

Biochemical genetics of some Indian fishes

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ABSTRACT

Studies on biochemical genetics of fishes, using electrophoretic methods, are relatively of recent origin. Earlier serum and eye lens protein were used to identify marine populations. This technique showed that closely related species have similarities in their protein make up, whereas these taxonomically apart showed striking differences. Thus, the usefulness of employing this method was clearly demonstrated in fish taxonomy. The study of genetic structure of fish populations through the analysis of enzyme polymorphism proved very promising. The advantage of isozyme technique was that genetic interpretations could be made directly from the raw data using simple genetic models.

Using these techniques, genetic variations within Indian mackerel, the oil sardine, the grey mullets, the sciaenids and the pomfrets were shown. The samples of these fishes were obtained from different locations of the marine environment. In addition to the genetic variation, genetic divergence among the species was also studied. The levels of genetic differentiation among the populations varied greatly. Probably these reflect the magnitude of gene flow among the populations. The differences recall the disparity in the spawning behavior and modes of larval dispersal among the species. It is concluded that the methodology of biochemical genetics can be applied to many areas of fisheries research with considerable success.

The model of the structure of the DNA molecule proposed by Watson and Crick (1953) led to an understanding of the direct relationship between the genes and the proteins. The starch-gel electrophoresis (Smithies 1955) enhanced by the application of histochemical staining methods (Hunter and Market 1957), added simplicity and sensitivity to the study of protein variation. These events led to an explosion of information on the protein variation and its significance. This started in the 1960s. Studies on genetic variations in diverse arrays of biologically oriented disciplines (Utter 1991). Many fishes in India and elsewhere were studied electrophoretically (deLigny 1969, Menezes and Qasim 1984a).

In this paper an attempt has been made to evaluate the present status of the biochemical genetic research in fishes. At the same time, we have given the importance of this field of study and also indicated what we regard as the most fruitful areas for future research.

BACKGROUND OF SEROLOGICAL AND BIOCHEMICAL STUDIES

Our research programme had its roots in the 1970s when it was decided to establish a new methodology to identify marine fish populations using serological and biochemical methods. The problem was approached using serum and eye lens proteins. In all these studies, electrophoresis was conducted on cellogel strips of 2.5 cm x 14 cm size (Menezes 1979). The distinctness of the patterns in different species became quite apparent, for the related species exhibited similarities. Eye lens protein variants were detected in the flying-fish *Gypsilurus speculiger* (Cuv. and Val.) which could be

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differentiated externally by the length of its pectoral fin and the depth of the body (Menezes 1984).

The soluble eye lens proteins of 10 fishes, viz. *Sardinella longiceps*, *S. fimbriata* (Clupeidae), *Hemirhamphus georgii* (Hemirhamphidae), *Lactarius lactarius* (Lactariidae), *Rastrelliger kanagurta* (Scombridae), *Parastromateus niger*, *Pampus argenteus* (Stromateidae), *Psettodes erumei* (Psettodidae), *Pseudorhombus arsius* (Bothidae) and *Brachirus orientalis* (Soleidae), were studied using immunoelectrophoresis. The lens antiserum of *Sardinella longiceps* was produced in a rabbit (Menezes 1980). Clear patterns of the precipitate were obtained with each of the antigen used. The immunoelectrophoretic patterns clearly showed a decrease in the number of shared components as the phylogenetic distance increased. Fishes more closely related on a morphological basis tended to have common antigens.

To determine whether any alteration occurs in the normal make-up of the electrophoretic patterns of the blood serum, haemoglobin and the eye lens proteins of the fishes on their exposure to adverse environmental pollutants, a study was conducted under controlled conditions. Long-term and short-term effects of low and high concentrations of mercury, on a euryhaline fish (*Tilapia mossambica*) were investigated (Menezes 1981). Inorganic mercury in the form of mercuric chloride (HgCl_2) was introduced as a toxicant. Collateral studies included the uptake of metal by the fishes. To select the threshold concentrations of mercury for the study, some preliminary tests were conducted on the acute toxicity and growth efficiencies of the fish in relation to some levels of the toxicant (Menezes and Qasim 1983, 1984b). The potential value of electrophoresis in this study was based on the hypothesis that stress conditions may cause significant changes in the

proteins of the blood serum and also in the haemoglobin and in the eye lens protein. Such changes might reflect an altered antibody synthesis or protein biosynthesis or cellular leakage or perhaps other events resulting directly or indirectly from the stress.

The electrophoretic patterns of the serum obtained after exposure for 48 and 72 hr to sublethal concentrations of mercury (0.4, 0.6 and 0.8 ppm) were similar to the one obtained in the low concentration of mercury (0.04 ppm) when the exposure was for 11 weeks. These features were regarded as the stress pattern of the serum of *T. mossambica* for the mercury (Menezes and Qasim 1984c).

During this exposure period, the electrophoretic pattern of haemoglobin showed an increase in the faster moving band whereas the eye lens proteins did not show much change in the components.

