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Morphometric and Genetic Analyzes of Indian Mackerel (*Rastrelliger kanagurta*) from Peninsular India

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Abstract

A holistic approach, combining one phenotypic and two genotypic methods, was adopted to analyze possible population differences in Indian mackerel (*Rastrelliger kanagurta*) from selected centers in the East and West coasts of India. Principal component analysis of truss landmark variables revealed that the area encompassing depth between the origin of anal and origin of second dorsal and caudal peduncle depth has high component loadings. Bivariate scatter plots of principal components showed a great degree of morphometric homogeneity between Indian mackerel populations from Mandapam, Kochi and Karwar. Clustering pattern of polypeptide markers revealed relatively greater population homogeneity among Mandapam fish (58%) than Kochi samples (33%). The three random amplified polymorphic DNA (RAPD) primers used in the present study have generated a total of 59 loci varying in size from 560 to 4500 bp. None of the populations from Mandapam, Kochi and Karwar showed RAPD fragments of fixed frequencies, to be treated as population-specific markers. No significant differences were found among the three populations.

Introduction

The Indian mackerel, *Rastrelliger kanagurta* (Cuvier) is a pelagic shoaling fish widely distributed in the Indo-west Pacific region. It is one of the major marine fishery resources of India. Mackerel fishery is characterized by its fluctuations. During the period 1993-99, annual average catch was 217,000 t forming 8.9% of the total marine fish catch (Yohannan and Nair 2002). The catch has declined to 90,000 t in 2001, constituting about 4% of total annual marine landings (NMLRDC CMFRI). About 70% of mackerel catches are landed along the West coast and the rest along the East coast and the Nicobar islands (Noble et al. 1992).

Indian mackerel is a migratory species. Only limited information on the migration pattern is available (Venkataraman 1970). Devaraj et al. (1994) have studied the dynamics of exploited stock of Indian mackerel from the West coast of India. However, no comprehensive attempt has been made to study the stock structure of Indian mackerel. Knowledge of the stock structure of the target species is fundamental to scientific resource management as well as marine stock-enhancement programs (Shaklee and Bentzen 1998), which entail genetic analysis of stocks over and above their phenotypic analysis.

Early attempts made to delineate the stock structure of Indian mackerel from the east and west coasts were based on the traditional morphometry and meristics (Seshappa 1985). Truss protocol system (Strauss and Bookstein 1982; Bookstein et al. 1985), which is more useful than the traditional morphometric methods to discriminate "phenotypic stocks" (Cadrin 2000) has not been so far applied in the case of Indian mackerel.

Examination of genetic variation by electrophoresis of the primary gene products (proteins) provides a powerful tool for the population discrimination and identification (Ferguson 1980; Shaklee and Bentzen 1998). Protein polymorphism in Indian mackerel from different localities of peninsular India and Andaman Sea was studied using isozyme electrophoresis (Menezes et al.1990a; Menezes et al. 1993; Verma et al. 1994; Verma et al. 2000).

Random Amplified Polymorphic DNA (RAPD) polymorphisms (Welsh and McClelland 1990; Williams et al. 1990) are relatively easy to generate and are increasingly used for population genetic studies in marine fishes (Dahle et al. 1997; Bielawski and Pumo 1997; Mamuris et al. 1998). Jayasankar and Dharmalingam (1997a and b) have made a preliminary study on RAPD polymorphisms of Indian mackerel from Mangalore, Kochi and Mandapam.

Earlier studies have, in general, revealed low regional genetic differentiation in Indian mackerel. A holistic or multiple approach, combining at least 1 phenotypic and 1 genotypic method can measure different biological processes and often allows apparent discrepancies implied by each method to be resolved (Begg and Waldman 1999). Hence the present study combined phenotypic and genotypic methods to determine stock relationships among Indian mackerel off east and west coasts of India.

Materials and Methods

Samples of Indian mackerel were collected from three locations situated in the East and West coasts of India (Fig. 1). Table 1 furnishes a summary of sample collection details.

Truss morphometrics

Morphological identification of *R. kanagurata* was based on the description of Fisher and Bianchi (1984). Samples were collected during the same

period from all the landing centers to avoid seasonal variation. Saila and Martin (1987) suggested a rule of thumb that 'n' (number of fish) should be at least three times 'p' (number of landmark positions). In the present study 'n' was 3x greater than 'p' (=10).

