

## Genetic and morphological variations in the Indian mackerel *Rastrelliger kanagurta* (Cuvier 1817) from the Goa region

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**Abstract.** The population structure of *Rastrelliger kanagurta* (Cuvier 1817) from two localities off Goa (Arabian sea) was assessed from genetic and morphological characters. Seventeen loci were examined from 8 enzymes, sarcoplasmic proteins and hemoglobins. The allele frequencies were not significantly different between the two localities. Nei's genetic distance value was 0.0017. The proportion of polymorphic loci at the 1% level was 47.1 and 52.9%. The level of genetic variation, estimated by average observed heterozygosity, was 5.8 and 6.6%. The sarcoplasmic protein-2 locus was found to be highly polymorphic. Significant ( $P < 0.001$ ) departures from Hardy-Weinberg expectations were observed at this locus. About 67-89% of the variation in meristic characters was found to be within the groups.

**Keywords.** Genetic structure; heterozygosity; meristic characters; *Rastrelliger kanagurta*.

### 1. Introduction

The Indian mackerel, *Rastrelliger kanagurta* (Cuvier 1817) is a pelagic shoaling fish, widely distributed in the tropical Indo-west Pacific region. Mackerel landings in Goa (Lat. 14° 54' and 15° 48'N, Long. 73° 41' and 74° 20'E) constitute 20% of the mackerel catch in India (Doiphode 1974). The fishery is seasonal, lasting from September to March, and is restricted to a narrow coastal belt along some 400 miles along the west coast from Ratnagiri to Quilon in the south (Banerji 1962).

The results obtained so far from tagging experiments show that the movements of mackerel are of two categories, one showing limited migration and the other showing long distance, north-south migration (Venkataraman 1970). Rao (1962) indicated the possible existence of mackerel spawning grounds in several areas along the coast of peninsular India and the Andaman Islands.

The methodology of protein electrophoresis is now available to examine the genetic structure of fish populations which was previously impossible to study. With this method, a large number of individuals can be analyzed for many protein loci, and allele frequencies for these loci can be reliably estimated. Having accurate estimates of the frequencies of detected allelic variants is important since, as far as is known, genetic differences between populations at the intraspecific level are due in large part to frequency differences of alleles, rather than to alleles unique to different populations. This technique is used in the present study to estimate the genetic structure of *R. kanagurta* from two localities off Goa (Arabian sea). The total length was measured and the fin rays and gill rakers were counted for the specimens analysed.

### 2. Materials and methods

Specimens of *R. kanagurta* were collected from the fish landings at Panaji (north)

and Colva (south), in Goa during October–November 1988. The sampling localities are marked in figure 1. The fish samples were kept frozen at  $-20^{\circ}\text{C}$  prior to analyses. Skeletal muscle, liver, heart and eye were dissected from individual specimens. The cell-lysate obtained by freezing and thawing was directly subjected to electrophoresis for phenotypic analysis. Starch gel electrophoresis was carried out by the procedure of Menezes and Taniguchi (1988). Detection of isozymes and nomenclature were as per the procedure of Allendorf and Utter (1979). The buffer used was citric acid aminopropylmorpholine (C-APM) of pH 6 (Clayton and Tretiak 1972) and Tris citric acid (T-C) of pH 8 (Shaw and Prasad 1970).

The list of enzymes, tissue specificity, buffer used and 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 the protein variants were inferred from the observed banding patterns: (i) banding patterns were consistent with known molecular structures of analogous proteins of other fish and (ii) whenever the same locus was expressed in two or more tissues, the banding patterns of variants were consistent among tissues.

Average heterozygosity was determined by totalling the number of observed heterozygotes for each locus, dividing this by the total number of individuals in the sample, and averaging over all loci. Genetic divergence between populations was expressed by Nei's (1972) indices of genetic similarity ( $I$ ) and genetic distance ( $D$ )

