has been observed in both the populations (Fig. 15).

3.11 *Phosphoglucomutase (PGM)*

The activity of PGM has been observed in brain, eye, heart,liver, kidney and muscle tissues extracts of Umiam reservoir population. PGM has shown two zones of activity. The slower zone or cathodal zone (PGM-1) has been observed in all the tissue and show a single banded pattern except liver tissue, where it shows two banded pattern. The fastest moving zone or anodal zone (PGM-2) has been observed in brain and liver tissues (Fig. 16 & 25). Unfortunately we did not get favourable result from the Pagladia river population.

3.12 *Xanthine dehydrogenase (XDH)*

Six tissues were used for XDH isozyme banding pattern analysis *i.e.*, brain, eye, heart, liver, kidney and muscle tissues. The maximum activity of XDH has been observed in liver tissue extract, while a three banded pattern has been obtained (Fig. 17). We did not observe the XDH activity in the Pagladia river population.

DISCUSSION

To day chocolate mahseer has lost much of their natural habitat in the North-Eastern region of India, due to the influence of man by constructing the dam/reservoir across the rivers. Many factors affect the success (*i.e.*, distribution and abudance) of fish species, it has been argued that reproductive adaptations is one of most important factor (Balon, 1975 & 1978). Since 1969 a power plant has been made near Umiam *i.e.*, Umiam reservoir. As a consequence most of the natural spawing site of the chocolate mahseer has been destroyed. *A. hexagonolepis* spawn on gravel beds of stone in continuing flowing river/streams and the spawing is governed by water temperature. Drastic changes in water level and temperature during the winter season in the Umiam reservoir may affect the reproductive sucess of the species. At the same time introduction of Japanese carp in 1975 in the reservoir led to the changes in mahseer population structure. This heightened the importance of determining the genetic structure of the chocolate mahseer in the reservoir. The Pagladia river is a continuing flowing water system with the bed gravel of stone. It is a good spawing site for the chocolate mahseer species. Keeping the overall time frame in mind, we selected these two sites because of their well contrasted features.

4.1 *Genetic Interpretation of Electrophoretic patterns:*

The product of twelve enzyme loci have been traced in the gels. Different loci coding for an enzyme with similar function (isozymes) are assigned capital letters with hyphenated number, the slowest migrating being "1", then "2" and so on, as the distance of migration towards the anode increases. Allozyme variants at each locus are given either as lower case supercripts, the most anodal being "a", then "b" etc. or as percentage of the mobility of the most common allele, which has been arbitrarily assigned the value of 100.

The main result of the electrophoretic analysis are compiled in Table 2. Banding patterns observed from these twelve enzymes represent the products of a minimum of eighteen gene loci. A minimum of ten enzyme loci yielded satisfactory gel resolution and are *AK-1*, ADH-1*, GDH-1* & 2*, G3PDH-2*, G6PDH*, LDH-1*, 2* & 3*, MDH-1*,*

*ME** and *PGM-1* & 2**. However, zones of activity observed for *G3PDH-1*, HK-1*, H6PDH-1**, and *XDH-1* & 2** banding patterns were either faint or poorly defined. In all the cases of our estimate, the number of monomorphic loci *i.e., GDH-1* & 2*, G3PDH-1*, G6PDH*, LDH-1* & 2*, ME*, PGM-2** and *XDH-1* & 2** is conservative and reflect the minimum number of loci controlling the expression of each particular enzyme (Fig. 5 to 25). For most of the enzymes products coded by specific loci are predominated in liver tissues.

4.1.1 *Adenylate kinase (AK)*

The enzyme adenylate kinase is present in most cells. It catalyzes the interconversion of ATP and AMP into ADP to release energy. A number of isozymes for AK has been reported. Allendorf *et al.* (1977) reported the presence of three isozymes in brown trout. Two isozymes were observed in large mouthbass and small mouthbass (Philipp *et al.*, 1979). Similar observation has been made by Winans (1980) in milk fish *Chanos chanos* and *Tilapia* species and *Sarotherodon* by McAndrew and Majumdar (1983). Andersson *et al.* (1983) obtained three isozymes in Arctic charr. Cruz *et al.* (1982) reported four isozymes in *Tilapia zilli*. These reports indicates variation in the distribution of the isozymes. For example according to Philipp *et al.* (1979) one of the isozymes which they named AK-B were present in all tissues with limited expression in white skeletal muscle whereas the other *viz.*, AK-A predominated in white skeletal muscle. We have detected the presence of two isozymes in our fish. As stated above AK is monomeric and may be encoded in one to four loci in fish. We presume that AK in *A. hexagonolepis* is encoded in a single locus with an allele variant. Both the isozymes appear in the cathodal zone and the allelic variant has been designated as *AK-1* 134* according to its relative mobility. The single banded phenotype is represented either by the homozygous AK-1 100 or AK-1 134. Obviously the double banded phenotype represented the heterozygous AK-1 100/134. In some of the individuals the two isozymes were detected in liver tissue whereas a single isozyme could be detected in other tissues.

We examined 130 individuals from Umiam reservoir population of which 57 individuals were found to be homozygous for AK-1 100, 20 individuals homozygous for AK-1 134, and 53 individuals heterozygous for AK-1 100/134. The allele frequencies of *AK-1* 100* (0.642) and *AK-1* 134* (0.358) were compared to the Hardy-Weinberg expectation. The Chi-square calculated value is 1.635, and is statistically insignificant. In the Pagladia river population only two individuals were found to be homozygous and both of them belonged to AK-1 134, and 20 individuals were heterozygous for AK-1 100/134. The overall ratio between homozygotes and heterozygotes were almost reverse. The allele frequency of *AK-1* 100* and *AK-1* 134* is 0.55 and 0.45. The Chi-square value of 15.42 is therefore, highly significant at the level of $p<0.001$ to the Hardy-Weinberg model. This clearly indicates a positive selection for the heterozygotes in the population.

4.1.2 *Alcohol dehydrogenase (ADH)*

Alcohol dehydrogenase exhibits a some what broad substrate specificity and mediates the conversion of primary alcohols to aldehydes and secondary alcohols to ketones, utilizing nicotinamide adenine dinucleotide (NAD) as the electron acceptor. ADH is known to be a liver-specific dimeric protein being encoded in the majority of fishes at a single locus (Hitzeroth *et al.*, 1961; Shaklee *et al.*, 1974; Frankel, 1978, 1980, 1981 & 1983). In the Cypriniformes *Hypernetelium nigricans* liver, ADH is under the apparent control of a single locus with two alleles (Buth, 1977). *A. hexagonolepis* showed two isozyme pattern for ADH, indicating that ADH is encoded in single locus with an allelic variant. The allele has been named as *ADH-1* 125* according to its relative mobility. Homozygous individuals show a single banded pattern. We observed only two out of 123 individuals examined from Umiam reservoir population showing a homozygous condition for the allele *ADH-1* 125*. In all we detected 101 individuals were homozygous for *ADH-1* 100*, two individuals homozygous for *ADH-1* 125* and 20 individuals heterozygous for *ADH-1* 100/125* out of 123 individuals examined from Umiam reservoir population. The allele frequencies of *ADH-1* 100* (0.902) and *ADH-1* 125* (0.098) and the Chi-square value (0.719)is insignificant to the Hary-Weinberg model. Of the 22 individuals examined from the Pagladia river population,

4 individuals were homozygous for *ADH-1* 100* and 18 individuals were heterozygous for *ADH-1* 100/125*. The allele frequencies of *ADH-1* 100* and *ADH-1* 125* is 0.59 and 0.41. The Chi-square value of 10.54 is highly significant at $p<0.001$ level to the Hardy-Weinberg model. From the above data it is tempting to suggest that the allele *ADH-1* 125* is of relatively recent origin and has not been fixed in both the populations. However, the heterozygotes definitely enjoy a selective advantage in the riverine population.

4.1.3 *Glucose 6-phosphate dehydrogenase (G6PDH)*

Glucose 6-phosphate dehydrogenase catalyzes the glucose 6-phosphate to 6-phosphogluconate via the formation of 6-phosphoglucolactone by the process of dehydrogenation. A relatively high G6PDH activity is generally indicative of an active pentose shunt. Appreciably high activity of G6PDH in brain, eye, liver and kidney tissues suggests the availability of a large number of sites in *A. hexagonolepis* for fatty acid synthesis.

The genetic basis of G6PDH is quite interesting. In mammals G6PDH is encoded in two separate loci, one is X chromosome-linked and the other is autosomal. The latter is presently designated as hexose 6-phosphate (H6PDH) and has a broader substrate specificity and catalyzes the oxidation of galactose 6-phosphate, 2-deoxyglucose 6-phosphate as well as glucose 6-phosphate and accept NAD as cofactor (Bautista *et al.*, 1984).

More than one activity zones of G6PDH has been reported in trout (Yamauchi and Goldberg, 1973; Diebig *et al.*, 1979), *Heteropneusteus fossilis* (Prakash, 1991). We have consistently observed a single banded pattern of G6PDH in *A. hexagonolepis* and we presume that G6PDH is encoded in a single locus without any allelic variant. Our findings seem to be similar to those in many other fish species *viz.*, *Xiphophorus xiphidium, X. helleri, Ctenopharyngodon idella, Hypophthlmichthys mobilis, Coregonus albula* (Scholl and Anders, 1973; Beck *et al.*, 1983; Vuorinen, 1984).

4.1.4 *Glutamate dehydrogenase (GDH)*

Glutamate dehydrogenase plays a major role in fish metabolism, that is channeling of carbon into Kreb's cycle and converts glutamate to oxyglutarate and ammonia ions (Hochachka and Somero, 1973). We could not detect GDH activity in brain, eye, heart, liver and kidney tissues, while skeletal muscle tissue exhibited somewhat moderate activity which resolved into two bands. A single invariable band has been observed in rainbow trout (Allendorf *et al.* (1977) and dermersal fish (Utter *et al.*, 1980). In *Chanos chanos* (Winans, 1980) and *Tilapia zilli* (Cruz *et al.*, 1982) GDH activity has been visualized as two bands. These authors infer that GDH in these fishes are coded by two loci because of its monomeric nature (Harris and Hopkinson, 1978). Comparing our observation with these data we would like to presume that GDH in *A. hexagonolepis* is encoded in two monomorphic loci.

4.1.5 *Glycerol 3-phosphate dehydrogenase (G3PDH)*

The trivial name of the glycerol 3-phosphate dehydrogenase is alpha-glycero phosphate dehydrogenase. It mediates the conversion of glycerophosphate into dihydroxy acetone phosphate in Emden-Mayerhof pathway. There is a controversy in tissue specific activity of G3PDH in fish species, *i.e.*, one group found G3PDH activity mainly in liver tissue (Utter *et al.*, 1973; Kimura, 1978; Cross and Ward, 1980; Andersson *et al.*, 1981; Taniguchi *et al.*, 1983) and the other group found that G3PDH is predominantely expressed in skeletal muscle tissue (Dando, 1970; Engel *et al.*, 1971; Stahl and Ryman, 1982). However, we have observed G3PDH isozymes in both liver and skeletal muscle, the latter showing relatively weaker activity. While two is the common number, four isozymes have been detected in some members of the Pagladia river population. The genetic nature of G3PDH in fish appears to be interesting. A single locus inheritance has been reported in sockey salmon, loach, Atlantic salmon, Atlantic herring, sea bream etc. (Utter *et al.*, 1973; Kimura, 1978; Cross and Ward, 1980; Andersson *et al.*, 1981; Taniguchi *et al.*, 1983). G3PDH is encoded in two polymorphic loci in *Moxostoma rhothoecuny* (Buth, 1977). A three loci pattern has been claimed in *Coregonus clupeaformis, Tilapia zilli, Salvenlineus* species (Clayton *et al.*, 1973; Clayton and Ihssen, 1980; Cruz *et al.*, 1982).

The expression in *A. hexagonolepis* draws parallel to that observed in *M.rhothoecuny* and *M. hamiltoni* (Buth, 1977). We presume that G3PDH in our fish is encoded in two loci *viz.*, *G3PDH-1** and *G3PDH-2**, the latter being polymorphic. Comparison of relative mobility indicates the absence of the locus *G3PDH-1** or its expression in the Umiam reservoir population though it was very much present in the Pagladia population. The G3PDH, as already stated, existed in two forms *viz.*, G3PDH-2 100 and G3PDH-2 109. In the Umiam reservoir population, of the 97 fish examined 40 were homozygous for *G3PDH-2* 100*, 27 were heterozygous and 30 homozygous for *G3PDH-2* 109*.

The allele frequency indicates both the alleles to be fixed in the two populations and a comparison to the Hardy-Weinberg expectation clearly indicates a selective advantage of the heterozygotes in the Pagladia population (Table 6), while strangely it becomes reverse in the Umiam population where the homozygotes enjoy selective advantage (Table 5).