These studies proved that while serum proteins may have some general usefulness in fish taxonomy, the eye lens proteins were preferable for precise differentiation of the species. Further, the serum proteins could be used with success as an additional tool to evaluate environmental stress on the animals.

The genetic structure of fish populations was studied also through the analysis of enzyme polymorphism using starch-gel electrophoresis (Menezes and Taniguchi 1988, Menezes *et al.* 1990a). This method was an advancement over previous methods used for the identification of genetic variation within and between the fish species.

Advantages of using the isozyme technology

The major advantage of the information obtained on biochemical genetics using the isozyme technique is that valid genetic interpretations can be made directly from the raw data. Codominant expression of most variant alleles occurs on the gels. This would mean that, in an individual containing different

alleles of a given locus, each of the alleles is expressed as a single, distinct protein. Such a situation commonly permits the designation of the genotypes of the individual samples based on the staining patterns on the gels. The frequency with which a given gene occurs in a population of individuals can directly be determined and the distribution of phenotypes can be tested for the deviations from the expected values based on simple genetic models, an important one being the Hardy-Winberg model. This model states that in a randomly mating population, in the absence of a variety of disturbing forces (e.g. selection and mutation), the expected distribution of genotypes is determined by the random combination of alleles. In alleles A and B the expected genotypic frequency, therefore, is

$$q^2 (AA) : 2q(1-q) (AB) : (1-q)^2 (BB)$$

where q is the portion of A allele in the population. Inferences can also be made directly from the raw data regarding the subunit structure of proteins based on the number of bands observed for a particular system in the heterozygous individuals.

The number and relative abundance of alleles in a population is a measure of genetic variation, sometimes termed heterozygosity. Genetic variation is a measure of the ability of populations to adapt to environmental change or stress, and thereby survive. It follows that the loss of genetic variation for whatever reason (e.g. prolonged selection, inbreeding and isolation) will result in a decrease in the potential adaptability of a population. In some species of fish, the individuals possessing the maximum genetic variation showed better survival rates or higher relative growth rates. Furthermore, the heterozygous individuals appear to be relatively more resistant to environmental perturbations during development. Clearly, genetically variable populations have many advantageous charac-

teristics that are absent in genetically impoverished species (FAO/UNEP 1981).

Species differentiation among the fishes is fairly prominent, but the degree or its magnitude varies in different forms. Until recently, the populational structure of the species was being studied predominantly using quantitative morphological traits, such as the number of vertebrae, the fin rays, and the gill rakers or the various other exterior indices (Menezes 1979). The earlier conclusions, however, were disputable and unconvincing in many cases. The genetic basis for the variation of these characters is complex, as these are probably under the influence of numerous environmental factors. Nevertheless, the use of the techniques of biochemical genetics has led to new and fruitful results in fish population studies. With this method, a large number of individuals can be analysed for many protein loci, and allele frequencies for these loci can be estimated reliably. Cumulative comparisons among the loci between the taxonomic groups were summarized using a variety of methods. These indicate the indices of similarity or conversely the genetic distance between them. A number of algorithms (Menezes 1990a) can be employed to convert the genetic data into the estimates of phylogenetic tree.

A total of 13 species (the Indian mackerel, the oil sardine, 4 grey mullets, 4 sciaenids and 3 pomfrets), were studied using the isozyme technology.

MATERIALS AND METHODS

Specimens of the Indian mackerel *Rastrelliger kanagurta* (Cuv.) were collected from the fish landing centres of Panaji and Colva in Goa (west coast) during October-November 1988, and from Ennur and San Thome in Madras (east coast) during June 1989. Some specimens were also collected

from Port Blair, Andaman, during the 41st cruise of ORV *Sagar Kanya* in April-May 1988.

Specimens of oil sardine, *Sardinella longiceps* (Val) were obtained from the fish landing centres at Panaji (Goa) in December 1988, October 1990 and January 1991, and from Malpe and Mangalore (Karnataka) during January 1989.

Specimens of grey mullet were collected by gill nets from a natural population (estuarine) at Caranzalem, Goa in September and December 1990 and March 1991, and from the Goa State Fisheries farm (brackish water) in August 1990. In the natural population, *Mugil cephalus* (Linn.) and *Liza subviridis* (Val.) specimens were obtained in all the 3 months of collections while *Valamugil cunnesius* (Val.) and *Liza macrolepis* (Smith) specimens were obtained in the sample collected in December 1990.

The sciaenid specimens were collected by trawling off Calangute, Goa. *Johnieops dussumieri* (Cuv) samples were collected from May 1989 to January 1990, *Kathala axillaris* (Cuv) from October to December 1989, *Pennahia macrophthalmus* (Blecker) during May 1989 and *Otolithes ruber* (Sch) during January 1990.

The pomfret species, *Parastromateus niger* (Bloch), *Pampus argenteus* (Euphrasen) and *P. chinensis* (Euphrasen) were collected from the Dona Paula Jetty, Goa, during October 1989 and November 1991.