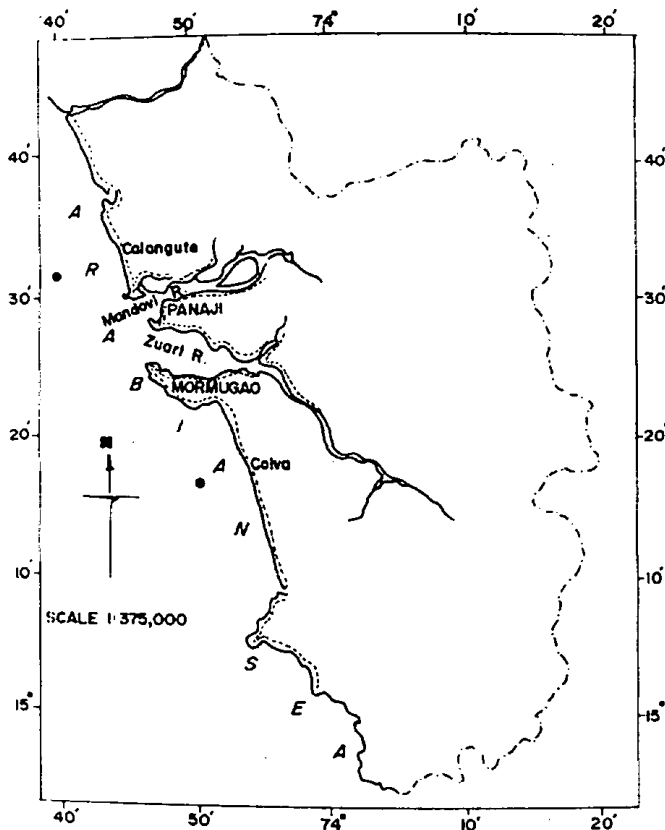


Figure 1. Map of Goa showing the sampling areas.

**Table 1.** List of enzymes examined, loci identified, buffer used and tissue assayed.

Enzyme	Enzyme number	Locus	Buffer	Organ
Glycerol-3-phosphate dehydrogenase (G3PDH)	1-1-1-8	G3pdh-B	C-APM	Liver
		G3pdh-A	C-APM	Skeletal muscle
Lactate dehydrogenase (LDH)	1-1-1-27	Ldh-C	TC	Eye
		Ldh-B	C-APM	Skeletal muscle, heart
		Ldh-A	C-APM	Skeletal muscle
Malate dehydrogenase (MDH)	1-1-1-37	Mdh-C	C-APM	Skeletal muscle
		Mdh-B	C-APM	Skeletal muscle
		Mdh-A	C-APM	Skeletal muscle
Isocitrate dehydrogenase (IDH)	1-1-1-42	Idh-B	C-APM	Liver
		Idh-A	C-APM	Skeletal muscle
Malic enzyme (ME)	1-1-1-40	Me	C-APM	Skeletal muscle
Phosphogluconate dehydrogenase (PGDH)	1-1-1-44	Pgdh	C-APM	Liver
Phosphoglucomutase (PGM)	5-4-2-2	Pgm	C-APM	Liver
Alcohol dehydrogenase (ADH)	1-1-1-1	Adh	C-APM	Liver
Sarcoplasmic protein (SP)		Sp-1	C-APM	Skeletal muscle
		Sp-2	C-APM	Skeletal muscle
Hemoglobin (HEM)		Hem	TC	Heart

C-APM, Citric acid aminopropylmorpholine pH 6; TC = Tris citric acid pH 8.

( $D = -\log_e I$ ). Genetic distances reflect estimates of net codon differences between pairs of populations (Menezes 1990).

The *G*-test for independence (Sokal and Rohlf 1981) was used to compare allele frequencies among the two localities.

The total length of the fish specimens were measured (Laevastu 1965). The number of first dorsal (D1), second dorsal (D2), anal (A) and pectoral (P) fin rays, and gill rakers (GR) of the lower limb of the first gill arch were counted for the fish analysed from the two localities.

### 3. Results

*R. kanagurta* specimens from Panaji were in the size range of 16.5–22.5 cm with a mode at 20.5 cm, specimens from Colva ranged from 20.5–22.5 cm with a mode at 21.5 cm. Table 2 gives the range and means of the meristic counts of the specimens analysed. There was a highly significant ( $P < 0.001$ ) added variance component (ANOVA, Sokal and Rohlf 1981) among the two groups for the first dorsal, second dorsal and pectoral fin rays (table 3). The proportion of variation among groups (coefficient of intraclass correlation) was 0.11, 0.31, 0.25, 0.55 and 0.33 for gill rakers, first dorsal, second dorsal, pectoral and anal fin rays respectively.