4.1.6 *Hexokinase (HK)*

Hexokinase is a major regulatory enzyme of glycolytic pathway. HK converts glucose into glucose 6-phosphate by the process of phosphorylation of glucose molecules. HK is liver specific in Arctic charr being encoded in a single locus (Andersson *et al.*, 1983). Allendorf *et al.* (1977) reported that HK is encoded in two loci in brown trout *Salmo trutta*. We have observed single banded as well as double banded phenotype in *A. hexagonolepis*. The isozymes could not be detected in eye and heart tissue. Strong activity was observed in skeletal muscle. In teleosts HK has been reported to be coded by one (Andersson *et al.*, 1983) or two loci (Allendorf *et al.*, 1977). We presume that HK is encoded in single locus in *A. hexagonolepis* with an allelic variant. The allele has been named as *HK-1* 80* according to its relative mobility. We have examined 90 individuals from the Umiam reservoir population from which 84 individuals were found to be homozygous for *HK-1* 100* and 6 individuals were found to be heterozygous *HK-1* 80/100*. The allele frequencies of *HK-1* 80* and *HK-1* 100* are 0.033 and 0.967 respectively. The Chi-square value with reference to Hardy-Weinberg expectation is 0.11. Of the 23 individuals collected from the Pagladia river population

16 were found to be homozygous for *HK-1* 100* and 7 were heterozygous *HK-1* 80/ 100*. The allele frequencies of *HK-1* 80* and *HK-1* 100* are 0.15 and 0.85 respectively. The Chi-square value is 0.761. We did not observe the homozygous for *HK-1* 80* in both the populations. The Chi-square value of both the population are insignificant to the Hardy-Weinberg model. Both the populations show almost same trends in the number of observed as well as expected number of individuals (Table 5 & 6). From the allele frequencies data, it may be suggested that the allele *HK-1* 80* perhaps originated recently and has not yet been fixed in both the populations. The absence of its homozygous state in either populations possess doubt about its surviability.

4.1.7 *Hexose 6-phosphate dehydrogenase (H6PDH)*

A second form of G6PDH found in human tissue has been designated as Hexose 6-phosphate dehydrogenase (Ohno *et al.*, 1966). H6PDH is slightly larger than the G6PDH molecule and also has broad substrate activity as compared to the specific activity of G6PDH. H6PDH oxidizes the galactose 6-phosphate and 2-deoxyglucose 6-phosphate as well as glucose 6-phsophate. H6PDH activity is restricted to echinoderm and vertebrates (Onishi and Horri, 1977; Horri *et al.*, 1977). We have observed a double banded pattern in *A. hexagonolepis*. Review of literature indicates that in teleosts H6PDH is encoded in two loci. However, the location of the two bands in the gel prompt us to infer the presence of a single polymorphic locus of H6PDH in our fish. It may be well to mention here that the appearance was same in all the tissues studied with moderate to weak activity. The allele has been designated as *H6PDH-1* 67* . In both the population the number of observed heterozygotes exceeds the Hardy-Weinberg expectation and the difference is significant in the Pagladia population indicating positive selection. We did not observe the homozygotes for the allele *H6PDH-1* 67* in either population.

4.1.8 *Lactate dehydrogenase (LDH)*

Lactate dehydrogenase isozymes have been studied extensively since Markert and Moller (1959) discovered and described the existence of isozymes. LDH catalyzes the pyruvate into lactate when Kreb's cycle is inoperative. As stated earlier, LDH is a tetrameric protein being encoded by three independent loci *i.e.*, *LDH-A*, LDH-B** and

*LDH-C**. *LDH-A** locus and *LDH-B** locus is mainly expressed in white skeletal muscle and heart muscle, however, it has been observed in other tissues also. *LDH-C** locus is expressed in a variety of tissue of Osteichthyes fishes (Whitt, 1975; Fisher *et al.*, 1980). In advanced teleosts the structure and expression of *LDH-C** locus is specialized. It is expressed in either retina or liver tissue of many species of various orders (Massaro and Markert, 1968; Callegarini and Vendamiati, 1975; Shaklee *et al.*, 1973 & 1974; Frankel, 1985). We detected three loci expression for LDH enzymes in *A. hexagonolepis*. For the sake of uniformity in nomenclature we have designated the three loci as *LDH-1* (A), LDH-2* (B)* and *LDH-3* (C)* Here, we observed the unique expression of *LDH-3** locus in all the tissues with strong activity in liver tissue. Similar observation has been made in *Carassius auratus* and *Punephalis notatus* (Markert *et al.*, 1975; Shaklee *et al.*, 1977; Leibel and Peairs, 1990) where *LDH-C* (LDH-3)* is expressed in all the tissues. However, in *A. sumatranius* (previously *Barbus sumatranius*) restricted expression of this locus has been reported (Whitt *et al.*, 1973). The relative mobility of LDH-3 banding pattern in *A. hexagonolepis* varies from tissue to tissue. On the whole, three positions/locations were noticed in the tissues examined. We presume that it is due to the expression of the allelic variant of the *LDH-3** locus. The allele was named as *LDH-3* 75* according to its relative mobility. Though five allozymes may result from the random association of the products of *LDH-3* 75* and *LDH-3* 100*, none of the tissues possessed more than one of these five. We detected 16 individuals to be homozygous for *LDH-3* 75*, 38 individuals were homozygous for *LDH-3* 100* and 52 were heterozygous from a total of 106 individuals studied from Umiam reservoir population. Among 23 individuals collected from the Pagladia river population, 5 were homozygous for *LDH-3* 75* and 4 were homozygous for *LDH-3* 100*, the rest being heterozygous. The Chi-square value of both the population (0.08 & 1.111) is insignificant with Hardy-Weinberg expectation. The allele frequencies of *LDH-3* 75* and *LDH-3* 100* is 0.40 & 0.60 for Umiam reservoir and 0.52 & 0.48 for the Pagladia river population. However, only three isozymes could be detected as the product of their association. We did not observe any allelic variant in either *LDH-1* (A)* or *LDH-2* (B)* their distribution follows a fairly constant pattern of tissue specificity. Heterotetramer formation between the products of *LDH-3** and *LDH-1*/LDH-2** has not been observed.

4.1.9 *Malate dehydrogenase (MDH)*

Malate is converted to oxaloacetate by malate dehydrogenase in the Kreb's cycle. MDH is usually present in two main form *i.e.*, supernatant MDH (sMDH) is present in the extramitochondrial cytoplasm and mitochondrial MDH (mMDH) is found in the mitochondrial matrix. Both have the same molecular weight but differ in amino acid composition, kinetic behaviour, and antigenic property. We studied only the cytoplasmic form, *i.e.* sMDH in *A. hexagonolepis*. sMDH exists as a dimer molecule and is encoded in two loci in many of fish species (Bailey *et al.*, 1970; Whitt *et al.*, 1973; Coppes *et al.*, 1987). However, it has also been reported that sMDH is coded by single locus in some of the fish species (Davidson and Cortner, 1967; Karig and Wilson, 1971). We detected either single or double banded pattern from the Umiam reservoir population. We infer from the banding pattern, sMDH is encoded in single locus with an allelic variant. The allele has been named as *sMDH-1* 75*. We examined 102 individuals from the Umiam reservoir, 15 individuals were homozygous for *sMDH-1* 75*, 39 individuals homozygous for *sMDH-1* 100* and 48 were heterozygous. The allele frequencies of *sMDH-1* 75* and *sMDH-1* 100* was 0.38 and 0.62 respectively. The Pagladia population revealed a sharp contrast being totally homozygous for *sMDH-1**. Of the 23 fish examined a uniform single banded pattern was noticed. Increasing the sample size in future study may confirm the fixation of the common allele in the population.

4.1.10 *Malic enzyme (ME)*

Malic enzyme, otherwise known as NADP Malate dehydrogenase, converts malate into pyruvate and during the reaction provides NADPH for lipogensis. ME exists in two form, one is mitochondrial (mME) and another supernatant (sME) form. The mitochondrial form is mainly expressed in muscle tissue and the supernatant form is expressed mainly in liver tissue. We observed the liver specific form *i.e.*,sME form. sME is a monomer and encoded in two loci in number of of fish species (Cross *et al.*, 1979; Stoneking *et al.*, 1979; Allendorf *et al.*, 1977; Utter *et al.*, 1980; McAndrew and Majumdar, 1983; Cross and King, 1983). We detected single banded pattern in both the populations. Similar observation has been made in *Barbus meridonalis*,

Tilapia zilli, Gadus morhua (Triantaphyllidis *et al.*, 1981; Mork *et al.*, 1982; Cruz *et al.*, 1982). Since we observed identical location in all the 111 fish (96 in Umiam and 15 in Pagladia), we are inclined to suggest the presence of a single locus for sME in *A. hexagonolepis*.

4.1.11 *Phosphoglucomutase (PGM)*

Phosphoglucomutase is a phospho transferase enzyme which catalyze glucose 6-phosphate to glucose 1-phosphate or vice versa. PGM is a monomer and encoded in a single locus in some fish (Smith *et al.*, 1981; Winans, 1980; Grant and Utter, 1980; Kruger, 1980; Utter *et al.*, 1983), while in many other fish species *e.g.*, trout, arctic charr, *Tilapia* spcies, it is coded by two loci (Utter *et al.*, 1973; Allendorf *et al.*, 1975 & 1977; May *et al.*, 1980; Allendorf *et al.*, 1982; Andersson *et al.*, 1982; Cruz *et al.*, 1982 & 1983; Basiao and Taniguchi, 1984). We suggest two gene loci *viz.*, *PGM-1** and *PGM-2** are responsible for PGM expression in our fish and the former *i.e.*, *PGM-1** is polymorphic due to the presence of *PGM-1* 100* and *PGM-1* 125* at the locus. The allele frequencies of *PGM-1* 100* and *PGM-1* 125* is 0.61 and 0.39 respectively and the phenotypes do not significantly differ from Hardy-Weinberg expectation. As stated earlier the Pagladia population could not be scored for PGM.

4.1.12 *Xanthine dehydrogenase (XDH)*

Xanthine dehydrogenase plays a major role in purine metabolism, and also in protein and amino acid catabolism. It contains molybdenum and plays an important role in conversion of purine bases to uric acid. XDH is a dimer and coded by a single locus in *Tilapia zilli* and a large number fish species (Allendorf *et al.*, 1977; Busack *et al.*, 1979; Cruz *et al.*, 1982; Andersson *et al.*, 1983, Padhi and Khuda-Bukhsh, 1990). We have observed a three banded pattern of XDH in the Umiam reservoir population. Comparison of the relative mobilities with available data forces us to presume the existence of two loci in our fish. Heterodimer formation along with the usual homodimers result in the production of three isozymes. We could not score XDH in the Pagladia river population due to technical reasons.

4.2 Population Structure

4.2.1 *Genetic Variation*

It is necessary to have some convenient quantitative measure of genetic variation for comparing the genetic constitution of the two population *viz.*, the Umiam reservoir population and the Pagladia river population. Genetic variation is usually studied in the form of multiple alleles of genes and can be quantified by using the concept of allele frequency. We selected the allozymes for calculating allele frequency which is simply the proportion of all alleles at particular locus. We observed polymorphic expression in seven loci out of a total 17 loci examined in the Umiam reservoir population and six out of the 14 loci from the Pagladia riverine population (Tables 3, 4, 5, 6 & 7). The allelic expression was visualized by difference in relative mobility of the allozymes on gel. A dimeric enzyme encoded in a bimorphic locus in heterozygous state is expected to be represented by three allozymes *e.g.*, the genotype AA' may produce allozymes AA, AA' and A'A'. Similarly a tetrameric isozyme like LDH may be resolved into atleast five allozymes due to association of the production of A and A' genes. Incase of a monomeric enzyme the genotype AA' will result in the formation of only two allozymes *viz.*, AA and A'A'. Difficulty in correctly interpreting allozymes (product of same gene locus - allelic form) and the isozymes which are product of different gene loci may be resolved by careful observation of the gel and scoring a large number of samples. The homomer isozyme products of different gene loci usually exhibit distinctly separate zone of activity. In case of an allele, we are likely to encounter some individuals with a single band which is due to the homozygous nature of the individuals concerned. In case of separate gene loci, the minimum number of bands will equal the number of gene loci. The polymorphic loci has been defined as where the frequency of the most common allele is less than or equal to 0.95.

