

Genetic divergence in the Indian Mackerel *Rastrelliger kanagurta* (Cuv) from the coastal waters of Peninsular India and the Andaman Sea

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ABSTRACT

Genetic divergence in the Indian mackerel *Rastrelliger kanagurta* (Cuv) from the west coast (Goa), east coast (Madras) and the Andaman Sea was estimated, using allozyme markers. Skeletal muscle and eye proteins were electrophoretically analysed for 5 enzymes and sarcoplasmic proteins. The sarcoplasmic protein-2 (*PROT-2*) locus of the west coast and east coast populations was highly polymorphic with 3 alleles (100, 125 and 150). Most of the individuals were homozygous for *PROT-2* (-100) allele. The *PROT-2* locus of the Andaman population was nearly monomorphic with the individuals being homozygous for *PROT-2* (-150) allele. Nei's mean genetic distances (D) ranged from 0.0003 to 0.0017 for the west and east coast samples and there was no geographic structure in the distribution of genetic distances. Between the west coast/east coast and the Andaman samples the average D was 0.059. This value lies on the higher range of values suggested for local races of a species.

The Indian mackerel, *Rastrelliger kanagurta* (Cuv), occurs along the east and west coasts of India. It is one of the most important commercial fishes in Indian waters. The bulk of the landings come from the west coast, where the active fishery region extends from Quilon (9°N lat.) to Ratnagiri (17°N lat). The mackerel fishery of the east coast and of the Andaman Islands are of a much lesser magnitude.

One important consideration in the commercial exploitation of a fishery resource is the identification of any discrete population or stock units. These stock units are usually self-maintaining groups of fish that are temporally or spatially isolated from one another. Because of their isolation it is assumed that they are genetically distinct (Booke 1981). Reproductive isolation between stocks of marine fishes may arise by homing to different spawning areas (Hourston 1982) or by hydrographic features which reduce or

prevent migration between areas (Illes and Sinclair 1982).

Examination of genetic variation by electrophoresis of the primary gene products (proteins), provides a powerful tool for population discrimination and identification (Ferguson 1980).

The population genetic structure of *R. kanagurta* (Cuv) from two localities off Goa (west coast) was assessed from genetic and morphological characters (Menezes *et al.* 1990a). Eight enzymes, sarcoplasmic proteins and haemoglobins encoded by 17 loci were examined by electrophoresis. The allele frequencies at the 17 loci 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%; at the 5% level it was 17.6% and 23.5%. The level of genetic variation, estimated by average heterozygosity, was 5.8% and 6.6%. The sarcoplasmic protein-2 locus from the skeletal muscle was highly polymorphic. Significant departures from Hardy-Weinberg expectations were

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observed at this locus. These may be due to natural selection, migration or gene flow and genetic drift. We assumed neutrality of allozymic variation to selection because it is a logical departure for testing hypotheses 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?

The meristic characters of *R. kanagurta* showed that 67–89% of the 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 the localities for these meristic traits may thus give an evidence of different environmental conditions, to which the developing fish were subjected.

The present study was conducted on *Rastrelliger kanagurta* from the east coast and the Andaman Sea, and compared with that of the west coast samples. Only 11 loci from the skeletal muscle and eye were analysed. If *R. kanagurta* is subdivided into regional stocks, then allele frequency differences between areas become apparent.

MATERIALS AND METHODS

Specimens of *R. kanagurta* 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. Specimens were collected from Port Blair, Andaman during the 41st cruise of ORV *Sagar Kanya* in April–May 1988. The specimens were stored at -20°C prior to analyses. Skeletal muscles and eyes 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 used for starch-gel electrophoresis was that of Menezes and Taniguchi (1988). Detection of isozymes and nomenclature of locus followed that of Shaklee *et al.* (1990). The enzymes, tissue specificity, buffers used and loci are given in Table 1. Allelic variants were designated according to their relative mobility, the most common allele being designated 100 and other alleles being given numbers that indicated their mobility relative to that of the common allele. Cathodal systems were designated in a similar way but these were given a negative sign. The genetic basis of the protein variants was inferred from the observed banding patterns: (1) banding patterns were consistent with the known subunit structures of homologous proteins in other fishes, and (2) whenever the same locus was expressed in two or more tissues, the banding patterns of variants were consistent among the tissues. The identity or difference of alleles at the same locus was decided by the banding positions of isozymes on the same gel.

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

Genetic divergence between the populations was expressed by indices given by Nei (1972) which states genetic similarity (I) and genetic distance (D) as $D = \log_e I$.

RESULTS

Total length of the specimens analyzed ranged from 16.8 cm to 22.5 cm (Panaji), 20.5 cm to 22.5 cm (Colva), 21.1 cm to 24.1 cm (Ennur), 15.5 cm to 25.3 cm (San Thome) and 16.2 to 28.3 cm (Andaman).