All the samples were immediately frozen and kept at -20°C prior to analyses. Skeletal muscle, liver, heart and eye were dissected out from the individual specimens. The cell lysate obtained by freezing and thawing was directly subjected to electrophoresis for phenotypic analysis. The procedure followed for the starch-gel electrophoresis was that of Menezes and Taniguchi (1988). Detection of isozymes and the nomenclature of locus

designation were made according to Shackle *et al.* (1990). Citric acid-aminopropylmorpholine (C-APM) buffer of pH 6.0 was used (Clayton and Tretiak 1972).

Gene-protein variation was studied by the straight forward procedure of electrophoresis (Menezes 1992). Filter-paper strips (4 mm x 10 mm) were soaked in the cell-lysate obtained by freezing and thawing of the concerned tissue and were inserted into a starch block, which was then subjected to an electric current. The migration of proteins in the starch matrix depend upon their size and net charge. Histochemical assays revealed the location of specific proteins as coloured band patterns of zymograms. Zymogram phenotypes are easily equated with genotypes. On a particular zymogram, the single bands of identical mobility in all the individuals are assumed to be the products of a single, non-varying locus. When variation exists among the individuals, the zymograms generally conform to those expected under the simple models of single-locus Mendelian inheritance with codominant expression. This means that the homozygotes show single-banded phenotypes while the heterozygotes appear as double-banded (Fig. 1a), triple-banded (Fig. 1b), or quintuple-banded (Fig. 1c) phenotypes depending on whether the active enzyme is composed of 1, 2 and 3 subunits respectively.

The examined enzymes, their tissue specificity and the identified loci are given in Table 1. A locus was considered polymorphic if the frequency of the most common allele was less than or equal to 0.99. The genetic basis of protein variants was inferred from the following observed banding patterns: (1) based on the consistency with known subunit structures of homologous proteins shown by other fishes, and (2) whenever the same locus was expressed in 2 or more tissues, the banding patterns of the variants were consistent among the tissues.

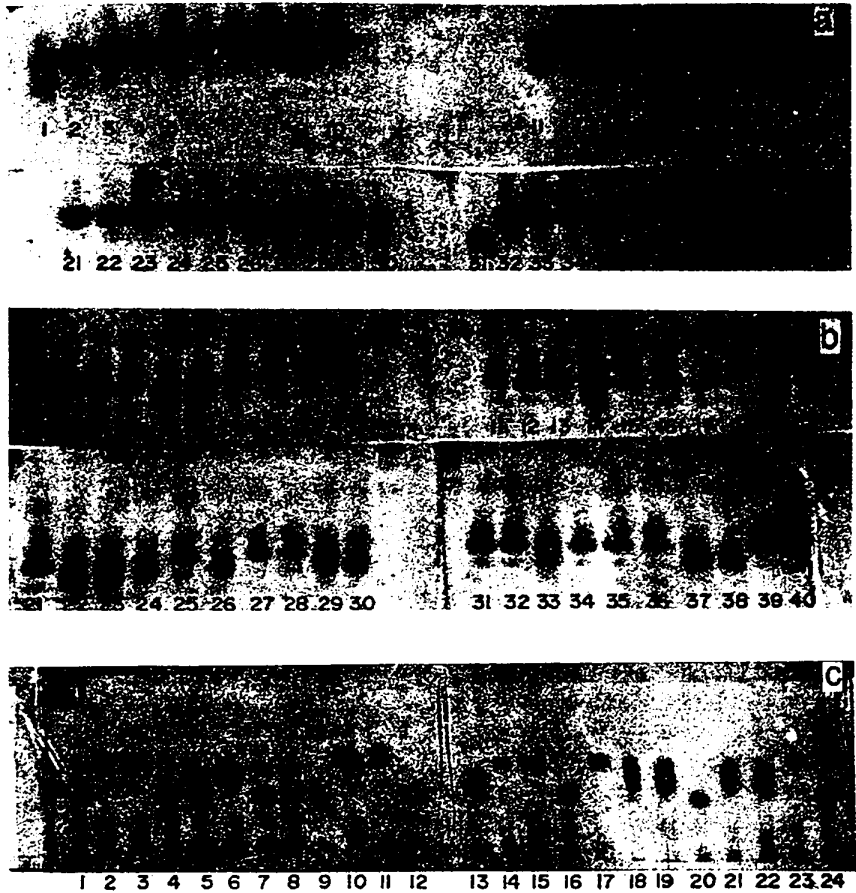


Fig. 1 Results of electrophoresis of the enzymes (a) Phosphoglucomutase in *Parastromateus niger*; (b) glycerophosphate dehydrogenase in *P. niger*; (c) malic enzyme in *Mugil* sp. The single-banded homozygotes and double-, triple- and quadruplebanded heterozygotes are shown in (a), (b) and (c) respectively.

Allele frequencies at each locus were calculated by a simple gene counting method assuming that a protein with the same mobility was controlled by the same allele even in different species and that its variation within the species was controlled by 2 or more codominant alleles (Nozawa *et al.* 1982).

