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Biochemical genetic polymorphism in Indian mackerel (*Rastrelliger kanagurta*)

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ABSTRACT

Six enzymes glucose 6 phosphate dehydrogenase (G6PD), xanthene de hydrogenase (XDH), alcohol dehydrogenase (ADH), peroxidase (PO), lactate dehydrogenase (LDH) and isocitric dehydrogenase (IDH) were examined to discover the genetic variation in Indian mackerel through polyacrylamide gel electrophoresis. A significant difference in allelic frequency at XDH locus was observed between the Cochin and Thotapally populations. Thotapally stocks exhibited a significant deviation ($P< 0.05$) from Hardy weinberg equillibrium. The proportion of polymorphic loci was 0.777. The coefficient of identity and the genetic distance estimated were 0.9262 and 0.076 respectively.

Key words: Biochemical genetic polymorphism, Indian mackerel, Polymorphism

The Indian mackerel (*Rastrelliger kanagurta*) is a pelagic fish. Though the fish is available all along the Indian coast, its commercial fishing is only along the West coast from Quilon to Ratnagiri. The mackerel fishery occurs during September-April, and September is supposed to be the peak season for mackerel fishing in Kerala (Noble 1979). The all India average annual landing of mackerel estimated from the 34 years of data (1950-83) was 66584 tonnes of which 58877 tonnes was along the West coast. In Kerala, mackerel forms 8.4% of total marine landings (James *et al.* 1991) and has been one of the most exploited fisheries.

The tagging experiments conducted by Venkatarama (1970) showed that mackerel is a migrating type fish and can cover long distances, North-South migration. The migrating nature of the fish can lead to a mixing of different stocks of population, if existing. In that case it becomes imperative to study the population structure for identification of different genetic stocks. The methodology of electrophoresis is generally applied to study the genetic make up of fish populations (Dhulkhed and Nagesh 1976, Menezes *et al.* 1990). The intraspecific genetic variations in fish populations are due to the differences in allelic frequencies at various loci. In the present study, the genetic variations at the loci controlling glucose 6 phosphate dehydrogeanase (G6-PD), xanthine dehydrogenase (XDH), alcohol dehydrogenase (ADH), peroxidase (PO), lactate dehydrogenase (LDH) and iso-citric dehydrogenase (IDH) enzymes were studied in the mackerel of offshore Cochin and Thottapally areas.

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MATERIALS AND METHODS

Specimens of *Rastrelliger kanagurta* were collected from the offshore Cochin and Thotapally areas. Samples were kept frozen at –20°C till processing. The total body length of each animal was recorded before dissecting it. Tissues like heart, liver, muscle and eye lens were dissected out from individual specimen. A weighed quantity of samples were macerated by means of an electric homogeniser in distilled water in the proportion 1 : 2 (weight : volume). The extracts were obtained by centrifuging the homogenate at 10 000 rpm for 20 min at 5°C.

Polyacrylamide gel electrophoresis (PAGE) was conducted using a continuous buffer system to analyse the tissue sample. For this, 20ml of running gel solution was prepared by mixing 5ml of small pore gel buffer (Shaw and Prasad 1970), 3.5ml 40% acrylamide, 2ml 2.1% N-N' methylene bisacrylamide, 5ml 0.14% ammonium per sulphate solution and 5ml distilled water. The gel tubes (8 × 75mm) were filled with the running gel solution with the help of syringe and the solution in the tubes was covered gently with few drops of water to avoid miniscus formation.

After polymerisation of the gel the overlying water was removed and 40μl of tissue extract and indicator dye (bromophenol blue) each was applied. The left out space in the tubes was filled with tank buffer solution. The electrophoretic tank was connected with a power pack and current supply was adjusted to 3mA/tube and 160-200 volts. After the completion of electrophoresis the gels were removed from the gel tubes carefully with the help of syringe filled with used buffer. The staining of the gels was done following the procedure of Shaw and Prasad (1970) and the gels were

Table 1. List of enzymes investigated and buffer and tissue used

Enzyme	Locus designated	Buffer	pH Tank	pH Gel	Tissue
Glucose 6 phosphate dehydrogenase	G6 PD	0.5 M Tris Versene borate	9.0	8.0	Liver
Xanthine dehydrogenase	XDH	0.5 Tris Versene borate	8.0	8.0	Liver
Alcohol dehydrogenase	ADH	0.5 Tris Versene borate	8.0	8.0	Liver
Peroidase	PO	0.3 M borate	8.0	8.5	Muscle
Lactate dehydrogenase	LDH	0.155 M Tris -0.043 M citric acid	7.0	7.0	Liver
Isocitric dehydrogenase	IDH	0.155 M Tris citric acid	7.0	7.0	Liver

incubated at 37°C till the bands appeared. The position and the colour intensity of the electrophoretic bands were recorded for further analysis.