Eight enzymes, sarcoplasmic proteins and hemoglobins encoded by 17 loci (table 1, figure 2) were examined by electrophoretic method. Among 17 loci, 9 were polymorphic in Panaji sample while 8 in Colva sample. Diagrammatic representation of the relative electrophoretic mobilities of the polymorphic enzymes are given in figure 3. Allele frequencies and chi-square values for the differences between

**Table 2.** Counts of meristic characters of *R. kanagurta* from two localities.

	Locality	
	Panaji	Colva
N	100	40
Dorsal		
D1	7-10 (9.02 ± 0.65)	7-9 (8.42 ± 0.55)
D2	9-13 (11.46 ± 0.89)	11-13 (12.1 ± 0.44)
Fin rays		
Anal	7-13 (11.75 ± 0.88)	11-13 (11.95 ± 0.50)
Pectoral	17-22 (19.25 ± 1.24)	18-24 (21.37 ± 1.59)
Gill rakers	28-38 (34.13 ± 2.47)	30-40 (35.25 ± 2.42)

Range with mean and SD in parentheses.

**Table 3.** Test of significance (ANOVA) for difference in meristic characters among the two localities.

Source of variation	df	MS	F <sub>s</sub>
<b>D1</b>			
Among the groups	1	10.63	26.88***
Within the groups	138	0.3955	
Proportion of variation among groups (coefficient of intraclass correlation)		= 0.31	
<b>D2</b>			
Among the groups	1	11.71	19.60***
Within the groups	138	0.5974	
Proportion of variation among groups		= 0.25	
<b>Anal</b>			
Among the groups	1	1.921	2.97*
Within the groups	138	0.6471	
Proportion of variation among groups		= 0.33	
<b>Pectoral</b>			
Among the groups	1	129.02	70.62***
Within the groups	138	1.8269	
Proportion of variation among groups		= 0.55	
<b>Gill rakers</b>			
Among the groups	1	46.08	7.78**
Within the groups	138	5.9196	
Proportion of variation among groups		= 0.11	