From the gene frequency data thus obtained, the genetic similarity (I) and the genetic distance (D) were calculated between every pair of species used for comparison. The measures used by Nei (1972) were followed for evaluating the divergence (Menezes 1990b). The D value will become zero when the allele fre-

Table 1. Enzymes examined, their identified loci and the assayed tissues (organs)

| Enzyme | Enzyme number | Locus | Organ |
|--|---------------|----------------|------------------------|
| Glycerol-3-phosphate dehydrogenase (G3PDH) | 1.1.1.8 | <i>G3PDH-2</i> | Liver |
| | | <i>G3PDH-1</i> | Skeletal muscle |
| Lactate dehydrogenase (LDH) | 1.1.1.27 | <i>LDH-C</i> | Eye |
| | | <i>LDH-B</i> | Skeletal muscle, heart |
| | | <i>LDH-A</i> | Skeletal muscle |
| Malate dehydrogenase (MDH) | 1.1.1.37 | <i>MDH-3</i> | Skeletal muscle |
| | | <i>MDH-2</i> | Skeletal muscle |
| | | <i>MDH-1</i> | Skeletal muscle |
| Isocitrate dehydrogenase (IDHP) | 1.1.1.42 | <i>IDHP-2</i> | Liver |
| | | <i>IDHP-1</i> | Skeletal muscle |
| Malic enzyme (MEP) | 1.1.1.40 | <i>MEP</i> | Skeletal muscle |
| Phosphogluconate dehydrogenase (PGDH) | 1.1.1.44 | <i>PGDH</i> | Liver |
| Phosphoglucomutase (PGM) | 5.4.2.2 | <i>PGM</i> | Skeletal muscle |
| Alcohol dehydrogenase (ADH) | 1.1.1.1 | <i>ADH</i> | Liver |
| Aspartate aminotransferase (AAT) | 2.6.1.1 | <i>AAT-1</i> | Skeletal muscle |
| | | <i>AAT-2</i> | Liver |
| Creatine kinase (CK) | 2.7.3.2 | <i>CK</i> | Skeletal muscle |
| Superoxide dismutase (SOD) muscle | 1.15.1.1 | <i>SOD</i> | Liver |
| Haemoglobin (HB) | | <i>HB</i> | Heart |
| Sarcoplasmic protein (PROT) | | <i>PROT-1</i> | Skeletal muscle |
| | | <i>PROT-2</i> | Skeletal muscle |
| | | <i>PROT-3</i> | Skeletal muscle |

quencies of the two species used for comparison are completely identical, and will reach infinity if the allele frequencies of the two species compared are completely divergent, i.e. they do not share any common allele. Average heterozygosity was determined by totaling the number of heterozygotes for each locus and dividing this by the total number of individuals in the sample and averaging the over all loci.

RESULTS AND DISCUSSION

Genetic variation within species

The Indian mackerel (Rastrelliger kanagurta)

Eight enzymes (G3PDH, LDH, MDH, IDHP, MEP, PGDH, PGM and ADH), sarcoplasmic proteins and haemoglobins encoded by 17 loci were examined using the electrophoretic method. Among these loci, the number of polymorphic loci (P), using the

0.99 criterion of polymorphism (i.e. when the frequency of the most common allele is less than or equal to 0.99) was found to be 9 for the *ADH*, *G3PDH-2*, *HB*, *IDHP-1*, *IDHP-2*, *MEP*, *PGDH*, *PGM* and *PROT-2* in the Panaji samples, and 8 for *ADH*, *G3PDH-2*, *HB*, *IDHP-1*, *IDHP-2*, *MEP*, *PGM* and *PROT-2* in the Colva sample. At the 0.95 level of polymorphism - P^* (i.e. when the frequency of the most common allele is less than or equal to 0.95), the loci polymorphic were 4 (*ADH*, *G3PDH-2*, *MEP* and *PROT-2*) in the Panaji samples and 3 (*G3PDH-2*, *HB* and *PROT-2*) at the Colva sample. In the samples of *R. kanagurta* from the east coast and the Andaman Sea, only 5 enzymes (*G3PDH*, *LDH*, *MDH*, *MEP* and *IDHP*) and sarcoplasmic proteins encoded by 11 loci were studied. Among these loci, the numbers of P were found to be 2 (*MEP* and *PROT-2*) in the Ennur samples and 3 (*LDH-B*, *MEP* and *PROT-2*) in the San Thome sample. In the Andaman sample 7 (*G3PDH-1*, *LDH-B*, *MDH-2*, *MDH-3*, *IDHP-1*, *MEP* and *PROT-2*) loci were polymorphic. The number of P^* in the Ennur and San Thome samples was 1 (*PROT-2*). No locus was polymorphic at the 95% level of polymorphism in the Andaman sample.

The average number of alleles per locus was 1.59 at Colva and 1.82 at Panaji. The average heterozygosity observed (H) per locus was 0.058 ± 0.098 at Colva and 0.066 ± 0.110 at Panaji. Since only 11 loci were studied in *R. kanagurta* from the east coast and from the Andaman sample, the genetic variability for these samples has not been given.