The allelomorphic frequencies at all loci were calculated from the electrophoretic data and the chi-square goodness of fit was applied to test the samples for Hardy Weinberg Equillibrium. The degree of genetic variations was estimated by the proportion of polymorphic loci (P). The genetic divergence between the 2 groups was calculated by applying Nei's index of genetic similarity and genetic distance (Nei 1972).

RESULTS AND DISCUSSION

The total length of *Rastrelliger kanagurta* collected from offshore Cochin ranged from 18.0 to 28.0 cm with a mean 21.41 ± 0.36 cm and that of Thotapally 11.0 to 24.0 cm with a mean of 19.26 ± 0.21 cm. Samples from the 2 localities exhibited 25.99% and 24.66% variation in their total lengths, respectively, and the difference was nonsignificant ($P>0.05$). The genetic basis of the morphometric variations is difficult to describe as the animals are also under the influence of numerous environmental factors (Ryman *et al.* 1984).

Six enzymes, viz. G-6 PD, XDH, ADH, peroxidase, LDH and IDH in mackerel were examined by electrophoretic method. The details of buffer systems and tissues selected for each enzyme are given in Table 1. The analysis of zymogram patterns indicated that except IDH other enzymes are polymorphic. Except XDH all other enzymes appeared to be controlled by 2 diallelic loci, whereas, the enzyme XDH was under the control of single diallelic locus. The allelic frequencies of the electromorphs of all the enzymatic loci are presented in Table 2. Chi-square values for the differences between observed and expected number of phenotypes of each polymorphic enzyme locus are also given in Table 2. The chi-square values were significant at G-6 PD, XDH and PO loci in Thotapally samples. Interestingly all the polymorphic loci in Cochin samples were in Hardy Weinberg equillibrium (Table 2). No slow

Table 2. Allelic frequencies of the electromorphs of enzymatic loci in Indian mackerel

Locus	Locality	Allelic frequency p	Allelic frequency q	X2
G6 PD1	Cochin 50	0.44	0.56	0.150
	Thotapally 25	0.40	0.60	11.10**
G6 PD2	Cochin 50	0.49	0.51	0.328
	Thotapally 25	0.60	0.40	2.77
XDH	Cochin 40	0.57	0.43	5.95
	Thotapally 36	0.21	0.79	6.08*
ADH1	Cochin 39	0.64	0.36	1.98
	Thotapally 35	0.43	0.57	5.19
ADH2	Cochin 39	0.49	0.51	0.231
	Thotapally 35	0.47	0.53	0.391
PO1	Cochin 47	0.62	0.38	3.34
	Thotapally 23	0.46	0.54	6.18*
PO2	Cochin 47	0.48	0.52	3.08
	Thotapally 23	0.41	0.59	7.04*
LDH	Cochin 40	0.5	0.5	3.33
IDH	Cochin 40	1.0	–	–

*$P<0.05$, **$P<0.01$.

homozygote was observed at G-6 PD locus in Thotapally samples. A significant difference in allelic frequency at XDH locus was observed between Cochin and Thotapally populations.

The deviation from Hardy Weinberg equillibrium observed in Thotapally samples appeared to be due to excess of either homozygotes or heterozygotes. Different reasons for excess of homozygotes (heterozygote deficiency) available from different reports may be such as Wahlund effect (Hedgecock 1977), mixing of genetically different populations, selection pressure (Smith and Jamieson 1978) and null allele (Grant *et al.* 1987). However, the neutrality of allozymic variation to selection can be assumed (Menezes *et al.* 1990) because it is a logical departure for testing hypothesis of genetic population structure (Grant *et al.* 1987). Apparent correlations between excess of homozygotes and heterozygotes in fishes and different environmental conditions have been reviewed by Ponniah (1989). Heterozygotic deficiency was also reported in marine catfish (Suzuki and Phan 1990) and Indian mackerel (Menezes *et al.* 1990).