From the table 5, 6 & 7 one can easily say that there is a large difference in allele frequency at presently examined loci. The changes in allele frequency in the population is dependant upon various factors, such as, population size, patterns of mating, geographical distribution of individuals, mutation, genetic drift, inbreeding, migration and natural selection. In the absence of any selection pressure, a population tends to

Table 3 Allele frequency and X^2 values with relation to their Hardy-Weinberg expectation in 17 isozyme loci in the chocolate mahseer *A hexagonolepis* from Umiam reservoir (Barapani), Shillong during the year 1993 - 1994

Locus	N	Distribution of phenotype			Allele frequency		X^2
*AK-1**	79	100	100/134	134	100	134	3 25
		36 (32 3)	29 (36 5)	14 (10 3)	0 647	0 353	
*ADH-1**	80	100	100/125	125	100	125	0 870
		63 (60 4)	15 (18 2)	2 (1 4)	0 881	0 119	
*G6PDH**	56				1 000		
*GDH-1**	58				1 000		
*GDH-2**	58				1 000		
*G3PDH-2**	58	100	100/109	109	100	109	6 145*
		22 (15 5)	16 (29 0)	20 (13 5)	0 517	0 483	
*HK-1**	53	80	80/100	100	80	100	0 053
		0 (0 1)	3 (3 1)	50 (49 9)	0 030	0 970	
*H6PDH-1**	69	67	67/100	100	67	100	0 512
		0 (0 4)	11 (10 2)	58 (58 4)	0 080	0 920	
*LDH-1**	72				1 000		
*LDH-2**	72				1 000		
*LDH-3**	72	75	75/100	100	75	100	0 556
		12 (10 5)	31 (33 9)	29 (27 5)	0 382	0 618	
*MDH-1**	63	75	75/100	100	75	100	0 867
		9 (7 3)	25 (28 3)	29 (27 5)	0 341	0 659	
*ME**	47				1 000		
*PGM-1**	45	100	100/125	125	100	125	0 005
		20	20	5	0 670	0 330	
*PGM-2**	45				1 000		
*XDH-1**	47				1 000		
*XDH-2**	47				1 000		

Significant level $p<0\ 05$

Table 4 Allele frequency and X^2 values with relation to their Hardy-Weinberg expectation in 17 isozyme loci in the chocolate mahseer, *A hexagonolepis* from Umiam reservoir (Barapani) Shillong during the year 1994 - 1995

Locus	N	Distribution of phenotype			Allele frequency		X^2
*AK-1**	51	100	100/134	134	100	134	0 046
		21 (21 4)	24 (23 3)	6 (6 4)	0 647	0 353	
*ADH-1**	43	100	100/125	125	100	125	0 170
		38 (38 2)	5 (4 7)	0 (0 2)	0 881	0 119	
*G6PDH**	76				1 000		
*GDH-1**	39				1 000		
*GDH-2**	39				1 000		
*G3PDH-2**	39	100	100/109	109	100	109	11 682**
		18 (14 9)	11 (18 7)	10 (6 2)	0 603	0 397	
*HK-1**	37	80	80/100	100	80	100	0 069
		0 (0 1)	3 (2 8)	34 (34 1)	0 040	0 960	
*H6PDH-1**	25	67	67/100	100	67	100	0 189
		0 (0 2)	4 (3 7)	21 (21 2)	0 080	0 920	
*LDH-1**	34				1 000		
*LDH-2**	34				1 000		
*LDH-3**	34	75	75/100	100	75	100	2 343
		4 (6 2)	21 (16 6)	9 (11 2)	0 426	0 574	
*MDH-1**	39	75	75/100	100	75	100	1 435
		6 (7 9)	23 (19 3)	10 (11 8)	0 449	0 551	
*ME**	49				1 000		
*PGM-1**	39	100	100/125	125	100	125	0 708
		10 (11 4)	22 (19 4)	7 (8 3)	0 540	0 460	
*PGM-2**	39				1 000		
*XDH-1**	49				1 000		
*XDH-2**	49				1 000		

Significant level $p<0\ 01$

Table 5 Allele frequency and X^2 values with relation to their Hardy-Weinberg expectation in 17 isozyme loci in the chocolate mahseer, *A hexagonolepis* from Umiam reservoir (Barapani) Shillong during the period of 1993 - 1995

Locus	N	Distribution of phenotype			Allele frequency		X^2
*AK-1**	130	100	100/134	134	100	134	1 635
		57 (16 7)	53 (59 8)	20 (53 6)	0 642	0 358	
*ADH-1**	123	100	100/125	125	100	125	0 719
		101 (100 1)	20 (21 8)	2 (1 2)	0 902	0 098	
*G6PDH**	132				1 000		
*GDH-1**	97				1 000		
*GDH-2**	97				1 000		
*G3PDH-2**	97	100	100/109	109	100	109	18 427***
		40 (29 6)	27 (47 9)	30 (19 5)	0 552	0 448	
*HK-1**	90	80	80/100	100	80	100	0 110
		0 (0 1)	6 (5 7)	84 (84 2)	0 033	0 967	
*H6PDH-1**	94	67	67/100	100	67	100	0 701
		0 (0 6)	15 (13 8)	79 (79 6)	0 080	0 920	
*LDH-1**	106				1 000		
*LDH-2**	106				1 000		
*LDH-3**	106	75	75/100	100	75	100	0 080
		16 (17 0)	52 (50 9)	38 (38 2)	0 400	0 600	
*MDH-1**	102	75	75/100	100	75	100	0 006
		15 (14 7)	48 (48 1)	39 (39 2)	0 380	0 620	
*ME**	96				1 000		
*PGM-1**	84	100	100/125	125	100	125	0 202
		30 (31 3)	42 (40 0)	12 (12 8)	0 610	0 390	
*PGM-2**	84				1 000		
*XDH-1**	96				1 000		
*XDH-2**	96				1 000		

Significant level $p<0.001$

Table 6 Allele frequency and X^2 values with relation to their Hardy-Weinberg expectation in 14 isozyme loci in the chocolate mahseer *A hexagonolepis* from Pagladia river, Subankhata, Assam during the month of March 1993, 1994 and May 1995

Locus	N	Distribution of phenotype			Allele frequency		X^2
*AK-1**	22	100	100/134	134	100	134	15 420***
		2 (6 7)	20 (10 9)	0 (4 5)	0 550	0 450	
*ADH-1**	22	100	100/125	125	100	125	10 540**
		4 (7 7)	18 (10 6)	0 (3 7)	0 590	0 410	
*G6PDH**	23				1 000		
*GDH-1**	18				1 000		
*GDH-2**	18				1 000		
*G3PDH-1**	23				1 000		
*G3PDH-2**	23	100	100/109	109	100	109	11 443**
		4 (8 0)	19 (11 1)	0 (3 9)	0 590	0 410	
*HK-1**	23	80	80/100	100	80	100	0 761
		0 (0 5)	7 (5 9)	16 (16 6)	0 150	0 850	
*H6PDH-1**	23	67	67/100	100	67	100	9 540**
		0 (3 5)	18 (10 9)	5 (8 6)	0 390	0 610	
*LDH-1**	23				1 000		
*LDH-2**	23				1 000		
*LDH-3**	23	75	75/100	100	75	100	1 111
		5 (6 2)	14 (11 5)	4 (5 3)	0 520	0 480	
*MDH**	23				1 000		
*ME**	15				1 000		

Significant level **p<0 01, ***p<0 001

Table 7. Allele frequencies of polymorphic loci in two population of chocolate mahseer, *A. hexagonolepis*.

Locus	Allele	Frequency	
		Umiam reservoir (N = 90 - 130)	Pagladia river (N = 15 - 23)
*AK-1**	100	0.642	0.550
	134	0.358	0.450
*ADH-1**	100	0.902	0.590
	125	0.098	0.410
*G3PDH-2**	100	0.552	0.590
	109	0.448	0.410
*HK-1**	80	0.033	0.150
	100	0.967	0.850
*H6PDH-1**	67	0.080	0.390
	100	0.920	0.610
*LDH-3**	75	0.400	0.520
	100	0.600	0.480
*MDH-1**	75	0.380	0.000
	100	0.620	1.000
*PGM-1**	100	0.610	-
	125	0.390	-

attain genetic equilibrium according to Hardy-Weinberg's theorem. Based on Hardy-Weinberg's principle we calculated the expected numbers of the different genotypes for a comparison to the actually observed data (Table 5 & 6). A standard statistical test *i.e.*, Chi-square test was applied to assess the closeness of fit to the model. The Chi-square value of each polymorphic locus in the Umiam reservoir population and the Pagladia river population are given the tables 3, 4, 5 & 6. The variations from the expected value in the Umiam reservoir population were insignificant for all the loci except the *G3PDH-2** (X^2 = 18.427; $p<0.001$). In the Pagladia river population, the variation of allele frequency of locus *AK-1**, *ADH-1**, *G3PDH-2**, and *H6PDH-1** are significant ($p<0.01$ - $p<0.001$) and the allele frequency for *HK-1** and *LDH-3** were statistically insignificant.

A wide difference in allele frequencies was observed between the Umiam reservoir and the Pagladia river population, as well as among the different year class of the Umiam reservoir population which has been presented in table 8. Riverine and reservoir samples differed most strikingly at *ADH-1* 125* and *H6PDH-1* 67* loci. These alleles were present in the Pagladia population at a frequency of 0.41 and 0.39 respectively. But in the Umiam reservoir population the respective frequencies were remarkably low (Table 8). The goodness of fit (G) was 5.3 and 4.72 for the two loci (with $p<0.05$) between the riverine and the reservoir samples. The *MDH-1* 75* allele was completely absent from the riverine population. Statistically significant difference between riverine and reservoir samples exists at *AK-1* 134* and *G3PDH-2* 109* loci with $p<0.01$. Summing up the G-value over all the polymorphic loci, the heterozygosity between the reservoir samples and reverine population is found to be highly significant (Table 8; Fig. 26).

A comparison between the two year classes indicates a clearcut heterozygosity in seven variable loci. At *G3PDH-2** the frequency of the allele 109 was reduced in 1994-1995 to the corresponding value of 1993-1994 (G = 8.99; $p<0.01$). On the contrary, the frequency of *HK-1* 80, LDH-3* 75* and *MDH-1* 75* alleles increased during the same periods. (Table 8 & Fig. 27).

Table 8. Heterogeneity between the Pagladia river and Umiam reservoir sample (d.f. = 1) and between reservoir sample(d.f. = 1) of chocolate mahseer, *A. hexagonolepis*.

Sample	Allele frequency						
	AK-1 134*	*ADH-1* 125*	*HK-1* 80*	*H6PDH-1* 67*	*LDH-3* 75*	*MDH-1* 75*	*G3PDH-2* 109*
Umiam reservoir							
1993 - 1994	0.353	0.119	0.030	0.080	0.382	0.341	0.483
1994 -1995	0.353	0.119	0.040	0.080	0.426	0.449	0.397
Combined	0.358	0.098	0.033	0.080	0.400	0.380	0.448
Pagladia river	0.450	0.410	0.150	0.390	0.520	0.000	0.410
Heterogeneity:							
Reverine Vs Reservoir stock	8.37**	5.32*	0.23	4.72*	0.56	0.00	14.74***
Between reservoir sample (1993-1994 and 1994-1995)	1.56	0.58	0.01	0.08	1.42	1.09	8.99**

Significant level: *p<0.05, **p<0.01, ***p<0.001

Both measures of genetic variation, *i.e.*, average heterozygosity and proportion of polymorphic loci, indicate an evident reduction from reverine to reservoir samples. The pooled reservoir samples have a 'H' value of 13.7% compared to 28.3% in the riverine samples. A high of 53% reduction in heterozygosity (Table 9) is noticed in the combined periods.

Apparently the variation in the number of observed homozygous and heterozygous individuals is the main cause for changing the allele frequency in the populations. Heterozygosity varies from locus to locus. Each locus has an optimum level of heterozygosity determined by its metabolic function and the substrate availability for the enzymes encoded. However, it has to be remembered that mutation is the major factor determining the level of heterozygosity at locus level. If there is a variation in the mutation rate, the correlation of heterozygosity may become larger than the genetic identity.

We presume that uneven distribution of individuals in the Umiam reservoir may lead to some sort of clumping or colony formation among the individuals. The clumping or colony formation of the species is mainly caused by the environmental factors, *i.e*, area of favourable habitat intermixed with unfavourable areas in the Umiam reservoir. These are the local interbreeding population. The observed high number of homozygous individuals in the Umiam reservoir population may be due to the inbreeding among the subpopulations. There may be another factor involving the reduction of heterozygosity. The unequal number of male and female in the population will reduce the effective population size, and can result in a loss of heterozygosity.

The low level of genetic variation in the Umiam reservoir population can also be explained by the process of genetic drift associated with a bottle neck effect, and selection in the subpopulation. Usually genetic drift enhances the divergence in the subpopulation, whereas migration between the population hinders its. It may be mentioned that the Umiam area experiences severe drop in temperature during the winter months and thus may facilitate bottle neck condition. Therefore, in our species, genetic variation produced by mutation is organized, maintained, eliminated or dispersed among subpopulation to the complex balances between natural selection, migration and random genetic drift.

Table 9.Genetic variability in chocolate mahseer,*A. hexagonolepis*

Sample	Polymorphic loci (%)	Heterozygosity		
		H. obs.	H. exp.	H^{δ}
Umiam reservoir				
1993 - 1994	41.2	12.7	14.9	55.1
1994 - 1995	41.2	15.4	14.8	45.6
Combined	41.2	13.7	13.3	51.6
Pagladia river	42.9	28.3	17.9	0.0

H.ob s - Oered heterozygosity; H.exp - Expected heterozygosity;

H^{δ} – Reduction of heterozygosity in percentage.