The truss protocol system of Indian mackerel was based on 10 homologous anatomical landmarks (Fig. 2): (1) anterior tip of snout on the upper jaw, (2) intersection of preopercle below posterior margin of eye, (3) nape above intersection of opercle, (4) origin of pelvic fin, (5) origin of first dorsal fin, (6) origin of anal fin, (7) origin of second dorsal fin, (8) insertion of anal fin, (9) dorsal origin of caudal fin and (10) ventral origin of caudal fin. Measurements were taken by placing the fish on water resistant paper sheet

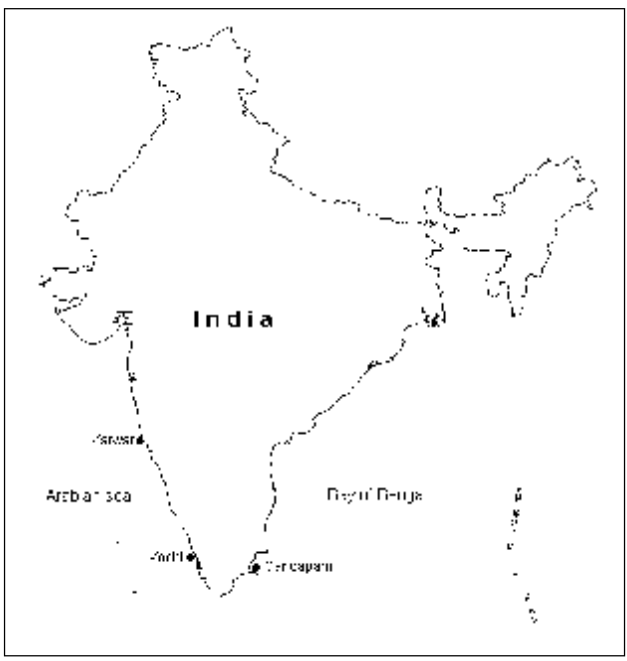

Fig. 1. Sample collection sites of Indian mackerel in the present study

Table 1. Summary of sample collection details

Method	Region	Sample size	Total length (mm)	Period of collection
Truss morphometrics	Mandapam (9°17'N, 79°22'E)	71	220-247	October-December 2001
	Kochi (9°58'N,76°22'E)	75	220-250	November-December 2001
	Karwar (13°48'N,74°31'E)	55	200-260	October-December 2001
Protein Polymorphisms	Mandapam (9°17'N, 79°22'E)	24	220-247	October-December 2001
	Kochi (9°58'N,76°22'E)	24	220-250	November-December 2001
RAPD	Mandapam (9°17'N, 79°22'E)	22	220-247	October-December 2001
	Kochi (9°58'N,76°22'E)	21	220-250	November-December 2001
	Karwar (13°48'N,74°31'E)	22	200-260	October-December 2001

and pricking the paper with a vertical dissection needle at points corresponding to the anatomical landmarks. The x and y coordinates of each landmark point were used to calculate the distance, D as:

$$D = \sqrt{(x1 - x2)^2 + (y1 - y2)^2}$$

Since size differences can hamper stock identification, a "size correction" method based on shear algorithm (Rohlf and Bookstein 1987) was used to analyze the truss morphometric data in the present study. This method adjusts for residual size effects in principal component PC 2 and PC 3, allowing comparisons of variation in shape of the fish among samples that differ in size distributions, PC 1 explains only the variations in size. Scatter plots of principal component scores were generated using SYSTAT 7.0 package.

Protein polymorphisms

Protein profiles of 24 individuals each from Mandapam and Kochi (Table 1) were compared using SDS electrophoresis of muscle proteins under denaturing and reducing conditions. Muscle tissue was homogenized in distilled water and centrifuged at 10,000 rpm for 20 min. The clear supernatant was mixed with buffer containing lauryl sulphate and â-mercaptethanol in equal volumes, heat digested at 100°C, cooled to room temperature and subjected to SDS PAGE at 30 mA for about 3 h. Following electrophoresis, the gel was stained with coomassie blue and the protein profiles were recorded with Microtek *Scanmaker 5*.

RAPD

About 200 to 400 mg of muscle and liver samples were collected from the freshly landed mackerel and stored in 95% ethanol. In the laboratory they were stored at -85°C till the extraction of DNA.