The most informative estimate of genetic variation in the natural populations is the average heterozygosity (H) per locus (Allendorf and Utter 1979). Both P and H vary non-randomly between the loci, between the populations and between the species. To avoid

any serious error in the estimation of average heterozygosity, a large number and a wide range of isozyme loci should be examined (Allendorf and Utter 1979). The average heterozygosity values in *R. kanagurta* were more or less similar to the mean heterozygosity calculated by Nevo (1978) for 51 species of fishes ($H = 0.051 \pm 0.034$) and for 82 species of fishes ($H = 0.048 \pm 0.33$) calculated by Winans (1980). According to Nevo (1978), significantly larger heterozygosity values are found in tropical vertebrates which he termed as ecological generalists (wide-spread, common, vagile, broad-niched mainland species). New genetic variation arises in a population from either spontaneous mutation of a gene or by immigration from a population of genetically different individuals (Gooch 1975).

All the loci studied in *R. kanagurta*, except *PROT-2*, were within the range of Hardy-Weinberg expectations. Significant departures from the Hardy-Weinberg expectations were detected at *PROT-2* locus in all the 4 samples from the west and east coasts (Panaji $X^2 = 21.78$, $df = 1$, $P < 0.001$; Colva $X^2 = 6.16$, $df = 1$, $P < 0.02$; Ennur $X^2 = 7.80$, $df = 1$, $P < 0.01$; San Thome $X^2 = 11.14$, $df = 1$, $P < 0.001$). The deviations in each case were due to a deficiency of the heterozygotes. In Andaman sample, all of the loci studied including the *PROT-2* were according to the Hardy-Weinberg expectations.

The significant departures from the Hardy-Weinberg expectations, due to deficiency of heterozygotes, observed at the *PROT-2* locus in all of the west and east coast samples may be explained by the mixing of individuals from the populations differing in the frequencies of the alleles (Menezes *et al.* 1990b). Alleles *A*, *B* and *C* were detected at this locus in the west and east coast samples, with the most common allele being *A*. In the Andaman sample, the *PROT-2* locus was

nearly monomorphic with the most common allele being C. A locus at which complete differentiation exists between the 2 populations can be used to diagnose the population to which an individual belongs (Ayala 1983). The occurrence of *PROT-2 (C)* allele in the west and east coast populations may indicate the migration of mackerel from the Andaman population to both east and west coasts.

Oil Sardine

Ten enzymes, viz. ADH, AAT, CK, G3PDH, IDHP, LDH, MDH, MEP, PGM and PGDH, sarcoplasmic proteins and hemoglobins encoded by 19 loci were examined. Among these loci, the number of P was 5 (*G3PDH-1*, *IDHP-1*, *MEP*, *PGDH* and *AAT*) in the Panaji sample, 3 (*LDH-B*, *G3PDH-1* and *G3PDH-2*) in the Malpe and 2 (*IDHP-1* and *PGDH*) in Mangalore samples. No locus was polymorphic according to the 95% criterion. Genotypes for the species from the 3 localities conformed to the Hardy-Weinberg proportions ($P > 0.05$) at all loci. This probably suggested that all the samples of *S. longiceps* came from a single Mendelian population.

The average number of alleles per locus was 1.26 for Panaji, 1.18 for Malpe and 1.12 for Mangalore. The average heterozygosity observed per locus was 0.006 ± 0.011 for Panaji, 0.009 ± 0.020 for Malpe and 0.006 ± 0.017 for Mangalore.

Biologists have long viewed genetic variability as a beneficial feature to a population because it provides the genetic material necessary for the evolutionary response to changing environments (Ryman 1981). The best estimate of genetic variation in the natural populations is the average heterozygosity per locus (Allendorf and Uter 1979). The average heterozygosity values in the oil sardine were less than half as compared to the Indian mackerel *Rastrelliger kanagurta* (Menczes *et al.*

1990b). Smith (1986) reported an evidence of comparatively low levels of electrophoretically detectable allelic variation in the shark species representing several different orders. He postulated that the bottlenecks in previous population, i.e. reduction in the small numbers of breeding individuals, might reduce the allelic variation in species.

A major factor in the loss of genetic variation is the fluctuation in population size from generation to generation. A greatly reduced population size for a single generation can have a drastic effect on genetic variation (Crow and Kimura 1970). The loss of genetic variability may result in a situation, where the population is no longer able to exploit its former economical niche (Ryman 1991). The need for conservation and management of genetic resources therefore has been recognized by fishery scientists for quite some time, especially in relation to overfishing of natural stocks and the effects of large-scale alterations in the population (FAO/UNEP 1981). As stated by Gall (1972): "We must make every effort to learn and understand both the biology and genetics of the organism before we attempt to tamper with the essential but perishable resource, genetic variability."