For discussing the causes for gene diversity, the common hypotheses point to local adaptation or genetic drift. The ecological variance can be tolerated by a multiplicity of the enzyme function. In the Umiam reservoir population *G3PDH-2* 109* alleles reduction in the 1994-1995 year samples, the increase in the *HK-1* 80, LDH-3* 75,* and *MDH-1* 75* from 1993-1994 to 1994-1995 may be indication of such variance. A selective advantage of a particular allele and subsequently natural selection acting upon the genotype changes the allele frequency. The presence of *MDH-1* 75* allele in the reservoir population, suggest that the allele may offer a selected advantage for the fish in this environmental conditions. But it raises the question to what level the biochemical and physiological mechanism are responsible for the selective advantage of the polymorphic loci. A detailed study is necessary to determine the enzyme catalytic efficiency relative with environmental factors, *i.e.*, effect of p^H, Km, activation enthalpies and thermal inactivation etc.

A selection experiment should preferably be started from a population showing performance for the character of interest, *e.g.* growth rate. It must be emphasized. however, that a population that is characterized by a fast growth rate does not necessarily harbour a high level of genetic variation for that character. Therefore, while searching among different natural populations for the one best suitable for future directional breeding for growth rate, one with good performance in the field and high genetic variance for growth rate should be selected. It is usually rather easy to identify different size groups but it is difficult to predict their growth rate in various field conditions. One possible way to circumvent this obstacle would be to assume that there is a correlation between average of genetic variation at the loci affecting the phenotype characters to be selected for.

We tried to correlate between mean heterozygosity of six polymorphic loci and the variation in body weight. The correlation coefficient 0.0497 is insignificant and the total variation is 0.24% (Table 10; Fig. 28). It is a very meagre value, and does not indicate a clear effect of any individual locus on weight (growth). However, heterozygosity at the *H6PDH-1** and *MDH-1** loci show a slight trend in positive direction. We, therefore, do subscribe to the view that heterozygosity of electrophoretically detectable allozymes

Table 10. Observed mean heterozygosity in six loci with mean weight in chocolate mahseer, *A. hexagonolepis* collected from Umiam reservoir during the year 1993 - 1995.

Locus	Mean observed heterozygosity	Mean weight (g)
*AK-1**	53	94
*ADH-1**	20	84
*H6PDH-1**	15	73
*LDH-3**	52	69
*MDH-1**	48	73
*PGM-2**	42	70

themselves affect growth/body weight and this may be substantiated by examining a larger number of gene loci. In our opinion enzyme heterozygosity, which obviously results due to over all genetic heterozygosity, may be conveniently linked to heterosis and variation in growth/body weight. It has to be remembered that ultimately this will depend more on developmental stability and overall viability in a particular ecological niche.

A number of recent studies has suggested that fish have a divesity of mechanisms which allow them to persist under fluctuating environmental conditions. As ectotherms, fish maintain body temperature very close to ambient environmental temperature (Block, 1991). Any change in the external environmental temperature directly affects their internal temperature. The isozymes play an important role in the thermal adaptation of fish. Changes in allele frequency at certain polymorphic loci and overall increase of heterozygosity may be due to temperature fluctuation (Hochachka and Somero, 1971 & 1973; Wilson *et al.*, 1973; Shaklee *et al.*, 1977; Andersson *et al.*, 1980).

We have found that there is no significant correlation between the minimum and maximum of water surface temperature with the heterozygosity at the polymorphic loci (Table 11 & 12; Fig. 29b & c). However, allele frequency of the polymorphic loci when correlated with the overall mean temperature recorded for the year 1993-1995 period is found to be highly significant $r = 0.635$, ($p<0.001$) (Table 13; Fig. 29a). It is not very likely that the few loci examined in this study are directly linked to a water surface temperature. The more plausible explanation seem to be that the loci studied reflect overall genetic heterozygosity at different water surface temperature.

4.2.2 *Allozyme differentiation between the population*

Chromosome formulas are known for a relatively large number of both new world and old world cyprinid fishes. While allozyme differentiation among cyprinid can be quite substantial, karyotypic differentiation has been minimal, with 2n values ranging from 44 to 52 in diploid form. The old world cyprinid exhibit a wider range of 2n values (96 - 100) in a significant number of species and it is only among the native old world cyprinids that tetraploid form are known. We have estimated the genetic similarity and genetic distance between the two populations of *A. hexagonolepis*.

Table 11. Mean observed heterozygosity at Minimum (16 - 17° C) water surface temperature in the Umiam reservoir population.

Locus	Mean observed heterozygosity
*AK-1**	2.00
*ADH-1**	1.00
*H6PDH-1**	2.00
*LDH-3**	2.67
*MDH-1**	1.67
*PGM-1**	1.50

Table 12. Mean observed heterozygosiy at Maximum (26 - 27° C) water surface temperature in the Umiam reservoir population.

Locus	Mean observed heterozygosity
*AK-1**	1.88
*ADH-1**	1.44
*H6PDH-1**	1.29
*LDH-3**	2.50
*MDH-1**	2.13
*PGM-1**	2.55

Table 13. Observed mean heterozygosity at mean water surface temperatur (23-24°C) in the Umiam reservoir population.

Locus	Mean observed heterozygosity
*AK-1**	1.82
*ADH-1**	1.42
*H6PDH-1**	1.38
*LDH-3**	2.03
*MDH-1**	1.97
*PGM-1**	2.08

Phenetic relationships between the Umiam reservoir and the Pagladia river population of chocolate mahseer, *A. hexagonolepis* are expressed through comparison of Nei's (1975) indices of genetic similarity (I) and genetic distance (D) and the coefficient of I = 0.918 and D = 0.121 between conspecific population are presented in the table 14. It is evident that though the two population share a common gene pool a gradual building up of distance at genetic level is already in progress.

4.3 Polyploidization

The duplication of the entire genome through polyploidization has been promoted as a major evolutionary events in many different organism (Ohno *et al.*, 1968; Ohno, 1970). The demonstration of tetraploid karyotypes and additional duplication of enzyme loci in certain fishes provided much of the early evidence in support of the polyploid hypothesis (Ohno *et al.*, 1967; Wolf *et al*, 1969; Engel *et al.*, 1971; Schmidtke *et al.*. 1976). Reports of electrophoretic data in support of cyprinid tetraploidy are listed in table 15. While the evidence in support of cyprinid tetraploidy is substantial, relatively few tetraploid cyprinids have been identified. Approximately only ten exhibit a tetraploid chromosome set and only five have been electrophoretically examined to demonstrate duplicate gene expression (Table 15). The taxonomic diversity of these ten species suggests that tetraploidy originated independently in several old world cyprinid lineages. A large number of species closely relative to these ten await karyotypic and electrophoretic investigation, so the number of cyprinid tetraploids known may increase with additional studies.

We detected the twelve enzymes coded by 18 loci in *A. hexagonolepis* from which the GDH, G3PDH, PGM and XDH isozymes are each coded by two loci (Table 16) and the rest of the enzymes is coded by single locus. According to Ferris and Whitt (1976b) 52% duplication exists among thirty five enzyme loci in *C. carpio*. Based on our study of twelve enzyme we have found about 29% duplication in the 18 loci studied in our fish. In this context it may be pertinent to draw attention to frequent occurrence of gene silencing resulting in functional rediploidization of duplicated genes (Buth 1977a,b & 1979; Ferris and Whitt 1977a,b). Moreover, cyprinids as a group do not express tetraploid expression to the degree that some other cypriniform groups do *e.g.*, catostomids (Ferris,

Table 14. Comparison of average genetic variability between Umiam reservoir and Pagladia river population of chocolate mahseer, *A. hexagonolepis*.

	Umiam reservoir	Pagladia river
No. of loci examined	17	14
No. of polymorphic loci	7	6
Proportion of polymorphic loci	0.412	0.429
No. of alleles per locus	1.412	1.429
Heterozygosity:		
Observed (H^o)	0.140	0.303
Expected (H^e)	0.154	0.192
H^o/ H^e	0.909	1.578
Genetic Identity (I)	0.918	-
Genetic distance (D)	-	0.121

1984; Buth, 1984). Retained duplicates through course of time may acquire new function (Ohno, 1970). Enough time has to be transpired since the polyploidization for duplicate genes to substantially diverge in their structure. Otherwise only a few amino acid substitution differences accumulate between the homologous isozymes and the probability of detecting them electrophoretically becomes low (Ferris and Whitt, 1977b)

The data in the table 16 suggest that approximately one-third of the gene loci in *A. hexagonolepis* express both loci and only one locus is expressed in the rest. The present study, therefore, has provided ample evidence that considerable gene duplication exists in some of the enzyme loci of *A. hexagonolepis*. It is tempting therefore, to suggest that *A. hexagonolepis* originated by tetraploidy as a result gene duplication could be detected only in a few enzyme loci due to rediploidization at many loci. It has been claimed that the *LDH-C** (*LDH-3**) gene has evolved by gene duplication in teleosts and in advanced teleosts the expression of *LDH-C** gene is very much specialized. The activity is seen either in liver or in eye and nerve tissue. It may be recalled that we have noticed the expression of this locus in all the six tissues studied. We have also detected allelic variant of this locus in both the population. This perhaps reflect a comparative primitive status of our fish among the cypriniformes. However, examination of a much higher number of enzyme loci is necessary to arrive at a definite conclusion.

Table 15. Karyotypic and Electrophoretic Evidence in support of tetraploidy in cyprinid fishes.

Species	Tetraploidy karyotype	Duplicate gene expression
Acrosscheilus hexagonolepis	Present investigation	Present investigation
A. sumatranus	Suzuki and Taki (1981)	___
Barbus barbus	Wolf *et al.* (1969)	Engel *et al.* (1971)
B. meridionalis	Cataudella *et al.* (1977)	Triantaphyllidis *et al.* (1981)
B. plebeius	Park (1974)	___
Carassius auratus	Ojima and Hitotsumachi (1967)	___
Cyprinus carpio	"	Ferris and Whitt (1977b)
C. carpio var *comminis*	Khuda-Bukhsh and Barat (1987)	___
Tor putitora	Khuda-Bukhsh (1980)	___
T. mosal mahanadicus	Khuda-Bukhsh *et al.* (1986)	___

Table 16. Comparison of gene loci in four species of Cyprinids.

Species	Enzyme locus														
	*AK-A**	*ADH**	*GDH**	*G3PDH**	*G6PDH**	*HK**	*H6PDH**	*LDH-A**	*LDH-B**	*LDH-C**	*MDH-A**	*MDH-B**	*ME**	*PGM**	*XDH**
Acrossocheilus hexagonolepis	1	1	2	2	1	1	1	1	1	1	1	-	1	2	2
Barbus barbus	-	-	-	-	-	-	-	2	2	2	1	1	-	-	-
B. meridionalis	-	-	-	-	-	-	-	2	2	2	1	1	2?	-	-
Cyprinus carpio	2	1	-	2	-	-	-	1	2	2	1	1	-	1	1

CONCLUDING REMARKS

CONCLUDING REMARKS

Cytogenetics and genetics have now acquired an important status in fishery science, which is evident from the increasing number of research papers suggesting the utility of application of cytogenetic and genetic principles in improving the fish breeding programme, for documenting genetic diversity in natural population and developing proper management programme to conserve genetic diversity. This is the basic premise upon which our study has been made.

The whole project has been accomplished in two section. In section I, cytogenetic study of *A. hexagonolepis* provides data indicating a diploid-tetraploid relationship among the cyprinids. In section II, the genetic background of *A. haxagonolepis* has been determined from isoenzymatic investigation.

As far as the cytogenetic study is concerned, we have reported for the first time the diploid chromosome number and the karyotype of *A. hexagonolepis* and have also shown that *A. hexagonolepis* evolved from the ancestral group containing 48 chromosome number by the process of polyploidization event. The karyotype (2n = 96; 7m + 15sm + 9st + 17t) was subsequently attained through Robertsonian alteration coupled with pericentric inversion. We would like to extend the work with the measurement of DNA content of the cells and analysis of the chromosome morphology by banding techniques.

In section II, the study of genetic background of *A. hexagonolepis* has been accomplished in four phases. In phase I, six tissues of *A. hexagonolepis* from two population have been electrophoretically surveyed providing data on 18 gene loci and their distribution in the two natural populations. In phase II, the genetic variation has been studied with emphasis on the tissues and the enzymes. In phase III, the correlation between the allele frequency of polymorphic loci and body weight, and ecological parameter *i.e.*, temperature has been studied with emphasis on the mean observed heterozygosity from the phase II. In phase IV, the genetic similarity/distance has been studied by comparison of allozymes between the populations.

We selected two well contrasted natural populations for our study. Our investigation clearly shows the adverse effect of human interference on genetic diversity. The Umiam reservoir population possesses a lower level of heterozygosity among the 17 gene loci studied. The stretch of the Pagladia river from where collection has been made is still free from human interference and embodies a population with higher level of genetic heterozygosity. Inclusion of a larger number of loci is expected to substantiate this inference.