Genomic DNA was extracted from the muscle tissue of 65 Indian mackerel specimens following the method of Jayasankar and Dharmalingam (1997a). Approximately 200 mg of tissue was homogenized in digestion

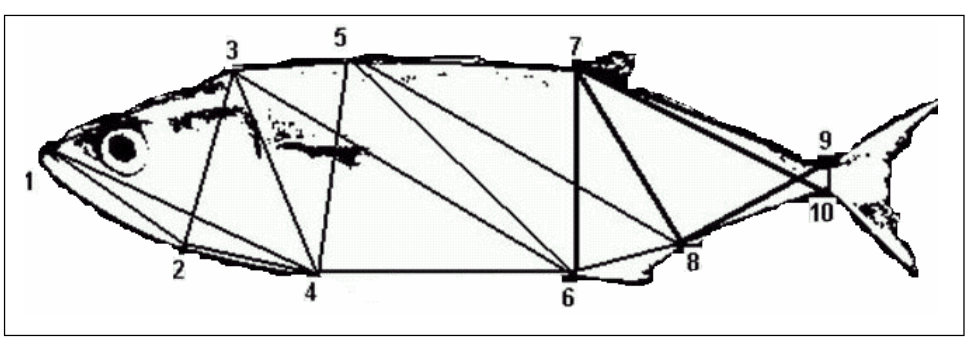

Fig. 2. Outline drawing of Indian mackerel showing the locations of the 10 anatomical landmarks (numbered points) and morphometric distance measures recorded on each individual

buffer (10 mM Tris HCl, 25 mM EDTA, 100 mM NaCl, 0.5% SDS and pH 8.0). Ten per cent SDS (1.0 g/ml) and proteinase-K (10 mg/ml) were added to the homogenate and incubated at 55^0C for 2 to 2 ½ h. After incubation, DNA was purified by successive extraction with buffered phenol, phenol: chloroform: iso-amyl alcohol (25:24:1) and chloroform: iso-amyl alcohol (24:1) respectively. DNA was precipitated with ice-cold ethanol and 3M sodium acetate (pH 5.2), washed with 70% ethanol, air dried and resuspended in 50 µl TE buffer (1M Tris HCl, pH 8.0; 0.5 M EDTA, pH 8.0). The quality and quantity of DNA extracts were checked using 0.8% agarose gel electrophoresis and a spectrophotometer.

Arbitrary primed PCR of the DNA samples were carried out with12 decamer primers (Operon Technologies Inc., USA) viz., OPA 01, OPA 02, OPA 03, OPA 04, OPA 05, OPA 06, OPF 01, OPF 02, OPF 03, OPF 04, OPF 05 and OPF 06 with a GC-content between 60 and 70 per cent. PCR was carried out in a total reaction volume of 25 µl containing 10 to 15 ng template DNA, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.2mM each of dATP, dCTP, dGTP and dTTP, 10 pM primer, and 1U *Taq* DNA polymerase (Bangalore Genei, India).

PCR was performed using thermal cycler, Perkin Elmer GeneAmp PCR System 2400 (Perkin Elmer, USA) programmed for an initial denaturation of 30s at 94ºC followed by 45 cycles each consisting of 30s at 94ºC (denaturation), 30s at 36ºC (annealing) and 120s at 72ºC (extension). A final extension was carried out at 72ºC for 7 min. The PCR products were separated in 1.5% agarose gels containing 1x TBE buffer at 100V for about 3 h. Gels were stained in ethidium bromide and documented using Bioprofil, a charge coupled device (CCD) video camera imaging system (Vilber Lourmet, France).

Data analysis of proteins and RAPD markers

The size of protein bands was determined by comparison with protein molecular weight markers, while that of RAPD bands was determined by comparison with a ë DNA digested with *Eco* RI / *Hin*d III molecular weight marker. Bioprofil (Bio-1D) was used to calculate the fragment sizes of the bands with reference to molecular size markers and the presence (1) or absence (0), of a fragment was scored. RAPD patterns of individuals were compared within and between populations.

A similarity index between all possible pair-wise comparisons of individuals was calculated using the formula:

$$S_{xy} = 2n_{xy}/(n_x + n_y),$$

where, n_x and n_y are the number of RAPD and protein fragments in individuals x and y, and n_{xy} is the number of fragments shared between those individuals (Nei 1978). Genetic distances between paired individuals or populations were calculated using Nei's distance (1978) and gene diversity was calculated using Nei (1973).