Grey mullets

Ten enzymes, viz. G3PDH, LDH, MDH, IDHP, MEP, PGDH, PGM, ADH, AAT and CK, and the sarcoplasmic proteins encoded by 19 loci were examined. Among these loci, the number of P was 8 (*ADH*, *AAT-1*, *G3PDH-1*, *IDHP-1*, *IDHP-2*, *LDH-C*, *PGM* and *MEP*) in the samples of *M. cephalus* collected from the natural population and 4 (*ADH*, *G3PDH-1*, *LDH-C* and *MEP*) from the farm population. In *L. subviridis* 6 loci (*MEP*, *IDHP-2*, *PGDH*, *ADH*, *LDH-C* and *G3PDH-2*) were polymorphic in the natural population and one locus, viz. *MEP*, was polymorphic in the 3 specimens studied from the fish farm. In *V.*

cunnesius 3 loci (*MEP*, *ADH* and *LDH-C*) were polymorphic in the specimens studied from the natural population. For *L. macrolepis* there was evidence of variability at 2 loci (*PROT-2* and *G3PDH-2*) in the specimen obtained from nature and at 1 locus (*MEP*) in the farm specimens. At the 95% level of polymorphism, the number of P* was 2 (*ADH* and *MEP*) in *M. cephalus* from the natural and farm population, and 1 (*MEP*) in *L. subviridis* from the farm population and *V. cunnesius* from the natural population. Genotypes for the species from the natural and farm samples conformed to the Hardy-Weinberg proportions ($P > 0.05$) at all loci, with one exception which is *M. cephalus* from the natural population. It had a deficit of heterozygotes at the *MEP* locus ($X^2 = 7.54$; $P < 0.05$). The reason for such an exception defies explanation and needs further study.

The average number of alleles per locus was 1.53 in *M. cephalus*, 1.42 in *L. subviridis* and 1.11 in *V. cunnesius*. The average heterozygosity was 0.038 ± 0.089 in *M. cephalus*, 0.014 ± 0.025 in *L. subviridis* and 0.035 ± 0.093 in *V. cunnesius*. As there were only 2 specimens of *L. macrolepis* in the samples for observation, the levels of genetic variation for this species have not been given.

From these studies it is clear that electrophoresis gives an independent estimate of the levels of variation within and between the species without making an extensive survey of morphological and other quantitative characters, and it reduces the likelihood of using highly inbred line for selection experiments (Tave and Smitherman 1980). The genetic variability, as measured by the proportion of polymorphic loci, average heterozygosity and the number of alleles per locus, was higher in *M. cephalus* than in the other species of grey mullets studied. The occurrence of many alleles at low frequencies

reflects a large effective population size (Andersson *et al.* 1981). The rate of loss of rare allele by the genetic drift is minimized by large population sizes whether the alleles are selectively neutral or not. Because the genic variability in the qualitative characters is an indicator of the genic variability throughout the genomes of the population (Allendorf and Utter 1979), the high average heterozygosity in this species probably indicates the possibility of high additive genetic variance in the quantitative traits which seem to be important for genetic improvement in future breeding stocks.

Sciaenids

In *J. dussumieri* and *K. axillaris* 11 enzymes (*ADH*, *AAT*, *CK*, *G3PDH*, *IDHP*, *LDH*, *MDH*, *MEP*, *PGM*, *PGDH* and *SOD*), sarcoplasmic proteins and haemoglobins encoded by 18 loci were examined while in *P. macrophthalmus* and *O. ruber* all the above enzymes and sarcoplasmic proteins, except *SOD* and hemoglobins, encoded by 16 loci were studied. Among the loci investigated, the number of P were 12 (*ADH*, *G3PDH-1*, *G3PDH-2*, *HB*, *IDHP-1*, *IDHP-2*, *LDH-A*, *MDH-2*, *MEP*, *PGM*, *PGDH* and *SOD*) in *J. dussumieri*, 10 (*G3PDH-1*, *G3PDH-2*, *IDHP-1*, *IDHP-2*, *MDH-1*, *MEP*, *PEM*, *PGDH*, *SOD* and *PROT-1*) in *K. axillaris*, 3 (*ADH*, *IDHP-2* and *MEP*) in *P. macrophthalmus* and 12 (*ADH*, *AAT*, *G3PDH-2*, *IDHP-1*, *IDHP-2*, *LDH-A*, *MDH-1*, *MDH-2*, *MEP*, *PGM*, *PGDH* and *PROT*) in *O. ruber*. The number of P* was 5 (*ADH*, *HB*, *MEP*, *PGM* and *SOD*) in *J. dussumieri*, 3 (*MEP*, *PGM* and *SOD*) in *K. axillaris*, 1 (*MEP*) in *P. macrophthalmus* and 4 (*ADH*, *G3PDH-2*, *IDHP-2* and *MEP*) in *O. ruber*. Genotypes for the species conformed to the Hardy-Weinberg proportions ($P > 0.05$) at all loci, with the exception of the *PGM* locus in *J. dussumieri* and *IDHP-2* locus in *O. ruber*, which had a deficit of heterozygotes.

The reason for these significant variations is unknown.