The proportion of polymorphic loci based on the 0.99 criterion of polymorphism (i.e. the frequency of the most common allele is less than or equal to 0.99) was 0.777. A high value of coefficient of identity (0.9262) was observed indicating that about 92% of the genes are identical in structure in the two populations. However, the genetic distance estimated was 0.076 and is higher than the reported value (0.020) between local fish population (Ayla and Kiger 1981). The value of genetic distance observed indicated that the allelic substitution might have occurred at 7.6% gene loci. The serological studies conducted by Dhulkhed and Nagesh (1976) indicated the genetic variation in the mackerel from the

Mangalore and Karwar areas.

Menezes *et al.* (1990) reported the genetic polymorphism in the Indian mackerel from Goa region but the results could not be compared as the enzymes studied were different from the enzymes taken for this study. A detail study of different populations of mackerel is essential to conclude about their population structure.

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REFERENCES

Allendorf F W and Utter F.M. 1979. *Population Genetics. In Fish Physiology* pp. 407-54. (Eds) W S Hoar, D J Randall and J R Brett. Academic Press, New York.

Ayala F J and Kiger J A (Jr). 1981. *Speciation and macroevolution: In Modern Genetics.* pp. 746. The Benjamin/Cummings Publishing Company Inc. Melano Park, California.

Dhulkhed M H and Nagesh C N. 1976. Serological studies on oil sardine (*Sardinella longiceps*) and Mackerel (*Rastrelliger kanagurta*). *Fish Technology* **13**: 9-12.

Grant W S, Zhang C I, Kobayashi T and Stahl G. 1987. Lack of genetic stock discretion in Pacific cod (*Gadius macrocephalus*). *Canadian Journal of Fisheries Aquatic Sciences* **44**: 490-98.

Hedgecock D. 1977. Biochemical genetic markers for broodstock identification in aquaculture. *Proceedings of World Maricultural Society* **8**: 523-31.

James P S B R, Kurup K N and Pillai N G K. 1991. A review of present status of marine fisheries of Kerala. *Marine Fisheries Information Services* **114**: 1-8.

Menezes M R, Naik S and Martins M. 1990. Genetic and morphological variations in the Indian mackerel *Rastrelliger kanagurta* (Cuvier 1817) from Goa region. *Proceedings of Indian Academy Sciences (Animal Sciences)* **99**: 457-65.

Nei M. 1972. Genetic distance between populations. *American Naturalist* 106: 283-92.

Noble A. 1979. The Indian mackerel in 1978. *Marine Fisheries Information Services* **8**: 1-11.

Ponniah A G. 1989. Functional relationship between biochemical genetic polymorphism and physiological variation in aquatic animals. *Proceedings of the Symposium on Conservation and Management of Fish Genetic Resources of India.* pp.**266**: 11-13 April 1986 New Delhi.

Ryman N, lagercrantz U, Anderson L, Chakraborthy R and Rosenberg R. 1984. Lack of correspondence between genetic mrophological variability patterns in Atlantic herring (*Clupea harrengus*). *Heridity* **53**: 687-704.

Shaw C R and Prasad R. 1970. Starch gel electrophoresis of enzymes A compilation of recipes. *Biochemical Genetics* **4**: 297-320.

Smith P J, Francis R I C C and Paul P J. 1978. Genetic variation and population structure in the Newzealand Snapper. *NewZealand Journal of Marine Freshwater Research* **12**: 343-50.

Suzuki H and Phan V N. 1990. Electrophoretic study on intraspecific variations and intraspecific relationships of marine Cat fishes (Siluri formes, Aridae) of Cananela (Sao Paulo, Brazil) 2. Isozymes of skeltic muscle. *Boletim DC Institute Occeanographico S.Paulo* **38**: 43-55.

Venkataraman G. 1970. The Indian mackerel 4. Bionomics and life history. *Bulletin of Central Marine Fisheries Research Institute* **24**: 17-40.

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