Phylogenetic relationships between individuals or populations of Indian mackerel were constructed using cluster analysis. For this, the unweighted pair-group method with arithmetic (UPGMA) (Sneath and Sokal 1973) contained in the NEIGHBOR program of PHYLIP ver 3.57c, based on Nei's (1978) genetic distance values was used. Data resampling (1000 replicates) and matrix calculations for bootstrap analysis were performed using WinBoot, a UPGMA-based program (Yap and Nelson 1996). Bootstrap values between 75 and 95 were considered to be significant (Hillis and Bull 1993). Percentage of polymorphic loci of RAPD markers was estimated using POPGENE version 1.31 (Yeh et al. 1999).

Statistical analysis

Test of differences in intra population and inter population genetic distance coefficients based on RAPD markers among the samples of Indian mackerel from Mandapam, Kochi and Karwar were made uisng one-way analysis of variance (ANOVA). Paired t-test was performed to test whether the intrapopulation genetic distance values differed from the interpopulaion values. The statistical analyzes were carried out using the Data Analysis option in Microsoft Excel.

Results

Truss morphometrics

Analysis of covariance matrix of size adjusted truss measurements for Indian mackerel from Mandapam and Kochi indicated that the first three PCs explained about 77.4% of variance of the morphometric characters (Table 2). PC 1, which represents size, explained 44.9% of the variation and exhibited component loadings that differed in magnitude with respect to characters. Strong positive loadings were associated with depth between origin of anal and origin of second dorsal (6-7), depth between origin of second dorsal and insertion of anal (7-8), distance between origin of second dorsal and dorsal origin of caudal fin (7-9) and distance between insertion of anal and dorsal origin of caudal (8-9). Strong negative loading was associated with caudal peduncle depth (9-10).

PC 2, which represents size-corrected shape, explained 19.6% of additional variation and has strong positive loadings for the distance between origin of second dorsal and dorsal origin of caudal fin (7-9), distance between insertion of anal and dorsal origin of caudal (8-9) and the distance between insertion of anal and ventral origin of caudal (8-10). Strong negative loadings were associated with the depth between origin of anal and origin of second dorsal (6-7) and caudal peduncle depth (9-10). The truss landmark variables in the body area encompassing the depth between origin of anal and origin of second dorsal and caudal peduncle depth contributed the maximum to the estimation of PC scores.

PC 3 explained 12.9% of additional variation. Bivariate scatter plots of PCs (Fig. 3) show great degree of morphological homogeneity among Indian mackerel populations from Mandapam, Kochi and Karwar.

Protein polymorphisms

The SDS PAGE resolved a total of 25 protein bands ranging in size from 13 kDa to >100 kDa. Three profiles were found, common pattern had 22 bands, and was found in both the Mandapam and Kochi samples. Two additional profiles resulted from three polymorphic bands, of which one was 15 kDa and the other two were >100 kDa in size. One variant profile in the Kochi samples had one additional band of e”100 kDa, the second in the variant profile of Mandapam samples had two additional bands of e”100 kDa and one additional15 kDa band. Cluster analysis of the distance matrix generated from protein profile data revealed no significant difference between Mandapam Kochi samples (Fig. 4).

RAPD

Of the 12 arbitrary primers initially screened, all except OPF 06 amplified mackerel DNA. However, for the population analyzes, OPA04, OPF02 and OPF05 produced repeatable, polymorphic and robust bands. Twenty two Indian mackerel from each of Mandapam and Karwar and 21 from Kochi were tested for 3 primers.

Table 2. Component loadings of the first three sheared principal components for truss morphometric characters in Indian mackerel

	Principal Component		
Character	1	2	3
1-2	0.086414	0.162668	0.136706
1-3	0.104550	0.168389	0.080505
1-4	0.080131	0.128513	0.083680
2-3	0.071344	0.151612	0.135883
2-4	0.066607	0.098382	0.049241
3-4	0.074738	0.131965	0.119046
3-5	0.070926	0.127824	0.151640
3-6	0.067379	0.121390	0.122943
4-5	0.084997	0.181508	0.171675
4-6	0.068932	0.151211	0.128659
5-6	0.069268	0.127348	0.136871
5-7	-0.021757	0.193701	0.184361
5-8	0.074952	0.144407	0.157544
6-7	0.878322	-0.373431	0.035325
6-8	0.056946	0.177519	0.262459
7-8	0.236255	0.084471	0.145665
7-9	0.134577	0.438859	-0.227558
7-10	0.089369	0.237986	0.014456
8-9	0.127853	0.300662	-0.032245
8-10	0.120753	0.399768	-0.085723
9-10	-0.200249	-0.195882	0.787647
Percent of variance explained	44.9	19.6	12.9