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

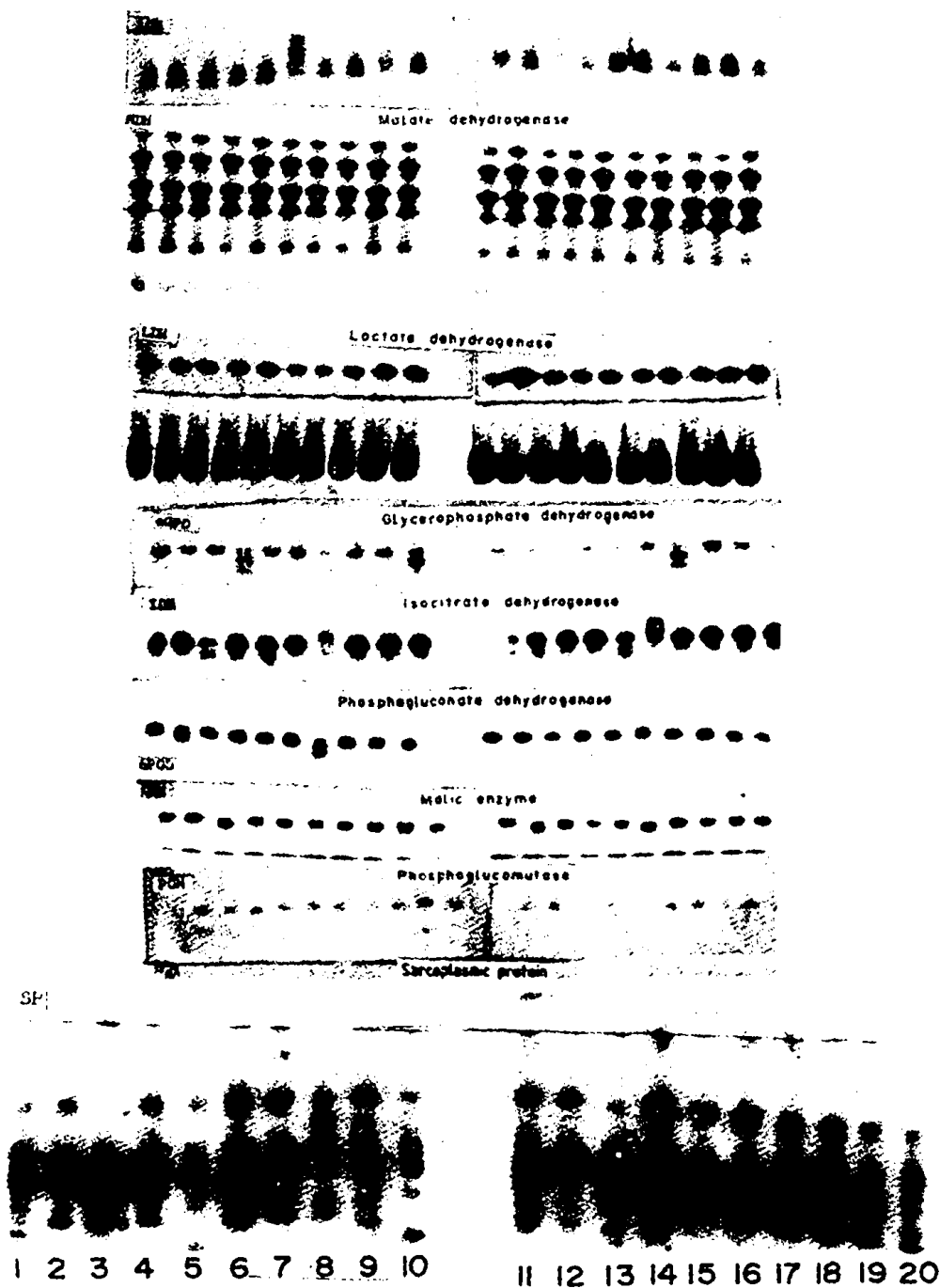


Figure 2. Gel photograph of isozyme patterns in 20 individuals of *R. kanaqurta* from Panaji.

observed and expected numbers of phenotypes of each polymorphic enzyme are given in table 4. All of the loci except sp-2 were found to be in Hardy-Weinberg equilibrium. Significant departures from Hardy-Weinberg expectations were observed at sp-2 locus in both the localities (Panaji sp-2  $X^2 = 21.78$ ,  $df = 1$ ,  $P < 0.001$ ;

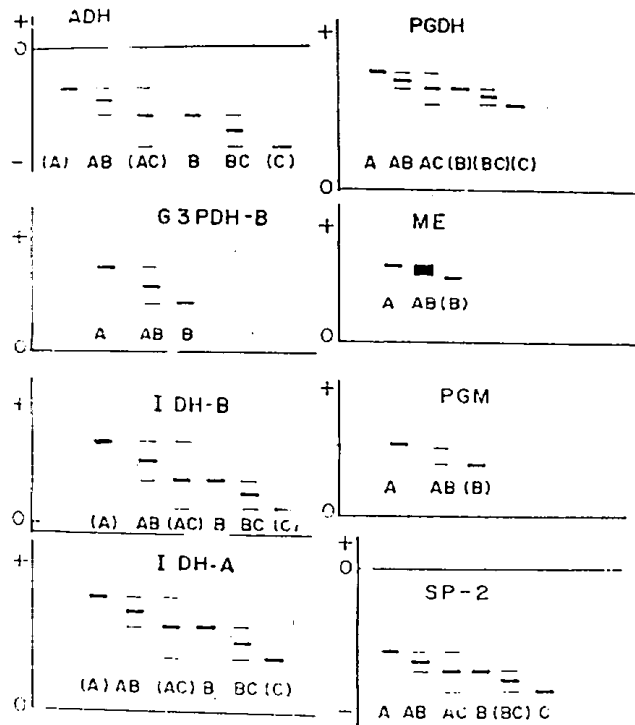


Figure 3. Diagrammatic representation of isozyme patterns of polymorphic enzymes in *R. kanagurta*, phenotypes in parentheses were not found.

Colva sp-2  $X^2=6.16$ ,  $df=1$ ,  $P<0.02$ ). The deviations reflected a deficiency of heterozygotes. Observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities at this locus were: Panaji  $H_o=0.4$ ,  $H_e=0.557$ ; Colva  $H_o=0.325$ ,  $H_e=0.384$ . This locus showed the presence of 3 alleles (A, B and C) with most of the individuals being homozygous for sp-2 (A) allele (figure 2). A slightly significant heterogeneity was observed only at the sp-2 locus ( $G_2=6.9$ ,  $P<0.05$ ) between the two localities that may be due to chance (table 5).

The average number of alleles per locus was 1.59 (Colva) and 1.82 (Panaji), the proportion of polymorphic loci ( $P$ ) using the 0.99 criterion of polymorphism (i.e. the frequency of the most common allele is less than or equal to 0.99) was 0.471 (Colva) and 0.529 (Panaji). At the 95% level of polymorphism the percentage of polymorphic loci was 17.6% (Colva) and 23.5% (Panaji). The average observed heterozygosity ( $H$ ) per locus was  $0.058 \pm 0.098$  (Colva) and  $0.066 \pm 0.110$  (Panaji). A very low genetic distance ( $D$ ) value was obtained between the two samples. The  $D$  value observed was 0.0017. This value lies in the lower range of the distribution of observed genetic distance values calculated between local populations of fishes which, in most cases, were not separated by as great a distance (Menezes *et al* 1990).