Chocolate mahseer has lost much of its natural habitat due to rampant destruction of its ecological niche and overfishing as a result its number is dwindling in many of its natural populations. It has to be remembered that it is one of the prized, endogenous game fish of North-Eastern India and can be sustained in reservoirs. A time has come to seriously consider the conservation and propagation of this great fish lest it completely disappears like many other fish species.

A well planned effort may be made to stock the reservoirs in this part of India with *A. hexagonolepis*, and this should not be done in a random careless way. Heterozygous populations will obviously be the ideal candidate for such venture and genetic studies on enzymic loci would provide the easiest but dependable tool for such work. We suggest a detailed survey to identify the natural populations of this fish should be undertaken and the genetic constitution of these population be studied. Attempt should be made to link some of the gene loci to various environmental factors and coupled with mating experiments suitable stocks can be produced and then released in the reservoir.

Looking at the overall qualities at this fish *viz.*, its large size, fast growth rate, sustainability in both reservoir and riverine population and its sporting nature, such a programme deserves top priority. The present study, in its own humble way, may serve as a stepping stone for such a project.

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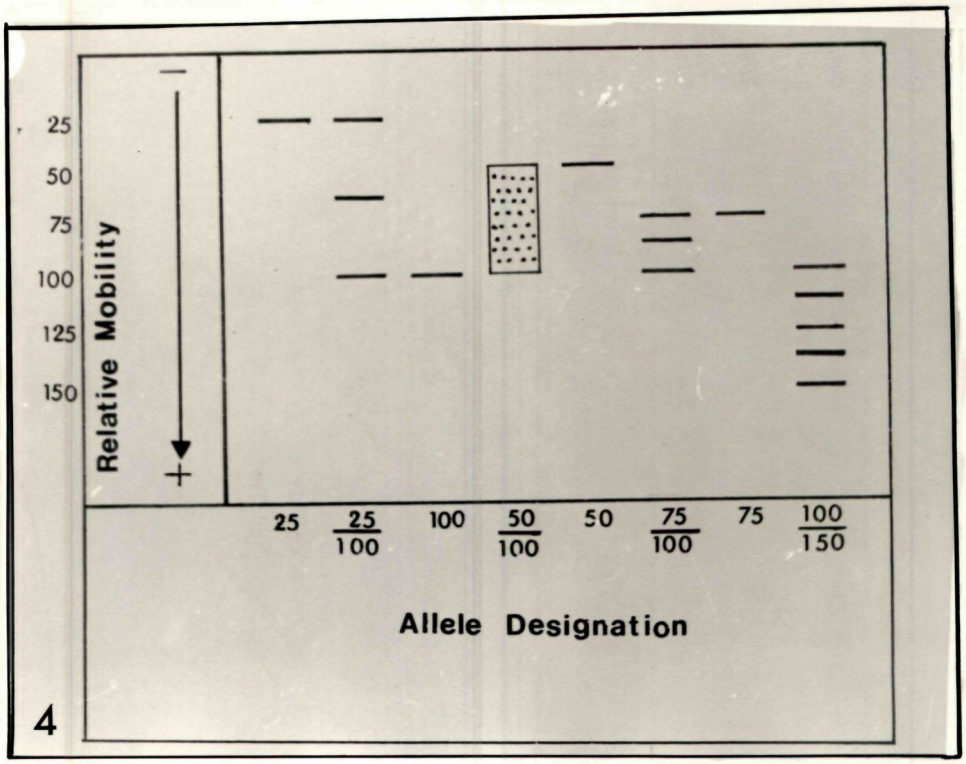
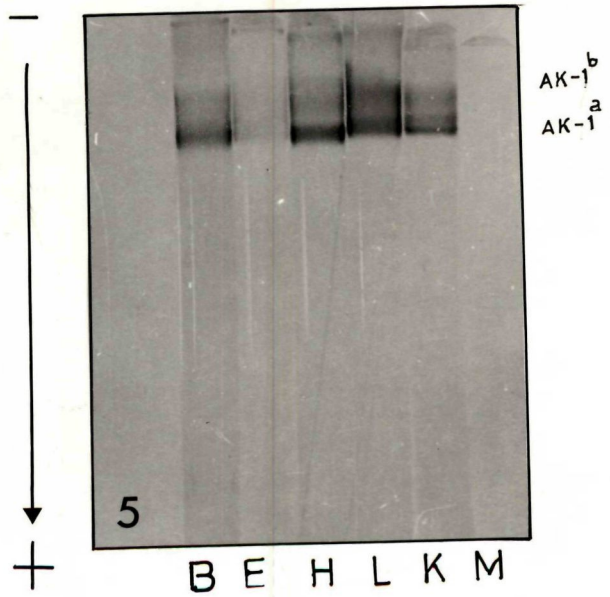
Fig. 4. Allele designation of allozymes

Fig. 5 & 6. AK and ADH isozymes pattern in *A. hexagonolepis*


25
50
75
100
125
150
Relative Mobility
−
+
25
25/100
100
50/100
50
75/100
75
100/150
Allele Designation
4


−
+
AK-1b
AK-1a
5
B E H L K M

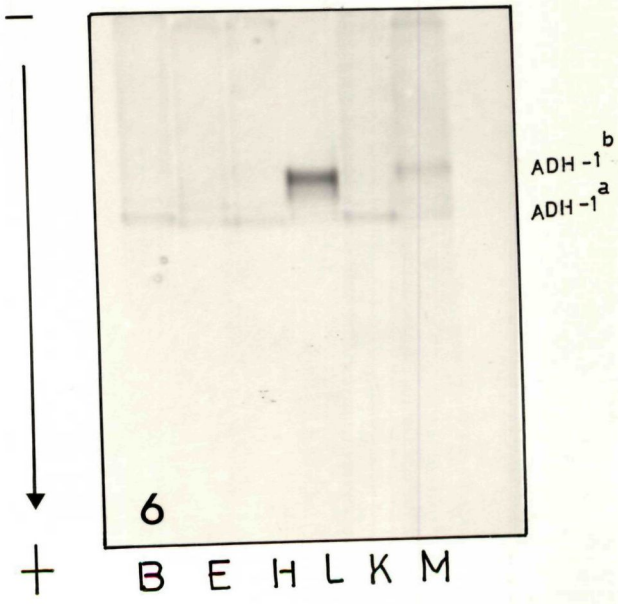
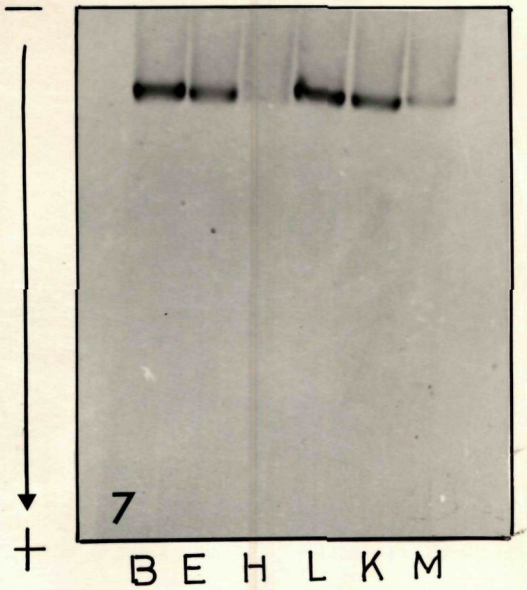
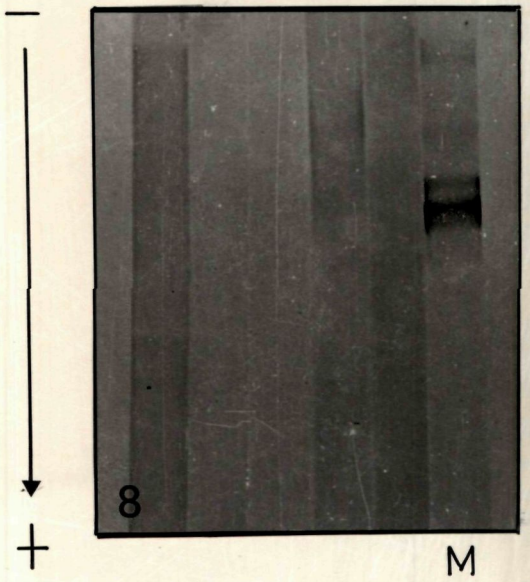
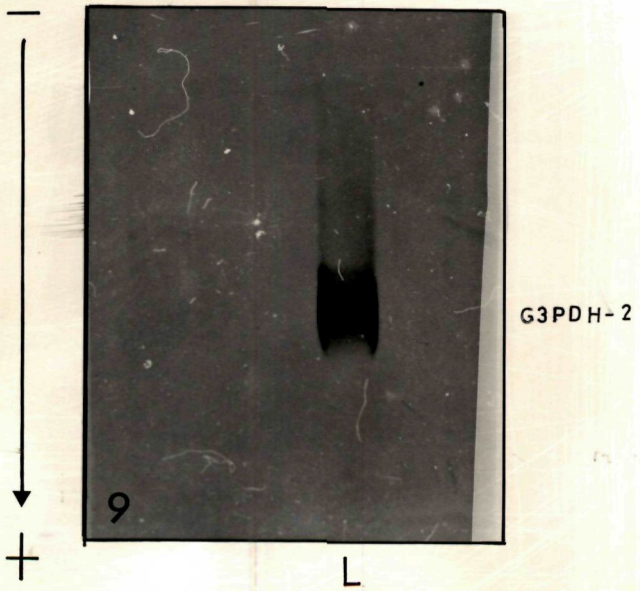

−
+
ADH-1b
ADH-1a
6
B E H L K M

Fig. 7, 8, 9 & 10 G6PDH, GDH and G3PDH isozymes pattern in *A. hexagonolepis*


7
B E H L K M


GDH-1
GDH-2
8
M


G3PDH-2
9
L

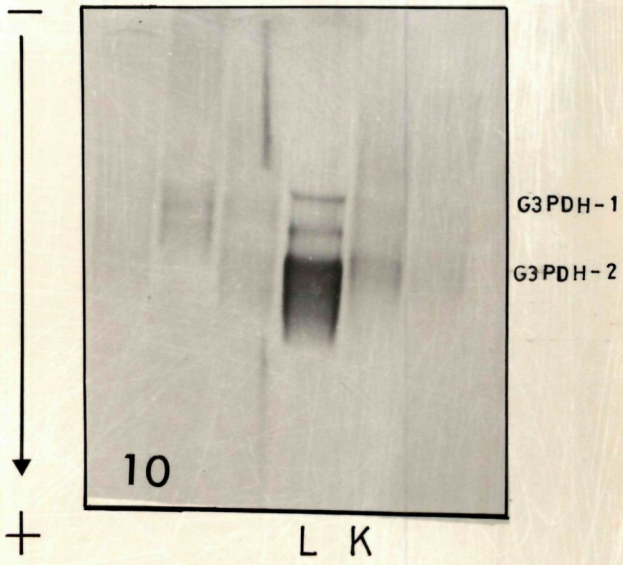
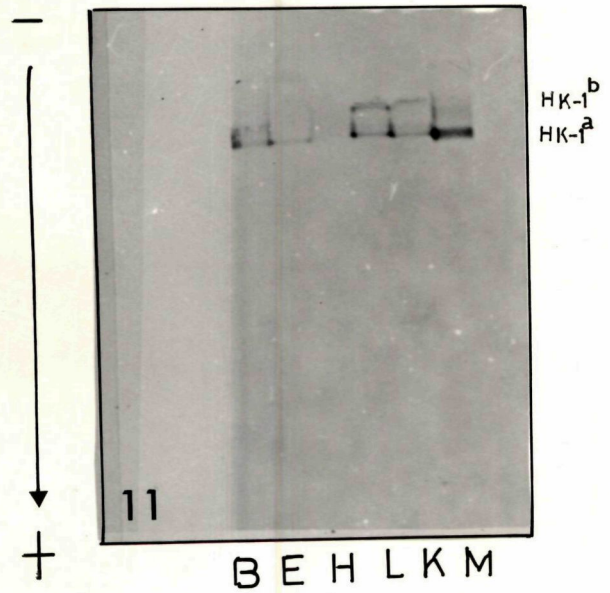
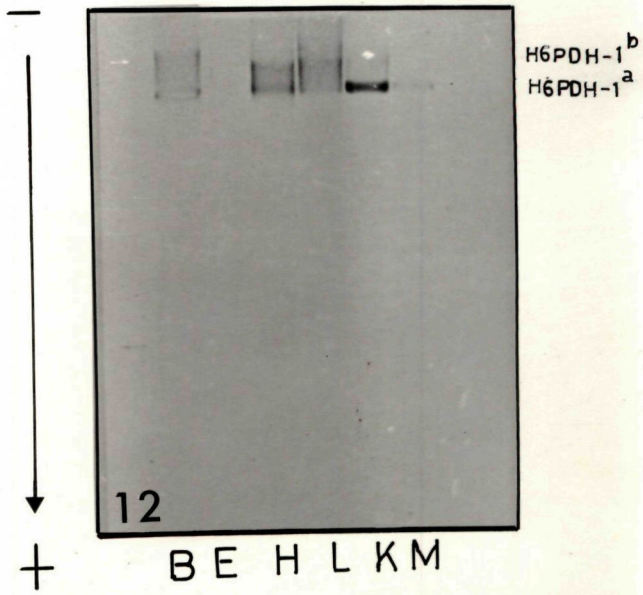
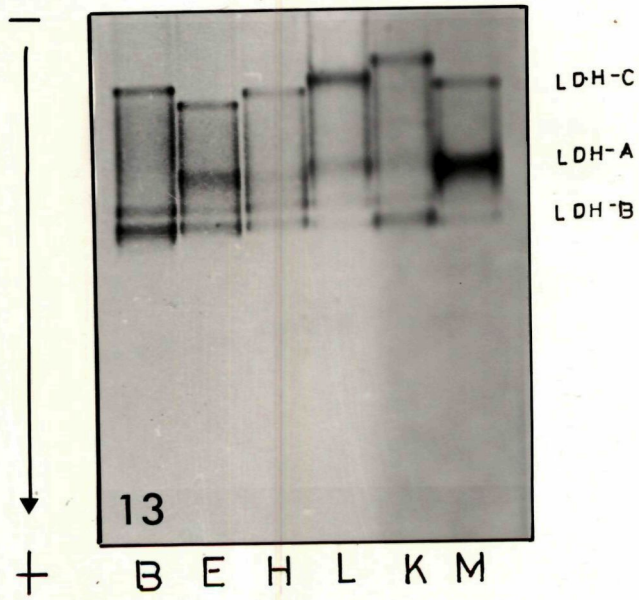