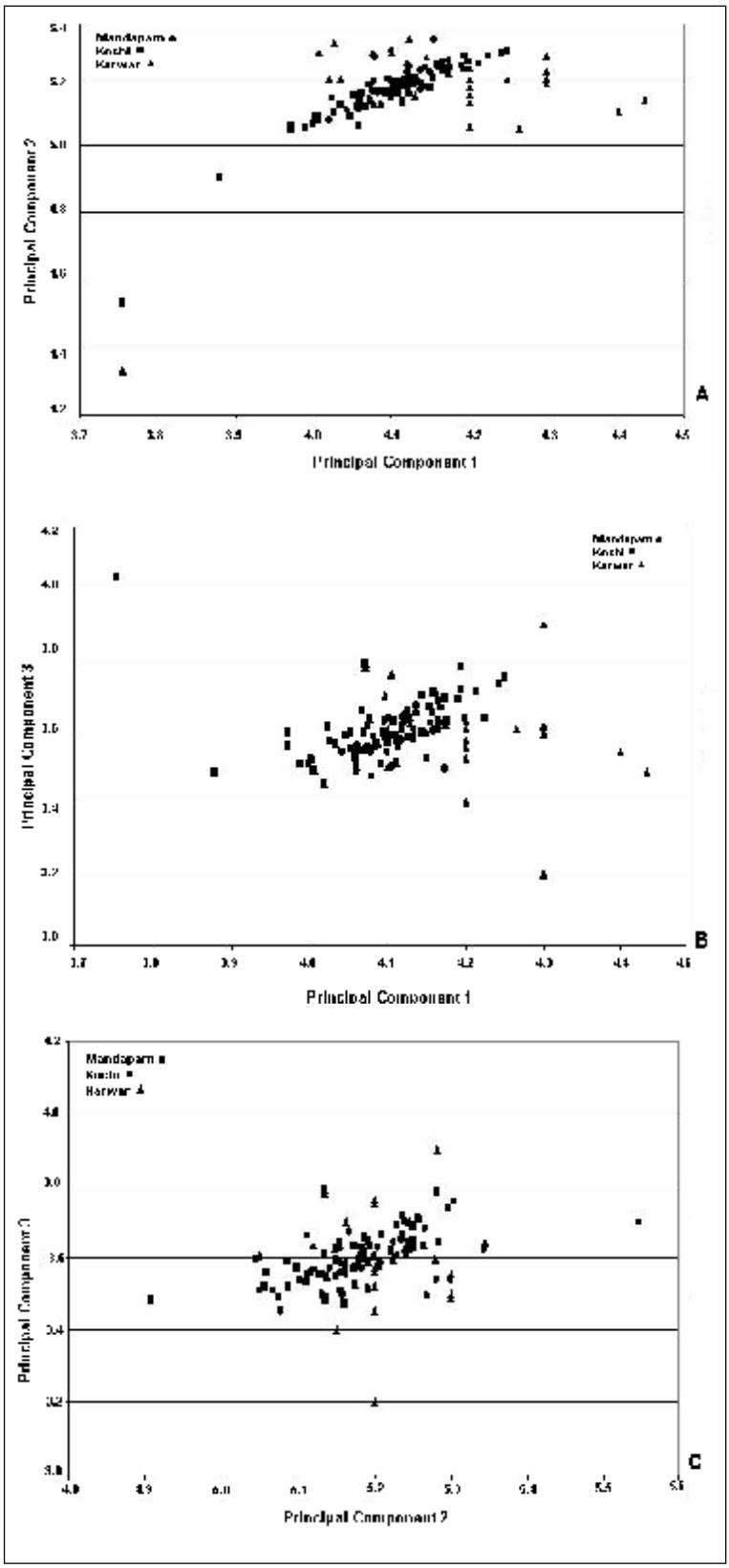

Fig. 3. Scatter plots of truss network landmarks of Indian mackerel from Mandapam, Kochi and Karwar showing relationships of PC 1 with sheared PC 2 and 3.

The three primers produced different RAPD fingerprint patterns and generated 59 clear and stable loci altogether with a size range of 560 to 4500 bp. Only those fragments having molecular weight ranging from 800 to 3500 bp were selected for analysis since they were more reproducible and robust. On an average, every primer generated 19.7 RAPD loci. None of the three populations showed RAPD fragments of fixed frequencies, to be treated as population-specific markers.