Five enzymes and sarcoplasmic proteins encoded by 11 loci (Table 1) were examined by electrophoretic methods. Among the 11

Table 1 Enzymes examined, loci identified, buffers used and tissues assayed.

Enzyme	Enzyme number	Locus	Buffer	Organ
Glycerol -3 phosphate dehydrogenase (G3PDH)	1.1.1.8	<i>G3PDH-1</i>	C-APM	Skeletal muscle
Lactate dehydrogenase (LDH)	1.1.1.27	<i>LDH-C</i>	TC	Eye
		<i>LDH-B</i>	C-APM	Skeletal muscle, heart
		<i>LDH-A</i>	C-APM	Skeletal muscle
Malate dehydrogenase (MDH)	1.1.1.37	<i>MDH-3</i>	C-APM	Skeletal muscle
		<i>MDH-2</i>	C-APM	Skeletal muscle
		<i>MDH-1</i>	C-APM	Skeletal muscle
Malic enzyme (MEP)	1.1.1.40	<i>MEP</i>	C-APM	Skeletal muscle
Isocitrate dehydrogenase (IDHP)	1.1.1.42	<i>IDHP-1</i>	C-APM	Skeletal muscle
Sarcoplasmic protein (PROT)		<i>PROT-1</i>	C-APM	Skeletal muscle
		<i>PROT-2</i>	C-APM	Skeletal muscle

C-APM, Citric acid aminopropylmorpholine, pH (6.0); TC-Tris citric acid, pH (8.0).

loci, 3 were polymorphic in the Panaji sample, 3 in Colva, 2 in Ennur, 3 in San Thome and 7 in the Andaman sample. The allele frequencies and chi-square (X^2) values for the differences between observed and expected Hardy-Weinberg counts of phenotypes of each polymorphic enzyme are given in Table 2. *G3PDH-1*, *LDH-B*, *MDH-2* and *MDH-3* were polymorphic for the Andaman sample, while they were monomorphic for the west coast and east coast samples except for the *LDH-B* locus which was polymorphic in the Santhome sample. Another interesting locus was the sarcoplasmic protein-two (*PROT-2*). Three alleles (-100, -125 and -150) were detected at this locus in the west coast and east coast samples, with the most common allele being -100. In the Andaman sample, the *Prot-2* locus was nearly monomorphic with the most common allele being -150. Significant departures from the Hardy-Weinberg expectations were detected for *PROT-2* locus in all the 4 samples from the west coast and east coast (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 heterozygotes. Observed (H_o) and expected (H_e) heterozygosities at this locus were: Panaji $H_o = 0.4$, $H_e = 0.557$, Colva $H_o = 0.325$, $H_e = 0.384$; Ennur $H_o = 0.4$, $H_e = 0.528$; San Thome $H_o = 0.257$, $H_e = 0.415$. In the case of the Andaman sample, all of the loci studied including *PROT-2* were in Hardy-Weinberg expectations (*PROT-2* $H_o = 0.033$, $H_e = 0.033$). A highly significant difference was detected with the G-test at the *PROT-2* locus between Peninsular India and Andaman islands population ($G_2 = 236.06$, $P < 0.001$) (Table 3). The degree of heterogeneity among all of the Peninsular samples was not significant ($G_6 = 11.68$, $P > 0.05$). But there was a slightly significant difference between the two west coast samples ($G_2 = 6.9$, $P < 0.05$); that may be due to chance.

The phenetic relationships among the samples were determined using Nei's (1972) index of genetic distance (D) (Table 4). Distinct genetic differentiation was evident

Table 2 Allelic frequencies in *R. kanagurta*

Locus	Allele	<i>R. kanagurta</i>				
		Panaji	Colva	Ennore	San Thome	Andaman
<i>G3PDH-1</i>	-100	1	1	1	1	0.988
	-50	0	0	0	0	0.013
	N	100	40	60	35	40
	X ²	0	0	0	0	0.006
<i>LDH-C</i>	100	1	1	1	1	1
	N	100	40	60	35	40
<i>LDH-B</i>	100	1	1	1	0.986	0.988
	50	0	0	0	0.014	0.013
	N	100	40	60	35	40
	X ²	0	0	0	0.007	0.006
<i>LDH-A</i>	-100	1	1	1	1	1
	N	100	40	60	35	40
<i>MDH-3</i>	100	1	1	1	1	0.988
	50	0	0	0	0	0.013
	N	100	40	60	35	40
	X ²	0	0	0	0	0.006
<i>MDH-2</i>	100	1	1	1	1	0.987
	50	0	0	0	0	0.013
	N	100	40	60	35	40
	X ²	0	0	0	0	0.006
<i>MDH-1</i>	-100	1	1	1	1	1
	N	100	40	60	35	40
<i>IDHP-1</i>	125	0.008	0.025	0	0	0
	100	0.967	0.975	1	1	0.975
	75	0.025	0	0	0	0.025
	N	60	40	40	35	40
	X ²	0.007	0.026	0	0	0.026
<i>MEP</i>	125	0	0	0.008	0	0
	100	0.95	0.975	0.967	0.975	0.975
	50	0.05	0.025	0.025	0.025	0.025
	N	60	40	60	35	40
	X ²	0.166	0.026	0.071	0.007	0.026
<i>PROT-1</i>	-100	1	1	1	1	1
	N	60	40	60	40	40
<i>PROT-2</i>	-100	0.60	0.763	0.638	0.743	0.017
	-125	0.242	0.175	0.175	0.143	0
	-150	0.158	0.062	0.187	0.114	0.983
	N	60	40	60	35	40
	X ²	21.78 ^{***}	6.16 [*]	7.80 [*]	11.14 ^{**}	0.009