#### 4. Discussion

The most informative estimate of genetic variation in natural populations is the average heterozygosity ( $H$ ) per locus (Allendorf and Utter 1979). The amounts of  $P$

**Table 4.** Allelic frequencies at polymorphic loci in *R. kanagurta* from two localities.

Locus	Locality	N	Allelic frequency			×2
			A	B	C	
Adh	Panaji	80	0.075	0.919	0.006	0.626
	Colva	40	0.025	0.962	0.013	0.061
G3pdh-B	Panaji	100	0.875	0.125	0	2.041
	Colva	40	0.838	0.163	0	0.004
Hem	Panaji	40	0.975	0.025	0	0.026
	Colva	40	0.950	0.050	0	0.111
Idh-B	Panaji	78	0.025	0.968	0.006	0.086
	Colva	40	0.013	0.988	0	0.006
Idh-A	Panaji	60	0.008	0.967	0.025	0.007
	Colva	40	0.025	0.975	0	0.026
Me	Panaji	60	0.950	0.050	0	0.166
	Colva	40	0.975	0.025	0	0.026
Pgdh	Panaji	100	0.990	0.005	0.005	0.010
	Colva	40	1.000	0	0	—
Pgm	Panaji	100	0.990	0.010	0	0.010
	Colva	34	0.956	0.044	0	0.072
Sp-2	Panaji	60	0.600	0.242	0.158	21.78**
	Colva	40	0.760	0.175	0.063	6.16*

\* $P < 0.05$ ; \*\* $P < 0.001$ ; N, number of individuals.

**Table 5.** Heterogeneity test (using *G*-statistic) at the polymorphic loci in *R. kanagurta* between two localities.

Locus	<i>G</i>	<i>df</i>
G3pdh-B	0.665	1
Pgm	2.730	1
Me	0.829	1
Idh-B	1.320	2
Idh-A	3.940	2
Adh	2.990	2
Hem	0.706	1
Sp-2	6.91*	2

\* $P < 0.05$ .

and *H* vary non-randomly between loci, populations and species. In order to avoid a serious error in the estimation of average heterozygosity, a large number and wide range of isozyme loci should be examined (Allendorf and Utter 1979). The average heterozygosity values in *R. kanagurta* are 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 ( $H = 0.048 \pm 0.033$ ) calculated by Winans (1980). According to Nevo (1978) significantly larger heterozygosity values are found in tropical vertebrates that are ecological generalists (widespread, 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). The high amount of genetic variability as measured by isozyme loci is an indicator of high genetic variability throughout the genomes of this population. Populations with high average heterozygosities should also demonstrate high additive genetic variance for phenotypic traits which are of importance to fish culturists (Allendorf and Utter 1979).

The meristic characters of *R. kanagurta* showed that 67–89% of variation is within the groups and only about 11–33% is among the groups. The genetic basis for the variation of these characters is complex and they are also under the influence of numerous environmental factors (Ryman *et al* 1984). The significant variation within localities for these meristic traits may thus be evidence of different environmental conditions, to which the developing fish were subjected. Iwate (1973, 1975a,b) found morphological differences among groups of pollock in the sea of Japan and the Okhotsk sea, but found no differences in the frequencies of biochemical genetic variants. In all cases, the distribution of morphological variants most likely reflects regional environmental differences and does not necessarily reflect genetic subdivision (Iwate 1975b). Genetic differences between populations within a defined body of water may conceivably decrease with time, due to gene flow between subpopulations. Alternatively, genetic differentiation could become accentuated through time (perhaps because subpopulations become increasingly associated with discrete breeding areas) (Felley and Avise 1980).

Significant departures from Hardy-Weinberg expectations may be due to natural selection, migration or gene flow and genetic drift. We assume neutrality of allozymic variation to selection because it is a logical departure for testing hypothesis of genetic population structure (Grant *et al* 1987). Also in fish species with aggregate spawning and external fertilization the effect due to genetic drift is assumed to be nil, thus reducing the interpretation to: Are the patterns due to migration or gene flow from some other genetically differentiated populations? Further studies on the genetic structure of *R. kanagurta* from distant areas will no doubt further our understanding.

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