Average pair-wise Similarity Index (SI) and Genetic Distance (GD) values were calculated for all the 3 primers together (Table 3). Data show relative genetic proximity of Mandapam and Kochi samples, while Karwar

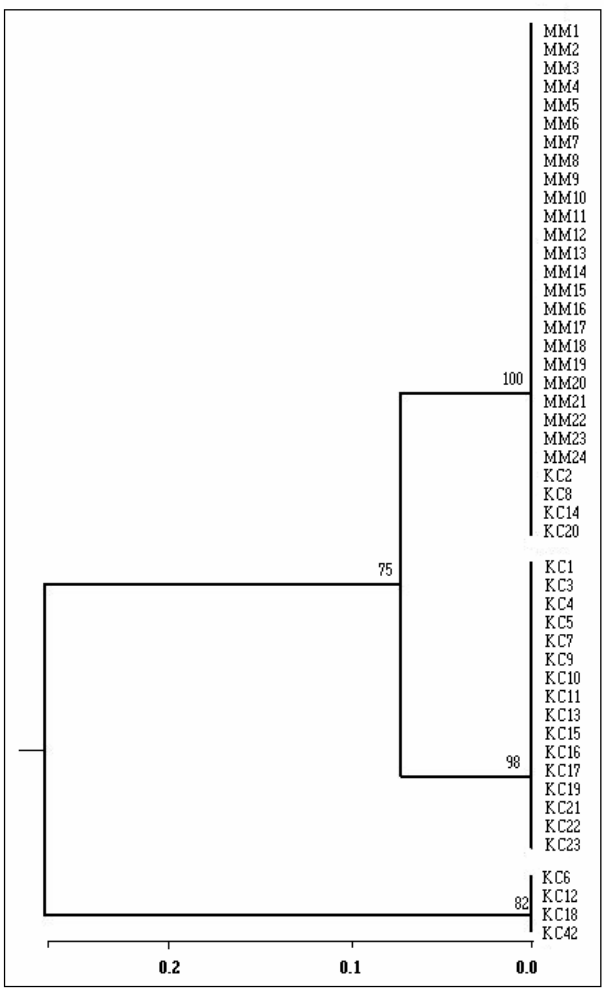

Fig. 4. UPGMA dendrograms constructed on the basis of the genetic distance calculated from protein electrophoresis, showing genetic relationship among 48 individuals of Indian mackerel from Mandapam and Kochi (MM, Mandapam; KC, Kochi).

Table 3. Data showing pair-wise comparison of SI (above diagonal) and GD (below diagonal) of Indian mackerel from Mandapam, Kochi and Karwar based on Nei (1978) calculated for primers OPA 04, OPF 02 and OPF 5

Centre	Mandapam (n=22)	Kochi (n=21)	Karwar (n=22)
Mandapam	—	0.8331	0.8124
Kochi	0.1826	—	0.8281
Karwar	0.2077	0.1886	—

sample is genetically most distant from Mandapam. UPGMA dendrogram constructed from the genetic distance values (Nei 1978) generated by three primers shows Mandapam marginally closer to Kochi (82%) than Karwar (80%) (Fig. 5 A).

The SI and GD within three populations were calculated and based on these values, dendrogram was constructed to show the genetic relationships among 65 different individuals of Indian mackerel. Though by and large the individuals from Mandapam, Kochi and Karwar have grouped with their respective center clusters, each center has shown multiple clusters with no clear pattern (Fig. 5 B).

To assess the level of agreement among the three primers, the correlation between the genetic similarities for each primer between different populations (Table 4) was calculated. Value (r = 0.9927) between OPA 04 and OPF 02 was highly significant (P < 0.01), but those between other two com-