N, Number of individuals; X², difference between observed and expected phenotypic distribution in each polymorphic enzyme

***P < 0.001; **P < 0.01; *P < 0.05.

Table 3 Genetic distance between *R. kanagurta* populations from different localities

	Panaji	Colva	Ennore	San Thome	Andaman
Panaji	—	0.0017	0.0004	0.0016	0.0525
Colva	—	---	0.0015	0.0003	0.0698
Ennore	—	---	---	0.0008	0.0508
San Thome	—	---	---	---	0.0632

between the west coast, east coast and the Andaman samples. Within the west coast and east coast samples, the phenetic relationship was very close. D values ranged between 0.0003 and 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).

DISCUSSION

The average heterozygosity values in *R. kanagurta* (0.058 ± 0.098 and 0.066 ± 0.110) (Menezes *et al.* 1990a) 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 ($H = 0.048 \pm 0.033$) by Winans (1980). In the sciaenids (Menezes *et al.* 1990b, 1993) the average heterozygosity ranged from 0.029 to 0.094, in the grey mullets (Menezes *et al.* 1992) from 0.014 to 0.038 and in the pomfrets from 0.013 to 0.042 (Menezes 1993). 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).

There was virtually no genetic differentiation among the west and east coast populations of *R. kanagurta*. This lack of divergence

among the populations is reflected in small average genetic distance between the samples ($D = 0.0003$). Between the peninsular India and the Andaman samples, the average genetic distance was relatively large ($D = 0.059$). 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 amount 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 display less geographic variation in allele frequencies than those which, for example, spawn demersally attached eggs (Johnson 1975). Another reason that marine fishes are characterized by a general lack of population subdivision is that genetic drift is negligible at the very large population sizes typical of marine fishes (Grant *et al.* 1987). Even for the populations that become completely isolated from one another, at least IV (=population size) generations must pass before any substantial divergence is likely (Kimura 1955). Nevertheless, genetic subdivisions among the regional stocks of marine fishes have been reported, which appear to be the result of ancient allopatric subdivision rather than the result of differentiation *in situ* (Grant 1987).

The highly significant heterogeneity at the *PROT-2* locus and the existence of polymorphic and monomorphic samples at

the same locus provides a further evidence of the differences between the Andaman and the west coast/east coast populations. The Andaman Island population of *R. kanagurta* also shows differences in many biological and fisheries characteristics from the populations in the coastal waters of the Indian peninsula (Jones and Silas 1962, Luther 1973).

The significant departures from Hardy-Weinberg expectations, due to a deficiency of heterozygotes, observed at the *PROT-2* locus in all the west and east coast samples may be explained by the mixing of individuals from populations differing in the frequencies of the alleles. In the Andaman population all the individuals showed the presence of the -150 allele at the *PROT-2* locus, whereas the most common allele at this locus in the west/east coast populations was the -100 allele. A locus at which complete differentiation exists between the two populations can be used to diagnose the population to which an individual belongs (Ayala 1983). The *sp-2* (-150) allele in the west and east coast populations may indicate the occurrence of migration from the Andaman population. The ultimate test of the genetic basis of a particular variant is actual breeding data where the phenotypic ratios of progeny for a particular protein system are consistent with the phenotypes of the parent. In the absence of inheritance studies, the expression of a predictable pattern of electrophoretic variation for a given protein system in a species is usually a sufficient evidence for simple genetic variation, provided that consistent individual phenotypes are obtained from the multiple tests of a tissue (Allendorf and Utter 1979).

Although electrophoretic differences provide unequivocal evidence of genetic isolation, the presence of similar allelic frequencies does not necessarily provide conclusive evidence to the contrary (Grant and

Utter 1980). Hence, it is important to examine the variation at as many informative polymorphic loci as possible, to maximize the likelihood of detecting geographical variation, if at all it exists.

Further studies should be conducted on more number of loci in *R. kanagurta* from the Andaman Sea in order to get a complete understanding of the subpopulation structure in this species.

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