The average number of alleles per locus was 1.89 (*J. dussumieri*), 1.67 (*K. axillaris*) 1.19 (*P. macrophthalmus*) and 1.94 (*O. ruber*). The average observed heterozygosity per locus was 0.077 ± 0.122 (*J. dussumieri*), 0.061 ± 0.104 (*K. axillaris*), 0.033 ± 0.100 (*P. macrophthalmus*) and 0.05 ± 0.077 (*O. ruber*).

The average heterozygosity, calculated for *J. dussumieri* (0.077) and *K. axillaris* (0.061) were relatively high. However, such estimates seem to be particularly dependent on the types and number of loci analysed (Allendorf and Utter 1979). In the sciaenids of Japan (Menczes *et al.* 1990a), based on 23 loci detected from 11 enzymes, haemoglobin and sarcoplasmic proteins, the average heterozygosities ranged from 0.029 to 0.094. It would probably be a reasonable assumption to indicate that the amount of isozyme variation reflects the relative amount of genetic variation found at the other loci in the genome (McAndrew and Majumdar 1983). High levels of polymorphism and average heterozygosity have also been reported in the other members of the family sciaenidae (Beckwith 1983).

Pomfrets

Nine enzymes (G3PDH, LDH, MDH, IDHP, MEP, PGDH, PGM, AAT and CK) and sarcoplasmic proteins encoded by 16 loci were examined. Among these loci, the number of P were 4 (*MDH-1*, *G3PDH-1*, *IDHP-2* and *PGM*) in *P. niger* and 7 (*LDH-A*, *LDH-C*, *MDH-1*, *MDH-2*, *PGM*, *MEP* and *IDHP-2*) in *P. argenteus*. The number of P* was 2 (*G3PDH-1* and *PGM*) in *P. niger*. No locus was polymorphic at the 95% level in *P. argenteus*. For *P. chinensis*, there was evidence of variability at 1 locus (*IDHP-2*). The genotypes for the species conformed to the Hardy-Weinberg proportions ($P > 0.05$) at all loci.

The number of alleles per locus was 1.25 for *P. niger* and 1.5 for *P. argenteus*. The average observed heterozygosity was 0.042 ± 0.100 in *P. niger* and 0.013 ± 0.018 in *P. argenteus*. As only 4 specimens of *P. chinensis* were studied, the level of genetic variation for this species was not considered reliable to be given.

The high average heterozygosity in *P. niger* showed the possibility of high additive genetic variance in its quantitative traits which are important for genetic improvement in any future breeding study. There are situations in which it may be desirable to electrophoretically estimate the genomic variability and then to use these data as a base-line for comparing the genetic effects of a particular pattern of stock breeding or exploitation (FAO/ UNEP 1981).

Genetic divergence among species

Indian mackerel (R. kanagurta)

The phenetic relationship among the samples was determined using Nei's (1972) index of genetic distance (D). Distinct genetic differentiation was evident between the west coast, east coast and the Andaman samples (Menczes *et al.* 1993a). Within the west coast and east coast samples, the phenetic relationship was very close; D values ranged between 0.0003 to 0.0017 and there was no geographic structure in the distribution of genetic distances between the samples. The Andaman sample, however, was more divergent (average D = 0.059). This value lies in the higher range of the levels suggested for local races of a species (Nei 1976).

The levels of genetic differentiation among the populations of marine teleosts vary greatly and appear to reflect the magnitude of gene flow produced by different reproductive strategies, especially spawning behaviour and mode of larval dispersal (Winans 1980). Species with pelagic larval stages tend to dis-

play fewer geographic variation in their allele frequencies than those which, for example, spawn demersally attached eggs (Johnson 1975). Another reason for the marine fishes to be characterized by a general lack of population subdivision is that the genetic drift in them is negligible because of the very large population sizes typical of marine fishes (Grant *et al.* 1987). Even for populations that become completely isolated from one another, at least several N generations (= population size) must pass before any substantial divergence is likely to appear in them (Kimura 1955). Nevertheless, genetic subdivisions among the regional stocks of marine fishes have been reported earlier. These appear to be the result of ancient allopatric subdivisions rather than the result of differentiation *in situ* (Grant 1987).

Oil sardine (*S. longiceps*)

The electropherograms of the isozymes were compared among the 3 localities. The allele frequencies were similar at all the loci (Menezes 1994). The genetic distance value in them was therefore taken as zero.

Grey mullets

The electropherograms of the isozyme patterns were compared among the 4 grey mullet species, viz. *Mugil cephalus*, *Liza subviridis*, *L. macrolepis* and *Valamugil cunnesius*. *DLH-A*, *MDH-1*, *PROT-2* and *PROT-3* were the most reliable markers for separating the 4 species (see Menezes *et al.* 1992a).

The average genetic distance (D) value was highest between *L. subviridis* and *V. cunnesius* (1.129), and the lowest between *M. cephalus* and *L. subviridis* (0.5230). Between *M. cephalus* and *V. cunnesius* the D value was 0.9274.