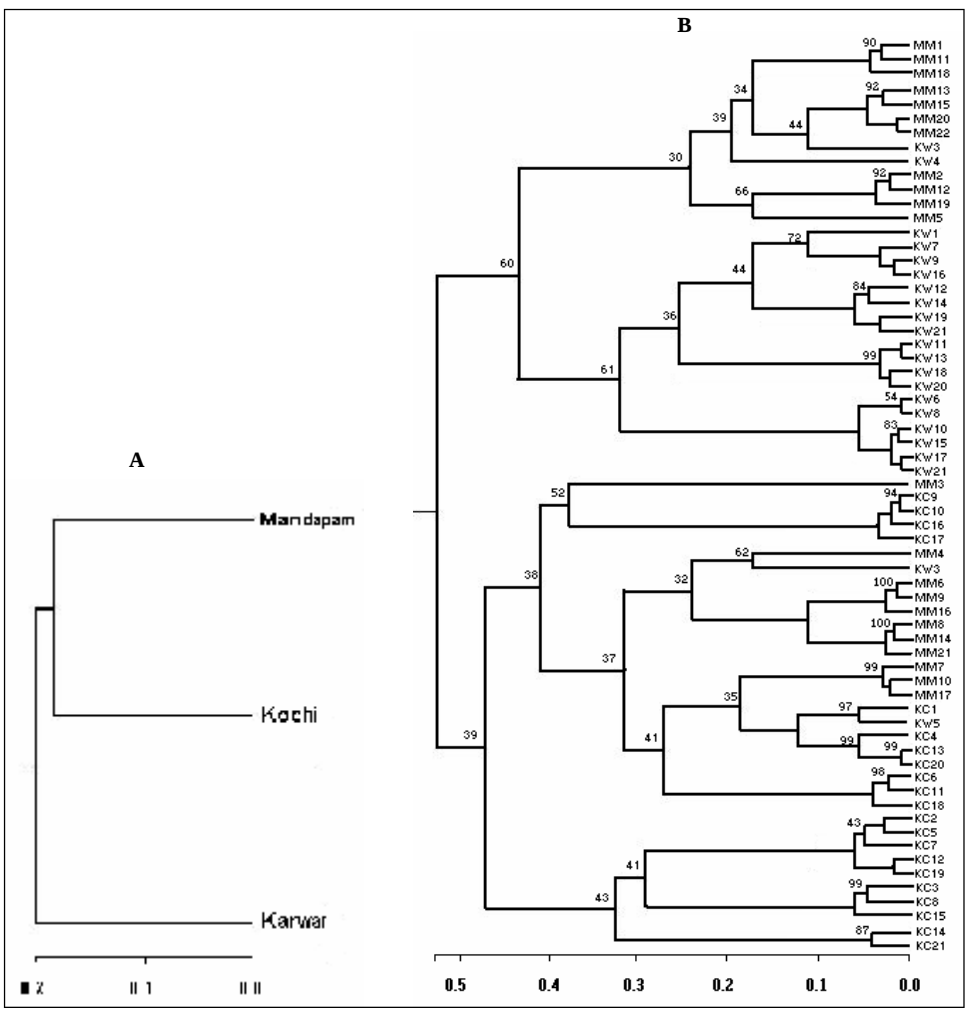

Fig. 5. UPGMA dendrograms constructed on the basis of the genetic distance calculated from RAPD assay (3 primers), showing genetic relationship among three populations of Indian mackerel (A) and 65 individuals representing them (B) (MM, Mandapam; KC, Kochi; KW, Karwar).

binations were insignificant, indicating no agreement across these primers. Intra-population and inter-population GD values were tested by one-way ANOVA and found not significant (Tables 5 and 6).

Statistical analysis was also carried out to test for differences in intrapopulation and inter-population GD values. Results of paired t-test showed that the intra-population GD (number of pair wise comparisons = 301, mean=0.314) was significantly lower ($t = 32.562$, $df = 300$, $P < 0.005$) than the inter-population GD values (number of pair-wise comparisons = 503, mean = 0.676).

Table 7 furnishes genetic information of Indian mackerel sampled from the selected locations. Gene diversity (Nei 1973) is equivalent to average heterozygosity (H) (Nei 1987) and is a measurement of genetic variation for randomly mating populations. Nei's (1978) genetic similarity index reveals virtually no variation among the three populations.

Table 4. Genetic similarity for each primer among the different samples of Indian mackerel

Primer	5' to 3' Sequence	Mandapam/ Kochi	Mandapam/ Karwar	Kochi/ Karwar
OPA 04	AATCGGGCTG	0.8982	0.9111	0.8132
OPF 02	GAGGATCCCT	0.6803	0.6801	0.8240
OPF 05	CCGAATTCCC	0.9349	0.8649	0.8644
Mean		0.8378	0.8187	0.8339
SD		0.1376	0.1222	0.027

Table 5. Summary of the results of one-way ANOVA to test for differences in intra population GD values calculated based on RAPD markers among Mandapam, Kochi and Karwar samples of Indian mackerel

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F	Probability
Within population	0.0056	2	0.0028	0.1080	0.8976*
Error	7.7470	298	0.0260		
Total	7.7526	300			

*Not significant

Table 6. Summary of the results of one-way ANOVA to test for differences in inter population GD values calculated based on RAPD markers among Mandapam, Kochi and Karwar samples of Indian mackerel

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F	Probability
Within population	0.0527	2	0.0264	2.7509	0.0648*
Error	5.0788	530	0.0096		
Total	5.1315	532			

* Not significant

Discussion

One of the important considerations in the management of a fishery resource is the identification of discrete populations or stock units which are generally defined as self maintaining groups, temporarily or spatially isolated from one another and considered 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 (Iles and Sinclair 1982). Failure to recognize or to account for stock complexity in management units has led to an erosion of spawning components, resulting into a loss of genetic diversity and other unknown ecological consequences (Begg et al. 1999).