G3PDH-1
G3PDH-2
10
L K

Fig. 11, 12, 13 & 14. HK, H6PDH, LDH and MDH isozymes pattern in *A. hexagonolepis*


HK-1b
HK-1a
11
B E H L K M


H6PDH-1b
H6PDH-1a
12
B E H L K M


LDH-C
LDH-A
LDH-B
13
B E H L K M

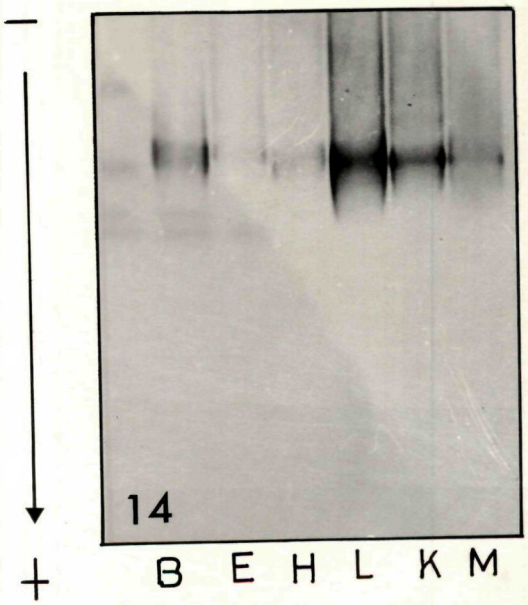
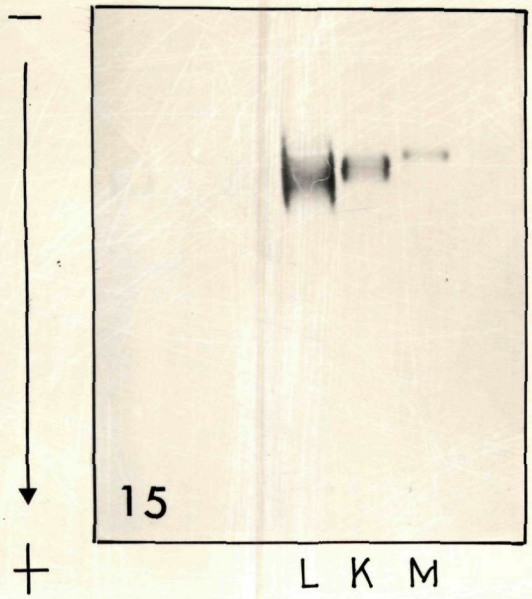
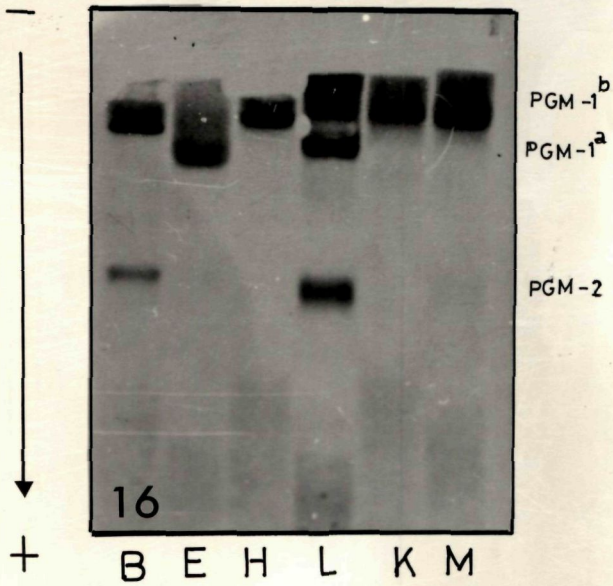

14
B E H L K M

Fig. 15, 16 & 17. ME, PGM and XDH isozymes pattern in *A. hexagonolepis*


15
L K M


PGM-1b
PGM-1a
PGM-2
16
B E H L K M

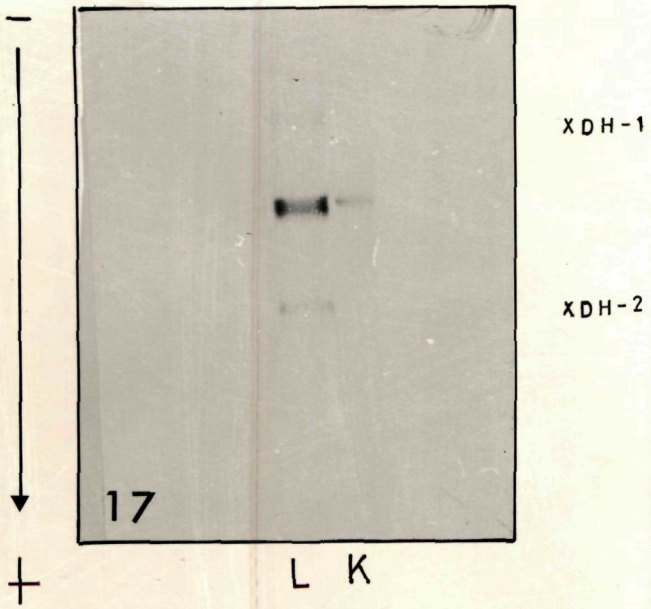

XDH-1
XDH-2
17
L K

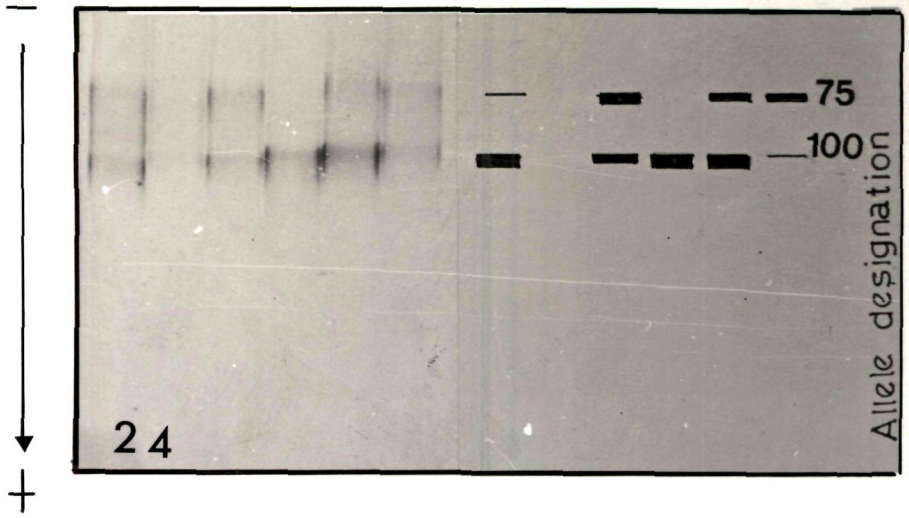
Fig. 18, 19 & 20. Polymorphism in AK, ADH and G3PDH isozymes in *A. hexagonolepis.*

18
100
134
Allele designation
19
100
125
Allele designation
20
100
109
Allele designation

Fig. 21, 22 & 23. Polymorphism in HK, H6PDH and LDH isozymes in *A. hexagonolepis.*

21
80
100
Allele designation
22
67
100
Allele designation
23
75
100
Allele designation

Fig.24 & 25. Polymorphism in MDH and PGM isozymes in *A. hexagonolepis.*


−
+
75
100
Allele designation
24

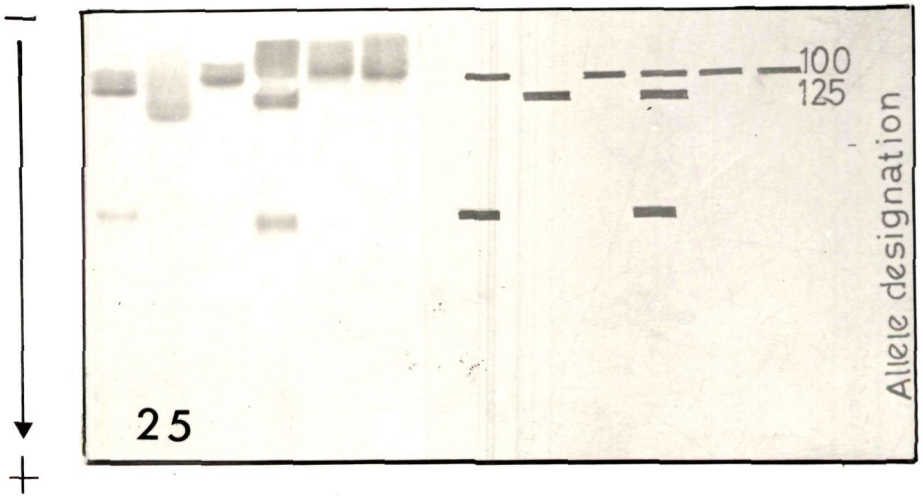

−
+
100
125
Allele designation
25

Fig. 26. Comparison between the allele frequency of polymorphic loci of *A.hexagonolepis* collected from Umiam reservoir and the Pagladia river population

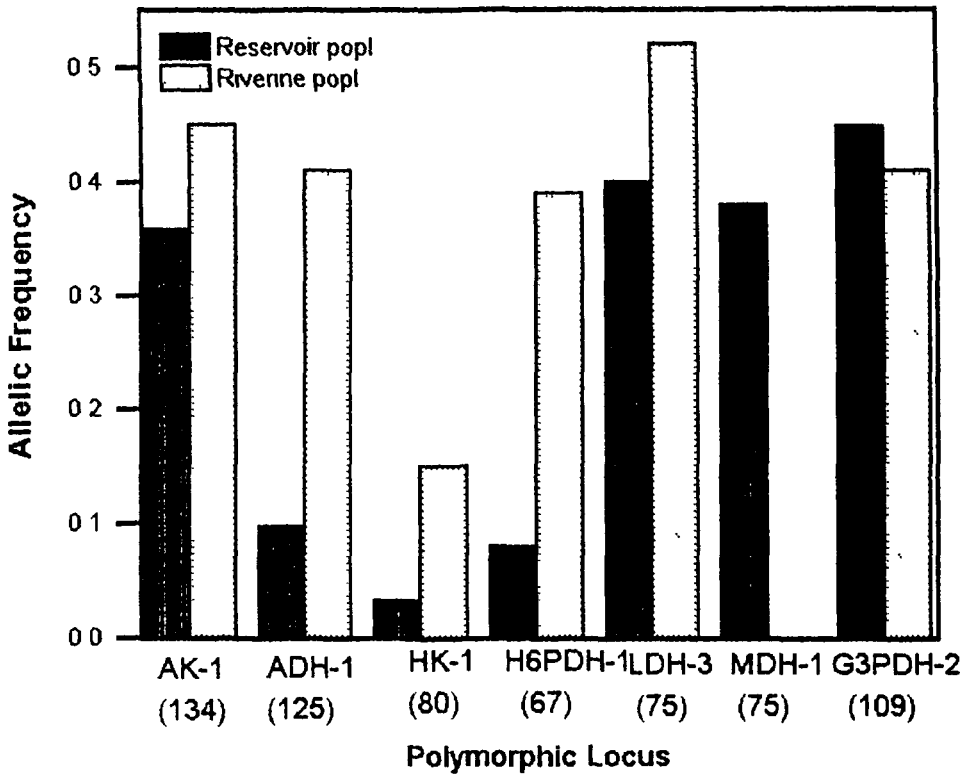

Reservoir popl
Riverine popl
0 5
0 4
0 3
0 2
0 1
0 0
Allelic Frequency
AK-1
(134)
ADH-1
(125)
HK-1
(80)
H6PDH-1
(67)
LDH-3
(75)
MDH-1
(75)
G3PDH-2
(109)
Polymorphic Locus