The Mugilidae, being a cosmopolitan family of fish, constitutes a serious taxonomic problem, particularly in the fish *Mugil*

cephalus (Oren 1975). The isozyme patterns in the present study clearly showed similarities and differences among the 4 mullets. These isozymes permit precise species assessment even at the very early life-history stages (Allendorf and Utter 1979, McAndrew and Majumdar 1983). These loci can thus be used as reliable markers to identify these 4 grey mullets.

Genetic divergence between the species was observed in many fishes using biochemical markers (Ayala 1993). Higher categories are on an average more different than the lower ones (Nei 1976). Even a single individual may be sufficient for obtaining the correct topology of a dendrogram if the genetic differences among the species are large (Nei 1978). The mullet species in the present study were clearly divisible by their biochemical genetic markers into 2 major groups, viz. *V. cunnesius* and the other two species (*M. cephalus* and *L. subviridis*) in which a clear differentiation was evident.

Sciaenids

The electropherograms of the isozymes were compared among the 4 sciaenid species, viz. *Johnieops dussumieri*, *Kathala axillaris*, *Pennahia macrophthalmus* and *Otolithes ruber*. These individuals are readily identifiable up to the species levels from the *LDH-B*, *MDH-2* and *G3PDH-1* loci (Menezes *et al.* 1993c).

To estimate the degrees of genetic divergence among the 4 species, the genetic distance (D) was calculated between every pair of species using the allele frequencies. The average genetic distance was greatest between *J. dussumieri* and *O. ruber* (D = 0.612), and the lowest between *O. ruber* and *P. macrophthalmus* (D = 0.334) followed by *J. dussumieri* and *K. exillaris* (D = 0.417).

The patterns clearly showed similarities and differences among the genera. These were in the positions of the common band for each

isozyme locus which permitted precise species assessment even at very early life-history stages. These loci can thus be used as reliable markers to identify these 4 sciaenid species.

In the family Sciaenidae, Menezes and Taniguchi (1988) reported the average genetic distance (D) among the congeneric species (*Nibea mitsukurii* and *N. albiflora*) as 0.092 which is quite small. Between the consubfamilial genera, on the other hand, it was 1.212, ranging from 0.861 to 1.668. In the bottom-living sparids also, the average genetic distance between the species tended to be small (Taniguchi *et al.* 1986). In the present study, the average D values between the different genera belonging to the family Sciaenidae were quite small. Between the consubfamilial genera (*Otolithes* and *Penahia*), the average genetic distance was 0.334 ($I = 0.716$) while between subfamilies (Johanniac, Kathalinac and Otolithinae) and average D was 0.523 ($I = 0.594$), ranging from 0.417 to 0.612 ($I = 0.543$ to 0.659). Typically, closely related species have I values close to 0.5 (Ayala 1983). It is possible that larger differences among these species may be found by increasing number of loci surveyed, but unless the loci examined here are entirely unrepresentative, it must be concluded that there is little genetical difference between these sciaenid species.

Pomfrets

The electropherograms of isozymes were compared among the 3 species of pomfret, viz. *Parastromateus niger*, *Pampus argenteus* and *P. chinensis*. Eight loci (*LDH-A*, *LDH-C*, *MDH-1*, *MDH-2*, *G3PDH-2*, *PGDH*, *MEP* and *PROT-2*) were divergent among the 3 species (Menezes 1993). When the allele frequencies were compared with every pair of species, a large number of divergent loci were

found between more distantly related taxonomic groups.

The average genetic distance value (D) was very high between *P. argenteus* and *P. niger* ($D = 10.27$), and there was no genetic similarity between them. This was followed by *P. chinensis* and *P. niger* ($D = 2.744$). The D value between *P. argenteus* and *P. chinensis* was, however, quite small ($D = 0.8371$).

The D value between *P. niger* and *P. chinensis* ($D = 2.74$) was slightly higher than that found at the subfamily level in the carangid fish (Menezes 1990b). The average D value between *Parastromateus niger* and *Pampus argenteus* ($D = 10.27$) was, however, very high. The 2 species of *Pampus* (*P. argenteus* and *P. chinensis*) ($D = 0.8371$) appear to be less diversified as was found at the species level in the carangid fish.

An objective way to test the morphological conclusions is to use the isozyme electro-phoresis to obtain a measure of the genetic distance, assuming that this is correlated with the closeness of their common ancestry (Makay and Miller 1991). The results presented here agree with the primary division of *Parastromateus niger* from the other 2 species *Pampus argenteus* and *P. chinensis*.

CONCLUSION

From the studies noted above, the following main conclusions can be drawn:

1. Electrophoresis of soluble enzymes and nonenzyme proteins is a very powerful but simple tool for the identification of species when morphological discrimination in them is either difficult or impossible. The technique will be particularly suitable for the identification of larvae, as the isozymes are known to permit precise species assessment even at the very early life-history stage.

2. The polymorphic loci detected within the species can be used as markers for racial studies and in stock typing to introduce the selected genetic traits into fish.
3. Biochemical genetic data may presently be applicable to several aspects of fish culture that include inbreeding, brood stock identification and registration, and estimations of genetic differences among the stocks.
4. The serum proteins can be used as an additional tool to evaluate the environmental stress on the animals.

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