In the truss protocol (Strauss and Bookstein 1982), it is essential to quantify shape difference among the individuals separately from size differences because sizes can be expected to vary with the time of the year, nutritional status, etc. The effects of size can mask subtle and more biologically interesting patterns of covariation among suites of variables (Rohlf and Bookstein 1987). Hence the 'shear' method of Rohlf and Bookstein (1987) was used in the present study to investigate probable population differences in Indian mackerel in the peninsular India.

Rohlf and Marcus (1993) have opined that phenotypic variation is more applicable to study short-term environmentally influenced differences between fish stocks. The morphometric data indicate that there is little differentiation of Indian mackerel populations from the East and the West coasts of India. It is advantageous to sample fish during the spawning season for phenotypic stock study, because spawning stocks are geographically separated at that time (Cadrin 2000). In the present study, Indian mackerel from all the sampling centers used for truss morphometric analysis showed predominance of mature/ripe/spent fish, thus fulfilling this requirement.

In the present study, RAPD analysis revealed more genetic variability among individuals compared to their protein profile. Since proteins are expressed traits subject to natural selection and culling, only very few variant isoforms which have any fitness value shall remain in the population. On the other hand, major portion of the DNA in the cell are non-coding regions, which can accumulate genetic variations, as they are not subjected to natural selection. RAPD analysis detects a lot of DNA variations accumulated in the genome, which are not transcribed and translated into proteins that can

Table 7. Gene diversity (Mean±SD), percent of polymorphism and within population, genetic similarity (Mean±SD) in Indian mackerel from three locations based on RAPD data

Parameter	Mandapam (n = 22)	Kochi (n = 21)	Karwar (n = 22)
Gene diversity (Nei 1973)	0.34 (±0.18)	0.30 (±0.21)	0.24 (±0.22)
No. of polymorphic loci	17	16	12
Percentage of polymorphism	80.95	76.19	57.14
Genetic similarity (Nei 1978)	0.739 (±0.12)	0.735 (±0.11)	0.744 (±0.12)

be detected through cellular protein profiling. Populations of hilsa shad (*Tenualosa ilisha*) and red mullet *Mullus barbatus* exhibited more genetic variation with RAPD than with allozymes when these two approaches were used (Dahle et al. 1997; Mamuris et al. 1998).

All the three locations of the present study had multiple clustering patterns, with some individuals not clustering with the rest of their respective samples or not placed within the corresponding geographical populations. One way-ANOVA has shown that the inter population variations in genetic distance among the three populations is not significant and thus discount any regional differences in populations of Indian mackerel. In an earlier study, Jayasankar and Dharmalingam (1997b) could not observe center-specific clusters in the dendrograms of genetic distances of Indian mackerel from Mandapam, Kochi and Mangalore.

Despite the variations observed in the growth parameters of Indian mackerel at various centers, including Ratnagiri, Karwar, Mangalore, Calicut, Cochin, Quilon and Vizhinjam along the southwest coast of India, the stock has been considered as a 'unit stock' primarily because of the continuity in the distribution of the population within the stock area (Devaraj et al. 1994). There was virtually no genetic differentiation in the allozyme patterns between the Goa and Tamil Nadu samples of Indian mackerel and the lack of divergence among these populations was reflected in small average genetic distance between the samples (Menezes et al. 1993). The present study has indicated that the populations from Karwar, Kochi and Mandapam region could belong to the same stock. The Andaman island population of *R. kanagurta* is reported to show differences in many biological and fisheries characteristics as well as in the allozyme patterns from the populations in the coastal waters of peninsular India (Jones and Silas 1962; Luther 1973; Menezes et al. 1993), which, however, could not be taken up in the present investigation.

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 genetic variation compared to species having demersally attached eggs (Johnson 1975). Indian mackerel belongs to the former category, hence showing no regional differences in its populations.

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