Fig. 26

Fig. 27. Comparison of allele frequency of polymorphic loci of *A.hexagonolepis* collected from Umiam reservoir

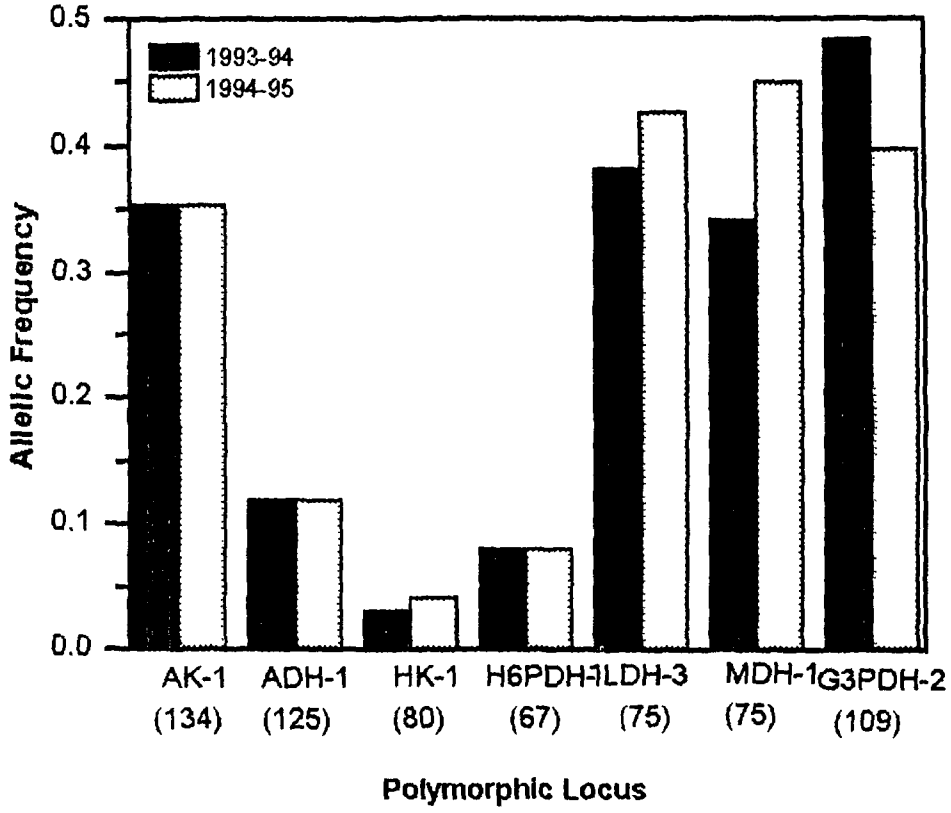

0.5
0.4
0.3
0.2
0.1
0.0
1993-94
1994-95
Allelic Frequency
AK-1
(134)
ADH-1
(125)
HK-1
(80)
H6PDH-1
(67)
LDH-3
(75)
MDH-1
(75)
G3PDH-2
(109)
Polymorphic Locus

Fig. 27

Fig. 28. Relationship between mean observed heterozygosity and mean weight of chocolate mahseer, *A. hexagonolepis* from Umiam reservoir

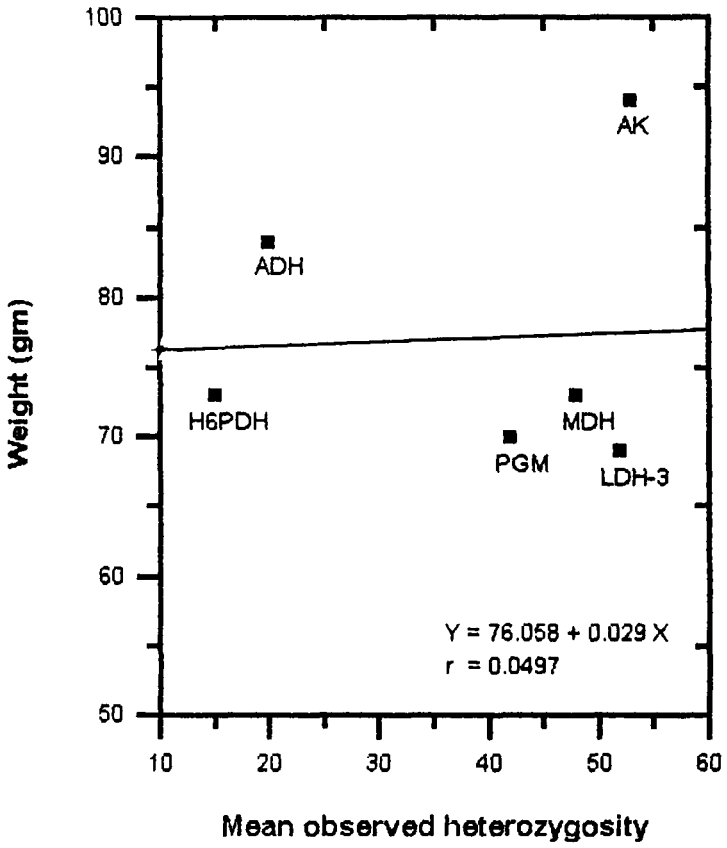

100
90
80
70
60
50
10
20
30
40
50
60
Weight (gm)
Mean observed heterozygosity
AK
ADH
H6PDH
MDH
PGM
LDH-3
Y = 76.058 + 0.029 X
r = 0.0497

Fig. 28

Fig. 29 a, b & c. Relationship between mean observed heterozygosity and water surface temperature from Umiam reservoir

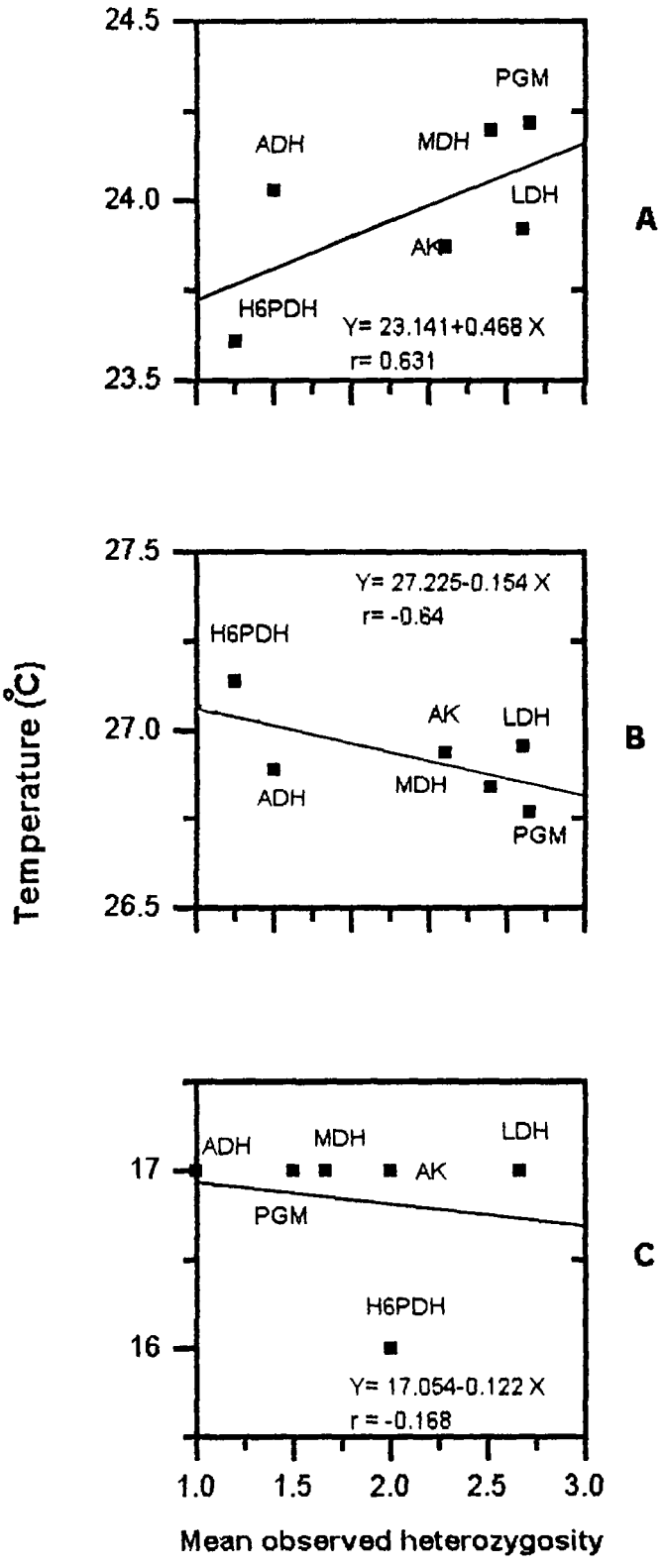

24.5
24.0
23.5
PGM
ADH
MDH
LDH
AK
H6PDH
Y= 23.141+0.468 X
r= 0.631
A
27.5
27.0
26.5
Y= 27.225-0.154 X
r= -0.64
H6PDH
AK
LDH
ADH
MDH
PGM
B
Temperature (°C)
17
16
ADH
MDH
LDH
AK
PGM
H6PDH
Y= 17.054-0.122 X
r = -0.168
C
1.0
1.5
2.0
2.5
3.0
Mean observed heterozygosity

Fig. 29

APPENDIX

Perspectives in Cytology and Genetics
(Eds. G.K. Manna and S.C. Roy) 8 (1995) 691-696

CHROMOSOMES OF A HILL-STREAM FISH, *ACROSSOCHEILUS HEXAGONOLEPIS*

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ABSTRACT

Acrossocheilus hexagonolepis, a hill - stream fish, is abundantly found in north-east India. The present report incorporates an account of the chromosome number and karyotype of this species. Chromosomes were prepared following colchicinization, hypotonic treatment, fixation, flame drying technique from kidney and gill epithelium cells of both sexes. The diploid chromosome number, as determined from 100 well-spread metaphase plates, is 96 in both the sexes. The karyotype is composed of 7 pairs of metacentric, 15 pairs of submetacentric, 9 pairs of subtelocentric and 17 pairs of telocentric chromosomes.

INTRODUCTION

Mahseers are important game fishes of India, they grow to a cosiderably large size and are generally found in mountainous streams and rivers. The chocolate mahseer belongs to the genus *Acrossocheilus*. The mahseers are essentially fish of the rocks, rapids and hills but they are also adapted to life in reservoirs (Dubey, 1986) and the chocolate mahseer has been reported to be suitable for culture in ponds (Jhingran, 1975). Review of literature reveals almost a total lacuna regarding the environmental genetic information about *Acrossocheilus*.

The present communication reports the karyotype and chromosome morphology of the chocolate mahseer *Acrossocheilus* (=*Neolissocheilus*) *hexagonolepis*.

MATERIALS AND METHODS

The specimens were mostly collected from Umiam reservoir (Barapani) about 15 Kms from Shillong, Meghalaya (25 34 N, 91"56' E). Specimens were also collected form the Pagaldia river near the Assam-Bhutan border. Fishes were brought to the laboratory alive and were injected with 0.02% of colchicine at the rate of 1ml per 100 gms. body weight. Chromosome preparations were obtained following the technique describe

by Chatterjee *et al* (1981). The diploid number of chromosomes has been determined by studying a minimum number of 100 well spread metaphase from both the sexes and the chromosomes classified according to Levan *et al.*, (1964).

RESULTS AND DISCUSSION

Cytogenetic studies in fish have been largely confined to ascertaining the chromosome number of a particular species and longlists of chromosome number have been published (Gyldenholm and Scheel, 1971; Prasad and Manna, 1971; Scheel, 1973; Ojima *et al.*, 1976; Manna and Khuda-Bukhsh, 1977; Gold *et al.*, 1979, 1980; Kirpichnikov, 1981; Sola *et al.*, 1981; Manna, 1984,1989; Ojima, 1985).

The diploid chromosome number as determined from a large number of metaphase nuclei seems to be 96±2 (Table 1). The table 2 shows chromosome measurement as well as chromosome classification. Karyotype analysis of A. *hexagonolepis* demonstrates the existence of four types of chromosome i.e. 7 pairs of metacentric,15 pairs of submetacentric, 9 pairs of subtelocentric and 17 pairs of telocentric chromosomes (Fig. 1) . The total genome length was found to be 219.48 µm approximately (Fig. 2), the individual length ranging from 8.67±0.03 µm to 2.17±0.02 µm.

We have not observed morphologically differentiated sex chromosomes.This is in accordance with the current concept that except a few species, morphologically distinguishable sex chromosomes are absent in fishes.

Table 1. Summary of karyotype analysis of *Acrossocheilus hexagonolepis* (2n=96)

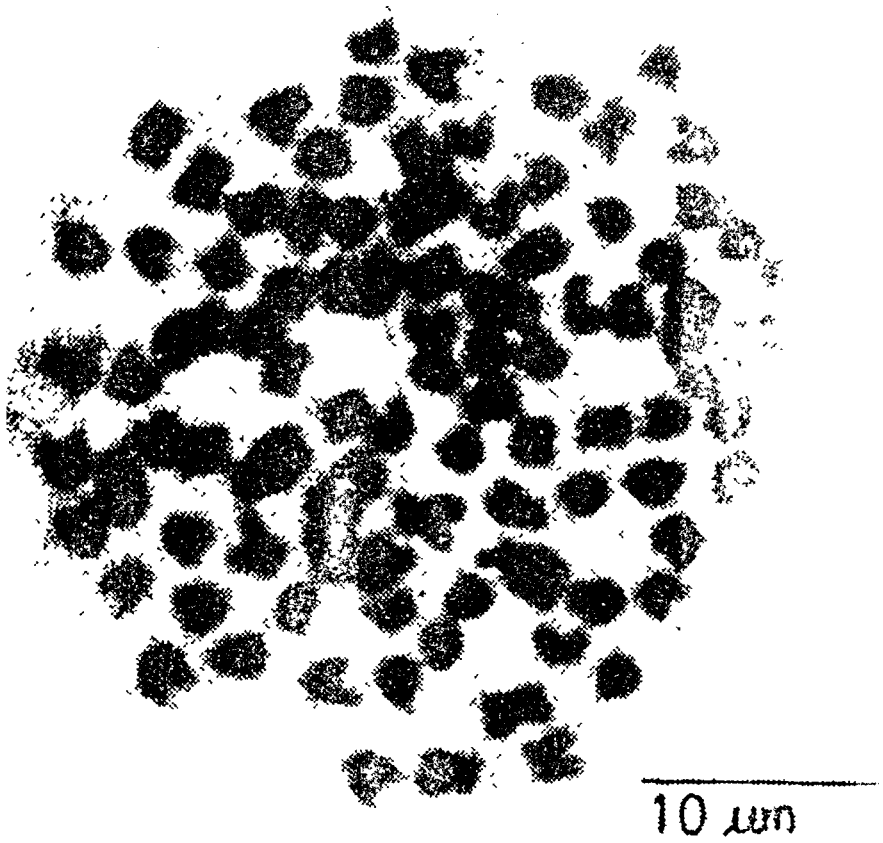
Total no. of specimens	Tissue	No. of cells scored	Frequency of Chromosome counts				
			92	94	96	98	100
10	Kidney	75	0	3	65	6	1
	Gill	25	2	2	17	4	0

ACKNOWLEDGEMENTS

The study was supported by financial grant by the Ministry of Environment and Forests, Government of India to Prof. K. Chatterjee. The authors are indebted to Head, Department of Zoology, NEHU for providing laboratory facilities.

Table 2. Morphometric data of the chromosomes of *A. hexagonolepis*.

Chromosome number	Absolute length (μm)	Relative length (% of TCL)	Centromeric index	Chromosome designation
1.	8.67±0.03	3.95±0.02	23.08±0.29	st
2.	7.33±0.04	3.34±0.02	18.18±0.21	st
3.	6.17±0.03	2.81±0.02	38.89±0.24	sm
4.	6.00±0.04	2.73±0.02	31.58±0.23	sm
5.	5.90±0.02	2.69±0.03	25.81±0.37	st
6.	5.83±0.02	2.66±0.03	38.89±0.34	sm
7.	5.67±0.04	2.58±0.04	26.67±0.21	st
8.	5.57±0.03	2.54±0.03	50.00±0.41	m
9.	5.50±0.03	2.51±0.02	28.57±0.28	st
10.	5.40±0.04	2.46±0.03		t
11.	5.33±0.02	2.43±0.03	44.00±0.41	m
12.	5.27±0.04	2.40±0.03		t
13.	5.17±0.03	2.36±0.02	33.33±0.27	sm
14.	5.13±0.03	2.34±0.02	38.46±0.24	sm
15.	5.07±0.02	2.31±0.03		t
16.	5.03±0.03	2.29±0.03		t
17.	4.93±0.05	2.25±0.03	45.45±0.41	m
18.	4.83±0.04	2.20±0.03	30.43±0.27	sm
19.	4.77±0.03	2.17±0.02	41.67±0.47	m
20.	4.73±0.07	2.16±0.01		t
21.	4.63±0.05	2.11±0.03	38.46±0.29	sm
22.	4.57±0.03	2.08±0.04	33.33±0.19	sm
23.	4.50±0.04	2.05±0.03	25.00±0.20	st
24.	4.43±0.04	2.02±0.03		t
25.	4.33±0.04	1.97±0.02	20.00±0.20	st
26.	4.30±0.03	1.96±0.02	33.33±0.27	sm
27.	4.23±0.03	1.93±0.03	30.77±0.24	sm
28.	4.20±0.04	1.91±0.02		t
29.	4.10±0.05	1.87±0.04		t
30.	4.04±0.07	1.85±0.04	30.77±0.21	sm
31.	4.03±0.08	1.84±0.03	30.00±0.28	sm
32.	4.00±0.05	1.82±0.04		t
33.	3.93±0.03	1.79±0.03	31.82±0.32	sm
34.	3.87±0.02	1.76±0.02		t
35.	3.83±0.04	1.75±0.02		t
36.	3.80±0.04	1.73±0.04	41.67±0.31	m
37.	3.77±0.04	1.72±0.04		t
38.	3.73±0.04	1.70±0.02	16.00±0.17	st
39.	3.67±0.04	1.67±0.03		t
40.	3.63±0.04	1.65±0.02		t
41.	3.53±0.03	1.60±0.02	42.11±0.29	m
42.	3.47±0.02	1.58±0.02	33.33±0.27	sm
43.	3.43±0.02	1.56±0.02		t
44.	3.40±0.01	1.55±0.02	45.83±0.40	m
45.	3.33±0.02	1.52±0.01	27.78±0.27	st
46.	3.23±0.03	1.47±0.02		t
47.	3.00±0.03	1.37±0.03	30.00±0.41	sm
48.	2.17±0.02	0.99±0.03		t



Legend of Figures.

Fig 1. Metaphase plate of *Acrossocheilus hexagonolepis*

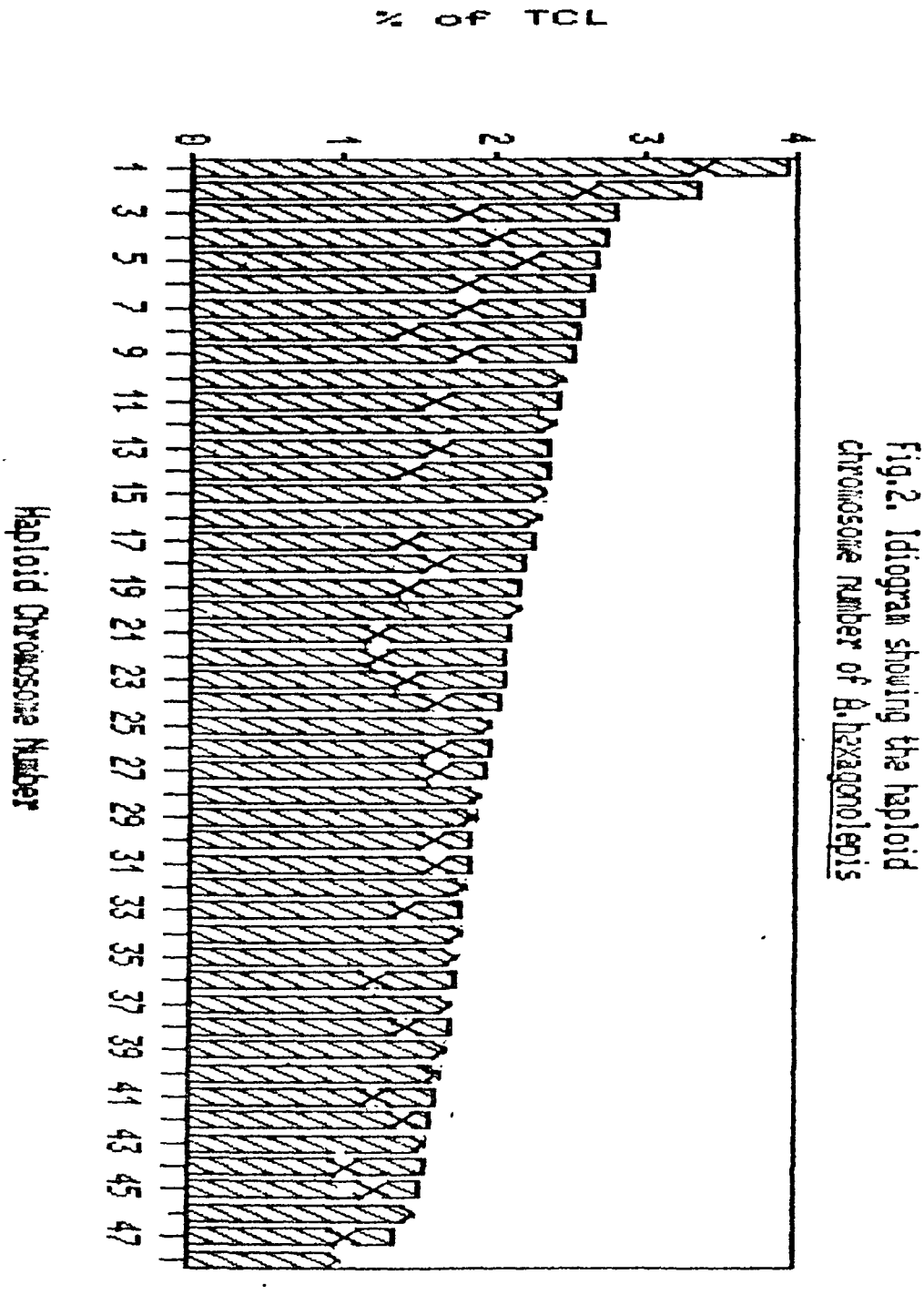


Fig.2. Idiogram showing the haploid chromosome number of A.hexagonolepis

Legend of Figure :

Fig . 2. Idiogram showing the haploid chromosome number of A. *hexagonolepis*. The bar represents 10 μm.

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BIO-DATA

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B. Sc.,	743992	Apr. 1989	First 74.3	Vivekananda College, M.K. Varsity
M. Sc.,	5210	Apr. 1991	First 72.3	"
M. Phil.,	91Z282	Apr. 1992	First 66.3	A.N.J.A. College, M.K. Varsity

9. Specialization in M. Sc.,	:	Bio-Technology
10. GATE Score	:	61.72

11. Research Experiences : 5 years 6 months

M. Sc., project work title : Acute oral toxicity of D-Alletherin on Drossophila melanogaster

M.Phil., Dissertation title : TobaccoCaterpillar, Spodoptera litura larval mortality: Operative effects of Bacillus thuringiensis kurstaki

12. List of Publications

a. Arunachalam, A and Palanichamy, M. (1993): Social Forestry, Biology Education, September Issue. UGC - New Delhi.

b. Palanichamy, M., Paramanandham, K and Alfred Mohandoss, (1995): Behavioural response of Spodoptera litura on foliage treated with Bacillus thuringiensis kurstaki. Geobios, 22: 53 - 54

c. Palanichamy, M and Arunachalam, A. (1995): Temperature depends feeding rate and the larval mortality ofSpodoptera litura treated with Bacillus thuringiensis kurstaki. New Agriculturist (Press)

d. Palanichamy, M., Bose, S. and Chatterjee, K. (1995): Chromosomes of a hill stream fish, Acrossocheilus hexagonolepis. In: Perspectives in Cytology and Genetics (Eds. Manna, G.K. and Roy, S.C.). Vol. 8. pp. 691 - 696

13. Seminar/Symposium/Conference/Workshop

i. Seminar on Recent Development in Biotechnology held at Vivekananda College, Tiruvedagam, 20th October 1990.

ii. National Symposium on Reproductive Biology and Comperative Endocrinology held at Department of Zoology, Sri Venkateswara University, Tirupathi. 29 - 30th January 1993.

iii. VIII All India Congress of Cytology and Genetics held at Department of Zoology, Berhampur University, Orissa. 15 - 18th October 1993.

iv. Regional Workshop on Electron Microscopy held at Regional Sophiscated Instrument Centre Shillong. 28 - 30th June 1994.

v. 2nd Arnold/O. Beckman European Conference on Toxicology held at Cannes, France. 1 - 2 June 1995. Abstract has been accepted.

14. Prizes/Awards

(a) G.K. Competition 2nd prizes in College level

(b) B Sc., Class 2nd Rank.

(c) M. Sc., Class 3rd Rank.

(d) N. S. S. Merit Certificate.

15. Extracurricular Activities

a. Certificate in Gandhian Thought

b. Diploma in Photography

c. NCC "C" Certificate

d. NSS, participated All India Camp at Port Blair, Andaman-Nicobar Island.